Monograph on Propylene Glycol & Derivatives 1/5/73

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MONOGRAPH ON PROPYLENE GLYCOL AND DERIVATIVES

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INFORMATICS INC.
6000 Executive Boulevard
Rockville, Maryland 20852

PROPYLENE GLYCOL
AND DERIVATIVES

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PROPYLENE GLYCOL
and
DERIVATIVES

Summary

Propylene glycol was discovered in 1859 by Wurtz, who prepared it from its diacetate, but it did not become commercially important until 1931, when it was first produced by the reaction of water with propylene chlorohydrin-derived propylene oxide. Since that time it has become increasingly important as a component of certain food and pharmaceutical products and as a possible carbohydrate substitute. As a result, numerous investigations of its pharmacology and toxicology have been performed.

In vivo studies on a variety of test animals, including man, have demonstrated that propylene glycol is rapidly absorbed from the gastrointestinal tract and promptly distributed throughout the body (109, 157, 190, 224, 257). In a study by Lehman and Newman (157) it was found that, although propylene glycol is rapidly absorbed in the dog, only slight absorption occurred when propylene glycol was introduced into the isolated stomach of a dog.

In vitro tests by Rudney (220) established that ^{14}C-1-propylene glycol can be split to give a 1-carbon unit entering the formate pool and in addition can be converted to a 3-carbon precursor (lactate) of the glycolytic cycle. By injecting rats i.p. with ^{14}C-labelled propylene glycol, Rudney (218) found that the ^{14}C-label was incorporated into the liver serine beta-carbon, the choline (isolated from the abdominal viscera) methyl groups, and the 2,5- and 1,6-carbons of the liver glycogen. Relatively high activity of the 3,4-carbons of the glycogen suggests that some of the propylene glycol is converted directly to lactic acid (218). Following intrarumenal administration of ^{14}C-labelled propylene glycol to a cow, Emery et al. (66) found that, during the first 24 hours following administration, the percentage of the dose recovered was 43.7 in expired CO_2, 12.4 in milk, 3.5-7 in urine, and less than 0.1 in feces. On the basis of the ^{14}C distribution it was concluded that propylene glycol is converted to glucose via carboxylation of pyruvate to oxalacetate and that the glucogenesis is primarily hepatic with oxidation primarily occurring in other tissues (66).

Long et al. found that propylene glycol distearate can be hydrolyzed by in vitro steapsin to yield free fatty acid at a rate of approximately 50% in 12 hours at 30°C (163).

Balls and Matlock found propylene glycol monostearate to be hydrolyzed by in vitro pancreatic enzyme to the extent of about 80% in 15 hours at 40°C (12).

Through in vitro studies on pancreatic enzyme homogenates, Fodor demonstrated that propylene glycol dibutyrate, propylene glycol

monobutyrate and propylene glycol dipropionate undergo hydrolysis at a high initial velocity which slows down rapidly after having brought about a 5-8% cleavage of the ester (76).

By administering ^{14}C-labelled propylene glycol distearate to rats, Long et al. (163) found that this compound is hydrolyzed to form propylene glycol, which is then rapidly absorbed and, for the most part, metabolized to CO_2. In a successive study by Long et al. (164), it was found that propylene glycol distearate is hydrolyzed in the gastrointestinal tract of the rat to yield propylene glycol monostearate, propylene glycol, and stearic acid.

On the basis of tests employing mice and rats, several investigators have reported that propylene glycol, which has a nutritive value of approximately 4 calories per gram (97), exhibits a moderate glycogenic action (224, 194, 107).

Van Winkle (257) found that rats given propylene glycol in their drinking water at concentrations of 0-20% excreted via the kidneys approximately one third of the amount ingested, regardless of the level consumed. Lehman and Newman (157) and Hanzlik et al. (109) reported similar results in dogs. Analysis of the urine of rabbits which had received propylene glycol orally revealed neither the unchanged dial nor any conjugated glucuronic acid (88).

Newman et al. (191) reported that propylene glycol inhibits the normal metabolism of isolated livers of cats. Intragastric doses of 5-10 ml of propylene glycol per kg body weight were found by Van Winkle (257) to significantly depress oxygen consumption of white rats. Giri (89) reported that rats treated with a high dose of propylene glycol have a drastically reduced capacity to synthesize or to store liver glycogen. Lehman and Newman (157) found that intragastric administration of propylene glycol to dogs produced a diuretic effect and an intoxicating action similar to that produced by alcohol.

Fommel and Vallette (79) reported that propylene glycol has a weak anti-acetylcholine effect and therefore is capable of modifying the action of pharmacodynamic substances in which it serves as vehicle or solvent.

In a series of studies on the reproduction of rats fed diets containing as much as 30% propylene glycol, Guerrant et al. (101) resolved that no untoward result on reproduction was effected by propylene glycol until the diet contained a level of 7.5% or more, whereupon it was observed that the treated rats consumed less food, grew more slowly, matured more slowly, produced smaller litters, and weaned fewer and smaller young than did the controls.

During investigation of the teratogenic effects of propylene glycol, Gebhardt (84) noted that 90% of the chick embryos injected

with 0.05 ml of propylene glycol in the air chamber on the fourth day of incubation died within 2 hours and 20% of those surviving had asymmetric malformations of the limbs. When 0.2 ml of propylene glycol was injected into the yolk sac on the fourth day of incubation a large liquid-containing cyst was observed on the dorsol side of 40% of the embryos (84). McLaughlin et al. (171) reported that injection of 0.05 ml of undiluted propylene glycol into the yolk sac of fertile eggs prior to incubation does not effect their viability.

Bowen and Waldroup (30) reported that feeding of 10% propylene glycol to chicks significantly increased the occurrence of toe deformities. In a later study, Bowen and Waldroup (31) found that there was a significant linear increase in the number of hock and toe deformities in chicks as the level of propylene glycol in the diet increased about 2.5%.

Seidenfeld and Hanzlik (230) reported that propylene glycol caused a moderate, though fleeting, local irritation of mucosa (tongue of man, conjunctiva of rabbits), and in the muscles and subcutaneous tissues of rats, rabbits, and man. Undiluted propylene glycol was reported to cause no irritation on the skin of the rabbit belly (184). Animal studies and repeated insult tests on human volunteers did not demonstrate development of skin sensitivity to propylene glycol (184). Flooding the rabbit eye with an excess of propylene glycol caused a reaction no more severe than moderate inflammation (184). Bucciardi (39) reported that, when subcutaneously injected into the gluteal area of human subjects in doses of 0.4 ml, propylene glycol causes an intense burning pain which subsides in 10-15 minutes. Application of pure propylene glycol on the conjunctiva of the rabbit caused blepharospasm and a violent inflammation of the conjunctiva (39). In the treatment of patients with leg ulcers, Huriez et al. (126) found that 16 of 38 patients developed an allergic reaction to propylene glycol after cutaneous treatment with a propylene glycol-containing product.

Extensive studies on the feeding of propylene glycol have been carried out in the last 40 years. These tests, which have been performed mainly on rats, have included feeding of dietary levels as high as 60% and have covered periods as long as 2 years. A table of acute toxicity is presented on page 00.

In three experiments on chicks, performed by Persons et al. (202), in which propylene glycol was fed at levels of 2.5, 5, and 10% for periods of 21 days, the higher levels of propylene glycol depressed body weight gain and feed efficiency. When hens were fed 5% propylene glycol for two 28-day periods, reductions were observed in feed consumption and egg production (202). Both the hens and chicks tolerated a level of 2.5% propylene glycol (202). Bayley et al. (15) reported that propylene glycol at levels up to 8% had no adverse effect on chicks when fed from day-old to 26 days of age but that at a level of 16% the diol adversely affected weight gain and the feed:gain ratio, lowered the fat content of the carcas, and increased the protein content. Three experiments by Waldroup and Bowen (264) indicate that propylene glycol can be utilized by chicks without adverse effects at levels not exceeding 5% of the diet - higher levels resulted in depressed body weight gains, reduced feed efficiency, diarrhea,

and the development of deformed toes and hocks. In a study by Yosida et al. (275) in which 40 male White Leghorn chicks were raised on diets containing propylene glycol at levels of 0, 5, 10 and 20%, the dietary level of 5% was found to produce normal growth while levels over 10% were reported to be less favorable.

In a feeding study by Guerrant et al. (101), groups of young growing rats were given dietary levels of propylene glycol as high as 60% for a period of 20 weeks. At dietary levels below 30% no marked difference in growth response was noted, but at the 30% level propylene glycol caused a significant depression of growth rate and at higher levels weaning rats invariably died within a few days after the start of the test (101). At 10%, pathological changes, mainly in the kidneys, were frequently found and at higher levels the frequency and severity of the changes became more pronounced (101).

For a period of 100 days, Weatherby and Haag (267) gave propylene glycol to male rats in concentrations up to 10% in their drinking water, thereby causing a reduction of growth rate (for the rats given 10%) during the first 10 days of the test, followed by apparent recovery and normal growth. Stoewsand et al. (242), while investigating the effect of feeding 30% propylene glycol on tissue lipids and the resistance of rats to extreme cold, found that epididymal adipose tissue was lowered in the treated group while liver lipids and liver cholesterol were increased and the resistance to intense cold was reduced. Lowered weight gains, lowered food intake, decreased epididymal adipose tissue, and increased total liver lipids and liver cholesterol were exhibited by male albino rats fed, by Stoewsand and Dymsza (243), for 4 weeks a diet containing 20% propylene glycol.

In a feeding study by Hanzlik et al. (109), in which 0, 25, 50, 75 or 100% of the carbohydrate of the diet of young growing rats was replaced with propylene glycol, the rats at the highest level maintained a fairly constant weight and died in about 1 month, while the growth rate of those rats given the lowest two levels indicated a lack of serious impairment. Van Winkle and Newman (260) found that replacing 25% of the carbohydrate of the diet or rats caused the treated rats to grow more rapidly, and larger, and exhibit a higher liver glycogen level than controls. According to Hanzlik (109), when 25% of the carbohydrate of the diet of 6 rats was replaced with propylene glycol, the treated rats showed a definitely greater average increase in body weight than did the controls.

The voluntary running activity of female white rats was found by both Hanzlik et al. (107) and Van Winkle and Kennedy (259) to be increased by the inclusion of 12% propylene glycol in their diets.

Of six 4.5-month-old female rats given a 3.58% propylene glycol solution as drinking water for 11 weeks, Holck (117) reported that 1 died after 10 weeks, 1 had lost considerable weight and the others

were beginning to lose weight at the end of the experiment. Studies by Asuncion-Vinals (9) indicate that rats can tolerate a 10% propylene glycol solution as their drinking water but a 50% solution causes death as a result of decreased food and water intake. Kesten et al. (140) found no demonstrable effect from the administration of propylene glycol to 39 rats in their drinking water in concentrations from 1 to 10% for from 11 to 234 days. Hunt (124) reported that young rats grew at the normal rate and reached maturity when the only liquid they received was a 5% solution of propylene glycol. Gaunt et al. (83) reported finding no significant differences between control rats and those given 5% propylene glycol for a period of 15 weeks. Slight liver damage was noted by Morris et al. (178) in 20 albino rats fed 2.45 or 4.9% propylene glycol for a period of 24 months. No statistically significant differences between treated and control rats were found when 60 rats were maintained for a period of 2 years on diets containing as much as 5% propylene glycol (83). On the basis of these findings, Gaunt et al. (83) suggested that a propylene glycol intake of 25 mg/kg/day would be acceptable in man.

Lepkovsky et al. (159) reported that the propylene glycol esters of the fatty acids of lard, when fed to six 21-day-old rats at a level of 60% of the diet, allowed normal growth.

While administering, intragastrically, 0-8.4 g of propylene glycol/kg/day to 18 rabbits for a period of 50 days, Braun and Cartland (31) noted satisfactory growth in the animals at the lower dosages while at the higher levels the rabbits exhibited no significant change in body weight throughout the test period.

In a 2-year feeding study in which beagle dogs were given 2-5 g of propylene glycol/kg/day Weil et al. (268) found that propylene glycol at the 5g/kg/day level produced increased weight gain, destruction of erythrocytes with accelerated replacement from the bone marrow, and a slight increase in bilirubin. No adverse effects were observed at the 2g/kg/day level (268). Van Winkle and Newman (260) reported that administration of 5-10% propylene glycol-solution to dogs in place of drinking water for 5-9 months produced no untoward effects.

Hanzlik et al. (107) reported that propylene glycol, in single oral doses of 50 ml, produced no effect on the basal metabolic rates of 3 human subjects.

Propylene Glycol

I. Nomenclature

A. Common Names

1. Propylene glycol
2. 1,2 Propanediol
3. 1,2 Dihydroxypropane
4. Methyl glycol
5. Methylethylene glycol
6. Trimethyl glycol

B. Chemical Name

(±) -1,2-Propanediol

C. Trade Names

(none)

D. Chemical Abstracts Registry Number

000057556

II. Empirical Formula

$C_3H_8O_2$

III. Structural Formula

$CH_2OH-CHOH-CH_3$

IV. Molecular Weight

76.10

V. Specifications

Food chemicals codex

Assay	Not less than 97.5%, by weight, of $C_3H_8O_2$
Distillation range	Between 185°-189°
Specific gravity	Between 1.035-1.037
Limits of Impurities	
Acidity	Passes test
Arsenic (as As)	Not more than 3 ppm
Heavy metals (as Pb)	Not more than 10 ppm
Residue on ignition	Not more than 0.07%
Water	Not more than 0.2%

VI. Description

A. General characteristics

Propylene glycol is a clear, colorless, odorless liquid with a slightly characteristic taste.

B. Physical Properties

Propylene glycol is miscible in water, ethanol, acetone, and chloroform. It is soluble in ether and will dissolve many essential oils, but is inmiscible with fixed oils. It is viscous and hygroscopic, has a density of 1.036, a freezing point of -59.0°C, and a boiling point of 188.2°C.

C. Stability

Under ordinary conditions, it is stable. But at high temperatures it tends to oxidize, giving rise to products such as propionaldehyde, lactic acid, pyruvic acid, and acetic acid.

VII. Analytical Methods

Propylene glycol occurs in many commercial products. The official method for the determination of propylene glycol in vanilla extract is by refluxing the sample with water and heptane for 8 hours. The aqueous layer in the receiver contains the propylene glycol (8).

After isolation, potassium periodate is added and then titrated with potassium arsenite to the disappearance of the blue color (8).

Eibl and Lands quantitatively determined vicinal diols using quantitative cleavage of dry alcohol solutions of diols with periodate, followed by coprecipitation of excess periodate with aluminum hydroxide. The supernatant is acidified and potassium iodide is added. Spectrophotometric determination of iodine is made at 350 nm. This method allows for accurate determination of 10-500 nanomoles of diol (65).

Propylene glycol has been quantitatively isolated from dentifrices using gas chromatography. The dentifrice sample was suspended in pyridine, silylated with trimethysilane, and injected. Prior to injection, erythritol was added as an internal standard. Using temperature programming, propylene glycol comes off the column at 105°C and was separated from glycerol and sorbitol. The percentage of propylene glycol present was then calculated (22).

DeSimone and Vicedomini have separated propylene glycol from several other diols, triols, and tetrols using thin-layer chromatography on lead (II) impregnated Silica Gel H plates. The liquid phase consisted of a mixture of ethanol, ammonia, and water. In an alkaline medium, a complex formation occurred between

lead (II) and polyols; this formation was affected by the number and position of the hydroxyl groups. The mobility of the polyols decreased with an increase in the number of adjacent hydroxyl groups. Propylene glycol was separated from 1,3-propanediol ethylene glycol, 1,2,5-trihydroxypentane, 1,2,4-butanetriol, glycerol, erythritol, mannitol, dulcitol, and sorbitol (60).

VIII. Occurrence

Synthetic

Propylene glycol is prepared industrially from propylene (obtained in the cracking of propane); the propylene is converted to the chlorohydrin by chlorine water and then converted to the glycol by Na_2CO_3 soln. It can also be made by heating glycerol with NaOH (Optically inactive form).

Propylene Glycol Monostearate

Chemical Information

I. Nomenclature

A. Common Name

Propylene glycol monostearate

B. Chemical Names

1,2 Propylene glycol monostearate
1,2 Propylene glycol stearic acid, monoester
2-Hydroxypropyl stearate
Stearic acid, 2-hydroxypropyl ester

C. Trade Names

(none)

D. Chemical Abstracts Registry Number

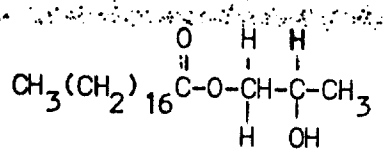
MX8028464

II. Empirical Formula

$CH_3(CH_2)_{16}CO\text{-}O\text{-}CH_2CH(OH)CH_3$

III. Structural Formula

Propylene glycol monostearate is a mixture of propylene glycol mono- and diesters of stearic and palmitic acids.



IV. Molecular Weight

(not applicable)

V. Specifications

Food Chemicals Codex

Total monoester content.	Not less than the minimum percent claimed by the vendor.
Hydroxyl value, Iodine value, and Saponification value.	Not greater than the values stated or within the range claimed by the vendor.

Limits of Impurities

Acid value.	Not more than 20.
Arsenic (as As).	Not more than 3 parts per million (0.0003 percent).
Free propylene glycol.	Not more than 1.5 percent.
Heavy metals (as Pb).	Not more than 10 parts per million (0.001 percent).
Soap (as potassium stearate).	Not more than 7 percent.

VI. Description

A. General characteristics

Propylene glycol monostearate occurs as white beads or flakes having a bland odor and taste.

B. Physical Properties

Propylene glycol monostearate is insoluble in water, but soluble in alcohol, ethyl acetate, and chloroform and other chlorinated hydrocarbons.

C. Stability

Store in well-closed containers.

VII. Analytical Methods

(see propylene glycol)

VIII. Occurrence

Synthetic

Prepared from 2 moles propylene glycol and 1 mole of hydrogenated soybean oil with an alkaline earth catalyst.

Biological Data

I. Acute toxicity

Substance	Animal	Sex & No.	Route	Dosage (g/kg)	Measurement	Ref.
Propylene Glycol	Mice	60 F	i.p.	9.73	LD_{50}	134
Propylene Glycol	Mice	24	s.c.	14.5	LD_0	154
Propylene Glycol	Mice	24	s.c.	19.2	LD_{50}	154
Propylene Glycol	Mice	24	s.c.	24.8	LD_{100}	154
Propylene Glycol	Mice	24	s.c.	10.4	MSD*	154
Propylene Glycol	Mice	24	s.c.	8.3	MND*	154
Propylene Glycol	Mice	24	p.o.	18.6	LD_0	154
Propylene Glycol	Mice	24	p.o.	22.8	LD_{50}	154
Propylene Glycol	Mice	24	p.o.	26.9	LD_{100}	154
Propylene Glycol	Mice	24	p.o.	8.3	MSD*	154
Propylene Glycol	Mice	24	p.o.	6.2	MND**	154
Propylene Glycol	Mice	24	i.v.	5.2	LD_0	154
Propylene Glycol	Mice	24	i.v.	8.3	LD_{50}	154
Propylene Glycol	Mice	24	i.v.	11.4	LD_{100}	154
Propylene Glycol	Mice	24	i.v.	2.1	MSD*	154
Propylene Glycol	Mice	24	i.v.	1.0	MND**	154
Propylene Glycol	Mice	70	p.o.	24.8	LD_{50}	153
Propylene Glycol	Mice	180	i.p.	7.0	LD_0	274
Propylene Glycol	Mice	180	i.p.	0.1	ED_0	274
Propylene Glycol	Mice	-	p.o.	31.8	LD_{50}	73
Propylene Glycol	Mice	-	p.o.	15.5-19.2	LD_0	155
Propylene Glycol	Mice	-	p.o.	21.8-31.1	LD_{100}	155
Propylene Glycol	Rats	15	p.o.	33.5	LD_{50}	267
Propylene Glycol	Rats	15	s.c.	22.5	LD_{50}	267
Propylene Glycol	Rats	20	i.m.	14	LD_{50}	267
Propylene Glycol	Rats	40	i.v.	6.8	LD_{50}	267
Propylene Glycol	Rats	-	p.o.	30	LD_{50}	172
Propylene Glycol	Rats	-	p.o.	26.3	LD_{50}	184
Propylene Glycol	Rats	18 M & 18 F	i.p.	14.7	LD_{50}	116
Propylene Glycol	Rats	19	i.m.	15.7	MLD	31
Propylene Glycol	Rats	20	s.c.	23.1	MLD	31
Propylene Glycol	Rats	89	p.o.	21.7	LD_{50}	153

Substance	Animal	Sex & No.	Route	Dosage (g/kg)	Measurement	Ref.
Propylene Glycol	Rats	25	i.p.	13.5	LD_{50}	248
Propylene Glycol	Rats	25	i.m.	20.7	LD_{50}	248
Propylene Glycol	Rats	25	s.c.	29	LD_{50}	248
Propylene Glycol	Rats	25	p.o.	29	LD_{50}	248
Propylene Glycol	Rats	-	p.o.	26.38	LD_{50}	234
Propylene Glycol	Guinea Pigs	-	p.o.	18.35	LD_{50}	234
Propylene Glycol	Guinea Pigs	40	p.o.	19.6	LD_{50}	153
Propylene Glycol	Guinea Pigs	-	s.c.	13	LD_{0}	155
Propylene Glycol	Guinea Pigs	-	s.c.	15.5	LD_{100}	155
Propylene Glycol	Rabbits	18	i.v.	6.5	LD_{50}	267
Propylene Glycol	Rabbits	22	p.o.	20	MLD	31
Propylene Glycol	Dogs	3	i.v.	22.0	AD***	109
Propylene Glycol	Dogs	3	i.v.	25.9	LD_{50}	109

* MSD - minimum symptomatic dose

** MND - maximum non-symptomatic dose

*** AD - anesthetic dose

Mice

Using 60 female, albino, Carworth Farms mice weighing between 18 and 27 g, Karel et al. determined the toxicity of intraperitoneally injected propylene glycol. Using 7-day mortality figures, the LD_{50} was calculated to be 9.73 g/kg of body weight. Gross and microscopic pathological studies were conducted on those mice which died during the first 7 days after injection and on an unspecified number sacrificed at the end of the 7-day observation period. Sections of heart, lung, liver, spleen, kidney, pancreas, adrenals, thymus, lymph nodes, bone marrow, sympathetic tissue, gastrointestinal tract, testes and ovary were subjected to microscopic examination. Slight kidney damage, both glomerular and tubular, was found. No other effects were noted (134).

Latven and Molitor investigated the toxicity of propylene glycol by i.v., s.c., and p.o. administration to white mice. The compound was tested at six dosage levels with at least four mice on each dose. The number of deaths during the first 24 hours was used to calculate the toxicity figures. The results of the test are presented in Table (154).

Table - Acute toxicity of propylene glycol in white mice (mg/kg body wt.).

Route	LD_0	LD_{50}	LD_{100}	MSD*	MND**
s.c.	14.0	18.5	24.0	10.0	8.0
p.o.	18.0	22.0	26.0	8.0	6.0
i.v.	5.0	8.0	11.0	2.0	1.0

* MSD - minimum symptomatic dose

** MND - maximum non-symptomatic dose

By means of a stomach tube, Laug et al. administered single doses of 20-30 cc of propylene glycol per kg body weight to 70 mice. Shortly after administration of large doses, the animals showed signs of loss of equilibrium, marked depression, analgesia, coma and finally death after a prolonged moribund state. Hemorrhagic areas in the small intestines were observed and the liver showed slight congestion and hyperemia. There were microscopic changes in the kidneys, with nuclear pyknosis and vascular degeneration of the cytoplasm, and a few cortical tubules contained protein debris or loose casts. The LD_{50} was calculated to be 23.9 cc/kg (153).

In a study using 18 groups of 10 male, Swiss albino, Caesarean-derived mice, 20-30 g in weight, Worthley and Schott determined the LD_0 and ED_0 of propylene glycol to be 6.8 and 0.1 ml/kg, respectively, when injected intraperitoneally (274).

Chickens

By intravenous infusion of a 50% propylene glycol solution into 3 chickens at a rate of 0.5 ml/minute, Lehman and Newman determined the fatal dose to be 25.7 ml/kg of body weight (157).

Rats

The acute toxicity of propylene glycol to rats was investigated by Weatherby and Haag. Adult white rats unselected as to age, source, weight or sex were used. The results of the test are as follows:

Method of Administration	No. of Rats	LD_{50} (g/kg)
p.o.	15	33.5
s.c.	15	22.5
i.m.	20	14
i.v.	40	6.8

With fatal doses death occurred from 2 minutes to 2 days following oral administration. Muscular tremors and mild clonic convulsions occurred frequently. The animals became comatose more or less rapidly depending on the method of administration (267).

Hickman injected, intraperitoneally, 36 adult SPF albino rats (in groups of 3 males and 3 females) with propylene glycol in doses of 9.4, 11.1, 13.0, 15.3, 18.0, or 21.1 ml/kg body weight. An LD_{50} value of 14.2 ml/kg was calculated from the mortality after 5 days (116).

The acute toxicity of propylene glycol was determined by Braun and Cartland by the intramuscular and subcutaneous injection of doses of 12.6-25.2 g/kg in rats (19 i.m. and 20 s.c.). With the larger doses all of the rats showed profound depression, analgesia and coma. The MLD for PG intramuscularly was found to be 15.7 g/kg and 23.1 g/kg subcutaneously (31).

By means of a stomach tube, Laug et al. administered single doses of 15-25 ml of propylene glycol per kg body weight to 89 rats. Shortly after administration of large doses, the animals showed signs of loss of equilibrium, marked depression, analgesia, coma and finally death after a prolonged moribund state. Hemorrhagic areas in the small intestines were observed and the liver showed slight congestion and hyperemia. There were microscopic changes in the kidneys, with nuclear pyknosis and vascular degeneration of the cytoplasm, and a few cortical tubules contained protein debris or loose casts. The LD_{50} was calculated to be 21.0 ml/kg (153).

Thomas et al. investigated the acute toxicity of propylene glycol in the rat by administering, by various means, the compound in various doses to groups of 5 adult male and female albino rats. Using the 24-hour mortality, the LD_{50}'s were calculated as follows (248):

Methods of Administration	No. of Rats	LD_{50} (ml/kg)
i.p.	25	13
i.m.	25	20
s.c.	25	28
p.o.	25	28

Symth et al. administered propylene glycol to groups of 10 albino male Wistar rats, weighing 90-120 g, by stomach tube in order to determine its acute toxicity. An LD_{50} of 26.38 g/kg body weight was calculated (234).

Ajazzi-Mancini reported the MLD of propylene glycol in rats to be 13.9 ml/kg intramuscularly and 11.6 ml/kg intravenously (2).

In an attempt to establish the acute toxicity of propylene glycol, Seidenfeld and Hanzlik obtained the following data:

*Toxicity of propylene glycol in white rats and in rabbits**

Dose per kilogram body weight		White rats			Rabbits		
		Number of animals used	Per cent mortality	Symptoms	Number of animals used	Per cent mortality	Symptoms
Intramuscular injections							
cc.	grams						
4	4.20	5	0	–	3	0	–
5	5.25	5	0	+	3	0	–
6	6.30	5	0	+	3	33	++
7	7.35	5	0	+	3	100	++
8	8.40	5	20	++	7	71	++
9	9.45	5	20	++	3	100	++
10	10.50	10	20	++			
11	11.55	10	20	++			
12	12.60	6	33	++			
13	13.65	10	40	++			
14	14.70	5	80	++			
15	15.75	5	100	++			
20	21.00	5	100	++			
Intravenous injections							
3	3.15	1	0	–	3	0	–
4	4.20	1	0	–	3	30	++
5	5.25	1	0	–	3	66	++
6	6.30	5	0	–	3	100	++
7	7.35	5	0	++	3	100	++
8	8.40	5	0	++			
9	9.45	10	30	++			
10	10.50	15	27	++			
12	12.60	15	20	++			
13	13.65	5	40	++			
14	14.70	5	40	++			
15	15.75	10	40	++			
16	16.80	5	60	++			
18	18.90	10	70	++			
19	19.95	5	100	++			
20	21.00	10	100	++			

* The minus sign in the table means absent; the plus sign, present, and the double plus sign, more marked.

The symptoms referred to in the table consisted of increased respiratory rate, loss of equilibrium, depression, and, after the highest doses, coma and death (230).

In an investigation of the acute toxicity of propylene glycol using 60-day-old male rats weighing 80 to 90 g, Bucciardi obtained the following results (39):

No. of rats	Propylene Glycol (ml/kg)	No. of Dead	% mortality
	intramuscular injection		
5	4	0	0
5	5	0	0
5	6	0	0
5	7	1	20
5	8	1	20
5	9	4	80
5	10	4	80
	intravenous injection		
5	4	0	0
5	5	0	0
5	6	0	0
5	7	2	40
5	8	4	80
5	9	5	100
5	10	5	100

Harvey et al. found that as much as 7.5 ml of propylene glycol per kg of body weight is well tolerated by male Wistar rats when injected intravenously (112).

The effect of propylene glycol on respiratory quotient was investigated in 13 fasting rats by Hanzlik et al. The respiratory quotient was determined before and after administration, by stomach tube, of 10 ml of propylene glycol/kg of body weight. No significant effect was noted (107).

Guinea Pigs

In order to determine its acute toxicity, Amyth et al. administered propylene glycol, by stomach tube, to groups of 10 guinea pigs weighing 250-300 g. An LD_{50} of 18.35 g/kg body weight was calculated (234).

By means of a stomach tube, Laug et al. administered single doses of 15-22.5 ml of proplyene glycol per kg body weight to 40 guinea pigs. Shortly after administration of large doses, the animals showed signs of loss of equilibrium, marked depression, analgesia, coma and finally death after a prolonged moribund state. Hemorrhagic areas in the small intestines were observed and the liver showed slight congestion and hyperemia. There were microscopic changes in the kidneys, with nuclear pyknosis and vascular degeneration of the cytoplasm, and a few cortical tubules contained protein debris or loose casts. The LD_{50} was calculated to be 18.9 ml/kg (153).

Rabbits

Weatherby and Haag reported an LD_{50} of 6.5 g/kg of body weight for propylene glycol in rabbits when administered intravenously. Eighteen adult rabbits unselected as to age, source, weight or sex were employed in the test (267).

Fifteen rabbits were given, by Kesten et al., propylene glycol intravenously in single doses from 2 to 5 ml/kg of body weight. One rabbit that received a 0.5 ml dose died the following night, but no abnormalities were observed except the presence of considerable quantities of protein within the tubules of the kidneys. The other rabbits were sacrificed from 2 to 46 days after the injection. Ten of the animals manifested no pathologic changes, but 4 of the rabbits exhibited evidence in the kidneys of hemolytic action by the compound. Granular, hyaline, and hemoglobin-containing casts were found and leakage of erythrocytes into glomerular spaces and convoluted tubules were sometimes seen (140).

By administering, by stomach tube, doses of 15.75 to 21.0 g/kg to 22 rabbits, Braun and Cartland determined the MLD of propylene glycol in rabbits to be 20 g/kg. Following administration of the material, the rabbits exhibited increased respiration rate, loss of equilibrium, profound depression, analgesia and coma. Death occurred in from 18 to 36 hours (31).

Ajazzi-Mancini reported the MLD of propylene glycol in rabbits to be 7.85 ml/kg intramuscularly and 4.50 ml/kg intravenously (2).

In an investigation of the acute toxicity of propylene glycol using rabbits weighing 1800-2100 g, Bucciardi obtained the following results (39):

No. of Rabbits	Propylene Glycol (ml/kg)	No. of Deaths
	Orally	
3	6	0
1	8	0
2	10	1
	Intramuscularly	
2	5	0
2	6	0
2	7	1

No. of Rabbits	Propylene Glycol (ml/kg)	No. of Deaths
	Intravenously	
2	4	0
2	5	0
2	6	1

Dogs

Hanzlik et al. administered a 50% solution of propylene glycol to 3 dogs by intravenous infusion at a rate of 0.5 ml/kg body weight/minute. The anesthetic dose (the quantity required to produce abolition of the corneal reflex) was determined at 21.2 ml/kg and the average fatal dose was 25.0 ml/kg (109).

Repeated gastric doses of propylene glycol to a total of 8, 9, or 20 ml/kg were reported by Hanzlik et al. to produce no evidence of toxicity in 3 dogs (109).

Man

Propylene glycol was administered orally by Hanzlik et al. in doses of 50 ml to 3 human subjects who had previously fasted for 12 hours. Measurements of basal metabolic rates were taken 0, 0.5, 2, and 3 hours after administration. No effect was noted as a result of propylene glycol ingestion (107).

II. Short Term Studies

Chicks

In a feeding study by Persons et al., propylene glycol was added to the diet of broiler chicks at levels of 0, 2.5, 5.0, and 10%. Each diet was fed to 10 groups (5 male and 5 female) of 10 chicks each from day-old to 3 weeks of age. At termination of the experiment, body weights and feed efficiencies were calculated. The two higher levels of propylene glycol depressed body weight of the chicks and the highest level depressed food efficiency. Mortality of the chicks was significantly greater among those fed the 10% propylene glycol diet (202).

Propylene glycol was fed by Persons et al. to 720 broiler chicks, half males and half females, at dietary levels of 0, 2.5, 5.0, and 10% from 1 to 21 days of age. At 21 days of age, body weights and feed efficiencies were determined and were found to be significantly depressed in the groups fed 5.0 and 10% propylene glycol. No significant effect on mortality was observed (202).

In an experiment by Persons et al. in which 60 broiler chicks were fed diets with or without 10% propylene glycol from day-old to 3 weeks of age, the growth rate and feed efficiency of the treated chicks was significantly depressed, but no significant effect on mortality was observed (202).

Propylene glycol was mixed in the diets of chicks at levels of 0, 4, 8, and 16% by Bayley et al. Each diet was fed to 5 groups of 10 male White Leghorn strain cross chicks from day old to 26 days of age. At the conclusion of the experiment feed consumption and live weights of the birds were recorded. Up to 8% propylene glycol caused no significant change in weight or feed:gain ratio, but 16% propylene glycol had an adverse effect on both parameters. Analysis of the carcasses of the birds showed that protein content tended to decrease as the level of propylene glycol increased to 8%. At 16% propylene glycol, the birds had a lower fat content and a higher protein content than the other birds (15).

In a 3-week feeding study by Waldroup and Bowen, eight groups of day-old broiler chicks, 4 groups of 10 males and an equal number of females, were given diets containing 0, 2.5, 5.0, and 10.0% propylene glycol. At 21 days of age, the chicks were individually weighed and group feed consumption determined. Body weight of the chicks fed 2.5 and 5.0% propylene glycol did not differ significantly from the controls, but those given 10% of the compound exhibited significantly lower body weight gains. Feed consumption of the group fed the highest level of the test material was 9% less than the control group. There were no significant differences in mortality among the groups, but the chicks fed the higher levels of propylene glycol had a high incidence of toe and hock deformities. Diarrhea resulted in the chicks given diets containing 5.0 and 10.0% propylene glycol (264).

Three groups of 10 day-old chicks were placed on each of 4 diets, by Waldroup and Bowen, an additional 3 groups were fed a basal diet to 7 days of age and then placed on each experimental diet. The diets employed contained propylene glycol at levels of 0, 2.5, 5.0, and 10.0%. When the chicks were 28 days old individual body weight gains of the chicks declined as the level of propylene glycol in the diet increased. An increase in feed consumption was observed in the test groups. The feed:gain ratio indicated significant increases in body weight gains resulting from feeding the diets with 5.0 or 10.0% propylene glycol. Mortality was unaffected but a high incidence of hock and toe deformities were noted at the higher dietary propylene glycol levels. Diarrhea also resulted at the higher levels. No statistically significant differences were noted as a result of starting treatment at different ages (264).

In an attempt to establish a metabolizable energy value of propylene glycol, Waldroup and Bowen fed diets containing 0, 1.9, 3.8, 5.7, and 7.6% propylene glycol to 300 broiler chicks from day-old to 28 days of age. The lower 3 levels of propylene glycol produced no adverse effects on body weight gains, feed utilization, or mortality.

However, the 7.6% level caused a significant depression in body weight gain and an increase in the feed:gain ratio. The results indicated a marked loss of energy from propylene glycol during digestion. No estimation of a metabolizable energy value was attempted (264).

Yoshida et al. investigated the chronic toxicity of propylene glycol using 40 newborn male White Leghorn chicks. After having received the control diet for 7 days, the chicks were randomly divided into 2 groups, of which one group remained on the control diet and the other group was given the experimental diet containing 5% propylene glycol. At 16 days of age, each group was further subdivided into 2 groups of 10 chicks, of which one group was given either the control diet or the 5% propylene glycol diet, and the other group was given a 10% propylene glycol diet. At 25 days of age, each group was further subdivided into 2 groups of 5, of which one remained on the same diet and the other was given a 20% propylene glycol diet, for 9 days. Records of weight gain and food consumption indicate that the chicks grew normally when given the 5% propylene glycol diet, but higher dietary levels inhibited growth (275).

Persons et al. fed 18 groups of 5 hens experimental diets containing 0, 2.5, or 5.0% propylene glycol for two 28-day periods. Records kept on feed consumption, egg production and body weight indicate a reduction of feed consumption and egg production at the 5% propylene glycol level (202).

Rats

Guerrant et al. fed groups of 10 young growing rats, equally divided as to sex, diets containing propylene glycol at levels of 0, 1, 3, 6, 10, 15, 20, 30, 40, 50, and 60% for a period of 20 weeks. In groups in which high mortality was experienced during the early stage of feeding trials, additional animals were subsequently added. Food and water were provided ad libitum and its consumption was recorded. The animals were observed daily and their body weights were recorded weekly. Hemoglobin determinations were made at regular intervals. Selected animals from each group were further observed for their inclination to exercise and other representative animals were tested for the effects of propylene glycol consumption on water intake and urinary output. Unspecified tissues of animals which succumbed during the test period as well as representatives from each dietary group were subjected to pathological examination. Other animals were sacrificed for the purpose of determining the effect of diet on dry matter, fat and liver glycogen. The results show no marked difference in the growth response when propylene glycol is fed at dietary levels below 30%; at 30% of the diet, propylene glycol caused a depression of growth rate and at dietary levels above 30%, weanling rats invariably died within a few days after being placed on the test diets. As the dietary level of propylene glycol increased, water intake of the rats increased, with a parallel increase in urinary output. Hemoglobin determinations indicate that propylene glycol does not exert any marked depressing effect on normal hemoglobin formation. The propylene glycol content of the diet apparently had no effect on the water content of the body tissues, the fat content or on the iodine number of the fat.

No consistent pathological changes were observed in the tissues from animals which had received propylene glycol at levels below 10%. However, at 10%, pathological changes were frequently found and at higher levels the frequency and severity of pathological changes were more pronounced. The most frequently observed changes were located in the kidney and consisted of degeneration of the tubular epithelium, accompanied by intestinal hemorrhage and edema, acute glomerular nephritis and calcified areas of the cortex (101).

Weatherby and Haag gave propylene glycol to groups of 5 male rats, about 55 g in weight, in concentrations of 0, 0.1, 0.3, 1.0, 3.0, and 10.0% by weight in their drinking water for a period of 100 days. At the 10% level this amounted to 9.6-15.8 g/kg/day. Food and drink were provided ad lib and its consumption, as well as the weight of the animals, was recorded at 5-day intervals. At the end of the experiment all surviving animals were sacrificed and all internal organs were subjected to microscopic examination. During the first 10 days of the test, the rats given 10% propylene glycol exhibited a slower rate of weight gain than the other animals, but after this period they apparently recovered and grew normally. Two of the rats given 10% propylene glycol and 1 given 1% died during the test period. No significant differences in consumption of food or drink were noted between the groups. Microscopic examination of the organs revealed no abnormalities (267).

Stoewsand et al. fed male weanling albino rats of the CFE Carworth strain, 40-60 g, diets containing 30% fat with or without (control) a 20% supplement of propylene glycol. These diets were fed, ad lib, to: 10 rats per each dietary treatment maintained for 4 weeks at 25°; 2) 10 rats per each dietary treatment maintained for 4 weeks at 5°; and 3) 18 rats per each dietary treatment maintained for 5 weeks at 25°, followed by exposure to -20° until each group had 9 surviving rats (50% survival). After 4 weeks, 5 rats in each of the dietary treatments of 25° and 5° were sacrificed. The liver was immediately excised and weighed and total lipids and liver cholesterol was determined. The epididymal fat pads were also excised and weighed. Weight gain was lower in the rats fed propylene glycol, but was similar to the controls when calculated on a per calorie intake basis. Epididymal adipose tissue was lower in the treated group while liver lipids and liver cholesterol were increased. However, cold exposure reduced the liver cholesterol in the animals given propylene glycol. A lowered resistance to intense cold was exhibited by the subject rats (242).

In a feeding study by Hanzlik et al. 0, 25, 50, 75, or 100% of the carbohydrate of the diet of young growing rats was replaced by propylene glycol. Therefore propylene glycol was present at levels of 0, 12.1, 24.2, 36.4, and 48.5% of the diet. At the highest level, the rats maintained a fairly constant weight and died in about one month. The rats fed 36.4% propylene glycol fared a little better, but when half of the carbohydrate was replaced with propylene glycol the rats gained weight and the first death did not occur until 5 months after the start of the experiment. No mortality was observed in the first 20 weeks in the group fed 12.1% propylene glycol and the rats gained weight nearly as rapidly as the controls. Although the growth of the rats on the two lower dosage levels was depressed at the beginning, a later increase in growth rate indicated a lack of serious

impairment. Food consumption was decreased somewhat with all of the treated rats (109).

Stoewsand and Dymsza fed male, weanling Carworth strain albino rats, unspecified number, diets containing 0 or 20% propylene glycol ad libitum for 4 weeks. In the rats fed the glycol, there were lowered weight gains with lowered food and energy intake. However, the food and caloric efficiencies of the test animals were similar to the control group. Removal of the epididymal fat pads after 4 weeks revealed a significant decrease in the epididymal adipose tissue. Analysis of the excised livers showed that total liver lipids and liver cholesterol were significantly higher in the animals fed propylene glycol (243).

Van Winkle and Newman conducted a paired feeding study using 10 rats, 61-74 g, in which one fourth of the carbohydrate of the diet was replaced with an equi-caloric amount of propylene glycol for half the rats. Records of body weight for 163 days indicated that the treated rats grew more rapidly and obtained greater body weights than the controls. Determinations of liver glycogen disclosed that the glycolized rats had 2 to 7 times as much liver glycogen as the controls. Two possibilities which might account for this effect were suggested: propylene glycol might be a more readily available source of energy than the carbohydrate of the diet; or the glycol might depress the animals enough to decrease their energy utilization and thus account for the difference (260).

Twelve rats of the same litter were divided into 2 groups of 6 and fed either a control diet or a diet containing 25% less carbohydrate than the controls. The latter group was given propylene glycol by stomach tube daily at a level equivalent to 12.8% of the total weight of food eaten the previous day. After a period of 5 months it was clear that the propylene glycol-treated rats showed a definitely greater average increase in body weight than did the controls. It was suggested that the greater caloric value of propylene glycol compared to carbohydrate was responsible for this effect (109).

The voluntary running activity of 5 female white rats, fed, for a period of 19 weeks, a stock diet in which 25% of the carbohydrate was replaced by propylene glycol, was found by Hanzlik et al. to be slightly higher than, or at least not lower than, the running activity of the control rats maintained on a stock diet (107).

The voluntary running activity of 5 female white rats was found by Van Winkle and Kennedy to be increased by the inclusion of 12% propylene glycol in the rats' diet during a period of 15 weeks (259).

In an experiment lasting 11 weeks, Holck gave 6 female rats, 4.5 months old, a 3.58% commercial propylene glycol solution (purity not given) in place of their drinking water. One of the rats died after the 10th week and one had lost considerable weight. The remaining rats were beginning to lose weight at the end of the experiment (117).

Vinals conducted a test in which 2 male and 2 female albino rats (6 months old) were given a 50% propylene glycol solution as drinking water and the same number of rats were maintained as controls. Ten days after the beginning of the experiment, all of the treated rats were dead. It was concluded that the deaths were not the result of direct toxicity of propylene glycol but were the result of decreased

food and water intake (9).

In a study by Vinals, 2 male and 2 female albino rats were given a 10% propylene glycol solution as drinking water for a period of 24 days. An equal number of animals were maintained as controls. No untoward effects attributable to the consumption of propylene glycol were observed. Determinations of the weight, at 24 days, of the heart, liver, kidneys, spleen, stomach, adrenal glands, thymus, and reproductive organs revealed no abnormalities (9).

Kesten et al. found no demonstrable effect from the administration of propylene glycol to 39 rats in their drinking water in concentrations from 1 to 10% for from 11 to 234 days (140).

Hunt reported that young rats grew at the normal rate and reached maturity when the only liquid they received was a 5% solution of propylene glycol. The compound was also well tolerated by animals whose livers and kidneys had been injured by various poisons (124).

Charles River CD rats were separated into groups of 15 males (120-150 g) and 15 females (120-140 g) and maintained for 15 weeks on diets containing 0 or 5% of propylene glycol. Renal concentration tests were performed during the 15th week. After 15 weeks, the rats were sacrificed and the brain, heart, liver, spleen, kidneys, adrenals, gonads, and pituitary were weighed. Hematological examinations were carried out on blood collected from the aorta at autopsy. No significant differences were found between the control rats and the treated rats. No abnormalities were seen at autopsy (83).

The propylene glycol esters of the fatty acids of lard, when fed to six 21-day-old rats at a level of 60% of the diet, allowed normal growth of the rats. Gross and microscopic examination of 2 of the animals, 40 days after the start of the experiment, revealed no untoward effects (159).

Rabbits

Braun and Cartland gave 18 rabbits propylene glycol by stomach tube in doses of 0-8.4 g/kg/day for a period of 50 days. Examination of the animals after 50 days revealed no gross pathology. Satisfactory growth was shown at the lower dosages (1.05-3.15 g/kg) while at the higher dosages (4.2 and 8.4 g/kg) the rabbits exhibited no significant change in body weight throughout the test period. No cummulative effects were observed (31).

Dogs

For a period of two years, Weil et al. fed groups of 5 male and 5 female beagle dogs, 10-14 months old, diets containing propylene glycol at levels of 2.0 and 5.0 g/kg body weight/day. Control groups were maintained either on basal diets or diets containing dextrose in amounts calorically equal to the amounts of propylene glycol administered. The dogs were weighed weekly and hematology tests, urine analysis, and studies of blood chemistry were performed after 0, 6, 12, and 23 months of feeding. These examinations consisted of total erythrocyte count, total and differential leucocyte counts, haemoglobin, haematocrit,

erythrocyte fragility (initial and complete hemolysis), urine volume and specific gravity, urine pH and micro-examination, serum alkaline phosphatase, total bilirubin, bromsulphthalein retention (5 mg/kg, 15 min after dose), blood-glucose level, serum propylene glycol level, serum glutamic-oxalacetic and glutamic-pyruvic transaminase activities and blood urea-nitrogen. At termination of the experiment, the following criteria were also assessed: anisocytosis and poikilocytosis, nucleated-erythrocyte count, reticulocyte count, direct and indirect bilirubin estimations (Van den Bergh), liver glycogen, metabolic rate of liver slices using glucose and propylene glycol as substrates, total liver lipids, liver triglycerides, water content of the liver (from constant weight of 1 g dried in a vacuum oven at 60°C and 21 mm Hg) and weights of liver, kidney and spleen. The organs examined, grossly and micropathologically, were liver, kidney, lung, heart, diaphragm, spleen, adrenal, thyroid, parathyroid, salivary and maxillary glands, tongue, lymph nodes, gall bladder, esophagus, stomach, duodenum, pancreas, ileum, jejunum, colon, urinary bladder, testicle, epididymis, prostate, uterus, ovary, skin, eye, brain, pituitary and bone marrow. At the lower propylene glycol level, none of the criteria measured were significantly altered. At the higher level, both the propylene glycol group and its respective dextrose control group gained more weight than the basal controls during the first part of the study. The rate of erythrocyte hemolysis was increased by propylene glycol at the higher concentration. At the 5 g/kg level, hemoglobin and hematocrit values and the total erythrocyte count were slightly low, while the incidence of anisocytosis, poikilocytes and reticulocytes was increased, indicating destruction of erythrocytes with accelerated replacement from the bone marrow. There was no evidence of damage to bone marrow or spleen and the liver was apparently unaffected except for a slight increase in bilirubin in the dogs consuming 5 g of propylene glycol/kg. It was concluded that propylene glycol fed to dogs at a concentration of 2 g/kg/day (approximately 8% of the diet) is utilized as a carbohydrate energy source without any adverse effects (268).

For 5 to 9 months, propylene glycol was administered by Van Winkle and Newman in the drinking water to 2 groups of 4 dogs at levels of 5 and 10%, resulting in consumption of 5.1 and 4.5 ml/kg/day, respectively. Quantitative excretions of galactose and uric acid and the rose bengal content of the blood indicated no impairment of liver function resulting from propylene glycol ingestion and phenolsulfonphthalein excretion and blood urea revealed no impairment of kidney function. Histological examination of the liver and kidneys disclosed no pathological changes (260).

III. Long Term Studies

Rats

In a long-term study by Gaunt et al., groups of 30 male (120-150 g) and 30 female (120-140 g) Charles River CD rats were maintained on diets containing 0, 6,250, 12,500, 25,000, or 50,000 ppm propylene glycol for a period of 2 years. Records were kept on body weight, food intake, and mortality throughout the test period. At weeks 13, 21, 52, and 80, blood samples were taken from 8 male and 8 female rats from the groups

given 0, 25,000, and 50,000 ppm propylene glycol. Blood samples were also taken from 8 rats from the control, 6,250, and 12,500 ppm groups at week 54. All the samples were examined for hemoglobin content, packed cell volume and counts of erythrocytes, total leucocytes and the individual types of leucocytes. Counts of reticulocyte, were also carried out on the samples obtained after 52, 54, and 80 weeks. At week 104, the hematological investigations were limited to measurements of hemoglobin concentration and examination of a stained blood smear from all surviving rats. At weeks 13, 30, and 52, a urine concentration test was conducted on selected rats from the control and two highest treatment groups. After 104 weeks, all surviving animals were sacrificed and subjected to autopsies, during which any macroscopic abnormalities were noted and the brain, heart, liver, spleen, kidneys, adrenals, gonads, stomach, small intestine and cecum were weighed. Samples of these organs and of the salivary gland, trachea, aorta, thymus, lymph nodes, pituitary, urinary bladder, colon, rectum, pancreas, uterus, muscle and any tissue that appeared to be abnormal were examined microscopically. No statistically significant differences between treated and control rats were found. On the basis of these findings, the authors suggested that an intake of 25 mg/kg/day would be acceptable in man (83).

For a period of 24 months, Morris et al. fed 20 albino rats diets containing 2.45 and 4.9% propylene glycol while a similar number were maintained on a basal diet as controls. Growth rate, food consumption, and survival time were analogous for the test and control rats. The only untoward effect revealed by microscopic examination of the lungs, heart, liver, spleen, kidneys, adrenals, testes, pancreas, stomach, intestines, and lymph nodes, was slight liver damage. No dose response difference was noted by the authors, however they stated that the animals receiving propylene glycol differed only slightly from the controls (178).

IV. Special Studies

Reproduction

Guerrant et al. carried out a series of studies on the reproduction of rats which were fed diets containing as much as 30% propylene glycol. The studies were continued through six generations of rats. No measurable effect on reproduction was observed until the diet contained 7.5% or more of propylene glycol. In general, the control rats consumed more food, grew faster, reproduced at a younger age, produced, on the average, larger litters, weaned a greater percentage of the young and weaned larger young than did the animals receiving corresponding propylene glycol-containing diets. Those animals receiving a diet containing 30% propylene glycol failed to wean third generation young. The females not only failed to breed normally but when they did breed and gave birth they were unable to feed the young satisfactorily (101).

Teratogenic

Gebhardt investigated the teratogenic effects of propylene glycol by injecting White Leghorn eggs with 0.05 ml of propylene glycol either in the air sac or the yolk. On the 15th day of incubation the chick embryos were studied for gross morphology. When propylene glycol

was introduced into the air chamber during the first day of incubation most of the chick embryos survived the treatment. However, injections on the 2nd day or later reduced the viability of the embryos. A maximum sensitivity toward propylene glycol was reached on the 4th day of incubation with a rapid decline thereafter. On the 4th day, 90% of the embryos injected died within 2 hours and 20% of those surviving had asymmetric malformations of the limbs. When 0.2 ml of propylene glycol was injected into the yolk sac on the 4th day of incubation, a large liquid-containing cyst was observed on the dorsal side of 40% of the embryos (84).

McLaughlin et al. reported that injection of 0.05 ml of undiluted propylene glycol into the yolk sac of fertile eggs prior to incubation does not affect their hatchability (171).

Deformities

Bowen and Waldroup reported the occurrence of a toe abnormality in chicks fed propylene glycol. The toes turned outward at each joint with the incidence of the condition increasing as the level of propylene glycol increased in the diet. At the maximum level fed, 10%, 57 out of 168 chicks exhibited the deformity while in the control group, 168 chicks, only 9 of the chicks were affected (29).

Bowen and Waldroup fed diets containing 0, 2.5, 5.0, 7.5, and 10.0% propylene glycol to a total of 840 male broiler chicks from day-old to 28 days of age. At 28 days of age the body weight gain and feed consumption were determined and observations were made of the incidence of hock and toe deformities. All of the chicks fed diets containing propylene glycol were significantly lighter in weight than those fed diets without propylene glycol. No significant differences in feed consumption were noted among any of the groups. As the level of propylene glycol in the diet increased, above 2.5%, there was a significant linear increase in the number of hock and toe deformities, indicating some defect in bone or cartilage metabolism (30).

Antitumor

The antitumor activity of the propylene glycol monoesters of oleic, palmitic and myristic acids were examined by Kato et al. in vivo using Ehrlich ascites tumor in mice. The ascitic tumor cells of Ehrlich carcinoma (2 x 106 cells) were implanted intraperitoneally in 5-week-old mice weighing 18-22 g. A saline solution of the samples was administered once daily for 5 successive days and the tumor growth and body weight gain after 7 days and life span were observed. The myristic ester showed a complete cure with prolonged survival time (136).

Lo' ' Irritant

Seidenfeld and Hanzlik reported that propylene glycol caused a moderate, though fleeting, local irritation of mucosa (tongue of man; conjunctiva of rabbits), and in the muscles and subcutaneous tissues of rats, rabbits and man. The irritant effect was attributed

to the hypertoxicity of the solutions used (230).

Undiluted propylene glycol caused no irritation on the tender skin of the rabbit belly (184).

Animal studies and repeated insult tests on human volunteers did not demonstrate development of skin sensitivity to propylene glycol (184).

Flooding the rabbit eye with an excess of propylene glycol caused a reaction no more severe than moderate inflammation (184).

Bucciardi reported that subcutaneously injected propylene glycol is strongly irritating; it causes vasodilation and local hyperemia, but does not prompt the formation of edemas. When injected into the gluteal area of human subjects in doses of 0.4 ml, propylene glycol caused an intense burning pain which subsided in 10-15 minutes. Application of pure propylene glycol on the conjunctiva of the rabbit caused blepharospasm and a violent inflammation of the conjunctiva. A 5% solution of the glycol was somewhat irritating (39).

Allergy

In the treatment of patients with leg ulcers, Huriez et al. found that some of the patients developed an allergic reaction to propylene glycol. After cutaneous treatment with a propylene glycol-containing compound (D.V. 511 or Synalor-propylene glycol), 16 of 38 patients exhibited a cutaneous intolerance to propylene glycol. Of 145 control patients, only 4% reacted positively (126).

pH

Three groups of twenty 14-day-old male broiler chicks were given diets containing propylene glycol at levels of 0, 5, and 10% for a period of 2 weeks. On days 5, 7, 10, and 14, five chicks on each diet were sacrificed and samples of crop, proventriculus, gizzard, duodenum, ileum, large intestine, and cecum were measured for pH. There was no significant differences in pH of the crop, proventriculus, gizzard, or duodenum between any of the groups, nor were there any differences due to day of sampling. The pH of the ileum and large intestine of the chicks fed 5% propylene glycol was significantly higher than the controls. With 10% propylene glycol, the chicks had a significantly higher ileum pH, but the pH in the large intestine decreased in the large intestine as the age of the chicks increased. It was suggested that the changes in pH may result from bacterial action (30).

Biochemical Aspects

I. Breakdown

No information available

II. Absorption - Distribution

The intestinal absorption of propylene glycol in mice was found by Salter et al. to be very rapid, 77.8% in 30 minutes and 93.7% in 5 hours (224).

Through extensive studies on rabbits, rats, and cats, Van Winkle found that propylene glycol is readily absorbed from the stomach, duodenum, jejunum and colon, the absorption being particularly rapid from the jejunum. Van Winkle also determined the following quantitative characteristics of intestinal absorption of propylene glycol: absorption was more rapid with low concentrations than high; rate of absorption was not affected by variations in the area of the intestinal surface; inhibition of absorption occurred during high blood concentrations of the glycol; rate of absorption decreased as the absolute amount of glycol in a given region increased; iodoacetic acid and chloral had no demonstrable effects on the absorption, thus differing from dextrose, glycerol, fats and lipoids; during absorption, large differences in concentration of the glycol occurred in different parts of the venous circulation of the gastrointestinal tract (257).

Newman and Lehman reported that the concentration of propylene glycol in the blood of dogs and rabbits following gastric administration indicates rapid absorption of the compound. Once it has entered the blood stream it escapes rapidly and apparently diffuses throughout the body. The rate of disappearance of propylene glycol from the blood is proportional to its concentration in the body (190).

The concentration of propylene glycol in the blood of 4 dogs was followed by Lehman and Newman following administration of doses of 2, 8, and 12 ml/kg body weight. Absorption and diffusion throughout the tissues occurred promptly. The maximum concentration of propylene glycol in the blood was reached in 30 minutes with the 2 ml dose, while 2-4 hours were required for complete absorption of the larger doses (157).

Six ml of propylene glycol per kg of body weight introduced into the isolated stomach of a dog gave a blood propylene glycol level of 124 mg% at the end of 3 hours indicating only slight absorption from the stomach (157).

Following oral administration of propylene glycol to 3 human subjects in doses of 1 ml/kg body weight, the maximum concentration of propylene glycol in the blood was reached in about one-half hour and

persisted for about 4 hours thereafter. At this dosage, 20-25% of the glycol was eliminated in the urine in 10 hours (109).

III. Metabolism and Excretion

Using rat liver slices in vitro, Rudney established that ^{14}C-1-propylene glycol can be split to give a 1-carbon unit entering the formate pool and in addition can be converted to a 3-carbon precursor (lactate) of the glycolytic cycle. He was unable to detect any incorporation of isotope into cholesterol (220).

In a test by Rudney, six fasted male rats weighing from 80 to 100 g were given 2 ml of 2.5 M glycine by stomach tube and 0.25 ml of 0.3 M propylene glycol intraperitoneally per 100 g. The dose of propylene glycol, which was labelled with ^{14}C in the C-1 position, was repeated at the end of 3, 6, and 10 hours and the animals were sacrificed after 14 hours. The choline of the abdominal viscera and the liver serine and glycogen were isolated and degraded. The ^{14}C label was incorporated into the serine beta-carbon, the choline methyl groups, and the 2,5- and 1,6-carbons of the glycogen, with higher labelling in the 1,6-positions. Relatively high activity of the 3,4-carbons of the glycogen suggests that some of the propylene glycol is converted directly to lactic acid (218).

Analysis of the urine of 4 rabbits which had received, orally, a total of 16 g of propylene glycol revealed neither unchanged diol nor any conjugated glucuronic acid (88).

Emery et al. monitored the metabolism of propylene glycol in a cow following intrarumenal administration of ^{14}C-2-propylene glycol with 400 g of carrier propylene glycol. During the first 24 hours following administration, the percentage of the dose recovered was 43.7 in expired CO_2, 12.4 in milk, 3.5-7 in urine, and less than 0.1 in feces. Some conversion of the propylene glycol to propionic acid occurs in the rumen although it is predominantly absorbed without alteration. Distribution of ^{14}C among the carbons of lactose and glutamic acid indicate conversion of propylene glycol to glucose via carboxylation of pyruvate to oxalacetate. The small concentrations of propylene glycol in peripheral blood and the 2-hour delay in attaining maximal specific activity in CO_2 after attaining maximal specific activity of blood glucose indicated that glucogenesis was primarily hepatic with oxidation primarily occurring in other tissues (66).

Long et al. found that propylene glycol distearate can be hydrolyzed by in vitro steapsin to yield free fatty acid at a rate of approximately 50% in 12 hours at 30°C (163).

Balls and Matlock found propylene glycol monostearate to be hydrolyzed by in vitro pancreatic enzyme to the extent or about 80% in 15 hours at 40°C (12).

Through in vitro studies on pancreatic enzyme homogenates, Fodor demonstrated that propylene glycol dibutyrate, propylene glycol monobutyrate and propylene glycol dipropionate undergo hydrolysis at a high initial velocity which slows down rapidly after having brought about a 5-8% cleavage of the ester (76).

Neubauer reported that paired glucuronic acid was found in the urine of rabbits after the oral administration of propylene glycol (187).

To investigate the metabolism of propylene glycol distearate, Long et al. administered orally to rats propylene glycol distearate labelled with ^{14}C in the propylene glycol moiety (PG* DS) or the stearate moiety (PGDS*), ^{14}C-labelled propylene glycol plus stearic acid (PG* +S), and propylene glycol plus ^{14}C-labelled stearic acid (PG + S*). The data obtained from the experiment is presented below:

	Recovery of Total Dose in 72 hr (%)			
	PDGS*	PG*DS	PG + S*	PG* +S
Feces	65.8	0.2	51.8	0.5
CO_2	17.5	94.0	42.6	76.4
Urine	6.4	0.4	1.6	2.3
Organs	6.2	2.7	3.2	3.3
Carcass	10.8	2.3	4.8	16.5
Recovery	106.8	99.6	104.0	99.0
	Alimentary Absorption (%)			
	34.2	99.8	48.2	99.5
	Recovery of Absorbed Dose (%)			
CO_2	51.3	94.8	89.0	76.4
Urine	18.9	0.4	3.4	2.3
Organs	18.5	0.9	4.2	4.5
Carcass	31.6	2.3	10.1	16.5
Recovery	120.3	98.4	106.7	99.7

The results of the experiment indicate that the initial limiting factor governing PGDS absorption is the hydrolysis rate of the ester. Hydrolysis of the compound allows rapid absorption of the PG moiety, leaving behind a slowly absorbed stearate. As shown in the table, most of the absorbed radioactivity is metabolized to $^{14}CO_2$ (163).

In a successive study by Long et al. it was determined that PGDS is hydrolyzed in the gastrointestinal tract of the rat to yield propylene glycol monostearate, propylene glycol, and stearic acid (164).

Salter et al. reported that ingestion of propylene glycol by fasted mice results in an increase of liver glycogen (224).

In an investigation of the effects of propylene glycol ingestion on liver glycogen formation, Opitz found that the oral administration of 100 mM of propylene glycol to previously fasted rats resulted in the production of a liver glycogen level of 739 mg% and the feeding of 5 mM of propylene glycol resulted in 779 mg%, while control rats given only a physiological saline solution exhibited a liver glycogen level of 135 mg% (194).

Hanzlik et al. administered, by stomach tube, propylene glycol in doses of 0.5-15 ml/kg to groups of 5 fasting white rats weighing from 80 to 150 g. At the end of 1, 2, 3, and 4 hours, the animals were sacrificed and their lives removed for determination of liver glycogen. Control rats were given equal volumes of water and handled similarly. The results are as follows:

Dose	Liver glycogen (mg%)					Maximum Increase
(ml/kg)	Controls	1 hr	2 hr	3 hr	4 hr	(mg/g liver)
0.5	148	211	156	208	135	0.63
1.0	123	333	336	330	294	2.13
2.0	113	241	371	465	720	6.07
5.0	241	331	425	570	852	6.11
10.0	75	72	224	673	726	6.51
15.0	212	359	581	699	627	4.87

Propylene glycol was found to possess a moderate glycogenic action, with a minimum effective dose of about 0.75 ml/kg in white rats. Doses of 2, 5, and 10 ml/kg gave optimum effects, while 15 ml/kg was less effective (107).

Determination of the liver glycogen of rats fed diets containing propylene glycol with reduced carbohydrate for a period of 5.5 months produced the following data:

Diet	No. of Rats	Medium Body Weight (g)	Ave. Liver Glycogen (mg%)	Increase Over Normal (mg/g liver)
Normal stock	5	223	694	
Carbohydrate free	4	253	812	1.18
25% of carbohydrate replaced with propylene glycol*	4	211	1,351	6.57
50% of carbohydrate replaced with propylene glycol**	4	182	1,909	12.15

* 12.5% of total diet
** 25% of total diet

It was concluded that propylene glycol is effective as a glycogen former under conditions of carbohydrate deficiency (107).

Hanzlik et al. reported that 4 rats given propylene glycol daily, for about 5 months, by intragastric intubation at a level of 12.8% of the diet showed a tendency toward an increase in liver glycogen (107).

Thirty-two rats given single oral doses of 1.5-10 ml of propylene glycol/kg body weight exhibited definite, though small, increases in whole body glycogen level (107).

Propylene glycol has a nutritive value of approximately 4 calories per gram (97).

Rats given propylene glycol in their drinking water at concentrations of 0-20% excreted through the kidneys, approximately one third of the amount ingested regardless of the level consumed. Van Winkle states that the remaining two thirds of the propylene glycol must have been metabolized by the body (257).

Following intragastric administration of propylene glycol to dogs in doses of 2, 8, and 12 ml/kg body weight, Lehman and Newman measured urinary excretion for 24-48 hours. They found that after administering 2 ml doses an average of 12% of the total dose was excreted in the urine in 48 hours. With the 8 ml dose, 39% was found in the urine at the end of 48 hours, and 36% of the 12 ml dose was recovered in 24 hours (157).

Approximately 24 hours were required for the elimination of propylene glycol from the body of a dog following intragastric administration of 8 ml of the compound (107).

Hanzlik et al. recovered approximately 30 ml of pure propylene glycol from 850 ml of urine excreted by a dog during the 24 hours following the gastric administration of 150 ml of propylene glycol (109).

IV. Effects on Enzymes and Other Biochemical Parameters

When the isolated livers of cats were perfused with propylene glycol in concentrations corresponding to toxic levels in intact animals, Newman et al. observed decreases in the oxygen consumption and carbon dioxide production of the livers. The glycogen level of the liver and the lactic acid content of the blood were increased by the perfusate. Dextrose utilization by the liver was decreased (191).

Intragastric doses of 5-10 ml of propylene glycol/kg of body weight, were found by Van Winkle to significantly depress the oxygen consumption of white rats. This depression was not due to a decrease in activity (258).

The administration of a high, nonlethal dose of propylene glycol (10 ml/kg) was found by Giri et al. to affect the carbohydrate metabolism of young rats. Blood glucose of the rats was markedly increased within 15 minutes following treatment. The maximum increase in blood glucose occurred in 1.5 hours while the overall increase lasted for a period of 6 hours. The amount of liver glycogen in the treated and the control animals was the same at 6 hours, however, a severe depletion in the liver glycogen was noted 12 hours after treatment. It was concluded that rats treated with a high dose of propylene glycol have a drastically reduced capacity to synthesize or to store liver glycogen (89).

Vinals reported that propylene glycol produces a significant decrease in the cholesterolemia of rats when administered in the drinking water at 10% (9).

In vitro experiments by Nagiwara showed that propylene glycol can activate nonspecific cholinesterase of human, cat, chicken, dog, hog, rat and mouse liver, and of human, horse and cat serum when benzoylcholine is used as substrate. However, propylene glycol caused the inhibition of benzoylcholinesterase of rabbit and guinea pig liver and of specific cholinesterase of cat brain (104).

To study the effect of propylene glycol on the isolated rat intestine, Hazard and Vaille added the substance to 100 ml of Tyrode solution in which a fragment of rat duodenum was immersed. Concentrations of propylene glycol from 1 to 5% gave rise to an initial loss of tonus followed by a renewed increase in tonus and then another progressive drop. Contractions were amplified by concentrations from 1 to 3%, either amplified or reduced at 4%, and reduced at 5% (114).

Lehman and Newman found that intragastric administration of propylene glycol to dogs produces a diurectic effect. Two ml/kg body weight doses produced no diuretic action, only 100 ml of urine being excreted during a 48-hour period. Eight ml of propylene glycol/kg, however, resulted in excretion of 850 ml of urine in the same amount

of time and two liters of urine were collected in the 24 hours following administration of 12 ml/kg (157).

An intoxicating action similar to that produced by alcohol was reported by Lehman and Newman to be produced in dogs by propylene glycol. The effectiveness of propylene glycol is one third that of alcohol (157).

V. Drug Interaction

Frommel and Vallette reported that propylene glycol has a weak anti-acetylcholine effect and therefore is capable of modifying the action of pharmacodynamic substances in which it serves as vehicle or solvent (79).

VI. Consumer Expsoure Information

Propylene glycol is added to manufactured food to promote the retention of the original quality of the food on aging and shipment, or to gain a texture or product quality not present in the original food. Propylene glycol and other polyols behave as, crystallization modifiers, hygroscopic agents, plasticizing agents, sweetness controllers, solvent agents, viscosity agents, rehydration aids and dietary agents. Propylene glycol is most used for its humectant and solvent characteristics.

Propylene glycol monostearate is a surface active agent and thus is used as an emulsifier. It is particularly useful as an additive to shortening used in cake batter. This addition increases the volume of the baked cake.

The following tables were compiled from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

Table 2 reports the usage levels of propylene glycol and propylene glycol monostearate in regular foods. Table 11 reports the annual poundage data for the various propylene glycol and derivatives. Table 13 reports the possible daily intake per food category and total dietary based on food consumption by total sample. Table 14 reports potential daily intakes in mg of NAS Appendix A substances (Groups I and II) per food category reported, based on food consumption by eaters only.

TABLE 2 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
PROPYLENE GLYCOL	01	BAKED GOODS(R)	44	.07903	.24409
NAS 0164 FEMA 2940	02	BREAK CERLS(R)	*	********	********
	03	OTHER GRAIN(R)	*	.00083	.00084
	04	FATS OILS(R)	14	.01429	.03179
	05	MILK PRODS(R)	*	.02299	.03771
	06	CHEESE(R)	7	.00711	.06199
	07	FROZN DAIRY(R)	30	.13749	.21258
	08	PROCSD FRUT(R)	5	.04941	.08202
	10	MEAT PRODS(R)	19	.02347	.05341
	11	POULTRY(R)	*	.00100	.01000
	12	EGG PRODS(R)	*	.00035	.00042
	14	PROCSD VEGS(R)	*	********	.50000
	16	SOFT CANDY(R)	40	.08945	.14408
	17	CONF FROSTY(R)	5	5.07337	5.08581
	19	SWEET SAUCE(R)	4	.30999	.42074
	20	GELATIN PUD(R)	17	.03609	.06854
	21	SOUPS(R)	4	.00003	.00004
	22	SNACK FOODS(R)	*	.00200	.09199
	23	BEV TYPE I(R)	35	.03775	.12390
	24	BEV TYPE II(R)	4	.06619	.50645
	25	NUT PRODS(R)	*	********	********
	26	RECONST VEG(R)	*	.00630	.00630
	27	GRAVIES(R)	4	.04901	.09803
	30	HARD CANDY(R)	23	.07150	.13531
	31	CHEWING GUM(R)	7	.06823	.29998
	48	SEAS FLAVRS(R)	*	14.90000	15.19395
	49	MISC UNCLAS(R)	*	.08098	.52750
PROPYLENE GLYCOL MSTEARAT	01	BAKED GOODS(R)	*	.47023	.72490
NAS 0309 FEMA 2942	04	FATS OILS(R)	*	10.00922	12.00000
	06	CHEESE(R)	*	.50000	1.00000
	07	FROZN DAIRY(R)	*	********	.00100
	19	SWEET SAUCE(R)	*	.02080	.02080
	20	GELATIN PUD(R)	*	.72760	.76659
PROPYLENE GLYCOL MSTEARAT(CONT.)	23	BEV TYPE I(R)	*	********	.00050
NAS 0309 FEMA 2942	24	BEV TYPE II(R)	*	********	.00050
	28	IMIT DAIRY(R)	*	.30000	.50000

TABLE 11, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
PROPYLENE GLYCOL NAS 0144 FEMA 2940	42/ 50	1,412,949	1,448,542	1,597,910	92	4,105,985	5,703,895
PROPYLENE GLYCOL MSTEARAT NAS 0309 FEMA 2942	6/ 7	2,005,500	6,756,889	7,056,889	*	33	7,056,922

TABLE 13, PART A --* POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
PROPYLENE GLYCOL NAS 0164	01 BAKED GOODS(R)	44	0-5 MO.	2.687520	3.555350	[illegible]
			6-11 MO.	20.073620	40.937540	61.[illegible]
			12-23 MO.	43.071350	70.968940	133.[illegible]
			2-65+ YR.	108.429160	161.063140	334.[illegible]
PROPYLENE GLYCOL NAS 0164	02 BREAK CERLS(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	**********	**********
			12-23 MO.	**********	**********	**********
			2-65+ YR.	**********	**********	**********
PROPYLENE GLYCOL NAS 0164	03 OTHER GRAIN(R)	*	0-5 MO.	.004150	.010110	.0[illegible]
			6-11 MO.	.050510	.227500	.[illegible]
			12-23 MO.	.136120	.314570	.[illegible]
			2-65+ YR.	.230740	.503620	.233520
PROPYLENE GLYCOL NAS 0164	04 FATS OILS(R)	14	0-5 MO.	.071450	.071450	.1[illegible]
			6-11 MO.	.400120	1.071750	.[illegible]
			12-23 MO.	.900220	1.714[illegible]	2.[illegible]
			2-65+ YR.	2.500750	4.515640	5.[illegible]
PROPYLENE GLYCOL NAS 0164	05 MILK PRODS(R)	*	0-5 MO.	1.241460	.919000	2.[illegible]
			6-11 MO.	14.[illegible]	60.[illegible]	23.[illegible]
			12-23 MO.	12.929550	40.[illegible]	[illegible]
			2-65+ YR.	9.[illegible]	21.723[illegible]	14.[illegible]
PROPYLENE GLYCOL NAS 0164	06 CHEESE(R)	7	0-5 MO.	**********	.007110	**********
			6-11 MO.	.191570	.5[illegible]	1.673[illegible]
			12-23 MO.	.5[illegible]	1.[illegible]	4.[illegible]
			2-65+ YR.	.668340	1.677940	5.[illegible]
PROPYLENE GLYCOL NAS 0164	07 FROZN DAIRY(R)	30	0-5 MO.	1.374900	5.637090	2.12[illegible]
			6-11 MO.	13.061950	36.207350	20.[illegible]
			12-23 MO.	19.798560	46.471620	30.611620
			2-65+ YR.	35.197440	84.[illegible]	54.[illegible]
PROPYLENE GLYCOL NAS 0164	08 PROCSD FRUIT(R)	5	0-5 MO.	2.322270	6.2[illegible]	5.[illegible]
			6-11 MO.	25.554380	63.735900	42.[illegible]
			12-23 MO.	49.706460	98.671770	82.512120
			2-65+ YR.	58.452030	123.821460	97.029660

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
PROPYLENE GLYCOL NAS 0164	10	MEAT PRODS(R)	19	0-5 MO.	.258170	.680630	.587510
				6-11 MO.	4.858290	13.096260	11.055870
				12-23 MO.	7.087940	12.181930	16.199920
				2-65+ YR.	18.400480	30.534470	41.573640
PROPYLENE GLYCOL NAS 0164	11	POULTRY(R)	*	0-5 MO.	.005000	.023000	.000000
				6-11 MO.	.039000	.132000	.390000
				12-23 MO.	.066000	.184000	.660000
				2-65+ YR.	.129000	.328000	1.290000
PROPYLENE GLYCOL NAS 0164	12	EGG PRODS(R)	*	0-5 MO.	**********	.003500	**********
				6-11 MO.	.001790	.000700	.000100
				12-23 MO.	.003150	.013690	.008700
				2-65+ YR.	.006650	.024150	.007980
PROPYLENE GLYCOL NAS 0164	14	PROCSD VEGS(R)	*	0-5 MO.	**********	**********	[illegible]
				6-11 MO.	**********	**********	[illegible]
				12-23 MO.	**********	**********	[illegible]
				2-65+ YR.	**********	**********	425.000000
PROPYLENE GLYCOL NAS 0164	16	SOFT CANDY(R)	40	0-5 MO.	.178900	1.789000	[illegible]
				6-11 MO.	[illegible]	[illegible]	[illegible]
				12-23 MO.	[illegible]	[illegible]	[illegible]
				2-65+ YR.	[illegible]	15.748200	[illegible]
PROPYLENE GLYCOL NAS 0164	17	CONF FROST(R)	5	0-5 MO.	**********	5.073370	**********
				6-11 MO.	5.073370	10.146740	[illegible]
				12-23 MO.	10.146740	[illegible]	[illegible]
				2-65+ YR.	[illegible]	[illegible]	[illegible]
PROPYLENE GLYCOL NAS 0164	19	SWEET SAUCE(R)	4	0-5 MO.	.929570	[illegible]	[illegible]
				6-11 MO.	2.789910	9.609690	[illegible]
				12-23 MO.	[illegible]	[illegible]	[illegible]
				2-65+ YR.	[illegible]	[illegible]	[illegible]
PROPYLENE GLYCOL NAS 0164	20	GELATIN PUD(R)	17	0-5 MO.	[illegible]	[illegible]	[illegible]
				6-11 MO.	[illegible]	[illegible]	[illegible]
				12-23 MO.	[illegible]	[illegible]	[illegible]
				2-65+ YR.	7.362360	18.947250	[illegible]
PROPYLENE GLYCOL NAS 0164	21	SOUPS(R)	4	0-5 MO.	.000000	[illegible]	[illegible]
				6-11 MO.	[illegible]	[illegible]	[illegible]
				12-23 MO.	.010440	.020830	[illegible]
				2-65+ YR.	.009510	.025350	.012660

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
PROPYLENE GLYCOL NAS 0164	22 SNACK FOODS(R)	*	0-5 MO.	**********	.002000	**********
			6-11 MO.	.008000	.022000	.307900
			12-23 MO.	.022000	.002000	1.011090
			2-65+ YR.	[illegible]	[illegible]	[illegible]
PROPYLENE GLYCOL NAS 0164	23 BEV TYPE I(R)	35	0-5 MO.	.906000	1.309000	[illegible]
			6-11 MO.	0.560250	20.331750	[illegible]
			12-23 MO.	20.460500	61.343750	67.133000
			2-65+ YR.	39.260000	104.831750	126.856000
PROPYLENE GLYCOL NAS 0164	24 BEV TYPE II(R)	4	0-5 MO.	.000000	.000000	[illegible]
			6-11 MO.	**********	[illegible]	**********
			12-23 MO.	**********	.132300	**********
			2-65+ YR.	21.511750	62.483360	191.[illegible]
PROPYLENE GLYCOL NAS 0164	25 NUT PRODS(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	**********	**********
			12-23 MO.	**********	**********	**********
			2-65+ YR.	**********	**********	**********
PROPYLENE GLYCOL NAS 0164	26 RECONST VEG(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.000000	.000000	[illegible]
			12-23 MO.	.000000	.000000	.000000
			2-65+ YR.	.012000	.037800	.012000
PROPYLENE GLYCOL NAS 0164	27 GRAVIES(R)	4	0-5 MO.	.049010	.147030	.097030
			6-11 MO.	.686140	1.911390	1.372420
			12-23 MO.	1.764360	[illegible]	[illegible]
			2-65+ YR.	4.067020	10.439130	[illegible]
PROPYLENE GLYCOL NAS 0164	30 HARD CANDY(R)	23	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.071500	.214500	.135310
			12-23 MO.	.214500	.643500	.405130
			2-65+ YR.	.429000	1.219500	[illegible]
PROPYLENE GLYCOL NAS 0164	31 CHEWING GUM(R)	7	0-5 MO.	**********	**********	**********
			6-11 MO.	.068230	.068230	[illegible]
			12-23 MO.	.068230	.204690	[illegible]
			2-65+ YR.	.136460	.272920	[illegible]
PROPYLENE GLYCOL NAS 0164	48 SEAS FLAVRS(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	1.490000	**********
			12-23 MO.	**********	2.980000	**********
			2-65+ YR.	1.490000	7.450000	1.519295
PROPYLENE GLYCOL NAS 0164	ALL CATEGORIES ********** ********** **********	141	0-5 MO.	10.750160	27.723740	30.109690
			6-11 MO.	102.507730	208.167970	[illegible]
			12-23 MO.	192.711660	422.105350	593.909770
			2-65+ YR.	348.[illegible]	752.627140	1379.636475

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH P
PROPYLENE GLYCOL MSTEARAT NAS 0309	01 BAKED GOODS(R)	*	0-5 MO.	15.987820	21.160350	24.646000
			6-11 MO.	119.438420	243.579140	184.124600
			12-23 MO.	256.275350	422.266540	395.070500
			2-65+ YR.	645.155960	959.326740	994.562000
PROPYLENE GLYCOL MSTEARAT NAS 0309	04 FATS OILS(R)	*	0-5 MO.	50.046100	50.046100	60.000000
			6-11 MO.	280.258160	750.691500	336.000000
			12-23 MO.	630.580960	1201.108400	756.000000
			2-65+ YR.	1751.613500	3162.913520	2100.000000
PROPYLENE GLYCOL MSTEARAT NAS 0309	06 CHEESE(R)	*	0-5 MO.	**********	.500000	**********
			6-11 MO.	13.500000	45.000000	27.000000
			12-23 MO.	39.000000	111.000000	78.000000
			2-65+ YR.	47.000000	118.000000	94.000000
PROPYLENE GLYCOL MSTEARAT NAS 0309	07 FROZN DAIRY(R)	*	0-5 MO.	**********	**********	.010000
			6-11 MO.	**********	**********	.090000
			12-23 MO.	**********	**********	.144000
			2-65+ YR.	**********	**********	.256000
PROPYLENE GLYCOL MSTEARAT NAS 0309	19 SWEET SAUCE(R)	*	0-5 MO.	.002400	.003200	.002400
			6-11 MO.	.187200	.624000	.187200
			12-23 MO.	.540000	1.800000	.540000
			2-65+ YR.	1.414400	3.722000	1.414400
PROPYLENE GLYCOL MSTEARAT NAS 0309	20 GELATIN PUD(R)	*	0-5 MO.	14.552000	19.645200	15.331000
			6-11 MO.	93.132000	252.308000	98.123520
			12-23 MO.	100.402000	244.473000	105.769420
			2-65+ YR.	148.430400	361.990000	156.3543[illegible]
PROPYLENE GLYCOL MSTEARAT NAS 0309	23 BEV TYPE I(R)	*	0-5 MO.	**********	**********	.012000
			6-11 MO.	**********	**********	.113000
			12-23 MO.	**********	**********	.271000
			2-65+ YR.	**********	**********	.520000
PROPYLENE GLYCOL MSTEARAT NAS 0309	24 BEV TYPE II(R)	*	0-5 MO.	**********	**********	.000000
			6-11 MO.	**********	**********	**********
			12-23 MO.	**********	**********	**********
			2-65+ YR.	**********	**********	.162000
PROPYLENE GLYCOL MSTEARAT NAS 0309	28 IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	4.200000	6.900000	7.000000
			12-23 MO.	2.400000	10.200000	4.000000
			2-65+ YR.	2.700000	4.500000	4.500000
PROPYLENE GLYCOL MSTEARAT NAS 0309	ALL CATEGORIES **********	7	0-5 MO.	80.649320	91.434850	100.052000
	**********		6-11 MO.	510.716580	1332.624240	652.643820
	**********		12-23 MO.	1029.205810	1990.627340	1339.815720
	**********		2-65+ YR.	2596.313860	4629.455460	3351.800000

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Baked Goods	Propylene glycol	44	0-5 mo	12.250	40.542	37.834	125.218
			6-11 mo	22.682	43.071	70.054	133.029
			12-23 mo	43.387	70.969	134.005	219.193
			2-65 yr	108.508	161.142	335.136	497.700
Other Grain	Propylene glycol	*	0-5 mo	.074	.154	.075	.156
			6-11 mo	.150	.344	.152	.349
			12-23 mo	.165	.330	.167	.333
			2-65 yr	.284	.559	.287	.565
Fats, Oils	Propylene glycol	*	0-5 mo	.700	1.986	1.558	4.419
			6-11 mo	.586	1.186	1.303	2.639
			12-23 mo	.929	1.729	2.066	3.847
			2-65 yr	2.544	4.544	5.659	10.109
Milk Prods	Propylene glycol	*	0-5 mo	12.001	26.048	19.685	42.725
			6-11 mo	30.186	91.868	49.513	150.689
			12-23 mo	19.404	70.694	31.827	115.958
			2-65 yr	13.058	35.726	21.419	58.601
Cheese	Propylene glycol	7	0-5 mo	.050	.085	.434	.744
			6-11 mo	.619	1.159	5.393	10.104
			12-23 mo	.818	1.770	7.129	15.436
			2-65 yr	.924	1.927	8.059	16.799
Frozen Dairy	Propylene glycol	30	0-5 mo	9.624	22.273	14.881	34.438
			6-11 mo	21.036	42.347	32.525	65.475
			12-23 mo	25.298	50.459	39.115	78.017
			2-65 yr	46.334	94.318	71.639	145.830
Procsd Fruit	Propylene glycol	5	0-5 mo	12.501	32.018	20.751	53.149
			6-11 mo	33.104	73.226	54.953	121.554
			12-23 mo	58.007	105.342	96.291	174.867
			2-65 yr	63.640	126.934	105.642	210.709
Meat Prods	Propylene glycol	19	0-5 mo	2.558	6.407	5.822	14.581
			6-11 mo	7.064	14.364	16.076	32.687
			12-23 mo	7.440	12.322	16.931	28.040
			2-65 yr	18.588	30.605	42.301	69.647

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Poultry	Propylene glycol	*	0-5 mo	.100	.250	1.000	2.500
			6-11 mo	.109	.206	1.090	2.060
			12-23 mo	.128	.218	1.280	2.180
			2-65 yr	.204	.392	2.040	3.920
Egg Prods	Propylene glycol	*	0-5 mo	.008	.012	.010	.014
			6-11 mo	.015	.026	.018	.031
			12-23 mo	.020	.042	.024	.050
			2-65 yr	.030	.056	.037	.067
Procsd Vegs	Propylene glycol	*	0-5 mo	---	---	41.000	78.500
			6-11 mo	---	---	151.000	293.500
			12-23 mo	---	---	202.000	328.500
			2-65 yr	---	---	427.500	717.000
Soft Candy	Propylene glycol	40	0-5 mo	5.001	8.408	8.068	13.544
			6-11 mo	6.709	15.207	10.806	24.494
			12-23 mo	6.619	14.312	10.662	23.053
			2-65 yr	10.197	22.094	16.425	35.588
Seas, Flavors	Propylene glycol	*	0-5 mo	---	---	---	---
			6-11 mo	2.980	5.960	3.040	6.080
			12-23 mo	4.470	7.450	4.560	7.600
			2-65 yr	14.900	29.800	15.199	30.398
Bev Type II	Propylene glycol	*	0-5 mo	.000	.000	.000	.000
			6-11 mo	.265	.397	2.354	3.531
			12-23 mo	.463	.794	4.119	7.061
			2-65 yr	88.867	218.427	789.700	1941.885
Reconst Veg	Propylene glycol	*	0-5 mo	---	---	---	---
			6-11 mo	---	---	---	---
			12-23 mo	---	---	---	---
			2-65 yr	.113	.189	.113	.189
Gravies	Propylene glycol	4	0-5 mo	.540	1.029	1.078	2.059
			6-11 mo	1.519	2.892	3.039	5.784
			12-23 mo	3.431	6.861	6.862	13.724
			2-65 yr	6.518	12.939	13.038	25.880

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Hard Candy	Propylene glycol	23	0-5 mo	.000	.000	.000	.000
			6-11 mo	.572	.858	1.083	1.624
			12-23 mo	1.430	3.003	2.706	5.683
			2-65 yr	3.504	7.293	6.630	13.802
Chewing Gum	Propylene glycol	7	0-5 mo	---	---	---	---
			6-11 mo	.546	.955	2.400	4.200
			12-23 mo	.409	.887	1.800	3.900
			2-65 yr	.478	1.024	2.100	4.500
Conf Frost	Propylene glycol	5	0-5 mo	10.147	10.147	10.172	10.172
			6-11 mo	40.587	111.614	40.686	111.887
			12-23 mo	131.908	284.109	132.231	284.805
			2-65 yr	172.495	314.549	172.917	314.320
Sweet Sauce	Propylene glycol	4	0-5 mo	25.419	65.098	34.501	88.355
			6-11 mo	7.749	15.189	10.518	20.616
			12-23 mo	14.879	26.659	20.195	36.184
			2-65 yr	38.129	75.638	51.751	102.660
Gelatin, Pud	Propylene glycol	17	0-5 mo	8.481	26.057	16.107	49.486
			6-11 mo	8.265	17.251	15.696	32.762
			12-23 mo	7.254	14.977	13.777	28.444
			2-65 yr	11.152	22.592	21.179	42.906
Snack Foods	Propylene glycol	*	0-5 mo	.006	.010	.276	.460
			6-11 mo	.020	.036	.920	1.656
			12-23 mo	.034	.070	1.564	3.220
			2-65 mo	.042	.092	1.932	4.232
Bev Type I	Propylene glycol	*	0-5 mo	12.307	51.114	40.391	167.761
			6-11 mo	17.780	39.789	58.357	130.591
			12-23 mo	30.653	70.479	100.607	231.321
			2-65 yr	53.718	120.385	176.310	395.117
Baked Goods	Propylene gycol mstearat	*	0-5 mo	72.886	241.228	112.360	371.834
			6-11 mo	134.956	256.275	208.046	395.071
			12-23 mo	258.156	422.267	397.970	650.960
			2-65 yr	645.626	958.799	995.288	1478.071

TABLE 14

Food Category	Substance	# of Firms	Age	Average	High A	High B	Very High
Fats, Oils	Propylene glycol mstearat	*	0-5 mo	490.452	1391.282	588.000	1668.000
			6-11 mo	410.378	830.765	492.000	996.000
			12-23 mo	650.599	1211.116	780.000	1452.000
			2-65 yr	1781.641	3182.932	2136.000	3816.000
Cheese	Propylene glycol mstearat	*	0-5 mo	3.500	6.000	7.000	12.000
			6-11 mo	43.500	81.500	87.000	163.000
			12-23 mo	57.500	124.500	115.000	249.000
			2-65 yr	65.000	135.500	130.000	271.000
Frozen Dairy	Propylene glycol mstearat	*	0-5 mo	---	---	.070	.162
			6-11 mo	---	---	.153	.308
			12-23 mo	---	---	.184	.367
			2-65 yr	---	---	.337	.686
Gelatin, Pud	Propylene glycol mstearat	*	0-5 mo	170.986	525.327	180.149	553.478
			6-11 mo	166.620	347.793	175.549	366.430
			12-23 mo	146.248	301.954	154.085	318.135
			2-65 yr	224.828	445.478	236.876	479.885
Bev Type I	Propylene glycol mstearat	*	0-5 mo	---	---	.163	.677
			6-11 mo	---	---	.236	.527
			12-23 mo	---	---	.406	.934
			2-65 yr	---	---	.712	1.595
Bev Type II	Propylene glycol mstearat	*	0-5 mo	---	---	.000	.000
			6-11 mo	---	---	.002	.003
			12-23 mo	---	---	.004	.006
			2-65 yr	---	---	.671	1.650
Imit Dairy	Propylene glycol mstearat	*	0-5 mo	.000	.000	.000	.000
			6-11 mo	49.800	62.700	83.000	104.500
			12-23 mo	24.900	45.000	41.500	75.000
			2-65 yr	17.400	51.000	29.000	85.000

PROPYLENE GLYCOL
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About the toxicity of the ethylene and propylene glycols.

By Professor M. Aiazzi-Mancini, Director.

After the introduction of ethylene glycol into the chemical-phamaceuthical industry as a solvent by SEINDELFELD and HANZLICK, its use became widespread insofar as an American business firm, with unpardonable levity, made an elixir based on Protonsil commercially available (para-amino-benzen-sulfamide); the solvent of said substance consisted of 40% ethylene glycol. This elixir was the cause of death in more than 92 cases in 1937. It is incomprehensible how such a disaster could have happened in America where pharmacologists are foresmost in the study of glycogen pharmacology and toxicology.

These intoxications caused alarm all over Europe:some attempted in good faith , and others in bad faith, to hold Protonsil responsible, while only the ethylene glycol was at fault. To reveal the truth about the toxicity of the glycol in question, I resumed a study of the same which I had already undertaken in 1935; I mention this study briefly here, while I refer to another of my publications which is in course of printing, for ample specifications and for the bibliography (1).

The resreach work of the above-mentioned American authors is much more interesting from the viewpoint of an eventual application in practice; said authors administered to numerous groups of rats ethylene glycol solutions at 10-5-3-2-1-0.5%; they were able to demonstrate that those groups which received from 0,70 to 2.20 of ethylene glycol per kg of body weight during 1.0 to 40 days, supplied each day grew as did the control groups, and remained entirely sound.

With higher doses than those mantioned above, the animals died within a more or less short term from acute nephritis, following the appearance of calcium oxalate, as a result of the transformation of the ethylene glycol into oxalic acid.

TABLE I.

Acute ethylene glycol intoxication: minimal lethal dose in cc per kg of body weight.

TABELLA I.

Intossicazione acuta da glicole etilenico: dose mortale minima in cc per kg di peso corporeo.

Via usata 1	Secondo Hanzlich e Seidenfeld e Johnson 2		Secondo Ajazzi-Mancini 3	
	Animali 4		Animali 4	
	Mus rattus 5	Coniglio 6	*Mus rattus* 5	Coniglio 6
Endomuscolare 7	3.8	4	3,50	3,70
Endovenosa 8	6	6	5,50	5,70

1. Method used
2. According to HANZLICK and SEIDENFELD and JOHNSON
3. According to AJAZZI-MANCINI
4. Animals
5. Rat
6. Rabbit
7. Endomuscular
8. Endovenous

This possible intoxication (which, however, occurs with doses that are absolutely unreachable in human therapy), together with the repercussions of the American hecatomb, discredited the ethylene glycol to some extent. I therefore focussed my attention on propylene glycol which, while having the same xcellent solvent properties in its ethylene homologus, is incomparably less toxic than ethylene glycol.

TABLE II.

Acute propylene glycol intoxication: minimal lethal dose in cc per kg of body weight.

TABELLA II.

Intossicazione acuta da glicole propilenico: dose mortale minima in cc per kg di peso corporeo.

Via usata per l'iniezione 1	Secondo Hanzlick e Seidenfeld 2		Secondo Ajazzi-Mancini 3	
	Coniglio 4	Ratto 5	Coniglio 4	Ratto 5
Endomuscolare 6	7.00	15	7.85	13,9
Endovenosa 7	5.00	19 (?)	4,50	11.6

1. Injection method used 2. According to HANZLICK and SEIDENFELD
3. According to AJAZZI-MANCINI
4. Rabbit 5. Rat
6. Endomuscular 7. Endovenous

With these high doses it is difficult to establish how much of the causa mortis can be attributed to the hydrodynamic disorder and how much to the intrinsic toxicity of the propylene glycol.

Chronic intoxication

According to the investigations of HANZLICK and SEIDENFELD, rats kept on standard food for 140 days and which were given solutions of propylene glycol to drink, showed a sound and robust development, with a weight increase from 50 to 175 g (i.e. a growth rate similar to the rate for the control animals), despite the administration of 13.30 g propylene glycol per kg of body weight each day.

My investigations are incomplete because--since propylene glycol is not manufactured in Italy--I had to discontinue the tests after having exhausted the product which was kindly given to me by Prof. WEESE of Elberfeld. The results are neverthless instructive: in fact those animals which received propylene glycol each day for 42 days reached the same weight as did the control animal group; no pathological element was ever found in the urine . During said period of time, the animals received 11,87 g propylene glycol daily per kg of body weight--a quantity certainly below the volume administered by my American colleague. But this is neverthless an always valuable data because it leads to highly important conclusions regarding applications in human therapy, whereby a man with an average weight of 70 kg could be given approximately 70 cc of propylene glycol per day for weeks without experiencing the slightest disorder.

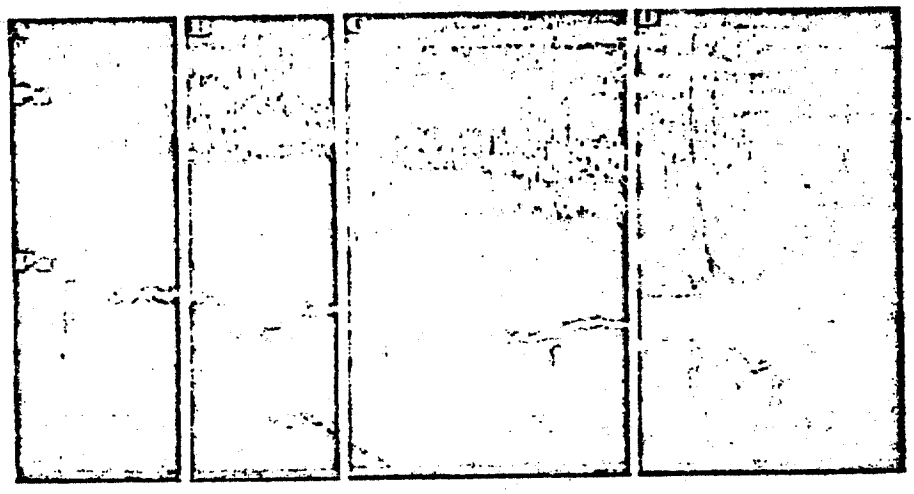

Fig. 1. - Rabbit, of 1850 g.-Pn = pneumogram; Pac = arterial carotid pressure.

A. Between the two arrows, injection of ½cc of propylene glycol.

B. 9 minutes after A: between the two arrows, 1 cc of propylene glycol. 1½ cc of propylene glycol was injected between A and B.

C. 20 minutes elapsed between B and C; in the meanwhile, 16 cc of propylene glycol was (still endovenously) injected. 6 cc of ethylene glycol was injected between the two arrows.

D. 8 minutes elapsed between C and D; in the meanwhile 7 cc of propylene glycol was injected. 7 cc of propylene glycol was injected bwetween the two arrows. The animal died of bulbar paralysis after having endovenously received 38 cc of propylene glycol.

I likewise intended to investigate the behavior of said compound in correlation with the circulatory and respiratory systems.
A rabbit with a weight of 1,850 kg was used for this test; it was prepared according to the customary technique. The propylene glycol was administered endovenously (margin of the ear); the substance has an agitating effect on the respiratory activity while the pression is slightly modified. The rabbit received enormous volumes of propylene glycol and its death as a consequence of bulbar paralysis occurred only when I administered 32 cc of propylene glycol (equal to 20 cc per kg of body weight) within 37 minutes (see Fig. 1.).

In view of the results yielded by my above-mentioned tests, which results coincide fully with those of the American authors, the following conclusions can be reached:

1) The ethylene glycol can be supplied without harmful effects, using any administration method, in daily doses of a few cc (from 0,70 cc to approximately cc); in stronger doses (10 to 15 cc per day) it can cause oxaluria; with extremely strong doses (40 to 50 cc daily) it causes death or serious acute nephritis intoxocations.

2) The difference between the toxicity of the propylene glycol and of the ethylene glycol is enormous; the use of propylene glycol can be prolonged for weeks and months, even with high doasges, without any inconveniences resulting from the same; this is likewise true for doses, with reference to results obtained with rats by the American authors, which (doses) could reach for humans up to 8 to 900 g per day.

3) Propylene glycol can therefore be used on a large scale in therapy as a solvent without any reasons for misgivings , because its toxicity rate is extremely low.

(From the Pharmacology and Toxicology Institute of the Royal University at Florence. - Meeting of November 15,1938 - XVII.

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PHARMACOLOGICAL ASPECTS OF PROPYLENE GLYCOL

by Maria Asuncion Viñals
J.C. Mutis Institute of Pharmacognosis

Introduction

It is intended, in this work, to gather a sum of data obtained from various experiments that let us deduce some conclusions relating to the pharmacological effects of propylene glycol and the modifications that propylene glycol exerts on pharmacological actions of other substances.

Taking into consideration all of the applications, more frequent every day, of the glycols in pharmaceutical preparations and continuing the numerous works, in this sense, we have carried out several tests, because the utilization of propylene glycol is of considerable importance and the problem of its tolerance and toxicity is of practical interest.

This is another contribution to the interesting professional problem of its utilization as a medium of medicinal substances.

The sum of its properties is unique and has immediate and wide applications.

Propylene glycol for pharmaceutical uses was first utilized as a substitute for glycerine for excipients in creams and ointments containing sulfamids, iodine, etc. Several tests were done for testing its toxicity and its effects, according to dosage, had been studied (34).

Its bacterial action has been described and utilized as a preservative in many pharmaceutical preparations, because its ability to inhibit the growth of microorganisms is similar to that of alcohol. The use of propylene glycol as a sterilizing agent is very useful for several preparations in which the method of sterilization by heat has to be avoided due to the heat capacity of the dissolved medicament (13).

Propylene glycol at 40% in water, prevents the fermentation of yeast. At

21%, it destroys pancreatic diastase and the oxidases of the crude potato, and at 20% quickly destroys penicillin (37).

The studies on bacterial spores proved that a vapor of propylene glycol destroys the vegetative form of the BACILLUS SUBTILIS in suspension in the air. In the culture medium, it follows the growth of the BACILLUS SUBTILIS in the presence of less than 20% of propylene glycol, requiring 80% to destroy living organisms. The spores are not destroyed in any case (1).

The bactericidal action is probably due to the hygroscopicity of the glycols, since the aqueous solutions of propylene glycol which contain large quantities of this substance have a low vapor pressure. According to Wells and Zappasode (46), the action comprises the condensation of propylene glycol on the bacteria, acting upon these as nuclei. The high humidity of the air inactivates, almost completely, the vapor of glycol because of saturation.

From 4 glycols studied, only the propylene glycol showed germicidal activity against STREPTOCOCCUS AUREUS. Heavy concentrations of glycol were required to inhibit the gas production by ESCHERICHIA COLI and AEROBACTER AEROGENES. The effect of the glycol is greater against the SACCHAROMYCES CEREVISIAE than against the ESCHERICHIA COLI or AEROBACTER AEROGENES.

The ethylene glycol has slight germicidal action against the SALMONELLA TYPHI.

The propylene glycol can be utilized as an aerosol for air sterilization. Tests conducted in air chambers with several microorganisms proved that the environment was quickly sterilized (39).

The vapor of propylene glycol protects mice from being infected by influenza virus, transported through the air and resistant to ordinary germicides, but not to ultraviolet rays. The mice were placed in a chamber where the propylene glycol vapors were introduced in the proportion of 1 g/2,000 - 3,000l of air; 0.2 - 1 ml of the solution at 10^{-2} of the virus was atomized. All the mice protected by

propylene glycol survived, but those in the control group were dead in 4-10 days(40).

No toxic consequence has been observed exposing animals to saturated areas of propylene glycol during long periods (12 - 13 months) (13).

As a dissolvent, it is excellent.

A temperature of 25° C dissolves the following products up to the concentrations (percentages) indicated: antypirina, 55; chloral hydrate, 89; ethylvarbamate, 57; sodium luminal, 49; urea, 22.2; sulfanilamide, 7.25; benzocaine, 12; camphor, 9.8; chlorothymol, 70; menthol and thymol, 50.

Strychnine is soluble at 90% and precipitates in cold. It also dissolves a number of colorants.

The possible use of glycol as a solvent for the chloromycetin for intramuscular injections and for penicillin and aureomycine has been studied.

It has been used as a solvent in the preparation of polivitamins for oral administration (drops) in pediatric use. The solution of Vitamins D_2 and D_3 has been successfully used in this case, when the glycol is substitutes for the the olive or peanut oil or the ethyl alcohol (13).

The incompatability in an acqueous solution between Vitamins B_{12} and C has been recently demonstrated. This can be resolved by dissolving the two vitamins in anhydrous propylene glycol.

The activity of Vitamin B_{12} appears three times greater if it is dissolved in glycol instead of oil; Vitamin B_6, riboflavin and chloral hydrate of thiamine are similarly dissolved. It has been used in intramuscular injections as a solvent of Vitamin D, progesterone and sodium luminal. For this use, its DL_{50} must be inferior to 10 ml/kg of anima, experimenting in rabbits or rats (35).

In 1931, this dissolvent was proposed for the preparation of stabilized solutions of barbiturates which are less soluble in water in free acid state; and that as a sodium salt, are quickly alterable. In chloral hydrate, an hypnotic substance used against insomnia of psychic origen, the problem of conservation

exists; this can be resolved by substituting the aqueous medium with propylene glycol. A classic example of stabilization is the solution of aspirin for oral administration (30).

The therapeutic activity of irradiated ergosterol dissolved in propylene glycol (0.03 g%) was proved to be 2 - 3 times greater to the one of Vitamin D crystalized and administered in oleosam solution; the propylene glycol does not result toxic (11).

As a solvent for steroid hormones, it can be used for extemporaneous preparation of mycrocrystalline suspensions of retarded absorption: since glycol, pure or mixed with variable quantities of the miscible or hydrosoluble solvents, permits the preparation of solutions, which when dissolved with water or dispersant solutions, produce precipitations with optimum physical characteristics for gradual and slow absorption of the medicinal ingredient (13).

In some preparations, it has protective action on medicaments easily oxidizable. Adrenalin can be protected from oxidation by anhydro glycol or glycol containing 30 - 40% of water; apomorphine, physostigmine and salvarsan, also are protected from oxidation with this dissolvent. For many substances easily hydrolized, with loss of activity, glycol is the most adequate medium for the preparation of stabilized solutions for parenteral use (13).

Studying its influence as a medium of medicaments, it has been demonstrated that propylene glycol produces pharmacological synergism between medicaments and medium; in some cases, also, propylene glycol can produce pharmacological antagonism between medicinal substance and medium (28).

Propylene glycol eventually added to TWEEN 20, can replace the alcohol and glycerin in pharmaceutical preparations. These products are stable and their acrid taste is easily discerned. Only the viscosity appears slightly accentuated (2).

Pentobarbital elixirs were prepared according to the formula N.F. IX, substituting all the glycerol for the following substances:

a) one volume equal to D-sorbitol at 80% (p/v)

b) one volume equal to a mixture of 2:1 (v/v) of sorbitol solution at 70% and propylene glycol

c) one volume equal to a mixture of 1:1 (v/v) of sorbitol solution at 70% and propylene glycol.

a) and b) are satisfactory in density, taste and approximate viscosity, but they do not conserve the solution of pentobarbital.

Substituting the glycerol in 27 N.F. elixirs by a 1:1 mixture of 70% sorbitol and propylene glycol, similar officinal products are obtained (19).

Even so, it is used as a solvent of volatile medicaments such as ethyl nitrite, obtaining solutions which still maintain their concentration in unfavorable conditions (13).

In some cases, this solvent can reinforce the local tolerance to medicinal substances particularly painful in hypodermic administration. For example, solutions of quinidine base (13).

In a free state, it has been proposed as an excipient for mediums of topical action medicaments (4); as a solvent for solutions of hypodermic use of steroid hormones (12) and adrenalin base; and for extract active ingredients of drugs containing saponin, tannin and anthraquinone derivatives, etc. (36).

In the utilization of the glycols as a solvent of medicinal substances for injectables, the solvent, due to its solubility in water, is easily absorbed by injection; on the other hand, the solution precipitates precisely at the moment of injection if its solubility in water is low, remaining more or less inert in the organism. The pharmacological action depends on the chemical solubility in water (23).

Jaros (18) has proposed the use of propylene glycol as a dissolvent of benzyl alcohol and of chloral hydrate in piperidinepropanedioldiphenylurethane for the preparation of a topical anesthetic useful in tonsillectomies, and Douglas and

Peyton (9) have successfully used a solution in glycol of paraldehyde as an anesthetic in obstetrical practices.

Clark (6) affirms that substituting 25% water in "antiseptic solution" of N.F. VI with propylene glycol, a preparation more acceptable for its organoleptic characteristics is obtained.

In high dosage, the propylene glycol has diuretic and narcotic action, similar to one-third of the action produced by ethyl alcohol (33): the anesthetic dose for a dog is almost 4/5 of the lethal dose (15). None of these effects is of importance to the dose normally utilized as a medicinal medium in the injectable form.

The "Leicester Pharmaceutical Committee" has called attention to the dangerous use of propylene glycol as a solvent for chloramphenicol when this medicine must be used in the treatment of ocular disturbances and it cannot be recommended for nasal applications (3).

Chloramphenicol solutions in propylene glycol can be prepared for the treatment of ear infections and for wounds. For colliriums, a moderate aqueous solution must be utilized and the same holds true for nasal drops.

Toxicity: the lethal dose for rats is 33.5 g/kg by oral administration; 22.5 g intracutaneous; 14 g intramuscular and 6.8 g intravenous. Several other investigators have found very similar results, except in the intravenous administration where they found it to be more than double. Studying its chronic toxicity by its addition to water swallowed by growing rats, it showed: a 3% concentration did not produce any change in the growing process; 10% produced a slow growing rate for 10 days, after which it normalized itself. The animals were killed after 100 days and their organs, histologically, did not show any significant change. In the rats, hematuria was observed after the administration of a sub-lethal dose. The hemolysis <u>in vitro</u> was produced with concentrations of more than 0.14 M, apparently due to the dilution of the isosmotic solution of ClNa

with the osmotically inactive solution of glycol, and not to the specific hemolytic dilution of glycol in solution (10).

Bucciardi (5) found that ethylene and propylene glycol had a slightly toxic action in rats and rabbits, but diethylene glycol and ethylene glycol monoethyl ether were very toxic. Small doses of diethylene and propylene glycol, injected intravenously, provoked a transient drop in blood pressure and large intramuscular doses caused the same effect. The continued administration of wthylene glycol to dogs produces oxaluria. If it is administered at 17% or more of ethylene glycol, there is hemolysis. In humans, the 2-3 ml intramuscular injection of ethylene or propylene glycol as a chemical solvent is harmful.

According to comparative tests, propylene glycol is less toxic than ethylene glycol. The toxicity of the glycols is extremely variable according to their administration. In one oral dose, the fatal mean dose for dogs is 25 l/kg of dipropylene glycol. Smaller doses, repeated daily, do not show any toxicity. Two doses of ethylene glycol of 2 ml/kg each produced death in rats in 4 days.

Six doses of 1.5 cm/kg each of diethylene glycol were required to cause death in rats. A remarkable degeneration of the liver and kidneys was observed. Propylene glycol and dipropylene glycol caused temperal depression of the central nervous system. Propylene glycol, added in large doses for a very long time to the food of rats, did not produce toxic effects (15).

From other tests, with rats and guinea pigs, the toxicity of glycols has been deduced; in ascendant order they are: 1. propylene glycol; 2. diethylene glycol; 3. ethylene glycol; 4. diethylene glycol monoethyl ether; 5. dioxane and ethylene glycol monoethyl ether (24).

From the mortality curves of white rats and guinea pigs produced by the administration of 60 glycol derivatives, the least toxicity of the α ethers against the β ethers of propylene glycol is deduced (42).

Providing a diet of 30% of glycerol to young rats, they grew normally; but a greater quantity of glycerol provoked retarded growth. During five consecutive generations a group was raised with a diet of 30% of glycerol. On the contrary, the young rats on a diet of 30% of propylene glycol grew less rapidly than normal; on a diet of 40%, 50% or 60%, they lost weight and died in a few days, having excreted in the urine most of the propylene glycol. The females on a diet of 30% of propylene glycol did not wean their brood (45).

Harold and Holks (16) observed that a concentration of 20% of glycerol in the rats' diet is harmless and does not stop their growing process or their development.

The tests on toxic, hypnotic and irritant properties of eight dissolvents, conducted on rabbits, guinea pigs, rats and frogs showed as a result that the least toxic of all of them was propylene glycol, followed by ethylene glycol which is already noticeably toxic because in the organism it is transformed into 3-4% in oxalic acid, triethylene glycol and glycerol.

When injected into rats, it provoked death due to respiration failure, while their hearts continued beating for a while thereafter. The glycerol produced chronic convulsions (25).

By means of 35 references of toxicity of glycols, the following conclusions can be drawn: Orally, for rats, diethylene glycol is the most toxic of all and propylene glycol is the least toxic; hypodermically, for guinea pigs, diethylene glycol is the most toxic of all, followed by propylene glycol. Orally, glycerol is less toxic than glycol, diethylene glycol and propylene glycol; hypodermically, glycerol is almost as toxic as propylene glycol. Rectally, the four irritate the intestinal mucose (26).

The rats which received 5-20% of ethylene glycol in their drinking water lost weight and finally died; 20% of glycerol and 1-2% of diethylene glycol did not produce that effect. Diethylene glycol can modify the action of other substances jointly administered (27).

The toxicity of trimethylene glycol, for rats, is almost twice that of propylene glycol. Cats are more sensitive and rabbits less. In rats, trimethylene does not show glucogenic action. Its employment in inadequate as a solvent of foods and medicinal substances (49).

In toxicity tests, it was found that diethylene glycol monoethylether and propylene glycol did not produce renal irritation in animals or humans. In the conjunctival bag of the cat, diethylene glycol monoethylether produced slight irritation, but a very dangerous one was produced in the rabbit, causing conjunctivitis with the production of iritis and corneal ulcer. Both glycols administered without dilution causes vomiting with a slight irritant effect on the intestinal tract. Administering both to rabbits during several weeks in a 20% solution, such irritant effects are no greater. Administering enough diethylene glycol monoethylether to cats produced chronic poisoning with hyperemia, kidney degeneration and later pneumonia. With 3 ml daily of propylene glycol, only some hyperemia and slight kidney degeneration was produced. The injection of several glycols produced the following results: Little infiltration without necrobiosis, with methyl glycol; great infiltration and small areas of necrobiosis, with diethylene glycol monoethylether and methyl glycol of acetate.

The following doses in ml/kg were fatal in 24 hours: Butane glycol, 1-2; ethylene glycol, 2; acetate of ethylene glycol, 3; methyl glycol, 3-4; acetate of methyl glycol, 4; diethylene glycol monoethylether, 7; propylene glycol, 20 (47).

The DL of propylene glycol as a solvent was determined by intraperitoneal injection in adult rats of 9.73 g/kg. It produces pulmonary congestion and edema, toxic reations in the spleen and thymus, degeneration of the glomeruli and renal tubules and slight damage in the liver. It does not produce nephritic hemorrage, cristallin oxaluria or damage to the suprarenal glands, osseous medulla, sympathetic system, gastrointestinal portion or gonads.

The rapid intravenous injection of 0.12 ml/kg or more of 1-2 propylene glycol

at 1-3 butylene glycol in anesthetised dogs produced a drop in blood pressure. Lesser quantities produced an increase in the peripheric blood flow and greater quantities, slowly administered by injection, did not produce any effect.

Ethylene glycol and propylene glycol are absorbed by subcutaneous, oral and rectal administration. Almost one-half of ethylene glycol and one-quarter of propylene glycol, injected into the blood stream are eliminated in the urine in 24 hours without alteration. Whatever the means of administration, the excreted quantities of ethylene glycol are 2-3 greater than those of propylene glycol (32).

According to Winkle (Jr.) (48), propylene glycol is absorbed by every part of the intestinal tract of rabbits, cats and rats. In distilled water solutions, the blood hemolizes at all concentrations, but in ClNa solution at 0.9%, the hemolysis does not begin until it reaches a concentration of 38%. The rats, drinking a 5% solution, were dehydrated. If they drank solutions at 15-20%, they excreted approximately one-third by the kidneys; the rest was metabolized. The concentration in the urine never exceeded 10%, although large quantities were fed to the rats.

Newman and Lehman, in 1937 (33), demonstrated that propylene glycol is easily absorbed and oxidated in the organism, similar to the ethyl alcohol and glycerol, the rapidness of combustion being independent of the dosage. One part is excreted in the urine, without alteration, up to 45% in 24 hours, with large dosages. Its rapid combustion and excretion help to explain its low toxicity, which is approximately the same as that of glycerine.

Nevertheless, Hunt ascribed the toxicity of propylene glycol to its oxidation in oxalic acid, but this seems to affect only a small fraction and he cannot explain the symptoms. The ethylene glycol monoacetate also produces a small quantity of oxalic acid which is not produced by dioxane, diethylenic ether of ethylene glycol and ethanolamide (Willy and collaborators, 1938).

All of the glycols damage the kidneys because of the accumulation without

excretion. Renal injury was observed in rats injected with 6 ml of ethylene glycol/kg, but it was not observed in mice injected with 6 ml of propylene glycol/kg. The toxicity increases if the glycol can not be metabolized or if it produces toxic metabolites. The destruction of the glycols is carried out with more celerity when the OH groups are contiguous. The propylene glycol CH_3-CHOH-CH_2OH is less toxic than the CH_2OH-CH_2-CH_2OH, and the CH_3-CHOH-CHOH-CH_3 is less toxic than the CH_2OH-CH_2-CH_2OH. The toxicity of ethylene glycol does not seem to be due to the formation of oxalic acid, COOH-COOH (41).

Administering to rats, during 2 years, propylene glycol at 1-3% in their diet, they did not suffer any apparent damage. The ethylene glycol and diethylene glycol produced numerous renal calculus constituted by calcic oxalate. Two-thirds of the rats to which ethylene glycol monoethylether was administered had an increase in the size of the testicles, edema and tubular atrophy. These symptoms were produced, in a lower proportion, in rats which were given similar compounds (31).

The studies on DL50 of propylene glycol by intraperitoneal injection in rats, calculated by unities of probability, was 9.33 to 9.70 ml/kg. Thus, its utilization is not convenient as a solvent in studies of toxicity, particularly in these animals(22).

The effect in vitro produced in the erythrocytes was studied and it was seen that they first disappeared from the bright field, but not from the dark field of the microscope, coinciding with the apparent total hemolysis of the blood-glycol solution. They reappeared, afterwards, on the bright field of the microscope and this reappearance depends upon temperature and glycol concentration. The spontaneous reappearance is attributed to an increase in the intracellular density with regard to the extracellular and it is probably associated with the coagulation, precipitate or other alteration of the cellular proteins. It concludes, finally, with the cellular destruction and the formation of a dark precipitate. Also, in high concentrations of propylene glycol, the alteration of the plasma, free of cells, is frequent and a dark precipitate is formed (44). Therefore, the physic-

chemical reactions produced by propylene glycol are not those which affect the erythrocytes specifically.

Mallery and Theron (29) observed a differential coloration of leucocytes, dissolving blood in a solvent constituted by 0.1% of eosin and 0.1% of methylene blue dissolved in equal parts of propylene glycol and water, obtaining an exact inventory of the leucocytes, the same in normal blood as in abnormal (leudemia). This coloration takes place only when the dissolvent is formed by propylene glycol and water in equal parts.

Also, they proved that the mentioned solvent has technical advantages over the use of acethic acid as a solvent liquid. These authors described the effect of several concentrations of water and propylene glycol on leucocytes in vitro.

Measuring the convulsive limen of rabbits non-anesthitised with the Tainter and collaborators method, remarkable disminution of excitability by bromures, alcohol, propylene glycol, paraldehyde and tribromoethanol was observed (43).

The isolated lung of the guinea pig was contracted by aerosols of histamine, acetylcholine or serotonin. Aerosols of isoproternol, procaine or propylene glycol were capable of expanding the lungs, but these were then resistent to the action of pharmaco -constrictors, including dosages much larger than normal. The pre-treatment with sympathomimetic aerosols prevents the action of the pneumoconstrictors (8)

Experimental Part

In the preceding pages, the most important bibliography related to our work has been selected. The authors who have studied the toxic effects of propylene glycol do not agree unanimously and there are not numerous references to the possible modifications that such glycol has on the chemical action of the drug for which it serves as a medium. Therefore, we have conducted our investigations as follows:

A) Toxicity of the propylene glycol in rats, as it can be deduced from the

study of their growth, weight of their organs, some hematic constants and the cholesterolemia.

B) Test on the entire animal: a) influence of the propylene glycol on the intestinal absorption; b) modification of the hypnotic action of luminal; c) modification of the mydriatic action of the athropine.

C) Modification of the chemical actions on isolated organs: a) acetyl coline b) athropine; c) camphor.

A) TOXICITY OF PROPYLENE GLYCOL IN RATS, AS CAN BE DEDUCED FROM THE STUDY OF THEIR GROWTH, WEIGHT OF THEIR ORGANS, SOME HEMATIC CONSTANTS AND THE CHOLESTEROLEMIA.

a) Influence of propylene glycol on growth.

a_1) Parenteral administration.

For this test, a group of 4 female Wistar rats, all 5 weeks old, were used; another group, under the same conditions, was used as a control group. The animals, in individual cages, were fed the same amount of food.

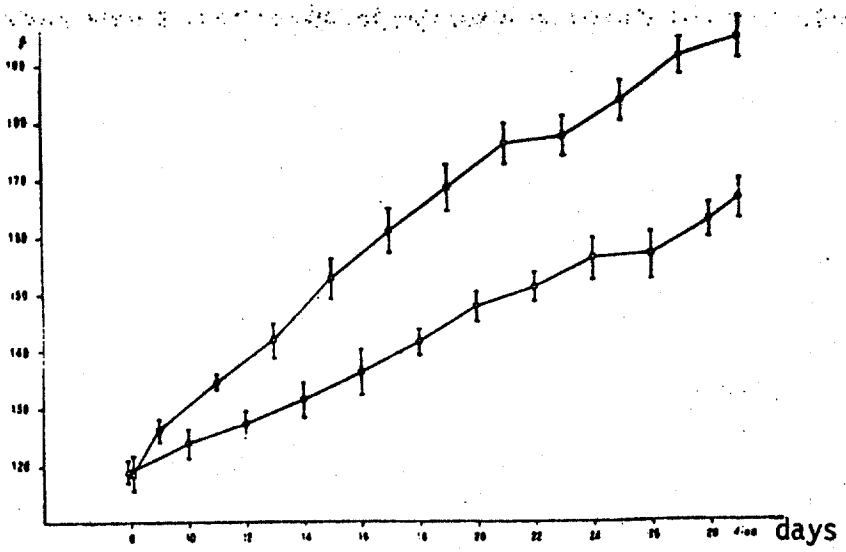


Figure 1. Upper curve: control group rats. Lower curve: treated rats.

The first group was injected intramuscularly daily for 22 days with 0.5 ml of propylene glycol. Every 24 hours the weight of each animal was recorded. The averages are collected in Fig. 1, where each value is accompanied by the variation limit, represented by a vertical segment. The statistical calculation of the results at the end of the experimental period is shown in Tables 1 and 2.

Conclusions. From the analysis of the results, we can conclude that the inhibition of growth provoked by propylene glycol in trial dosage is highly significant.

Table 1. Maximum weights reached

Rats treated with 0.5 ml of propylene glycol

1ª	2ª	3ª	4ª
176	165	162	161

$$\text{Media } M_1 = \frac{664}{4} = 166$$

$$\text{Deviations} \begin{cases} 1^a = 176 - 166 = 10 \\ 2^a = 165 - 166 = -1 \\ 3^a = 162 - 166 = -4 \\ 4^a = 161 - 166 = -5 \end{cases}$$

$$d^2 = \begin{cases} 1^a = 100 \\ 2^a = 1 \\ 3^a = 16 \\ 4^a = 25 \end{cases} \qquad \Sigma d^2 = 142$$

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}} = \sqrt{\frac{142}{4-1}} = 6{,}88$$

$$\varepsilon = \frac{\sigma}{\sqrt{n}} = \sqrt{\frac{\Sigma d^2}{n(n-1)}} = \frac{6{,}88}{\sqrt{4}} = 3{,}44$$

$$Cv = \frac{\sigma}{M} 100 = \frac{6{,}88}{166} \cdot 100 = 4{,}1445$$

$$t = \frac{M_1 - M_2}{\sqrt{\varepsilon_1^2 + \varepsilon_2^2}} = \frac{193{,}75 - 166}{\sqrt{3{,}40^2 + 3{,}44^2}} = \frac{27{,}75}{4{,}83} = 5{,}74$$

$\sigma = 6{,}88$

$\varepsilon = 3{,}44$

$Cv = 4{,}1445$

$t = 5{,}74$

Table 2. Maximum weights reached

Control Group Rats

1ª	2ª	3ª	4ª
189	187	201	198

$$\text{Media } M_c = \frac{775}{4} = 193{,}75$$

$$\text{Deviations} \begin{cases} 1^a = 189 - 193{,}75 = -4{,}75 \\ 2^a = 187 - 193{,}75 = -6{,}75 \\ 3^a = 201 - 193{,}75 = 7{,}25 \\ 4^a = 198 - 193{,}75 = 4{,}25 \end{cases}$$

$$d^2 = \begin{cases} 1^a = 22{,}5625 \\ 2^a = 45{,}5625 \\ 3^a = 52{,}5625 \\ 4^a = 18{,}5625 \end{cases}$$

$$\Sigma\, d^2 = 138{,}7500$$

$$\sigma = \frac{\Sigma\, d^2}{n-1} = \sqrt{\frac{138{,}75}{4-1}} = 6{,}80$$

$$\varepsilon = \frac{\sigma}{\sqrt{n}} = \sqrt{\frac{\Sigma\, d^2}{n(n-1)}} = \frac{6{,}80}{\sqrt{4}} = 3{,}40$$

$$Cv = \frac{\sigma}{M}\, 100 = \frac{6{,}80}{193{,}75} \cdot 100 = 3{,}5096$$

$$\sigma = 6{,}80$$

$$\varepsilon = 3{,}40$$

$$Cv = 3{,}5096$$

The relation between the difference of averages divided by the square root of the errors is the "t" of Student. Margin of error=7.

a_2) Oral administration of drinking water.

This second test was verified in 2 parts: first, from our laboratory colony, we utilized albino adult rats, all of them 6 months old,and divided them into 2 groups: 2 males and 2 females in the first group (experimental) and 2 males and 2 females in the second group.

All of the rats were isolated in different cages.

The drinking water for the experimental group of rats contained 50% propylene glycol.

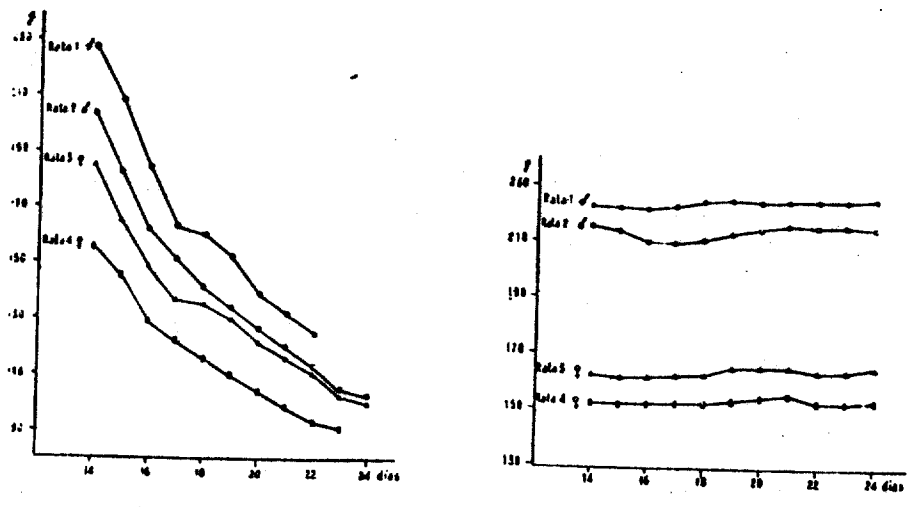


Figure 2. a) Treated rats. b) Control group rats.

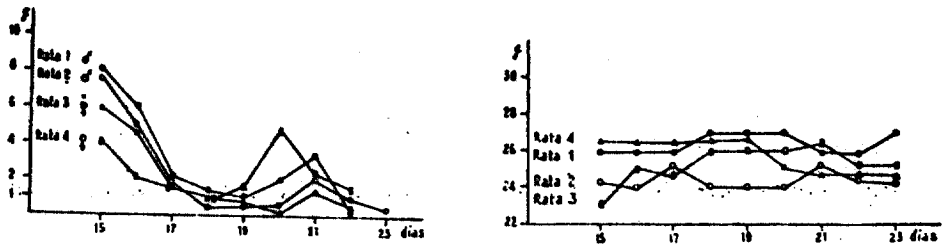


Figure 3. a) Treated rats. b) Control group rats.

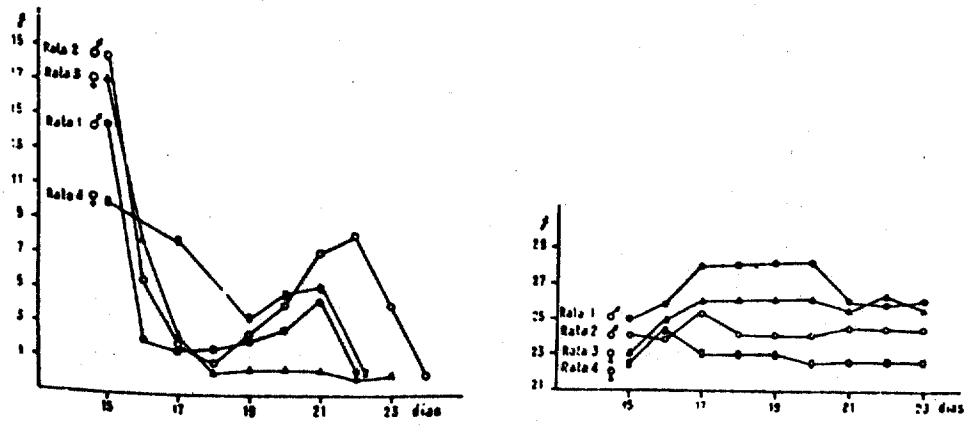


Figure 4. a) Treated rats. b) Control group rats.

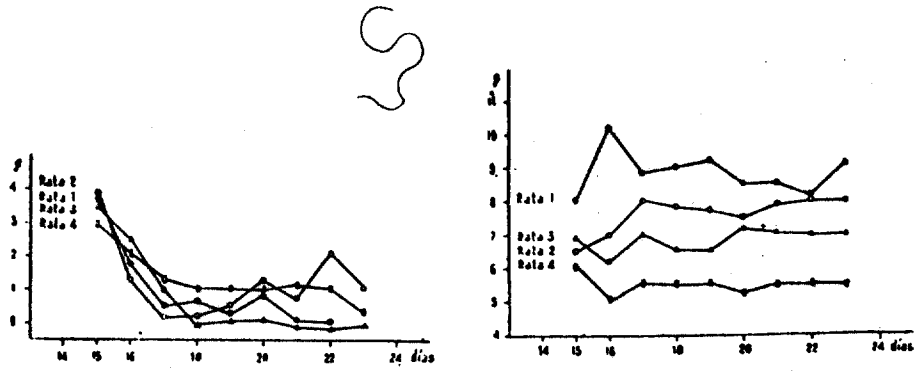


Figure 5. a) Treated rats. b) Control group rats.

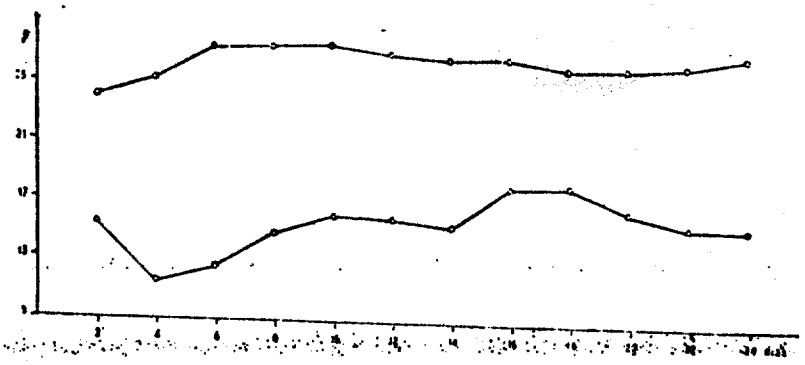


Figure 6. Upper curve: Control group rats.
Lower curve: Treated rats.

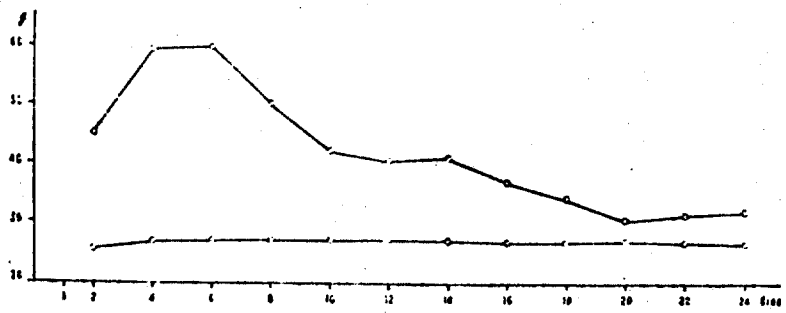


Figure 7. Upper curve: Treated rats.
Lower curve: Control group rats.

The weight of the animal was recorded every 24 hours, and also, the weight of the food and water, and the weight of the excreted feces air-dried for 24 hours. Ten days after the beginning of the experiment, all the rats in the first group were dead. A loss of weight up to 48%, as is shown in fig. 2, was observed during that period; it was due to the exclusion of liquid (fig. 3) and to the disminution of food (fig. 4). Weight of the feces: fig. 5.

It cannot be deduced from this test that propylene glycol is directly toxic for rats in the administered dose; it seems more likely due to the decrease of water and food ingested.

The test was repeated with 2 groups of albino rats under the same conditions. The first group was not treated. The second was treated with 10% of propylene glycol in the drinking water.

The following were controlled: Ingested food/day (fig. 6)
Swallowed water/day (fig. 7)
Feces/day (air-dried for 24 hours)(fig. 8)

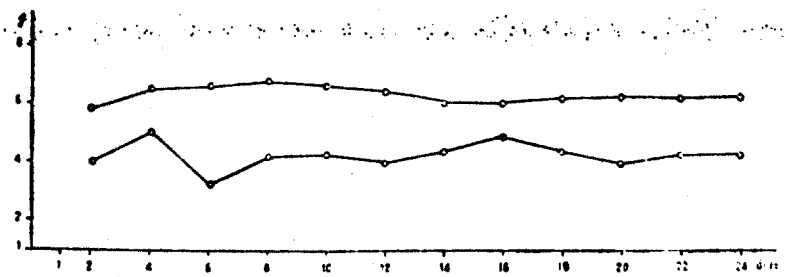


Figure 8. Upper curve: control group rats.
Lower curve: treated rats.

Curves of daily weight (fig. 9).

The temperature between 19° and 22° C during all tests.

Experimental duration: 24 days.

The tested rats, after a period of heavy liquid intake, were stabilized after 18 days at the same level of the control group. The weight variations do not seem to be significant.

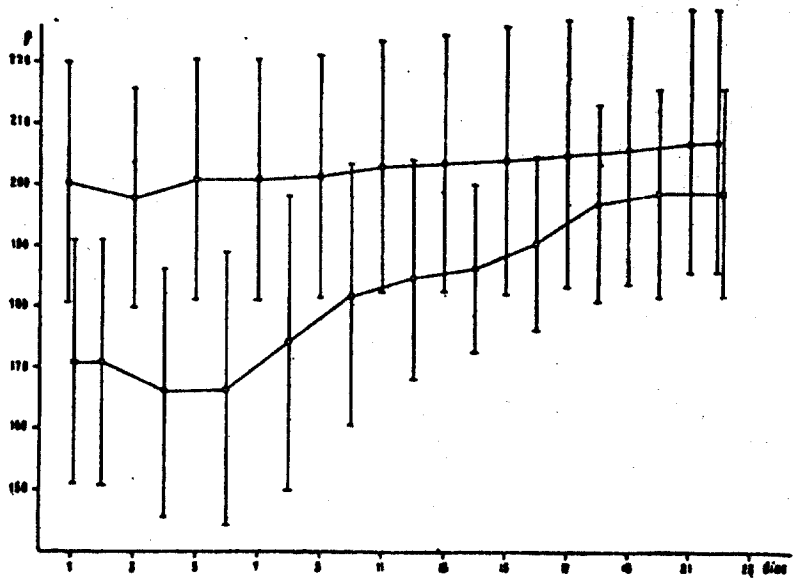


Figure 9. Upper curve: Control group rats.
Lower curve: Treated rats.

Comparing these results with those of the other test, the hypothesis that the animals' death was due to the decrease of water and food ingested in reinforced.

b) Weight of the organs.

After 24 days, and studying the results obtained on the growth of rats treated with 10% of propylene glycol in the drinking water, we decided to observe its action on the final per cent weight of several organs: heart, liver, kidneys, spleen, stomach, adrenal glands, thymus, sexuales; all of them were weighed, after previously having been dried with a filter paper (Tables 3 and 4).

Also, we obtained the mean per cent weight shown in Table 5, where no notable differences are shown. The data gathered is the result of dividing the weight of the animal by the weight of the organ.

These results agrees with the publications of different authors who didn't find any important organic alteration after 100 days of oral treatment in rats

under similar conditions.

Table 3. Per cent weight of the organs in treated rats.

	Organo	Rata 1 ♀ 160 g	Rata 2 ♀ 151 g	Rata 3 ♂ 208,5 g	Rata 4 ♂ 210 g
Heart	Corazón (Heart)	0,340	0,310	0,330	0,300
Liver	Hígado (Liver)	3,760	3,640	3,860	4,090
Capsules	Cápsulas	0,034	0,018	0,019	0,011
Spleen	Bazo	0,220	0,210	0,170	0,170
Kidneys	Riñones	0,900	0,850	0,940	0,890
Uterus	Utero	0,290	0,320		
Testicles	Testículos			1,200	1,230
Stomach	Estómago	0,680	0,610	0,520	0,550
Thymus	Timo	0,074	0,090	0,070	0,080
Vesicles & Prostate	Vesículas y próstata			0,940	0,990

Table 4. Per cent weight of the organs in control group rats.

	Organo	Rata 5 ♀ 150 g	Rata 6 ♀ 157,5 g	Rata 7 ♂ 228 g	Rata 8 ♂ 211 g
Heart	Corazón	0,310	0,260	0,300	0,310
Liver	Hígado	4,400	4,060	4,200	3,460
Capsules	Cápsulas	0,026	0,030	0,013	0,014
Spleen	Bazo	0,300	0,260	0,200	0,260
Kidneys	Riñones	0,790	0,930	0,910	0,880
Uterus	Utero	0,320	0,340		
Testicles	Testículos			1,220	1,230
Stomach	Estómago	0,760	0,740	0,610	0,660
Thymus	Timo	0,070	0,074	0,070	0,079
Vesicles & Prostate	Vesículas y próstata			0,900	0,950

Table 5. Average per cent weight of several organs of rats treated with 10% of propylene glycol in drinking water.

	Organ	Control Group	Experimentals
Heart	Corazón	0,29	0,32
Liver	Hígado	4,03	3,90
Kidneys	Riñones	0,90	0,90
Capsules	Cápsulas	0,02	0,02
Spleen	Bazo	0,20	0,19
Stomach	Estómago	0,70	0,59
Thymus	Timo	0,07	0,08
Testicles	Testículos	1,22	1,21
Uterus	Utero	0,33	0,30
Vesicles & Prostate	Vesículas y próstata	0,92	0,96

c) Inventory and leucocytarian formula. Percentage of hemoglobin.

Many authors have studied the variation in the hematic picture as a consequence of the treatment with propylene glycol. In this sense, our results are the following: a group of 4 female Wistar rats was injected daily with 0.5 ml of propylene glycol for 22 days, using a control group of 4 rats. The results for the treated rats are shown parallel to those of the control group in Table 6.

After the statistical analysis (Tables 7-8), we can conclude that the hemolytic effect of propylene glycol is not significant.

When the rats received propylene glycol orally (10% in the drinking water), the erythrocyte count after 20 days of treatment was the same as that of the control group. In this test, 8 animals were used (4 test, 4 control). The number of leucocytes, the percentage of hemoglobin (Hellige) and the leucocytarian formula (methanol-Giemsa) were also determined. Although the number of erythrocytes was not significantly lower than normal, there was a notable decrease in the hemoglobin. The leucocytarian formula caused modifications in by simultaneously increasing the number of neutrophils and decreasing the number of lymphocytes (Table6).

Table 6. Hematic constants

Inventory

		Control Group	Experimentals
Erythrocytes	Eritrocitos	7.040.000 — 8.740.000	5.940.000 — 7.360.000
Leucocytes	Leucocitos	9.200 — 10.400	8.020 — 10.200
Hemoglobin percentage	Hemoglobina, porcentaje . .	86 — 92	78 — 86

Leucocytarian formula

		Control Group	Experimentals
Neutrophils	Neutrófilos	26 — 34	40 — 42
Eosinophils	Eosinófilos	1 — 3	5 — 6
Basophils	Basófilos	0 — 1	0 — 10
Lymphocytes	Linfocitos	60 — 65	44 — 48
Monocytes	Monocitos	2 — 7	5 — 8

Table 7. Inventory of erythrocytes

Control Group

1ª	2ª	3ª	4ª
10.160.000	8.680.000	8.080.000	8.160.000

$$\text{Media } M_{,} = \frac{35.080.000}{4} = 8.770.000$$

$$\text{Deviations} \begin{cases} 1^a = (1.016 - 877) \,.\, 10^4 = 1.390.000 \\ 2^a = (\ 868 - 877) \,.\, 10^4 = -\ 90.000 \\ 3^a = (\ 808 - 877) \,.\, 10^4 = -690.000 \\ 4^a = (\ 816 - 877) \,.\, 10^4 = -610.000 \end{cases}$$

$$d^2 = \begin{cases} 1^a = 19.321 \,.\, 10^8 \\ 2^a = \quad 81 \,.\, 10^8 \\ 3^a = \ 4.761 \,.\, 10^8 \\ 4^a = \ 3.721 \,.\, 10^8 \end{cases}$$

$$\Sigma\, d^2 = 27.884 \,.\, 10^8 = 22.788.400.000.000$$

$$\sigma = \sqrt{\frac{\Sigma\, d^2}{n-1}} = \sqrt{\frac{27.884}{4-1}} \,.\, 10^4 = 964.088$$

$$\varepsilon = \frac{\sigma}{\sqrt{n}} = \sqrt{\frac{\Sigma\, d^2}{n\,(n-1)}} = \frac{964.088}{\sqrt{4}} = 482.044$$

$$Cv = \frac{\sigma}{M}\, 100 = \frac{964.088}{8.770.000} \,.\, 100 = 10.993$$

Table 8. Inventory of erythrocytes

Rats treated with 0.5 ml of propylene glycol

1ª	2ª	3ª	4ª
8.520.000	5.880.000	7.040.000	7.360.000

$$\text{Media } M_1 = \frac{28.800.000}{4} = 7.200.000$$

$$\text{Deviations} \begin{cases} 1^a = (852 - 720) \cdot 10^4 = 1.320.000 \\ 2^a = (588 - 720) \cdot 10^4 = -1.320.000 \\ 3^a = (704 - 720) \cdot 10^4 = -160.000 \\ 4^a = (736 - 720) \cdot 10^4 = 160.000 \end{cases}$$

$$d^2 = \begin{cases} 1^a = 17.424 \cdot 10^8 \\ 2^a = 17.424 \cdot 10^8 \\ 3^a = 256 \cdot 10^8 \\ 4^a = 256 \cdot 10^8 \end{cases}$$

$$\Sigma d^2 = 35.360 \cdot 10^8 = 3.536.000.000.000$$

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}} = \sqrt{\frac{35.360}{4-1}} \cdot 10^4 = 1.085.664$$

$$\varepsilon = \frac{\sigma}{\sqrt{n}} = \sqrt{\frac{\Sigma d^2}{n(n-1)}} = \frac{1.085.664}{\sqrt{4}} = 542.832$$

$$Cv = \frac{\sigma}{M} 100 = \frac{1.085.664}{7.200.000} 100 = 15.078$$

$$t = \frac{M_1 - M_2}{\sqrt{\varepsilon_1^2 + \varepsilon_2^2}} = \frac{877 \cdot 10^4 - 720 \cdot 10^4}{\sqrt{482.014^2 + 542.832^2}} = \frac{157 \cdot 10^4}{725.970} = 2.162$$

d) Cholesterolemia

There are not many bibliographical citations referring to the modification of cholesterolemia caused by propylene glycol, and most of them refer to the effect of propylene glycol when parenterally administered. It is interesting to study the hypocholesterolemizing effect of this glycol when it is orally administered in drinking water at 10%.

In two groups each of six six-month old male rats, one received propylene glycol in the indicated dosage and the other was used as the control group.

The cholesterolemia of each animal was determined at the beginning of the experiment and again after 15, 30, 45 and 60 days. One of the experimental animals died between 15 and 30 days after the beginning of the experiment.

After anesthetising the animal with ether, blood was taken from the jugular vein through an opening in the depilated skin. The hypodermic syringe and the needle utilized were absolutely dry; the needle was 25 mm long, 0.8 mm wide and of short bevel angle. The blood was transferred to conic tubes of centrifugate in portions of 4 ml; the centrifugation was applied immediately at 1,000 r/m for 12 minutes, which is the minimum time for completing hte coagulation process. The serum obtained was separated by decantation and again centrifugated. It was kept under refrigeration at 2°C for later use (24 hours as a maximum).

The method utilized for determination of cholesterol in the blood was Hange and Nicolaysen (14), modified by us as explained below.

Such a method utilizes 50 µl of serum for human clinical experimentation (its normal value is 150 mg per 1,000) but in the serum for rats such an amount can not be utilized because in rats it is found in a smaller proportion (0.5-0.8 g per 1,000).

Four times the amount of serum was used.

Varying the quantity of the reagent each time, it was proved that the best way to separate the cholesterol was to utilize 400 mg of SO_4Mg instead of 200mg of the original. In the method, the reagents were in excess, permitting a four-fold increase in the quantity of serum without altering the results.

Hange's reagent admits an very small amount of water: 1 ml admits 1 drop as a maximum. Acetyl chloride in large quantities of free acetic acid gives with the small amount of water of the reagent according to the reaction:

$$ClCO\text{-}CH_3+H_2O = ClH+CH_3\text{-}COOH$$

the great concentration of hydrogen ions necessary for the reaction in the liquid.

Technique: We prepared, previously, a double boiler and numbered all the test tubes. In each one, 0.2 ml of serum was deposited making sure that the entire amount went directly to the bottom without touching the walls of the test tube.

Then, 1 ml of ethanol acetone and a drop of KOH were added (this separates the cholesterol from its combinations, helped by the ethanol acetone).

The test tubes (uncovered) were placed in the double boiler at 80-85°C for 10 minutes in order to verify the reaction.

After being cooled, 5 ml of chloroform were added (dissolving the free cholesterine) and the test tubes were recovered in order to avoid the loss of chloroform.

Next we added 400 mg of SOMg (previously dried in a stove for 24 hours). As it is hydrophilic, it gives more volume to the hydrophic surface and permits a better extraction.

It was shaken very well for 3 minutes and not centrifugated. Three test tubes were taken apart and numbered:

First tube, patron = 1 ml CL_2Zn (Hange prep.) + 2 ml cholesterine solution.

Second tube, 0=1 ml CL_2Zn (Hange prep.) + 2 ml chloroform solution.

Third tube, problem = 1 ml Cl_2Zn (Hange prep.) + 2 ml of centrifugated solution.

Using a buret, 1 ml of acetyl chloride was added to each and then well shaken.

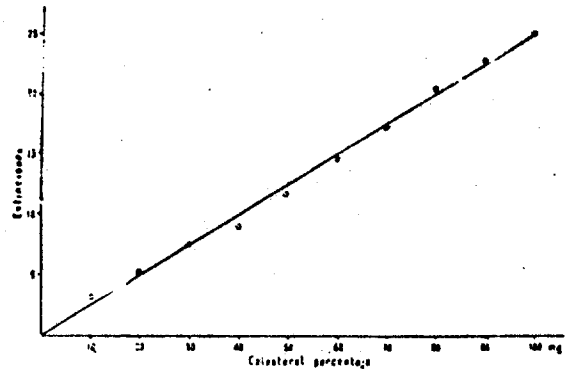
Putting the double boiler at 55°C, the tubes were put inside for 18 minutes. Each one was shaken and the color became darker. Then they were cooled with ice.

When placed in the photocolorimetric cuvette, more chloroform must be added for leveling at 7 ml.

It was read in the photocolorimetric Eel with the Evans photoelectric cell of direct lecture, placing the green filter complementary to the red one.

Valuing the cholesterine colorimetrically, with a patron curve obtained in our laboratory utilizing concentrations of cholesterol exactly measured and following the same technique as that used for the patron curve, we have obtained the following

as represented in Figure 10:



This calibration curve was obtained with progressive values of cholesterol, corresponding to increases of the percentages from 10 to 10 mg.

The reagents are:

- KOH at 10% in aqueous solution.
- Ethanol-acetone 1:1.
- Anhydro chloroform.
- SO_4Mg anhydro (the night before, in stove at 15°-200°C).
- Acetyl chloride

Reagent Cl_2Zn. Dissolving 120 g granular anhydrous Cl_2Zn, in 359 ml of acetic glacial acid, warmed to 80°C and shaking occasionally during 2-3 hours, cooling and filtering by glass, keeping in a dark bottle with burnished cap, it can be kept for more than 3 months. It is enturbiated with chlorform by humidity, afterwards.

Chloroform patron. 100 mg of cholesterol in 100 ml of chloroform, dissolved at 1/20 for use.

Results. See Figure 11.

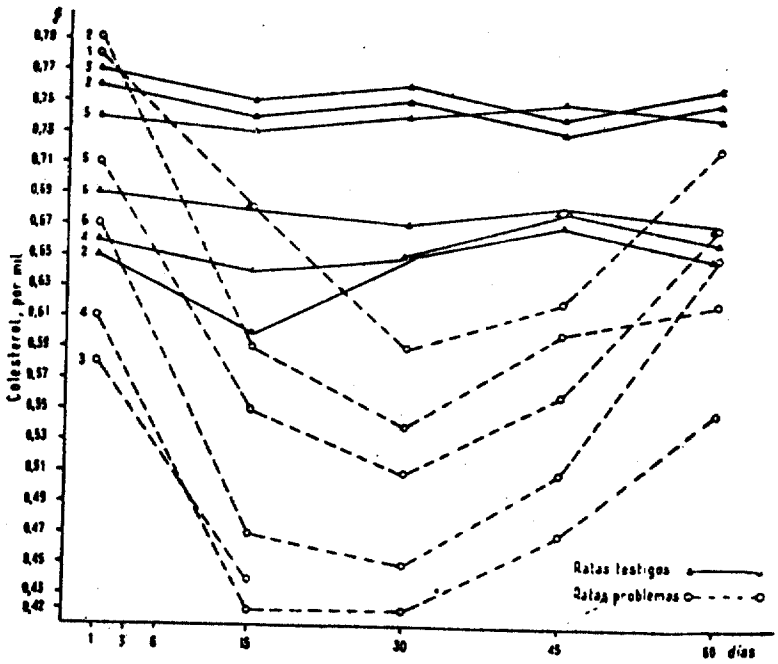


Figure 11
Control Group Rats _____

Problem Rats - - - - - - - - - - - -

It can be observed that the propylene glycol produces a significant decrease of the cholesterolemia in 100% of the animals. This reaches 30% of the initial value 15 to 30 days after the beginning of the test and has a tendency toward normal level at the interruption of the treatment. The reversibility of the effect is a phenomenon in the greatest part of the hypocholesterolemizing pharmacus.

B) TEST ON ENTIRE ANIMAL

a) Influence of propylene glycol on the intestinal absorption.

This effect was studied in rats, utilizing a uranyl salt on a group of rats by a gastric catheter and determining, afterwards, the uranium recuperation in the feces.

Parallely, another group was administered propylene glycol along with the uranyl salt, in order to to find out if this increased the absorption.

We worked with uranyl nitrate and uranyl acetate. A solution of the uranyl salts in distalled water was prepared at a concentration of 50 mg/1 ml.

The quantity of uranyl administered and excreted was determined by the radioactivity:

In 5 capsules of the machine, the following quantities of the solution and the uranyl salt were placed at the same time:

Solution Milliliters	Salt Milligrams
0.05	2.5
0.10	5.0
0.20	10.0
0.40	20.0
0.80	40.0

The volume was completed up to 1 ml with distilled water in order that the uranyl salt was homogenously distributed at the bottom of the capsule. Then the capsules were placed on the stove until total evaporation occured.

The radioactivity was read on a radiation counter, type E-10.

The corresponding graph shows milligrams of uranyl and impulse/min.(Fig.12).

Another graph was prepared, similarly, for the uranyl acetate (Fig. 13).

In the sample of the feces, the radioactivity was measured to determine the number of impulse/min. of a known weight of feces. Applied to the graph, the number of milligrams of uranium of the tested sample were obtained.

The determination of the uranium mixed with normal feces was previously tested:

1) The uranium mixed with feces cannot be extracted with water, nitric acid or ether.

2) The measurement of radioactivity in feces presents the problem of low penetrable particles emitted by the uranium and therefore the radioactivity is partially retained by the feces residue.

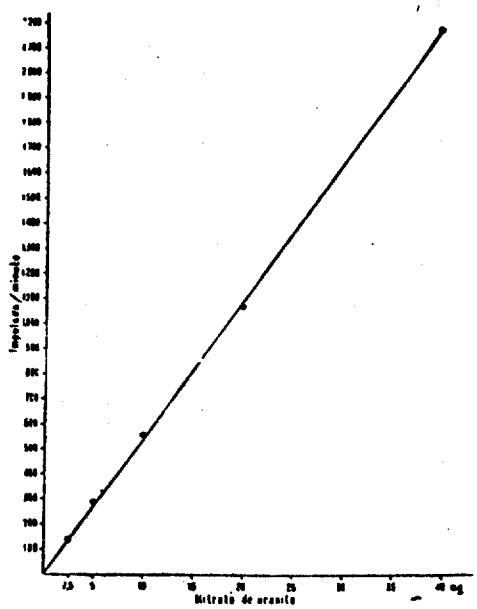


Figure 12. Uranyl Nitrate

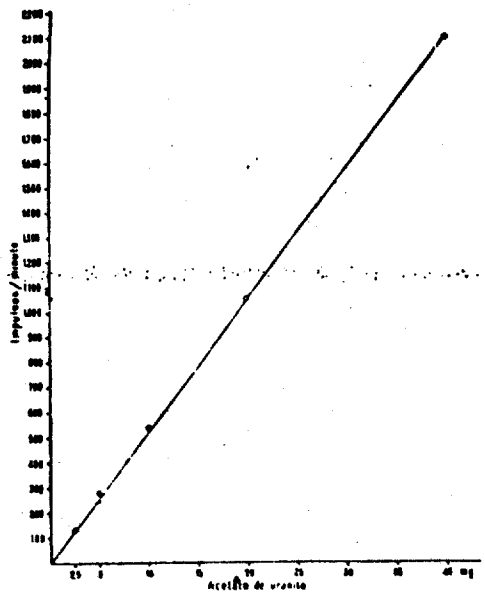


Figure 13. Uranyl Acetate

In the tests in which dried, normal feces were mixed and triturated with nitrate of dried uranium, the recuperation was 84% of the theoric uranium.

In order to avoid this error, the feces were calcined. The recuperation in ashes was 96% of the theoric uranium.

In tests with recuperated feces of animals treated with uranyl salts, it was proved that the percentage of recuperation depended on the density of the sample (working with feces powder); this was due to the greater or lesser retention of the particles α.

Working with feces ashes, the percentage of recuperation is the same for all samples when their weight is between 10 and 50 mg.

Therefore, the radioactivity measurements must be done in ashes.

The quantity of ashes of the capsule cannot exceed 50 mg and cannot be less than 10 mg; thus, the absorption of the α particles by the density of the layer of ashes.

The minimum detectable quantity is 2 mg of uranyl nitrate/100 mg of ashes, corresponding to 0.4 mg of dried feces and an administration of 20 mg/day.

The experiments were carried out with male adult Wistar rats.

Uranyl salt was administered to the rats by gastric catheter, after they were slightly anesthetized with ether.

Some groups animals were given 100 mg of uranyl salt. The others were given 100 mg of uranyl slat + 1 ml of propylene glycol.

Two diet types were tested: one with ample brute fibre; for the tests of recuperation of uranium, the feces were collected after 24 hours. They were dried on the stove at 95°C and were weighed, then pulverized. One sample of the feces powder, as homogeneous as possible, was weighed, then it was calcinated and the radioactivity in ashes was measured. Almost 100% was recuperated in the feces of the first 24 hours. The feces of the second day had very low quantities of uranium, not readily determinable based on its radioactivity.

For diminishing the quantity of the feces, another diet with very small amount of brute fibre was tested. The animals were deprived of food for 24 hours and then given 20g of the diet; 24 hours later, the uranyl salt was administered to them. For the tests on recuperation of uranium, the feces were collected every 24 hours. It was necessary to collect the feces after 24, 48, 72 and 96 hours. Apparently, due to the lack of brute fibre, the uranium was eliminated very slowly.

Due to the scarce quantity of feces, they were calcinated directly, separating those of each 24 hours. The ashes were homogenized in mortar and the radioactivity of three samples was determined, obtaining the arithmetic media.

Using this diet, it was observed that the percentage of recuperation of uranium in feces was hardly homogeneous, due to the slow elimination of uranyl salt. Finally, this diet was eliminated and the other, with ample brute fibre, had to be used again.

In the tests with uranyl nitrate, in rats treated with the first diet, it was observed that the recuperation of uranyl nitrate in feces ashes was 98.8%. Almost all of the administered uranium was eliminated in the feces.

In the tests with uranyl acetate, two groups of 5 rats each were used and administered 100 mg of uranyl acetate and 100 mg of uranyl acetate + 1 ml of propylene glycol, respectively. Later, two groups of 3 rats each were administered 50 mg of uranyl acetate and 50 mg of uranyl acetate + 1 ml of propylene glycol, respectively.

The tests were carried out, exclusively, with the diet lacking in brute fibre. Therefore, the results were not concordant and it cannot be affirmed that the acetate functions in the same form as the nitrate.

Result. The propylene glycol aids slightly in the intestinal absorption of the uranium when it is administered in the form of nitrate.

b) Modification of the hypnotic action of luminal.

The synergism between medicinal substance and vehicle is very important pharmacologically, as was studied by Hasleton and Helleman in 1946. Therefore, we have analyzed the different potential results between the utilization of water and propylene glycol as a solvent in the administration of phenolethyl barbituric acid (luminal).

Ten male rats and ten females, of approximately the same weight, were chosen; 5 of the males and 5 of the females were subcutaneously injected with 0.2 ml of luminal solution in propylene glycol (10mg/l) per 20g of animal.

The same was done with the other 5 males and 5 females, substituting the propylene glycol by distilled water. This was the control group.

The injected animals were observed through individual crystal bottles, controlling the prehypnotic time. The determinations were repited 4 times on different days and the number of anesthetized animals in each of the four determinations on 10 rats constituted the response to the dosage.

The time which elapsed between the total anesthesia, determined by the incapacity of the animals to return to normal posture when placed on their backs and the return to normalcy is the duration of the effect.

According to Tables 9-11, when the luminal solution in propylene glycol was injected, the responses were incremented. The hypnosis duration was more than 4 times greater than the one produced by the luminal solution in water; this proves that propylene glycol empowers the action of luminal.

No toxic consequence was observed afterwards.

c) Modification of the mydriatic action of atropine.

It is intended, in this test, to study the possible influence of propylene glycol on the quantitative response of the eye to atropine.

The general actions of atropine have been determined in different studies

measuring the activity of the medicinal substance.

Nevertheless, examining experimental data from various authors, we have not found anyone who has carried out a test in this sense. Therefore, we decided to perform this test, hoping it would be of clinical interest.

Technique of measuring pupilar diameter of rats. The fundamental technique is very similar to that used in microscopy for micrometic measures, but with some variations which can accomodate the system to the particular end, of measuring the pupilar diameter. In order to avoid the optical effect of the corneal curvature, we have employed objectives of very large focal distance, using an inverted-image binocular microscope magnifying glass, like those used in dissections.

Rat	1ª determinación	2ª determinación	3ª determinación	4ª determinación
	Hours slept			
1	0	0	0	0
2	0	4	0	0
3	0	0	0	8
4	0	0	0	0
5	0	0	0	0
6	0	4	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0

Table 9. Duration of anesthesia in rats treated with luminal solution in distilled water.

Table 10.

Rat	1ª determinación	2ª determinación	3ª determinación	4ª determinación
	Hours slept			
1	6	0	6	8
2	0	6	0	0
3	0	0	0	0
4	2,30	6	6	9
5	3,30	0	0	0
6	0	0	2	2
7	0	2	1	0
8	5,45	0	0	0
9	0	0	0	0
10	6	0	0	0

Duration of anesthesia in rats treated with luminal solution in propylene glycol.

Table 11

Anestesiados	Número de determinaciones	Número de animales	Anestesiados positivos	Duración de la anestesia (acumulativa)	Duración de la anestesia (media)	Desviación patrón
With propilenglicol . .	40	10	13	71,45 h	107,5 min	± 152,315
W/O propilenglicol . .	40	10	3	16,00 h	24,0 min	± 90,900

The objectives of 2.4 proper magnifying power, combined with pleriplanatic oculars of 6 magnifying power, give a total amplification of 14.4 diameters which permits one to observe the totality of the iris at one time. The illumination used has been that of the incidence by a lateral lamp with an orange filter in order to obtain the optimum grade of contrast.

One of the oculars has on its field diaphragm a micrometric, glass engraved scale of 10 mm divided into tens of millimeters.

During the test it is probed, manually, until the pupilar diameter coincides with the center of the scale, reading the extent visually; the number of divisions on the ocular micrometer scale corresponding to this measurement represents a relative value that is transformed into absolute value taking into consideration the representative value of each division of the scale. In order to determine this representative value, we have employed a micrometric objective with a photographic scale 2 mm long, subdivided into hundreths of a millimeter. The equivalent in the experimental conditions that we have utilized has been the following: one division of the ocular micrometer scale is equal to 0.04 mm = 40μ.

Tests. Three different tests were conducted.

In the first test, 6 adult rats were used. Three were injected subcutaneously with 0.3 ml of solution of atropine sulfate (50μl/ml) + 0.3 ml of propylene glycol.

The starting moment of effect, magnitude and duration were controlled.

From the data shown in Tables 12-13, it can be deduced that in the rats injected with atropine + propylene glycol, the initiation has no effect until 15 minutes later.

The response is greater in extent and duration of its maximum effect which is more than double, although it begins later. Without propylene glycol, the effect does not last two hours. With propylene glycol, it lasts more than 4 hours.

The second test consisted of the injection of 2 rats, subcutaneously, with

0.25 ml of atropine sulfate solution (100μg/ml).

As in the first test, the moment initial effect, its magnitude and its duration were controlled.

The initial effect was the same in both cases. The magnitude was greater in the experimentals and the duration was doubled.

In this test, we have to take into consideration that less atropine was injected (0.3 ml of 50μg/ml) and, proportionally, less propylene glycol than in the first test because of 0.3 ml of 50μg/ml = o.15 of the solution of 100μg/ml and only 0.125 ml of propylene glycol were injected.

Third test: The rats were injected subcutaneously with 0.25 ml of solution of atropine sulfate (100μg/ml) + 0.25 ml of propylene glycol.

As in the other tests, the moment of the initial effect, magnitude and duration were controlled. Immediately, two other rats were injected with 0.25 ml of solution of atropine sulfate (100μg/ml + 0.25 ml of propylene glycol).

The dilations of the rats treated with atropine were complete, but not as great as those treated with propylene glycol (Table 14).

The treatment of rats with atropine and propylene glycol permits a clear differentiation between mydriasis caused by atropine and the one obtained when propylene glycol is added.

Differences in pupilar sizes were highly significant.

Table 12. First Test

Rat	Without propylene glycol. W/ 0.30ml of atropine sulfate solution. (100µg/ml)				With 0.3 ml of propylene glycol + 0.3 ml of atropine sulfate solution. (50µg/ml)			
	A Iniciación del efecto, minutos	B Magnitud, milímetros	C Duración del efecto máximo, minutos	D Duración total, horas	A Iniciación del efecto, minutos	B Magnitud, milímetros	C Duración del efecto máximo, minutos	D Duración total, horas
1	10	0,5	15	1	15	2,25	30	4
2	7	0,6	20	1	15	2,25	50	4
3	10	0,7	20	1	15	3,00	65	4

Key: (for all three tests)
A - Initiation of effect, minutes
B - Magnitude, millimeters
C - Duration of maximum effect, minutes
D - Total duration, hours

Table 13. Second Test

Rat	Without propylene glycol. W/ 0.125ml of atropine sulfate solution. (100µg/ml)				With 0.125 ml of propylene glycol + 0.125 ml of atropine sulfate solution. (100µg/ml)			
	A Iniciación del efecto, minutos	B Magnitud, milímetros	C Duración del efecto máximo, minutos	D Duración total, horas	A Iniciación del efecto, minutos	B Magnitud, milímetros	C Duración del efecto máximo, minutos	D Duración total, horas
1	10	0,5	15	1	10	2	20	4
2	10	0,5	15	1	10	2	20	4

Table 14. Third Test

Rat	Without propylene glycol. W/ 0.25ml of atropine sulfate solution. (100µg/ml)				With 0.25 ml of propylene glycol + 0.25 ml of atropine sulfate solution. (100µg/ml)			
	A Iniciación del efecto, minutos	B Magnitud, milímetros	C Duración del efecto máximo, minutos	D Duración total, horas	A Iniciación del efecto, minutos	B Magnitud, milímetros	C Duración del efecto máximo, minutos	D Duración total, horas
1	12	3,8	85	4	7	4	110	4
2	12	3,8	80	4	7	4	120	4

Figure 14

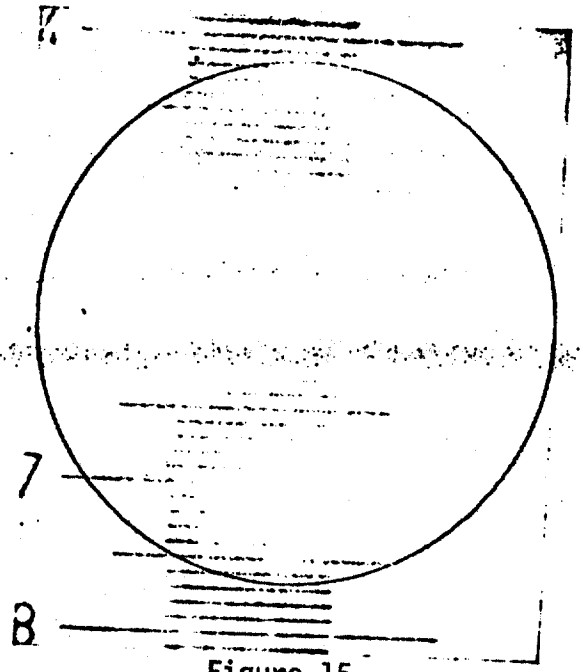


Figure 15

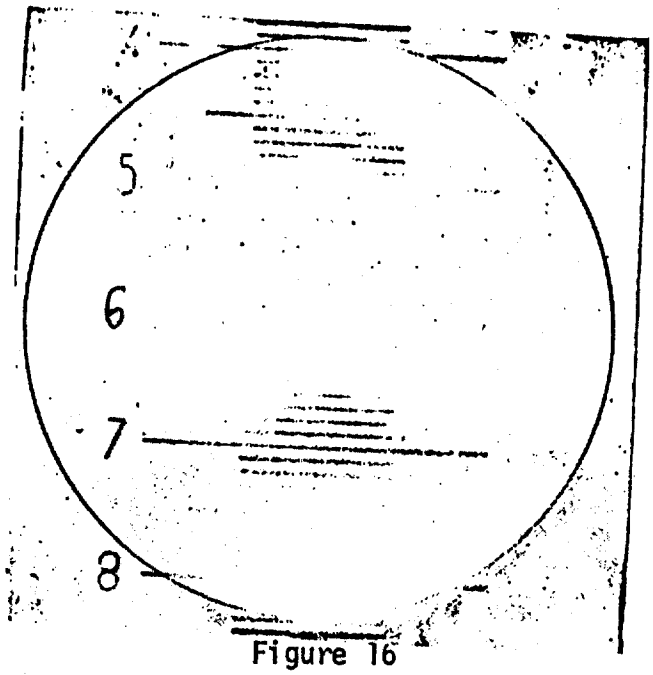


Figure 16

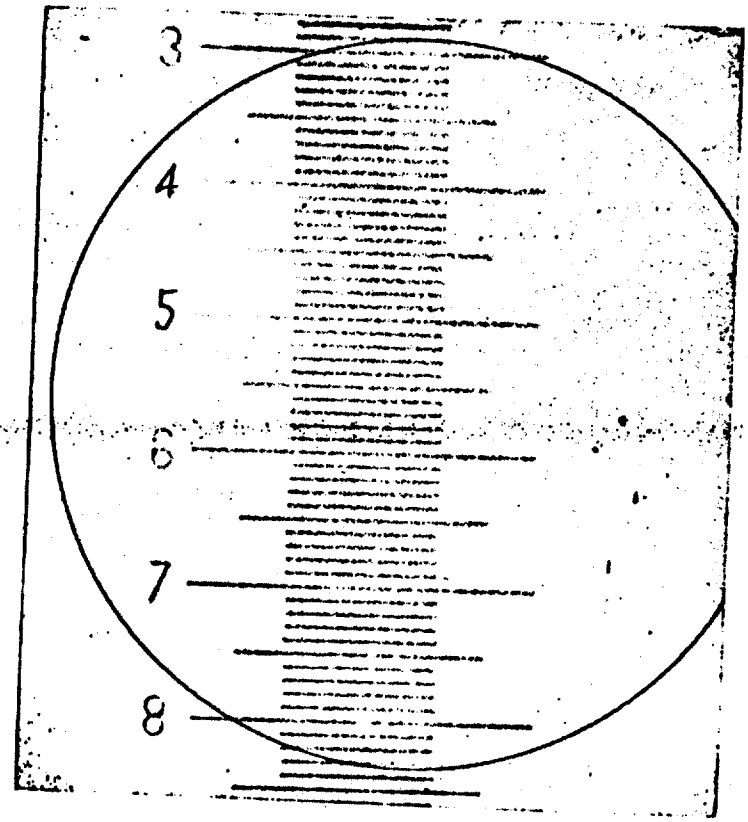


Figure 17

Pupilar aspect of the rat and observed diameter during third test. As it has been deduced previously, a scale division of the ocular micrometer is equal to 0.04 mm = 40μ; therefore, the dilations will have the following diameters:

Figure	Divisions	Millimeters
14	4.4 - 6.7 = 23	0.90
15	4.2 - 7.6 = 34	1.36
16	4.0 - 8.4 = 44	1.76
17	2.9 - 8.4 = 55	2.20

It is necessary to observe that Figure 14 is slightly tilted.

C) MODIFICATION OF THE PHARMACEUTICAL ACTIONS IN ISOLATED ORGANS

a) Effects of propylene glycol on intestinal motility of acetyl-choline stimulated rats.

Cogni and Morvillo (7) studied the inhibition of spontaneous activity of the isolated intestine of a rabbit by TWEEN 80 dissolved at 1:100,000, finding that its reactivity did not diminish with acetylcholine injections. Similar results were obtained with an isolated uterus.

We have studied the action of acetylcholine in the presence of propylene glycol on the isolated intestine of rats after a certain time.

In the first series of tests, the effect of propylene glycol when it is extemporaneously added to the ambient fluid of the organs, moments before the addition of acetylcholine, was studied.

Description of the method. A portion of jejunum of the white rat, deprived of food for 24 hours, was isolated. This portion was placed in the ambient fluid of the organs of 20 ml capacity with Tyrode solution at 37°C and the air was continuously bubbled through the saline solution.

In Figure 18, it was stimulated with acetylcholine and a series of contractions was obtained. After they ceased, the acetylcholine was permitted to act

for 10 minutes without washing. During the first minute, the chemigraph was working with minimum velocity. During the remaining 9 minutes, the chemigraph worked for the first 15 seconds, then it stopped for the last 45 seconds of each minute. The entire process was repeated from the beginning. Immediately, the action of the acetylcholine was observed in the presence of propylene glycol, adding 0.2 ml of propylene glycol during 1 minute to the ambient liquid; immediately thereafter, the acetylcholine was added and acted for 10 minutes without washing as explained above.

In Figure 19, the same process as the first was repeated, except that 0.5 ml of propylene glycol was added to the ambient liquid, and in Figures 20-21, 1 ml of propylene glycol was added.

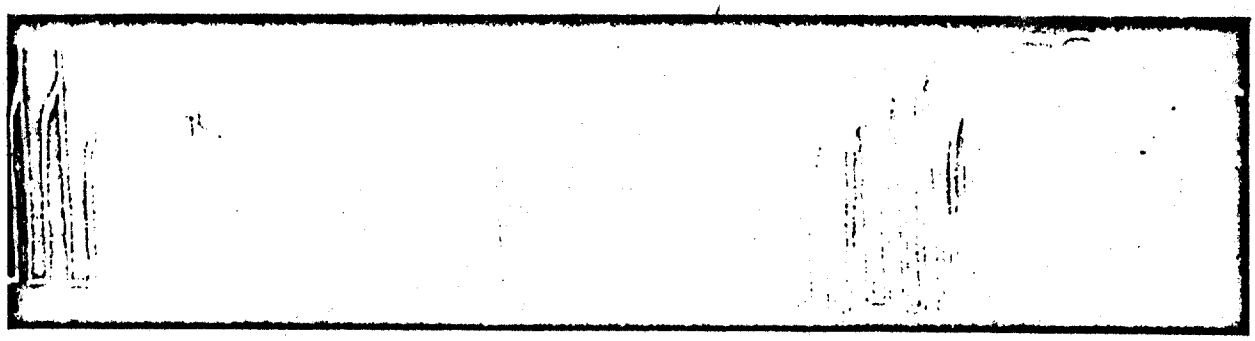
In other tests (described below) the propylene glycol was added like any other component to the Tyrode liquid in the proportion indicated and with this perfused liquid, the entire experiment was carried out.

Results. Propylene glycol reduces the intestinal response to acetylcholine. The inhibiting effect is larger with larger doses. The effect is reversible; 0.2 ml of propylene glycol in the ambient liquid (20 ml) shows an inhibiting action very limited or non-existent. The persistence of the effect of acetylcholine is modified by propylene glycol.

Propylene glycol, added to Tyrode, inhibits the intestinal activity due to acetylcholine, as shown in Figures 22-23. First the intestine was submerged in normal Tyrode and immediately in Tyrode with 10 ml of propylene glycol (1%).

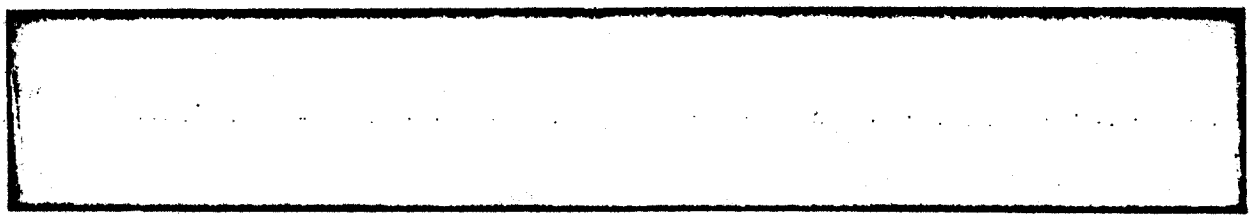
In Figure 24, as in the others, normal Tyrode was used and then Tyrode with 2.5% of propylene glycol.

Results. With Tyrode + propylene glycol at 1%, the effect is transitory, but diminishes the response to acetylcholine. With Tyrode + propylene glycol at 2.5%, the response of the intestine to acetylcholine diminishes, but the effect is persistent; nevertheless, it is rapidly reversible when it is substituted by normal Tyrode.



a b a

Figure 18. a) Acetylcholine. b) Propylene glycol



a b a

Figure 19. a) Acetylcholine. b) Propylene glycol

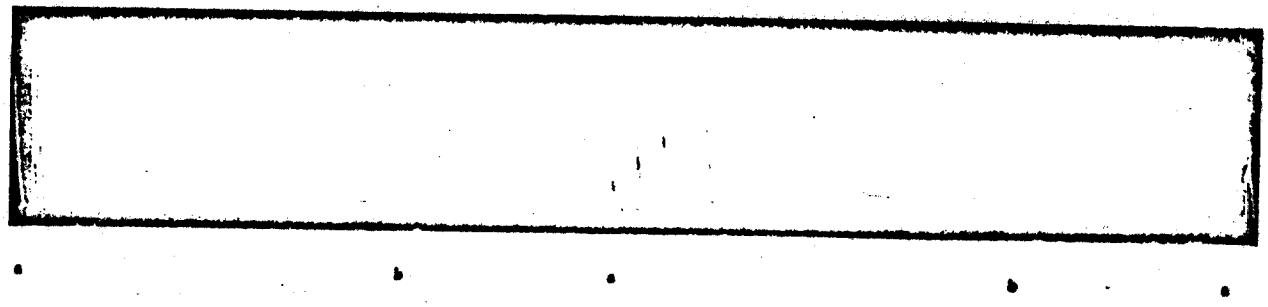


Figure 20. a) Acetylcholine b) Propylene glycol

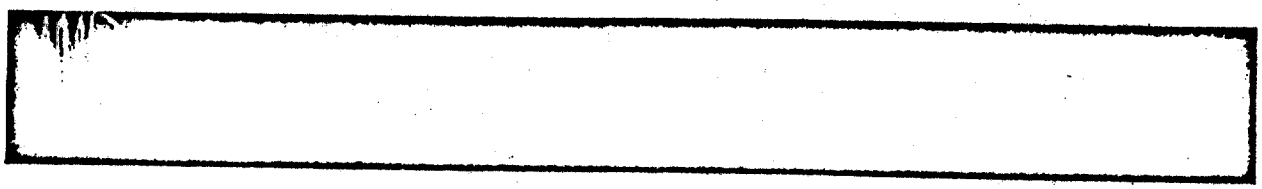


Figure 21. a) Acetylcholine b) Propylene glycol

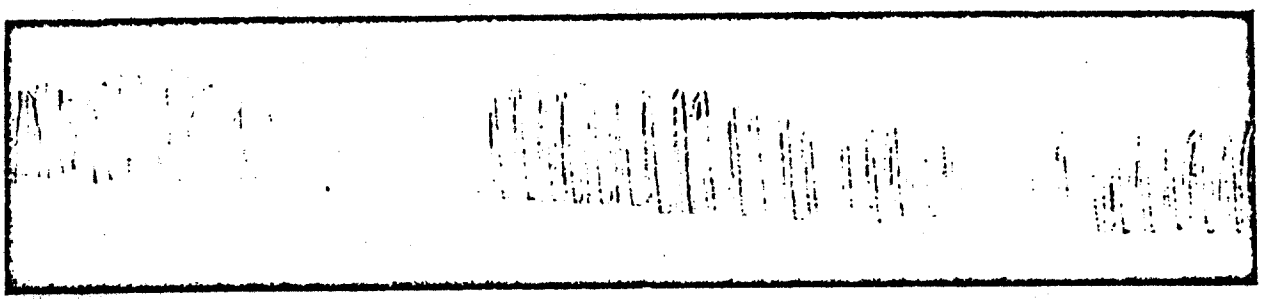


Figure 22. a) Normal Tyrode b) Tyrode + Propylene glycol

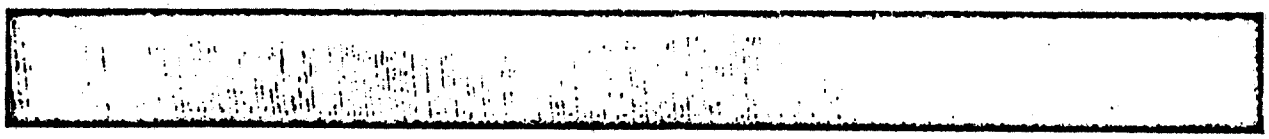


Figure 23. a) Normal Tyrode b) Tyrode + Propylene glycol

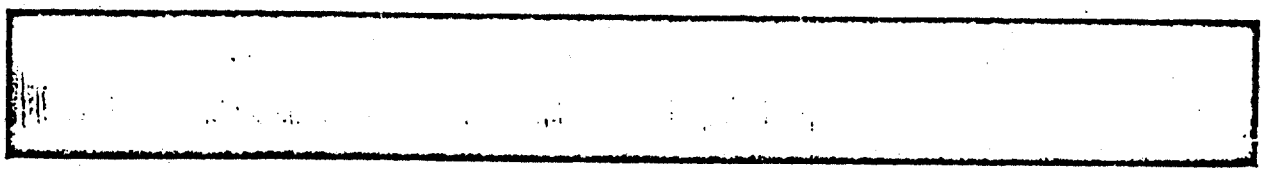


Figure 24. a) Normal Tyrode b) Tyrode + Propylene glycol

b) Influence of propylene glycol on the pharmacological action of atropine in the intestine of white rats.

A portion of jejunum of rats was utilized. It was placed in ambient liquid of organs of 20 ml capacity with Tyrode solution at 37°C and oxigenation by air.

The preparation was stimulated periodically by the addition of acetylcholine with the dose measured according to the organ sensibility in each test.

Obtaining equal responses, atropine was added (0.1 ml of solution which contained 0.1μg/ml) and, after 1 minute, the effect of acetylcholine was retested according to the systematic march of stimulus with acetylcholine and the recuperation of the initial effect.

Immediately, the normal Tyrode was substituted by Tyrode with 1% of propylene glycol. Working with this perfused liquid, all of the anterior sequences were repeated.

The principal results obtained in this series of experiments were collected in graphs that permits the comparison of different reactions of the flat muscle to the atropine, before and after the presence of propylene glycol.

Stimulating with acetylchlorine, a series of equal contractions were obtained. After the addition of atropine, there were smaller responses. The atropine decreased the intestinal activity or rats to acetylcholine, as was recorded in Figure 25, approximately 2/3, and 1/2 in Figure 26.

This inhibition does not subsist for a long period of time and the responses increased and normalized when washed with Tyrode and immediately stimulated with acetylcholine.

Later on, washing with Tyrode with 1% of propylene glycol, a weak decline was observed for the first time since the initial response, increasing the loss of organ sensibility to the acetylcholine in the following responses. Adding atropine at the same time as the propylene glycol action, its inhibiting effect is much greater than that when propylene glycol was not present, and a comparative study

indicates that the normalization of the response of the muscle to acetylcholine is, also, slower. The total normalization has no effect in any case.

Conclusions. Propylene glycol intensifies the antiacetylcholinic effect of the atropine on the isolated intestine.

c) Effect of propylene glycol on the inhibiting effect of camphor on the isolated intestine of rats, stimulated periodically, with acetylcholine.

In this test, it is intended to show whether or not the intensification of the effect of atropine by propylene glycol is a specific phenomenon or not. We selected an inhibitor of the action of acetylcholine on the intestine's effect which is due to the non-specific depression of the flat muscle, as camphor.

A solution of camphor at 1.6/800 was prepared, dissolving 0.2 g in 15 drops of alcohol and filling up with water to 100 ml.

The tests were carried out under the same conditions explained above. Camphor acts for 1 minute before the addition of acetylcholine again.

In the first series of tests, normal Tyrode and Tyrode with 2.5% of propylene glycol (Fig. 27) were used and it was observed that besides the diminishing responses to acetylcholine, the inhibition exercised by camphor in the presence of propylene glycol is more intense.

Fiugres 28-29 were selected among those that formed the second series of tests. When working with normal Tyrode, the camphor was added with and without propylene glycol. Also, the effect of camphor was more intense when administered utilizing propylene glycol as a vehicle.

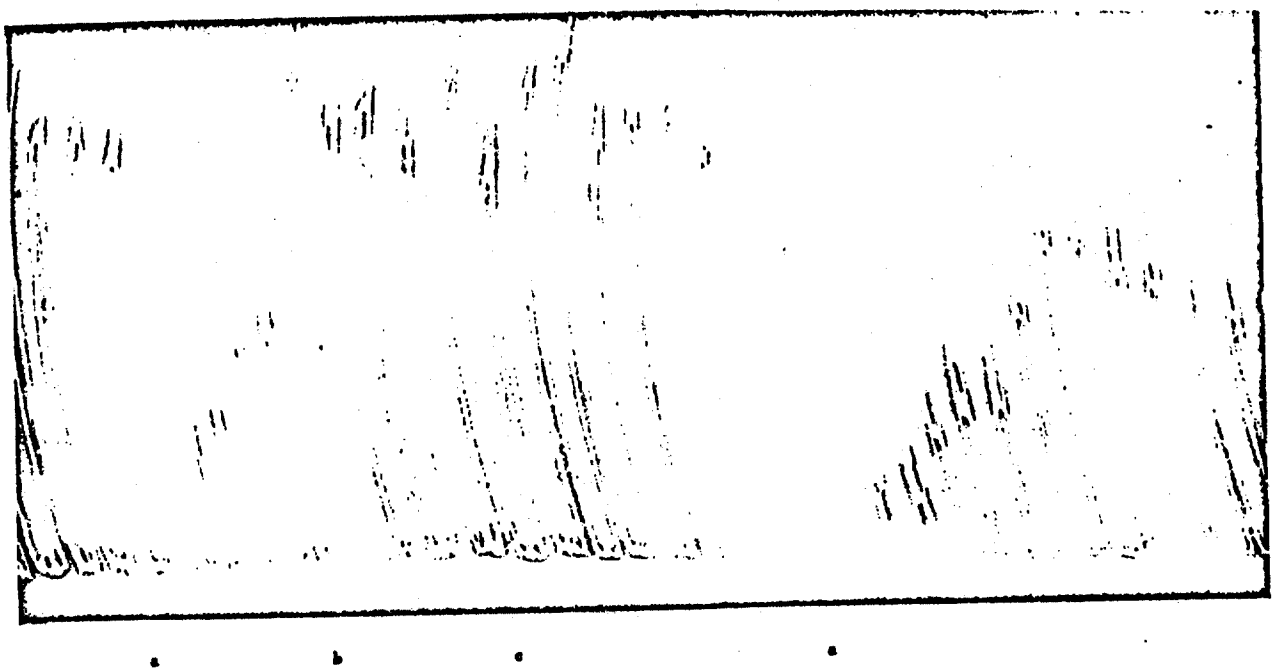


Figure 25. a) Atropine b) Normal Tyrode c) Tyrode + Propylene glycol

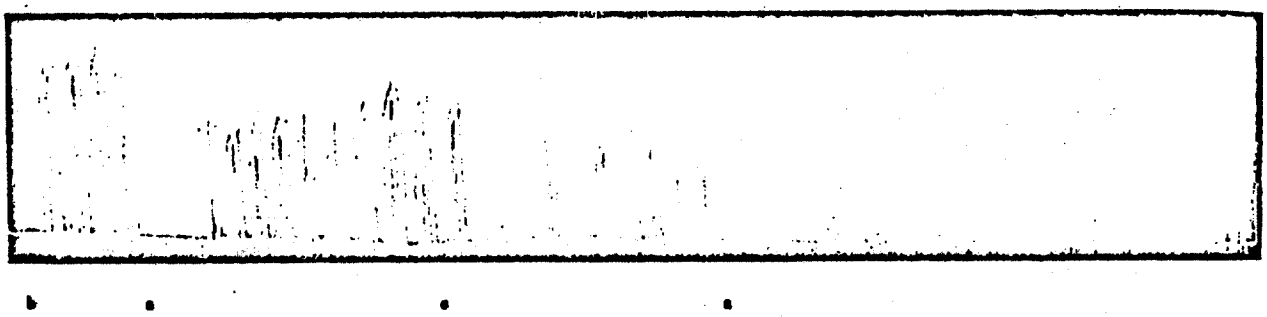


Figure 26. a) Atropine b) Normal Tyrode c) Tyrode + Propylene glycol

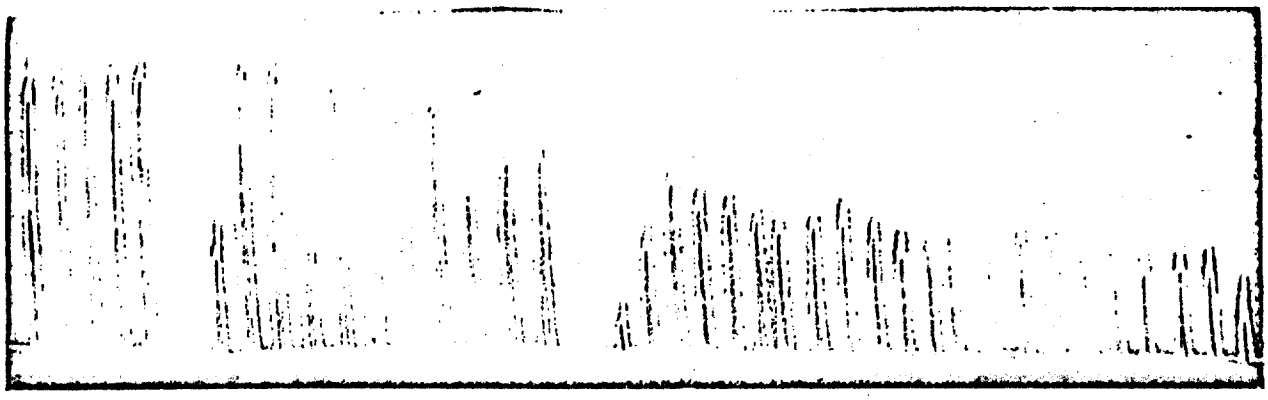


Figure 27. a) Camphor b) Normal Tyrode c) Tyrode + Propylene glycol

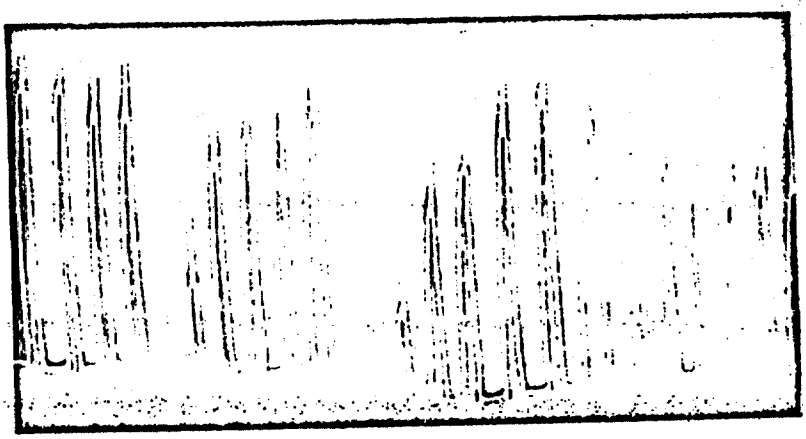


Figure 28. a) Camphor b) Normal Tyrode c) Tyrode ± Propylene glycol

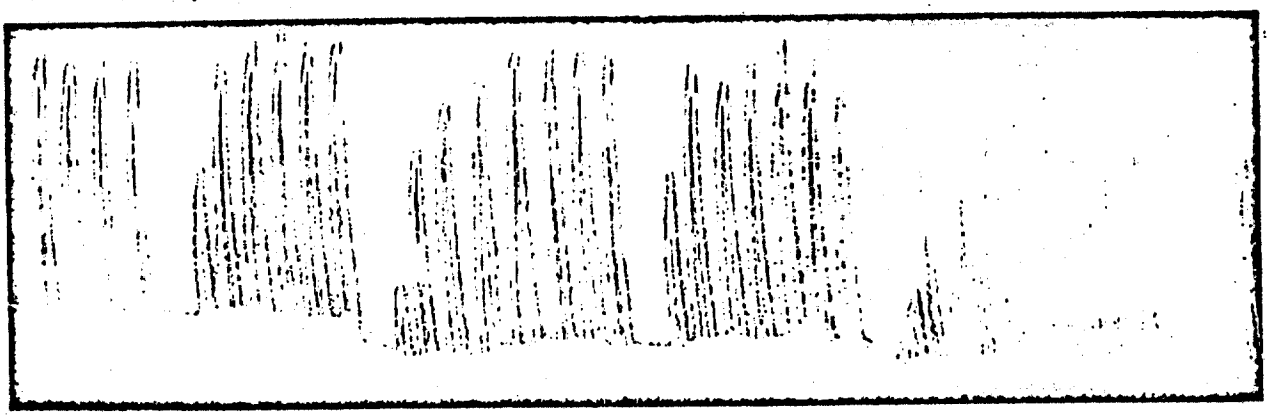


Figure 29. a) Camphor b) Normal Tyrode c) Tyrode + Propylene glycol

Conclusions

1st. Propylene glycol, injected intramuscularly for 22 days in doses of 0.5 ml will provoke inhibition in the growth of rats, a highly significant result.

2nd. When propylene glycol is administered in the drinking water at a 50% proportion, the animales die as a result of the decrease in the consumption of water and food. If the proportion of propylene glycol in the water is 10%, the alteration is not significant.

3rd. Oral administration of propylene glycol does not provoke notable differences in the weight or external morphology of several organs in rats: heart, liver, capsules, spleen, kidneys, uterus, testicles, stomach, thymus, vesicle and prostate.

4th. The parenteral administeration of propylene glycol (0.5 ml/day, for 22 days) does not modify the figure of the erythrocytes, but notably decreases the quantity of hemoglobin. The leucocytarian formula shows an increase in the neutrophiles and a parallel decrease of the lymphocytes.

5th. The presence of 10% or propylene glycol in the drinking of rats causes a significant decrease of cholesterolemia, down to 30% of the initial value, 15-30 days after the beginning of the test, and a tendency to the normal level if the treatment is interrupted.

6th. Propylene glycol aids the intestinal absorption of uranium when this metal is administered in the nitrate state.

7th. The subcutaneous injection of propylene glycol, causes an hypnotic effect of luminal in white rats, as shown in percentage increase of animals that suffer hypnosis with a fixed dose of luminal and during anesthesia.

8th. The pupilar dilation, provoked by atropine in rats, and the modification caused by propylene glycol on this effect when it is administered simultaneously, considering the moment of the initial response, magnitude and duration were studied.

9th. On the isolated intestine of rats, propylene glycol inhibits reversibly the response of the organ to acetylcholine.

10th. The inhibiting action of atropine on the response of the isolated intestine to acetylcholine is intensified by propylene glycol.

11th. Also, propylene glycol intensifies the pharmacological actions of camphor on the isolated intestines of rats, due to a non-specific reactive capacity of the organ.

SUMMARY

The influence of propylene glycol, administered orally and by intramuscular injection, on growth, relative weight of organs and some hematic constants in white rats was studied. It was observed that, possibly, propylene glycol dissolves fats and can destroy the cell.

Nevertheless, its hypocholesterolemizing effect was studied, when administered orally with the drinking water given to white rats, assuming that propylene glycol increases the permeability of the renal glomerulus.

The influence of propylene glycol on intestinal absorption, administering uranyl salt to a group of rats by gastric catheter and determining later the recuperation of uranium in the feces, was studied.

The pharmacological importance of potential synergism between drug and medium, examining different potentials between the use of water and propylene glycol as a solvent in administration of phenylethyl barbituric acid was observed.

The treatment of rats with atropine and propylene glycol permits a clear differentiation between mydriasis caused by atropine and that obtained by the addition of propylene glycol.

Working with isolated organs, several tests were verified examining the pharmacological actions of acetylcholine with propylene glycol on the isolated intestine of rats and, if this effect is studied, whether it is a specific phenomenon or not. An inhibitor of acetylcholine, whose effect is due to the non-specific depression of the reactive capacity of the organ, like camphor, on

on the intestine, was selected. Also, in this case, the effect of camphor is more intense when it is administered using propylene glycol as a medium. All of these effects seem to be originated by a non-specific depression of the reactive capacity of the organ.

The results obtained with respect to the effect of the product on membrane permeability were judged and the practical application of these results has been considered.

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MODE OF ACTION OF PANCREATIC LIPASE*

By A. K. BALLS and M. B. MATLACK

(From the Food Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington)

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In the past the specificity of lipase action for fats has been to some extent taken for granted. Pancreas extracts were known to hydrolyze all the ordinary glycerides (except possibly the stearins) and this behavior defined the enzyme lipase. There have been good reasons for supposing that stearins constituted no exception in principle, but were merely attacked very slowly. Actually, tristearin was split so poorly by otherwise powerful preparations of pancreas lipase that its digestion, when observed at all, was entirely out of line with what might have been expected for a commonly occurring edible fat.

We have recently shown, however, that the hydrolysis of mono-, di-, or tristearin can be carried out at a rate quite comparable to that found with the other fats, provided special conditions are maintained involving a high temperature and a good emulsion (1). The temperature of digestion is best kept at 40° or over. The emulsion is made by dissolving the stearin in a hot mixture of bile and glycerol, cooling down the solution until it is pasty, and then diluting with the required buffer.[1] This technique, as proved by the rapid digestion of tristearin, ought to give a more complete picture of the range of lipase activity on other substrates than has hitherto been obtainable.

* Food Research Division Contribution No. 358.

Presented before the American Chemical Society at Rochester, New York, September 8, 1937.

[1] An explanation of the effect of increased temperature is perhaps found in the work of Clarkson and Malkin (2), who have shown that the internal arrangement of higher glycerides is affected by heating. It is not improbable that such changes in molecular configuration can produce a form of the substrate vulnerable to lipase. The lower glycerides may already be a mixture of several forms in equilibrium.

We have, therefore, experimented with the action of lipase on a number of esters which could not perhaps have been fairly tested by other methods, and whose splitting, so far as we are aware, has not been previously observed. These attempts have been justified by the finding that many esters of higher fatty acids other than the glycerides are saponified by crude lipase at a rate of the same order of magnitude as that shown by the saturated fats.

Most of the substrates tested throughout the work have been synthesized or at least purified in this laboratory.[2] In some instances well known commercially available chemicals were used after testing. No attempt was made at this time to purify the crude glycerol extract of pig pancreas employed as an enzyme preparation. Control experiments, however, eliminated the possibility that the observed hydrolyses were due to the digestion of protein or other constituents of the enzyme system, or to the spontaneous decomposition of the substrates.

Effect of Fatty Acid Component of Ester on Its Rate of Hydrolysis

Pancreas extracts hydrolyze all the triglycerides of N saturated fatty acids. Respectively, however, they are split at very different rates, as shown in Fig. 1. The fats that are split most rapidly are the glycerides of the fatty acids occurring midway between acetic acid and stearic acid. The possibility exists, however, that such results merely portray the degree of emulsification attained. Nevertheless, a similar experiment with various esters of a monohydric alcohol shows the same relationship. The lipase is best adapted to the splitting of esters of fatty acids of intermediate chain length, whether the esters in question are triglycerides or esters of ethyl alcohol.

In no case do the ethyl esters hydrolyze as rapidly as the tri-

[2] We have not found records of the following compounds in the literature: tertiary butyl stearate, m.p. 33°; neopentyl stearate, m.p. 35°; tertiary amyl stearate, m.p. 27.5°; and secondary propyl stearate, m.p. 25°. They were all made without difficulty by the well known quinoline method with stearyl chloride and the appropriate alcohol. They all crystallized readily. We are indebted to Professor F. C. Whitmore for the neopentyl alcohol. Melting points differing materially from those recorded in Beilstein were observed for isobutyl stearate, found to melt at 28.5° instead of at 25° as recorded, and for benzyl stearate, found to melt at 45° instead of at 28° as recorded.

glycerides, but the difference in speed is not very startling. Even ethyl palmitate and ethyl stearate are split rapidly. It is also evident that a separate esterase, at least as found in the liver, plays no significant part in the hydrolyses reported here, for the reason that horse liver extract rich in esterase (splitting ethyl butyrate) was without effect on the stearates used in our experiments.

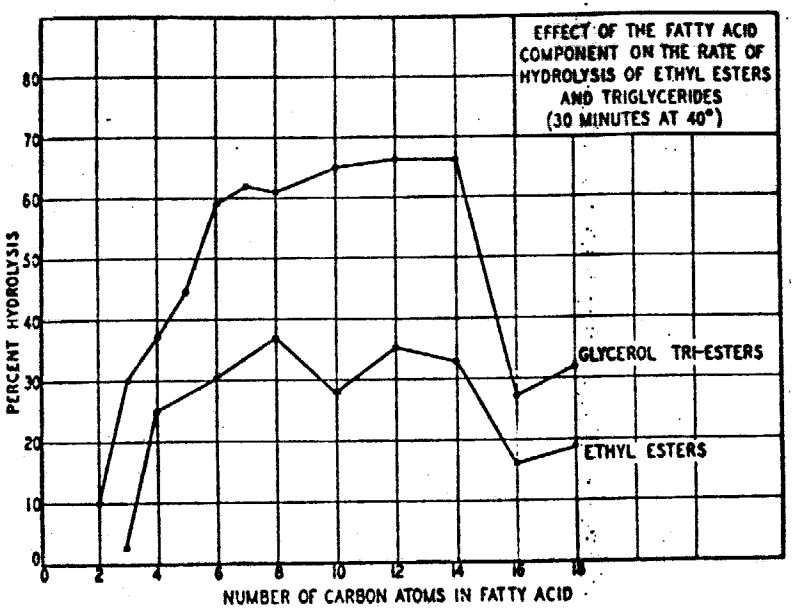

FIG. 1

The specificity of the liver system as contrasted to that of the pancreas is shown in Table I. In general, both liver and pancreas extracts split the lower esters (such as the butyrates) of a variety of alcohols, but only the pancreas enzyme splits the stearic esters. A differentiation noted by Loevenhart (3) between the substrates of pancreas enzyme and liver enzyme is clearly shown in Table I by the hydrolysis of the triglycerides. Liver extract hydrolyzed tributyrin very rapidly, trivalerin slowly, and tricaproin practically not at all. The upper limit of the liver esterase is here the fat of the straight chain C_8 acid.

TABLE I

Action of Liver and Pancreas Extracts on Several Esters

Substrate	Concentration	Per cent splitting in 2 hrs.*	
		Liver extract	Pancreas extract
	mM		
Ethyl butyrate	0.2	95	55
" propionate	0.2	72	15
" myristate	0.2	6 (18 hrs.)	115†
Methyl stearate	0.2	1.3 (18 ")	64
Ethyl "	0.2	3.2 (18 ")	45
Propyl "	0.2	−1.0 (18 ")	46
Allyl "	0.2	−1.9 (18 ")	85
Butyl "	0.2	3.2 (18 ")	75
Isobutyl stearate	0.2	0 (18 ")	118†
Isoamyl "	0.2	0 (18 ")	91
Neopentyl "	0.2	4.7 (18 ")	23 (18 hrs.)
Benzyl stearate	0.2	4.6 (18 ")	101
Cetyl "	0.2	10.7 (18 ")	23
Cholesterol stearate	0.2	0 (18 ")	0.0 (18 hrs.)
Secondary propyl stearate	0.2	5.5 (18 ")	9 (18 ")
" butyl "	0.2	0.4 (18 ")	13 (18 ")
Tertiary " "	0.2	4.7 (18 ")	±3 (18 ")
" amyl "	0.2	0 (18 ")	4 (18 ")
Ethylene glycol dibutyrate	0.2	66	56
Trimethylene glycol dibutyrate	0.2	71	57
Ethylene glycol dicaprate	0.1		99 (30 min.)
" " distearate	0.1	−0.5 (18 hrs.)	2.1 (14 hrs.)
Trimethylene glycol distearate	0.1	1.3 (18 ")	27
Propylene glycol monostearate	0.2	−0.5 (18 ")	37

* The amount of substrate as stated in the table plus 0.33 cc. of glycerol extract per titration. Temperature 40°.

† Because with some esters a part of the undigested substrate is apt to cling to the glass container during the digestion, aliquot portions of the liquid removed for titration toward the end of an experiment may contain more than their proportionate share of free acid. The end-results may therefore show more digestion than they should. This error is not important for a qualitative interpretation of the results, but it must be considered in any experiment designed to differentiate between complete and incomplete splitting. It may be avoided by titrating the entire contents of a container at each time interval.

TABLE I—*Concluded*

Substrate	Concentration	Per cent splitting in 2 hrs.*	
		Liver extract	Pancreas extract
	m.u.		
Tripropionin	0.2	33	44
Tributyrin	0.2	33	59
Trivalerin	0.1	62	70
Tricaproin	0.2	1.5	73
Trimyristin	0.1	0.8 (18 hrs.)	91
Tripalmitin	0.1	−1.0	83
Tristearin	0.1	1.3	80
Triisovalerin	0.1	30	30

Effect of Alcohol Component of Ester on Its Rate of Hydrolysis

This has been examined with a fairly long list of stearic acid esters, and the observed velocities of hydrolysis under comparable conditions are exhibited in Fig. 2. The results show a surprising indifference on the part of the enzyme to the constitution of the alcohol, as long as it is of the primary variety. The length of the carbon chain, the occurrence of free hydroxyl groups, the presence of a double bond or of secondary or tertiary carbon atoms may all affect the rate of splitting, but do not change the qualitative or even the general quantitative aspect of the result.[3] It is evident then that the configuration of the alcohol is without effect on the enzyme, except in so far as the configuration of the hydroxyl-bearing carbon atom is concerned.

On the other hand the splitting of secondary and tertiary esters occurs with great difficulty. The results obtained with secondary propyl stearate, secondary butyl stearate, tertiary butyl stearate, and tertiary amyl stearate are in marked distinction to those with the corresponding esters of *n*-propyl, *n*-butyl, isobutyl, *n*-amyl, and isoamyl alcohols. The splittings observed with these refractory substances were barely greater than the maximum experiment al error over a period of 18 hours at 40°. The slight hydrolysis nay possibly be due to a trace of primary alcohol in the

[3] It must be remembered that small differences in speed are probably due to differences in the fineness of the emulsion which, in spite of the best efforts, must vary from one substance to another.

material used for the ester synthesis. It is therefore difficult to say whether these substances are split very slowly or not split at all. It is safe to assume, however, that the splitting of a secondary ester linkage takes place at an almost negligible velocity, in comparison with the splitting of a primary ester linkage.

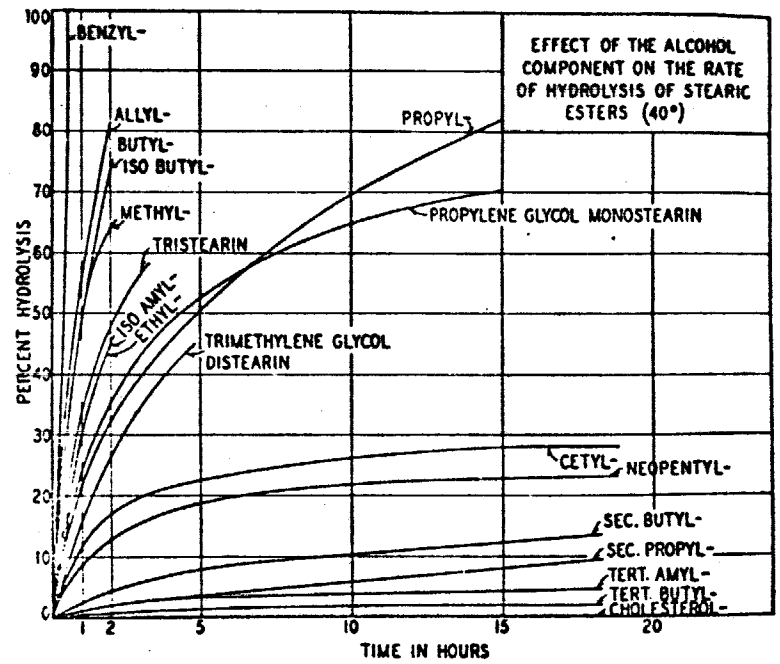

FIG. 2

DISCUSSION

The liver esterase is known to be distinguished from the pancreas enzyme by its specificity for esters of the lower fatty acids, whether esters of glycerol or of simple monohydric alcohols. The pancreas enzyme, however, has been described as digesting the higher fats only. It is now apparent by the use of more inclusive methods that the higher esters of primary monohydric alcohols are also vulnerable. Our data do not indicate whether pancreas lipase also splits the lower esters attacked by the liver enzyme, or whether this was due to an accompanying esterase. The net

result, however, was that crude pancreas extract rapidly hydrolyzed all the primary esters tried except ethylene glycol distearate and dipalmitate.

The anomalous behavior of these two glycol esters might seem very significant, were it not for the fact that until the present technique was adopted, tristearin was found as resistant to lipase action as these esters now appear to be. Further attempts to split them therefore seem advisable before drawing any conclusions. It may in any event be pointed out that while all primary esters have not been hydrolyzed by lipase, primary esters only have been split.

The configuration of the ester-bearing carbon atom of the alcohol must therefore be of great importance to the action of lipase. Either the enzyme requires 2 haptophore hydrogen atoms on this carbon atom or else the replacement of one of these hydrogens by a larger group prevents the approach of the enzyme. The latter view seems more probable because there is no independent evidence to show that the hydrogen atoms in question are specially active. In either case, however, the alcoholic carbon atom of the ester linkage or its neighboring alcoholic oxygen atom is clearly indicated as an anchoring point of the lipase.

The conclusion that only primary ester linkages can be split with reasonable velocity by lipase is of importance in considering the mechanism of enzymic fat hydrolysis. The fats contain one secondary ester linkage, yet are split rapidly and completely. Complete splitting can therefore occur only if the secondary ester group in a triglyceride possesses properties essentially different from the secondary groups in the substrates reported here, or if the migration of the β ester group to an α position takes place before it is hydrolyzed. Further experiments aided by the cooperation of Professor C. G. King of the University of Pittsburgh are in progress on this point.

SUMMARY

An investigation of the effect of pancreas lipase on a series of monoesters shows that ethyl esters of the higher fatty acids are split almost as easily as are the fats themselves. The rate of splitting of a series of ethyl esters exhibits the same characteristic variation with the length of the fatty acid carbon chain as was observed with a series of corresponding triglycerides.

A change in the alcohol component of the ester materially affects the rate of hydrolysis only when the configuration of the carbon atom bearing the hydroxyl group is concerned. Evidence is presented pointing to the conclusion that only primary ester groups are split by lipase. One point of attachment for the enzyme on the substrate would therefore appear to be the C—O— on the alcohol side of the ester linkage. It also follows that either the β ester group in a fat has entirely different properties from those of other secondary esters, or else enzymic hydrolysis must be accompanied by a migration of the β group to an α position.

The authors wish to acknowledge their indebtedness to Mr. I. W. Tucker, of this laboratory, for his assistance throughout the work.

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The Use of Propylene Glycol as a Source of Energy for the Chick

H. S. Bayley, S. J. Slinger and J. D. Summers
Departments of Nutrition and Poultry Science, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada

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Hanzlick *et al.* (1939) showed that propylene glycol could replace some of the carbohydrate in diets for dogs. At low levels there were no ill effects on the dogs and the addition of propylene glycol caused an increase in the levels of blood sugar. When higher levels of propylene glycol were used some was lost in the urine.

Rudney (1950) showed that propylene glycol is converted to lactic acid and hence to glucose in the rat. Miller *et al.* (1953) proposed a metabolic pathway for the conversion of phosphorylated propylene glycol to lactic acid with the generation of a high energy phosphate bond and the reduction of two moles of NAD. Maplesden (1954) described the use of propylene glycol in the treatment of ketosis in dairy cattle. Increasing amounts of this material are being made available and with a gross energy value of 5650 Kcal./kg. as compared with 4000 Kcal./kg. for starch, its use in poultry

TABLE 1.—*Basal diet*

Diet	%
Corn starch	16
Ground corn	42
Soybean meal (50% protein)	35
Supplement	7

The supplement supplied the following to the complete diet: calcium 0.9%, phosphorus 0.35%, iodized salt 0.4%, santoquin 0.0125%, DL-methionine 0.1%, manganese 70 mg./kg., zinc 50 mg./kg., iron 20 mg./kg., copper 2 mg./kg., cobalt 0.1 mg./kg., molybdenum 2 mg./kg., selenium 0.1 mg./kg., vitamin A 6000 I.U./kg., vitamin D_3 700 I.U./kg., vitamin E 4 mg./kg., choline chloride 300 mg./kg., menadione sodium bisulphite 5 mg./kg., calcium pantothenate 10 mg./kg., riboflavin 4 mg./kg., folic acid 2 mg./kg., vitamin B_{12} 0.01 mg./kg., niacin 20 mg./kg.

0.3% chromic oxide was added to the diet in the supplement to allow the metabolizable energy value to be determined.

The diet contained 21% protein (N×6.25) and had a metabolizable energy value of 2840 Kcal./kg.

diets was considered worthy of investigation.

EXPERIMENTAL PROCEDURE

An experiment was designed to compare propylene glycol with corn starch and corn oil, two well defined feeds, in the diets of young chicks. A control diet was prepared containing 16% of corn starch, the formula for which is shown in Table 1. Two series of diets were prepared; in the first series 2, 4, 8 and 16% of corn oil replaced equivalent amounts of corn starch in the control diet and in the second series the same levels of propylene glycol were added at the expense of corn starch. Each diet was fed to five groups of ten male White Leghorn strain cross chicks from day old to twenty-six days of age. Chromic oxide was included in the diets to allow the metabolizable energy values of the complete diets to be determined. At the conclusion of the experiment feed consumption and live weights of the birds were recorded. Two collections of droppings were made; one on the 21st and the second on the 24th day of the experiment. The droppings were freeze dried to constant weight and then ground for analysis.

At the conclusion of the experiment the birds were weighed, deprived of feed for twenty-four hours and then killed with ether. The carcasses were frozen, and while still frozen were ground in a meat grinder. A sample of the ground material was freeze-dried to constant weight and ground in a laboratory mill. The carcass samples were analyzed for nitrogen, ether extract and gross energy values.

RESULTS

The final live weights and feed:gain ratios are shown in Table 2. As expected, the addition of corn oil caused an increase in weight and a decrease in feed:gain ratio with the exception of the diet containing 16% corn oil; the protein content of this later diet may have been inadequate to allow the birds to utilize this high energy diet. Replacing up to 8% of corn starch by propylene glycol caused no significant change in either weight or feed:gain ratio. The droppings from the birds receiving the diet with 16% propylene glycol were very wet and had a strong smell. Some of the

TABLE 2.—*The effect on final live weight, and feed:gain ratio of replacing corn starch with corn oil or propylene glycol*

Diet No.	Level of corn starch replaced	Final live weight	Feed:gain ratio
	%	(g.)	
		Corn oil	
1	0	269abd	2.24
2	2	278bc	2.15
3	4	280c	2.09
4	8	290	2.00
5	16	264bd	1.99
		Propylene glycol	
6	0	269abd	2.24
7	2	273abcd	2.24
8	4	274abc	2.23
9	8	266ad	2.35
10	16	200e	2.65f

Figures followed by the same letters do not differ at the 5% level of probability.

propylene glycol in the diet may have escaped absorption and been partially oxidized by the intestinal microflora or it could have been absorbed and excreted in the urine.

The results of the carcass analysis are shown in Table 3; up to 8% of corn oil caused a decrease in the protein content and an increase in the fat content with a resulting increase in the gross energy value. The carcasses from the birds receiving the diets containing the various levels of propylene glycol did not show such a consistent pattern, but again, the protein content tended to decrease as the level of propylene glycol was increased to 8 percent. The carcasses of the birds receiving 16% propylene glycol had a lower fat content and a higher protein content than the other birds, but at the 16% level the propylene glycol depressed the growth of the birds to subnormal levels.

The metabolizable energy values of the diets increased as the level of corn oil increased. By using a value of 3800 Kcal./kg. for the metabolizable energy value of starch and using the determined metabolizable energy values for the control and experimental diets, it was possible to calculate the contribution of either the corn oil or the propylene glycol to the diet. For the diet with 16% corn oil, the corn oil had a metabolizable energy value of 9100 Kcal./kg., a result in agreement with accepted values.

The same calculations applied to the diets containing propylene glycol gave metabolizable energy values which exceeded its gross energy value. In subsequent work it was found that propylene glycol was volatile in the freeze drier and thus the gross energy values of the freeze dried droppings underestimated the true loss of energy in the excreta and so the metabolizable energy values were overestimated.

The present experiment has shown that up to 8% of corn starch can be replaced in the diet of growing chickens by propylene glycol without adversely affecting weight gain, feed efficiency or carcass characteristics. The volatile nature of propylene glycol, precluded an estimation of its metabolizable energy content by the method used. Attempts will be made to overcome the problem imposed by its volatility.

TABLE 3.—*The effect on carcass composition of replacing corn starch with corn oil or propylene glycol*

Level of corn starch replaced by corn oil	Protein (N×6.25)	Fat	Gross energy
	(dry weight basis)		
%	%	%	kcal./g.
0	64.0a	21.2	5.70ab
2	62.5a	23.7	5.74abc
4	59.3a	27.7	5.90abc
8	57.0	33.0	6.02ab
16	57.6	30.8	5.98abc
Level of corn starch replaced by propylene glycol			
0	64.0a	21.2	5.70b
2	62.3a	22.4	5.79abc
4	63.0a	22.7	5.67b
8	61.9a	24.1	5.70ab
16	65.5a	19.2	5.60

Figures followed by the same letter do not differ at the 5% level of probability.

SUMMARY

Propylene glycol, corn oil and corn starch were compared as energy sources for young chickens. Rations containing up to 16% of these materials were fed from day old to 26 days of age. Liveweight gain and feed consumption were recorded and the carcasses of the birds analyzed at the end of the experiment. It was found that up to 8% of propylene glycol could replace an equivalent amount of corn starch. An attempt was made to estimate the metabolizable energy value of the propylene glycol but its volatility prevented this.

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The Determination of Humectants in Dentifrices by Gas Chromatography

by **Jaime Blum** and **Walter R. Koehler**, Lever Brothers Research Center, Edgewater, New Jersey 07020

Abstract

A rapid, quantitative procedure has been developed for determination of humectants used in dentifrices. The procedure, which is based on conversion of the humectants to their TMS derivatives, requires a minimum of sample preparation. Applications are described for the analysis of three polyols—glycerol, sorbitol, and propylene glycol—commonly used as humectants in toothpaste, with erythritol as the internal standard.

Introduction

Humectants are incorporated in the liquid phase of dentifrices so as to retain moisture when the paste is exposed to air and to prevent its hardening (1). The most common humectants utilized in toothpaste are glycerol, sorbitol, and propylene glycol.

Several procedures have been reported for the analysis of polyol mixtures in samples other than dentifrices. Wheaton and Baumann (2) have published a procedure using nonionic exclusion chromatography. Quantitation was accomplished by refractometry, although a number of other procedures were also mentioned as suitable to monitor the effluent: colorimetry, titration, and density. Of these, the dichromate oxidation colorimetric procedure of Sargent and Rieman (3) offers the most sensitive detection. All of these procedures are time consuming and require a constant supervision on the part of the operator.

A few years ago, Sweeley (4) reported a simple, rapid procedure for the conversion of sugars into volatile trimethylsilyl (TMS) derivatives suitable for gas chromatography. These derivatives are formed by the following reaction:

$$2\,ROH + (CH_3)_3SiNHSi(CH_3)_3 \xrightarrow[]{(CH_3)_3SiCl} 2\,ROSi(CH_3)_3 + NH_3$$

It was only natural to attempt to extend the application of this procedure to polyols such as dentifrice humectants. Sawardeker (5) found that it was impossible to resolve isomeric mixtures of hexitols as the TMS derivatives. This is generally no problem in the analysis of dentifrices because the humectants present differ in chain length rather than configuration.

We have now developed a gas chromatographic procedure based on converting the humectants to the TMS derivatives, which is rapid, quantitative, and requires a minimum of sample preparation.

Experimental

Apparatus

A Barber-Colman gas chromatograph model Selecta 5000 with flame ionization detector was used. The U-shaped glass columns, 6-ft x 3-mm i.d., were packed with silanized Chromosorb W (Applied Science Laboratories) coated with 6% SE 30 and conditioned as recommended by Wells (6). The runs were started at 75°C and temperature programmed at the rate of 6°C/min to 225°C.

Reagents

1. Hexamethyldisilazane (HMDS), Peninsular Chem-Research, Gainesville, Florida.
2. Trimethylchlorosilane (TCS), Peninsular Chem-Research.
3. Pyridine, reagent grade (kept over KOH pellets).
4. l-Erythritol, reagent grade, Mann Research Laboratories, New York, N.Y. 10006.
5. Glycerol, 99.45% pure, Lever Brothers Company.
6. Sorbitol, Fisher Scientific, New York, N.Y.
7. Propylene glycol, reagent grade.

Materials

1. Vials, 2-dram, with plastic tops, Scientific Glass Co., Bloomfield, New Jersey.
2. Micromagnetic stirrer bars, 10 x 3-mm, Cole Parmer Co., Chicago, Illinois.
3. Dentifrice sampling device. Obtain a dentifrice-tube cap identical to the sample cap. Using a No. 3 cork borer, make a hole and insert a medicine dropper through it, rubber tip removed. Screw this cap onto the dentifrice tube.

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Samples

Attach the dentifrice sampling device to the sample tube. Extrude and discard the first portion of dentifrice. Wipe the tip of the medicine dropper and extrude about 80-mg of dentifrice into a tared 2-dram vial, being careful to deposit it in the bottom of the vial and to avoid smearing it on the vial side walls. Reweigh the vial and calculate the dentifrice weight by difference. Place into the vial one micromagnetic stirrer and add 3-ml of pyridine. Stir on the magnetic plate until a homogeneous suspension is obtained.

To the stirring dentifrice suspension, add by pipette 1.00-ml of an internal standard solution containing 250-mg of erythritol in 50-ml of pyridine and stir for another minute. Then, while stirring, add 1.2-ml of HMDS and 0.4-ml of TCS, in that order. On adding TCS, an immediate white precipitate will form. Keep stirring for another 30 minutes.

Chromatographic Procedure

Inject 0.5-mcl of the supernatant into the column and immediately start the program. The elution temperatures of the polyol TMS derivatives are approximately as follows: propylene glycol, 105°C; glycerol, 130°C; erythritol, 160°C, and sorbitol, 195°C.

Calculations

Calculate the area ratios of the pairs glycerol/erythritol and sorbitol/erythritol. Calculate the glycerol and sorbitol levels in the dentifrice using this equation:

$$\text{Humectant, \%} = \frac{\text{area ratio x mg internal standard added x 100}}{\text{sample weight}}$$

The results described below show that if a flame detector is used in the gas chromatograph, no correction factor is needed to convert relative peak areas to relative weights, since the TMS derivatives of glycerol, erythritol, and sorbitol give essentially identical responses on a weight basis. If other detectors are used or if other components such as propylene glycol are found, suitable calibration factors (K) must first be determined. In the case of propylene glycol, after calculating the propylene glycol/erythritol area ratio, its level is determined using this equation

$$\text{Propylene glycol, \%} = \frac{\text{area ratio x K x mg internal standard added x 100}}{\text{sample weight}}$$

where K is the relative response of the detector to propylene glycol and erythritol.

Results

Calibration

For each of the three humectants, two-component calibration mixtures were prepared by weight, varying the ratio of humectant/internal standard while keeping the internal standard weight unchanged. After analysis peak areas were calculated by triangulation and the area ratios compared with the weight ratios. A correction factor, K, was then calculated.

Tables I and II show the correction factors obtained with mixtures of glycerol/erythritol and sorbitol/erythritol. The first columns indicate the weight

Table I. Area Ratio Correction Factor for Glycerol/Erythritol Standard Solutions

Glycerol/Erythritol Weight Ratio	Ratio	Factor K
0.287	0.292	0.982
	0.289	0.993
	0.300	0.956
0.667	0.666	1.001
	0.660	0.989
	0.674	1.010
1.001	1.044	0.997
	1.000	1.001
	1.048	0.955
	1.054	0.949
1.334	1.417	0.941
	1.344	0.992
	1.354	0.985
	1.282	1.040
	1.327	1.005
1.916	1.960	0.977
	1.876	1.021
	1.830	1.046
	1.949	1.017
	Average	0.992

Table II. Area Ratio Correction Factor for Sorbitol/Erythritol Standard Solutions

Sorbitol/Erythritol Weight Ratio	Peak Area Ratio	Correction Factor, K
0.330	0.318	1.038
	0.324	1.018
	0.337	0.979
0.660	0.641	1.029
	0.645	1.023
	0.633	1.042
	0.657	1.004
	0.658	1.003
0.991	1.011	0.980
	0.960	1.032
	0.991	1.000
	1.014	0.977
1.320	1.312	1.006
	1.304	1.012
	1.333	0.990
1.651	1.628	1.014
	1.606	1.028
	1.663	0.992
	Average	1.009

ratios. The second columns list the observed peak area ratios while the third columns show each individual correction factor obtained. The means of all the correction values were 0.992 and 1.009, respectively.

An analysis of variance was conducted to determine whether the weighing errors and other manipulations associated with the dilution procedure contributed significantly to the error of the method. It was found that the errors so introduced were not significant in comparison to the variability of the GLC method itself.

It was also found that no significant error is committed when it is assumed that these correction factors are equal to 1 for glycerol/erythritol and sorbitol/erythritol calibration mixtures (Tables I and II). This statistical analysis showed that the standard deviation of a single determination was ±2.5 per cent.

A similar, although less complete study, was performed with propylene glycol/erythritol mixtures (Table III). The correction factor was found to be 1.17. It is not known whether this is due to the effect of the methyl group of propylene glycol or to measurement errors since the propylene glycol peak is "sitting" on the solvent tail.

Toothpaste Samples

Table IV presents the results obtained when this procedure was applied to dentifrice samples. Data obtained with this procedure are listed under the heading GLC. All but one of these samples were also analyzed by a procedure based on a combination of nonionic exclusion chromatography (2) with a dichromate colorimetric procedure (3). These data are listed under NEC. Each of the values given in Table IV represents a single determination.

Figure 1 shows a chromatogram obtained on a commercial dentifrice (dentifrice "H"). No significant peaks other than those derived from glycerol and sorbitol could be observed. No internal standard was used in this case. Figure 2 is the chromatogram obtained with another dentifrice "D" to which erythritol had been added as internal standard. In this case all three common humectants were noticed. There is a particular feature in this dentifrice in that lactose was found towards the end of the chromatogram.

Recovery

This analytical method was shown not to be affected by any of the other constituents present in most dentifrices. The low water levels present in dentifrices had no effect on the recovery. A sample of dentifrice containing 21 per cent of water was analyzed by the above procedure, then freeze-dried and again analyzed. Glycerol levels were found to be 6.8 per cent and 6.6 per cent before and after freeze-drying, while the sorbitol levels were 12.5 per cent and 12.1 per cent, respectively, after correction for weight losses due to

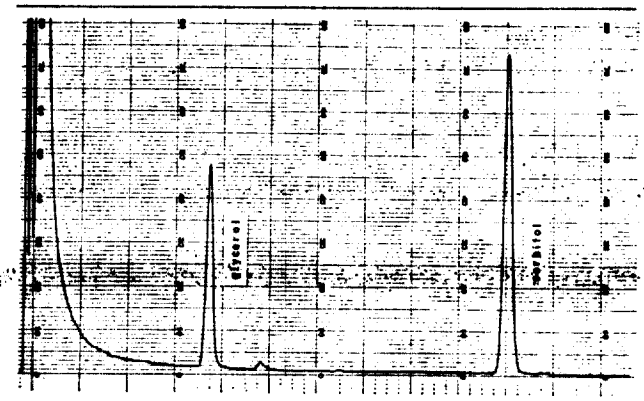

Figure 1. Chromatogram of Silylated Dentifrice "H".

Table III. Area Ratio Correction Factor for Propylene Glycol/Erythritol Standard Solutions

Propylene Glycol/Erythritol Weight Ratio	Peak Area Ratio	Correction Factor, K
0.75	0.640	1.178
1.50	1.340	1.150
3.00	2.586	1.160
3.75	3.155	1.188
	Average	1.169

Table IV. Humectant Levels in Dentifrices by Gas Liquid Chromatography (GLC) and Nonionic Exclusion Chromatography (NEC)

Dentifrice	A		B		C		D		E		F	
Procedure	GLC	NEC	GLC	NEC	GLC	NEC	GLC	NEC	GLC	NEC	GLC	NEC
Glycerol %	21.9	20.8	9.7	10.3	18.9	16.7	9.9	(1)	6.2	6.6	40.9	39.4
Sorbitol %	—	—	12.4	11.4	4.1	4.8	5.7	(1)	7.9	7.8	—	—
Propylene Glycol %	—	—	—	—	—	—	4.3	(1)	—	—	—	—

Dentifrice	G		H		I		J	
Procedure	GLC	NEC	GLC	NEC	GLC	NEC	GLC	NEC
Glycerol %	7.0	6.2	7.1	6.3	4.8	3.8	6.8	6.7
Sorbitol %	12.8	12.2	12.9	11.2	13.6	12.6	12.4	12.4

(1) Not determined

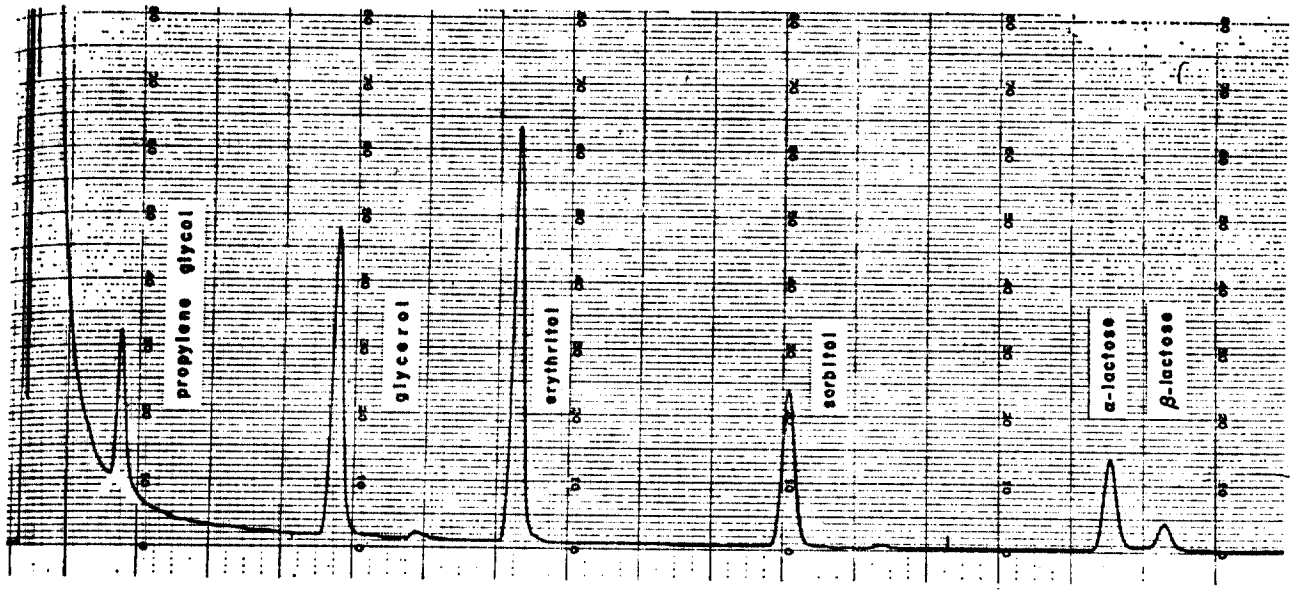

Figure 2. Chromatogram of Silylated Dentifrice "D."

lyophillization. This points out that the water present in the dentifrice does not affect the silylation reaction, provided sufficient silylating reagent is present to react with all water and hydroxy compounds present in the mixtures.

Other inorganic constituents would not be expected to present any problem since they do not form volatile derivatives. Organics, other than the humectants, are generally present at low levels and they or their silylated derivatives do not appear in the chromatograms.

A commercial dentifrice was used as a model for a recovery experiment. Its glycerol and sorbitol levels were determined by the proposed procedure. To a known weight of this dentifrice, known weights of glycerol and sorbitol (in pyridine solution) were added. This enriched sample was assayed by the same procedure in triplicate. Average recoveries calculated from the chromatograms so obtained were 95.1 per cent for glycerol and 98.8 per cent for sorbitol.

A time study on the silylation reaction showed that 30 minutes is enough for a complete silylation. Chromatograms of the same sample obtained after 1.5 and 3.0 hours of silylation did not show any variation in the results obtained originally. The silylated mixture can be keept at room temperature for a number of days with no decomposition. This was found with calibration mixtures or silylated dentifrices which, when [illegible]ing run 20 days after preparation, produced the same results as when originally silylated.

Discussion

It can be seen in Figure 2 that propylene glycol TMS is eluted before the elution of the solvent (pyridine) is complete. Although satisfactory precision in measuring the levels of all components was achieved, several attempts were made to reduce or eliminate the solvent tail. One of these approaches was to increase the concentration by augmenting sample size while keeping the other reagents at the same level. The net effect of this approach was that the polyols were not completely silylated and, therefore, what should have been one peak per humectant became a pair of overlapping peaks. Therefore, in all subsequent runs, the solvent proportions indicated by Sweeley (4) were strictly adhered to.

Other solvents mentioned in the literature as good silylation media (dimethyl formamide, methyl pyrrolidone, and diethylamine) either tailed worse than pyridine or were not sufficiently good proton acceptors to promote silylation. Other workers (7) working with sugars have shown that the solvent tailing can be eliminated by extracting the TMS derivatives into chloroform and removing the pyridine with water washes and evaporating the chloroform at 100°C. The sugar TMS derivatives vaporize at temperatures high enough not to be evaporated under these conditions. When this technique was applied to the silylated dentifrice mixture, the propylene glycol TMS derivative was totally lost in the evaporation process while the glycerol TMS evidenced serious, although not complete, losses.

The propylene glycol peak could be well separated from pyridine by using the more polar chloromethyl dimethylsilyl derivative (8-10). However the corresponding erythritol and sorbitol derivatives could not be eluted from the column at a reasonable temperature. We are, therefore, recommending that the TMS derivatives be prepared in pyridine and injected directly. ■

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DIURETIC ACTION OF MULTIVALENT ALCOHOLS

Bornmann, G. & K. Ritter (Pharmacological Institute, Munster University). Arch. exper. Path. u. Pharmakol. 219, 57-63 (1953).
Diuresis was observed in the experimental animals in several experiments after oral administration of glycerine, 1,3-butanediol and other multivalent alcohols. A similar effect of glycerine was reported as early as 1876 by Ustimovitsch (13), it has been confirmed in several instances; diuresis occurring following the oral administration of 1,2-propyleneglycol and diethyleneglycol was reported by Van Winkle (15) and Laug et al. (9). Experiments were performed on male rates in order to determine whether multivalent alcohols in general have diuretic effects and to what extent compounds of this class, i.e. ethanediol, 1,2-panediol, 1,3-butanediol, glycerine, 1,2,4-butanetriol and the ether alcohol diethyleneglycol, are effective.

Methodology

The multivalent alcohols were adminstered in all experiments orally (pharyngeal probe), because preliminary experiments indicated that no acute substantial increase in the volume of urine occurred following parenteral administration. This observation is in agreement with the findings of Ustimowitsch in testing glycerine and the report by Ginsburg (7) concerning enhanced diuresis after oral than after parenteral administration of water.

The rats, which were without food or water for 12 hours prior to and during the experiment, were given 0.5 or 1.0 cm^3 of the alcohol to be tested per 100 g of body weight. The alcohols were given either undiluted or diluted. Fire cm^3 per 100 g body weight of 10 or 20% solutions prepared with distilled water or a physiological table salt solution were given with pharingeal probes. The solutions were thus adjusted so that the amount of glycol tested was the same as in the experiments without dilution. Each experimental group consisted of 5

animals. Control groups of equal numbers received corresponding amounts of water or physiological salt solutions. Following the administration of the liquids to be examined, the urine volume of each animal was measured 6 times in intervals of 2 hours, and once 12 hours later. In addition to the determination of the urine volume, the Cl content of the urine was also determined at regular intervals, generally of 4 hours (by the Volhard method).

Date of the experiments: June to September 1952.

Results:

All of the multivalent alchols in the undiluted state clearly induced a certain diuresis, i.e. the volume of urine produced by the experimental animals under their infiuence was substantially higher than that of the control animals. Diuresis was apparent as early as the first reading, i.e. 2 hours after the administration of the test substances and lasted -- with the exception of the glycerine diuresis -- at least for 12 hours. Table 2 shows the amount of urine produced during 12 hours by the 5 animals of each experimental group and the progress of diuresis in time. The effect of 1,3-butanediol and, less clearly, of diethylene glycol -- both substances were given in small (0.5 cm^3 per 100 g of body weight) and large (1.0 cm^3) amounts -- proved to be dependent on dosage. Of the other alcohols, the effect of a single dose only was investigated, a high dose of 1,2-propanediol, glycerine, 1,2,4-butanediol (3) and, in accordance with its stronger infiuence on the general condition of the body, a lower dose of ethanediol. A comparison of urine volumes produced under the effect of different multivalent alcohols yields the following series in order of decreasing effectiveness: ethanediol, diethyleneglycol, 1,3-butanediol, 1,2,4-butanetriol, 1,2-propaneiol, gylcerine (Fig. 1).

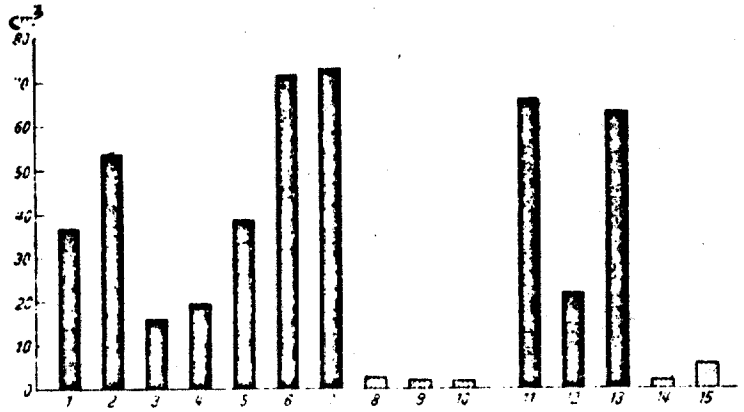

Abb. 1. Harnmengen von Ratten nach oraler Zufuhr von 1,0 cm³ 1,2-Propandiol (1), 1,3-Butandiol (2), Glycerin (3, 4), 1,2,4-Butantriol (5), Diäthylenglykol (6, 7) oder Aqua dest. (8–10) bzw. von 0,5 cm³ Äthandiol (11), 1,3-Butandiol (12), Diäthylenglykol (13) oder Aqua dest. (14, 15) je 100 g Körpergewicht. Jede Säule entspricht dem 12 Std-Wert von je 5 Tieren. Ordinate (Säulenhöhe): Harnmenge in Kubikzentimeter.

FIGURE 1. Urine Volumes of Rats following Oral Administration of 1.0 cm^3 1,2-Propanediol (1), 1,3-Butanediol (2), Glycerine (3,4), 1,2,4-Butanetriol (5), Diethyleneglycol (6,7) or Distilled Water (8-10) and of 0.5 cm^3 Ethanediol (11), 1,3-Butanediol (12), Diethyleneglycol (13) or Distilled Water (14, 15) per 100 g of Body Weight. Each column corresponds to the 12-hour value for 5 animals. Ordinate (height of column): urine volume in cm^3.

TABLE 1. Urine Volumes of Five Rats Following Oral Administration (Pharingeal Probe) of Multivalent Alcohols (Undiluted):

Prüfsubstanz	Dosis je 100 g Körpergewicht (cm³)	Gewicht 5 ♂ (g)	Harnmengen (cm³) 1.–4.	5.–8.	9.–12. Std	Harnmenge in 12 Std (cm³)	24 Std (cm³)
Äthandiol	0,5	792	33,9	20,8	11,2	65,9	72,3
1, 2-Propandiol	1	754	20,1	11,2	5,5	36,8	39,5
1, 3-Butandiol	1	752	23,6	18,3	11,5	53,4	58,6
1, 3-Butandiol	0,5	787	7,9	9,1	4,2	21,2	23,7
Glycerin	1	747	11,4	3,3	1,2	15,9	16,1
Glycerin	1	955	11,7	6,6	1,3	19,6	21,1
1, 2, 4-Butantriol	1	790	22,5	10,1	6,2	38,8	48,0
Diäthylenglykol	1	885				71,9	84,9
Diäthylenglykol	1	950	30,8	27,5	14,7	73,0	87,9
Diäthylenglykol	0,5	925	37,7	19,1	5,3	62,1	66,2
Aqua dest.	1	752	2,2	0,2	0,3	2,7	4,3
Aqua dest.	1	885				1,8	3,2
Aqua dest.	1	955	0,7	0,9	0,3	1,9	4,3
Aqua dest.	0,5	792	0,4	1,2	0,3	1,9	3,8
Aqua dest.	0,5	925	2,4	2,3	0,5	5,2	8,3

Dosage: 0.5 or 1.0 cm^3 per 100 g of Body Weight.

Control: Distilled water.

Key: 1. Test substance; 2. Dose per 100 g of body weight (cm^3); 3. Weight; 4. Urine volume (cm^3); 5. Urine volume in 12(24) hours.

The particularly intensive diuretic effect of undiluted ethanediol and diethyleneglycol also became apparent when the period of time needed to eliminate the volume of liquid introcued was applied as a criterion of efficiency. Thus, following the administration of these glycols, a urine volume corresponding to a multiple of the amount of glycol given was produced in 2 hours. In the case of glycerine, on the other hand, the urine volume corresponding to the amount of liquid given, was evacuated in only 4 hours.

The diuretic effect was less intensive when the multivalent alcohols were intoruced diluted with physiological salt solutions in the form of 10 or 20% per volume solutions, in doses of 5.0 cm^3 per 100 g of body weight. Although here again the experimental animals produced substantial amounts of urine, they were generally even higher than in the case of undiluted substances, but -- in comparison with the urine volumes of the control animals -- the difference was less impressive. While for the control groups 8.5 to 16 cm^3 urine was measured in 12 hours, the corresponding values for the experimental animals were 26 to 75 cm^3. The diuretic effect of not all of the compounds lasted the full 12 hours; thus, the effect of propanediol (20% solution) and butanediol (10% solution) ceased after just 10 hours and that of glycerine even sooner. Arranging the alcohols in order of their effectiveness -- with consideration of the dosage -- results in the following series: diethyleneglycol, ethanediol, 1,2-propanediol, 1,3-butanediol, 1,2,4-butabetriol and, finally, glycerine (Fig. 2).

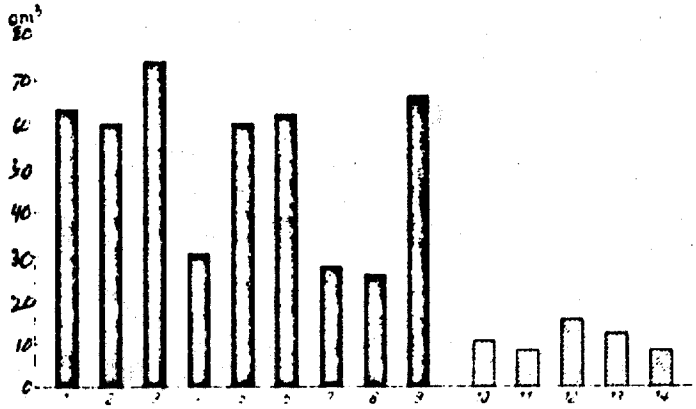

Abb. 2: Harnmengen von Ratten nach oral Zufuhr von 5,0 ml einer 20%igen Lösung von 1,2-Propandiol (2), 1,3-Butandiol (3), 2,3-Glycerin (4), 1,2,4-Butantriol (5) bzw. von 5,0 ml einer 10%igen Lösung von Äthandiol (6), 1,3-Butandiol (7), 2,3-Diäthylenglykol (9) je 100 g Körpergewicht (Verdünnungsmittel: physiologische Kochsalzlösung). Die Säulen 10–14 geben die Harnmengen der Vergleichsgruppen wieder, denen je 100 g Körpergewicht 5,0 ml physiologische Kochsalzlösung verabfolgt wurde. Jede Säule entspricht dem 12 Std-Wert von je 5 Tieren. Ordinate (Säulenhöhe): Harnmenge in Kubikzentimeter.

FIGURE 2. Urine Volumes of Rats Following the Oral Administration of 5.0 cm^3 of a 20% Solution of 1,2-Propanediol (1), 1,2-Butanediol (2,3), Glycerine (4), 1,2,4-Butanediol (5), and 5.0 cm^3 of a 10% Solution of Etahendiol (6), 1,3-Buatnediol (7, 8), Diethyeleneglycol (9) per 100 g of body weight (Dilutant: physiological salt solution). Columns 10-14 show the volume of urine for the control groups receiving 5.0 cm^3 of the physiological salt solution per 100 g of body weight. Each column represents the 12 hour-value per 5 animals. Orinate (height of column): urine volume in cm^3.

TABLE 2. Urine Volumes of Groups of 5 Rats Each Following the Oral Administration (Pharingeal Probe) of 10% and 20% Solution of Multivalent Alcohols (Dilutant: physiological salt solution).

Dose: 5 cm^3 per 100 g of body weight. Control: physiological salt solution

Prüfsubstanz	Dosis je 100 g Körpergewicht (cm³)	Gewicht 5 Tiere (g)	Harnmengen (cm³) 1. 4.	5. 8.	9. 12. Std	Harnmenge in 12 Std (cm³)	24 Std (cm³)
Äthandiol	0,5	635	34,6	21,2	7,0	62,8	68,2
1, 2-Propandiol	1	635	51,7	9,9	2,1	63,7	64,4
1, 3-Butandiol	1	700	38,2	17,8	4,7	60,7	62,7
1, 3-Butandiol	1	757	43,8	22,2	8,6	74,6	78,9
1, 3-Butandiol	0,5	700	17,4	7,2	2,5	27,1	29,7
1, 3-Butandiol	0,5	750	14,4	8,1	3,2	25,7	29,0
Glycerin	1	732	29,5	0,9	0,3	30,7	32,5
1, 2, 4-Butantriol	1	735	32,5	17,1	10,7	60,3	72,8
Diäthylenglykol	0,5	910	44,5	16,4	5,3	66,2	69,5
physiol. Kochsalzlösung		710	9,2	3,6	3,0	15,8	23,9
physiol. Kochsalzlösung		635	5,6	1,5	3,0	10,1	13,7
physiol. Kochsalzlösung		755	1,2	1,9	2,3	8,4	14,6
physiol. Kochsalzlösung		734	7,5	0,9	3,9	12,3	20,5
physiol. Kochsalzlösung		910	5,2	2,2	1,1	8,5	14,9

Key: 1. Rest substance; 2. Dose per 100 g body weight; 3. Weight; 4. Urine volume; 5. Urine volume in 12 hours - 24 hours; 6. Ethanediol; 7. Diethyleneglycol; 8. Physiological salt solution.

The last series of experiments served to determine whether the multivalent alcohols would cause diuresis when diluted with distilled water. In a manner similar to the investigation of

alcohols diluted with a physiological salt solution, here again the volume of urine of the experimental animals was greater than that of the control animals, only the difference was still smaller. (Table 3). Urine volumes determined in the 9-10th hour after the administration of an aqueous glycerine solution were within the range of volumes measured for the control animals. In the 11-12th hour the urine volumes of rats treated with propanediol (20$ solution) or butanediol (10% solution) were approximately equal to those of the control animals, while in the case of the experimental groups the increase in volume was still appreciable. In this experimental arrangement ethanediol and diethylenegylcol have the strongest effect, 1,2,4-butanetriol, 1,3-butanediol and 1,2-propanediol are weaker, while gylcerine has only a slight effect (Fig. 3).

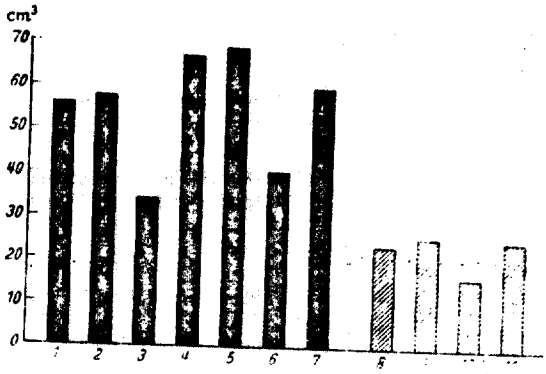

FIGURE 3. Urine Volumes of Rates Following the Oral Adminstration of a 20% Solution (5.0 cm^3) of 1,2-Propanediol (1), 1,3-Butanediol (2), Glycerine (3), 1,2,4-Butanetriol (4) and of 5.0 cm^3 of a 10% Solution of Ethanediol (5), 1,3-Butanediol (6), Diethyleneglycol (7) per 100 g of Body Weight. (Dilutant: Distilled Water). Columns 8-11 show the urine volumes for the control groups, which received 5.0 cm^3 distilled water per 100 g of body weight. Each column represents the 12-hour value per 5 animals. Ordinate (height of column): urine volume in cm^3.

TABLE 3. Urine Volumes of Groups of 5 Rats Following the Oral Administration (Pharingeal Probe) of 10% and 20% Solutions of Multivalent alcohols (Dilutant: Distilled Water):
Dose: 5 cm^3 per 100 g body weight (Control: distilled water)

Prüfsubstanz	Dosis je 100 g Körpergewicht (cm^3)	Gewicht 5 ♂ (g)	Harnmengen (cm³) 1.–4.	5.–8.	9.–12. Std	Harnmengen in 12 Std (cm³)	24 Std (cm³)
Äthandiol	0,5	665	41,3	20,1	7,2	68,6	74,7
1, 2-Propandiol	1	665	42,7	11,3	2,0	56,0	57,0
1, 3-Butandiol	1	702	32,3	18,2	6,8	57,3	61,2
1, 3-Butandiol	0,5	697	32,2	6,3	1,6	40,1	43,4
Glycerin	1	701	30,4	3,4	0,2	34,0	36,5
1, 2, 4-Butantriol	1	665	39,2	16,3	11,2	66,7	76,6
Diäthylenglykol	0,5	915	32,2	20,2	7,2	59,6	64,2
Aqua dest.		702	22,2	1,1	1,8	25,1	28,7
Aqua dest.		665	19,5	1,6	2,0	23,1	27,4
Aqua dest.		730	20,5	2,7	1,8	[illegible]	29,0
Aqua dest.		920	13,9	1,3	[illegible]	[illegible]	18,4

Key: (As for Table 2)

In spite of adhering to Burn's specification for using only male animals of equal eight, maintained under identical conditions, comparable measured values show a wide scatter. The fundamental result, i.e. the diuretic effect of both undiluted and diluted glycols, in comparison with the administration of similar volumes of liquids (water or physiological salt solution) in control experiments, was, however, significant in each case, even in the mathematical treatment of the 12-hour urine volumes.

The determination of chlorides yielded higher contents on the average in the urine of the experimental animals than in that of the control animals (14), while the mean values of chloride concentrations showed no difference.

The solution to the problem of how diuresis caused by miltivalent alcohols actually occurs, may be facilitated by experiments in which the inhibition of 1,3-butanediol diuresis by small amounts of pituitary gland extract was tested. (10).

In fact, such treatments did substantially restrict the diuresis -- hourly values of 2.8, 3.9, 4.5, 6.4 cm^3 instead of 4.8, 8.3, 10.1, 10.5 cm^3 -- the results are simiar to the findings og Struuss et al., (12) in an investigation of the iduretic effects of ethylalcohol. It seemed logical therefore to assume an inhibition of reabsorption as the cause of the diuretic effect, both in the case of the multivalent alcohol 1,3-butanediol and the univalent alcohol ethanol. This concept is in agreement with the fact that numerous multivalent alcohols are evacuated with the urine. Most of the glycols mentioned above were also detected in the bile (4, 11), however, the volume of the bile did not increase (1, 2). It is known, however, that urine and bile secretion are controlled by difference laws.

Summary:

Ethanediol and diethylenegylcol possess very vigorous diuretic effects, 1,3-butanediol, 1,2-propanediol and 1,2,4-butanetriol appreciable effects, while glycerine causes only a moderate increase the volume of urine.

The diuretic effect of multivalent alcohols remains detectable when 10 or 20% solutions of these substances (diluted with distilled water or physiological salt solutions) are used.

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*The calculation is based on the following expression:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}, \quad \varepsilon = \frac{\sigma}{\sqrt{n}}, \quad \frac{m_1 - m_2}{\sqrt{\varepsilon_1^2 + \varepsilon_2^2}} = R$$

R 2 is taken as significant (see Burns)

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THE OCCURRENCE OF A TOE ABNORMALITY IN CHICKS FED PROPYLENE GLYCOL

T. E. Bowen and P. W. Waldroup
Department of Animal Sciences, University of Arkansas, Fayetteville, Arkansas 72701

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Propylene glycol (1, 2-dihydroxy propane) is unique among the glycols because its low toxicity permits it to be taken internally. In recent years it has been shown to be effective in the prevention of bovine ketosis (Maplesden, 1954). Bayley *et al.* (1967) reported that up to 8% propylene glycol could replace an equivalent amount of corn starch in the diet of chicks.

During the course of feeding trials conducted at this laboratory to evaluate propylene glycol as a feed ingredient for poultry diets, a peculiar deformity of the toe was observed (Figure 1). The toes turned outward at each joint with the incidence of the condition increasing as the level of propylene glycol increased in the diet (Table 1). These chicks were maintained in electrically heated battery brooders with raised wire floors and a small incidence of deformed or crooked toes were observed even in the control group with no propylene glycol. However, the severity was not as great as that which occured at the higher levels of propylene glycol. Increasing the level of the propylene glycol resulted in statistically significant increases in toe defects.

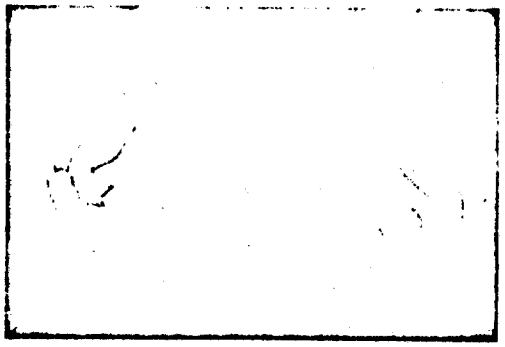

Fig. 1. Typical toe deformity resulting from feeding propylene glycol to broiler chicks.

Table 1.—*Influence of propylene glycol on the incidence of toe abnormalities of broiler chicks*

% Propylene glycol	Incidence of toe defects[1]
0	9/168[a]
2.5	19/168[ab]
5.0	29/168[bc]
7.5	39/168[c]
10.0	57/168[d]

[1] Indicates the number of chicks having a toe defect out of total number examined. Means having the same superscript do not differ significantly ($P = <.05$).

It is interesting to note that Rutter *et al.* (1953) reported a lactose-induced crooked toe deformity in broiler chicks which appears to be similar to that observed in trials in our laboratory. Propylene glycol is converted to lactic acid and hence to glucose in the rat (Rudney, 1950; Miller *et al.*, 1953). Therefore, these compounds may be broken down to a common metabolite responsible for the toe defect.

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The Influence of Propylene Glycol on pH of the Gastrointestinal Tract and the Incidence of Leg Abnormalities in Broiler Chicks

T. E. Bowen and P. W. Waldroup
Department of Animal Sciences, University of Arkansas, Fayetteville, Arkansas 72701

(Received for publication September 5, 1968)

PREVIOUS reports from this laboratory (Bowen and Waldroup, 1968; Waldroup and Bowen, 1968) have indicated that propylene glycol (1, 2-dihydroxy propane) may be included as a synthetic energy source in broiler diets at levels up to 5 percent. Higher levels resulted in decreased growth rate, diarrhea and the development of a leg deformity characterized by an outward curling of the toes along with a perosis-like hock disorder.

This condition is similar to that reported by Rutter *et al.* (1953) for chicks fed diets high in lactose. Since propylene glycol has been reported to be metabolized to lactic acid in the rat (Rudney, 1950; Miller *et al.*, 1953), it is possible that some common metabolite may be responsible for this condition.

The objectives of the trials reported herein were to determine if the inclusion of propylene glycol in the diet of broiler chicks would alter the pH of the digestive tract and to see if the addition of higher levels of trace mineral supplementation would aid in overcoming the hock and toe disorder. It is possible that propylene glycol may be altering the gastrointestinal pH or have a chelating effect inhibiting the absorption of some of these minerals.

INFLUENCE ON INTESTINAL pH

Experimental Procedure. One hundred day-old male broiler chicks were placed on the standard University broiler diet. On the fourteenth day, the chicks were randomly divided into three equal weight groups of twenty chicks each. The remainder of the chicks were discarded. Three experimental diets were compared as to their influence on the pH of the gastrointestinal tract. One diet consisted of a standard chick diet without propylene glycol,[1] while in the other two diets propy-

[1] Sirlene, produced by Dow Chemical Company, Midland, Michigan.

lene glycol was added at 5 and 10 percent by weight (Table 1). Five battery pens of four chicks were fed each of the diets.

After 5 days on the experimental diets, five chicks (one per pen) were selected at random from each treatment. The chicks were sacrificed by use of ether and samples were taken from various segments of the gastrointestinal tract as follows and diluted with 3 cc. of boiled distilled water.

a. *crop*—a sample of approximately 2 cc. of the contents was removed and diluted.
b. *proventriculus*—the entire contents were washed out and diluted.
c. *gizzard*—a sample of approximately 2 cc. of the contents was removed and diluted.
d. *duodenum*—a section about 5 cm. long was removed from the center, the contents removed and diluted.
e. *ileum*—a section about 5 cm. long was removed about 10 cm. above the ceca and the contents diluted.
f. *large intestine*—a section about 5 cm. long was removed about 5 cm. below the ceca and the contents diluted.
g. *ceca*—a sample of about 2 cc. of the cecal contents were removed from one of the ceca and diluted.

Samples were taken at the fifth, seventh, tenth, and fourteenth day on treatment using five chicks from each diet for each determination. Cecal samples were not taken on the fifth day. No measurements were made of body weight gains or feed consumption.

Results and Discussion. There were no significant differences in pH of the crop, proventriculus, gizzard, or duodenum between chicks fed any of the experimental diets, nor were there any differences due to day of sampling (Table 2).

Chicks fed the diets containing 5 percent propylene glycol had a significantly higher pH in the ileum and the large intestine than those fed the control diets. Chicks fed the diet with 10 percent propylene glycol also had a significantly higher ileum pH, but the pH in the large intestine was lower than in those fed the control diet. The pH increased in the ileum but decreased in the large intestine as the age of the chick increased.

TABLE 1.—*Composition of diets*

Ingredient	1	2	3
		% of Diet	
Yellow corn	42.00	42.00	42.00
Soybean meal (50% protein)	38.10	38.10	38.10
Corn oil	7.04	3.62	0.20
Propylene glycol	—	5.00	10.00
Washed sand	9.44	7.86	6.28
Defluorinated phosphate	1.62	1.62	1.62
Ground limestone	0.80	0.80	0.80
Iodized salt	0.40	0.40	0.40
Microingredients[1]	0.50	0.50	0.50
Trace mineral mix[2]	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated analysis			
% Protein	23.00	23.00	23.00
% Calcium	1.00	1.00	1.00
% Phosphorus	0.70	0.70	0.70

[1] Supplies per kg. of diet: 6,600 I.U. vit. A; 220 I.C.U. vit. D_3; 22 I.U. vit. E; 1.1 mg. vit. K; 6.6 mg. riboflavin; 17.6 mg. pantothenic acid; 44 mg. niacin; 1870 mg. choline; 11 mcg. vit. B_{12}; 125 mg. ethoxyquin.

[2] Supplies in mg./kg. of diet: 100 iron, 100 zinc, 100 manganese, 10 copper, 1 cobalt, 3 iodine.

These increases in the pH of the tract were quite unexpected since the propylene glycol has been reported to be metabolized to pyruvic, lactic, and proprionic acids (Anonymous, 1966).

A possible explanation for this increase in pH of the lower gastrointestinal system is that the diarrhea which occurs on the propylene glycol may possibly be caused by increased bacterial fermentation, resulting in gases and end products which may alter the pH. Rutter *et al.* (1953) attributed the chick diarrhea on diets high in lactose to this cause. Thus, it is possible to state that propylene glycol in broiler diets does significantly alter the pH of the lower gastrointestinal tract.

INFLUENCE ON LEG ABNORMALITIES

Experimental Procedure. It had been noted in previous trials at this laboratory (Bowen and Waldroup, 1968; Waldroup

TABLE 2.—*Influence of propylene glycol on pH of various segments of the gastrointestinal tract*

Propylene glycol (%)	Sampling time (days)	Crop	Prov.	Gizzard	Duod.	Ileum	Large int.	Ceca
0.0	5	5.10	3.75	2.47	5.46	6.46	6.98	—
	7	4.60	3.54	2.66	6.21	7.87	7.38	6.00
	10	4.73	4.00	2.70	5.32	7.13	7.03	6.20
	14	5.88	3.42	2.93	6.46	7.65	7.32	5.97
	Av.	5.07	3.67	2.81	5.86	7.41	7.16	6.06
5.0	5	5.19	3.71	3.06	6.42	7.93	7.62	—
	7	5.35	4.02	2.87	6.65	7.93	7.62	5.60
	10	5.16	3.53	2.88	6.17	7.99	7.69	6.19
	14	4.74	2.96	2.68	6.45	8.24	7.30	5.97
	Av.	5.11	3.56	2.87	6.42	8.02*	7.56*	5.92
10.0	5	5.70	3.88	2.92	6.33	7.95	6.72	—
	7	5.32	3.61	2.86	6.34	7.74	7.31	6.04
	10	5.59	3.52	2.98	6.20	8.22	6.93	5.97
	14	4.66	3.75	2.65	5.81	8.14	6.28	6.14
	Av.	5.13	3.69	2.85	6.17	8.01*	6.81**	6.05

* pH significantly higher than on control diet (P= <.05).
** pH significantly lower than on control diet (P= <.05).

and Bowen, 1968) that propylene glycol in broiler diets caused a peculiar leg abnormality characterized by an outward curling of the toes.

The objectives of this trial were to determine if the degree of leg abnormalities induced by feeding propylene glycol could be reduced by trace mineral supplementation. Noland (1966) stated that the addition of zinc to swine diets containing propylene glycol improved performance.

Five levels of propylene glycol were compared in the experiment (0, 2.5, 5.0, 7.5 and 10.0 percent). The composition of the diets containing 0, 5.0 and 10.0 percent is shown in Table 1. The other diets were obtained by blending appropriate amounts of these diets. These diets were fortified with a commercial trace mineral mix which provided 100 p.p.m. iron, 100 p.p.m. zinc, 100 p.p.m. manganese, 10 p.p.m. copper, 1 p.p.m. cobalt, and 3 p.p.m. iodine.

In addition to the trace minerals added to the basal diets, the diets were fortified with additional amounts of iron (0 to 100 p.p.m.), zinc (0 and 100 p.p.m.) and the commercial trace mineral mix (0 and twice normal amounts). The iron and zinc were both added in the sulfate form.

The combinations of these factors resulted in a 5×2×2×2 factorial arrangement of treatments for a total of 40 experimental diets. Eight hundred and forty day-old male broiler chicks were randomly divided into 120 lots of 7 chicks each. Each lot was assigned to a compartment in electrically heated battery brooders with raised wire floors. Each of the diets was fed to 3 lots of chicks.

At 28 days of age the experiment was terminated and the body weight gains and feed consumption determined. Observations were made of the incidence of toe and hock deformities. The data were subjected to the analysis of variance as outlined by Steel and Torrie (1965) with significant differences between treatment means determined by the multiple range test of Duncan (1955).

Results and Discussion. The chicks fed diets containing any of the levels of propylene glycol were significantly lighter in weight than those fed the control diet

TABLE 3.—*Effect of supplemental levels of iron, zinc, and a trace mineral mix added to diets containing different levels of propylene glycol on growth rate of broiler chicks (Experiment 5)*

Supplements[1]	Amount added (parts per million)								
Iron	0	100	100	100	0	100	0	0	
Zinc	0	0	0	100	100	100	0	100	
Trace mineral mix[2]	0	0	2,000	2,000	0	0	2,000	2,000	Average[3]
% Propylene glycol	28 day body weight (grams)								
0.0	451	472	454	477	462	471	480	464	466[a]
2.5	442	436	421	416	428	434	418	442	430[b]
5.0	407	431	422	397	421	454	388	399	415[b]
7.5	411	437	349	452	441	397	388	399	409[b]
10.0	350	337	389	386	353	322	375	391	363[c]
Average	412	423	407	426	421	416	410	419	

[1] In addition to the amounts supplied by the trace mineral mix in the basal diet (Table 1).
[2] Composition listed in Table 1.
[3] Means having the same superscripts do not differ significantly (P=0.05).

without propylene glycol over all levels of trace mineral supplementation (Table 3). There was some variation observed within the various levels of mineral supplementation; however, the only significant interaction which existed was expressed by an alteration in the linearity of the depression due to propylene glycol.

No significant differences were noted in feed consumption among any of the experimental treatments (Table 4). The reduced body weights along with the relatively constant feed intake resulted in significant increases in the feed:gain ratios of the diets containing 7.5 and 10.0 percent propylene glycol (Table 5).

As the level of propylene glycol in the diet increased, there was a significant linear increase in the number of deformed toes (Table 6). Chicks fed the diet with 2.5 percent propylene glycol did not differ significantly from those fed the control diet. However, those fed levels of 5.0 percent or more suffered a significantly greater number of deformed toes than those fed the control diet. The addition of the various individual trace minerals, alone or in combination, or the commer-

TABLE 4.—*Effect of supplemental levels of iron, zinc, and a trace mineral mix added to diets containing different levels of propylene glycol on feed consumption of broiler chicks*

Supplements[1]	Amount added (parts per million)								
Iron	0	100	100	100	0	100	0	0	
Zinc	0	0	0	100	100	100	0	100	
Trace mineral mix[2]	0	0	2,000	2,000	0	0	2,000	2,000	Average
% Propylene glycol	28 day feed consumption (grams)								
0.0	788	771	809	833	768	845	804	765	798
2.5	750	725	864	721	751	816	679	854	768
5.0	735	734	815	682	751	791	698	720	741
7.5	743	753	761	831	854	756	742	780	778
10.0	695	693	799	801	756	715	810	837	764
Average	738	735	810	774	776	785	747	791	

[1] In addition to the amounts supplied by the trace mineral mix in the basal diet (Table 1).
[2] Composition listed in Table 1.

TABLE 5.—*Effects of supplemental levels of iron, zinc, and a trace mineral mix added to diets containing different levels of propylene glycol on feed utilization of broiler chicks*

Supplements[1]	Amount added (parts per million)								
Iron	0	100	100	100	0	100	0	0	
Zinc	0	0	0	100	100	100	0	100	
Trace mineral mix[2]	0	0	2,000	2,000	0	0	2,000	2,000	Average[3]
% Propylene glycol	28 day feed per gain ratio								
0.0	1.75	1.63	1.78	1.75	1.70	1.79	1.68	1.65	1.71[a]
2.5	1.65	1.66	2.05	1.73	1.75	1.88	1.62	1.93	1.79[a]
5.0	1.81	1.70	1.93	1.72	1.78	1.74	1.80	1.80	1.78[a]
7.5	1.81	1.72	2.18	1.84	1.94	1.90	1.91	1.95	1.90[b]
10.0	1.99	2.06	2.05	2.08	2.14	2.22	2.16	2.14	2.10[b]
Average	1.79	1.74	1.99	1.82	1.84	1.89	1.82	1.89	

[1] In addition to the amounts supplied by the trace mineral mix in the basal diet (Table 1).
[2] Composition listed in Table 1.
[3] Means having the same superscript do not differ significantly (P=0.05).

cial trace mineral mix had no effect on the number of deformed toes.

There was a significant increase in the number of deformed hocks, similar to perosis, in chicks fed diets containing 7.5 or 10.0 percent propylene glycol. Again there were no beneficial response from any of the trace mineral supplements.

The results of this trial indicate that there is apparently no interaction or relationship between the levels of trace minerals considered in this study and the adverse effects of high levels of propylene glycol. Diets containing the propylene glycol induced hock and toe deformities in broiler chicks which indicates some defect in bone or cartilage metabolism.

SUMMARY

Feeding propylene glycol to broiler chicks resulted in an abnormal toe disorder, expressed by an outward curling

TABLE 6.—*Effects of supplemental levels of iron, zinc, and a trace mineral mix added to diets containing different levels of propylene glycol on leg abnormalities of broiler chicks*

Supplements[1]		Amount added (parts per million)								
Iron		0	100	100	100	0	100	0	0	
Zinc		0	0	0	100	100	100	0	100	
Trace mineral mix[2]		0	0	2,000	2,000	0	0	2,000	2,000	Total[3]
% Propylene glycol		Deformities[4]								
0.0	Toe	2/21	0/21	0/21	2/21	0/21	2/21	2/21	1/21	9/168[a]
	Hock	2/21	1/21	2/21	0/21	1/21	0/21	1/21	2/21	9/168[a]
2.5	Toe	3/21	1/21	3/21	1/21	3/21	3/21	1/21	4/21	19/168[ab]
	Hock	2/21	1/21	0/21	2/21	0/21	1/21	0/21	0/21	6/168[a]
5.0	Toe	1/21	5/21	4/21	7/21	4/21	5/21	3/21	1/21	29/168[bc]
	Hock	1/21	2/21	1/21	1/21	2/21	2/21	0/21	1/21	10/168[a]
7.5	Toe	5/21	7/21	1/21	6/21	5/21	5/21	5/21	5/21	39/168[c]
	Hock	1/21	1/21	1/21	5/21	4/21	5/21	1/21	1/21	18/168[b]
10.0	Toe	8/21	6/21	7/21	8/21	7/21	8/21	9/21	4/21	57/168[d]
	Hock	3/21	3/21	1/21	5/21	1/21	4/21	4/21	5/21	25/168[b]
Totals	Toe	19/105	19/105	15/105	24/105	18/105	23/105	20/105	15/105	
	Hock	9/105	8/105	5/105	13/105	7/105	11/105	6/105	9/105	

[1] In addition to the amounts supplied by the trace mineral mix in the basal diet (Table 1).
[2] Composition listed in Table 1.
[3] Figures having the same superscripts do not differ significantly (P=0.05).
[4] Indicates number of deformities out of total number of chicks.

of the toes. Studies were conducted to determine if propylene glycol might have some effect on nutrient absorption either by altering the pH of the gastrointestinal tract or by chelating some of the trace minerals. A level of 5 percent propylene glycol significantly increased the pH of the ileum and the large intestine. Chicks fed a diet with 10 percent propylene glycol had an increased ileum pH but a decreased pH in the large intestine, possibly due to increased bacterial fermentation.

The addition of increased levels of iron, zinc, or a commercial trace mineral premix to the diet had little effect on the occurence of propylene glycol induced leg abnormalities. Increasing the level of propylene glycol in the diet significantly increased the incidence of these leg disorders, suggesting some derangement of bone or cartilage metabolism.

ACKNOWLEDGMENTS

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THE TOXICITY OF PROPYLENE GLYCOL.*

BY HERBERT A. BRAUN AND GEORGE F. CARTLAND.

In recent years propylene glycol has assumed a position of somewhat increasing pharmaceutical interest as a vehicle and solvent. Seidenfeld and Hanzlik (1) have reported extensive toxicity studies in which propylene glycol is compared with glycerol and ethylene glycol. Hunt (2) has discussed the use of ethylene and propylene glycols as medicinal solvents and has shown that systemically propylene glycol is much less toxic than ethylene glycol.

We have compared the acute toxicities of propylene glycol and glycerol in rats by intramuscular, subcutaneous and intravenous injection and have obtained results which show satisfactory agreement with those obtained by Seidenfeld and Hanzlik (1). In addition, the acute and chronic toxicities of propylene glycol administered orally in rabbits have been studied.

The propylene glycol used in these studies is the Alpha Propylene Glycol (1, 2 Propane Diol).

Acute Toxicity.—Propylene glycol was injected in undiluted form intramuscularly and subcutaneously in rats. Parallel groups of rats were similarly injected with undiluted glycerol. With the large doses administered to establish the minimum fatal dose, all of the rats showed profound depression, analgesia and coma. With sub-lethal doses, the toxic symptoms persisted for a longer time in the glycerol-injected animals than in those injected with propylene glycol. In the larger doses, glycerol produced greater local tissue damage than was observed with propylene glycol in corresponding percentages of the M. L. D. The results of these experiments given in Table I show that propylene glycol is much less

* From the Research Laboratories, The Upjohn Company, Kalamazoo, Michigan.

toxic than glycerol. Intramuscularly, the M. L. D. for propylene glycol is 15.7 Gm. per Kg.; for glycerol, 7.6 Gm. per Kg. Subcutaneously, the M. L. D. for propylene glycol is 23.1 Gm. per Kg.; for glycerol, 15.1 Gm. per Kg.

The acture oral toxicity was determined, in rabbits, by administering a 20 per cent aqueous propylene glycol solution by stomach tube giving divided doses over a period of one hour. The toxic symptoms consist of increased respiratory rate, loss of equilibrium, profound depression, analgesia and coma. Death occurred in from 18 to 36 hours. The results given in Table I indicate that the M. L. D. of propylene glycol orally in rabbits is 20 Gm. per Kg.

TABLE I.—ACUTE TOXICITY OF PROPYLENE GLYCOL AND GLYCEROL IN RATS AND RABBITS.

Propylene Glycol. Dose per Kg. Cc.	Gm.	No. of Animals Used.	Per Cent Mortality.	Glycerol. Dose per Kg. Cc.	Gm.	No. of Animals Used.	Per Cent Mortality.
			Intramuscular Injections in Rats.				
12	12.60	3	0	4	5.04	5	0
13	13.65	3	0	6	7.56	5	60
14	14.70	3	0	8	10.08	4	100
15	15.75	5	60	10	12.60	3	100
16	16.80	5	80	12	15.12	3	100
			Subcutaneous Injections in Rats.				
16	16.80	2	0	8	10.08	3	0
18	18.90	3	0	12	15.12	5	60
20	21.00	5	20	14	17.64	3	100
22	23.10	5	60	16	20.16	3	100
24	25.20	5	80	18	22.68	3	100
				20	25.20	2	100
			Oral Administration in Rabbits.				
15	15.75	2	0				
18	18.90	9	33				
19	19.95	7	86				
20	21.00	4	100				

The intravenous injection of propylene glycol in 20 rats indicated a minimum fatal dose of 18 cc. (18.90 Gm.) per Kg. This value shows satisfactory agreement with that given by Seidenfeld and Hanzlik of 16 cc. (16.8 Gm.) per Kg. intravenously in rats. The intravenous injection of undiluted propylene glycol produced rapid obliteration of the veins making administration very difficult. At doses of 16 to 19 cc. per Kg. intravenously, all the rats showed marked muscular tremors and later became comatose. Some of these symptoms may be due simply to the hypertoxicity of the undiluted propylene glycol.

Since the use of undiluted glycerin intravenously proved impracticable, a 50 per cent dilution was used. A series of 24 rats indicated that the minimum fatal dose for glycerol at this dilution was 6 cc. (7.56 Gm.).

Chronic Toxicity.—The toxicity of propylene glycol was studied by chronic oral administration in rabbits. Daily doses of 1 to 8 cc. (1.05 to 8.40 Gm.) per Kg. were administered by stomach tube as a 20 per cent aqueous solution of propylene glycol. The animals were weighed daily and the average body weights at successive 10-day periods are given in Table II. The experiment was continued for 50 days,

at the end of which time the animals were killed by the intravenous injection of magnesium sulfate. Comparison of the experimental animals with their controls showed no gross pathology which could be ascribed to the drug.

TABLE II.—CHRONIC ORAL TOXICITY OF PROPYLENE GLYCOL IN RABBITS.

Rabbit Number.	Dose per Kg. Daily. Cc.	Gm.	Initial Body Weight. Kg.	Average Body Weight at Successive 10-Day Periods. 1–10. Kg.	11–20. Kg.	21–30. Kg.	31–40. Kg.	41–50. Kg.
1	1	1.05	1.59	1.59	1.61	1.64	1.70	1.65
3	2	2.10	1.70	2.15	2.52	2.66	2.75	2.77
4	2	2.10	1.70	1.92	2.30	2.53	2.65	2.68
6	3	3.15	1.76	1.81	2.15	2.26	2.41	2.34
25	4	4.20	2.32	2.36	2.48	2.56	2.58	2.57
26	4	4.20	2.53	2.56	2.55	2.59	2.58	2.66
39	4	4.20	1.72	1.78	1.87	1.86	2.05	2.21
17	4	4.20	2.42	2.46	2.47	2.48	2.52	2.58
27	8	8.40	1.70	1.74	1.83	1.92	1.94	2.01
28	8	8.40	2.59	2.52	2.48	2.51	2.47	2.51
38	8	8.40	2.36	2.31	2.23	2.11	2.26	2.36
2	Control		1.81	1.88	1.86	1.88	1.89	1.83
11	"		1.81	1.89	2.13	2.45	2.64	2.75
7	"		1.81	2.00	2.40	2.59	2.69	2.68
5	"		1.70	1.92	2.30	2.20	2.69	2.70
24	"		1.98	2.12	2.31	2.45	2.60	2.79
55	"		1.94	2.04	2.06	2.00	2.00	2.07
50	"		2.00	2.16	2.17	2.24	2.32	2.54

The absence of any significant weight loss at the highest dosage and the satisfactory growth observed at the lower dosages indicate that daily doses of 4 to 8 cc. per Kg. are tolerated by the rabbit for a period of 50 days without any toxic symptoms other than slight anorexia. The highest dosage of 8 cc. per kilo represents the daily administration of 40 per cent of the fatal dose with no demonstrable cumulative effects. This lack of cumulative action in rabbits with a high percentage of the fatal dose of propylene glycol administered daily over a long period of time agrees with similar observations made in rats by Seidenfeld and Hanzlik (1) in which propylene glycol was added to the drinking water.

Local Irritation.—The low systemic toxicity observed for propylene glycol would indicate it to be a solvent of choice for certain medicinal preparations. It is an excellent solvent for a number of substances which are now administered in oil solution. However, the local pain caused by the injection of undiluted propylene glycol constitutes a serious handicap to its use in hypodermic preparations. Experiments on human subjects in our laboratories have shown that the subcutaneous injection of as little as 0.1 cc. of undiluted propylene glycol produces a very marked local burning sensation which, however, disappears in 5 to 10 minutes. When injected intramuscularly the local irritation appears to be no greater than that produced by oil.

SUMMARY.

The acute toxicity of propylene glycol has been compared to that of glycerol by intramuscular and subcutaneous injections in rats. The acute and chronic oral toxicity of propylene glycol has been studied in rabbits.

The M. L. D. for propylene glycol intramuscularly and subcutaneously in rats is 15.7 and 23.1 Gm. per Kg., respectively, as compared to corresponding values of 7.6 and 15.1 Gm. per Kg., respectively, for glycerol.

In rabbits, the acutely fatal dose of propylene glycol by oral administration is 20 Gm. per Kg. Daily oral doses up to 8 cc. per Kg. administered for 50 days are tolerated by rabbits with no observed cumulative effects.

The low systemic toxicity of propylene glycol would recommend it as a solvent for certain medicinals. However, the severe, although transient, local irritation produced by its subcutaneous injection would appear to preclude its use in hypodermic preparations in undiluted form.

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From: Pharmacology Laboratory Dr. Recordati - Coreggio
Director: Dr. G. Recordati

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Prof. Dr. Giulio BUCCIARDI
Director, Section for Biology

The glycols in therapy

Concise experimental memorandum.

The news that in the United States of America numerous deaths occurred at the end of 1937 among persons who had used the Sulfamide Elixir made by S.E. MASSENGILL of Bristol (Tenn.Val), was extensively discussed.

It results from the report of the Secretary of Agriculture, submitted to the Senate on November 16,1937 and to the House of Representatives in October 1937 that 73 cases of death were certainly due to the use of said Sulfamide Elixir, and 20 additional deceases can probably be attributed to the same fatal Elixir (1).

The sad consequences of the Massengill Elixir use were subject to large-scale publicity, necessary during a first period to prevent other possible victims from increasing the list of the deceased. However, new reasons emerged occasionally later , prompting the intensification of said publicity which developed into a real campaign against the diethylene glycols. The latter were in fact considered to be the principal cause of the serious toxic phenomena, since the fatal Elixir contained a considerable amount of diethylene glycol, as found likewise during the analysis carried out by SCHOFFEL and others from the A.M.A. Chamical Laboratory (2).

Following said publicity campaign, praiseworthy in view of its other humanitarian aims, the toxic properties of the diethylene glycol were of course exaggerated. Understandably, a state of alarm resulted also concerning substances such as for example, the ethylene glycol and the propylene glycol, much used as medicament vehicles; they are related to diethylene glycol in nomenclature, and regarding properties as well as characteristics.

Since the glycols had for a prolonged period of time been used in this Laboratory as vehicles or as solvents of some substances, it does not seem out of context to me at this time to supply the press with the protocols of the tests carried out to establish those conditions under which the introduction of the glycols for therapeutic use is admissible.

However, in view of the fact that the glycols are not among the best-known substances, I conisider appropriate to disclose first some notions concerning the chemical-physical properties and characteristics of certain glycols.

Chemical-physical records.

The glycols (diols, alcandiols) are bivalent alcohols, with the two alcoholic hydroxides bound to two different carbon atoms. The two alcoholic functions can be primary, secondary or tertiary, and depending on the second of the two alcoholic functions, the glycols are specified as (α -) o 1,2, (β -) o 1,3, (γ -) 1,4,etc. (3)

The first known term of the bihydroxide alcohols in the bicarbonate series is the standard glycol CH_2OH-CH_2OH; from its definition all those belonging to the subsequent sries are derived (tricarbonic, tetracarbonic,etc.). Starting from the tricarbonic series, numerous cases of isomerism are found which depend on the position held by the hydroxides relatively to various carbon atoms in the molecule.

By now numerous glycols are known and are prepared in the laboratory. Some time ago, the industrical manufacturing of certain glycols has been started on a large scale; the ethers, esters, diesters etc. of some are known and used. Numerous dissertations report tables classifying the glycols and indicate their principal characteristics (4).

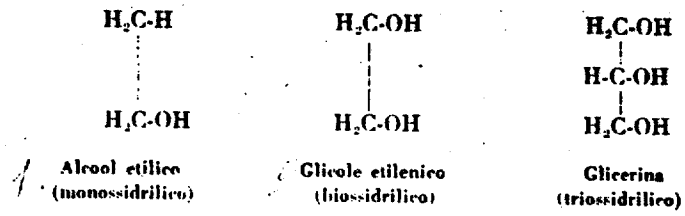
For the purposes of this work, however, only the glycols of the first terms are of interest, which, on account of their characteristics (liquid condition at ambient temperature)and their properties (strong solvent capacity) are already widely used in pharmaceutical industry.

Ethylene glycol.

The ethylene glycol (1,2 dihydroxide ethane, Ethanediol, or simply glycol), discovered by WURTZ, is a homologue of glycerine , obtainable in various ways (5). Its synthetic production is considerable because said substance (as well as its ethers and esters) is used for various industrial purposes (as solvent, for explosives, as anti-freeze liquid of radiators, etc.).

At ambient temperature, the glycol appears as a rather thick liquid; it is colourless and odorless; its taste is slightly sweetish; it is non-flammable.

Some properties and characteristics of the glycols are intermediate between those of the alcohol and the glycerine.



1) Ethyl alcohol (monohydroxide) 2) Propylene glycol (bihydroxide) 3) Glycerine (trihydroxide)

<u>Density</u>. - Intermediate between the density of water and glycerine (1,26), but there are minor differences between the reported values of the various AA. A 0°C has a density of 1,125 (WURTZ(6)), 1,1297 (DE FORCHAND(7)), 1,1260 (SCHWERS(8)), 1,1274 (WALDEN(9)); at a temperature of 15°, it has a density of 1,11757 according to TAYLOR and RINKENBACH (10); of 1,11068 at 20° according to RUBER, SRENSEN and THORKELSEN (11); of 1,1110 according to DUNSTAN (12) and of 1,098 according to WALDEN(9) at 25°C; of 1,0919 at 50°C according to WALDEN (9).

The density value of the glycols is influenced by its more or less high degree of purity, depending principally on the preparation method, the purification systems and also depending on the eventual water content (very easy, given the strong hygroscopicity of the glycol).

Table 1, published by LAWRIE (13) shows the summarized aspect of density variations of the glycol as a function of temperature; for said Table, the specific weight was computed on the basis of a temperature of 4°C.

TABLE 1 - Specific weight of glycol as a function of temperature.

TABELLA 1 — Peso specifico del glicole in funzione della temperatura

Temperatura (T) °C	Peso specifico T°/°C	Temperatura (T) °C	Peso specifico T°/4 °C	Temperatura (T) °C	peso specifico T°/4 °C
0,0	1,12700	70,0	1,07585	140,0	1,02063
5,0	1,12326	75,0	1,07198	145,0	1,01668
10,0	1,12019	80,0	1,06803	150,0	1,01273
15,0	1,11665	85,0	1,06408	155,0	1,00878
20,0	1,11320	90,0	1,06015	160,0	1,00483
25,0	1,10970	95,0	1,05618	165,0	1,00088
30,0	1,10601	100,0	1,05223	170,0	0,99693
35,0	1,10246	105,0	1,04828	175,0	0,99298
40,0	1,09883	110,0	1,04433	180,0	0,98003
45,0	1,09513	115,0	1,04088	185,0	0,98508
50,0	1,09137	120,0	1,03643	190,0	0,98113
55,0	1,08757	125,0	1,03248	195,0	0,97718
60,0	1,08363	130,0	1,02853	197,5	0,97520
65,0	1,079675	135,0	1,02458	—	—

Fusion point. - For BOUCHARD (14) and DE FORCHAND (7) the glycol solidifies between -13°and -25°C; for LADENBURG and KRUGEL (15) it settles at -17°C; for PARKS and KELLEY (16) is settles at -12°C; for TAYLOR and RINKENBACH it settles between -17°, 4 and -11°, 5.

Steam pressure. - According to LOUGUININE (18) the boiling point is lowered by 0°,048 C for each millimetre of Hg baromatric pressure reduction. Table 2 reports the variations of steam pressure for glycol at various temperatures, as found by TAYLOR and RINKENBACH (10).

TABLE 2.- Steam pressure of the glycol at various temperatures.

TABELLA 2 — Pressione di vapore del glicole a varie temperature

Temperatura °C	Pressione di vapore mm Hg	Temperatura °C	Pressione di vapore mm Hg
105	27	155	183
110	30	160	220
115	34	165	263
120	42	170	313
125	53	175	369
130	68	180	434
135	84	185	514
140	103	190	605
145	125	195	708
150	152	197,2	760

The values reported on Table 2 come very close to those obtained by DE FORCHAND (19) during a series of precise research concerning the steam pressure of glycol at various temperatures.

Boiling point.- According to WURTZ (6) the glycol boils between 197° and 197,°5 C, at 765,5 mm. of Hg pressure. For LOUGUININE (18), it boils between 197°,03 and 197°,37 C; for FORCHAND (19) at 197°C, and for TAYLOR and RINKENBACH, at 197°,2, with a Hg pressure of 760 mm.

Refraction index. - Table 3 shows the values reported by EYKMAN (20) for the refraction index of glycol at temperatures of 19°,3 and 138°,8 C (lines A and B of the hydrogen and atmosphere A).

TABLE 3.- Refraction index of the glycol.

TABELLA 3 - - Indice di refrazione del glicole

°C	Indice di refrazione (corr. al vuoto) D	T° Vol. 4° Mol.	H_B	H_a	A	Refrazione molecolare (n—1) VM H_B	H_a	A
19,3	1,1134	55,68	1,43828	1.43087	1.42209	24,41	24,00	23,50
138,3	1,0230	60,61	1,60063	1,39406	1,38606	24,28	23.88	23,40

1) Refraction index (corresponds to vacuum)
2) Molecular refraction . .

RUBER, SÖRENSEN and THORKELSEN (11) determined the molecular refraction on the D line of the sodium; they found, for M_g and M_l respectively, the values of 24,03 and 14,19, at a temperature of 20°C, with a density of 1,113068 at 20/40°C, corresponding to values of 1,4311 and 1,4331.

Viscosity. - The glycol is more viscous than water and less so than glycerine. For DUNSTAN (12) n = 0,1733 at 23°C. For PRIBRAM and HANDL (21) the specific viscosity of the glycols is 350 at 55°C and 300 at 60°C.

Solubility. -The glycol mixes to any ratio: with water, glycerine, alcohol, ethyl, methyl and amyl , acetone, frigid acetic acid, piridine. It does not mix with chloroform, benzol, xilol, toluol ,tetrachloride of carbon, sulfur of carbon, petroleum ether. In particular, it is insoluble in ether. KNORR (22) however admits that 100 parts of ethyl ether dissolve 1,1 parts of glycol.

The glycol is strongly hygroscopic. As a matter of course, its watery mixtures have a boiling point which is proportionately lower as the water content increases.

The above is demonstrated in detail by the graphs of Figure 1 (reported by LAWRIE) which indicate the boiling points of glycol-water mixtures as a function of the glycol content percentage.

Figures 1 and 2. :

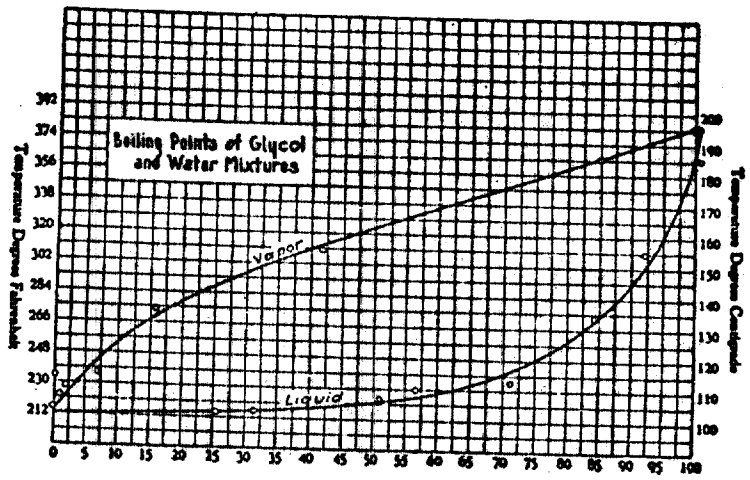


Fig. 1 - (da Lawrie)

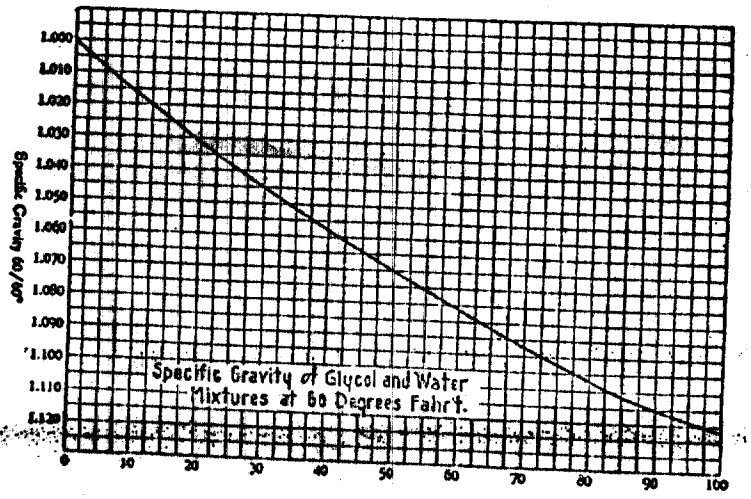


Fig. 2 - da Lawrie)

The variations of the specific weight for glycol-water mixtures as a function of the glycol percentage variations in the mixture are shown on Fig. 2 (also reported by LAWRIE), in which the curve was deduced from the results of observations made at a temperature of 15°,56 C.

The glycol is a valuable solvent because it shares the properties of water and alcohol ; it is in fact able to dissolve. fair quantities of several salts, in particular the chlorides and also the potassium carbonate, cuastic potassium, calciums, etc.

Chemical behavior. - Depending on conditions, the glycol,with oxidants, transforms into glycolic acid and oxalic acid (23). With caustic potassium when cold,it decomposes almost completely into oxalic acid and hydrogen. With potassium lye and bioxide of lead when hot, it yields hydrogen and formic acid (24).

With chlorine it forms various chloride compounds. With hydrochloric and bromochloric acids it yields the corresponding ethers; with hydroiodine acid, it is reduced to ethylene iodide. When heated with much water,at 200 to 210°C it yields aldehydes (25). With sulfuric acid, it yields glycosulfuric acid. With zinc chloride, it dissolves into water, aldehyde and crotonaldehyde at 250°C. (26).

Through the glycolysis of glycols to which sulfuric acid was added, formic acid , glycolic acid and trioxymethilene are formed. When heated with ammonia chloride in dry condition, it generates collidine at 190°. It absorbs the vapours of cyanic acid and yields the allophanous ether of the glycol (27).

With sodium, it develops hydrogen and generates monosodium glycol, which-- through fusion with excess sodium at190°C--is transformed into bisodic glycol. From these sodic glycols,several compounds specified by DE DORCHARD (28) are obtained with the aid of various alcohols.

When treated with nitric acid and sulfuric acid (in various proportions at at various temperatures according to the methods of HENRI (29) , CHAMPION (30), NEF (31) and others (32) dinitrate of glycol forms; it has a high explosite potential, almost similar to the explosive value of nitro-lycerine. Its physical-chemical characteristics and properties are described in detail by RINKENBACH (33) and others (34).

Ethers derived from glycol. - The monoalkylic ethers are of a certain importance among the glycol derivates on account of their optimal solvent quality. The characteristics of some monoalkylic ethers are shown on Table 3..

TABLE 4. -Boiling point, specific weight and refraction index of some ethers of the ethylene glycols.

TABELLA 4 — Punto di ebollizione, peso specifico ed indice di refrazione di alcuni eteri del glicol etilenico

Eteri monoalchilici 1	2 Punto di ebollizione °C (743) mm di Kg)	3 Peso specifico 15°/15°C Glicol etilenico	4 Indice di refrazione (n_g) a 20 °C
5 Metilico	124,5	0,9748	1,4004
6 Etilico	134,8	0,9360	1,4042
7 Isopropilico	144 0	0,9139	1,4080
8 n-Propilico	150,0	0,9110	1,4125
9 Isobutilico	158,8	0,9130	1,4135
10 n Butilico	170,6	0,9188	1,4177
11 Isoamilico	181,0	0,9000	1,4190

1) Monoalkylic ethers
2) Boiling point . .
3) Specific weight $15^{0}/15^{0}C$ Ethylene glycol
4) Refraction index . . .
5) methyl (ether)
6) ethyl
7) Isopropyl
8) n-propyl
9) isobutyl
10) n butyl
11) isoamyl

All these monalkylic ethers are colorless, fluid and almost odorless. They are soluble in organic solvents and they dissolve very well in water, where however their solubility decreases proportionately to the increase of the molecular weight of the alkylic group.

The boiling point likewise rises simultaneously with the increasing weight of the alkylic group, while on the other hand the specific weight and the refraction index decrease, as evident from the values shown on Table 4.

Simple and mixed di-ethers of the glycole have also been prepared, such as for example: di-methyl, di-ethyl, do-propyl of the di-butyl, the methyl-propyl, the ethyl-propyl, the methyl-butyl, the ethylbutyl, etc.

The elimination of a water molecule from a glycol molecule (or of a single glycol molecule during the formation of the polyglycols) results in the formation of cyclic ethers, usually defined as oxides, the first term of which is represented by the ethylene oxide $H_2C \diagdown O \diagup CH_2$, having a boiling point at 10°,5C.

The elimination of a water molecule from two glycol molecules results in the formation of a polyglycol according to the following diagram:

$$\underset{\text{glicole}}{CH_2CH - CH_2O} \boxed{H + HO} \underset{\text{glicole}}{CH_2 - CH_2OH} = \underset{\text{glicole dietilenico}}{CH_2OH - CH_2 - O - CH_2 - CH_2OH} + H_2O$$

The elimination of a water molecule in the union between an ethylene glycol molecule and a diethylene glycol molecule results in the formation of a triethylene glycol molecule:

$$\underset{\text{glicole dietilenico}}{CH_2OH - CH_2 - O - CH_2O} \boxed{H + HO} \underset{\text{glicole}}{CH_2 \; CH_2OH} = \underset{\text{glicole trietilenico}}{CH_2OH - CH_2 \; O \; CH_2 - CH_2 \; O - CH_2 - CH_2OH} + H_2O$$

This extensive reaction finally led to the formation of decaethylene glycol. In addition, mixed polyglycols were prepared (for example: from the union of a methylene glycol molecule with a propylene glycol molecule and elimination of one water molecule). Only the diethylene glycol is of interest for the purposes of our study among said polyglycols which are widely used in industry as solvents, in particular for the production of synthetics such as card film and celluloid for cigarette paper.

Diethylene glycol.

It results, as stated, from the union of two ethylene glycol molecules, and the elimination of a water molecule . It can be prepared with various methods (35) and is today obtained on a large scale as the by-product of the ethylene glycol manufacturing.

The diethylene glycol is a colorless and odorless liquid, with a sweet taste, slightly irritating.

Density. - According to WURTZ (35) its density is = 1,132 at 0°C. Density variations as a function of the temperature were determined by RINKENBACH (36) and are reported in Table 5.

TABLE 5. - Density of the diethylene glycol at various temperatures.

TABELLA 5 - Densità del glicol dietilenico a varie temperature

Temperature °C	Specific Weight Peso specifico 15 °C	Temperature °C	Peso specifico 15 °C
0,0	1,1318	20,0	1,1177
1,0	1,1283	25,0	1,1141
10,0	1,1248	30,0	1,1106
15,0	1,1212	35,0	1,1071

Steam pressure. - The values (of LAWRIE) are shown on Table 5.

TABLE 6. - Steam pressure of the diethylene glycols.

TABELLA 6 — Pressione di vapore del glicol dietilenico

Temperature °C	Pressione di vapore mm Hg	Temperature °C	Pressione di vapore mm Hg
130,0	8	185,0	116
135,0	12	190,0	135
140,0	16	195,0	180
145,0	21	205,0	228
148,5	25	210,0	268
150,0	27	215,0	311
155,0	34	220,0	370
160,0	43	222,0	430
165,0	54	230,0	499
170,0	66	235 0	577
175,0	80	240,0	669
180,0	96	245,0	760

Boiling point. - According to WURTZ (35) the diethylene glycol boils at 250°C, according to RINKENBACH (36) at 244°,5 C, according to LAWRIE (13) at 245°, with a Hg pressure of 760 mm.

Solubility. -At normal temperature, the diethylene glycol is mixable to any proportion with water, methyl alcohol, ethyl alcohol, ethylene glycol, frigid acetic acid, acetone, piridine, diacetate of glycol, chlororform and aniline. It does not mix with ethylene ether, benzole, toluol, disulphide and terachloride of carbon.

It is flammable at 130°C and burns with a bluish flame. It is strongly hygroscopic.

The monoethyl ether is the most important among the monoalkylic ethers derived from ethylene glycol. Its following chemo-physical characteristics are well-known: boiling point at 198°C (Hg: 760 mm); refraction index (r_p) = 1,4240; specific weight + 0,9996 at 15°C according to LAWRIE, = 1,033 at 15°,5 C according to BROWN (37).

Propylene glycol.

The propylene glycol (1,2- propanediol) appears at normal temperature as a very dense liquid, colorless and almost odorless. Its formula is as follows: ($CH_3CH(OH).CH_2OH$).

It is easily obtainable by synthetic means (38), but its production at this time is much more restricted than the production of the ethylene glycol, since it is little used for industrial purposes.

Boiling point. - At 188°C (at a Hg pressure of 760 mm) according to BROWN (37), between 188 and 189°C according to others (38).

Density. - = 1,040 (at 20°C) according to BROWN; = 1,051 at 0°C according to others (38).

Solubility. - At normal temperature the propylene glycol is mixable to any ratio with water, acetone, chloroform and ethyl alcohol.

The adding of water to propylene glycol - acetone and propylene glycol - ethyl alcohol mixtures does not result in any separation. The adding of water to chloroform - propylene glycol mixtures causes the separation of two components; the speration of two layers results; the top layer of these contains the added water and the propylene glycol, while the bottom layer contains the chloroform.

The propylene glycol is partially soluble in ether: 1 volume of propylene glycol in 39 volumes of ethyl ether according to BROWN (37), 1 volume of glycol in 12 to 13 volumes of ether according to THORPE (39). The adding of water results in the complete senaration of the propylene glycol from the ether; the added water and the glycol form a lower layer, while the the ether forms a top layer.

The propylene glycol is not mixable with petroleum ether.

Ethers of the propylene glycol. - Alkylic ethers can likewise be obtained from the propylene glycol. The most important characteristics of the foremost among them, i.e. of some monoalkylic ethers, are shown on Table 7.

TABLE 7. - Boiling point, density and refraction index of some monoalkylic ethers of the propylene glycol.
(For translation see Table 4), p. 8 of translation)

TABELLA 7 — Punto di ebollizione, densità ed indice di refrazione di alcuni eteri monoalchilici del glicol propilenico

Eteri monoalchilici	Punto di ebollizione a 736 mm di Hg	Peso specifico a 15 °C	Indice di refrazione (n_D) a 20 °C
Metilico	122,8	0,9335	1,4035
Etilico	133,0	0,9090	1,4061
Isopropilico	141,5	0,8900	1,4062
n—Propilico	150,0	0,8970	1,4102
Isobutilico	161,3	0,8810	1,4112
n—Butilico	170,6	0,8990	1,4151
Isoamilico	183,3	0,8835	1,4182

Several alkaloids find optimal solubility conditions in the propylene glycol, and so do several organic and inorganic coloring substances, stable and volatile oils, tinctures,etc. In a specific monograph, BROWN (37 supplies the solibility terms in propylene glycol for numerous substances which are of interest for medicine and therapy.

It is to be pointed out that the adding of glycol to saturated watery saline solutions results in the separation of the salts in the form of crystals.

Pharmacological records.

BACHEM (40) proposed in 1916 the use of glycol as medicinal solvent and vehicle. In the following year MENDEL (41), resuming the research work of BACHEM, obtained identical results which made it foreseeable that the use of the glycols in therapy could provide optimal results.

However, 20 years passed before the question concerning the use of glycols in pharmaceutical preparations was raised once more by PAGE (42) and HAAG and BOND (43). Since that time, researchers focussed their attention on the argument; numerous investigations followed, in particular regarding the ethylene glycol and the propylene glycol. Results obtained by almost all observers indicateed that the toxicity of the ethylene glycol is quite minimal (40,42,43,44,45), while some voiced contrasting opinions on the point at issue (45,47,48).

It is principally the merit of HANZLIK and collaborators (49) to have precisely determined the physiological properties of the ethylene glycol by comparing its effect to the effect of other solvents in the course of a brilliant research series; they determined its toxicity grade with precision and started the preparation of medicinal specialties, using it as solvent and vehicle.

HANZLIK stressed that the toxicity of glycol is extremely low and that it is very well tolerated by the animal organism in high doses; he pointed out that the continued administration of high glycol doses does not cause noteworthy disorders, except for a condition of conjunctive irritation (conjunctivitis). He disproved the conclusions reached by HUNT(48) which attribute a toxic effect to the glycol, on the basis of an oxalaria (ossularia) during chronic intoxication conditions

According to HUNT, the <u>oxalaria</u> was allegedly due to the formation of oxalic acid through a series of consecutive glycol oxidations according to the following diagram:

$$\begin{array}{c} CH_2OH \\ | \\ CH_2OH \end{array} \rightarrow \begin{array}{c} COHH \\ | \\ CH_2OH \end{array} \rightarrow \begin{array}{c} COOH \\ | \\ CH_2OH \end{array} \rightarrow \begin{array}{c} COOH \\ | \\ CH(OH)_2 \end{array} \rightarrow \begin{array}{c} COOH \\ | \\ COH \end{array} \rightarrow \begin{array}{c} COOH \\ | \\ COOH \end{array}$$

However, to obtain said <u>oxalaria</u> , it would be necessary to administer for a prolonged period of time very high glycol doses, enormously in excess of doses used for therapeuthic purposes according to HANZLIK, MEHRTENS, GURGOT, JOHNSON (49) and DILLE (50).

With tests for comparing ethylene glycol to propylene glycol, SEIDENFELD and HANZLIK (49) confirmed the statement of HUNT (48) according to whom the propylene glycol is even less toxic than the ethylene glycol. They recommend, however, that ethylene glycol be used for pharmaceutical preparations, in view of its advantage as compared to the propylene glycol: the ethylene glycol has a much less strong locally irritating effect (subcutaneously and intramuscularily) than does the propylene glycol.

The question of the irritating effect of the glycols was the subject of a study implemented by OETTINGEN and JIRONCH (45) who carried out tests with glycol and some of its derivatives (diethylene glycol, ethyl-diethylene glycol, ethyl- ethylene glycol, acetate of ethyl-ethylene glycol, butyl-ethylene glycol, (1-4) dioxane). They found that the locally irritating effect of the derivatives is more pronounced than the effect due to glycol; furthermore, they observed a major toxicity in the derivatives. The toxic effect was demonstrated by muscular and nervous depression and by a hemolytic effect (endovenously).

The intensity of the depressing effect seems parallel to the precipitating action of the proteins, the oil-water separation coefficient, the chemical stability and in particular to more or less resistance against oxidizers .

LEMATTE, BOINOT and KAHANE (51) stated that the metoxyethanol, the etoxyethanol, the diethylene glycol, the monoformic, the monoacetate, the monopropionate, the monochlorineacetate of the glycol have a definitely less locally irritating effect as compared to the irritating effect of alcohol, ether, chloroform, benzole and glycerine; but it is still stronger than the irritating effect of the ethylene glycol.

On the whole, the results of various authors confirm that the ethylene glycol rarely acts as a local irritant, insofar as it can be injected undiluted, while its derivatives and the propylene glycol must be diluted: otherwise, their toleration encounters difficulties.

Another fact which interested the pahmacologists is a transient hypertensive phenomenon which follows the endovenous administration of glycols (45). But the effect becomes evident on the cardio-circulatory apparatus only with high doses, or with doses much in excess of the customary therapeutic doses; it was not believed therefore that the above fact could advise against the use of the glycol. On the other hand, the use of glycol is indicated on the basis of some of its important properties, such as: a marked antibacterial and antiseptic action, an antifermentation effect (it slightly inhibits the activity of amigdaline (tonsilline?)). I understood that these were the best-known pharmacological facts when I started my experimental research concerning the glycols.

Experimental research.

My reseach related to the pharmacology and toxicity of the glycols originated in 1935 at which time Dr. RECORDATI appointed me to test on animals the first preparations of Bismjol (iodinebismuth of sodium and iodide of sodium, which was subject to study in his Laboratory.

My task required me to establish which was the most suitable solvent from the pharmaceutical viewpoint among those suggested by HANZLIK for bismuth soluble in the form of anion (49).

The aim of my research was the determination of the toxicity rate and pharmacological action of the various preparations (with specific regard to the absorption and elimination time), as well as of tollerability as a consequence of the more or less strong locally irritating effect (intramuscular)in case of a specific solvent (*).

(*) The tests were started in the Physiology Institute of the Modena University where I was assistant; they were continued at Ferrara in the Physiology and Biological Chemistry Institute, of which I was Director in charge; they were terminated in this Pharmacological Laboratory.

I restrict myself here to report the experimental part only from the study of glycols as solvents, and specifically the study of the ethylene glycol, the propylene glycol, the diethylene glycol and the ethylic ether of the ethylene glycol; these substances were taken into consideration by me, and I carried out separate research work for each.

Toxicity research.

Initially, I carried out tests concerning the toxicity of the glycols, investigating:

1) the minimal lethal dose, intramuscularly and endovenously administered to rats and orally, intramuscularly and endovenously to rabbits, given in a single dose (acute toxicity research);

2) the eventual detrimental manifestations caused by a prolonged administration of sub-lethal doses to rats (chronic intoxication research).

1). I was able to utilize a large number of animals for the testing of acute toxicity in rats, since large numbers were available from our breeding establishment. Male rats were exclusively used for the tests because the females were needed for other purposes (biological control of the estrogen action of Estrofol and Estrofollicoline in castrated rats).

I first carried out orientation research with rats; I injected into each member of a group of animals a different dose of the substance under investigation and I observed which doses resulted in the survival of the animals, and which proved to be lethal.

After having with the above method obtained an approximate notion of the lethal dose per kilogram of body weight, I carried out more accurate tests concerning minimal lethal doses of each glycol per kilogram of rat weight.

For the purpose of subdividing the test animals into various groups (of 5 rats each) I selected subjects of the same age ,having an almost identical weight (60 days old, weight 80 to 90 g). All animals of each groups received uniform diet during the period preceding the tests, and food was withdrawn from them 12 hours prior to the start of the tests; however, they were given water ad libitum.

All animals, weighed and placed into separate cages for each group, were kept under identical conditions during the testing period. The same dose of the substance under investigation (dose per kg of weight) was administered to each animal within a group, and different doses were given to animals in the various groups. Using this method, I researched the toxicity of the four substances under examination, administered by intramuscular and endovenous injection, (the latter into the tail vein) to young male rats.

The results of each test are shown on Tables 8,9,10 and 11.

TABELLA 8 - Tossicità del glicol etilenico nei ratti

1 Iniezione intramuscolare				2 Iniezione endovenosa			
N. ratti 3	Glicole cc. Kg. 4	N. morti 5	Mortalità % 6	N. ratti 3	Glicole cc./Kg. 4	N. morti 5	Mortalità % 6
5	4	0	0	5	4	0	0
5	5	0	0	5	5	1	20
5	6	0	0	5	6	1	20
5	7	2	40	5	7	3	60
5	8	2	40	5	8	5	100
5	9	4	80	5	9	5	100
5	10	5	100				

TABELLA 9 — Tossicità del glicol propilenico nei ratti

Iniezione intramuscolare				Iniezione endovenosa			
N. ratti	Glicole cc. Kg.	N. morti	Mortalità %	N. ratti	Glicole cc./Kg.	N. morti	Mortalità %
5	4	0	0	5	4	0	0
5	5	0	0	5	5	0	0
5	6	0	0	5	6	0	0
5	7	1	20	5	7	2	40
5	8	1	20	5	8	4	80
5	9	4	80	5	9	5	100
5	10	4	80	5	10	5	100

TABELLA 10 — Tossicità del glicol dietilenico nel ratto

Iniezione intramuscolare				Iniezione endovenosa			
N. ratti	Glicole cc. Kg.	N. morti	Mortalità %	N. ratti	Glicole cc. Kg.	N. morti	Mortalità %
5	2	0	0	5	2	2	1
5	3	1	20	5	3	3	3
5	4	4	80	5	4	5	5
5	5	5	100	5	5	5	5
5	6	5	100	5	5	5	5

TABELLA 11 - Tossicità dell'etere etilico del glicol etilenico nel ratto

Iniezione intramuscolare				Iniezione endovenosa			
N. ratti	Glicole cc. Kg.	N. morti	Mortalità %	N. ratti	Glicole cc./Kg.	N. morti	Mortalità %
5	0.5	1	20	5	0.5	4	4
5	1	4	80	5	1	5	5
5	2	5	100	5	2	5	5

TABLE 8 - Toxicity of the ethylene glycol in rats.

TABLE 9 - Toxicity of the propylene glycol in rats.

TABLE 10 - Toxicity of the diethylene glycol in rats.

TABLE 11 - TOXICITY of the ethyl ether of glycol in rats.

1) Intramuscular injection
2) Endovenous injection
3) Number of rats
4) Glycol, cc/kg
5) Number of dead
6) Mortality %

(text identical for tables 8 to 11)

Glycols purified according to the recommendations of HAZLIK (49) were used for the toxicity tests in rats and for other tests which will be described. The commercial glycols were distilled; only the fraction (which is in fact the most abundant) was conserved which distilled between 195,5° and 197°; the distillate was placed into the exsiccator under vacuum over calcium chloride and conserved protected from air (access) until the moment when it was used.

For our tests when investigating the toxicity rate we always used pure glycols and never any watery glycol solutions.

The results concerning the mortality percentages observed in rats with various glycols are summarized in Figure 3.

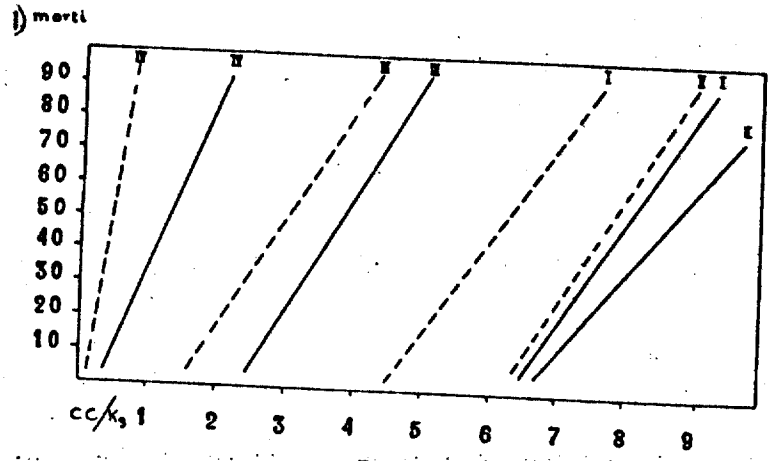


Fig. 3

FIGURE 3

1) Died

Tests concerning the acute toxicity of the glycols were repeated with rabbits; however, these tests were limited to the ethylene glycols, propylene glycols and diethylene glycols since it was not believed appropriate to continue tests with the ethyl ether of glycol, considering the considerable toxicity which was demonstarted. The technical methods of the tests with rabbits were the same as those applied to rats (however, the endovenous injection was given into the marginal vein of the ear).

Male and female animals were used, with a weight of 1,800 to 2,100 Kg. In these animals, the d.m.m. was likewise tested with oral administration. The obtained results are reported on Tables 12,13 and 14.

TABELLA 12 Tossicità del glicol etilenico nel coniglio

1 Via orale			2 Via intramuscolare			3 Via endovenosa		
N. conigli 4	Glicole cc./Kg. 5	N. morti 6	N. conigli 4	Glicole cc./Kg. 5	N. morti 6	N. conigli 4	Glicole cc./Kg. 5	N. morti 6
3	6	0	2	5	0	2	4	0
2	8	1	2	6	1	2	5	1
2	10	1	2	7	2	2	6	2

TABELLA 13 — Tossicità del glicol propilenico nel coniglio

Via orale			Via intramuscolare			Via endovenosa		
N. conigli	Glicole cc./Kg.	N. morti	N. conigli	Glicole cc./Kg.	N. morti	N. conigli	Glicole cc./Kg.	N. morti
3	6	0	2	5	0	2	4	0
1	8	0	2	6	0	2	5	0
2	10	1	2	7	1	2	6	1

TABELLA 14 — Tossicità del glicol dietilenico nel coniglio

Via orale			Via intramuscolare			Via endovenosa		
N. conigli	Glicole cc./Kg.	N. morti	N. conigli	Glicole cc./Kg.	N. morti	N. conigli	Glicole cc./Kg.	N. morti
2	3	1	2	2	1	2	2	1
2	4	2	2	3	2	2	3	2

TABLE 12 - Toxicity of the ethylene glycol in the rabbit

TABLE 13 - Toxicity of the propylene glycol in the rabbit.

TABLE 14 - Toxicity of the diethylene glycol in the rabbit.

1) Orally
2) Intramuscularly
3) Endovenously
4) Number of rabbits
5) Glycol, cc/kg
6) Number of dead

(Text 1) to 6) identical for Tables 12 to 14

Since only a limited number of animals was tested, the results are hardly adequate for a definite statement : it has to be supposed that when the number of the animals is increased, fluctuations of the mortality rate per single dose would very probably result. We neverthless believed that the tests should be continued concerning toxicity in rabbits, because these tests had already yielded results sufficient for the confirmation of the results obtained with rats.

FIGURE 4. 1) Died morti

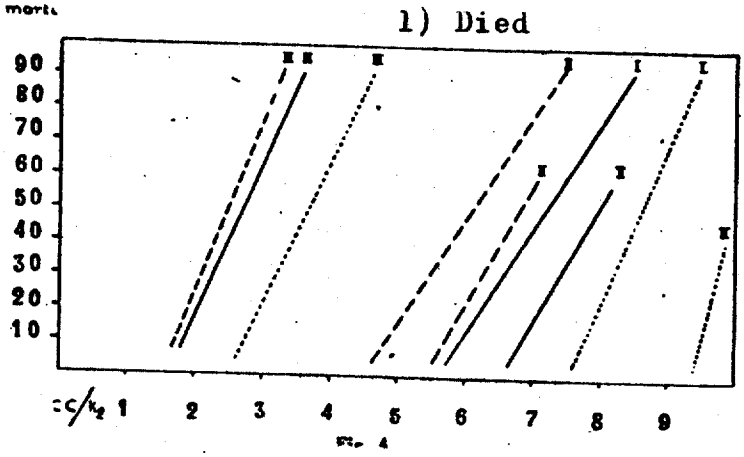


Fig. 4

On Figure 4, we show a diagram of the results obtained during the toxicity tests with rabbits, to facilitate a comparison with the results obtained for rats.

2) Research concerning chronic toxicity was carried out with rats only. Young males, with a weight of 80 to 90 g were likewise used for this test series.

The test animals were fed a uniform diet. At the start of the testing period the rats were divided into groups of 5 rats each. When forming the groups, care was taken to do so in a way that the sum total of the body weight of the animals in the various groups did not differ by more than 10 g from each other.

The same dose of glycol was administered intramuscularly each day to all animals within each group; different doses were given to animals in different groups. During the entire test period each group received identical quantities of food and water ad libitum. Twice per week all animals were weighed and thoroughly examined.

Tests were started for 9 groups of 5 rats each.

Two groups were used for researching the chronic toxicity of the ethylene glycol, two for the propylene glycol and two for the diethylene glycol; three groups served for control. In this way, two sub-lethal doses of each glycol were tested. For the ethylene glycol and for the propylene glycol, the tests were continued during 28 days.

Tables 15,16 and 17 show the results obtained.

TABLE 15 - Chronic toxicity of the ethylene glycol in the rat.

TABELLA 15 — Tossicità cronica del glicol etilenico nel ratto

Giorno[1] di prova	Gruppo controllo peso medio di 5 animali gr.	Gruppo A 0,3 cc. Kg. pro die di glicol per animale		Gruppo B 0,7 cc. Kg. pro die di glicol per animale	
		Peso medio di 5 animali gr.	Osservazioni	Peso medio di 5 animali gr.	Osservazioni
0	88	90	—	91	—
3	94	96	—	97	irritazione congiunticale
7	101	105	irritazione congiuntivale	105	"
10	106	113	"	112	"
14	115	118	"	117	"
17	120	125	"	122	"
21	128	132	"	130	vivacità dimin.
24	133	138	vivacità diminuita	135	"
28	139	147	"	142	"

1) Day of test
2) Control group -average weight of 5 animals, g
3) Group . .
4) 0,3 cc kg per day of glycol per animal
5) 0,7 cc.kg per day of glycol per animal
6) Average weight of 5 animals, g
7) Remarks
8) Conjunctival irritation
9) Vivacity decreased

Tabella 16 — Tossicità cronica del glicol propilenico nel ratto

Giorno di prova 1	Gruppo controllo 2 peso medio di 5 animali gr.	Gruppo A 3 — 0,3 cc. Kg. pro die di glicol propilenico per animale 4		Gruppo B 3 — 0,7 cc. Kg. pro die di glicol propilenico per ratto 5	
		Peso medio di 5 animali gr. 6	Osservazioni 7	Peso medio di 5 animali gr.	Osservazioni 7
0	84	81	—	93	8
3	89	81	—	99	irritazione congiuntivale
8	97	93	irritazione congiuntivale 8	105	
10	103	100	"	111	"
14	119	108	"	120	"
27	116	115	"	127	"
21	123	124	"	135	9 "
24	129	131	"	143	vivacità dimi
28	132	137	"	152	"

Tabella 17 — Tossicità cronica del glicol dietilenico nel ratto

Giorno di prova	Gruppo controllo peso medio di 5 animali gr.	Gruppo A — 0,3 cc. Kg. pro die di glicol dietilenico per animale		Gruppo B — 0,7 cc. Kg. pro die di glicol dietilenico per ratto	
		Peso medio in gr. di 5 ratti	Osservazioni	Peso medio in gr. di 5 ratti	Osservazioni
0	86	88	—	00	—
3	91	95	1 morto 10	97	4 morti 10
8	99	102	2 "	—	1 morto
10	104	—	2 "	—	— 10

TABLE 16 - Chronic toxicity of the propylene glycol in the rat.
(Text 1) to 9): see translation for Table 15)
TABLE 17 - Chronic toxicity of the diethylene glycol in the rat.
(Text 1) to 9): see translation for Table 15)
10) Died

Hypertensive effect of the glycols.

In other research series, I carried out some tests to evaluate the arterial hypertension rate following the endovenous administration of ethylene glycol, reported by OETTINGEN (45) and confirmed by AIAZZI-MANCINI (52).

For this type of research work I used rabbits to test the arterial tension changes and the variations of the rythm and amplitude of cardiac pulsation after the injection of ethylene glycol into the marginal vein of the ear. Whenever necessary I prepared the carotide of the test animal and connected the central stump with a mercury-recording manometer and I recorded the movements of the mercury surface on the smoke-stained paper of a rotating cylinder.

Simultaneously, I connected a tracheal cannula to T, one branch of which served for regulating air intake; the other was connected with a MAREY drum receiver equipped with a recording pen, to obtain a tracing of the respiratory movements.

I tested the effect of various doses and found that the endovenously injected ethylene glycol constantly causes a marked decrease of the arterial pressure.

The decrease is rapid and temporary, and within a period of 2 to 3 minutes the arterial pressure returns to the initial value. The rate of the hypertension is not proportionate to the quantity of the introduced ethylene glycol; it shows nearly the same value with ethylene glycol doses of 5 cc per kg as well as with doses of 3 cc per kg; the same applies to the recovery time. Consecutive injections given to the same animal in short intervals of time (10 within 10 minutes) cause an almost constant hypertension, regarding its intensity and duration.

The decrease of the arterial pressure caused by ethylene glycol occurs simultaneously with a slow-down of the cardiac pulsations which however become much more ample.

During the hypertensive condition the respiratory actions become more frequent and ample. In Figures 5 and 6 I report two tracings of the pressure and of the respiration obtained from the same rabbit which weighed 2,400 kg.

2 cc of ethylene glycol were injected into the animal; an immediate decrease of the arterial pressure, a slow-down and increased amplitude of the cardiac pulsations and increased frequency of respiratory actions was observed (see Figure 5). After 5 minutes and 45 seconds the tracings of the pressure and of the respiration had returned to normal. 10 minutes later 3 cc of glycol was injected, resulting in an almost identical tracing variation for pressure and respiration as observed with the previous injection. After an additional period of 10 minutes , 4 cc of glycol was injected. The arterial pressure decreased to the same extent as previously observed; only the slow-down and the amplitude increase of the cardiac pulsations were more evident, and the hyperpnea condition was more conspicuous (see Figure 6). One minute and 32 seconds after said third injection the cardio-circulatory and respiratory conditions had become completely normal.

I applied this type of research to the propylene glycol as well; I tested it in rabbits, using the same technique as for the ethylene glycol.

FIGURES 5 and 6. 1) 2 cc (4cc) of glycol 2) Time : 1 sec.

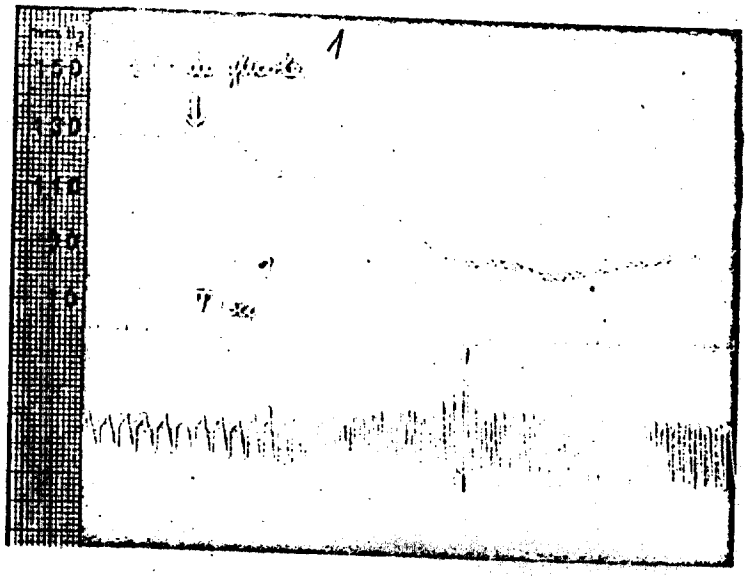


Fig. 5

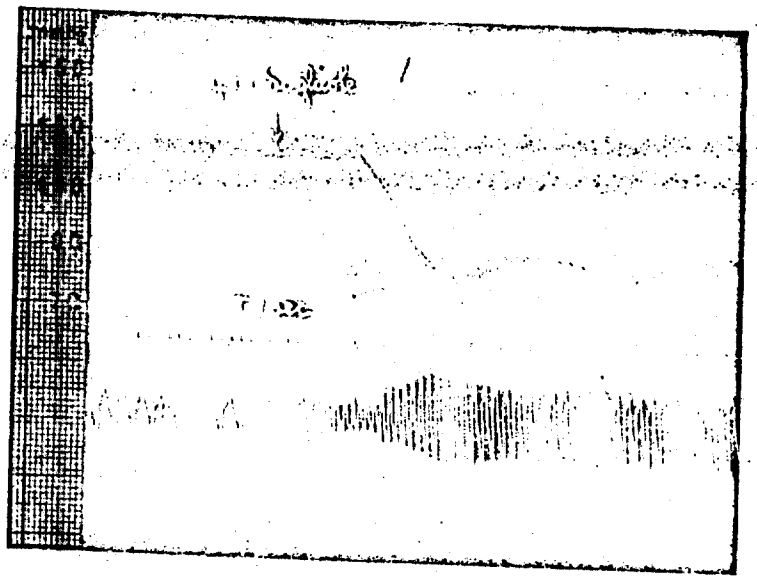


Fig. o

I observed a rapid and temporary hypertension following endovenous injection of propylene glycol as well. It becomes immediately evident from the implemented control tests that the hypertensive condition resulting from the propylene glycol has an intensity nearly identical with the condition observed as a result of ethylene glycol action. It is noteworthy, however, that with propylene glycol the return of the arterial pressure to the initial values is delayed as compared (to its return) with ethylene glycol. The frequency of respiratory movements and their amplitude increase while the arterial pressure falls, but the respiratory amplitudes soon decrease (see Figure 7).

Consecutive injections of propylene glycol do not cause a major hypertension; however, a dispnoic condition can occur, as shown for example in Figure 8.

The tracings of Figures 7 and 8 were obtained from the same rabbit, which weighed 2250 g. 2 cc of propylene glycol was injected into the marginal ear vein of this animal. Then , after 10 minutes, an additional dose of 3cc propylene glycol was injected (Fig. 7), and 10 minutes later 4 cc of propylene glycol was injected (Fig.8).

I likewise conducted some investigations to obtain evidence concerning some of the most pronounced effects attributed to the glycols, which I will briefly summarize.

FIGURES 7 and 8 1) Propylene glycol, 4 cc 2) Time: 1 sec

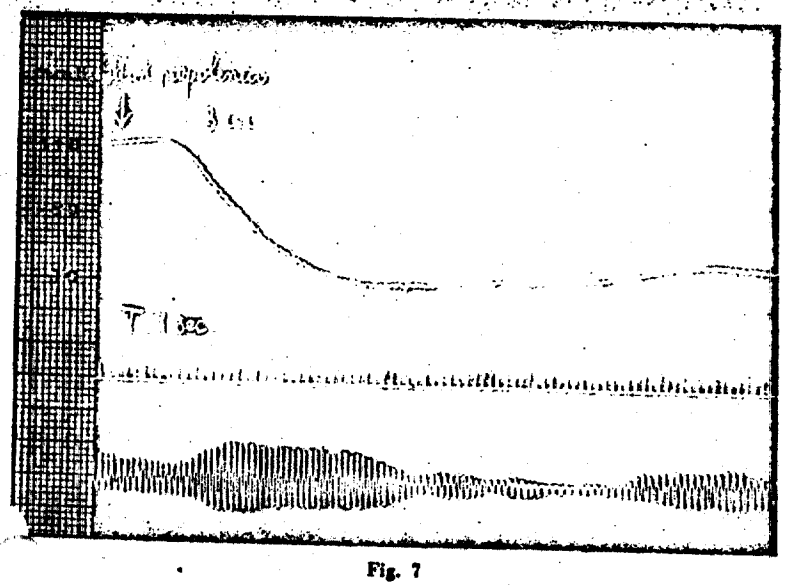


Fig. 7

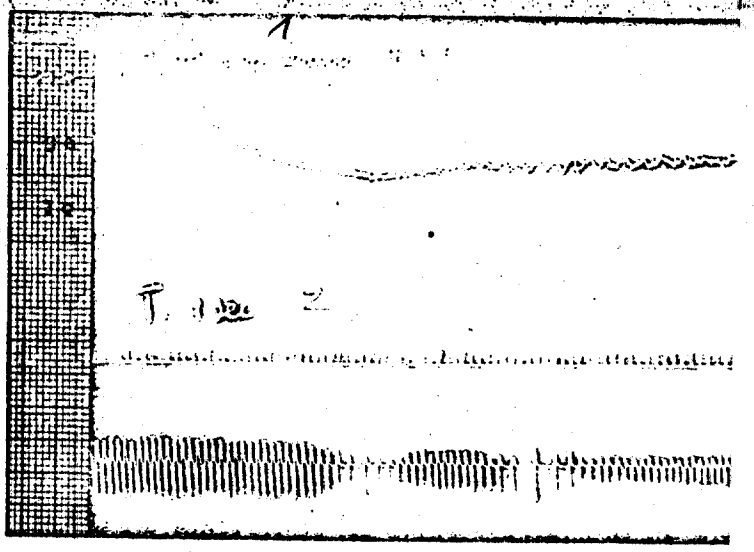


Fig. 8

1) Investigation of oxaluria caused by ethylene glycol. A dog and a rabbit served as test animals. 1 cc of glycol was intramuscularly injected each day for 10 consecutive days into a dog weighing 9 kg, while a rabbit weighing 1800 g likewise received intramuscular injections of 0,5 cc glycol during 9 days. The animals were normally fed. The oxalic acid test and the calcium oxalate test was applied to the 24-hour urine according to the method of AUTENRIETH and BARTH. My oxalic acid and oxalate tests proved to be negative for the entire test period; the oxalic calculi test implemented in the vescica of bothanimals after the autopsy was also negative.

2) Examination of the organs of the poisoned animals. - The following was found during the microscopic examination of the toxicity test animals which had died as a result of glycol action (acute intoxication): evident indications of renal and spleen hyperemia for all glycols; hemorrhage in the urines, especially intensive for the ethyl ether of the ethylene glycol. No evidence of organic changes was found in the animals subjected to chronic intoxication tests with propylene and ethylene glycol (the animals survived), while symtoms of hyperemia and spleen enlargement and indications of an acute glomerulonefritis were found in the animals which had received diethylene glycol (the animals had succumbed to the effect of the glycol).

3) Research concerning the hemolytic capacity of the ethylene glycol. I found that drops of blood suspended in pure glycol rapidly progress towards hemolysis. Watery solutions of glycol with 9% of NaCl are hemolytic in mixtures having a glycol percentage over and abive 17%.

4) Local irritation caused by glycols. - The subcutaneously injected glycols are strongly irritating; they case vasodilatation and local hyperemia, but do not prompt the formation of edemas. According to the subjective evaluation of persons to whom I gave subcutaneous injections into the gluteal area of equal amounts of propylene and ethylene glycol (0,4 cc per injection), pure propylene glycol is very painful: it causes an intensive burning pain which subsides in 10 to 15 minutes; on the other hand, ethylene glycol is much less irritating.

The intramuscular administration is almost painless with ethylene glycol and slightly painful with propylene glycol. Applications of pure ethyele and propylene glycol on the conjunctiva of the rabbit causes blepharospasm and a violent conjunctiva inflammation. Glycols diluted in water are less irritating. A 10% solution of ethylene glycol is harmless; a 5% solution of propylene glycol is somewhat irritating.

5) Local anesthetic effect of the ethylene glycol . - 5 to 7 % solutions of the glycol in water have a slight local anesthetic effect on the cornea of the rabbit with the LEVY method (53). However, the anesthetic effect is weak and transient; it is by no means comparable to the effects of the better-known local anesthetics.

On the whole, the results of our research concerning the pharmacology and toxicology of the glycols do not differ from those previously reported in the literature, nor from those made public in the meanwhile.

On the basis of the few tests carried out with the ethyl ether of the ethylene glycol we can immediately state that said substance is strongly toxic and can therefore not be taken into consideration for therapeutic use. The minimal lethal dose of the ethyl ether of the ethylene glycol is comparatively small. The intoxicated animals die rapidly, within 2 to 3 hours after the intramuscular injection (with doses listed on Table 11), and they die 1 to 2 hours after endovenous injection. The principal symptoms shown by the animals are: strong conjunctiva irritation, with abundant lachrymation and blepharospasm, a pronounced general debility, especially in the rear, which part of the body seems to be paretic. The animals show intense thirst and drag themselves painfully to drink. The respiration is frequent and superficial; however , before death occurs the respiratory movements become very intermittent.

The diethylene glycol also proved to be slightly toxic; its toxicity during my tests was much stronger than the toxicity observed by HAAG and AMBROSE (54) who state that the minimal lethal dose of diethylene glycol for rats is 7 cc/kg with intramuscular injection, 5 cc/kg when given endovenously, and 15 cc/kg when orally or subcutaneously administered; according to the above authors, the minimal lethal dose for the rabbit is 4 cc/kg intramuscularly, and 2 cc/kg endovenously.

Furthermore, HAAGE and AMBROSE affirm that the adding of diethylene glycol to the drinking water does not harm the rats in any way if the concentration rate of the glycol is very low; also, a prolonged administration of small glycol quantities with drinking water (0,5 to 1%) increases the body weight as a function of time, which possibly results from the fact that the diethylene glycol has a nutritious effect similar to the effect attributed to glycerine by JOHNSON, CARLSON and JOHNSON (55). However, concentrations over and above 3% prove to be rapidly lethal.

According to HOLCK (56) the diethylene glycol, when orally administered together with drinking water, is less toxic than the ethylene glycol.

The tests of HANZLIK, MERTENS and SPAULDING (57) as well as my own tests show in each case that the toxicity of the diethylene glycol is definitely stronger than the toxicity of the ethylene glycol. The results are certainly influenced by numerous factors, last but not least by the quality of the animals and the method used for the administration of the substance.

Recent research work concerning the toxicology of the diethylene glycol does in fact endeavor to obtain knowledge on its toxicity in cases when sereval doses are repeatedly administered orally(for example: the Chemical Laboratory of the American Medical Assication undertook an attempt to reproduce experimentally the toxic aspects of the MASSENGILL Elixir (2)). In general, my own investigations and those of HANZLIK and collaborators are based on the method which involves injections of pure glycol. My rabbits which I tested in the course of the research work, using oral administration, received a single dose of pure undiluted diethylene glycol.

Identical chronic toxicity tests were carried out by me with daily intramuscular injections of glycol. The high mortality rate among rats during said chronic toxicity tests (see Table 17) prompted us to exclude the diethylene glycol from use as solvent of Bismjol(*)

(*) I wish to point out incidentally that Bismjol is intramuscularly injected; it was our purpose to establish whether glycol injections with the above method showed toxic effects through cumulative action. Furthermore the Bismjol was submitted for registration on March 13, 1935, and the distribution of samples to physicians began on August 1, 1935; at that time, much less was known concerning the toxicity of the diethylene glycol than today.

Regarding the chronic toxicity tests: we found no modification whatsoever of the growth curve of rats under the effect of ethylene glycol and propylene glycol.

The weight differences observed during 4 weeks of testing are within the limits of variations which usually occur even among different groups of animals receiving standard food which (animals) were not subjected to any treatment whatsoever.

For both glycols, the only noted symptoms are: an evident conjunctiva irritation, and a considerably muscular debility which appeared as the tests progressed, whenever the daily doses, while sub-lethal, were considerable (i. .e 100 times in excess to the doses administered to humans with intramuscular injection, proportionately to the body weight).

Accordingly, we had to make a choice between the tested glycols, for the pre-established purpose (vehicle and solvent of Bismjol): a choice had to be made between the ethylene glycol and the propylene glycol; the toxicity tests for both had yielded results which fully guarantee innocuity for therapeutic use, in consideration of doses to be administered

In fact the toxicity of the propylene glycol proved to be minor, and in this respect our results had confirmed the findings of HANZLIK and collaborators (48), of HUNT (48) and of BROWN (37) who also obtained subsequent confirmations from the studies of HOLCK (56), LEHEMAN and NEWMAN and LEHMAN (59), etc.

Said advantage, i.e. the minor toxicity of the propylene glycol, however, was ephemeral insofar as there was no reason whatsoever even to mention eventual toxic phenomena related to the ethylene glycol in doses of 2 to 3 cc, with intramuscular injection.

AIAZZI-MANCINI (51 & 61) actually stipulates that the toxic doses of ethylene glycol are astronomically remote from the customary therapeutic dosages; his above statement is based on personal research (62 and on the testing with iodinebismuthol (solvent and vehicle ethylene glycol) in the dermosyphilopathic clinic of Stanford University, at San Francisco, California; it is based on the tests of BACHEM (63) who even subjected himself to tests and who on one occasion drank 25 cc of glycol, on another occasion, he drank 45 cc of glycol within 2 days without experienceing any detrimental effects.

The above statement is likewise based on the fact that the only cases of ethylene glycol intoxication were described by HANSEN (47); they were caused by the ingestion of 100 and 150 cc of glycol .

Since there was no cause for concern regarding the toxicity of ethylene glycol or propylene glycol, our choice was based on the demonstrated properties and characteristics.

Our choice of the ethylene glycol as vehicle and solvent was prompted by the fact that its locally irritating effect is much less intense than the irritating effect of propylene glycol. This influenced our decisions also because a new preparation, the Tefamin , was subject to study in these Laboratories; it was planned to use the ethylene glycol as solvent for Tefamin as well. Since the alkalinity of the new product (Tefamin) and the same components of the Bismjol tend to make the intramuscular injection painful, we considered it appropriate to use the glycol as solvent which proved to be the more easily tolerated of the two when introduced with the above method.

The obtained data, however, show in each case that the ethylene glycol can be used to advantage in therapy and that no harmful effect whatsoever is foreseeable (provided that its use is maintained within the limits of our preparations.).

The reported effects resulting from the use of ethylene glycol in particular , based on the feared hemolytic potential, on the occurence of oxaluria, on a hypertensive condition, have already been disproved by HANZLIK (49) and principally by AIAZZI-MANCINI (52); my investigations confirm the statements of the latter. Oxaluria does in fact not occur even after repeated strong dosages; hemolysis occurs only when the glycol concentration in the blood exceeds 17% (this could certainly not be reached in humans with the intramuscular injection of 2 cc). The hypertensive condition which definitely manifests itself in animals with high glycol doses introduced endovenously does not result with identical dosages injected intramuscularly. The minimal intramuscular dose can cause a transient and minor prressure decrease in the rabbit; said dose is 50 times larger than the dose customarily injected into normal humans (2 cc).

The above are therefore not factors which could advise against the use of ethylene glycol. The argumentation likewise applies to propylene glycol, as it does to ethylene glycol; it should be regarded as an excellent solvent.
Finally, we have to report that clinical experience--in support of the laboratory results-- indicates that ethylene and propylene glycol are innocuous and can be used to great advantage. A recent report of BARNETT and KULCHAR (65) informs us that 125.000 injections of iodinebismuthol (first ethylene, then propylene glycol as vehicle and solvent) were administered to 3000 patients without causing any disorders. KULCHMAR also claims to have given 300 consecutive intramuscular injections to the same patient without observing any symptoms.

We may add that thousands of Bismjol and Tefamin samples (ethylene glycol as vehicle and solvent) have been experimentally used by Italian and foreign clinical doctors and practicioners. In the reports received by us no detrimental effects whatsoever are mentioned, resulting from the action of glycol. In this case, the clinic confirmed the Laboratory results, approving the therapeutic use of ethylene glycol and of propylene glycol.

Conclusions.

1) Comparative research work was implemented concerning the toxicity of the ethylene glycol, the propylene glycol, the diethylene glycol and the ethyl ether of the ethylene glycol, using rats and rabbits. The obtained results show that the toxicity of the propylene glycol and of the ethylene glycol is minor. The diethylene glycol, on the other hand, is strongly toxic; the toxicity of the ethyl ether of the ethylene glycol is very powerful.

2) The glycols injected into veins cause a sudden decrease of the arterial pressure, which soon (in 1 to 2 minutes) returns to normal values. A slight and transiemt hypertensive condition with intramuscular injections of glycols is obtainable only with doses per kg enormously in excess of the doses currently used in human therapy for the ethylene glycol and for the propylene glycol.

3) The prolonged administration of ethylene glycol to rabbits and dogs did not result in the occurence of oxaluria.

4) The ethylene glycol has a hemolytic effect in concentrations over and above 17% in the blood (a concentration which cannot be reached with the customary therapeutic doses).

5) No contra-indication advises against the use of the ethylene glycol and of the propylene glycol in customary doses (2 to 3 cc) as solvent and vehicle of drugs to be injected intramuscularly into humans.

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The separation of polyols by thin-layer chromatography*

Many procedures have recently been proposed for the separation of polyols by thin-layer chromatography on silica gel, alumina or Kieselguhr G plates using various developing solvents[1]. In some cases, adsorbents modified with complexing agents such as boric acid[2] or ammonium borate[3] have also been tried.

In previous work[4-6] it has been shown that in an alkaline medium complex formation takes place between lead(II) and polyols (P) containing at least two adjacent hydroxyl groups, according to the following equilibrium:

$$Pb(OH)_3^- + P \leftrightharpoons PbP^- + nH_2O.$$

Because the complex formation is affected by the number and position of the hydroxyl groups and the equilibrium constants are quite different, lead(II) has been used here as a complexing agent in the adsorbent layer to separate polyols by thin-layer chromatography. The influence of complex formation with lead(II) and of eluent composition on the mobility of polyols has been studied.

Experimental

Thin-layer plates were prepared according to the following procedure: 30 g of Silica Gel H (Merck) were mixed with 70 ml 0.1 *M* $Pb(NO_3)_2$ (Erba RP, Milan) or 70 ml water (untreated plates) by shaking in a stoppered conical flask, and were then transferred to the spreader (Chemetron, Milan). The thickness of the layer was set at 0.25 mm. All the plates were activated by heating at 110° for 30 min. Samples were applied to the starting line of the chromatograms as dilute solutions in alcohol or water–alcohol, in chromatography tanks. Alcohol–ammonia or alcohol–ammonia–water mixtures were used as developing solvents, and the polyols were detected by spraying with potassium permanganate containing sodium carbonate solution. Experiments carried out with Silica Gel G plates have not given satisfactory results. This seems to be due to the reaction of lead(II) with calcium sulfate present in the Silica Gel G.

TABLE I

FORMATION CONSTANTS WITH LEAD (II) OF POLYOLS IN ALKALINE MEDIUM

Polyol	*log K*
1,3-Propanediol	−0.20
Ethylene glycol	0.30
1,2-Propylene glycol	0.30
1,2,5-Trihydroxypentane	0.40
1,2,4-Butanetriol	0.45
Glycerol	1.15
meso-Erythritol	1.93
d-Mannitol	2.78
Dulcitol	2.90
d-Sorbitol	3.42

* Work carried out with the aid of the "Consiglio Nazionale delle Ricerche".

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Results

The polyols listed in Table I were examined, their formation constants, K, with lead(II) in alkaline medium (0.1 M OH^-) having been previously determined.

Typical chromatograms of some polyols on untreated Silica Gel H and lead(II)-impregnated Silica Gel H for three developing solvent systems are reproduced in Fig. 1. The influence of lead(II) is evident: the mobility of the polyols decreases with an increase of the number of adjacent hydroxyl groups, as the formation constant between lead(II) and polyols become larger.

In Table II the R_F values measured for the polyols investigated are collected. The R_F values for glycols are slightly affected by the presence of lead(II) in the adsorbent layer, while the mobility of other polyols is considerably decreased by

TABLE II

R_F VALUES × 100 OF THE INVESTIGATED POLYOLS ON SILICA GEL H (A) AND LEAD(II)-IMPREGNATED SILICA GEL H (B)

Developing solvents: (I) ethanol saturated with gaseous ammonia; (II–VII) ethanol–conc. ammonia (32%)–water, respectively: II (21:2:0), III (23.5:2.2:1), IV (23:2.2:2), V (21:2:2.4), VI (21:2:3.5) and VII (20:2:4).

Polyols	*I*		*II*		*III*		*IV*		*V*		*VI*		*VII*	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1,3-Propanediol	76	68	82	75	80	78	86	79					82	82
Ethylene glycol	62	59	67	60	64	60	66	62	66	62	74	68	71	66
1,2-Propylene glycol	69	65	73	67	72	67	73	60	72	70	80	76	77	74
1,2,5-Trihydroxypentane	65	61	69	64	68	64		66	70	65	78	[illegible]	76	[illegible]
1,2,4-Butanetriol	60	53	62	60	64	60	66	62	65	62	73	68	71	67
Glycerol	48	22	53	42	50	46	56	48	57	52	62	54	63	55
meso-Erythritol	33	6	39	22	38	30	45	34	49	39	51	43	50	45
d-Mannitol	7	0	14	3	15	6	22	9	30	14	27	17	37	22
Dulcitol	4	0	12	0	14	3	21	3	29	8	24	10	35	18
d-Sorbitol	4	0	11	0	12	2	17	5	26	7	22	9	34	14

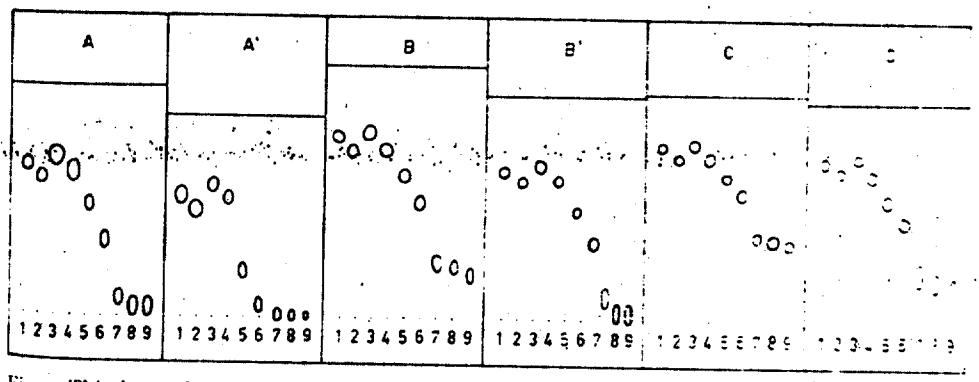

Fig. 1. Thin-layer chromatograms on Silica Gel H (A, B, C) and lead(II)-impregnated Silica Gel H (A', B', C') of the following polyols: (1) 1,2,5-trihydroxypentane; (2) 1,2,4-[illegible]; (3) 1,2-propylene glycol; (4) ethylene glycol; (5) glycerol; (6) *meso*-erythritol; (7) [illegible]; (8) [illegible]; (9) *d*-sorbitol. Developing solvents: (A,A') ethanol saturated with gaseous ammonia; (B,B') ethanol–conc. ammonia (32%)–water (23:2.2:2); (C,C') ethanol–conc. ammonia (32%)–water (20:2:4).

the presence of the metal. A marked influence of the complexing ability of the added metal can be observed by running compounds that do not bind lead(II) on the two types of plates. Amines such as benzylamine yield identical R_F values when 95% ethanol–25% ammonia (4:1) is used as eluent.

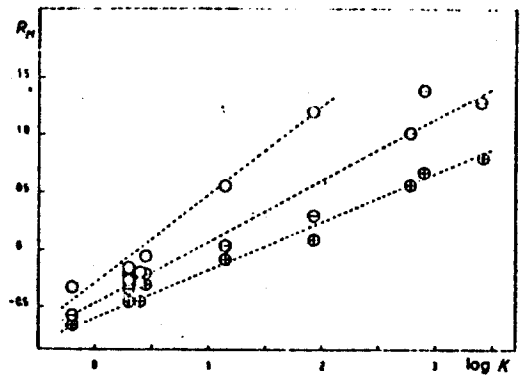

Fig. 2. R_M values plotted *versus* logarithm of the formation constants with lead(II). Developing solvents: (○) ethanol saturated with gaseous ammonia; (⊖) ethanol–conc. ammonia (32%); water (23:2.2:2); (⊕) ethanol–conc. ammonia–water (20:2:4).

The mobility of the polyols changes noticeably with the eluent composition. Since the polyol–lead(II) complexes are polar, their migration is affected by the polarity of the elution medium consisting of ethanol and ammonia, with and without water. The migration of glycols is slightly affected by the eluent composition. Glycerol and erythritol exhibit a large difference in the R_F values when they are chromatographed on untreated and lead(II)-treated silica gel plates when water is present in the eluent. Higher polyols show a larger difference in the R_F values on the two types of plates when the eluent does not contain water.

More favourable conditions for the separation of triols and higher polyols from diols are obtained when anhydrous ethanol saturated with ammonia is used. In this medium the higher polyols have a smaller mobility because their complexes with

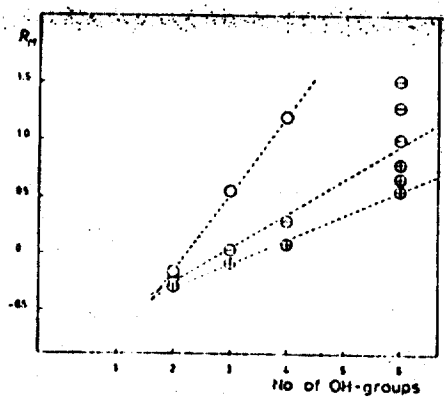

Fig. 3. R_M values plotted *versus* number of hydroxyl groups of various polyols. Developing solvents: (○) ethanol saturated with gaseous ammonia; (⊖) ethanol–conc. ammonia (32%); water (23:2.2:2); (⊕) ethanol–conc. ammonia–water (20:2:4).

lead(II) are stronger and very slightly soluble. It is possible to separate glycerol from erythritol and the latter from hexahydric alcohols by increasing the eluent polarity by adding water.

The dependence of the chromatographic behaviour on the formation constant of lead(II)-polyol complexes is readily observed in Fig. 2, where the R_M values obtained for three different eluents have been plotted *versus* the logarithm of the formation constants reported above. The plot is only of a qualitative nature because the formation constants were obtained under different experimental conditions (0.1 *M* OH^- and 1 *M* Na^+). It clearly shows, however, that the R_M values increase gradually as log *K* increases. From this plot the effect of the eluent is quite clear because the slopes of the hypothetical curves drawn through the experimental points are different.

Since R_M is a linear function of the number of equal groups responsible for the chromatographic behaviour, the R_M values obtained on lead(II)-treated plates have been plotted *versus* the number of hydroxyl groups of the polyols investigated. There is a linear relationship for diols, triols and tetrols, whereas deviations are observed for the hexahydric alcohols; this is probably due to interactions among -OH groups which influence each other.

Istituto di Chimica Analitica,
Università di Roma (Italy)

VINCENZO DE SIMONE
MARIO VICEDOMINI

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A New Spectrophotometric Micro Determination of Vicinal Diols[1]

HANSJÖRG EIBL AND WILLIAM E. M. LANDS

The Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104

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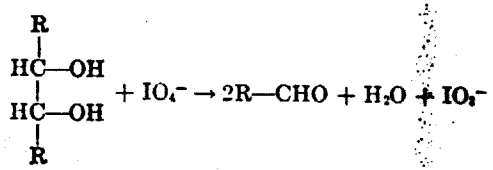
The quantitative determination of vicinal diols by periodate was introduced by Malaprade (1). One mole of a vicinal diol reacts with one mole of periodate and forms two moles of aldehyde and one mole each of iodate and water:



Generally an excess of periodate is used to ensure complete reaction of the glycol (2). Thus, periodate and iodate are always present in the reaction mixtures along with other reaction products, depending on the kind of diol or polyol which is analyzed. Some of the products may be quantitatively measured, particularly formic acid, formaldehyde, and micro analyses have been developed for certain types of compounds (3).

A general method for the quantitative determination of various diols should, however, be concerned with measuring either the disappearance of periodate or the formation of iodate. Potassium iodide reacts quantitatively with periodate in solutions buffered with bicarbonate at pH 8 to form iodine, but the corresponding reaction with iodate does not occur. Thus, periodate is easily determined in the presence of iodate but not vice versa. It is not surprising then, that the disappearance of periodate has been used in the quantitation of vicinal diols rather than the appearance of iodate (1–5).

The iodometric method of determining periodate with sodium arsenite at pH 8 is much less sensitive than that at acid pH values where iodate

[1] This work was supported in part by a research grant from the United States Public Health Service (AM05310) and by a fellowship from the Max Planck Gesellschaft.

also forms iodine. Since we wished to analyze the very small amounts (10–200 nmoles) of vicinal diols produced in enzymic reaction mixtures, we examined the possibility that iodate could be measured directly after the selective removal of periodate. Rather than attempt a quantitative removal of periodate as the insoluble potassium salt, we examined the recent report that quantitative coprecipitation of periodate with aluminum hydroxide is possible in solutions containing small amounts of periodate and an excess of iodate (6). If relatively small amounts of iodate could be quantitatively retained in solutions having an excess amount of periodate, a direct determination of iodate formation should be possible in the reaction mixtures after removing the periodate by precipitation and centrifugation. The present report indcates that a sensitive iodometric assay for glycols can be developed using such a procedure.

REAGENTS

Sodium periodate solution (37 m*M*): 40 mg $NaIO_4$ (Mallinckrodt analytical reagent) was dissolved in 5 ml 0.1 *M* boric acid. The solution was always prepared immediately before use.

Potassium iodate standard solution (9.5 m*M*): 203 mg KIO_3 (Mallinckrodt analytical reagent) was dissolved in 100 ml distilled water.

Aluminum potassium sulfate solution (21 m*M*): 10 gm $K_2Al_2(SO_4)_4 \cdot 24H_2O$ (Mallinckrodt analytical reagent) was dissolved in 500 ml distilled water.

Potassium iodide solution (0.2 *M*): 3.3 gm KI (Baker analyzed reagent) was dissolved in 100 ml distilled water.

D-Mannitol was purchased from Nutritional Biochemicals Corporation.

1,2-Propanediol was a product of Matheson, Coleman & Bell.

1-Stearoylglycerol (90%) was purchased from California Corporation for Biochemical Research.

APPARATUS

A Cary model II recording spectrophotometer and a Beckman DU spectrophotometer were used for the measurements.

RECOMMENDED PROCEDURES

(*A*) The diol (0.1 to 1 μmole) was dissolved in absolute alcohol (0.2 ml) and 0.2 ml of the periodate solution was added. The reaction mixture was shaken and kept in the dark. Aliquots of 0.1 ml were taken after 20, 40, and 60 min and transferred to 6.3 ml of distilled water in a test tube. 2 ml of the aluminum potassium sulfate solution (42 μmoles) was added and then 0.5 ml of ammonia (0.72 *M* solution in distilled

water). After each addition, the contents of the tubes were mixed thoroughly. Centrifugation in a small centrifuge for 2 min resulted in a tight pellet. The supernatant was decanted into a tube containing 1 ml of 1 *N* sulfuric acid; then 0.1 ml of the potassium iodide solution was added and the contents were mixed again. The tubes were flushed with nitrogen and the absorbance at 350 nm of the solution was read after 10 min of standing in the dark. A blank without added diol was always included in the determinations. The final volume was 10 ml. The diol cleavage was finished normally after 40 min, and the determination after 60 min was only necessary to confirm that the reaction was, in fact, complete.

(*B*) The diol (50 to 600 nmoles) was treated as described in *A*. After 20, 40, and 60 min, 0.1 ml of the reaction mixture was removed and added to 5.4 ml of distilled water and treated with aluminum hydroxide as described above. Finally 1.0 ml of the potassium iodide solution was added and the absorbance read at 350 nm.

RESULTS

The reaction of periodate with diol was always performed in the dark as recommended in earlier review articles (2, 5). Blanks without added diol were always used to correct for the small reaction of periodate with alcohol. Alcohol was used in the reaction medium to get full solubility of lipoidal substrates, and was necessary especially for the analysis of monoacylglycerols.

The absorbance of iodine produced by adding potassium iodide to acidic solutions of iodate was linear with respect to concentration, and the absorption maximum at 350 nm was chosen for further determinations.

Table 1 shows that the removal of periodate by coprecipitation with aluminum hydroxide was essentially complete. The periodate in these determinations was dissolved in solvent mixtures which are commonly used in periodate determinations of vicinal diols (2, 5). For reasons, which will be discussed later, we have chosen solvent system *B*—0.1 *M* boric acid 95% alcohol (1/1)—for the quantitative determination of diols.

Table 2 shows the simulation of the products of a diol reaction mixture with periodate. No interference in the determination of iodate was observed, whether we analyzed iodate alone, iodate after treatment with aluminum hydroxide, or iodate after precipitation of the periodate with aluminum hydroxide. Although the molar ratio of periodate to iodate in part C ranged from 35 to 11, the determination of iodate in the supernatant was not influenced by the large excess of periodate, indicating that the precipitation of periodate was quantitative.

TABLE 1

Iodine Produced after Removal of Periodate with Aluminum Hydroxide

The solutions were kept in the dark for the indicated time periods and aliquots were analyzed according to the recommended procedure. The amount of oxidant remaining in the supernatant was determined as iodine and is expressed in parentheses as per cent of the total amount added.

	Periodate added, μmoles				
	0	0.58	1.15	2.30	4.60
Maximum possible iodine	0	(2.32)	(4.60)	(9.20)	(18.40)
Experiment	Iodine observed in supernatant, nmoles				
A (0.1 *M* boric acid)					
20 min	8	23 (1.0)	60 (1.3)	92 (1.0)	166 (0.9)
B (0.1 *M* boric acid/95% alcohol (1/1))					
20 min	8	32 (1.4)	60 (1.3)	120 (1.3)	239 (1.3)
40 min	8	32 (1.4)	68 (1.5)	123 (1.3)	272 (1.5)
60 min	8	34 (1.5)	68 (1.5)	126 (1.4)	284 (1.5)
C (0.1 *M* boric acid/dioxane (1/1))					
20 min	8	77 (3.3)	148 (3.2)	292 (3.2)	515 (2.8)

A standard curve for the transformation of absorbances in nmole of iodate is presented in Figure 1. The molar absorbance calculated from this graph for the absorbance of iodine is 1.2×10^4 (A) or 2.3×10^4 (B), depending upon the iodide content of the solution.

TABLE 2

Absorbances Produced in a Periodate Reaction Assay System[a]

Exptl. condition	Added KIO_3, nmoles			
	0	66.5	133	199.5
A	(0.030)	0.239	0.489	0.721
B	(0.040)	0.235	0.485	0.734
C	(0.150)	0.236	0.483	0.729

[a] Three different amounts of iodate were checked in three experimental conditions: A, Iodate was analyzed after adding the indicated amounts to 8.4 ml of distilled water; 0.5 ml of ammonia (0.72 *M*) was then added and continued as described in the recommended procedure. B, Iodate was analyzed exactly as described in the standard procedure. C, Iodate in the presence of 2.3 μmole of added periodate was determined by the standard procedure. Absorbances were corrected for the blank values (shown in parentheses) obtained when iodate was omitted.

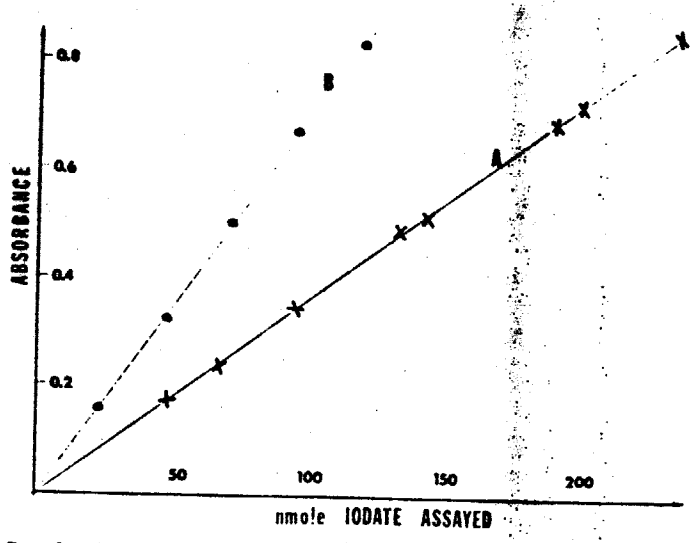


FIG. 1. Standardization curve measuring absorbance at 350 nm of iodine produced. The amounts of iodate in nmole per tube were as indicated: 8.4 ml (A) or 7.5 ml (B) of distilled water, 0.5 ml of ammonia (0.72 *M*), 1 ml of 1 *N* sulfuric acid, and 0.1 ml (A) or 1.0 ml (B) of 0.2 *M* potassium iodide were added and mixed with a final volume of 10 ml.

As a final test of the method we checked the assay system with some vicinal diols of known structure. In all cases, the estimated content of vicinal diol which was determined by measuring the formation of iodate was close to the theory—one mole of iodate formed per mole of diol (shown in Table 3). The final readings after 45 min were always taken

TABLE 3
Assay of Vicinal Diols of Known Structure

The amount of iodate formed in relation to the equivalents of added diol is described also as a percentage value.

Compound	Amt., nmoles	Diol-equivalent, nmoles	Iodate formed, nmoles	%
D-Mannitol	50	250	244	98
	100	500	478	96
	150	750	750	100
1,2-Propanediol	130	130	127	98
	260	260	262	101
	390	390	380	97
1-Stearoylglycerol (90%)	312	281	280	100
	625	562	550	98
	937	843	840	100

for the calculations, although the increase in product during the last 15 min was almost negligible as shown in Table 4 and Figure 2.

Figure 2 presents the reaction of 1-acylglycerol and 2-acylglycerol with periodate. Even after 45 min the formation of iodate in the case of 2-acylglycerol was negligible—in contrast to 1-acylglycerol, which had almost completely reacted as expected within 15 min.

DISCUSSION

The method described in this paper for the quantitative determination of vicinal diols is simple and allows the accurate measurement of diols in 60 min. In contrast to many methods of measuring the disappearance of periodate, we were able to directly analyze the iodate produced in the reaction mixtures. Excess amounts of periodate used in fairly high concentrations allowed a complete reaction in relatively short time periods. The molar ratio of periodate/diol was usually 10 to 100.

The results in Table 1 indicate that the coprecipitation of periodate with aluminum hydroxide was quantitative. The iodine produced in the supernatant was a constant percentage value (e.g., approximately 1.4% for solvent B) of the added periodate. This suggests that these amounts were produced by impurities in the periodate rather than by unprecipitated periodate since the solubility of aluminum periodate in the supernatant would be expected to be constant. A constant solubility would lead to decreasing percentage values of iodine found in the supernatant with increasing amount of periodate, such as: 1.4, 0.7, 0.35, and 0.175. A possible contaminant in the periodate reagent could simply be iodate. The absorbances were always corrected for these control values obtained without added diol. In addition, the almost constant control readings over a time period of 60 min indicate that unspecific side reactions of periodate with alcohol are negligible.

TABLE 4
Time Dependence of Iodate Formation

Absorbance at 350 nm was measured after 15, 30, and 45 min, and corrected for a blank without added diol (shown in parentheses). The 50, 100, and 150 nmole of D-mannitol analyzed corresponded to 250, 500, and 750 nmole of diol. One fourth of the total sample was used at each time point.

D-Mannitol, nmoles	15 min	30 min	45 min
0	(0.150)	(0.153)	(0.157)
50	0.210	0.220	0.223
100	0.416	0.430	0.436
150	0.641	0.661	0.668

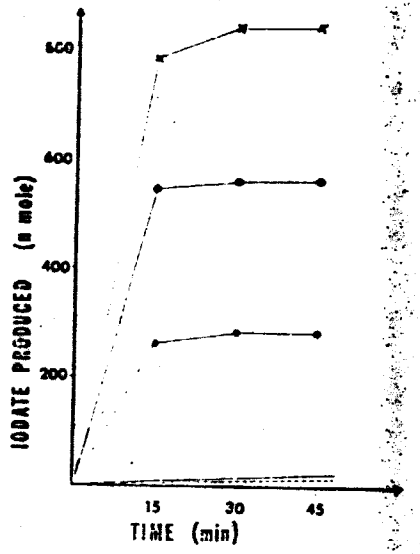


FIG. 2. Reaction of 1- and 2-acylglycerols in periodate assay system: 280 (●), 560 (○), and 810 (×) nmoles of 1-stearoylglycerol were compared with 370 (—) and 650 (—) nmoles of 2-acylglycerol.

Our interest in positional isomers of acylated glycerols led us to regard a mixture of 0.1 *M* boric acid/alcohol (1/1) as the most desirable solvent system. As reviewed recently by Serdarevich (7), boric acid stabilizes the monoacyl glycerols and prevents migration of the fatty acids. We were able to show that a borate system which hinders acyl migration did not interfere with glycol determinations.

When analyzing monoglycerides, the periodate reagent in mixtures of 0.1 *M* boric acid/alcohol (1/1) should not be added to the dry solid. Rather, the monoglycerides should first be dissolved in absolute alcohol and then the periodate in boric acid added. Otherwise there is an underestimation of 1-acylglycerols, which are not completely dissolved, especially with monoglycerides containing saturated fatty acids.

Negligible amounts of iodate were formed in the presence of 2-acylglycerols even after reaction times of 45 min. The amount of vicinal diol in the reaction mixture after 45 min was calculated to be about 2%. Extrapolation of the small absorbances measured after 30 and 45 min to time 0, however, would indicate that there was almost no contamination initially of the 2-acyl isomer by 1-acylglycerol, but that some slight acyl migration as noted by Mattson and Volpenhein (8) had occurred. The 2-acylglycerol studied had been freshly prepared from 2-acylglycerophosphorylcholine by treatment with phospholipase C. Thus monoglycerides with fatty acids in the 1- or 3-position react quantitatively with periodate to produce iodate whereas 2-acylglycerols do not react. This finding is now being successfully applied to the analysis of isomeric mixtures of monoglycerides derived from monoacylphosphoglyceride precursors (9).

Table 5 presents a comparison of our new method for the quantitative determination of diols with other procedures described in the literature, where generally the disappearance of periodate was measured. This method of determining directly the iodate produced is more sensitive than most reported procedures by a factor of 100. The method described by Frommhagen (15) allowed the determination of diols in amounts of 100 nmole. This method, however, as well as other procedures analyzing the disappearance of periodate spectrophotometrically at 220 nm, has some limitations (2). For instance, incubation times of 3 to 23 hours were needed because of low periodate concentrations and many inorganic materials also absorb in this region (15). The method of spectrophotometrically measuring formaldehyde has been developed to handle 80 to 800 nmoles (3), but this is not always applicable to all vicinal glycerols.

TABLE 5
Comparison of Different Methods for Determination of Diols

Authors	Method	Amounts used, μmoles
Van Lohuizen and Verkade (10)	Titration	800
Smith and Willeford (11)	Titration	400–600
Martin (12)	Titration	0.9–27
Karnovsky and Brumm (13)	Chromotropic (CH_2O)	2–10
Belcher *et al.* (14)	Chromotropic (CH_2O)	0.08–0.80
Frommhagen (15)	Spectrophotometric (220 nm)	0.100–1.0
New method	Spectrophotometric (350 nm)	0.01–0.250

The present method has a wide range of applications. Most buffers and many organic compounds do not absorb at 350 nm, and the iodine is produced by a product found in all diol oxidations with periodate. An added flexibility comes from the fact that the absorbance of iodine is very much dependent on solvents or ionic concentrations. For instance, increase in the concentration of iodide by a factor of 10 (procedure B in Fig. 2) resulted in an almost twofold increase in the observed absorbance from 1.2×10^4 to 2.3×10^4. This change is a reminder of the fact that the absorbance of iodine is markedly influenced by both the equilibrium between iodine and triiodide and the dielectric constant of its environment.

In developing this method we were influenced by the sensitivity of the iodine measurements obtained in the method for alkenyl ethers as

described by Gottfried and Rapport (16). That method provides iodine absorbancies of 2.75×10^4 in 76% ethanol solutions. Our method A (Fig. 2) gave a value of 1.2×10^4 and with increased iodide (20 m*M*) we observed 2.3×10^4, which agrees more closely with that reported by others (16, 17).

The chance of selecting other solvent systems containing less polar solvents for the iodine produced offers a still wider range of sensitivities. The sensitivity of the described method, however, is sufficient to allow the accurate determination of 10 to 500 nmoles of diol. Even amounts as small as 1 nmole can be determined by reducing the final total volume in our procedure from 10 ml to 1 ml.

While this manuscript was being prepared for publication, we found a recent article by Nisli and Townshend (18), who regarded the same considerations described in this paper and devised a procedure for determining iodate in the presence of excess periodate by using the molybdate masking phenomenon described by Burnel (19). That phenomenon is sensitive to pH and to the presence of other ions, and requires small absorbance corrections for the molybdate and periodate, but does not require a centrifugation, since the periodate remains in the system. The precipitation procedure is not particularly difficult, and it allows greater flexibility by removing the major interference, periodate. Thus, two universal techniques are now available to investigators for the determination of nmole amounts of vicinal glycols whereas none were available a year ago.

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Metabolism of DL-1,2-Propanediol-2-^{14}C in a Lactating Cow [1,2]

R. S. EMERY, R. E. BROWN AND A. L. BLACK
Departments of Dairy Science and Physiological Sciences, Michigan State University, East Lansing, Michigan, University of Illinois, Urbana, Illinois and University of California, Davis, California

ABSTRACT Since propanediol is glucogenic and is extensively used in therapy of bovine ketosis, its metabolic fate was investigated. DL-1,2-propanediol-2-^{14}C with 400 g of carrier propanediol was administered intraruminally to a lactating cow. During the next 24 hours, the percentage of the dose recovered was 43.7 in CO_2, 12.4 in milk, 3.5 to 7 in urine, and less than 0.1 in feces. The propanediol was predominantly absorbed from the rumen without alteration, although some conversion to propionic acid in the rumen was detected. The maximal level of propanediol in milk was 0.04 mg/ml. Distribution of ^{14}C among the carbons of lactose and glutamic acid indicated conversion of propanediol to glucose via carboxylation of pyruvate to oxalacetate. These results demonstrate that propanediol is glucogenic in the classical sense of that term; namely, that it is metabolized via intermediates, probably pyruvate and oxalacetate, which can lead to net synthesis of glucose. The small concentrations of propanediol in peripheral blood and the approximately 2-hour delay in attaining maximal specific activity in CO_2 after attaining maximal specific activity of blood glucose indicated that glucogenesis was primarily hepatic with oxidation primarily occurring in other tissues.

A previous investigation showed that intraruminal doses of 0.9 kg of DL-1,2-propanediol produced maximal levels of propanediol in peripheral blood of 57 mg/100 ml at 2 hours after administration (1). This concentration in blood was only 11% of the ruminal concentration which contrasts sharply with the results from gastric and intravenous doses to nonruminants where peripheral blood concentrations of propanediol were equivalent for both routes of administration in 2 hours (2). Since 1,2-propanediol was resistant to ruminal destruction, it was suggested that the rumen acted as a diluting pool permitting slow absorption (1). Only traces of propanediol appeared in the urine or feces of cows fed 1,2-propanediol for 22 days in increasing amounts to 2.1 kg/day (16.7% of the ration). Maximal concentrations in the blood were attained in 8 days and then declined, which suggested adaptive metabolism (1). Hanslik et al. (2, 3) obtained efficient utilization and apparent adaptive metabolism of 1,2-propanediol in growing rats.

1,2-Propanediol is glycogenic and a precursor of lactic acid in the rat (3, 4). The distribution of ^{14}C in glycogen following tracer doses of propanediol-^{14}C and its continued glycogenesis after enolase inhibition have been considered evidence for propanediol conversion directly to glucogenic triose-phosphates (4, 5). However, the published distributions of ^{14}C in glycogen are consistent with gluconeogenesis via the pyruvate carboxylase-phosphoenol pyruvate carboxykinase route (4–8). Recent reviews on the metabolism of 1,2-propanediol indicate its oxidation to lactaldehyde and then lactic acid by several enzymes with low specificity (7, 8).

The present report describes the fate of a nutrient-size dose of 1,2-propanediol-2-^{14}C in a lactating cow. To clarify the pathway of glucogenesis, milk lactose and glutamic acid were degraded. The radioactivities of rumen fermentation products and of essential amino acids in casein were examined to determine the extent of ruminal metabolism of propanediol.

EXPERIMENTAL

A 653-kg cow in the eighth month of pregnancy and producing 12.2 kg of milk per day was placed in an open-circuit

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respiration apparatus equipped for continuous monitoring of respiratory CO_2 and radioactivity. Its ration was 7.7 kg alfalfa hay plus 12.5 kg of a soybean meal-corn grain mixture (16% crude protein). It had an established rumen fistula with plastic closure through which 400 g of DL-1,2-propanediol-2-^{14}C (217 μCi/g atom carbon) in 1.8 liters of aqueous solution was introduced in a single dose. This solution was manually mixed with the rumen contents. Respiratory CO_2 and radioactivity were measured continuously for 6 hours after which the cow was returned to its stall for feed and water until 18 hours after dosing. Respiration data were again collected during the 18- to 24-hour period of the experiment.

Milk was removed with the aid of oxytocin (15 U, iv) immediately before administration of the propanediol and at 1.1, 3.2, 6.0 and 14.5 hours thereafter. Feces were collected continuously for 24 hours and urine was collected while the cow was in the respiration apparatus. Blood samples were taken from an indwelling jugular catheter, mammary vein, or tail (essentially arterial) at the times indicated in the figures. Since there were no obvious arterio-venous differences for radioactivity or propanediol, the blood samples were pooled. Rumen contents were sampled at the times indicated in figure 1 and 3.6 liters of water were added to the rumen contents 3.5 hours after starting the experiment. A 2.4-g sample of the liver was taken 1.5 hours after administering the propanediol using the biopsy technique of Anderson et al. (9).

Upon collection, all samples were frozen until further processing, and blood samples contained NaF as an additional pre-

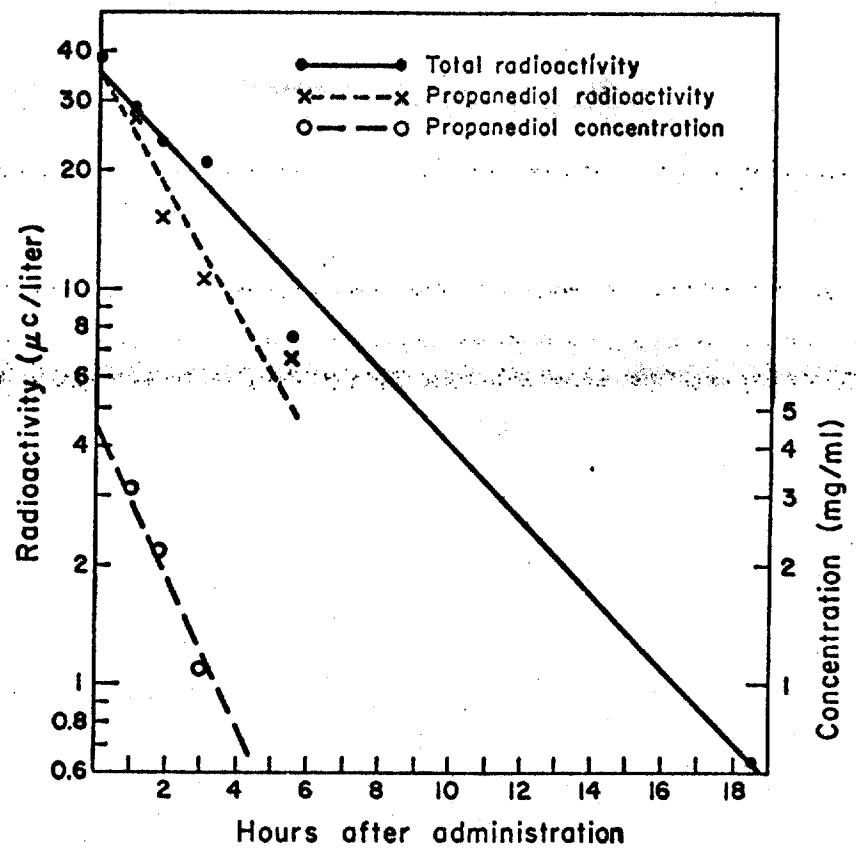

Fig. 1 Disappearance of radioactivity and propanediol from the rumen of a 653-kg cow following administration of 400 g of DL-1,2-propanediol-2-^{14}C (217 μCi/g atom carbon) at zero hours.

servative and anticoagulant. Milk fat, casein and lactose were separated as described by Wood et al. (10) and galactose was isolated as mucic acid as described by Baxter et al. (11). Lactose was assayed by a phenol sulfuric acid procedure (12) and hydrolyzed by autoclaving 1 g with 10 ml 0.5 M HCl in a sealed tube for 2 hours. The hydrolysate was desalted with BioRad AG11A8 ion-retardation resin.[3]

Glucose, derived from lactose, and lactose were fermented to lactic acid with *Lactobacillus casei* (ATCC7469) grown on glucose and lactose, respectively (13). Preliminary experiments showed that the culture fermented only the sugar to which it was adapted. The lactic acid derived from glucose (1.7 mmoles) was oxidized to acetic acid with 1.5 g CrO_3 plus 1 ml H_2SO_4 at 95° for 90 minutes in a volume of about 30 ml H_2O and the SO_2 was collected as described by Bernstein and Wood (13). The lactic acid derived from lactose was recrystallized as zinc lactate before oxidizing to acetic acid with $KMnO_4$ (14). The Schmidt reaction was used to degrade acetic acid (14). Amino acids were isolated from casein and the glutamic acid was degraded step-by-step (15).

Blood glucose was determined by a commercial glucose oxidase procedure[4] before it was isolated as potassium gluconate (16). These isolations were repeated until replicate determinations of the specific activity of blood glucose agreed within 70%.

The liver sample was extracted successively with acetone, ethanol, ether and 10% trichloroacetic acid at room temperature. Carrier glycogen (500 mg) was added to the trichloroacetic acid extract and the glycogen precipitated with an equal volume of ethanol. These liver fractions were dried in a vacuum oven, weighed, and redissolved for determination of radioactivity.

One milliliter of 50% H_2SO_4 was added to each 100 ml of rumen supernatant fluid obtained by centrifugation. Urine samples were concentrated to 50% of their original volume on a steam bath. This procedure gave over 90% recovery of added propanediol. Protein-free filtrates were prepared from blood by adding 2 volumes of 6% $HClO_4$ to 3 volumes of blood. These protein-free filtrates, the rumen samples, urine samples, and the supernatant after removal of casein from milk were subjected to gas chromatography for determination of volatile fatty acids and the propanediol as described previously (1). The carrier gas-flow rate and column length were increased and 86% of the column effluent was diverted to a heated, gas phase proportional radioactivity monitor.[5] Radioactivities of other samples were determined by counting at infinite thickness with a gas-flow, end-window Geiger counter or in a liquid scintillation counter. Internal standards were used to correct for counting efficiency.

RESULTS AND DISCUSSION

The half-life for propanediol in rumen contents was 1.7 hours (fig. 1) which was similar to the 1 hour reported previously (1). The specific activity of propanediol in the rumen during the first 3 hours remained at 217 μCi/g atom carbon. As calculated from figure 1, the 400 g of administered propanediol was distributed in 83 liters, which is a reasonable volume for

[3] Calbiochem, Los Angeles.
[4] Glucostat, Worthington Biochemical Corporation, Freehold, New Jersey.
[5] Nuclear Chicago, Des Plaines, Illinois.

TABLE 1

Concentrations of volatile fatty acids in rumen contents

Time [1]	Proportion of total				Total conc
	C_2	C_3	C_4	C_5 and iso C_5	
hours	*moles/100 moles*				*mM*
1	56	19	13	11	166
2.1	57	23	14	6	183
3	56	25	13	6	168
5	56	25	14	5	59
18.5	59	23	12	6	73

[1] Time after intraruminal administration of 400 g of DL-1,2-propanediol-2-^{14}C (217 μCi/g atom carbon) to a 653-kg cow.

the rumen contents of this cow. The total radioactivity was absorbed more slowly, with a 3.2-hour half-life, indicating retention of some labeled product in the rumen. All of the radioactivity in the 18.5-hour rumen sample was chromatographically identical with propionic acid. Propanediol was absorbed from the rumen at a rate of 41.6%/hour and converted to propionic acid in the rumen at a rate of 19.7%/hour. If this rate is multiplied by the propanediol concentration at 1 hour it can be calculated that 10 μmoles of propionic acid should be formed per ml rumen contents between 1 and 2.1 hours. The actual increase in propionic acid was 11 μmoles which can be calculated from the data in table 1. The small value for total radioactivity at 5 hours may have been due to poor mixing after adding water to rumen at 3.5 hours. These data support the conclusion of Emery et al. (1) that propanediol is primarily absorbed as such from the rumen and also support the partial conversion of propanediol to propionate, as reported by Waldo and Schultz (17).

The propanediol concentration in peripheral blood attained a maximal concentration in 0.5 hours at which time it accounted for 90% of the blood radioactivity (table 2). Glucose gradually accounted for an increasing fraction of the blood radioactivity, attaining a maximum of 50% at 3 hours. After 3 hours, the major portion of blood radioactivity was present in substances other than glucose or propanediol. Blood glucose attained a maximal specific activity of about 61.4 μCi/g atom carbon (fig. 2) between 3 and 5 hours after the administration of propanediol. Since the administered propanediol had a specific activity of 217 μCi/g atom carbon, it can be estimated that about 28% of the blood glucose at 3 hours had been formed from propanediol. Expired CO_2 did not attain maximal specific activity until after 6 hours. The low peripheral blood levels of propanediol and the time delay between the maximal specific activity of blood glucose and expired CO_2 suggests that a major portion of the propanediol was converted to glucose by the liver before oxidation to CO_2. For comparison, glucose injected intraduodenally yielded maximal specific activity for blood glucose within 1 hour and for respiratory CO_2 within 1.5 hours (18). If propanediol had contributed to CO_2 without conversion to some product such as glucose, CO_2 should have reached its maximal specific activity at about 3 hours since the blood was largely cleared of propanediol before this time.

The 24-hour CO_2 excretion contained 43.7% of the administered radioactivity. Assuming that all 3 carbons of propanediol contributed equally to CO_2, 6.9 moles of CO_2 were formed from propanediol out of 140.64 total. Thus, 4.9% of the expired CO_2 came from the propanediol which made up only about 3.3% of the ingested digestible dry matter. Since less than 0.1% of the radioactivity was recovered in feces during the 24-hour period in which 61% of the dose was recovered in other products, propanediol was considered entirely digestible. This conclusion was further supported by the rapid disappearance of radioactivity from the rumen and agrees with data from previous digestion trials (1).

The liver biopsy taken at 1.5 hours contained 0.11 μCi/g. This radioactivity was

TABLE 2

Distribution of radioactivity and concentration of metabolites in blood

Time [1]	Propanediol	Glucose	% of blood radioactivity		
			Propanediol	Glucose	Unidentified
hours	*mg/ml*	*mg/100 ml*	%	%	%
0.5	0.32	66	90	10	0
1.0	—	61	—	13	—
2.0	0.16	82	42	34	24
3.0	0.05	80	21	50	29
3.5	—	80	—	30	—
6.0	0.04	71	16	37	47
18.0		117	—	21	—

[1] Time after intraruminal administration of 400 g of DL-1,2-propanediol-2-^{14}C (217 μCi/g atom carbon) to a 653-kg cow.

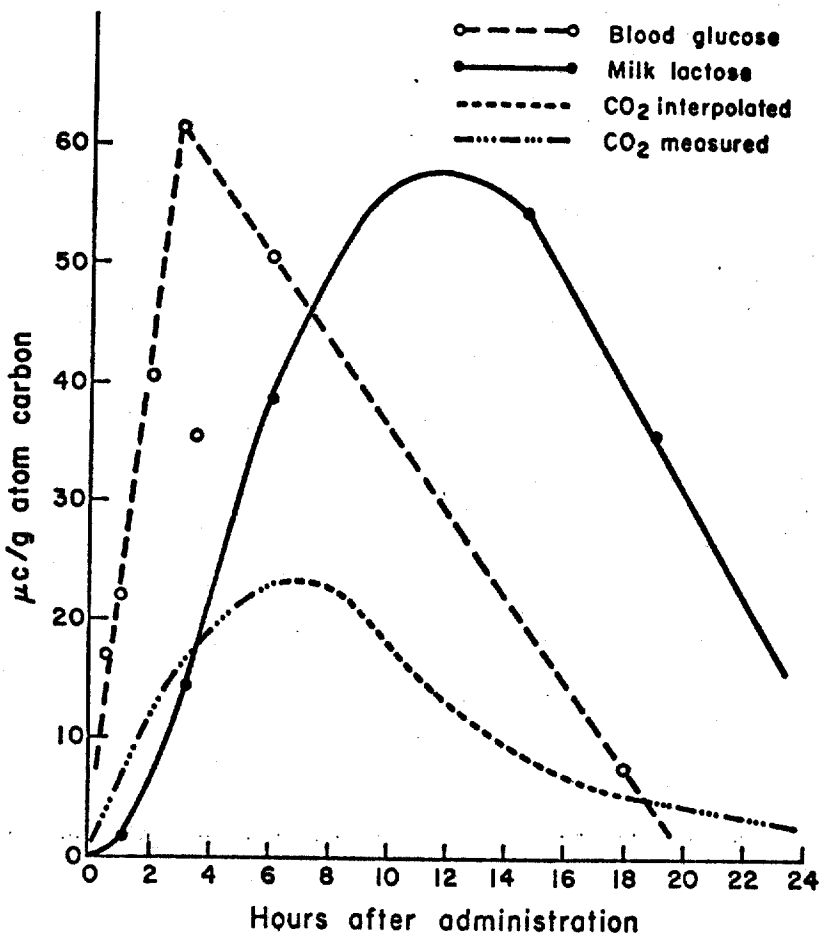

Fig. 2 Specific activities of blood glucose, milk lactose and respiratory CO_2 as a function of time after intraruminal administration of 400 g of DL-1,2-propanediol-2-^{14}C (217 μCi/g atom carbon) to a 653-kg cow at zero hours.

distributed 18% in glycogen, 2% in lipid, 7% in protein residue, and 73% in a cold trichloroacetic acid-soluble fraction, which was not hydrolyzed glycogen because the carrier glycogen was completely recovered. Propanediol at a concentration of 9.5 mg/g liver would account for this trichloroacetic acid-soluble radioactivity but propanediol should have been extracted with the lipid in ethanol-acetone. This trichloroacetic acid-soluble radioactivity may represent metabolic intermediates between propanediol and glucose.

Glycogen accounted for 5.9% of the dry weight of the liver sample. Its specific activity of 0.01 μCi/g was much lower than the 0.03 μCi/g found in blood glucose at the corresponding time. Moreover, liver glycogen could not have contained more than 0.2% of the ^{14}C which had been absorbed at this time. This excludes glycogen as a storage pool for the propanediol activity in this experiment but does not exclude a flux through a small portion of the liver glycogen.

Urine collected during this experiment (3.3 liters) accounted for 3.5% of the ^{14}C dose. However, urine was not collected during a 12-hour period in the middle of the experiment and previous experience suggests about 7 liters should have been excreted during the 24-hour experiment. A more reasonable figure for urine excretion of ^{14}C might be 7% of the dose. Urine collected at 0.4, 2.5 and 5.2 hours contained 0.46, 7.8 and 5.8 mg of free pro-

panediol per ml which accounted for 80, 42 and 57% of the radioactivity in these samples, respectively. Miura (19) reported that propanediol was excreted in urine as a glucuronide. Although the blood (table 2) and urinary radioactivity in unidentified products could have been conjugated propanediol, these products probably were intermediates between glucose and CO_2. Supporting this conclusion, the proportion of radioactivity in unidentified products was small until glucose approached maximal specific activity from which time this proportion increased continuously.

Lactose accounted for 64 to 93% of the radioactivity in milk as shown in table 3. The maximal specific activity of milk lactose was 54.5 μCi/g atom carbon which approached the 61.4 μCi/g atom carbon measured in blood glucose (fig. 2). This close agreement between the specific activities of lactose and blood glucose precluded cycling of glucose through other tissues before conversion to lactose as could be predicted from the conclusion of Bartley and Black (18) that the mammary gland has first priority on blood glucose. Propanediol accounted for only 2.3% and 0.05% of the milk radioactivity at 1.1 and 6.0 hours, respectively. These radioactivities correspond to 1 and 4 mg propanediol per 100 g milk. On the basis of the average amino acid composition of casein and the specific activities of the amino acids isolated from the 6-hour casein sample, 8% of the milk radioactivity was in casein. Casein plus lactose accounted for 76% of the total 6-hour milk radioactivity. Similarly, less than 2% of the 14.5-hour milk radioactivity was present as milk-fat. But, blood acetate is a good carbon precursor for milk fat and hence any acetate derived from propanediol must not have equilibrated with the blood acetate pool. Acetyl-CoA formed in the liver would not be expected to enter the blood acetate pool. The cumulative excretion of radioactivity in milk was 12.4% of the dose. Isotope excretion in milk remained high at termination of the experiment (table 3) and milk was probably a major source of isotope excretion during the next 24 hours.

The specific activities of amino acids from casein reported in table 4 are similar to those found by Black et al. (20), 3

TABLE 3

Distribution of radioactivity in milk

Time [1]	Milk		% of milk radioactivity	
	Amount	Activity	Propanediol	Lactose
hours	*kg*	*μCi/kg*	%	%
1.1	0.68	3.3	2.30	80
3.2	0.95	22.0	—	93
6.0	1.13	69.8	0.05	68
14.5	2.63	86.7	—	66
19.0	2.18	59.4	—	64

[1] Time after intraruminal administration of 400 g of DL-1,2-propanediol-2-^{14}C (217 μCi/g atom carbon) to a 653-kg cow.

TABLE 4

Specific activity of the amino acids from the 6-hour casein [1]

Essential amino acids		Nonessential amino acids	
	μCi/g atom C		*μCi/g atom C*
Methionine	0.04	alanine	24.83
Lysine	0.08	serine	7.93
Leucine	0.08	glutamic acid	13.46
Isoleucine	0.20	aspartic acid	7.96
Valine	0.08	glycine	4.83
Histidine	0.36	proline	0.55
		arginine	0.67

[1] Casein isolated from the milk formed between 3.2 and 6.0 hours after intraruminal administration of DL-1,2-propanediol-2-^{14}C (217 μCi/g atom carbon).

hours after intravenous injection of uniformly labeled glucose. To whatever extent propanediol entered the carbon pools of the rumen bacteria, it would be expected to contribute ^{14}C to the essential amino acids synthesized by these bacteria (19). The low specific activities of essential amino acids relative to the nonessential amino acids is further evidence for a lack of significant propanediol fermentation in the rumen.

Alcohol dehydrogenases which oxidize DL-propanediol to lactaldehyde with reduction of NAD have been isolated from rat and rabbit liver (7, 8). These enzymes had Michaelis constants for propanediol of about the same size as the maximal blood concentration of 4.2 mM observed in this study. The equilibrium constants and low activities of these enzymes were also unfavorable for rapid propanediol metabolism. The characteristics of the propanediol, NADP specific dehydrogenases which have been isolated from several tissues were even less favorable for propanediol utilization (21, 22). These enzymes might account for the slow rate of propanediol metabolism observed in this trial and by Hanzlik et al. (2) following a single dose but would not appear adequate to explain the efficient metabolism of the large amounts used in feeding trials (1, 2). Lactaldehyde is as glycogenic as lactic acid and enzymes accounting for its rapid utilization have been characterized (5, 8). With these enzymes, propanediol-2-^{14}C could proceed to pyruvate-2-^{14}C via lactaldehyde and lactic dehydrogenase. Pyruvate-2-^{14}C could then be carboxylated to oxalacetate as discussed by Utter et al. (6) and, via the tricarboxylic acid cycle, yield glutamate labeled in carbon 3. Equilibration of the oxalacetate with fumarate would cause equal labeling in carbons 2 and 3 and complete equilibrium within the tricarboxylic acid cycle would yield about 50% as much activity in carbon 1 as in carbons 2 and 3 but no labeling in carbons 4 or 5 (23, 24). If pyruvate-2-^{14}C is decarboxylated and enters the cycle as acetate-1-^{14}C, the label appears as glutamate-5-^{14}C. During gluconeogenesis through phosphoenolpyruvate, glucose would be labeled in the 1-, 2-, 5- and 6-position to twice the extent of the labeling in the 3- and 4-position, assuming complete equilibration with the tricarboxylic acid cycle. This model accounts for the intramolecular ^{14}C distribution in glutamate and sugars presented in table 5, if it is assumed that about one-third of the carbon from propanediol entered the tricarboxylic acid cycle as acetate.

Results for the glucose portion of lactose (table 5) were considered less reliable than the results for lactose because glucose standards degraded simultaneously showed about 10% randomization. Glucose standards degraded simultaneously with lactose showed less than 3% randomization. The slightly greater specific activity for lactose compared with its glucose moiety suggested preferential labeling of galactose. However, mucic acid prepared from the galactose moiety had a specific activity of 31 μCi/g atom carbon compared with 38 for the lactose prepared from the 6-hour milk sample and it was concluded that within experimental error glucose and galactose were equally labeled. Although it has been shown that lactaldehyde can combine with triosephosphate to form glycogenic methylpentoses such as fucose, it is doubtful if such a pathway would yield the labeling patterns shown in table 5 (5,25,26). Dilution of glutamic acid carbon

TABLE 5

Distribution of radioactivity in glucose from lactose, and glutamic acid from the 6-hour milk sample

Compound	Carbon no.					
	1	2	3	4	5	6
	μCi/g atom carbon					
Glucose [1]	47.3	49.1	15.4	15.4	49.1	47.3
Lactose [2]	54.1	55.9	10.0	10.0	55.9	54.1
Glutamic acid	10.8	16.6	18.6	4.8	15.2	—

[1] Carbons 1 and 6, 2 and 5, 3 and 4 were determined together.
[2] Glucose and galactose fermented together and carbon pairs degraded as in footnote 1.

by entry of acetate and other tricarboxylic acid cycle intermediates would be expected to cause a lower specific activity in glutamate than in glucose (or lactose) if most of the gluconeogenesis proceeded via oxalacetate without traversing the cycle to glutamate. Glutamic acid would have been much lower in specific activity than lactose if glucogenesis had proceeded by any pathway resulting in direct transfer of propanediol to a triose-phosphate intermediate of glycolysis since glutamate would be further diluted by the flux of carbon through glycolysis (23). To the extent that propanediol was converted to propionic acid in the rumen, some randomization between carbons 2 and 3 of propionic acid would have occurred and the labeling of lactose or glutamic acid would not have differed perceptibly from the model presented above.

These data support the concept of propanediol absorption from the alimentary tract with some ruminal conversion to propionic acid. Absorption of propanediol was apparently followed by nearly complete cor ersion to glucose predominantly in the ' ver and via the established pathway of pyruvate carboxylation (6).

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Chemical Structure and Pharmacological Effects of Glycols, Particularly 1,3-Butylene Glycol

Article by L. Fischer, R. Kopf, A. Loeser and G. Meyer of the Pharmacological Institute, Munster University, in Zeitschrift fur die Gesamte Experimentelle Medizin 115:22-39 (1949).

The divalent alcohols ethylene glycol, propylene glycol, butylene glycol, etc. grouped together under the summary designation of glycols, have in common with the univalent alcohols their narcotic effect, and with the most important representative of the trivalent alcohols, glycerine, the sweet taste and solubility in water. They differ from glycerine in that their effect on the central nervous system is generally stronger. While certain glycols cause partial or permanent damage to the body even in small doses, this does not appear to be valid for others, or possibly under special conditions only.

As regards the true glycols, toxic effects have been reported to date for ethylene glycol (2,3,6,9,10) and, as reported recently by the authors, for 1,4-buthylene glycol (5,7). Comparison of average lethal doses (LD50) of the best known glycols shows that they differ strongly with respect to magnitude and that they cannot be correlated either with the length of the carbon chain or the position of the hydroxyl groups with respect to each other. Rather, they exhibit all transitions from a practical lack of toxicity (1,2-propylene glycol, 1,3-butylene glycol) to the highest effectiveness of very small doses (1,4-butylene glycol, Table 1). With the exception of values given for 1,3-propylene glycol and 2,3-butylene glycol (10), all of the data originate in the authors' own toxicity determinations. The average lethal dose (LD50) determined by the method developed by Karber (1) indicates the amount of the substance per kg of body weight which kills 50% of the experimental animals within 24 hours. The deviations found from numerous literary data (3, 10) should be attributed in part to the higher degree of purity of the glycols used. In part, they are certainly due to the fact that the authors' investigations were conducted on a very large animal material.

In this connection, mention should be made of a fact, which, although generally known, has not been considered sufficiently, according to the authors' knowledge, in the pharmacological and toxicological evaluation of glycols. As is known, univalent alcohols are classified as primary, secondary and tertiary. Accordingly, glycols also differ according to the type of the alcoholic hydroxyl group. If one therefore considers not only the number but also the quality of the hydroxyl groups, three subgroups may be established within the common class of glycols. Group 1 includes glycols which contain the CH2OH group twice (diprimary glycols, e.g. ethylene glycol), Group 2 those which contain the CH2OH group of primary alcohols and the CH.OH group of secondary alcohols (primary-secondary glycols, e.g. 1,2-propylene glycol) and, finally, Group 3, in which the CH.OH group of the secondary alcohols appears twice (disecondary glycols, e.g. 2,3-butylene glycol). Let us mention for the sake of completeness that primary-tertiary, secondary-tertiary and ditertiary glycols are also possible.

TABLE 1. Chemical Structure and Toxicity of Glycols Arranged by the Number of Carbon Atoms

1 Substanz	2 Konstitution	3 LD 50 per os cm³/kg Maus (24 Stunden)
4 Äthylenglykol	$CH_2(OH)-CH_2(OH)$	13,79
1,2-Propylenglykol	$CH_3-CH(OH)-CH_2(OH)$	30,76
1,3-Propylenglykol	$CH_2(OH)-CH_2-CH_2(OH)$	6,0
1,3-Butylenglykol	$CH_3-CH(OH)-CH_2-CH_2(OH)$	23,31
1,4-Butylenglykol	$CH_2(OH)-CH_2-CH_2-CH_2(OH)$	2,14
2,3-Butylenglykol	$CH_3-CH(OH)-CH(OH)-CH_3$	9,0

Key: 1. Substance, 2. Structure, 3. LD50 per os, cm3/kg mouse (24 hours), 4. Ethylene glycol.

The arrangement of glycols listed in Table 1 by these aspects yields two remarkable facts (Table 2). First, it is seen from Table 2 that the toxicity of the individual compounds within the same group appears to be dependent on the length of the carbon chain. This observation is especially obvious in the group of diprimary glycols. If the extension of the investigation to other glycols should result in a repetition of this observation, it would perhaps be possible to establish a rule similar to that found with primary alcohols, where i is known that their toxicity also increases with the number of carbon atoms (12, 13). It should be noted in this context, however, that with divalent alcohols other effects, such as the position of OH groups with respect to each other and other factors can create exceptions from such a schematic interpretation. The second fact derived from Table 2 is the observation that glycols which contain both the CH2OH group of the primary alcohols and the CHOH group of the secondary alcohols, display an astonishingly low toxicity in comparison with all of the other groups. Thus, it is known that 1,2-propylene glycol greatly ressembles in its toxicological behavior the most prominent representative of trivalent alcohols, glycerine (Table 3). Without trying to establish a connection, attention is called to the fact that glycerine also contains a primary and a secondary alcoholic hydroxyl group; it should be noted, however, that the CH2OH group of primary alcohols is represented twice in the compound.

TABLE 2. Chemical Structure and Toxicity of Glycols Arranged by the Type of Hydroxyl Groups

1 Substanz	2 Konstitution	3 LD 50 per os cm³/kg Maus (24 Stunden)
4 I. Zweiwertige diprimäre Alkohole.		
5 Äthylenglykol	$CH_2(OH)-CH_2(OH)$	13,79
1,3-Propylenglykol	$CH_2(OH)-CH_2-CH_2(OH)$	6,0
1,4-Butylenglykol	$CH_2(OH)-CH_2-CH_2-CH_2(OH)$	2,14
6 II. Zweiwertige primär-sekundäre Alkohole.		
1,2-Propylenglykol	$CH_3-CH(OH)-CH_2(OH)$	30,76
1,3-Butylenglykol	$CH_3-CH(OH)-CH_2-CH_2(OH)$	23,31
7 III. Zweiwertige disekundäre Alkohole.		
2,3-Butylenglykol	$CH_3-CH(OH)-CH(OH)-CH_3$	9,0

Key: 1. Substance, 2. Structure, 3. LD50 per os, cm3/kg of mouse (24 hours), 4. Divalent diprimary alcohols, 5. Ethylene glycol, 6. Divalent primary-secondary alcohols, 7. Divalent disecondary alcohols.

TABLE 3. Chemical Structure and Toxicity of Di- and Trivalent Primary-Secondary Alcohols

1 Substanz	2 Konstitution	3 LD 50 per os cm³/kg Maus (24 Stunden)
4 I. Zweiwertige primär-sekundäre Alkohole.		
1,2-Propylenglykol	$CH_3-CH(OH)-CH_2(OH)$	30,76
1,3-Butylenglykol	$CH_3-CH(OH)-CH_2-CH_2(OH)$	23,31
5 II. Dreiwertige primär-sekundäre Alkohole.		
1,2,3-Propantriol (Glycerin)[1]	$CH_2(OH)-CH(OH)-CH_2(OH)$	20,71

6 [1] wasserfreies Glycerin.

Key: 1. Substance, 2. Structure, 3. LD50 per os, cm3/kg of mouse (24 hours), 4. Divalent primary-secondary alcohols, 5. Trivalent primary-secondary alcohols, 6. Anhydrous glycerine.

The exceptional behavior of 1,2-propylene glycol has been known for a long time and has been the subject of numerous investigations (3, 10). It is explained by the fact that the molecule is loosened by the adjacent position of the OH groups and made susceptible to decomposition in the body (10).

A similar behavior is exhibited by 1,3-butylene glycol, which should also be interpreted as a primary-secondary alcohol (Table 2). The authors have investigated the effects of the latter.

1,3-Butylene glycol is a colorless, viscous liquid of a bitter-sweet taste and an odor vaguely ressembling butter. It is miscible with water and alcohol in all proportions and insoluble in ether, lipoid solvents and fatty oils. The boiling point of the pure substance is given differently in the literature, e.g. 185-195o, 203-204o (760 mm) or 207-208o (769 mm). The specific gravity is given as 1.0258 at 20o.

According to Gross (3), 1,3-butylene glycol is used in technology occasionally as a lubricant. Based on its physical and chemical properties, it has been proposed as a substitute for glycerine in the cosmetic, tobacco, paper and textile industries. The material is readily available as an intermediate of synthetic rubber (Buna) production.

The effect of 1,3-butylene glycol, following the oral and subcutaneous administration of sublethal amounts to rats and mice is characterized by the fact that the animals become sluggish and tired after 10-20 minutes, lie on their bellies, lose muscle tone, exhibit accelerated respiration, later they lie on their sides and show respiratory spasms. The phenomena gradually disappear after 4-5 hours following the administration of the substance, their duration and intensity being a function of the amount given.

The same obvious changes occurring as the result of sublethal doses, also appear in cases of lethal intoxication. Here, however, the narcosis deepens rapidly, reflexes disappear and the animals perish because of respiratory paralysis. The mean lethal dose (LD50) for mice was found to be 23.31 cm3 per kg of body weight (per os). The dose depends on the purity of the product and amounts to 19.16 cm3/kg using the commercial grade with oral administration. LD50 for a purified grade of 1,3-butylene glycol is 22.52 cm3/kg and, finally, for the high-purity grade 23.31 cm3/kg (Table 4). The variation of LD50 is the same for rats. The degree of purity also affected the dosage following subcutaneous injection (Table 4).

A comparison of the mean lethal dose of 1,3-butylene glycol with that of glycerine, which generally is considered nontoxic, shows that the LD50 for 1,3-butylene glycol given to mice per os is approximately the same as the LD50 of anhydrous glycerine. With subcutaneous administration, 1,3-butylene glycol was even somewhat less toxic in mice and rats than hydrous glycerine (DAB VI).

TABLE 4. Comparison of the Toxicity of 1,3-Butylene Glycol of Different Grades of Purity in Mice and Rats (cm3/kg, 24 hours)

Reinheitsstufe	Maus		Ratte	
	per os	subcutan	per os	subcutan
1,3-Butylenglykol, technisch[1] . . .	19,16	15,80	28,38	19,42
1,3-Butylenglykol, gereinigt	22,52	—	—	—
1,3-Butylenglykol, Reinprodukt . .	23,31	16,51	29,42	20,06

[1] Nach den Angaben der Herstellerfirma Chemische Werke Hüls sind die Konstanten:

Key: 1. Degree of purity, 2. Mice, 3. Rats, 4. Commercial grade, 5. Purified, 6. High-purity product, 7. According to data provided by the manufacturer, the Huls Chemical Works, the constants are the following:

Technisches Produkt: d_4^{20} = 1,0035, $n_D^{20°}$ = 1,4399, Kp = 202,5°—206,2° (768 mm);

1,3-Butylenglykol: d_4^{20} = 1,0046, $n_D^{20°}$ = 1,4398, Kp = 203,0°—206° (760 mm). (Reinprodukt)

It is remarkable that with rats the intervals of mean lethal doses in case of the oral and subcutaneous administration of glycerine (DAB VI), are far apart (Table 5).

TABLE 5. Comparison of the Toxicity of 1,3-Butylene Glycol and Glycerine in Mice and Rats (cm3/kg, 24 hours)

Substanz	Maus		Ratte	
	per os	subcutan	per os	subcutan
1,3-Butylenglykol (Reinprodukt). .	23,31	16,51	29,42	20,06
1,3-Butylenglykol (Reinprodukt, als 90%ige wäßrige Lösung) . .	24,44	—	—	—
Glycerin, wasserfrei	20,71	—	—	—
Glycerin, DAB VI[1].	30,21	10,78	46,72	16,69

[1] Glycerin DAB VI (83%—87% reines Glycerin).

Key: 1. Substance, 2. Mice, 3. Rats, 4. Pure product, 5. Pure product as a 90% aqueous solution, 6. Anhydrous glycerine, 7. Glycerine DAB VI (83%-87% pure glycerine).

The blood pressure of dogs and rabbits is not changed by the intravenous injection of 0.01-0.2 cm3 1,3-butylene glycol in a physiological table sale solution as 1%, 5%, 10% and 50% solutions.

Only administrations of 0.5 cm3 (50% solution) or 1.0 or 2.0 cm3 pure, undiluted 1,3-butylene glycol depress the blood pressure of dogs by a maximum of 12 mm Hg and that of a rabbits by 58 mm Hg. The degree of depression is a function of the magnitude of the dose given. After 45 and 100 seconds the blood pressure always returns to normal. If glycerine is given in place of 1,3-butylene glycol intravenously in the same concentrations, again only doses of 0.5-2.0 cm3 cause temporary blood pressure depressions in 45-165 seconds, which in the authors experiments amounted to a maximum of 50 mm Hg in dogs and 36 mm Hg in rabbits. A similar behavior was observed by Schubel (11) in cats, following the intravenous injection of glycerine.

The respiration of dogs and rabbits is temporarily accelerated only by doses which also depress the blood pressure. In mice and rats, high doses of both substances also increase the frequency of respiration, which, with a rising severity of intoxication, may develop into convulsive breathing.

Investigation of the sugar content of the blood of sober rabbits by the Hagedorn-Jensen method showed, after a single administration of 3 cm3 of a 50% solution of 1,3-butylene glycol in water per kg of body weight (per os, pharingeal probe), the same fluctuations during a 24-hour observation period as that produced by the ingestation of glycerine (3 cm3/kg, 50% glycerine solution in water, Fig. 1).

The effect of 1,3-butylene glycol on organs with smooth muscles is different. The isolated small intestine of rats is only slightly affected by a 1:400 1,3-butylene glycol solution. Higher concentrations of 1:100 to 1:40, however, result in a strong decline in tone and a cessation of contractions. This inhibiting effect cannot be eliminated by subsequent doses of Prostigmin. As found in agreement with Schubel (11), the isolated small intestine is also calmed by the effect of corresponding glycerine concentrations. However, in this case the intestine tends to recover spontaneously and remains responsive to Prostigmin.

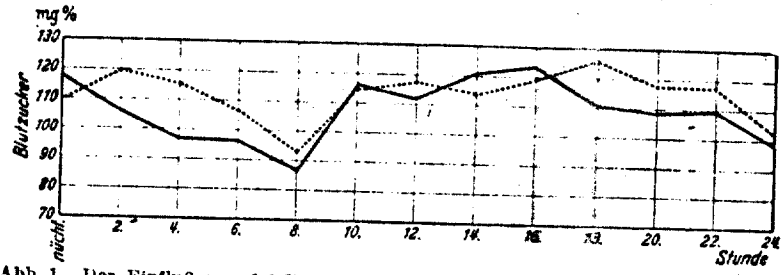

Abb. 1. Der Einfluß von 1,3-Butylenglykol und Glycerin auf den Zuckergehalt des Blutes von Kaninchen. Abszisse: Zeitpunkt der Blutentnahmen in Stunden. Ordinate: Zuckergehalt des Blutes in mg-%. Ausgezogene Linie: 1,3-Butylenglykol (3 cm³/kg, 50%ige Lösung in Wasser). Gestrichelte Linie: Glycerin (3 cm³/kg, 50%ige Lösung in Wasser). Jeder Punkt der Kurven entspricht den Mittelwerten von je 5 nüchternen Kaninchen, denen nach Bestimmung der Ausgangswerte die angegebenen Mengen 1,3-Butylenglykol bzw. Glycerin per os (Schlundsonde) zugeführt worden waren.

FIGURE 1. The effect of 1,3-Butylene glycol and Glycerine on the Sugar Content of Rabbit Blood. Abscissa: Time of blood sampling, hours. Ordinate: sugar content of the blood, mg-%. Solid line: 1,3-butylene glycol (3 cm3/kg, 50% aqueous solution). Broken line: glycerine (3 cm3/kg, 50% aqueous solution), Each point on the curves corresponds to the mean values of 5 sober rabbits, which had been given, following the determination of initial values, the amounts of 1,3-butylene glycol or glycerine per os (pharingeal probe)

Key: 1. Blood sugar, 2. Hour

Natural movements and movements reinforced by a pituitary extract ("Physormon" Promonta) of the uterus of guinea pigs were also inhibited by concentrations of 1,3-butylene glycol sufficient to eliminate the mortility of intestines. Lower concentrations, e.g. 1:400, produce more numerous and more productive contractions. The effect of 1,3-butylene glycol on the tone of the uterus depends on its initial position; it declines when the uterus is initially contracted, but remain unaffected if moderately or completely relaxed.

The special position of 1,3-butylene glycol within the common class of glycols is derived not only from the above investigations, but also from local effects on the skin and mucous membranes and from the behavior of the body in case of continued administration of the substance.

Body Behavior in the Case of Localized Action of 1,3-Butylene Glycol

1. Animal Experiments:

Application of 1,3-butylene glycol for 2 hours to the shorn belly of rats in urethane sleep produces no visible irritation. The condition of the skin, whether intact or artifically injured, has no effect. The same result was obtained with guinea pigs. The experimental procedure consisted of applying once daily 19.16 cm3 of undiluted 1,3-butylene glycol (commercial) to the shorn, whole (14 days) or sacrified (4 days) skin of the belly of guinea (per kg of body weight), i.e. an amount corresponding to the mean

lethal dose of mice and sufficient, if given subcutaneously, to induce narcosis in guinea pigs. The licking of the substance was prevented by immobilizing the animals. After a 2-hour exposure, the glycol was washed off with warm water, the animals dried under an electric dryer and placed in a thermostat. On the 5th or 15th experimental day the animals were killed and their kidneys, livers and bladder histologically examined.

No harmful effect of 1,3-butylene glycol was found during the entire treatment period. All of the guinea pigs behaved normally. Narcotic phenomena were specifically absent. As seen from daily weight measurements, body weights remained unaffected. No protein was found in the urine. No macroscopic or microscopic pathological changes were found in the organs.

In a similar experimental procedure, ethylene glycol (13.79 cm3 per kg of body weight, corresponding to the LD for mice) caused no pathological changes in guinea pigs. In contrast, 1,4-butylene glycol (2.14 cm3 per kg of body weight, corresponding to the LD50 for mice) produced a clear narcotic effect when applied to the sacrificed skin. The depth of narcosis was a function of the extent of the artificially inflicted injuries of the skin. About 75-90 minutes after the application of the substance the animals appeared to be dazed, lay on their bellies and exhibited initially superficial and accelerated breathing, followed by deep and slow respiration. After about 100-110 minutes they turned on their sides and breathed spasmodically. Reflexes were maintained. In the case of one animal, hyperactive reflexes and spasmodic breathing was observed clearly after 330 minutes. After about 480 minutes the narcotic phenomena in general disappeared and the animals resumed normal behavior. The narcotic effect did not occur when 1,4-butylene glycol was applied to the intact skin. 1,4-butylene glycol is thus not capable of penetrating normal skin. It penetrates, however, injured skin in amounts sufficient to cause obvious absorptive remote phenomena.

The sensitive mucous membranes again do not show harmful effects of 1,3-butylene glycol. Thus, a drop of the undiluted substance was applied to the eyes of rabbits and dogs without observing changes in the conjunctive membrane and the cornea. Similarly, visible damage was absent when 1,3-butylene glycol was applied with a brush once or several times to the mucous membrane in the mouth of dogs. 1,3-butylene glycol had no effect on the mucous membrane of the colon. Enema of 1,3-butylene glycol were ineffective in the case of rats, guinea pigs and dogs, in contrast to glycerine enema. The nerves of mucous membranes again showed no reaction to the effect of 1,3-butylene glycol in the sense of irritation -- no vomiting in dogs after the feeding of a probe of 1 cm3/kg, nor any paralysis -- and no anesthesia of the cornea in rabbits. The absence of effects on peripheral nerves in the case of local application is confirmed by the observation that 1,3-butylene glycol, used as eye drops, does not affect the pupilla reaction.

Following the intracutaneous injection of 0.15 cm3 and the subcutaneous injection of 0.8 cm3 of 1,3-butylene glycol in dogs, reactions at the locations of application are no more visible than after the subcutaneous or intramuscular administration of 0.2 cm3. In a similar manner, no changes were found at the vein in the ear of rabbits following the intravenous administration of 1 cm3 of the substance diluted 50% with water.

2. Observations on Humans:

Negative results, similar to those obtained in the animal experiments, resulted from investigations performed with members of the institute, who volunteered their services. In agreement with Gross (3), no local irritation was observed following the application of 1,3-butylene glycol to the intact skin of the upper of forearms. Similarly, no local irritation resulted when a cotton wad saturated with approx. 5 g undiluted 1,3-butylene glycol was applied for 16 days for 24 hours every other day to the skin (3 x 3 cm), covered with Billroth fabric, to expose the skin to the action of 1,3-butylene glycol. The skin also tolerates a repeated exposure to 1,3-butylene glycol after a pause of 12 days, without irritation. None of the experimental persons experienced interference with general well being, the occurrence of protein or blood pigment in the urine, or pathological components in urine sediments. Application to the mucous membrane of the mouth caused no disagreeable sensations or local anesthesia, with the exception of a slight, rapidly passing sensation of heat (heat of solution at the location of the application which is common, more or less, to all glycols). Similarly, no irritation of the mucous membranes of the mouth was observed following the continuous use (4 weeks) of 1,3-butylene glycol-containing toothpaste (menthol 1, 0, Sapo medicat. 10.0, calc. carb. 35.0, magn. carb. 10.0, saccharin 0.05, ol. menth. gtt. XXX, ol. caryoph. gtt. V, 1,3-butylene glycol ad 105.0). Intracutaneous (0.2 cm3) and subcutaneous (0.1 cm3) injection of 1,3-butylene glycol sterilized at 100o causes no irritation at the location of the injection, except for a rapidly passing sensation of burning. No difference was observed in this case between the commercial product and the pure substance. The urine of all the persons was free of protein during the entire experimental period.

Summarizing the results of the above investigations, it may be stated that 1,3-butylene glycol causes no detectable changes of the skin and mucous membranes under the given conditions. This observation results both from animal experiments and observations of humans.

Behavior of Organisms During Continuous Application of 1,3-Butylene Glycol.

The necessity for conducting extended experiments is the result of the empirical fact that substantial differences may exist between the toxicity of a substance in single or multiple applications, which cannot or not always be determined by the results of a

toxicity test. The authors therefore agree with Scholz (10) that "it is not permissible to declare a substance to be harmless on the basis of a single toxicity determination".

1. Experiments on Animals:

In order to study the reaction of the body to the repeated administration of 1,3-butylene glycol, 180 young male rats weighing 75-110 g, maintained under identical conditions and fed kitchen scraps, bran and oats, were divided into three groups. The first group received a daily dose of 1 cm3 of a solution of 1,3-butylene glycol solution per 100 g of body weight, the second 1 cm3 of a solution of glycerine in water, and the third 1 cm3 of distilled water (20 rats). The experimental period was 44 days in each case. 1,3-butylene glycol and glycerine was given in the form of 1%, 5%, 10% and 20% solutions to 20 rats in each group, pharingeal probe. Following the completion of the experiments, the animals were killed and their kidneys, livers and bladders examined histologically.

Although the greater part of the rats in their treatment with 1,3-butylene glycol or glycerine in the given concentrations received approximately a single, double or triple mean lethal dose, their behavior did not differ from that of animals receiving only water. Narcotic effects or diarrhea were not observed. Daily weight controls revealed an increase in body weight in every case. (Fig. 2). This finding deserves special emphasis, because in the experiments young, growing animals were used of which it is known that interference with their normal growth is an especially sensitive indicator for chronically acting poisons. No difference was found with respect to lethality between the treated and the control animals. It amounted to approx. 15% for all groups of animals.

The compatibility of the substance is also supported by the observation that a young dog (4.3 kg) tolerated without reaction repeated doses of a total of 23 cm3 undiluted 1,3-butylene glycol within a period of 14 days. No precipitation of protein in the urine or hematuria was observed.

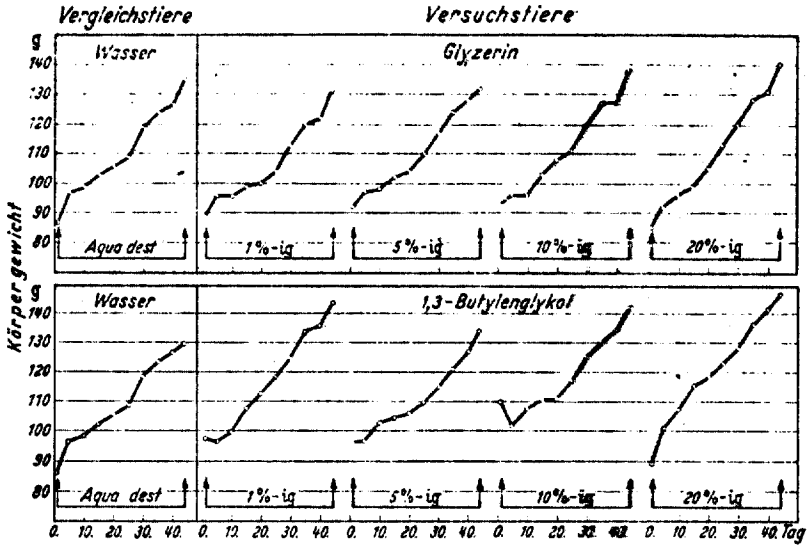

Abb. 2. Wachstumskurven männlicher Ratten bei täglicher Einwirkung von 1,3-Butylenglykol und Glycerin verschiedener Konzentration (per os, Schlundsonde). Links Vergleichstiere (1 cm³ Aqua dest. je 100 g Körpergewicht und Tag). Rechts Versuchstiere (1 cm³/100 g 1,3-Butylenglykol bzw. Glycerin als 1%ige, 5%ige, 10%ige und 20%ige Lösung in Aqua dest.). Abszisse: Behandlungszeit in Tagen. Ordinate: Körpergewicht in Gramm. Jeder Punkt der einzelnen Kurven entspricht den Mittelwerten von je 20 Ratten. Die Pfeile geben die Dauer der Behandlung an.

FIGURE 2. Growth Curves of Male Rats Under the Daily Effect of 1,3-Butylene Glycol and Glycerine of Different Concentrations (per os, pharingeal probe). Left: Control animals (1 cm3 of distilled water per 100 g of body weight and day). Right: Experimental animals (1 cm3/100 g of 1,3-butylene glycol or glycerine as 1%, 5%, 10% or 20% aqueous solutions in distilled water). Abscissa: treatment period in days. Ordinate: body weight in grams. Each point on the curves represents the average value for 20 rats. The arrows indicate the duration of the treatment.

Key: 1. Control animals, 2. Experimental animals, 3. Water, 4. Glycerine, 5. Distilled water, 6. Body weight, 7. Days.

The histological investigation of livers, kidneys and bladders showed no pathological changes.* Specifically, the kidneys lacked any indication of damage. The possibility that with other animal species or following the administration of larger amounts of 1,3-butylene glycol the organs may suffer damage cannot be excluded. Such an occurrence is known in the case of other glycols. They may also be observed when glycerine is used. It should be noted here that the liver, kidneys and bladders of animals which perished during toxicity determinations within 24 hours also lacked pathological changes.

* The authors thank Professor Dr. Giese, Pathological Institute, Municipal Hospitals, City of Bremen, for his review of the histological preparations.

In all experimental groups, in some rats worm eggs were found in the bladder and ocassionally worms in the liver.

During the investigation of the behavior of the organism following the repeated administration of 1,3-butylene glycol, the consumption of oxygen and the blood count were also tested continuously. (Fig. 3).

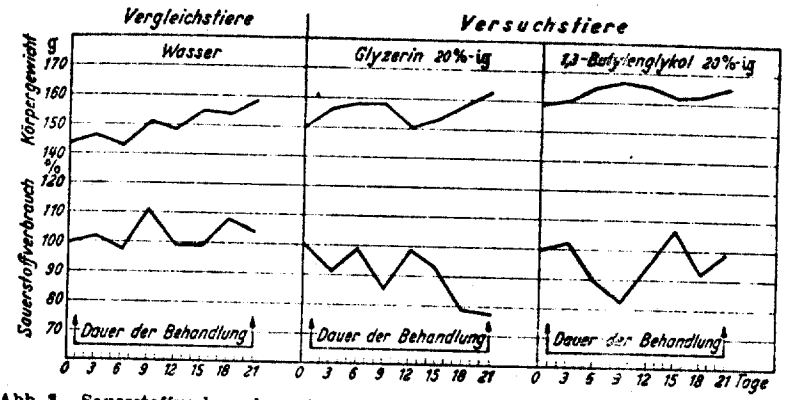

Abb. 3. Sauerstoffverbrauchswerte männlicher Ratten bei täglicher Einwirkung von 1,3-Butylenglykol und Glycerin (per os, Schlundsonde). Links Vergleichstiere (1 cm³ Wasser je 100 g Körpergewicht und Tag). Rechts Versuchstiere (1,3-Butylenglykol bzw. Glycerin; 1 cm³/100 g Körpergewicht und Tag als 20%ige Lösung in Wasser). Abszisse: Behandlungszeit in Tagen (Pfeile). Ordinate: Sauerstoffverbrauch je g/Stunde in Prozent des gleich 100 gesetzten mittleren Ausgangswertes. Obere Kurven Körpergewicht in Gramm. Jeder Punkt der Kurven der Vergleichs- und der mit 1,3-Butylenglykol behandelten Ratten entspricht den Mittelwerten von je 8 Tieren. Die Punkte der Kurven von den Ratten, welche Glycerin erhalten hatten, entsprechen am 6. Versuchstage den Mittelwerten von 7, am 9. denen von 6, am 12. denen von 5, am 15. denen von 4 und am 18. und 21. Tage denen von 3 Tieren.

FIGURE 3. VAlues of Oxygen Consumption in Male Rats Under the Daily Effect of 1,3-Butylene Glycol and Glycerine (per os, pharingeal probe). Left: Control animals (1 cm3 water per 100 g body weight and day). Right: Experimental animals (1,3-Butylene Glycol or glycerine; 1 cm3 per 100 g body weight and day as a 20% aqueous solution). Abscissa: treatment period in days (arrows). Ordinate: oxygen consumption in g/hour as percentage of the average initial value set equal to 100. Upper curves, body weight in grams. Each point on the curves of the control animals and the rats treated with 1,3-butylene glycol corresponds to the mean value of 8 animals. The points of the curves of rats receiving glycerine represent the mean values of 7 rats on the 6th experimental day, of 5 rats on the 12th day, of 4 rats on the 15th and of 3 rats on the 18th and 21st days.

Key: 1. Control animals, 2. Experimental animals, 3. Body weight, 4. Consumption of oxygen, 5. Water, 6. Duration of treatment, 7. Days.

Oxygen Consumption:

Twentyfour male rats weighing 140-160 g were divided into 3 groups of 8 animals each. The rats of Group 1 received daily per os (pharingeal probe) 1 cm3 of tap water and served as the control animals. The animals of Group 2 were given a daily dose

of 1 cm3 of a 20% aqueous solution of glycerine, and the rats of Group 3 1 cm3 of a 20% aqueous solution of 1,3-butylene glycol in water per 100 g body weight. The experimental period was identical for all groups and amounted to 21 days. The consumption of oxygen was determined by the method described by Loeser & al. (8).

The values of oxygen consumption determined show that neither the 20% glycerine nor the 20% 1,3-butylene glycol solutions have clear effects on metabolism in the amounts given. The oxygen consumption of the control animals varied around the mean initial value (set 100%). The oxygen values of rats treated with the 20% glycerine solution exhibited a slight temporary decline on the 3rd and 9th day of treatment. Since, due to the great mortality in this group, only 2 animals remained alive on the 18th day of the experiment, further evaluations had to be discontinued. Following the administration of the 20% 1,3-butylene glycol solution, the oxygen consumption of rats declined temporarily only on the 6th, 9th and 12th day by 10, 17 and 5% (Fig. 3 and Table 6).

Blood Count:

Ten rats weighing approx. 75-100 g received for 44 days a daily dose of 1 cm3 of a 10% aqueous solution of 1,3-butylene glycol per 100 g body weight per os (pharingeal probe). Simultaneously, a second group of 10 male rats of equal weight were given a daily dose of 1 cm3 of a 10% aqueous glycerine solution, and a third group of 10 male rats received daily 1 cm3 of distilled water per 100 g of body weight for the same period of time, per os. The first examination of the blood took place on the 2nd day of treatment, i.e. 7 days after the determination of initial values. The examination was repeated in 7-day intervals. The blood was taken from the tail vein of the animals and examined by conventional clinical hematological methods: hemoglobin determination by the Sahli method, erythrocite and leucocyte count with conventional pipettes in the new Thoma-Zeiss chamber. Differential blood picture: staining in accordance with Pappenheim, evaluation according to Schilling.

As is evident from Fig. 4, the hemoglobin content, the number of erythrocytes and leucocytes, and the values of the white blood cells behaved in a similar manner with respect to both experimental and control animals. Only individual fluctuations were observed. The increase in eosinophiles in animals treated with 1,3-butylene glycol and glycerine is presumably the result of a worm infection. The presence of worms was confirmed in the dissection of the animals.

To complete the above experiments, the hemolytic effect of 1,3-butylene glycol was compared with that of glycerine. It was shown that 1,3-butylene glycol -- and glycerine -- does not cause hemolysis up to a concentration of 40% in a 0.9% table salt solution. Only an 80% concentration of 1,3-butylene glycol caused an incomplete hemolysis after 1 1/2 hours, while glycerine

animals treated with the 10% aqueous solution of 1,3-butylene glycol or the 10% aqueous glycerine solution. No such indication was found in a cat after the administration for 3 days of 5 cm3/kg 1,3-butylene glycol per day.

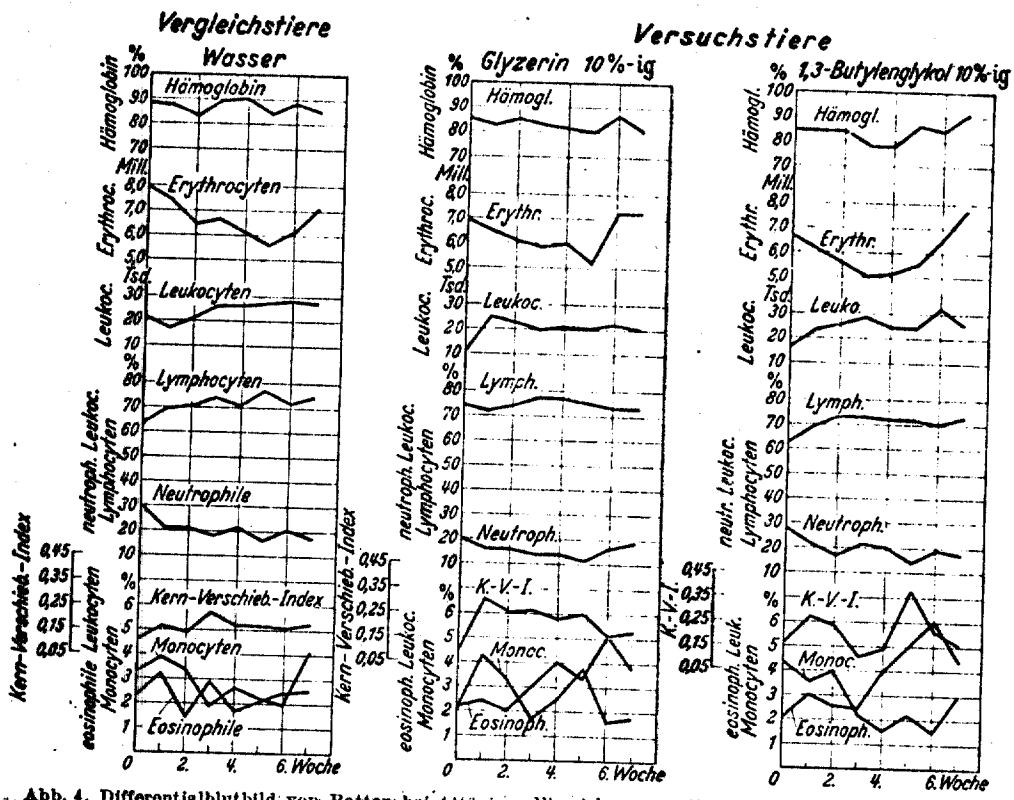

Abb. 4. Differentialblutbild von Ratten bei 44tägiger Einwirkung von täglich 1 cm³ 1,3-Butylenglykol bzw. Glycerin (10 %ige Lösung in Aqua dest.) bzw. 1 cm³ Aqua dest. je 100 g Körpergewicht per os (Schlundsonde). Blutentnahme in 7tägigen Abständen. Die Punkte der einzelnen Kurven entsprechen den Mittelwerten von je 10 Ratten. Abszisse: Zeit in Wochen. Ordinate: Hämoglobin in Prozent, Erythrocyten in Millionen, Leukocyten in Tausend, neutrophile und eosinophile Leukocyten, Lympho-

FIGURE 4. Differential Blood Picture of Rats During a 44-Day Administration of a Daily Dose of 1 cm3 1,3-butylene glycol or Glycerine (10% solution in distilled water) or 1 cm3 distilled water per 100 g of body weight per os (pharingeal probe). Blood sampling in 7-day intervals. The points of the individual curves correspond to the median values of 10 rats in each group. Abscissa: time in weeks. Ordinate: hemoglobin, %. Erythrocites in millions, leucocytes in thousands, lymphocytes and monocytes in % and nuclear displacement index

Key: 1. Control animals, 2. Experimental animals, 3. Water, 4. Nuclear shift index, 5. Weeks.

Observations on Humans:

Auto experiments showed that 1,3-butylene glycol, as a 50% aqueous solution taken on 3 consecutive days internally, does not cause changes in the general condition in doses of 1 cm3 or 2 cm3. Daily urine examinations indicated that the urine was free of protein or other pathological components, both during and after the experimental period.

With respect to the absorption, the destination in the body and the elimination of 1,3-butylene glycol it may be stated, based on observations during the determination of toxicity, that -- measured by the occurrence in time of the narcotic effect -- the substance is absorbed relatively rapidly by the mucous membrane of the digestive tract and the subcutaneous cell tissue. Nothing is known concerning its destination in the body and its location. It is conceivable that in the decomposition of 1,3-butylene glycol in the organism beta-oxybutyric acid and acetoacetic acid are formed as intermediate products. However, these compounds were not detected by the use of conventional clinical investigation methods in the urine.

These investigations were based on the fact that the mean lethal doses of 1,2-propylene glycol and 1,3-butylene glycol are remarkably high in comparison with other glycols, i.e. the toxicity of these bivalent alcohols is astoundingly low. Both substances contain both the CH2OH group of primary alcohols and the CHOH group of secondary alcohols. Based on these approximations, it was initially assumed that the compounds are similar in other effects as well. The assumption has been confirmed by the investigations of the authors. They show that 1,3-butylene glycol, under the given conditions and amounts, does not cause changes in humans and animals indicating any damage to the body. In this respect, the results of the authors are in agreement with the results of Hildebrandt (4), who, based on a few preliminary experiments considered the substance to be practically nontoxic, although he observed a slight precipitation of protein in the case of a dog fed undiluted 1,3-butylene glycol (0.2 g/kg) 14 times in 2 weeks.

A review of the divalent alcohols grouped together in the common pharmacological class of glycols shows that the former unique position of 1,2-propylene glycol with respect to its toxicity cannot be maintained, as shown on the example of 1,3-butylene glycol. As in other groups of substances, there are certain representatives of the glycol series, which on the basis of their effects may be considered practically nontoxic. It is therefore not correct to speak of the toxicity of glycols in general. Every one of them must be evaluated individually. Particular attention must be paid to the glycol derivatives -- esters, ethers, etc. -- which, according to the literature (3, 10), have in general proved to be especially toxic.

The authors have emphasized repeatedly that both in 1,2-propyelen glycol and in 1,3-butylene glycol the CH2OH group

of the primary alcohols and the CHOH group of the secondary alcohols are present. If further investigation should reveal that other primary-secondary alcohols also exhibit such low toxicities, it may be possible to establish a regular relationship between chemical structure and pharmacological and toxic effects. Results obtained to date are inadequate for this purpose; they can serve only as an incentive for additional study.

Summary:

Arrangement of the divalent alcohols ethylene glycol, propylene goycol, butylene glycol and their isomers by the type of hydroxyl groups, they contain indicates that 1,2-propylene glycol as a representative of the primary-secondary alcohols differs from other glycols by its remarkably low toxicity.

According to the present investigation, 1,3-butylene glycol occupies a similar position.

Its repeated application to the skin and mucous membranes of humans and animals for short periods of time did not result in harmful effects. Given per os under the conditions of the experiments, even the repeated administration of aqueous solutions of 1,3-butylene glycol (1, 5, 10 and 20%) left the general condition, growth oxygen consumption and blood count of rats unaffected. Specifically absent are pathological changes in kidneys, livers and bladders, such as caused by other glycols. In humans, the urine remains free of protein after the repeated ingestion of small amounts of 1,3-butylene glycol (50% aqueous solution, per os).

Being an alcohol, 1,3-butylene glycol has a narcotic effect when given in high doses. The mean lethal dose (LD50) was found to be 23.31 cm3/kg given per os and 16.51 cm3/kg subcutaneously for mice, 29.42 cm3/kg per os and 20.06 cm3/kg subcutaneously for rats. The values are a function of the purity of the substance. The effect of 1,3-butylene glycol on respiration, blood pressure, blood sugar and the isolated intestine and uterus were investigated.

The potential significance of the type of hydroxyl groups with respect to the effect of glycols is discussed.

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Inactivation Studies on Pancreatic Lipase. II. The Relation Between Substrate Structure and Inactivation Effect

Paul J. Fodor [1]

From the National Cancer Institute, National Institutes of Health, Bethesda, Maryland

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Introduction

Studies on the differential inactivation of hog pancreas homogenates and of fractions thereof by pretreatment of the enzymes with heat, alkali, and by digestion of a pancreas fraction with crystalline trypsin have shown the presence of an enzymatic component which is specific for the monovalent alcohol esters and another which is specific for the triesters of glycerol (1,2). While the former undergoes a decrease to a very low level of activity by any of the aforementioned inactivation procedures, the activity loss of the second is of a much smaller order of magnitude. The aim of the present study is to establish the relation between the inactivation effect and the substrate structure by employing substrates which represent a series of intermediate compounds between monovalent alcohol and trivalent alcohol esters with monocarboxylic acids. In addition, the enzymatic susceptibility of monovalent alcohol esters of some polycarboxylic acids has been studied.

As in the previous investigations the procedures have included the following: (*a*) differential heat inactivation of the glycerolated homogenates, (*b*) differential alkali inactivation of the aqueous homogenates, and (*c*) determination of the activity of fraction P_L of pancreas obtained by digestion and differential inactivation with crystalline trypsin. All the inactivation experiments were performed as described previously (1).

Experimental

The Substrates

The following three-carbon-chain alcohol esters have been used: tributyrin, α, β-dibutyrin, monobutyrin, α, β-propylene glycol dibutyrate, α-propylene glycol monobutyrate, *n*-propyl-*n*-propionin, α,β-propyleneglycol dipropionate, *n*-propyl *n*-propionate, and in addition tributyl citrate, tributyl aconitate, dibutyl malonate, and dibutyl succinate. In Table I analyses and boiling points of those esters have been given which are not reported in the literature. The esters were prepared by heating corresponding amounts of the acid and alcohol to the boiling point of their mixture for 16 hr. in the presence of an excess of anhydrous $CuSO_4$ and 1 ml. of H_2SO_4/150 ml. of reaction mixture, followed by the usual purification procedure, *i.e.*, washing with bicarbonate solution, drying over Na_2SO_4, and distillation *in vacuo* (3). While this method gave satisfactory results in most instances, the esters of propylene glycol were prepared by the interaction of the acyl chlorides with the alcohols in the presence of an excess of pyridine (2). Monopropionin was prepared by interaction of sodium propionate with glycerol-α-chlorohydrin at 180°C.[2]

TABLE I

Analyses and Boiling Points of Esters

Substrate	Calcd.		Found		Collected at	
	C	H	C	H	Temperature	Pressure
					°C.	mm. Hg
Propylene glycol dibutyrate	61.02	9.32	61.33	9.40	89–91	3
Propylene glycol monobutyrate	57.50	9.65	57.54	9.55	63–65	3
Propylene glycol dipropionate	57.37	8.56	57.50	9.00	65–67	3
Monopropionin	48.65	8.09	48.53	8.27	109–11	3
Tributyl citrate	59.97	8.92	59.39	8.79	178–80	3
Tributyl aconitate	63.07	8.82	63.27	8.96	167–68	3
Dibutyl succinate	62.55	9.62	62.56	9.69	116–17	3.5

The boiling points of the other esters were compared with those reported in the literature and their purity was ascertained by elemental analysis. For use in the enzyme tests all were emulsified in phosphate buffer of pH 8 with half their weight of gum arabic (with the exception of monobutyrin and monopropionin) and their concentration was adjusted so as to contain, respectively, 1 mmole of the acyl or the alcohol radical in 10 ml. of the emulsions of the polyvalent alcohol or polycarboxylic acid esters. To each 10 ml. of emulsion was added 3 ml. of the enzyme preparation.

Method of Assay

The titration method described before (1) was employed. Since many of the substrates possess a very high initial rate of hydrolysis the zero points were estimated by introducing first the ester emulsion and then the enzyme into neutralized formol in the same proportion as they are present in the final enzyme–substrate mixture. This precaution was necessary since it was observed that in the short time interval that

[1] Research Fellow of the National Cancer Institute. Present address: New York Medical College and Flower Hospital, New York, N. Y.

[2] α,β-Dibutyrin was prepared from redistilled α,β-dichlorohydrin (Eastman Kodak Co.) and sodium butyrate in a similar manner as monopropionin.

elapsed between the mixing of the substrate emulsion with the enzyme and the removal of an aliquot (about 15 sec.) some hydrolysis had already occurred, thus rendering the zero points too high.

The Enzymes

Glycerolated and aqueous hog pancreas homogenates and the fraction P_L obtained from pancreas by differential inactivation with crystalline trypsin (1) were employed as before.[3]

The results of the pretreatment with heat, crystalline trypsin, and alkali are given, respectively, in Tables II, III, and IV.[4]

Discussion

With pancreatic preparations two types of ester hydrolysis under comparable experimental conditions may be observed and, consequently, must be attributed to two different enzymes. The first one, which hydrolyzes the triglycerides, acts upon these substrates with a high initial rate of hydrolysis, which, however, slows down very soon and never, under the present conditions, brings about more than a 40% hydrolysis of the esters. This enzyme possesses a relatively high resistance toward inactivation by either one of the procedures mentioned above. The inactivation seems rather to be concerned with a decrease in the capacity to hydrolyze the substrates at their former high *initial* velocity, than to fail to bring about their hydrolysis at all. The other, more labile enzyme, hydrolyzes the monohydric alcohol esters with a lower initial rate of velocity which is maintained until the hydrolysis has reached 30%; thereafter, the time curves of the hydrolysis cease to remain linear, but continue at least to 90% cleavage. Treatment of this enzyme with heat, alkali, or cyrstalline trypsin results in an almost complete destruction of the hydrolytic capactiy.

[3] The enzymatic behavior of the alkali-treated crude homogenate is similar to that of the purified fraction P_I (1) except at the highest alkalinity used (pH 10–11) where the activity loss of P_I exceeded that of the crude homogenate. Since fraction P_I suffers a decrease in activity after 3 days storage at 4°C. the more stable crude homogenate has been used instead.

[4] It has been found in later experiments that P_L preparations obtained by increasing the trypsin from 156 mg./1300 mg. of enzyme nitrogen (260 ml. of P_I suspension) to 234 mg. fail completely to hydrolyze monovalent alcohol esters. In this case, however, the general decrease in enzymatic activity renders the estimation of smaller activity differences in the above substrate series too difficult. Moreover, incubation of fraction P_I with t[illegible]n should not be extended over the 3-hr. limit owing to its not inconsiderable thermal lability which causes about 30% of activity loss in the trypsin-free control if incubation is extended beyond this time.

All the polyhydric alcohol esters used in this study are attacked by the enzymes with a rather high initial rate of hydrolysis which, however, slows down rapidly after having brought about a 5–8 per cent cleavage of the esters. In this respect the course of their hydrolysis is very similar to that of the triglycerides. This is best exemplified in Fig. 1, presenting the enzymatic behavior of the heat treated glycerolated homogenates towards α-propylene glycol monobutyrate on the one hand and towards *n*-propyl *n*-butyrate on the other. Although the decrease in hydrolysis by the heated homogenate as calculated from the linear part of

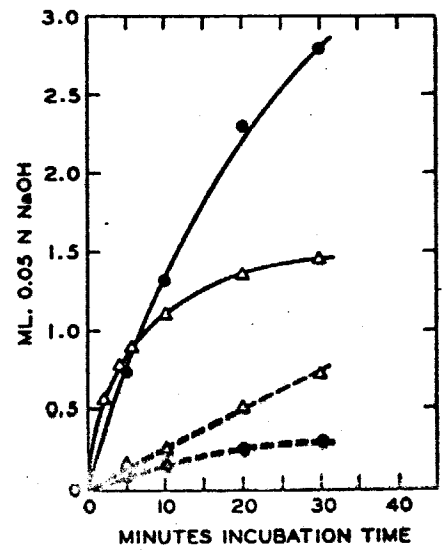

FIG. 1. Hydrolysis of α-propylene glycol monobutyrate and of *n*-propyl *n*-butyrate by heat treated and by the nontreated glycerolated homogenates. Concentration of the enzymes: 0.777 mg. N/ml. Broken lines indicate hydrolysis by homogenate after heat treatment. △ α-Propylene glycol monobutyrate, ● *n*-propyl *n*-butyrate.

the curves is of the same order of magnitude for both esters (Table II) the extent of the cleavage of the propylene glycol ester is considerably higher than that of the propyl alcohol ester even in a rather short time interval. The rather prolonged linear part of the curves produced by the hydrolysis of the propylene glycol ester by the heat-treated homogenate is shared by all the polyhydric alcohol esters used, and is not characteristic of the heat-treated enzymes only but also of the alkali- and trypsin-treated preparations as well.

Table II shows a steady decrease in the residual activity of the heat-treated homogenate toward the esters of the polyhydric alcohol series employed. This decrease corresponds to the decrease of ester linkages in the glycerol and propylene glycol ester molecules and, moreover, shows that of all the homologous esters employed, the triglycerides suffer the smallest percentage losses in the hydrolysis by the heat-

TABLE II

Hydrolysis of the Esters by Heated and Nonheated Glycerolated Homogenates

Activities are calculaetd from the linear part of the time curves and are expressed in μmoles of NaOH consumed/hr./mg. of enzyme nitrogen. Glycerol extract was diluted with phosphate buffer so as to contain 1.554 mg. N/ml. for monopropionin and *n*-propyl *n*-propionate, and 0.777 mg. N/ml. for all the other esters.

Substrate	Nonheated homogenate	Heated homogenate	Decrease in hydrolysis
	activity	*activity*	*per cent*
Tributyrin	1333	512	62
α,β-Dibutyrin	1601	356	78
α,β-Propylene glycol dibutyrate	2257	356	84
Monobutyrin	945	135	86
α-Propylene glycol monobutyrate	1395	210	85
n-Propyl *n*-butyrate	670	42	96
Tripropionin[a]	2657	741	72
α,β-Propylene glycol dipropionate	1445	236	84
Monopropionin	194	0	100
n-Propyl *n*-propionate	150	0	100
Dibutyl malonate	337	0	100
Dibutyl succinate	377	0	100

[a] The activities of both the heat-treated and the nontreated homogenates towards tripropionin and monobutyrin are considerably higher than those given earlier (1). As was noted only recently, the rate of hydrolysis of these esters is extremely high in the first 30 sec. of their enzymatic hydrolysis. The zero values were therefore estimated as described under *Method of Assay*, and the author wishes to correct herewith the values given earlier.

treated homogenate. The results obtained by alkalinization and trypsin treatment (Fraction P_L) of the homogenates are essentially similar (Tables III and IV).

Comparison of the enzymatic susceptibility of the butyl alcohol esters of malonic and of succinic acid with that of the butyric acid esters of dihydric alcohols shows that the activity of the heat-, alkali-, or trypsin-

TABLE III

Hydrolysis of the Esters by Aqueous Homogenates and by Fraction P_L

Activities are expressed as in Table II. Concentration of P_L: For *n*-propyl *n*-butyrate, propylene glycol monobutyrate, α,β-dibutyrin, and dibutyl succinate 0.915 mg. N/ml.; for α,β-propylene glycol dibutyrate 0.591 mg. N/ml.; for dibutyl malonate and tributyrin 0.660 mg. N/ml.; for monobutyrin 1.000 mg. N/ml.; and for monopropionin and propyl *n*-propionate 2.000 mg. N/ml. Concentration of homogenate: 0.460 mg. N/ml.

Substrate	Pancreas homogenate	Fraction P_L	Decrease in hydrolysis
	activity	*activity*	*per cent*
Tributyrin	2723	980	64
α,β-Dibutyrin	2986	658	78
α,β-Propylene glycol dibutyrate	4019	766	81
α-Propylene glycol monobutyrate	2151	311	85
Monobutyrin	1741	243	85
n-Propyl *n*-butyrate	1464	50	97
Tripropionin	5044	2000	60
α,β-Propylene glycol dipropionate	3094	533	83
Monopropionin	386	40	90
n-Propyl *n*-propionate	319	29	91
Dibutyl malonate	840	0	100
Dibutyl succinate	840	0	100

TABLE IV

Hydrolysis of the Esters by Homogenates Treated to pH 10.5 and by the Nontreated Control

Activities are calculated as in Table II. Enzyme concentration: For dibutyl malonate and dibutyl succinate 1.020 mg. N/ml.; for monopropionin and *n*-propyl *n*-propionate 2.012 mg. N/ml.; for all other esters 0.500 mg. N/ml.

Substrate	Nontreated homogenate	Treated to pH 10.5	Decrease in hydrolysis
	activity	*activity*	*per cent*
Tributyrin	2723	1842	32
α,β-Dibutyrin	2968	1456	51
α,β-Propylene glycol dibutyrate	4019	2630	35
Monobutyrin	1741	617	65
α-Propylene glycol monobutyrate	2151	1430	40
n-Propyl *n*-butyrate	1464	237	84
Tripropionin	5044	4641	7
α,β-Propylene glycol dipropionate	3094	2506	19
Monopropionin	386	141	64
n-Propyl *n*-propionate	319	108	66
Dibutyl malonate	685	0	100
Dibutyl succinate	795	78	91

treated homogenates has been reduced toward the former either to zero or to a very low level. Moreover, tributyl citrate and tributyl aconitate fail to be hydrolyzed at all by hog pancreas homogenates.

The residual activity in the heat-, alkali-, and trypsin-treated homogenates is thus seemingly concerned with the polyhydric alcohol and not with the polycarboxylic acid esters.

Acknowledgment

The author is indebted to Mr. R. J. Koegel for the elemental analyses.

Summary

The relation between the substrate structure and the rate of hydrolysis of various polyhydric and monovalent alcohol esters with butyric and propionic acid by differentially inactivated hog pancrease homogenates has been investigated.

Percentage losses in the initial rates of the hydrolysis of the 3-carbon-chain alcohol esters increased in the following order: tributyrin, α,β-propylene glycol dibutyrate, *n*-propyl *n*-butyrate; and in the butyrin series: tributyrin, dibutyrin, and monobutyrin. Similarly, the loss in hydrolysis suffered by α-propylene glycol monobutyrate exceeded that of α,β-propylene glycol dibutyrate. Analogous results have been obtained with the propionic acid ester homologs.

Butyl alcohol diesters of malonic and of succinic acid fail to be hydrolyzed by pancreas homogenates heated for 25 min. to 60°C. and by pancreas fractions treated with crystalline trypsin. Their enzymatic susceptibility is thus markedly different from that of the diesters of dihydric alcohols. Moreover, tributyl esters of citric and of aconitic acid are not hydrolyzed by hog pancreas homogenates. The comparatively high residual activity of the differentially inactivated homogenates is thus specifically concerned with the polyhydric alcohol esters.

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Anticholinergic Effect of Propylene Glycol

by

Edouard Frommel and Florence Vallette

Propylene glycol is a less toxic solvent than ethylene glycol.

Toxicity

Propylene glycol: s.c. injection to mice for 5 days. Dose 10 cc/kg, 5 mice. None died

Ethylene glycol: s.c. injection of 10 cc/kg. Three mice died after the first injection, 2 after the second injection.

However, propylene glycol is a central nervous system depressant (vasomotor function, respiratory centers) and appears to have pharmacodynamic properties of its own.

Relationship of propylene glycol to mediators of excitation in the vegetative nervous system

A. Cholinergic tests.

Propylene glycol has a definite anticholinergic effect against an acetylcholine aerosol (guinea pigs, method of Halpern).

Propylene glycol inhibits the acetylcholine contraction of the mouse ileum (method of Chang and Gaddum).

Propylene glycol has a slightly anticholinergic effect on acetylcholine hypotension in rat (method of Bayo).

B. Histamine tests.

Propylene glycol does not have a neutralizing effect on histamine aerosol (method of Halpern, guinea pig).

C. Adrenalin tests.

Propylene glycol has no effect on adrenalin hypertension (method of Bayo, rat).

Conclusion

Propylene glycol has a weak anti-acetylcholine effect and is thus capable of modifying the action of pharmacodynamic substances in which it serves as vehicle or solvent.

University of Geneva,
Institute of Therapuetics.

LONG-TERM TOXICITY OF PROPYLENE GLYCOL IN RATS*

(Research Report No. 5/1971)

I.F. Gaunt and F.M.B. Carpanini —— Toxicology

and

P. Grasso and A.B.G. Lansdown —— Pathology

*These studies were carried out between November 1966 and December 1968.

Abstract—Groups of 30 male and 30 female weanling rats were fed for 2 yr on diets containing 0 (control), 6250, 12,500, 25,000 or 50,000 ppm propylene glycol. The treatments were found to have no effect on mortality, body-weight gain, food consumption, haematology, urinary cell excretion, the urine-concentrating ability of the kidneys, organ weights or pathological findings, including the tumour incidence. In a parallel short-term study in groups of 15 males and 15 females fed 0 or 50,000 ppm propylene glycol in the diet for 15 wk, haematological indices, serum and urine analyses and organ weights were again found to be comparable in the control and test groups.

No carcinogenic potential was detected with dietary levels of propylene glycol up to 50,000 ppm, which was also established as the no-untoward-effect level in this study. This level is equivalent to an intake of approximately 2·5 g/kg/day.

INTRODUCTION

Propylene glycol (monopropylene glycol, propane-1,2-diol) of pharmaceutical grade (British Pharmacopoeia) is one of nine solvents permitted for use in food in the UK (The Solvents in Food Regulations 1967, Statutory Instrument 1967, no.1582). In its Report on Solvents, the Food Additives and Contaminants Committee (1966) considered that the evidence of safety for propylene glycol was sufficient to allow its use in food to continue for up to 5 yr, after which further information would be required.

The metabolism of propylene glycol has been studied by several workers. Hiura (1911) isolated propylene glycol monoglucuronic acid from the urine of a rabbit which had received a subcutaneous dose of an aqueous solution of propylene glycol. However, after giving oral doses of 4 m-moles propylene glycol/kg to rabbits Gessner, Parke & Williams (1960) found that only 0-3% of the dose was excreted as the glucuronide and no other urinary metabolites were detected. Lehman & Newman (1937) found, in the dog, that propylene glycol was rapidly absorbed from the gastro-intestinal tract but not from the stomach. They reported a rapid fall in the blood level of the solvent particularly after high oral doses and related this to excretion in the urine which accounted for up to 45% of the dose. They thought that the remainder was converted completely to carbon dioxide and water with lactic and pyruvic acids as possible intermediary metabolites. After giving a single oral dose of propylene glycol amounting to 5 g/kg to a dog, Weil, Woodside, Smyth & Carpenter (1971) found peak blood levels of 560 mg/100 ml at 6-8 hr with these levels being halved by 12 hr.

Propylene glycol can provide a source of energy for animals. Rats grew normally when fed on diets in which up to 25% of the carbohydrate was replaced by propylene glycol and there was an increased glycogen storage in fasted rats given doses as low as 0·5 ml/kg propylene glycol (Hanzlik, Lehman, Van Winkle & Kennedy, 1939a; Hanzlik, Newman, Van Winkle, Lehman & Kennedy, 1939b).

The utilization of propylene glycol in energy-forming pathways was confirmed by Rudney (1950) who showed that it is converted to lactic acid and thence to glucose. Miller, Huggins & Arai (1953) demonstrated a similar pathway for phosphorylated propylene glycol.

Various pharmacological effects have been reported for propylene glycol. Diuresis was seen in dogs after intravenous (Bost & Ruckebusch, 1962) or oral (Lehman & Newman, 1937; Weil et al. 1971) administration. Weil et al. (1971) attributed the increased urine flow to the water produced during the metabolism of the high dietary concentration (20%) of propylene glycol used in their study. A central nervous depressant activity was demonstrated by Hanzlik et al. (1939b) following slow intravenous infusion of a 50% solution in dogs. However, anaesthesia was only induced by doses equivalent to 80-90% of the lethal dose.

After intravenous injection in cats, Seidenfeld & Hanzlik (1932) found decreased arterial blood pressure and a reduced respiratory rate but increased depth of respiration. Following intramuscular doses in rats and rabbits the respiratory rate was increased and there was a loss of equilibrium. At lethal doses these signs were followed by a period of coma.

Several workers have reported haematological effects following the intravenous injection of propylene glycol. Lehman & Newman (1937) reported haemolysis, which they attributed to exposure of the erythrocytes to a high concentration of propylene glycol at the injection site.

The haemolytic action of propylene glycol has also been described by Vaille, Debray, Souchard, Roze & Chariot (1968). They reported haemolysis in the rat after intravenous administration of 10% propylene glycol in normal saline an effect which was minimized by replacing the normal saline with sorbitol.

After intravenous administration to rabbits of propylene glycol in concentrations of up to 50% in normal saline, Brittain & D'Arcy (1962) found no effect on the erythrocyte count, haemoglobin concentration, packed cell volume or fragility of the erythrocytes. They reported an increase in the number of polymorphonuclear leucocytes although the total leucocyte count was unaffected. Weil et al. (1971) found reduced erythrocyte counts and increased numbers of circulating reticulocytes and nucleated erythrocytes together with an increased marrow activity in dogs given oral doses of 5 g propylene glycol/kg/day for 2 yr but not in those given 2 g/kg. They considered that this effect was due to haemolysis caused by the relatively high blood levels of propylene glycol generated by their treatment.

Spector (1956) lists LD_{50} values for propylene glycol in a number of species. These are summarized in Table 1 and show that propylene glycol has a low order of acute toxicity.

Seidenfeld & Hanzlik (1932) administered propylene glycol to groups of five male and five female rats at 1, 2, 5 and 10% of the drinking water for 140 days. The only effect seen was a moderate vacuolation of the centrilobular cells of the liver in the rats given drinking water containing 10% propylene glycol, though this was stated to be little different from the controls. The body weight and consumption of food and water at all levels of treatment were similar to those of the controls. In addition, rats were given drinking water containing 25 or 50% propylene glycol, but this was not consumed and the rats died in 6-9 days.

Morris, Nelson & Calvery (1942) studied the chronic toxicity of propylene glycol in the rat. Groups of ten rats received 0, 2·45 and 4·9% propylene glycol in the diet for 2 yr. No differences between test and control rats were reported other than "slight liver damage".

In a 2-yr study, Weil et al. (1971) gave groups of five male and five female dogs dietary concentrations providing doses of 2 or 5 g propylene glycol/kg/day. They found no histopathological damage but the dogs at the higher treatment level gained more weight than the untreated controls. However, their weight gain was similar to that in dogs given isocaloric amounts of dextrose and was attributed to the calorific value of propylene glycol. The only other effects also occurred at the 5-g/kg level and consisted of the slightly increased urine flow and haemolysis referred to previously. These authors considered the no-effect level in dogs to be 2 g/kg.

There is some indirect evidence of safety from the use of propylene glycol as a solvent for vitamin D for human use (Abrams & Bauer, 1938; Albright, Butler & Bloomberg, 1937).

The present paper describes a long-term feeding study on propylene glycol in rats, carried out as part of the BIBRA safety evaluation programme.

EXPERIMENTAL

Materials. Monopropylene glycol was obtained from the Shell Company Ltd., and was stated to conform to the following specification:

Colour (platinum-cobalt), max 10; specific gravity (20/20°C), 1·038-1·040; distillation range, min 185°C (initial boiling point) to max 189°C (dry point); acidity (as acetic acid), max 0·005% w/w; sulphates, nil; chlorides, max 0·001% w/w; arsenic, max 1 ppm; heavy metals, max 5 ppm.

This conforms to the Standards for propylene glycol BP (British Pharmacopoeia).

Animals and diet. Rats of the Charles River CD strain obtained from an SPF breeding colony (Charles River, France) were fed ground Spillers' Laboratory Small Animal Diet and water ad lib. The animal room was maintained at 21 ± 1°C with a relative humidity of 50-60%.

Experimental design and conduct

Long-term study. Groups of 30 male (body weight 120-150 g) and 30 female (body weight 120-140 g) rats were fed on diets containing 0 (control), 6250, 12,500, 25,000 or 50,000 ppm propylene glycol for 2 yr.

The animals were checked, at least once daily, for deaths or signs of ill health. Any animal which appeared to be ill was isolated, to be returned to its cage if its condition improved or to be killed and autopsied, with preservation of tissue samples if its condition deteriorated. Individual body weights were recorded at 2-wk intervals throughout the study and the food intake was measured over the preceding 24 hr.

At wk 13, 21, 52 and 80 of the study, blood was collected from the tail veins of eight male and eight female rats from each of the groups which had been fed on diets containing 0 (control), 25,000 or 50,000 ppm propylene glycol. An additional bleed of eight rats from the control, 6250 and 12,500 ppm groups was made at wk 54. All the samples were examined for haemoglobin content, packed cell volume and counts of erythrocytes, total leucocytes and the individual types of leucocytes. Counts of reticulocytes were also carried out on the samples obtained after 52, 54 and 80 wk. At wk 104 the haematological investigations were limited to measurements of haemoglobin concentration and examination of a stained blood smear from all surviving rats.

At wk 13, 30 and 52, a urinary concentration test was conducted on selected rats from the control and two highest treatment levels (25,000 and 50,000 ppm). Measurements were made of the specific gravity and volume of urine produced during a 6-hr period of water deprivation, during a 2-hr period following a water load of 25 ml/kg and during a 4-hr period commencing 16 hr after the water load. Urinary cell counts were carried out on the 6-hr samples.

After 104 wk all the surviving animals were killed by exsanugination under barbiturate anaesthesia. An autopsy was conducted during which any macroscopic abnormalities were noted and the brain, heart, liver, spleen, kidneys, adrenals, gonads, stomach, small intestine and caecum were weighed. Samples of these organs and of salivary gland, trachea, aorta, thymus, lymph nodes, pituitary, urinary bladder, colon, rectum, pancreas, uterus, muscle and any tissue that appeared to be abnormal were preserved in 10% buffered formalin. Paraffin wax sections of these samples and of those from rats dying during the study were stained with haematoxylin and eosin for microscopic examination.

Short-term study. Groups of 15 male and 15 female rats of similar age and body weight to those in the long-term study were fed concurrently with the long-term study, on diets containing 0 (control), or 50,000 ppm propylene glycol for 15 wk. Body weight and food consumptions were not recorded as the dietary level was the same as one of those in the long-term study. During the last week of treatment renal concentration tests were carried out as described previously and, in addition, the activity of glutamic-oxalacetic transaminase was estimated (Karmen, 1955) in the 6-hr urine samples. Blood was collected from the aorta at autopsy and a haematological examination was carried out as described previously. Serum was separated and measurements were made of urea concentration (Marsh, Fingerhut & Miller, 1965) and of the activities of glutamic-oxalacetic and glutamic-pyruvic transaminases (Karmen, 1955). The brain, heart, liver, spleen, kidneys, adrenals, gonads and pituitary were weighed at autopsy.

RESULTS

Long-term study

No abnormalities were seen in the appearance or behaviour of the rats. There were no statistically significant differences between treated and control rats in cumulative death rate (Table 2), body-weight gain (Table 3) or food consumption (Table 4). The calculated daily intakes of propylene glycol are shown in Table 4, and in Table 5 the average total amounts consumed per rat up to wk 64 and wk 104 are summarized. Most of the rats survived to wk 64 and figures shown up to this time are the minimum amounts consumed by any of the rats examined and the figures up to wk 104 represent the maximum amounts consumed. There were no significant differences between treated and control rats in the results of the haematological examination (Tables 6 and 7), urinary cell excretion or renal concentration tests (Table 8).

Values of absolute organ weights and weights of organs relative to the terminal body weight were similar in all treated and control groups (Table 9). There was a wide range of histological abnormalities, particularly in kidney, liver and lung, though the incidence was similar in both test and control groups of rats (Table 10). The kidney changes varied in severity and consisted of tubular degeneration, stromal and periglomerular fibrosis and inflammatory-cell infiltration mainly with lymphocytes. Occasional foci of calcification were also seen. The pigment in the kidneys of treated and control animals was found within the cells of the proximal tubules and resembled lipofuscin. The chronic changes found in the lungs were, generally, peribronchial and perivascular lymphocyte cuffing and, in some cases, thickening and collapse of the alveoli. The animals with

pneumonia showed definite polymorphonuclear leucocyte infiltration of the alveoli. Most (86%) of the cases of pneumonia were found in animals which died due to ill health during the course of the study.

The neoplasms found are shown in Table 11. There was a high incidence of mammary fibroadenomas and pituitary adenomas mainly affecting female rats, but the incidence was similar in test and control groups. Only a few of the neoplasms were malignant. The most numerous were subcutaneous fibrosarcomas but these occurred in both treated and control rats. The abdominal fibrosarcoma found in a control male rat was large and occupied the upper part of the abdomen involving the pancreas and liver but not the stomach or bile duct. Numerous small nodules (1-3 mm in diameter) were scattered in the remainder of the pancreatic tissue and mesentery. Larger masses (2 cm in diameter) were found in the lower abdominal cavity on the right vas deferens. The lung contained one metastasis.

Short-term study

There were no significant differences between the control rats and those fed a diet containing 50,000 ppm propylene glycol in the analyses of serum and urine (Table 12), haematological indices (Table 13) or organ weights (Table 14). No abnormalities were seen at autopsy.

DISCUSSION

There were no statistically significant differences between control rats and those fed on diets containing up to 50,000 ppm propylene glycol in the examinations made during the course of the long-term study or in the rats killed after 15 wk or 2 yr.

There may be some doubt concerning the validity of parameters such as organ weights in the 2-yr-old rats due to the small number of survivors examined and the large degree of variance of the results obtained in animals of this age. However, in all cases, the negative findings were substantiated by observations made at earlier periods in the study indicating that at dietary levels of up to 50,000 ppm, propylene glycol has no adverse effect on body-weight gain, mortality, haematology, urinary cell excretion, renal function, serum chemistry or organ weights.

The histopathological changes were consistent with those expected in ageing rats. Their incidence and severity were not related to the level of propylene glycol in the diet. The incidence of chronic infective changes in the lung was higher than expected. Furthermore, a number of rats which died, or were killed due to ill-health, in the early stages of the study, were found to have acute pulmonary infections. This unusually high incidence of pulmonary disease probably accounts for the higher than usual mortality.

The most common tumours found were mammary fibroadenomas, which have been shown to occur spontaneously in a high proportion of 2-yr-old rats of this strain (Pollard & Kajima, 1970; Crain, 1958). Malignant mammary tumours occurred less frequently. The numbers found (in the control group and in animals on the lowest level of treatment only) were similar to the expected incidence (Pollard & Kajima, 1970). Pituitary adenomas were also

common, but the incidence was not related to treatment and such lesions have been described in untreated ageing rats (Simms, 1967). The remaining tumours were infrequent in their occurrence and were found either in both treated and untreated animals or occurred only in rats given low doses of propylene glycol and thus showed no dose-relationship in their incidence. This random distribution and lack of any dose-related effect show that the tumours could not be related to the presence of propylene glycol in the diet of the rats.

Thus no carcinogenic potential has been detected with dietary levels of up to 50,000 ppm propylene glycol (equivalent to an intake of approximately 2·5 g/kg/day) given over most of the lifespan of rats. The no-untoward-effect level established is also 50,000 ppm. This is higher than the level of 24,500 ppm reported by Morris et al. (1942). However, their only finding was undefined "slight liver damage" at a level of 49,000 ppm of the diet, an effect which has not been substantiated in the present work. The no-untoward-effect level found in the present study is similar to the 2 g/kg level established by Weil et al. (1971) in dogs.

Application of a 100-fold safety factor to these results would suggest that an intake of 25 mg/kg/day would be acceptable in man. This is equivalent to a daily intake of 1·75 g/day in a 70-kg adult and proportionately less in children.

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Table 1. Summary of the acute toxicity of propylene glycol

Route of administration	LD_{50} (g/kg) values in				
	Rat	Mouse	Rabbit	Guinea-pig	Dog
Oral	21-32	22-24	19	18-19	-
Intraperitoneal	13	10	-	-	-
Intravenous	-	5-8	4-6	-	25*
Subcutaneous	25-28	19	-	-	-
Intramuscular	13-20	-	-	-	-

* Lethal dose.

Table 2. Cumulative death rate of rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Week no.	Total no. of deaths									
	In males fed dietary levels (ppm) of					In females fed dietary levels (ppm) of				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
20	2	0	0	0	1	0	1	1	0	0
30	2	0	1	0	2	0	1	1	0	0
40	4	0	2	3	4	0	1	1	0	0
48	5	1	3	3	4	0	1	1	0	0
56	5	1	3	3	4	1	2	1	0	1
64	6	4	3	8	4	2	4	2	1	1
72	9	4	4	10	6	2	5	4	3	4
80	10	6	9	13	7	4	7	6	4	5
88	12	7	10	14	11	6	8	8	8	7
96	16	12	12	17	13	11	13	10	11	11
104	18	14	16	19	19	15	18	11	16	15

The values represent the number of rats dead or killed *in extremis* from groups of 30.

The values are shown at 4-weekly intervals in Appendix IV.

Table 3. Mean body weight of rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Week no.	Body weights (g)									
	In males fed dietary levels (ppm) of					In females fed dietary levels (ppm) of				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
0*	141	140	141	141	143	126	126	121	128	126
3	297	284	294	290	293	196	195	196	205	198
13	487	466	482	479	473	288	285	280	293	275
26	601	576	615	583	568	335	344	329	344	314
38	673	659	681	652	652	383	396	377	392	370
52	724	695	729	695	715	451	461	451	458	434
64	778	742	793	728	775	482	511	481	520	472
78	815	784	798	775	804	546	550	507	563	497
90	856	828	837	809	834	590	537	564	594	522
104	926	841	827	810	819	663	588	598	618	595

* Day 1 of feeding.

Results are the means of all surviving animals in each group. (Weights at 2-weekly intervals are shown in Appendix I).

Table 4. Food consumption and propylene glycol intake of rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Sex and dietary level (ppm)	Food consumption and propylene glycol intake at wk									
	0*	3	13	26	38	52	64	78	90	104
	Food consumption (g/rat/day)									
Male										
0	18·9	20·7	17·7	20·8	21·1	19·5	24·4	19·8	22·9	20·6
6250	18·9	20·7	16·2	22·6	19·0	17·0	20·3	21·4	20·6	18·7
12,500	18·9	21·6	15·3	25·8	21·7	18·5	23·9	21·3	22·9	18·3
25,000	19·1	20·9	16·2	26·3	19·6	18·7	20·8	18·7	20·8	16·6
50,000	20·1	21·2	17·3	22·8	20·6	19·0	23·1	20·5	21·1	17·3
Female										
0	14·2	17·0	9·5	13·9	14·7	15·0	18·4	17·9	18·5	18·4
6250	14·2	16·2	12·3	16·0	15·8	15·7	20·2	18·4	18·1	16·4
12,500	15·5	16·3	12·7	15·3	15·4	15·3	17·7	15·8	19·8	16·8
25,000	15·4	17·5	11·6	15·8	15·5	16·2	18·7	17·0	18·0	19·4
50,000	14·2	17·0	11·7	15·1	14·8	15·2	17·1	15·8	15·9	17·8
	Propylene glycol intake (g/kg/day)									
Male										
6250	0·84	0·46	0·22	0·25	0·18	0·20	0·18	0·17	0·14	0·14
12,500	1·67	0·92	0·40	0·52	0·40	0·32	0·38	0·33	0·34	0·28
25,000	3·39	1·80	0·85	1·13	0·75	0·67	0·71	0·60	0·64	0·51
50,000	7·03	3·62	1·83	2·01	1·58	1·33	1·49	1·27	1·26	1·15
Female										
6250	0·70	0·52	0·27	0·29	0·25	0·21	0·25	0·21	0·21	0·17
12,500	1·60	1·04	0·57	0·58	0·51	0·42	0·46	0·39	0·44	0·35
25,000	3·00	2·13	0·99	1·15	0·99	0·88	0·90	0·75	0·76	0·78
50,000	5·63	4·29	2·13	2·40	2·00	1·75	1·81	1·59	1·52	1·50

* Day 1 of feeding.

The values of food consumption represent the means for six cages of five rats. (The values at 2-weekly intervals are shown in Appendix II).

The values for propylene glycol intake are calculated from the data on body weight and food intake. (The values at 2-weekly intervals are shown in Appendix III).

Table 5. Calculated total propylene glycol intake by rats fed on diets containing 6250-50,000 ppm propylene glycol for 2 yr

Week no.	Approximate total intake of propylene glycol (g/rat)							
	In males fed dietary levels (ppm) of				In females fed dietary levels (ppm) of			
	6250	12,500	25,000	50,000	6250	12,500	25,000	50,000
0-64	56	120	230	470	45	86	181	333
0-104	91	193	369	754	76	147	304	556

Table 6. Haematological findings in rats fed on diets containing 0-50,000 ppm propylene glycol for 80 wk

Sex and dietary level (ppm)	No. of animals	Hb (g/100 ml)	PCV (%)	RBC (10^6/mm^3)	Retics (% of RBC)	Leucocytes: Total (10^3/mm^3)	Differential (%): N	E	L	M
Wk 13										
Male										
0	12	16·2	50	8·89	-	15·9	13	2	85	0
25,000	12	16·1	50	8·81	-	17·0	12	1	87	0
50,000	12	16·1	51	8·65	-	15·6	13	1	86	0
Female										
0	12	16·4	49	7·29	-	13·9	10	1	89	0
25,000	11	16·3	51	7·60	-	13·7	11	1	88	0
50,000	12	15·2	48	7·31	-	14·1	12	1	87	0
Wk 21										
Male										
0	12	15·7	49	8·82	-	18·3	17	1	82	0
25,000	12	15·7	50	8·00	-	18·0	18	2	80	0
50,000	12	15·5	49	9·07	-	15·1	17	1	82	0
Female										
0	12	15·2	45	8·11	-	12·0	15	2	83	0
25,000	12	15·0	45	8·13	-	12·9	14	1	85	0
50,000	12	14·1	45	7·80	-	12·5	12	2	86	0
Wk 52										
Male										
0	8	15·7	50	8·59	0·72	16·2	19	3	74	4
25,000	8	14·8	52	8·73	1·05	15·1	15	2	80	3
50,000	8	14·8	52	8·02	1·25	12·4	16	4	77	3
Female										
0	7	16·0	50	7·70	0·96	11·6	17	1	78	4
25,000	8	15·6	46	7·53	1·02	10·8	15	1	79	5
50,000	8	15·5	46	7·33	1·32	9·8	20	3	75	2
Wk 54										
Male										
0	8	15·0	48	7·52	1·54	15·0	-	-	-	-
6250	8	15·0	49	8·38	0·77	15·2	-	-	-	-
12,500	8	14·1	47	8·14	1·48	12·9	-	-	-	-
Female										
0	8	15·1	47	7·54	1·61	12·4	-	-	-	-
6250	8	14·7	45	7·49	1·09	9·5	-	-	-	-
12,500	8	14·7	46	7·50	1·61	11·8	-	-	-	-

Cont'd/....

Table 6. Continued

Sex and dietary level (ppm)	No. of animals	Hb (g/100 ml)	PCV (%)	RBC (10^6/mm^3)	Retics (% of RBC)	Leucocytes: Total (10^3/mm^3)	Leucocytes: Differential (%) N	E	L	M
				Wk 80						
Male										
0	8	15·9	47	8·22	1·43	16·4	29	2	62	7
25,000	8	15·2	47	8·72	1·23	16·6	19	3	71	7
50,000	8	16·4	49	8·72	1·45	14·7	21	3	70	6
Female										
0	8	15·2	45	7·29	1·41	9·6	29	3	64	4
25,000	8	14·3	46	6·87	1·62	9·7	25	3	67	5
50,000	8	14·3	45	7·05	1·36	7·7	28	2	64	6

Hb = Haemoglobin PCV = Packed cell volume RBC = Red blood cells
Retics = Reticulocytes N = Neutrophils E = Eosinophils
L = Lymphocytes M = Monocytes

The figures are means for the numbers of rats shown.

Basophils were not encountered in any of the differential counts at more than 0·5% of the leucocytes.

Table 7. Haematological values for rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Dietary level (ppm)	No. of rats	Hb (g/100 ml)	Differential leucocyte count (%)			
			N	E	L	M
		Males				
0	9	13·3	34	1	65	0
6250	14	13·6	35	1	64	0
12,500	8	15·1	37	2	59	2
25,000	9	13·6	34	1	65	0
50,000	7	13·6	36	2	62	0
		Females				
0	11	15·3	37	1	59	3
6250	8	14·3	37	1	60	2
12,500	14	13·9	44	2	53	1
25,000	11	14·4	42	1	55	2
50,000	11	13·7	44	1	54	1

Hb = Haemoglobin N = Neutrophils E = Eosinophils
L = Lymphocytes M = Monocytes

Figures are the means for the numbers of rats shown.

Basophils were not encountered at more than 0·5% of the leucocytes.

Table 8. Renal concentration tests and urinary cell excretion in rats fed on diets containing 0-50,000 ppm propylene glycol for 1 yr

Sex and dietary level (ppm)	No. of rats examined	Cells (10^3/hr)	Concentration test: Specific gravity 0-6 hr	Concentration test: Specific gravity 16-20 hr	Concentration test: Volume (ml) 0-6 hr	Concentration test: Volume (ml) 16-20 hr	Dilution test (2 hr): Specific gravity	Dilution test (2 hr): Volume (ml)
				Wk 13				
Male								
0	6	1·6	1·044	1·054	3·1	0·9	1·010	4·9
25,000	6	4·0	1·043	1·059	2·5	1·0	1·014	5·3
50,000	6	1·2	1·033	1·058	2·9	1·2	1·008	4·5
Female								
0	6	2·0	1·055	1·060	1·2	0·7	1·007	5·0
25,000	6	3·9	1·038	1·080	2·2	0·6	1·010	3·8
50,000	6	2·2	1·038	1·071	2·4	0·7	1·011	3·5
				Wk 30				
Male								
0	8	6·6	1·029	1·057	3·1	1·5	1·005	8·7
25,000	8	-	1·041	1·059	1·7	1·8	1·009	6·2
50,000	8	-	1·033	1·067	2·5	1·9	1·006	6·2
Female								
0	6	7·6	1·041	1·073	1·5	0·6	1·007	5·5
25,000	6	5·7	1·045	1·060	1·0	0·4	1·010	3·7
50,000	6	9·2	1·032	1·070	1·9	0·6	1·010	6·2
				Wk 52				
Male								
0	10	15·3	1·034	1·052	4·2	1·3	1·005	8·7
25,000	10	13·9	1·039	1·046	3·2	0·7	1·009	8·8
50,000	10	15·7	1·031	1·060	4·3	1·5	1·006	8·8
Female								
0	10	6·8	1·038	1·072	2·0	0·9	1·004	8·8
25,000	10	10·4	1·032	1·066	2·6	0·6	1·007	8·1
50,000	10	10·6	1·028	1·065	1·9	0·8	1·003	8·0

Results are means for the numbers of rats shown.

Table 9. Absolute and relative organ weights of rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Sex and dietary level (ppm)	No. of rats examined	Brain	Heart	Liver	Spleen	Stomach	Small intestine	Caecum	Kidneys	Adrenals*	Gonads†	Terminal body weight (g)
						Organ weight (g)						
Male												
0	9	2·15	2·01	19·86	1·06	2·87	10·42	1·41	4·83	95	3·07	817
6250	14	2·30	1·90	18·37	1·15	2·61	9·29	1·51	5·12	84	3·40	715
12,500	8	2·26	2·08	19·37	1·27	2·76	9·66	1·53	4·98	95	2·97	770
25,000	9	2·17	1·85	18·02	1·23	2·49	11·04	1·54	4·15	77	3·59	749
50,000	7	2·29	2·06	18·18	1·01	3·03	10·68	1·41	4·51	87	2·93	662
Female												
0	11	2·00	1·47	14·04	1·19	1·98	8·44	1·13	2·88	112	119	518
6250	8	2·03	1·56	15·43	1·37	1·86	9·04	1·18	2·[illegible]	122	130	558
12,500	14	2·10	1·48	14·27	1·07	2·09	8·45	1·13	2·86	90	90	546
25,000	11	2·02	1·63	15·29	0·91	2·08	9·30	1·28	3·37	99	101	553
50,000	11	2·10	1·43	13·46	0·87	2·13	7·57	1·21	2·83	104	101	524
						Relative organ weight (g/100 g body weight)						
Male												
0	9	0·28	0·25	2·15	0·13	0·35	1·29	0·18	0·59	10	0·41	
6250	14	0·32	0·27	2·57	0·16	0·37	1·30	0·21	0·72	12	0·48	
12,500	8	0·29	0·27	2·51	0·16	0·36	1·25	0·20	0·65	12	0·39	
25,000	9	0·29	0·25	2·41	0·16	0·33	1·51	0·21	0·55	10	0·48	
50,000	7	0·37	0·31	2·84	0·16	0·50	1·67	0·21	0·68	13	0·44	
Female												
0	11	0·42	0·30	2·87	0·21	0·39	1·62	0·22	0·57	22	26	
6250	8	0·37	0·28	2·76	0·25	0·33	1·62	0·21	0·53	22	23	
12,500	14	0·39	0·27	2·61	0·20	0·38	1·55	0·21	0·52	16	22	
25,000	11	0·36	0·29	2·76	0·16	0·38	1·68	0·23	0·60	18	18	
50,000	11	0·40	0·27	2·57	0·17	0·41	1·44	0·23	0·54	20	19	

* Values of the absolute and relative weights of this organ are expressed in mg and mg/100 g body weight respectively.

† Values of absolute and relative weights of female gonads are expressed in mg and mg/100 g body weight respectively.

Values are the means of the numbers of rats shown.

Table 10. Incidence of histological findings (excluding neoplasms) in rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Organ and histological finding	Incidence of finding									
	In males fed dietary levels (ppm) of					In females fed dietary levels (ppm) of				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
No. of rats examined...	26	27	27	23	24	28	25	27	28	27
Kidney										
Yellow-brown pigment	9	9	7	10	8	10	4	11	13	2
Nephropathy	23	22	22	18	18	18	18	20	23	16
Liver										
Fatty change	19	13	15	10	13	24	19	24	21	19
Portal lymphocyte infiltration	9	6	7	6	7	7	5	7	6	6
Nodular hyperplasia	2	4	3	0	1	4	5	2	4	2
Hepatocyte vacuolation	3	7	8	4	9	4	2	3	0	5
Bile duct proliferation	9	11	10	10	8	11	10	9	14	9
Cirrhosis and fibrosis	0	1	0	0	1	0	0	0	0	0
Lung										
Chronic infection	15	17	19	15	18	23	12	12	9	20
Pneumonia	5	5	3	6	7	3	2	5	3	3
Heart										
Focal myocardial fibrosis	7	2	2	1	4	5	1	0	1	2
Focal myocardial lymphocyte infiltration	1	3	2	1	1	2	0	0	1	0
Focal areas of calcification	3	2	3	1	1	1	0	0	0	0
Pancreas										
Acinar degeneration	4	0	1	0	3	0	0	0	0	0
Granulomatous reaction	0	0	0	1	0	2	0	0	0	0

Figures represent the numbers of animals affected out of the total numbers of rats shown.

Table 11. Incidence of neoplasms in rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Organ and neoplasm	Incidence of neoplasms									
	In males fed dietary levels (ppm) of					In females fed dietary levels (ppm) of				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
No. of rats examined...	26	27	27	23	24	28	25	27	28	27
Kidney										
Adenocarcinoma	0	1	0	0	0	0	0	0	1	0
Lung										
Adenoma	1	1	0	0	0	0	2	1	2	0
Brain										
Malignant astrocytoma	1	0	0	0	0	0	0	0	0	0
Pituitary										
Adenoma	2	2	4	1	1	9	7	13	12	4
Adrenal										
Adenoma	1	1	1	2	3	1	0	2	0	1
Pancreas										
Islet cell adenoma	0	1	1	0	1	0	0	0	0	0
Thyroid										
Adenoma	1	1	0	0	0	1	0	0	0	0
Carcinoma	0	0	0	0	0	1	0	0	0	0
Testis										
Interstitial cell tumour	0	1	0	0	1	-	-	-	-	-
Ovary										
Follicular adenoma	-	-	-	-	-	0	0	1	0	0
Salivary gland										
Malignant tumour	0	0	0	0	0	0	0	0	1	0
Subcutaneous tissue										
Fibroma	0	0	0	0	0	0	1	0	0	0
Fibrosarcoma	1	0	1	2	3	2	0	0	0	3
Rhabdomyosarcoma	0	1	0	0	0	0	0	0	0	0

Cont'd/.....

Table 11. Continued

Organ and neoplasm	Incidence of neoplasms									
	In males fed dietary levels (ppm) of					In females fed dietary levels (ppm) of				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
No. of rats examined...	26	27	27	23	24	28	25	27	28	27
Mammary gland										
Fibroadenoma	2	3	2	1	1	17	16	15	18	13
Adenocarcinoma	1	0	0	0	0	0	3	0	0	0
Skin										
Squamous cell carcinoma	0	0	1	0	0	0	0	0	0	0
Lymph tissue										
Lymphoma	0	1	0	1	1	0	0	0	0	0
Abdomen										
Fibrosarcoma	1	0	0	0	0	0	0	0	0	0

Figures represent the numbers of rats with tumours out of the total numbers of rats shown.

Table 12. Analyses of urine and serum of rats fed on diets containing 0 or 50,000 ppm propylene glycol for 15 wk

Dietary level (ppm)	Serum			Urine							
						Concentration test				Dilution test (2 hr)	
						Specific gravity		Volume (ml)			
	Urea (mg/100 ml)	GOT (IU)	GPT (IU)	GOT (IU)	Cells (10^3/hr)	0-6 hr	16-20 hr	0-6 hr	16-20 hr	Specific gravity	Volume (ml)
					Males						
0	18·8	47·3	13·3	4·8	3·7	1·028	1·075	5·0	0·9	1·005	9·3
50,000	22·2	50·8	14·0	5·8	1·3	1·028	1·072	5·0	1·0	1·011	6·3
					Females						
0	20·3	46·3	13·0	4·6	2·2	1·038	1·063	1·8	0·9	1·009	4·8
50,000	23·3	51·5	13·7	5·8	4·0	1·036	1·076	2·3	0·7	1·008	4·1

GOT = Glutamic-oxalacetic transaminase GPT = Glutamic-pyruvic transaminase

The figures are means for groups of nine rats.

Table 13. Haematological findings in rats fed on diets containing 0 or 50,000 ppm propylene glycol for 15 wk

Dietary level (ppm)	No. of rats examined	Hb (g/100 ml)	PCV (%)	RBC ($10^6/mm^3$)	WBC ($10^3/mm^3$)
		Males			
0	14	14·2	44	8·02	7·6
50,000	14	14·3	44	7·97	7·4
		Females			
0	15	13·3	40	7·14	4·5
50,000	14	13·8	42	7·48	4·2

Hb = Haemoglobin PCV = Packed cell volume
RBC = Red blood cells WBC = Leucocytes

Figures are means for the numbers of rats shown.

Table 14. Organ weights and relative organ weights of rats fed on diets containing 0 or 50,000 ppm propylene glycol for 15 wk

ex and ietary level (ppm)	Brain	Heart	Liver	Spleen	Kidneys	Adrenals*	Gonads+	Pituitary*	Terminal body weight (g)
				Organ weights (g)					
ale									
0	1·99	1·29	10·63	0·69	2·70	61	3·20	16·9	443
50,000	1·97	1·42	11·97	0·78	2·90	63	3·37	21·0	473
emale									
0	1·87	0·93	8·64	0·56	1·95	72	153	22·0	307
50,000	1·81	0·90	8·12	0·52	1·92	74	155	20·7	292
				Relative organ weights (g/100 g body weight)					
ale									
0	0·45	0·29	2·40	0·16	0·61	14	0·73	3·8	
50,000	0·43	0·30	2·53	0·17	0·63	14	0·74	3·8	
emale									
0	0·62	0·31	2·81	0·18	0·64	23	51	7·4	
50,000	0·53	0·28	2·67	0·17	0·64	24	53	7·0	

Values of absolute and relative weights of this organ are expressed in mg and mg/100 g body weight respectively.

Values of the absolute and relative weights of female gonads are expressed in mg and mg/100 g body weight respectively.

alues are the means for 15 animals.

Appendix I. Mean body weight (g) of rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Week on test	Sex and dietary level (ppm)									
	Male					Female				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
0*	141	140	141	141	143	126	126	121	128	126
2	248	241	247	245	246	176	178	178	186	178
4	321	312	321	314	318	211	210	210	222	210
6	374	359	369	365	367	236	234	237	248	232
8	414	405	412	406	407	251	249	249	264	246
10	440	431	438	437	430	263	268	267	283	254
12	476	462	472	470	466	281	276	271	285	272
14	500	488	501	491	488	287	291	283	297	281
16	523	507	519	508	505	296	298	293	309	288
18	534	518	532	525	520	304	305	298	314	284
20	557	533	553	539	532	312	312	318	319	300
22	576	551	571	555	548	322	324	319	332	313
24	586	560	581	564	557	329	333	324	340	320
26	601	576	615	583	568	335	344	329	344	314
28	618	589	595	612	586	345	350	335	347	345
30	639	608	628	617	604	357	362	350	362	342
32	647	625	643	624	612	361	372	357	370	348
34	654	635	654	626	638	369	381	352	376	356
36	663	647	671	640	643	375	389	368	385	364
38	673	659	681	652	652	383	396	377	392	370
40	693	674	696	665	669	397	411	389	403	377
42	698	676	701	672	676	402	421	400	413	398
44	705	672	695	676	682	410	428	410	425	393
46	715	677	695	684	691	426	457	418	443	405
48	723	686	692	694	701	437	456	430	457	416
50	731	691	724	705	688	444	461	442	461	425
52	724	695	729	695	715	451	461	451	458	434
54	741	706	745	708	727	453	472	460	479	438
56	743	713	756	708	742	466	475	461	486	443
58	758	712	764	720	745	469	481	466	487	454
60	755	719	777	725	755	471	490	471	495	463

Appendix I. Continued

Week on test	Sex and dietary level (ppm)									
	Male					Female				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
62	767	729	775	705	765	479	489	473	512	472
64	778	742	793	728	775	482	511	481	520	472
66	772	750	804	744	786	491	520	490	525	495
68	800	762	822	751	788	500	527	501	526	476
70	814	782	820	757	800	514	537	511	542	487
72	824	784	838	772	805	517	549	517	554	491
74	820	783	826	776	801	529	548	520	557	492
76	806	780	828	765	802	536	548	512	569	494
78	815	784	798	775	804	546	550	507	563	497
80	818	809	832	783	815	559	539	515	566	503
82	849	819	848	801	825	566	558	529	578	507
84	845	790	860	801	803	583	578	535	591	517
86	852	829	857	812	821	577	569	547	599	528
88	852	841	868	808	822	581	573	562	589	538
90	856	828	837	809	834	590	537	564	594	522
92	868	836	863	829	837	588	556	588	607	528
94	878	843	887	824	833	618	581	599	607	524
96	934	857	866	825	829	635	588	609	588	548
98	929	850	848	822	826	639	583	609	613	554
100	945	850	869	810	819	641	581	614	619	571
102	926	853	834	815	793	659	598	612	610	588
104	926	841	827	810	819	663	588	598	618	595

* First day of feeding.

The figures are means for all surviving rats.

Appendix II. Mean values of food consumption (g/rat/day) in rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Week on test	Sex and dietary level (ppm)									
	Male					Female				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
0*	18·9	18·9	18·9	19·1	20·1	14·2	14·2	15·5	15·4	14·2
2	20·0	19·5	18·0	21·1	21·3	14·4	15·6	16·2	16·4	15·8
4	21·7	21·3	22·3	21·3	22·6	15·1	14·7	14·3	15·2	13·8
6	19·0	18·6	19·7	19·6	19·3	14·9	14·9	14·0	15·4	14·3
8	19·8	20·1	20·1	20·2	20·2	14·0	15·2	13·6	14·5	12·1
10	21·4	20·8	23·3	13·6	22·4	14·0	12·7	10·4	13·6	11·7
12	18·8	17·3	17·2	17·3	19·0	11·3	13·6	13·0	12·6	10·8
14	19·7	18·4	19·0	18·8	18·5	14·7	13·5	12·8	13·1	12·8
16	20·9	19·5	19·8	20·0	19·1	14·2	13·8	13·5	14·5	13·0
18	20·3	18·2	19·0	20·3	18·9	13·9	13·3	13·0	13·8	12·5
20	20·6	18·6	18·9	19·5	19·1	14·3	13·2	13·3	13·6	13·9
22	22·6	20·4	22·1	21·1	21·0	15·8	16·1	15·2	15·8	15·0
24	19·2	18·7	20·1	19·0	17·8	13·6	14·1	12·6	13·7	12·3
26	20·8	22·6	25·8	26·3	22·8	13·9	16·0	15·3	15·8	15·1
28	23·2	23·4	22·9	22·7	22·4	15·5	15·7	15·9	15·4	15·2
30	23·2	22·0	23·0	22·6	22·7	14·5	15·6	15·6	15·7	16·2
32	22·8	20·2	20·0	19·9	19·4	15·2	15·8	15·5	15·8	14·9
34	22·3	20·6	22·0	20·4	21·9	15·0	16·0	15·8	15·3	14·9
36	20·4	19·2	21·0	19·9	20·4	14·3	15·6	15·1	15·7	14·8
38	21·1	19·0	21·7	19·6	20·6	14·7	15·8	15·4	15·5	14·8
40	19·9	20·2	21·2	20·3	20·6	15·9	15·4	15·3	15·7	13·6
42	24·4	22·1	23·8	23·4	22·5	15·5	17·8	16·8	17·6	16·2
44	21·2	18·8	22·2	21·1	21·6	16·3	17·1	17·3	18·0	18·2
46	24·7	15·8	24·4	24·8	24·4	18·9	18·3	18·0	20·6	18·0
48	25·2	23·5	24·4	24·7	23·0	19·0	18·8	18·9	20·2	17·4
50	21·1	20·7	21·3	19·6	21·2	17·0	17·4	16·9	16·9	16·8
52	19·5	17·0	18·5	18·7	19·0	15·0	15·7	15·3	16·2	15·2
54	20·5	20·4	22·3	21·6	20·5	17·4	18·0	18·2	22·6	17·2
56	20·4	19·6	21·9	19·3	22·0	17·6	18·1	16·4	17·1	15·0
58	22·0	19·8	21·6	19·8	20·7	17·9	18·5	16·4	16·2	16·5
60	21·7	19·5	22·0	18·8	20·9	16·0	17·3	17·3	17·9	17·0

Cont'd/.....

Appendix II. Continued

Week on test	Sex and dietary level (ppm)									
	Male					Female				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
62	23·6	23·0	24·4	21·2	22·9	18·6	19·6	18·3	20·7	18·1
64	24·4	20·3	23·9	20·8	23·1	18·4	20·2	17·7	18·7	17·1
66	20·8	21·3	22·6	21·2	21·2	17·4	18·8	17·9	18·1	16·9
68	23·0	21·5	23·0	20·6	19·9	17·8	19·2	18·2	18·7	15·9
70	22·9	21·5	21·0	19·8	21·3	17·4	19·2	17·7	17·8	15·7
72	22·3	21·0	21·3	20·8	20·4	16·3	18·0	15·5	18·3	15·4
74	18·3	19·8	20·4	21·2	21·6	15·7	16·2	15·3	17·4	15·8
76	18·6	17·8	20·1	16·8	21·7	16·5	15·8	14·9	16·5	14·0
78	19·8	21·4	21·3	18·7	20·5	17·9	18·4	15·8	17·0	15·8
80	22·8	21·6	23·4	19·8	22·0	18·5	18·0	17·2	17·5	16·7
82	19·8	15·8	18·5	18·5	16·6	15·7	15·5	15·0	15·6	12·4
84	20·9	20·7	24·6	22·4	24·4	18·8	18·3	18·5	19·4	17·0
86	20·7	21·0	22·1	20·9	20·0	18·3	17·6	19·7	19·7	16·1
88	18·4	20·8	21·6	18·8	20·3	16·5	16·6	18·1	18·8	15·7
90	22·9	20·6	22·9	20·8	21·1	18·5	18·1	19·8	18·0	15·9
92	24·4	18·8	20·0	21·1	22·6	17·8	17·7	18·6	16·0	15·3
94	18·9	20·2	21·0	19·6	20·5	15·8	17·3	18·2	16·1	16·7
96	24·0	22·2	20·2	19·9	20·4	17·4	18·7	17·5	15·6	13·6
98	22·9	23·2	21·0	22·6	19·3	18·2	16·2	18·6	17·9	16·2
100	18·3	17·1	15·8	18·8	18·3	15·8	15·4	15·9	17·1	16·6
102	20·1	20·3	18·0	18·3	17·5	18·7	17·8	16·8	16·9	17·6
104	20·6	18·7	18·3	16·6	17·3	18·4	16·4	16·8	19·4	17·8

* First day of feeding.

The figures are means for six cages initially each containing five rats.

Appendix III. Mean values of solvent intake (g/kg/day) of rats fed on diets containing 6250-50,000 ppm propylene glycol for 2 yr

Week on test	Sex and dietary level (ppm)							
	Male				Female			
	6250	12,500	25,000	50,000	6250	12,500	25,000	50,000
0*	0·84	1·67	3·39	7·03	0·70	1·60	3·00	5·63
2	0·51	0·91	2·15	4·33	0·55	1·13	2·20	4·44
4	0·43	0·87	1·70	3·55	0·44	0·85	1·71	3·29
6	0·32	0·67	1·34	2·63	0·40	0·74	1·55	3·08
8	0·31	0·61	1·24	2·48	0·38	0·68	1·37	2·46
10	0·30	0·66	0·78	2·60	0·30	0·49	1·20	2·30
12	0·23	0·46	0·92	2·04	0·31	0·60	1·11	1·99
14	0·24	0·47	0·96	1·90	0·29	0·57	1·10	2·28
16	0·24	0·48	0·98	1·89	0·29	0·58	1·17	2·26
18	0·22	0·45	0.97	1·82	0·27	0·54	1·10	2·20
20	0·22	0·43	0·90	1·80	0·27	0·53	1·07	2·32
22	0·23	0·48	0·95	1·92	0·31	0·60	1·19	2·40
24	0·21	0·43	0·84	1·60	0·26	0·49	1·01	1·92
26	0·25	0·52	1·13	2·01	0·29	0·58	1·15	2·40
28	0·25	0·48	0·93	1·91	0·28	0·59	1·11	2·20
30	0·23	0·46	0·92	1·88	0·27	0·56	1·08	2·37
32	0·20	0·39	0·80	1·58	0·27	0·54	1·07	2·14
34	0·20	0·42	0·81	1·72	0·26	0·56	1·02	2·12
36	0·19	0·39	0·78	1·59	0·25	0·51	1·02	2·03
38	0·18	0·40	0·75	1·58	0·25	0·51	0·99	2·00
40	0·19	0·38	0·76	1·54	0·23	0·49	0·97	1·80
42	0·20	0·42	0·87	1·66	0·26	0·53	1·07	2·04
44	0·23	0·40	0·78	1·58	0·25	0·53	1·06	2·32
46	0·22	0·44	0·91	1·77	0·25	0·54	1·16	2·22
48	0·19	0·44	0·89	1·64	0·26	0·55	1·11	2·09
50	0·17	0·37	0·70	1·54	0·24	0·48	0·92	1·98
52	0·20	0·32	0·67	1·33	0·21	0·42	0·88	1·75
54	0·19	0·37	0·76	1·41	0·24	0·49	1·18	1·96
56	0·19	0·36	0·68	1·48	0·24	0·49	0·88	1·69
58	0·19	0·35	0·69	1·39	0·24	0·50	0·83	1·82

Cont'd/.....

Appendix III. Continued

Week on test	Sex and dietary level (ppm)							
	Male				Female			
	6250	12,500	25,000	50,000	6250	12,500	25,000	50,000
60	0·21	0·35	0·65	1·38	0·22	0·46	0·90	1·84
62	0·20	0·39	0·75	1·50	0·25	0·48	1·01	1·92
64	0·18	0·38	0·71	1·49	0·25	0·46	0·90	1·81
66	0·18	0·35	0·71	1·35	0·23	0·46	0·86	1·71
68	0·17	0·35	0·69	1·26	0·23	0·45	0·89	1·67
70	0·17	0·32	0·65	1·33	0·22	0·43	0·82	1·61
72	0·16	0·32	0·67	1·27	0·20	0·37	0·83	1·57
74	0·14	0·31	0·68	1·35	0·18	0·37	0·78	1·61
76	0·17	0·30	0·55	1·35	0·18	0·36	0·72	1·42
78	0·17	0·33	0·60	1·27	0·21	0·39	0·75	1·59
80	0·12	0·35	0·63	1·35	0·21	0·42	0·77	1·66
82	0·16	0·27	0·58	1·01	0·17	0·35	0·67	1·22
84	0·16	0·36	0·70	1·52	0·20	0·43	0·82	1·64
86	0·15	0·32	0·64	1·22	0·19	0·45	0·82	1·52
88	0·17	0·31	0·58	1·23	0·18	0·40	0·80	1·46
90	0·14	0·34	0·64	1·26	0·21	0·44	0·76	1·52
92	0·15	0·29	0·64	1·35	0·20	0·40	0·66	1·45
94	0·16	0·30	0·59	1·23	0·19	0·38	0·66	1·59
96	0·17	0·29	0·60	1·23	0·20	0·36	0·66	1·24
98	0·13	0·31	0·69	1·17	0·17	0·38	0·73	1·46
100	0·15	0·23	0·58	1·12	0·17	0·32	0·69	1·45
102	0·14	0·27	0·56	1·10	0·19	0·34	0·69	1·50
104	0·14	0·28	0·51	1·15	0·17	0·35	0·78	1·50

* First day of feeding.

The figures are calculated from the data in Appendices I and II.

Appendix IV. Cumulative death rate of rats fed on diets containing 0-50,000 ppm propylene glycol for 2 yr

Week on test	Sex and dietary level (ppm)									
	Male					Female				
	0	6250	12,500	25,000	50,000	0	6250	12,500	25,000	50,000
10	0	0	0	0	1	0	0	0	0	0
20	2	0	0	0	1	0	1	1	0	0
30	2	0	1	0	2	0	1	1	0	0
40	4	0	2	3	4	0	1	1	0	0
44	4	1	3	3	4	0	1	1	0	0
48	5	1	3	3	4	0	1	1	0	0
52	5	1	3	3	4	0	2	1	0	0
56	5	1	3	3	4	1	2	1	0	1
60	6	2	3	6	4	1	2	1	1	1
64	6	4	3	8	4	2	4	2	1	1
68	9	4	3	9	5	2	5	2	3	3
72	9	4	4	10	6	2	5	4	3	4
76	10	4	6	11	7	3	6	5	4	5
80	10	6	9	13	7	4	7	6	4	5
84	11	6	10	14	8	5	7	6	5	6
88	12	7	10	14	11	6	8	8	8	7
92	13	8	10	16	12	10	12	9	9	10
96	16	12	12	17	13	11	13	10	11	11
100	17	13	14	18	16	11	15	10	14	13
104	18	14	16	19	19	15	18	11	16	15

Values represent the number of rats dead or killed *in extremis* from groups of 30.

The Teratogenic Action of Propylene Glycol (propanediol-1,2) and Propanediol-1,3 in the Chick Embryo

D. O. E. GEBHARDT
National Institute of Public Health, Utrecht, Holland

ABSTRACT When 0.05 ml propylene glycol or a related glycol was introduced into the air chamber during early development most chick embryos survived the treatment. When a glycol was injected on the second day of incubation or later the viability of the embryos was reduced. A maximal sensitivity towards glycols was observed on the fourth day of development, when 90% of the embryos died within two hours and 20% of the surviving embryos had asymmetric malformations of the limbs. Of all the compounds tested in the air chamber, only propanediol-1,3 caused a specific type of malformation (micromelia) in a high percentage of the embryos.

When injections were made into the yolk sac it was found that the injection of a needle prior to incubation caused a high mortality even in the controls. This sensitivity towards a mechanical disturbance diminished rapidly after 24 hours of incubation. With the exception of propanediol-1,3 none of the glycols appeared to be teratogenic when injected into the yolk sac in amounts not exceeding 0.05 ml. When 0.2 ml propylene glycol was introduced into the yolk on the fourth day of incubation a large liquid-containing cyst was observed on the dorsal side of many of the embryos.

The high toxicity of glycols when injected into the air chamber on the fourth day may be attributed to their capacity of destroying the vascular system of the yolk sac.

Recently the question arose whether certain water insoluble organic substances were teratogenic for the chick embryo. It was thought advisable to inject the substances in a soluble form as Williamson et al. ('63) and Mitolo ('63) had shown that nonspecific lesions could be induced in embryos following the administration of insoluble products such as sand. According to the literature propylene glycol is an excellent solvent for this purpose. McLaughlin et al. ('63) and Verret et al. ('64) showed it to be nontoxic when injected prior to incubation in amounts not exceeding 0.05 ml. Furthermore it is completely miscible with albumen, thereby facilitating the transport of dissolved substances to the embryo.

Before using this solvent it nevertheless seemed advisable to study more systematically its embryonic effects. Most authors, such as Karnofsky ('55), Dagg ('55), Landauer and Clark ('62), and Pierro and Spiggle ('66), inject the substance under investigation into the yolk sac. Beaudoin and Wilson ('58), however, did not obtain consistent results when they injected trypan blue into the yolk. Besides, Schlesinger ('58) demonstrated that injection of dye into the yellow yolk yielded different results than those following injection into the white yolk latebra. On the other hand, Freericks ('54) showed that the air chamber is in general more suitable for the introduction of foreign substances.

In the present study a comparison was made between the effect of introducing propylene glycol into the yolk sac and into the air chamber on different days of development. When it was found that 0.05 ml of the solvent injected into the air chamber was highly toxic for the four-day-old embryo it was decided to test other related glycols in a similar manner.

METHODS

Eggs from a White Leghorn strain of known high fertility, supplied by the firm of Van Agten in Leidschendam, were used for all experiments. The average weight of the eggs was 59 gm with a standard deviation of 4 gm. All solvents were sterilized by filtration through an L3 Chamberland candle. The hypodermic syringes and needles were sterilized by heating in an oven for two hours at 180°C.

In general 0.05 ml of a solvent was injected either into the air chamber or the

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yolk. For this purpose a hole was drilled into the large end of the egg with a dental drill, after the air chamber had been located by candling. When the solvent was injected into the yolk, a needle 2 cm long and with an external diameter of 0.5 mm was inserted horizontally through the air chamber. For injection into the air chamber the needle was held at right angle to the long axis of the egg. As soon as the needle was removed from the egg the hole was covered with melted paraffin. The eggs were incubated with the air chamber pointing upward. The incubator, which rotated once every hour, was set at a temperature of 38°C and a relative humidity of 55%. Candling was done on the fourth and sixth days of incubation; all unfertilized and dead embryos were removed from the incubator. In general the gross morphology of the chick embryos was studied on the fifteenth day of incubation. The skeleton was cleared and stained with alizarin S (Dawson, '26).

Of the solvents used in this investigation, propylene glycol and glycerol were supplied by Merck, Darmstadt. The purity of the propylene glycol, determined by gas chromatography, was 99.4%. In addition, propylene glycol of Baker (Baker analyzed reagent) was used. It gave similar results in the chick embryo to those obtained with the Merck product. Propanediol-1,3 (Eastman grade) was obtained from Eastman Organic Chemicals, Rochester, N. Y., ethylene glycol from Baker, Philipsburg, N. J., butanediol-1,3, butanediol-2,3, butanediol-1,4, and diethyleneglycol from the British Drug House, Poole, and 3-hydroxypropionic acid from Koch-Light Laboratories Ltd., Colnbrook, England.

RESULTS

The mortality rate among eggs, following injection of 0.05 ml propylene glycol into the yolk sac on different days of development, is shown in table 1. In figure 1 are given the results of injecting 0.05 ml propylene glycol into the air chamber on different days of development. The unexpected, high mortality following injection on the fourth day of incubation made it desirable to repeat the experiments with larger numbers of eggs and with different dilutions of propylene glycol (table 2).

Surprisingly enough, surviving embryos often had asymmetric malformations of the extremities (plate 1, fig. 2) such as were not observed in more than 1000 control embryos used during these and other experiments. The occurrence of hematoma on the site of the malformation was quite common. Once the toxicity of propylene glycol on the fourth day of incubation had been established, it was decided to determine whether other glycols

TABLE 1

Mortality of embryos following the injection of 0.05 ml propylene glycol into the yolk sac on different days of incubation

Day	Treatment	No. of eggs	Mortality [1]	Malformations
			%	
	None	30	7	0
0	Control [2]	38	55	0
0	0.05 ml Propylene glycol	20	45	0
1	Control [2]	20	20	0
1	0.05 ml Propylene glycol	28	14	0
2	Control [2]	20	10	0
2	0.05 ml Propylene glycol	20	5	0
3	Control [2]	19	5	0
3	0.05 ml Propylene glycol	25	0	0
4	Control [2]	18	5	0
4	0.05 ml Propylene glycol	27	11	0

[1] Per cent embryos that died within the first 15 days of development.
[2] Eggs in which a needle of 2 cm length and 0.5 mm diameter was inserted into the yolk sac for two seconds.

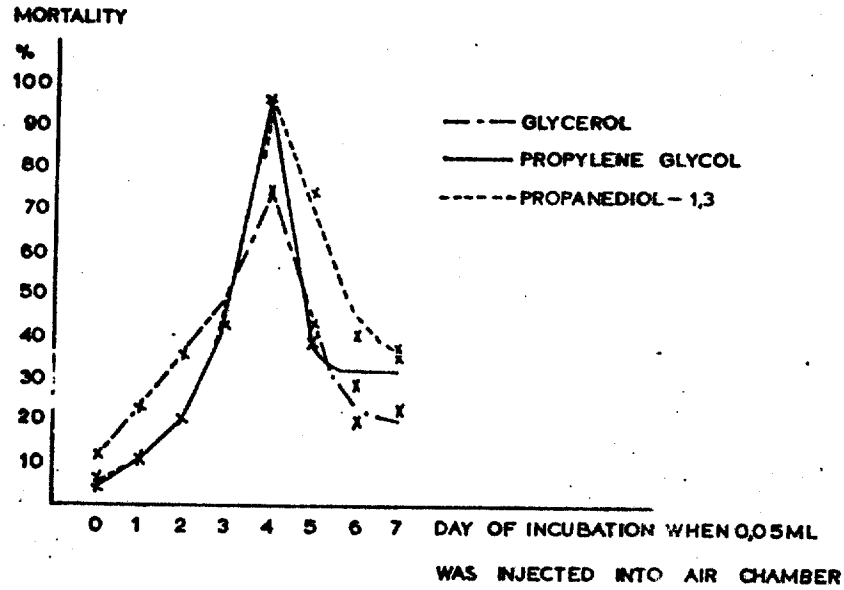

Fig. 1 The mortality of chick embryos when 0.05 ml propylene glycol, propanediol-1,3, or glycerol was injected into the air chamber on different days of incubation. Each point represents the mortality frequency of about 20 eggs. Mortality of control eggs injected with saline never exceeded 10%.

TABLE 2

The teratogenic effect of 0.05 ml propylene glycol of different dilutions injected into the air chamber of four-day-old chick embryos

Dilution [1]	No. of eggs	Mortality [2]	Malformed [3] surviving embryos
		%	%
Undiluted	227	90	21
Diluted 1:1	165	82	27
Diluted 1:2	144	57	8

[1] The propylene glycol was diluted with saline.
[2] Although the mortality was determined on the fifteenth day, most embryos were dead within two hours after injection of the solvent.
[3] Unilateral micromelia. 75% left- and 25% right-sided.

were also toxic in the air chamber. Table 3 shows the results of injecting certain glycols into the air chamber either prior to incubation or on the fourth day of incubation. It follows from this table that most glycols are not toxic when injected prior to incubation but become so when the injection takes place on the fourth day. The mortality rates of eggs treated with glycerol or with propanediol-1,3 on different days of development are also shown in figure 1. They are similar to the rate found for propylene glycol. Likewise table 3 shows that other glycols are capable of inducing asymmetric types of micromelia in embryos.

Another interesting result was that only propanediol-1,3 caused a very specific type of malformation (plate 1, fig. 3) known as micromelia, whose main symptoms were reduction and torsion of the lower limbs often associated with parrot beak. A closer analysis of the micromelic legs by clearing of the skeleton (plate 1, fig. 4) revealed a flexure of the distal part of the diaphysis of the tibia and of the metatarsals, a relative increase in size of the fibula, and a broadening of the distal epiphysis of the tibia. In severe cases a hydropic type of feather was also observed.

The effect of introducing certain glycols and especially propanediol-1,3 into the yolk sac was also studied (table 4). When the latter solvent was injected in this manner, particularly on the third, fourth, or fifth day (table 5), the mortality was lower and the yield of micromelic embryos was higher than after injection into the air chamber. In fact all embryos were micromelic if a high dose, viz. 0.2 ml, of propanediol-1,3 was injected into the yolk on the fourth day of development. On

TABLE 3

The effect on chick embryos of 0.05 ml of different glycols injected into the air chamber either prior to incubation or on the fourth day of incubation

Day of injection	Solvent	No. of eggs	Mortality [1]	% and type malformation in surviving embryos
			%	
0	Saline	20	10	none
0	Butanediol-1,3	17	12	none
0	Butanediol-1,4	17	35	none
0	Butanediol-2,3	18	17	none
0	Propanediol-1,3	26	5	81% micromelia
0	Diethyleneglycol	18	6	none
0	Ethyleneglycol	18	6	none
0	Glycerol	20	10	none
4	Saline	15	0	none
4	Butanediol-1,3	18	89	10% asymmetric micromelia
4	Butanediol-1,4	18	89	not determined [2]
4	Butanediol-2,3	18	89	10% asymmetric micromelia
4	Propanediol-1,3	19	95	100% micromelia
4	Diethyleneglycol	19	79	not determined [2]
4	Ethyleneglycol	27	37	none
4	Glycerol	18	72	not determined [2]

[1] It should be noted that if 10% of a sample of 20 elements react, the 95% confidence interval of this percentage is 3–33%. For a sample of 15 elements this is 2–[illegible]%.
[2] The few surviving embryos were not malformed. Whether these substances also give asymmetric malformations can only be determined if larger number of eggs are used.

TABLE 4

The effect on the chick embryo of 0.05 ml of different glycols injected into the yolk sac on the fourth day of incubation

Solvent	No. of eggs	Mortality	% and type malformation
		%	
Saline	19	10.5	0
Butanediol-1,3	18	22.1	0
Butanediol-1,4	18	5.6	0
Butanediol-2,3	18	0	0
Propanediol-1,3	19	11.0	65% micromelia
Diethyleneglycol	18	0	0
Ethyleneglycol	18	5.6	0
Glycerol	18	11.1	0

TABLE 5

The teratogenic effect of 0.05 ml propanediol-1,3 when injected on different days of incubation into the yolk sac

Day of incubation	No. of eggs	Mortality	Micromelic embryos
		%	%
1	19	11	88
2	20	5	63
3	20	5	53
4	19	11	65
5	19	16	69
6	19	0	79
7	19	16	56
8	20	20	38
9	20	20	19

the other hand a similar dose of propanediol-1,2 never induced micromelic malformations. However, 40% of the embryos treated with 0.2 ml propylene glycol in the yolk on the fourth day developed a large liquid-containing cyst on the dorsal side (plate 1, fig. 5). Such a cyst was only rarely observed when 0.2 ml propanediol-1,3 was administered.

An attempt was also made to protect the embryos from the teratogenic action of propanediol-1,3. Karnofsky ('65) divided substances causing micromelia in the chick embryo into two groups, viz.,

those whose effects are overcome by the simultaneous administration of nicotinamide and those remaining teratogenic in the presence of this vitamin. Table 6 shows that nicotinamide had no protective value for chick embryos even if the minimal teratogenic dose of propanediol-1,3 (0.05 ml of a solution of one part propanediol-1,3 and two parts physiological salt) was used. [Care was taken in these experiments not to inject more than 5 mg nicotinamide per egg, since Landauer ('48) found that in amounts greater than this it is itself a teratogen.]

DISCUSSION

The present results have raised a number of questions. In the first place an explanation must be sought for the high mortality in control eggs injected in the yolk sac prior to incubation (table 1). Most authors, such as Zwilling and de Bell ('50), Beaudoin ('61), Landauer and Clarke ('63), Grabowski ('66), etc., injected substances into the yolk after a day or more of incubation without explicitly stating why unincubated eggs were not used. On the other hand, Carter ('65) also found a mortality of 55% in control eggs injected in the yolk sac prior to incubation.

The difference in results presented here and those of McLaughlin et al. ('63), who injected many thousands of eggs prior to incubation without apparent loss, can probably be attributed to differences in the genetic background of the White Leghorn eggs used. Why piercing the vitelline membrane before incubation is not compatible with survival whereas eggs of later stages of development appear to be insensitive to such an injury is still an open question.

Another problem concerns the lack of toxicity of propylene glycol and most other glycols (fig. 1) when injected into the air chamber during early development and the high toxicity of these substances on the fourth day of incubation. The apparent lack of toxicity of the glycols when injected into the yolk (table 4) also needs explaining. The lethal action of propylene glycol on the fourth day is rapid. Within a few hours many of the extraembryonic blood vessels showed large hemorrhages, which were always situated directly under the site of deposition of the solvent, and were probably the direct cause of death. On the other hand, when solvent is introduced into the air chamber before the development of the blood circulation, it will be diluted with albumen. This diluting effect may be sufficient to prevent damage of the blood vessels when the latter are formed on the second, third, or fourth day. Likewise, the glycols are nontoxic when injected into the yolk, since by this route they are unable to reach the extraembryonic circulation. The reason why ethylene glycol was less toxic than the other glycols in the air chamber on the fourth day remains obscure at present.

A possible explanation for the reduction in sensitivity of the embryo towards glycols in the air chamber from the fifth day of incubation onwards (text fig. 1) may be the following. Romanoff ('60), referring to the work of Cohn and Lange ('30), stated that during the course of incubation the walls of the blood vessels in the area vasculosa undergo a certain amount of histological development. In the four-day-old embryo the arteries consist of a single layer of flat or spindleshaped endothelial

TABLE 6

The effect of propanediol-1,3 alone or in combination with nicotinamide on the chick embryo

Day of injection in yolk	Substance(s) injected	No. of eggs	No. of micromelic embryos
2	0.05 ml propanediol-1,3 (diluted with saline 1:2)	15	2
2	0.05 ml propanediol-1,3 (diluted with saline 1:2) + 5 mg nicotinamide	15	3
4	0.05 ml propanediol-1,3	17	11
4	0.05 ml propanediol-1,3 + 5 mg nicotinamide	17	14

cells. By the tenth day, however, the walls have become about five cell layers thick. Therefore the older blood vessels are probably resistant to the solvents because of their improved structural integrity.

The next point of inquiry concerns the relatively high incidence (ca. 20%) of asymmetric micromelia among those embryos surviving an injection of propylene glycol in the air chamber on the fourth day of incubation (table 2). According to Ancel ('50), these malformations only rarely occur spontaneously. He was unable to detect more than one case of hemimelia among 2000 untreated eggs. It appears that very little work has been done on the induction of asymmetric malformations. Only one reference was found that could be used in interpreting the predominance of leftsided malformations in our experimental material (table 2). Cairns ('41), in a study of the homozygous creeper factor, came to the conclusion that structural disturbances were caused by anomalies of the circulatory system. He obtained leftsided asymmetric malformations experimentally by blocking the extraembronic circulation. Cairns (loc. cit., p. 493) stated "The lack of yolk sac circulation in the creeper embryos suggests itself as the immediate cause for the general cessation of growth, for the degeneration of tissues and the subsequent death. Lack of oxygen or nutriment or an accumulation of carbon dioxide or other metabolites are the consequences of circulatory failure. The asymmetries indicate that the tissues and organs adjacent to the shell are affected later than those farthest from the shell. Lack of oxygen suggests itself as the immediate cause for the asymmetries." On the other hand, Ancel ('50) and Salzgeber ('63, '66) obtained mainly rightsided malformations in their studies on the effect of certain teratogenic agents in the chick embryo. The authors explain these malformations by pointing out that the embryo lies on its left side on the fourth day of development. This side is therefore protected from the action of agents which are dropped on the embryo. The intraembryonic hemorrhages observed in the vicinity of the micromelic limbs are similar to those described by Ancel ('42, '50). The same author suggested that this pathological condition is a contributory factor in the etiology of malformations of the extremities.

In discussing the malformations obtained by injecting propanediol-1,3 into the yolk (table 5), it should be remembered that a number of unrelated substances, such as thallium (Ridgway and Karnofsky, '51), triazenes (Dagg and Karnofsky, '55), insulin (Landauer, '47; Erhard, '59), and sulfonamides (Ancel, '50; Zwilling and de Bell, '50; Erhard, '59), cause a macroscopically similar defect. On histological and histochemical examination, however, Erhard ('61) came to the conclusion that micromelia induced by insulin differs from that obtained by sulfonamides. Therefore, it should be stressed that only macroscopic criteria have been used in describing the embryos treated with propanediol-1,3 as micromelic. So far no histological work has been done to establish whether this malformation is identical with the one described by Landauer as chondrodystrophy. According to Landauer ('27, '31, '47), malformed fowls known as creepers are characterized by (1) shortening of all long bones, (2) curvature and thickening both of the tibia and the fibula in the distal part of their diaphyses, (3) relative increase in the length of the fibula to that of the tibia, (4) parrot beak, (5) a defect in the formation of the feathers, (6) normal periosteal ossification but no formation of endochondral bone, and (7) synostosis of the basisphenoid and basisoccipital bones. Of these characteristics, the first five have all been observed in chicks treated with propanediol-1,3. The hydropic feathers have already been described by Erhard ('61), who suggested that they reflect a malfunctioning of the thyroid.

Finally the question should be discussed as to why only propanediol-1,3 is teratogenic when injected into the yolk and not propylene glycol, which is structurally so similar. A possible explanation for this difference could reside in the separate pathways by which the two glycols are metabolized. Williams ('59) mentioned that glycols break down more readily in vivo if the hydroxyl groups are vicinal. Although the exact metabolic fate of propanediol-1,3 is not known, it is believed to be oxidized via 3-hydroxypropionic acid to malonic acid. On the other hand, pro-

pylene glycol is excreted in rabbits partly unchanged, partly oxidized to lactic acid, and partly conjugated to glucuronic acid.

Preliminary results of work done on this problem in our department indicate that malonic acid is not teratogenic in the chick embryo. 3-Hydroxypropionic acid, however, does give rise to micromelic embryos when 0.05 ml is injected into the yolk on the fourth day of development. As the incidence of malformation is nevertheless much lower than when propanediol-1,3 is used, it seems likely that the glycol and not one of its metabolites was the causative agent of micromelia in the chick embryos.

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PLATE 1

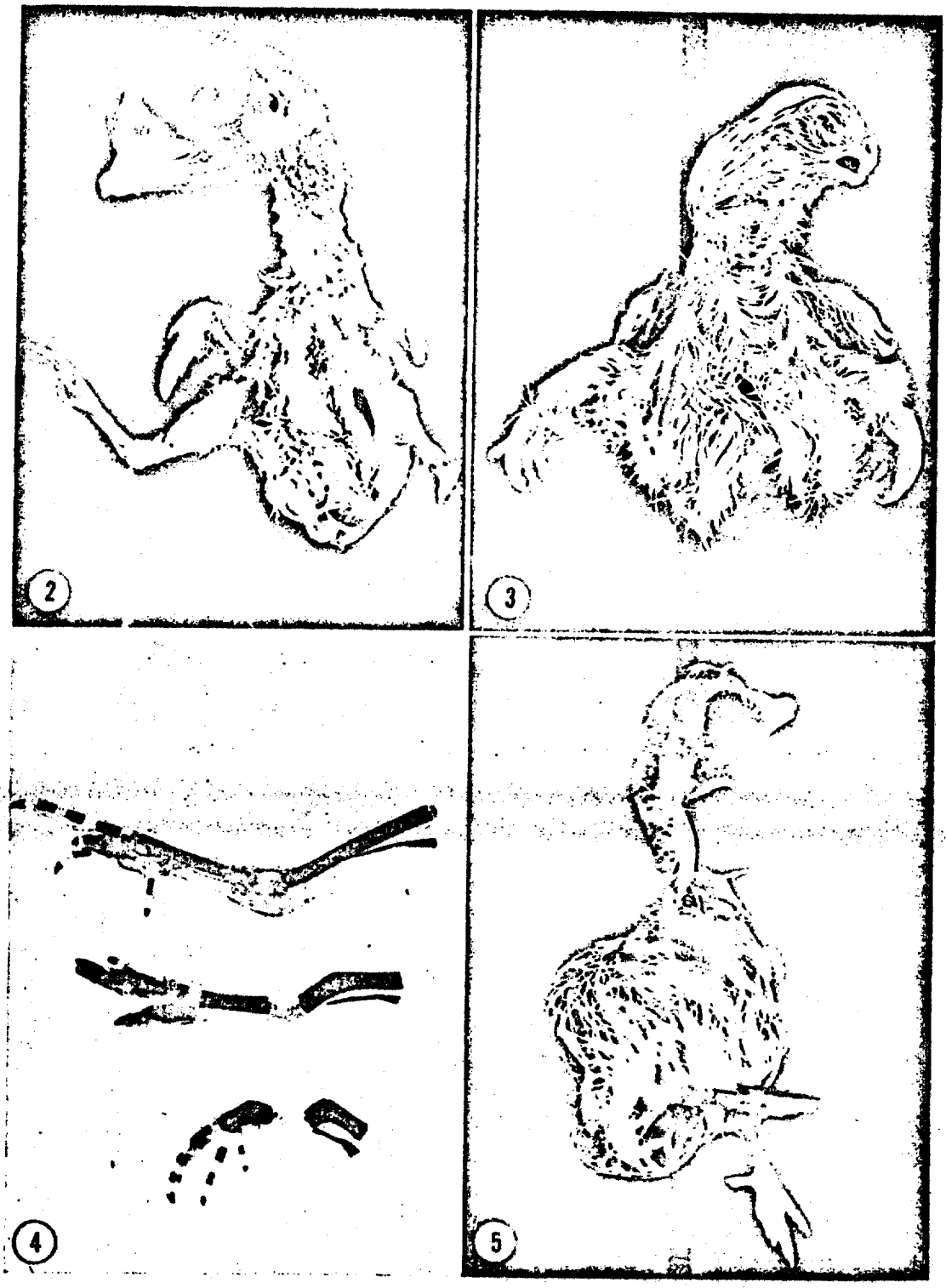
EXPLANATION OF FIGURES

2 Fourteen-day-old embryo injected on fourth day with 0.05 ml propylene glycol into the air chamber. Micromelia of left wing and leg.

3 Twenty-one-day-old micromelic embryo injected with 0.1 ml propanediol-1,3 in the yolk sac on the fourth day of development.

4 Alizarin S-stained and cleared leg bones (from top to bottom) of (1) a control chick; (2) a moderate form of micromelia following the injection of 0.05 ml propanediol-1,3 into the yolk sac. Note the curvature in the distal part of the tibia and the relative increase in length of the fibula; and (3) an extreme case of micromelia following the injection of 0.1 ml propanediol-1,3 into the yolk sac. Note also the curvature of the metatarsals.

5 Fifteen-day-old chick embryo treated with a high dose (0.2 ml) of propylene glycol on the fourth day of incubation in the yolk sac. Note lack of micromelia, but large liquid-containing cyst on dorsal side.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and recrystallization of the product from ethanol, gave phenacyl glutarate, m.p. and mixed m.p. 104° (yield 136 mg. or 0·5% of dose).

3-Methylpentane-1:5-diol. A total of 10 g. of the glycol (1·5 g./kg.) was fed to two rabbits. The ether extract of the acidified urine was extracted with 2N-Na_2CO_3 (3 × 20 ml.) to remove acids, and was then evaporated to dryness to leave a pale-brown liquid (1·45 g.). This was distilled (b.p. 224–225°) and the distillate was identified as 3-methylpentane-1:5-diol, n_D^{25}, 1·453 [Wojcik & Adkins (1933) give n_D^{25}, 1·452]. Treatment of the diol with 3:5-dinitrobenzoyl chloride gave the bis-3:5-dinitrobenzoate of 3-methylpentane-1:5-diol, m.p. and mixed m.p. 121° from aqueous ethanol (cf. Dische & Rittenberg, 1954). The Na_2CO_3 extract was now acidified and extracted with ether. Evaporation of the extract gave a partially crystalline solid (3·5 g.; 30% of the dose) which was dried over P_2O_5 *in vacuo* and then triturated with benzene to yield crystals (1·9 g.) which were identified as 3-methylglutaric acid, m.p. and mixed m.p. 87° after recrystallization from benzene. The acid was further identified as its anhydride (m.p. and mixed m.p. 45°) after refluxing with acetic anhydride and distilling (Darbishire & Thorpe, 1905), and as 3-methylglutaric monoanilide, m.p. and mixed m.p. 120°, after heating the anhydride with aniline in benzene solution.

Hexane-1:6-diol. The diol (2·8 g.) was fed to a rabbit. None of the unchanged diol was isolated from the 2-day urine. The carboxylic acid fraction of the urine in Na_2CO_3 was acidified with an excess of conc. HCl and kept overnight at 0°. A crystalline deposit (320 mg.; 9% of the dose) separated, which was purified by dissolution in 2N-Na_2CO_3 (charcoal) and precipitation with HCl. The crystals were identified as adipic acid, m.p. and mixed m.p. 152°, from which the *p*-nitrobenzyl ester, m.p. and mixed m.p. 106°, was prepared.

2-*Substituted propane*-1:3-*diols*

Crystalline metabolic products could not be isolated from the urine of rabbits receiving 2-ethyl- or 2:2-diethylpropane-1:3-diol, although Borger & Ludwig (1950) have shown that 2:2-diethylhydracrylic acid is a metabolite of the 2:2-diethyldiol in man.

2:2-Dimethylpropane-1:3-diol. This diol is highly conjugated (62%) with glucuronic acid in the rabbit (see Table 1). The glucuronide and its triacetyl methyl ester were readily obtained as gums by the lead acetate procedure (cf. Kamil, Smith & Williams, 1951), but these gums could not be crystallized.

The 24 hr. urine of four rabbits given a total of 8 g. of the diol was brought to pH 10·0 with NH_3 soln. and then continuously extracted with ether for 6 hr. Evaporation of the extract and sublimation of the residue yielded unchanged 2:2-dimethylpropane-1:3-diol, m.p. and mixed m.p. 127° (yield, 56 mg. or 0·7% of the dose). The residual urine was brought to pH 4·0 with 2N-HCl and again continuously extracted with ether for 6 hr. Evaporation of the extract gave an acidic gum which partly crystallized on keeping at 0° for a week. Repeated crystallization of this material from ethanol–ether mixtures gave white needles (150 mg.) of hydroxypivalic acid (3-hydroxy-2:2-dimethylpropionic acid), m.p. and mixed m.p. 126° (Found: equiv. 121. Calc. for $C_5H_{10}O_3$; equiv. 118). The authentic acid was prepared according to Wessely (1901).

2-Methyl-2-n-propylpropane-1:3-diol. Four rabbits were given a total of 22 g. of this diol, and the subsequent 24 hr. urine was continuously extracted with ether as described above, first after making alkaline with NH_3 soln. and again after making the residual urine acid. Neither of these extractions yielded crystalline products. The residual urine after the second extraction was therefore saturated with $(NH_4)_2SO_4$ and then extracted by shaking with a mixture of ethanol and ether (1:2). Evaporation of the solvents yielded a glucuronide gum (9·4 g.) which did not crystallize. The gum was therefore methylated with diazomethane and then acetylated with pyridine and acetic anhydride as described by Kamil *et al.* (1951), but again the triacetyl methyl ester was obtained as a gum. This gum was taken up in $CHCl_3$ and the solution washed three times with 2N-HCl and then thrice with saturated aq. $NaHCO_3$ solution. The washed solution, after drying over anhydrous Na_2SO_4, was evaporated to yield a gum (2·3 g.), which crystallized with considerable difficulty to yield 170 mg. of a compound, m.p. 116° and $[\alpha]_D^{20}$ – 25° in $CHCl_3$ (*c*, 1), which analysed as *methyl (2-acetoxymethyl-2-methyl-n-pentyl tri-O-acetylglucosid)uronate* (Found: C, 53·95; H, 6·9; CH_3CO, 35·9. $C_{22}H_{34}O_{12}$ requires C, 54·0; H, 6·8; CH_3CO, 35·2%).

The glucuronide gum (0·5 g.), which had been prepared by ethanol–ether extraction as described above from the 24 hr. urine of a rabbit dosed with 5 g. of the diol, was heated under reflux for 2 hr. with 5N-HCl (10 ml.). The resulting solution was extracted with ether, and the extract was dried over anhydrous Na_2SO_4 and then evaporated. The dark semi-crystalline residue was treated with a solution of phosgene in toluene according to Ludwig & Piech (1951) to form a carbamate, and the product was recrystallized from hot water. There was obtained 2-methyl-2-*n*-propylpropane-1:3-diol dicarbamate (meprobamate), m.p. and mixed m.p. 105°.

Other diols

Unsuccessful attempts were made to isolate crystalline glucuronides or other metabolites with propane-1:2-diol (of which 300 g. was fed in one experiment), 2-methyl- and 2-ethyl-pentane-1:3-diol, 2-methylpentane-1:4- and -2:4-diol, and 2:3-dimethylbutane-2:3-diol (pinacol). Most of these diols contain one or two asymmetric carbon atoms and therefore more than one glucuronide could be formed; this would tend to make the isolation of crystalline glucuronides difficult.

Butane-2:3-diol. This compound could be expected to give rise to at least two glucuronides of the diol itself, to acetoin and its glucuronides and to diacetyl. Neither diacetyl nor acetoin was detected (by spectra and colour tests; see Feigl, 1956) in the expired air or urine of rabbits dosed with 1·2–1·5 g. of this compound. However, a glucuronide was isolated from the urine of four rabbits which had been dosed with 2 g. each of the diol. The glucuronide gum was prepared by the lead acetate method and was methylated and acetylated in the usual manner. The triacetyl methyl ester was obtained in poor yield from ethanol as white needles of m.p. 151° and $[\alpha]_D^{20}$ – 18° in $CHCl_3$ (*c*, 1). It analysed as *methyl (2-hydroxybutyl-3-tri-O-acetylglucosid)uronate* (Found: C, 49·9; H, 6·2; CH_3CO, 32·4. $C_{17}H_{26}O_{12}$ requires C, 50·2; H, 6·45; CH_3CO, 31·8%).

Table 1. *Glucuronic acid conjugation and metabolites of glycols in rabbits*

The results for glucuronic acid conjugation are given as means for three animals with ranges in parentheses.

Glycol fed	Dose (m-moles/kg.)	Glucuronic acid conjugation (% of dose)	Urinary metabolites characterized
1. Glycol type: $(CH_2)_n \cdot (CH_2 \cdot OH)_2$			
Ethane-1:2-diol.	4	0 (0)	CO_2 in expired air, traces of oxalate in urine
Propane-1:3-diol	4	1 (0–2)	None found
Butane-1:4-diol	4	1 (0–2)	Succinic acid
Pentane-1:5-diol	2	0 (0)	Glutaric acid
3-Methylpentane-1:5-diol	2	7 (3–11)	3-Methylglutaric acid
Hexane-1:6-diol	2	7 (4–9)	Adipic acid
2. Glycol type: $CRR' \cdot (CH_2 \cdot OH)_2$			
2-Ethylpropane-1:3-diol (R = H)	2	23 (18–28)	—
2:2-Dimethylpropane-1:3-diol	2	62 (53–67)	3-Hydroxy-2:2-dimethylpropionic acid
2:2-Diethylpropane-1:3-diol	2	35 (11–66)	—
2-Methyl-2-propylpropane-1:3-diol	2	33 (23–49)	Glucuronide of the glycol
3. Glycol type: $R \cdot CH(OH) \cdot CHR' \cdot CH_2 \cdot OH$			
Butane-1:3-diol (R′ = H)	4	1 (0–2)	None found
2-Methylpentane-1:3-diol	2	22 (21–23)	None isolated
2-Ethylpentane-1:3-diol	2	48 (46–52)	—
2-Ethylhexane-1:3-diol	2	7 (4–11)	None isolated
Heptane-1:3-diol (R′ = H)	2	9 (7–13)	—
2-Ethylheptane-1:3-diol	2	9 (8–10)	—
4. Glycol type: $R \cdot (CH \cdot OH)_2 \cdot R'$			
Propane-1:2-diol (R = H)	4	1 (0–3)	None found
Butane-1:2-diol (R = H)	2	12 (7–17)	None found
Butane-2:3-diol (R = R′ = CH_3)	4	22 (20–26)	Glucuronide of the glycol
5. Miscellaneous glycols			
2:3-Dimethylbutane-2:3-diol	2	27 (25–29)	Non-crystalline glucuronides not characterized
2-Methylpentane-2:4-diol	1	67 (49–93)[5]	
2-Methylpentane-1:4-diol	1·5	37 (32–43)[4]	

RESULTS AND DISCUSSION

The results of our experiments are summarized in Table 1. The first group of glycols have the general formula $CH_2(OH) \cdot [CH_2]_2 \cdot CH_2 \cdot OH$ and do not undergo glucuronic acid conjugation unless they contain six carbon atoms (i.e. 3-methylpentane-1:5-diol and hexane-1:6-diol), and even then the conjugation is less than 10% of the dose. The metabolic fate of ethane-1:2-diol has been briefly reported by Gessner & Williams (1959). The main metabolite of the ^{14}C-labelled compound in rats and rabbits is carbon dioxide. Oxidation to oxalic acid is a very minor reaction of the glycol, but rats and cats produce more oxalate than do rabbits and guinea pigs. Ethane-1:2-diol is oxidized in liver slices to glycolaldehyde and is then converted into carbon dioxide (Gessner, 1958). No malonic acid was detected in the urine of rabbits receiving propane-1:3-diol even with doses of 4 g./kg. Three oxidation products of propane-1:3-diol are possible, β-hydroxypropionic aldehyde, β-hydroxypropionic acid (hydracrylic acid) and malonic acid. Malonic acid can apparently be metabolized through the tricarboxylic acid cycle (Lee & Lifson, 1951) and recently malonyl-coenzyme A has been found to be an intermediate in fatty acid synthesis (Brady, 1958; Gibson, Titchener & Wakel, 1958). Hydracrylic acid has also been reported to be metabolized in animal tissues (Green, Dewan & Leloir, 1937). It appears probable that propane-1:3-diol is oxidized completely to carbon dioxide in the body.

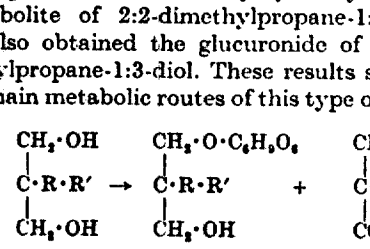
Butane-1:4-diol appears to be metabolized via succinic acid, since we were able to isolate this acid (yield, 7% of the dose) from the urine of rabbits given 0·75 g. of the diol/kg. Succinic acid is readily metabolized unless administered in large doses (Weitzel, 1947*a*, *b*; Emmrich, Neumann & Emmrich-Glaser, 1941). Similarly, pentane-1:5-diol appears to be metabolized via glutaric acid, since we were able to isolate small amounts of glutaric acid (0·5% of the dose of 0·75 g./kg.) from the urine after feeding with the glycol. Glutaric acid is known to be readily metabolized to carbon dioxide (Hobbs & Koeppe, 1958; Rothstein & Miller, 1952). As much as 30% of the dose (1·5 g./kg.) of 3-methylpentane-1:5-diol was isolated from the urine as 3-methylglutaric acid. It appears that the

3-methyl group hinders the further oxidation of the dicarboxylic acid. Our results suggest that the main outlines of the metabolism of these $\omega\omega'$-diols are:

$$(CH_2)_n(CH_2\cdot OH)_2 \rightarrow \begin{matrix}\text{intermediate}\\ \text{oxidation}\\ \text{products}\end{matrix} \begin{matrix}\nearrow CO_2\\ \\ \rightarrow (CH_2)_n(CO_2H)_2 \rightarrow CO_2.\end{matrix}$$

The second group of glycols in Table 1 are 2-substituted propane-1:3-diols, which are diprimary alcohols. These compounds are of interest for a number of them possess anticonvulsant and tranquillizing properties. In this group, however, glucuronic acid conjugation is appreciable, and dicarboxylic acids (i.e. substituted malonic acids) were not found as metabolites. Berger & Ludwig (1950) isolated 2:2-diethyl-3-hydroxypropionic acid as a metabolite of 2:2-diethylpropane-1:3-diol in man, but found no diethylmalonic acid. We have isolated 3-hydroxy-2:2-dimethylpropionic acid (hydroxypivalic or 2:2-dimethylhydracrylic acid) as a metabolite of 2:2-dimethylpropane-1:3-diol, and we also obtained the glucuronide of 2-methyl-2-propylpropane-1:3-diol. These results suggest that the main metabolic routes of this type of glycol are:

$$\begin{matrix}CH_2\cdot OH\\ |\\ C\cdot R\cdot R'\\ |\\ CH_2\cdot OH\end{matrix} \rightarrow \begin{matrix}CH_2\cdot O\cdot C_6H_9O_6\\ |\\ C\cdot R\cdot R'\\ |\\ CH_2\cdot OH\end{matrix} + \begin{matrix}CH_2\cdot OH\\ |\\ C\cdot R\cdot R'\\ |\\ CO_2H\end{matrix}$$



The third group of glycols in Table 1 are derivatives of propane-1:3-diol with one primary and one secondary alcohol group. Butane-1:3-diol is a compound of low toxicity. No metabolite of it is known and none was found in this work; it is probable that it is completely oxidized in the body via β-hydroxybutyric acid, which should be its primary oxidation product. 2-Methylpentane-1:3-diol and 2-ethylhexane-1:3-diol were also examined in some detail, but no recognizable metabolic product was isolated. The other glycols in this group were available only in small quantities and detailed examination of their metabolites was not possible. At present we have no clear idea of the metabolic fate of this type of glycol.

The fourth group of glycols are derivatives of propane-1:2-diol. No urinary metabolites of propane-1:2-diol or of butane-1:2-diol were isolated. Propane-1:2-diol is relatively non-toxic (its LD_{50} for rats is about 26 g./kg.; Smyth, Seaton & Fischer, 1941) and it is probably oxidized to lactic acid, through which it can enter normal metabolic processes; it is, in fact, converted into glycogen in rats (Rudney, 1950). Butane-2:3-diol is not oxidized to butane-2:3-dione (diacetyl) in dogs (Westerfeld & Berg, 1943) and we have found no diacetyl in the urine or expired air of rabbits dosed with the glycol. Neuberg & Gottschalk (1925) reported that it formed a glucuronide in rabbits. We have isolated this glucuronide as a triacetyl methyl ester and from elementary analysis it appears to be a monoglucuronide. Since butane-2:3-diol contains two asymmetric carbon atoms, monoglucuronides corresponding to the *dextro-*, *laevo-* and *meso*-glycols are possible.

The three glycols 2:3-dimethylbutane-2:3-diol (pinacol), 2-methyl-pentane-2:4-diol and 2-methyl-pentane-1:4-diol are conjugated with glucuronic acid to an appreciable extent. None of these glucuronides was isolated in a crystalline form.

SUMMARY

1. The fate of oral doses of 22 glycols has been investigated in the rabbit.

2. Glycols of the general formula,

$$CH_2(OH)\cdot[CH_2]_n\cdot CH_2\cdot OH,$$

where $n = 0$–6, do not form appreciable amounts of conjugated glucuronic acid. Ethane-1:2-diol is mainly oxidized to carbon dioxide. Butane-1:4-diol, pentane-1:5-diol and hexane-1:6-diol yield small amounts of the corresponding dicarboxylic acids in the urine, but appear to be mainly destroyed *in vivo*. 3-Methylpentane-1:5-diol yields large amounts of 3-methylglutaric acid in the urine.

3. Glycols of the general formula

$$CH_2(OH)\cdot CRR'\cdot CH_2\cdot OH$$

form appreciable amounts of conjugated glucuronic acid. These appear to be glucuronides of the unchanged diols, for the monoglucuronide of 2-methyl-2-*n*-propylpropane-1:3-diol was isolated. These glycols do not form dicarboxylic acids *in vivo*, but monocarboxylic acids. 3-Hydroxy-2:2-dimethylpropionic acid was isolated as a metabolite of 2:2-dimethylpropane-1:3-diol.

4. No definite information about the other glycols in Table 1 was obtained, except that some are highly conjugated with glucuronic acid (2-methyl- and 2-ethyl-pentane-1:3-diol, butane-2:3-diol, 2:3-dimethylbutane-2:3-diol, and 2-methyl-pentane-1:4- and -2:4-diol). The monoglucuronide of butane-2:3-diol was isolated.

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Effects of 1,2-Propanediol on Blood Glucose and Liver Glycogen of Young Rats (34524)

S. N. GIRI, S. A. PEOPLES, AND R. L. MULL

Department of Physiological Sciences, School of Veterinary Medicine, University of California, Davis, California 95616

1, 2-Propanediol (propylene glycol) is extensively used as a vehicle in many pharmacological preparations (1, 2). This is because it has low toxicity (3) and because of its ability to dissolve many compounds not soluble in water. Ambrose (4) reported that administration of undiluted propylene glycol dipropionate (8 ml/kg) in rabbits did not produce any local or systemic reactions and concluded that within reason the ingestion or handling of propylene glycol does not involve any significant health hazards. Harvey *et al.* (5) reported that a single dose of propylene glycol as high as 7.5 mg/kg was well tolerated by the intravenous route in rats. Hickman (6) reported that the intraperitoneal (ip) administration of propylene glycol in mice resulted in the lack of coordination of movement followed by deep narcosis, but none of the mice died within 7 days of injection. In an earlier study, using weanling rats 50–60 g, it was noticed that propylene glycol as a carrier vehicle for phenylthiourea had produced an enormous increase in the blood glucose. This prompted us to study the effects of propylene glycol on blood glucose and liver glycogen in the weanlings in regard to the onset and duration of its effects on blood glucose and liver glycogen.

Propylene glycol behaves potentially as a carbohydrate in the metabolic pathway. It is glycogenic in starved rats (7), to an extent comparable to pyruvic acid (8); it has been used as a substitute for carbohydrate in the treatment of bovine ketosis (9). It is likely that a small amount of propylene glycol is readily metabolized via the lactate propanediol pathway (10) without causing any systemic metabolic upsets. The present report shows that a high nonlethal dose of propylene glycol is associated with severe disturbances in carbohydrate metabolism. Thus, the possible consequences of metabolic upsets caused by propylene glycol as such should be borne in mind while using it as a carrier vehicle for drugs.

Materials and Methods. Sprague-Dawley male rats weighing 50–60 g were used throughout the experiment. They were fed Purina rat chow and water *ad libitum*. The dose-response curve for lethality of propylene glycol was determined. Mortality was checked every 3 hr for a period of 12 hr and at 24 hr after administration of propylene glycol. The maximum tolerated dose (10 ml/kg) having no lethal effect was used in the subsequent metabolic studies.

One dose of propylene glycol (10 ml/kg) was administered ip. The control rats were injected with an equivalent amount of saline. The control and experimental rats were then transferred into metabolic cages in order to determine the food intake. The animals were decapitated at different times and blood samples were collected in heparinized tubes. Duplicate samples were run for each rat to measure the level of blood glucose according to the method of Krebs *et al.* (11, 12). For the determination of liver glycogen, a piece of liver was removed immediately after decapitation, weighed quickly, and transferred into a test tube containing hot 30% KOH. Liver glycogen was measured according to the method of Hensen *et al.* (13). The determination of blood glucose and liver glycogen at any time interval after propylene glycol treatment was done simultaneously with a saline control.

In order to judge the capacity of animals to synthesize liver glycogen after propylene

TABLE I. Toxicity of Propylene Glycol (PG) in Sprague-Dawley Male Rats Weighing 50–60 g.

Mortality	Dose of PG (ml/kg) ip				
	10	15	20	25	30
Number of deaths/number of animals used	0/50	1/10	7/10	9/10	8/10

glycol treatment, the experiments were carried out as follows: Rats were injected with propylene glycol (10 ml/kg) ip. The control rats received at the same time an equivalent amount of saline. Soon after injection they were transferred into separate cages, where they had access to water *ad libitum*, but not to food. Six hours after the administration of propylene glycol or saline, a 10% dextrose solution was injected ip hourly for 6 hr to provide 50 mg of dextrose/hr/rat in one series of experiments and 100 mg of dextrose in the second series. Thereafter, the determination of liver glycogen was performed as described previously.

Data are reported in terms of average values with their standard error of mean (SE). The Student *t* test was applied to test the significance of differences between means.

Results and Discussion. The maximum nonlethal dose of propylene glycol by the intraperitoneal route was found to be 10 ml/kg in the young rats. This dose caused a mild central nervous system (CNS) depression which lasted for 2 or 3 hr. The minimum lethal dose was 15 ml/kg, and the dose producing the lethal effect close to LD_{100} was between 25 and 30 ml/kg (Table I). Death in all cases of propylene glycol toxicity resulted from a severe CNS depression and respiratory failure.

Propylene glycol at the dose of 10 ml/kg seems to have a biphasic effect on the blood glucose level. A marked increase in blood glucose occurred after 15 min with a maximum increase after 1½ hr. The increase in blood glucose lasted for a period of 6 hr. Thereafter, a slight decrease at the 12-hr interval was noticed. However, the significance of the decrease at 12 hr is questionable since these values fall within a broad normal range (Table II). The liver glycogen content in rats treated with propylene glycol or with saline was just about the same for a period of 6 hr (Table II). This indicate strongly that the increase in the blood glucose level after propylene glycol is not mediated through the mobilization of liver glycogen, but that propylene glycol itself is a direct precursor of blood glucose. This is in agreement with the work of Emery *et al.* (14) that intraruminal administration of propylene glycol in the cow was glucogenic in the classical sense of that term, namely, that it is metabolized via intermediates and lead to net synthesis of glucose.

A consistent depletion of liver glycogen was noticed 12 hr after propylene glycol administration (Table II). This depletion could possibly be accounted for by a reduced food intake, as the propylene glycol-treated rats during the day consumed only one half the amount of food in 12 hr as the control group (Table II). However, food consumption by propylene glycol-treated rats during the night in 12 hr was twice as much as during the day (Table II), and, because of diurnal variation in the food intake, practically no difference in the liver glycogen content was observed. This suggested the possibility that the rats treated with propylene glycol somehow lack the ability to synthesize the liver glycogen. This was substantiated in the following experiment: Rats, 6 hr after propylene glycol treatment, when subjected to 100 mg of dextrose ip hourly for 6 hr, showed a profound depression in their ability to synthesize liver glycogen. The livers of control saline-treated rats under the identical conditions, contained three times more liver glycogen than those of the rats treated with propylene glycol (Table III).

The actual mechanism as how propylene glycol (10 ml/kg) administration decreases the liver glycogen content is not clear at the present. Such possibilities as the inhibition of glycogen synthetase due to a high amount of glucose resulting from propylene glycol

TABLE II. Effect of a Single Dose (10 ml/kg) of Propylene Glycol (PG) on Blood Glucose, Liver Glycogen, and Food Intake at Different Times in Young Rats.

Time after PG-administration (hr)	Glucose (mg/100 ml of blood, avg ± SE)		Glycogen (% of liver weight, avg ± SE)		Food intake (g/rat, avg ± SE)	
	Control	Propylene glycol	Control	Propylene glycol	Control	Propylene glycol
0.25	129.2 ± 3.88 (5)	182.4 ± 5.21[a] (5)	4.18 ± 0.24 (5)	3.75 ± 0.65 (5)	0.0	0.0
1.5	131 ± 2.66 (5)	245.3 ± 11.55[a] (5)	3.30 ± 0.43 (5)	2.47 ± 0.35 (5)	0.0	0.0
3.0	120.9 ± 9.25 (16)	180.2 ± 14.74[b] (9)	3.59 ± 0.79 (13)	4.50 ± 0.72 (7)	0.0	0.0
6.0	121.4 ± 6.68 (5)	188.2 ± 15.20[b] (5)	3.01 ± 0.29 (5)	2.95 ± 0.32 (5)	0.0	0.0
12.0[c]	134.0 ± 2.77 (5)	117.0 ± 6.04[d] (5)	3.61 ± 0.39 (5)	1.61 ± 0.34[b] (5)	2.23 ± 0.18 (4)	1.05 ± 0.26 (5)
12.0[e]	134.0 ± 2.39 (5)	119.3 ± 5.05[d] (5)	5.17 ± 0.34 (5)	1.54 ± 0.22[a] (5)	7.30 ± 0.46 (4)	2.53 ± 0.67 (4)

The figures in parentheses are the number of animals used.

[a], [b], [d] t values between propylene glycol and control saline are significant at 0.1%, 1%, and 5% level, respectively.

[c] Propylene glycol was injected at 8:00 AM; blood glucose and liver glycogen were determined on the same day at 8:00 PM.

[e] Propylene glycol was injected at 8:00 PM; blood glucose and liver glycogen were determined on the next day at 8:00 AM.

the CNS-depressant effect of propylene glycol (15) as such might well be involved, remain to be tested. However, the possibility of such factors as hypertonicity in the intraperitoneal cavity or pain from glycol irritation influencing the metabolic balance of young rats cannot be ignored.

Summary. The administration of a high nonlethal dose of propylene glycol (10 ml/kg) was found to affect the carbohydrate metabolism of young rats. There was a marked increase in the blood glucose in 15 min, with the maximum increase in 1½ hr; the overall increase in blood glucose lasted for a period of 6 hr. The amount of liver glycogen in propylene glycol and saline control was the same for 6 hr. A severe depletion in the liver glycogen was noticed 12 hr after propylene glycol treatment. Evidence has been provided that the rats treated with a high dose of propylene glycol have a drastically reduced capacity to synthesize or to store liver glycogen. Some of the possible mechanisms for the inhibitory effects of propylene glycol on synthesis of liver glycogen have been discussed. The present work provides the proof for the metabolic upsets associated with a large dose of propylene glycol used either as such or as a carrier vehicle in a drug study.

TABLE III. Effect of a Single Dose (10 ml/kg) of Propylene Glycol (PG) on Synthesis of Liver Glycogen in Young Rats.[a]

Treatment	No. of animals	Glucose (mg)	Glycogen (% of liver wt, avg ± SE)
Saline control	4	50	0.638 ± 0.115
Propylene glycol	4	50	0.537 ± 0.132
Saline control	4	100	3.620 ± 0.680[b]
Propylene glycol	6	100	1.23 ± 0.174[b]

[a] Design of the experiment is discussed in the Methods section.

[b] A t value between PG and saline control is significant at the 1% level.

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CONTRIBUTED ARTICLES

RESPONSE OF RATS TO DIETS CONTAINING VARYING AMOUNTS OF GLYCEROL AND OF PROPYLENE GLYCOL*

By N.B. Guerrant, G.P. Whitlock, M.L. Wolff and R.A. Dutcher

Glycerol has long been used in foods, beverages, flavoring extracts, and as a vehicle in pharmaceutical preparations. While glycerol is a normal constituent of both plant and animal fats and in this respect cannot be regarded as a toxic material, all writers are not in complete accord regarding its potential toxicity when ingested in large amounts. Deichmann (1) has published an excellent review relative to the behavior of glycerol in the animal organism and cites conditions under which excessive doses of glycerol might possess harmful effects. On the other hand, Johnson, Carlson and Johnson (2) described experiments with rats, dogs and men in which relatively large amounts of glycerol were ingested without apparent harmful effects.

The extensive use of glycerol has been due primarily to its unique physical properties and to its relative freedom from toxicity. Among its many desirable physical characteristics are: its sirupy consistency, its hygroscopic nature and its characteristic solvent properties. Owing to the temporary scarcity of glycerol for domestic uses during and following the World War II, glycerol substitutes have aroused increased interest. Of the various glycols investigated to date, propylene glycol (propanediol-1,2) has received, perhaps, the most favorable consideration as a glycerol

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substitute. This is due to the fact that propylene glycol is apparently the least toxic of the more commonly produced glycols (3) and possesses physical characteristics somewhat similar to those of glycerol.

While certain similarities in the physical properties of glycerol and propylene glycol have been well established, there remains a variance of opinion regarding the pharmacological properties of the two compounds. The literature pertaining to their relative toxicities and to the potential dangers of glycerol and propylene glycol in relation to their chemical constitution and their fate in metabolism has been adequately reviewed by von Oettingen (4). In an attempt to summarize the scientific literature bearing on this question, Laug and associates (5) state that ".... It is concluded that ethylene glycol, diethylene glycol, ethylene glycol monoethyl ether, diethylene glycol monoethyl ether and dioxan should be entirely omitted from foods and drug preparations and that propylene glycol must be avoided except in very small concentrations." The studies reported do not, however, appear to have answered satisfactorily several pertinent questions which arise when the possible consequences to animals of ingesting materials of questionable toxicity are considered. Among these questions are:

(a) What is the maximum daily intake of glycerol or of propylene glycol for a given species of animal which may be tolerated without evidence of biological injury?

(b) Are there any harmful effects arising from ingesting submaximum daily intakes of these compounds over an extended period and are there any cumulative effects which become evident when several generations of experimental animals have subsisted on diets containing these compounds?

The investigations reported in the present paper were undertaken with the view of throwing further light on the above questions as they concern glycerol and propylene glycol.

Experimental

Growth studies. - The studies reported at this time consist of two phases: growth studies and reproduction studies. The first phase of the investigation was concerned with a study of the effects of varying intakes of glycerol and of propylene glycol on the growth rates of young rats, the glycerol or the propylene glycol each being provided as a partial constituent of the energy component of a basal diet which, prior to supplementation, had proved adequate for growth. The basal diet consisted, in parts per 100, of purified casein 20, salts mixture (McCollum 185) 4, Cell-U-Flour 2, filtered butter fat 6, medicinal grade cod liver oil 2, dehydrated brewers' yeast 6, and corn starch 60. This basal diet was modified by replacing corn starch with glycerol and with propylene glycol, respectively, at the levels of 1, 3, 6, 10, 15, 20, 30, 40, 50 and 60 parts per 100 Gm. of total diet (see Table I). In consequence of these substitutions, this series of

TABLE I. - Composition of Diets Employed in a Series of Comparative Growth Studies Conducted Over a Period of 20 Weeks Using Young Growing Rats as the Test Subjects

	Ingredients - Per cent									
Diet No.	Casein	Salt Mixture	Cell-U-Flour	Butter Fat	Cod Liver Oil	Yeast	Corn Starch	Glycerol	Propylene Glycol	Total
Control	20	4	2	6	2	6	60	--	--	100
1	20	4	2	6	2	6	59	1	--	100
2	20	4	2	6	2	6	59	--	1	100
3	20	4	2	6	2	6	57	3	--	100
4	20	4	2	6	2	6	57	--	3	100
5	20	4	2	6	2	6	55	5	--	100
6	20	4	2	6	2	6	55	--	5	100
7	20	4	2	6	2	6	50	10	--	100
8	20	4	2	6	2	6	50	--	10	100
9	20	4	2	6	2	6	45	15	--	100
10	20	4	2	6	2	6	45	--	15	100
11	20	4	2	6	2	6	40	20	--	100
12	20	4	2	6	2	6	40	--	20	100
13	20	4	2	6	2	6	30	30	--	100
14	20	4	2	6	2	6	30	--	30	100
15	20	4	2	6	2	6	20	40	--	100
16	20	4	2	6	2	6	20	--	40	100
17	20	4	2	6	2	6	10	50	--	100
18	20	4	2	6	2	6	10	--	50	100
19	20	4	2	6	2	6	0	60	--	100
20	20	4	2	6	2	6	0	--	60	100

growth studies involved 21 dietary groups, each group consisting initially of ten young rats. All rats constituting the series were from the same genetic source and were of approximately the same age and weight when placed on experiment. Efforts were made to distribute males and females equally among the several experimental groups. When high mortality of test animals was experienced among certain groups during the early stage of the feeding trials, additional animals were subsequently added to those groups.

Except during the course of certain special tests, all animals of the series were maintained in individual all-metal cages of the Wahmann Lc-28 type, which were provided with raised screen floors preventing access to excreta. The test diets were fed ad libitum and fresh distilled water was accessible to each animal at all times. The animals were observed daily and body weight changes and food consumption were recorded at weekly intervals. Hemoglobin determinations were made at regular intervals on bloods from representative animals from each group by the Newcomer method and blood glucose was determined by the Shaffer-Somogyi method (6). Other animals were transferred to special exercise cages for definite periods in order to observe the comparative effects of the composition of the diet on the inclination of the animals to exercise. Likewise, representative animals from the respective groups were transferred to special metabolism cages at specified intervals for the purpose of determining the effect of glycerol and propylene glycol intake on water intake and urinary output. Samples of urine were analyzed for glycerol or for propylene glycol by the method of Lehman and Newman (7).

Animals which survived the dietary treatments were maintained under observation for at least 20 weeks. Animals which succumbed, as well as representatives of those which survived the growth tests, were subjected to pathological examination.

In order to study the effect of this series of diets on reproduction and lactation in the rat, typical animals, which survived the 20-weeks experimental period, were maintained on the respective diets and were mated. Other animals were slaughtered for the purpose of determining the effect of diet on dry matter, fat and liver glycogen. Dry matter was determined by desiccation under partial vacuum, fat by Soxhlet extraction of the desiccated material with ethyl ether, and glycogen was determined by the method of Good, Kramer and Somogyi (8). The iodine number of the extracted body fat was determined by the method of Wijs (9). All remaining animals were submitted to pathological examination.

In a second series of feeding tests, groups of young rats were fed, respectively, four basal diets identical in composition with that of the first series with the exception that in three of the four diets the corn starch was replaced by equal weights of

glucose, sucrose or dextrin[1]. In this series, glycerol and propylene glycol were fed only at the 30 per cent level, replacing, in each case, an equal weight of the carbohydrate. The purpose of this series of tests was to determine whether glycerol or propylene glycol functioned differently, biologically, when fed in combination with different types of carbohydrates.

When it became apparent that young rats which received the glycerol-containing diets invariably grew at a faster rate than did other young rats receiving comparable propylene glycol-containing diets, it was decided to determine the part that palatability of the respective diets played in bringing about these differences in growth rates. Consequently, a group of young rats was fed diets containing the same amount of glycerol and of propylene glycol, during alternate weeks. A careful record was made of the amount of the respective diets consumed and of changes in body weight. This feeding test continued for twelve weeks. A similar group of rats was offered a choice of glycerol and of propylene glycol-containing diets by offering both diets simultaneously. This experiment continued for six weeks, during which time a record was made of the amounts of each of the diets consumed, as well as changes in body weight.

Since in each of the above series of growth studies, glycerol and propylene glycol replaced an equal weight of the carbohydrate component of the diets and since both of these alcohols possessed greater potential caloric value than the replaced carbohydrate, an additional series of feeding tests was conducted in which starch, glycerol and propylene glycol were fed in isocaloric amounts at a level approximating 30 per cent of the diet. The test animals employed in these studies remained under observation for eight weeks.

Reproduction studies. - When attempts to obtain reproduction on the 21 diets of the first series were extremely disappointing regardless of whether these diets contained glycerol, propylene glycol or starch, it became evident that failure was not due alone to the energy component of the diets. Consequently, an attempt was made to improve reproduction by supplementing the various diets of the series with certain mineral salts or with mineral salts and dried yeast. When these supplementary measures did not produce the desired results, attempts to conduct reproduction studies with purified diets were abandoned.

Other reproduction studies were initiated in which select groups of young rats were maintained on diets consisting of 70

[1]Prepared by mixing 10 Kg. of commercial corn starch with 10 liters of 1/10 per cent aqueous citric acid solution, transferring to six shallow enamel pans and then autoclaving for four hours under 15 pounds pressure. The autoclaved starch was then dehydrated and pulverized.

parts of the breeding colony diet[1], and 30 parts of glycerol or propylene glycol. These experimental groups consisted of 3 male and 6 female rats, with one male to two females to a cage. Pregnant females were removed from the breeding cage and placed in individual cages before the litters arrived. Females failing to breed were remated with other males which had received the respective diets. As a last resort, those females which remained sterile were remated with normal males from the breeding colony. All dietary treatment of the females remained the same during growth, reproduction and lactation. Attempts were made to obtain at least two litters from each female, unless evidence was obtained which indicated that the female was incapable of producing young. When a litter was weaned the female was returned immediately to the breeding cage. Young animals selected from the litter were continued on the same diet while the remaining young were discarded. At the conclusion of each generation, representative adult animals were submitted to pathological examination. These studies were continued for approximately two years before being terminated.

Reproduction studies with the diets composed of 70 per cent of the breeding colony diet and 30 per cent of glycerol or 30 per cent of propylene glycol did not yield data which were comparable to those data obtained in the breeding colony over the course of many years. However, some interesting observations were made during the course of the feeding tests and some of these will be described later in this report. The difference was probably due to the diluting effect of the glycerol and of the propylene glycol on essential nutrients other than energy. Consequently, a third and more comprehensive series of reproduction studies were initiated. The composition of the diets employed in this series is shown in Table II.

These diets differed from those employed in the previous series of reproduction studies in that a special effort was made to formulate diets which retained optimal amounts of proteins, minerals, vitamins, etc., after replacement of starch by isocaloric amounts of glycerol or of propylene glycol.

Fourteen groups of selected young rats (3 males and 6 females) were used as experimental subjects. To facilitate the recording of data on growth and reproduction, each experimental group was divided into three subgroups of three animals each (1 male and 2 females). All animals were weighed at weekly intervals and a record was made of the average amount of diet consumed by the three animals comprising each subgroup. On reaching 70-80 days of age

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[1]For composition of breeding colony diet see Table II, Diet 31

[2]Since starch, glycerol and propylene glycol possess different caloric values (4.19, 4.32 and 5.67 Kg. calories per Gm. respectively), adjustments were made in the amounts of these substances incorporated in the various diets so as to yield diet pairs of isocaloric or equal caloric values.

TABLE II. - Composition of the Various Diets Employed in a Series of Reproduction Studies in Which Rats Were Continued Under Observation Through Three Successive Generations

	INGREDIENTS - Grams															
Diet Number	Yellow Corn Meal	Rolled Oats	Linseed Meal	Wheat Bran	Meat Scrap Meal	Skim Milk Powder	Sodium Chloride	Calcium Carbonate	Ferric Citrate	Alfalfa Leaf Meal	Ground Whole Wheat	Wheat Bran, Germ, etc.,[1]	Corn Starch Commercial	Glycerol	Propylene Glycol	Total
31 (Stock)	680	196	320	100	200	400	40	60	4	200	1800	---	--	--	---	4000
32 (Control)	680	196	320	100	200	400	40	60	4	200	--	540	1260	--	---	4000
33	680	196	320	100	200	400	40	60	4	200	--	540	1162	98	---	4000
34	680	196	320	100	200	400	40	60	4	200	--	540	1186	--	74	4000
35	680	196	320	100	200	400	40	60	4	200	--	540	1064	196	---	4000
36	680	196	320	100	200	400	40	60	4	200	--	540	1113	--	147	4000
37	680	196	320	100	200	400	40	60	4	200	--	540	966	294	---	4000
38	680	196	320	100	200	400	40	60	4	200	--	540	1040	--	220	4000
39	680	196	320	100	200	400	40	60	4	200	--	540	868	392	---	4000
40	680	196	320	100	200	400	40	60	4	200	--	540	966	--	294	4000
41	680	196	320	100	200	400	40	60	4	200	--	540	476	784	---	4000
42	680	196	320	100	200	400	40	60	4	200	--	540	672	--	588	4000
43	680	196	320	100	200	400	40	60	4	200	--	540	83	1177	---	4000
44	680	196	320	100	200	400	40	60	4	200	--	540	378	--	882	4000

[1] From 1800 Gm. of ground whole wheat.

females were examined at frequent intervals for indications of pregnancy in order that all pregnant rats could be removed to individual cages before the litter was delivered. When litters arrived, the date, the number of young born, and the average weight of the young were recorded. If the number of young in a litter exceeded six, the less thrifty young were discarded on the fifth or sixth day. Litters were weighed at weekly intervals until they were weaned. When the young animals reached a suitable size and age for weaning, the female was weighed and returned to the mating cage. Nine choice young (3 males and 6 females) were retained on the same diet as second or subsequent generation animals. These were carefully selected and, so far as possible, were chosen from first litter animals. The remaining young were either submitted to pathological examination or were discarded.

In this series of reproduction studies as in the preceding series, an attempt was made to obtain at least two weaned litters from each female. Where reproduction failures were experienced, the hitherto unreproductive females were mated to other males which were subsisting on the corresponding diet. As a last resort, the remaining unproductive females were mated to normal males from the breeding colony. At the completion of each of the several reproduction tests, the mature males and females were subjected to pathological examination. This latter series of reproduction studies was continued through three successive generations.

During the course of the studies with the first three generations there were observed some marked differences in the biological responses of different groups of animals which apparently were attributable to dietary origin. Attempts were made, therefore, to ascertain whether these differences would become more markedly manifested in succeeding generations and whether the differences would disappear or become less marked as the result of maintaining the progeny on more favorable diets. Three groups of young rats (each consisting of 9 males and 18 females) were selected from the third generation young of Groups 9, 10 and 12, (see Table III) respectively, for use in these studies. The animals of each of the groups were divided into three subgroups, each subgroup containing 3 males and 6 females. (See groups 15-23 in Table III). The animals of one subgroup were continued on the original diet. The animals of the second subgroup were changed to the corresponding glycerol or propylene glycol-containing diet. The animals of the third subgroup received the control diet (Diet No. 32). These experiments were continued through three additional generations.

Thus at the end of these tests, data had been obtained on effect of feeding glycerol or propylene glycol-containing diets through six generations, glycerol or propylene glycol-containing diets through three generations followed by the control diet for three generations, propylene glycol-containing diets for three generations followed by the corresponding glycerol-containing diet fed through three generations followed by the corresponding propylene glycol-containing diet for three generations.

TABLE III. - Composite Responses of Three Generations of Female Rats Produced on Each of Several Diets

Group No.	Diet[1] of Previous Three Generations	Diet[1] During Test-Period (Three Generations)	Substitution[2] in Test-Period Diet Per cent	No. of Females Involved	No. of Females Producing Litters	No. of Unproductive Females	Weight of Females at 100 days Gm.	Weight of Females at 200 days Gm.	Av. Age of Female at First Litter days	No. of Litters Born	No. of Young Born	Av. Wt. of Young at Birth Gm.	No. of Young Weaned	Av. Wt. of Young at 21 Days Gm.	Av. No. of Young Per Female	Av. No. of Young Born Per Litter	Total Young Weaned Percent
1	--	31	Stock	18	18	0	183	233	115	54	418	6.2	275	31.2	23.1	7.7	66
2	--	32	Control	36	36	0	183	242	117	91	689	6.0	422	29.9	19.1	7.4	61
3	--	33	G* 2.5	18	17	1	187	217	118	35	253	5.9	165	29.8	14.9	7.2	65
4	--	34	PG 2.5	18	16	2	176	218	124	38	260	5.5	147	29.3	16.3	6.8	57
5	--	35	G 5.0	18	18	0	202	234	111	43	325	5.4	212	30.2	18.1	7.6	65
6	--	36	PG 5.0	18	18	0	192	233	120	40	315	5.4	193	26.5	17.5	7.8	61
7	--	37	G 7.5	18	18	0	221	249	103	39	287	5.5	165	31.3	16.0	7.4	58
8	--	38	PG 7.5	18	18	0	175	230	121	40	229	5.8	144	27.9	12.7	5.7	62
9	--	39	G 10.0	18	15	3	178	242	131	49	349	6.0	170	30.3	23.3	7.1	49
10	--	40	PG 10.0	18	16	2	160	242	160	46	280	5.8	158	29.6	17.5	6.1	54
11	--	41	G 20.0	18	18	0	169	228	130	51	392	5.9	228	27.7	21.8	7.7	58
12	--	42	PG 20.0	18	16	2	159	204	159	38	204	6.0	120	27.8	12.7	5.4	59
13	--	43	G 30.0	18	17	1	165	227	132	51	361	6.0	239	25.2	21.2	7.1	66
14	--	44	PG 30.0	18	9	9	150	215	175	18	113	6.0	77	23.9	12.5	6.3	68
15	39	32	Control	18	17	1	184	253	109	34	267	5.7	179	30.2	14.8	7.8	67
16	39	39	G 10.0	18	18	0	168	200	101	37	278	5.6	200	28.5	15.4	7.7	62
17	39	40	PG 10.0	18	18	0	155	201	150	36	234	5.5	157	27.1	13.0	6.4	67
18	40	32	Control	14	14	0	177	206	98	32	226	5.5	158	29.5	16.1	7.1	69
19	40	39	G 10.0	16	16	0	167	198	114	34	236	5.6	153	28.8	14.8	7.0	65
20	40	40	PG 10.0	16	16	0	158	204	121	35	223	5.4	158	28.1	14.0	6.4	68
21	42	32	Control	18	18	0	203	241	111	39	361	5.6	197	31.1	20.6	9.3	54
22	42	41	G 20.0	18	18	0	200	260	100	40	333	5.5	191	30.4	18.5	8.3	57
23	42	42	PG 20.0	18	18	0	178	218	124	35	237	5.6	154	28.6	13.2	6.8	65

[1]See Table II for composition of diets.

[2]Calculated on the basis of an isocaloric amount of starch.

*G = glycerol; PG = propylene glycol.

Data

Since space does not permit a full presentation of all the data obtained in the course of this investigation, only typical data are presented. Inasmuch as the investigation involved female responses primarily, the data presented concern particularly this sex. The data presented in Table III and Figures 1-5 inclusive, typify some of the findings of this investigation. Some of these data have been subjected to statistical analyses and where the findings were statistically significant this has been pointed out.

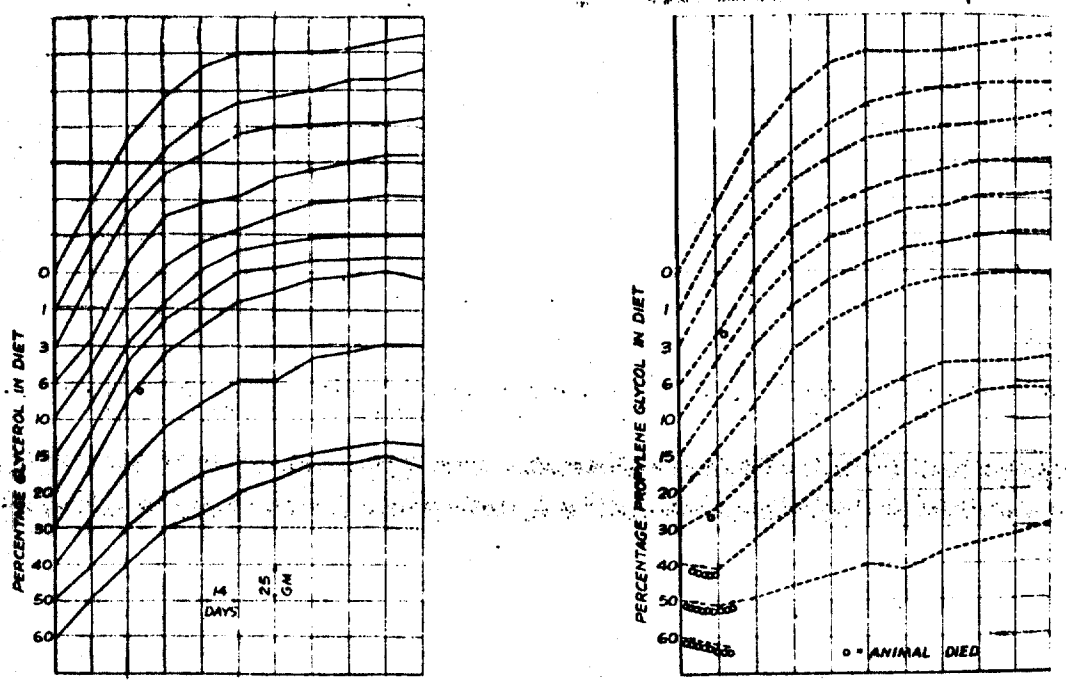

Figure 1 - The effect of replacing varying percentages (by weight), of the carbohydrate component of the diet by equal weights of glycerol or propylene glycol on the rate of growth of young female rats (for composition of diets, see Table I).

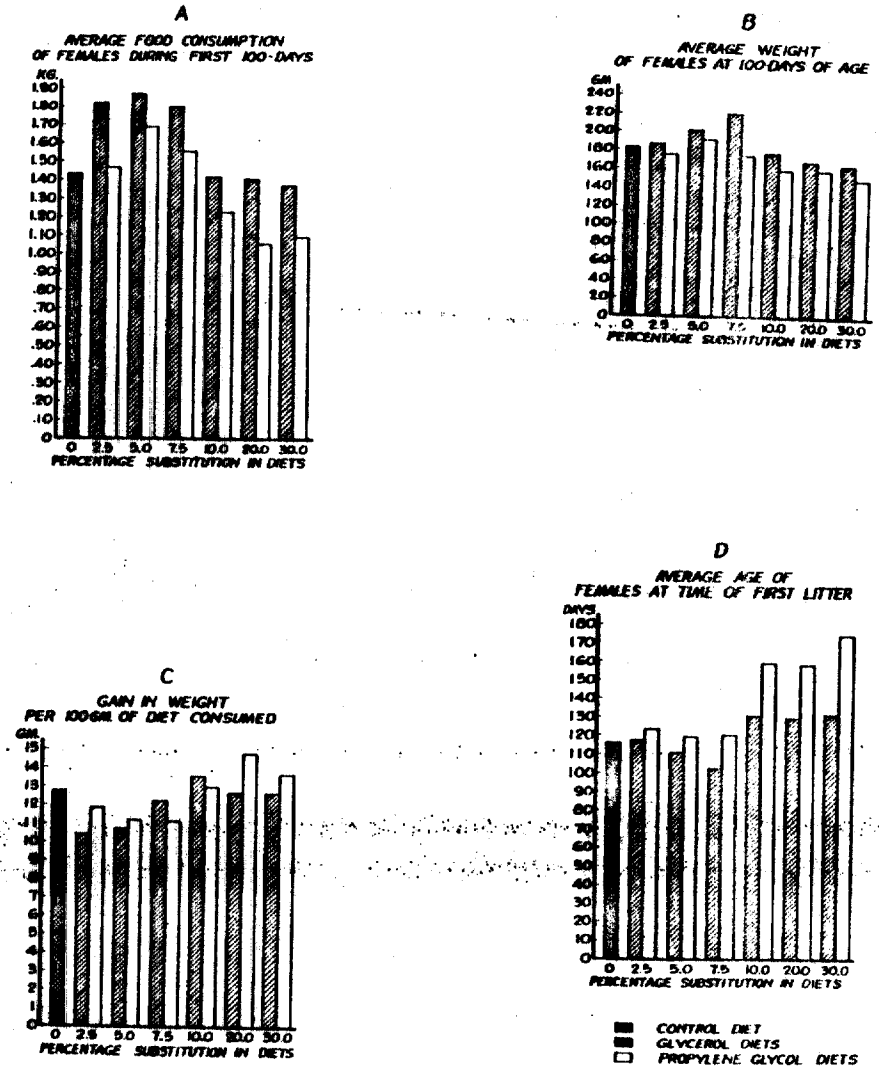

Figure 2. - The relative effects of the glycerol and of the propylene glycol content of the diet on the food intake, the rate of growth and the reproductiveness of young female rats. (The glycerol and propylene glycol were incorporated in the respective diets in equal caloric amounts. See Table II).

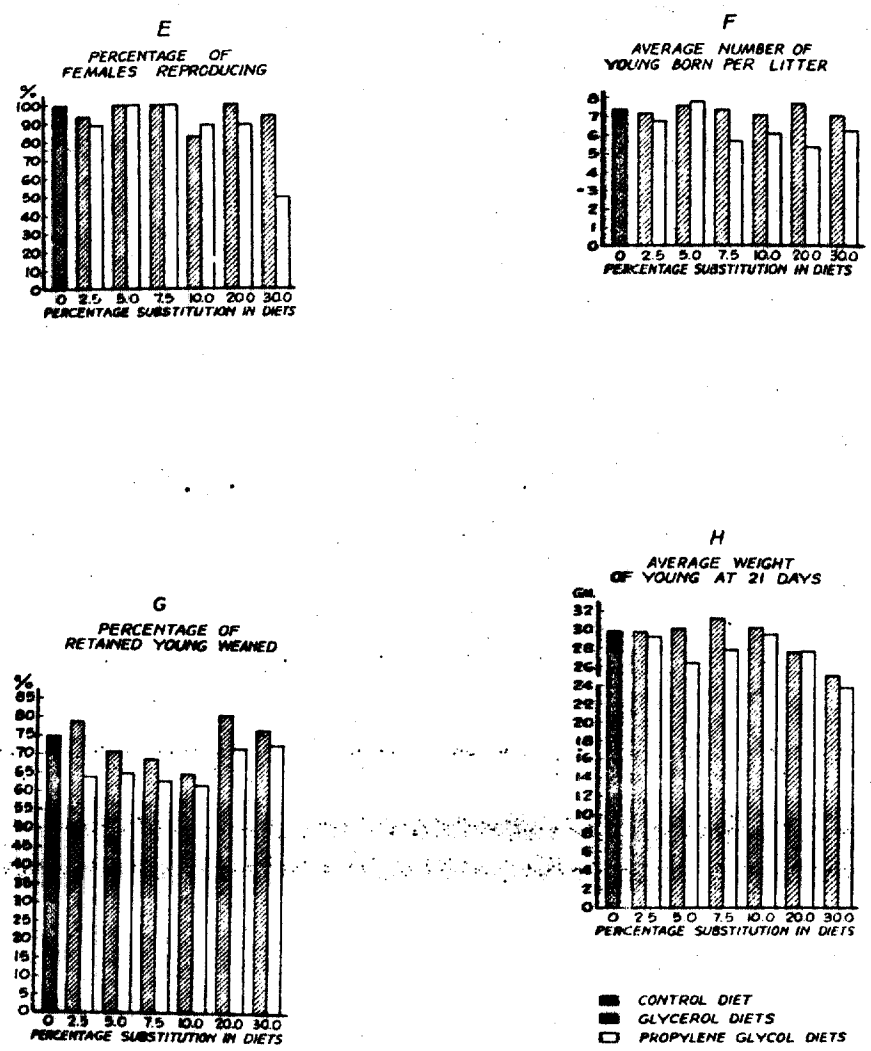

Figure 3. - The relative effects of the glycerol and of the propylene glycol content of the diet on reproduction by young female rats, with special reference to the number of litters born, the size of litters, the percentage of young weaned and the rate of growth of young. (See Table II for the composition of the various diets employed).

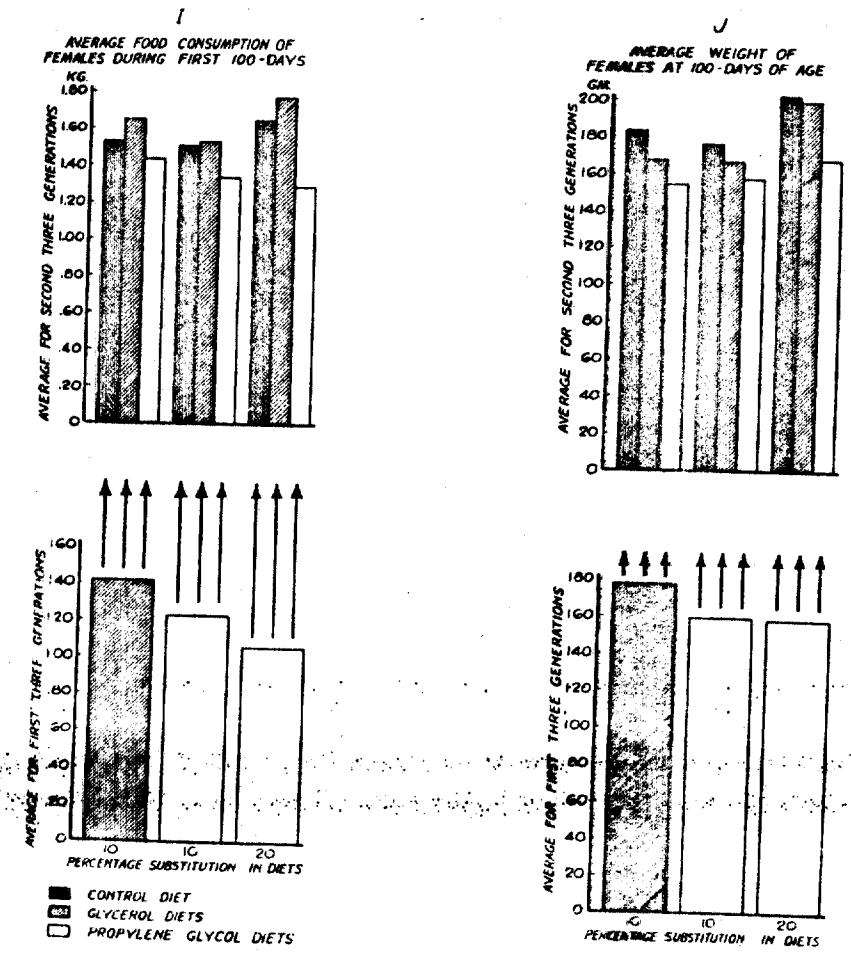

Figure 4. - The effect on food consumption and on growth of transferring growing rats which had been reared for three generations on glycerol or on propylene glycol-containing diets to the control diet and to the corresponding glycerol or propylene glycol diet, and continuing them under observation for three additional generations. (See Table II for the composition of the various diets employed).

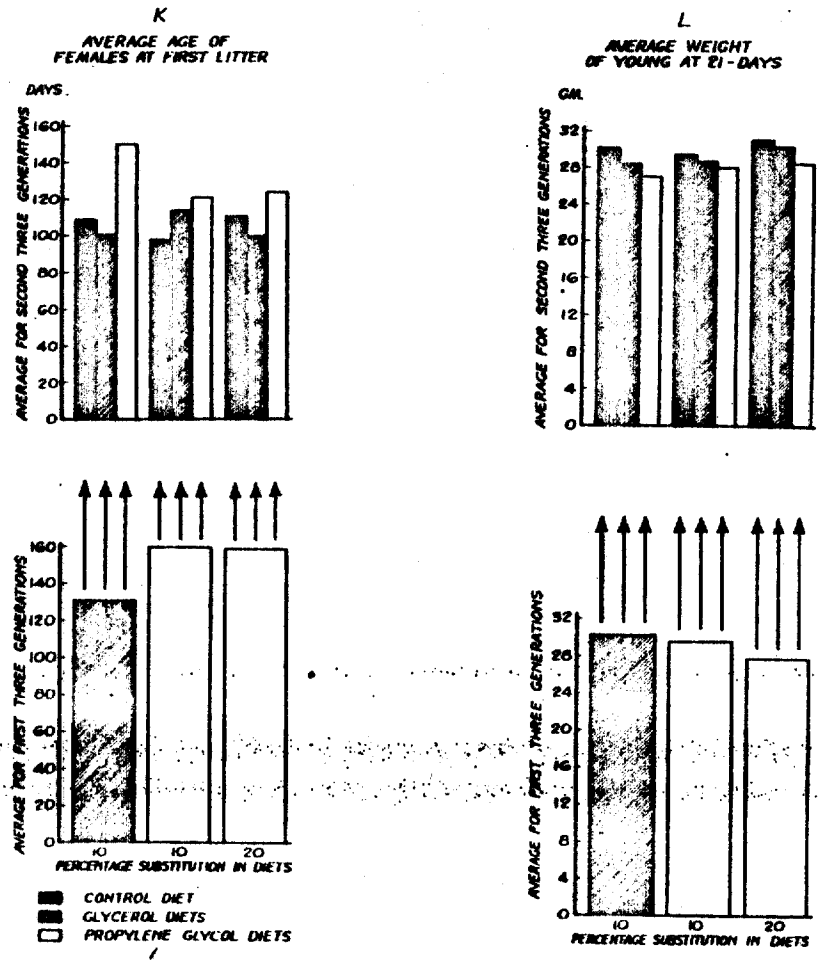

Figure 5. - The effect on reproduction and on growth of the progeny of transferring growing rats which had been reared through three generations on glycerol or on propylene glycol-containing diets to the control diet and to the corresponding glycerol or propylene glycol diet and continuing them under observation for three additional generations. (See Table II for the composition of the various diets employed).

Discussion

Some of the more important findings of the early phases of the investigation have been reported briefly in a previous publication (10). These findings, together with those obtained through subsequent experimentation, will be discussed more fully at this time.

Food Intake and Growth

In the initial experiments in which a series of purified diets containing ten different increments of glycerol and propylene glycol were fed to growing rats (males and females) no marked differences in the growth responses could be detected until the alcohols were fed at the 30 per cent level. At this level the glycerol animals grew at approximately normal rates, while the propylene glycol animals grew less rapidly (see Figure 1). Weanling rats invariably died within a few days after being placed on diets containing 40, 50 and 60 per cent of propylene glycol. When comparable groups of young rats were fed diets containing similar percentages of glycerol, growth was depressed somewhat but practically all of the test animals survived a 20-week feeding period.

A statistical examination of the growth data revealed that where the concentration of the respective alcohols in the diet was 7.5 per cent or more, the glycerol-fed rats made significantly greater gains in body weight (P = .01 - .05)[1] than did the corresponding propylene glycol-fed rats (Figures 1 and 2B). An examination of the growth data of the young females used in the reproduction studies (Figure 2B, also see Table III) reveals that first generation rats made somewhat greater gains in body weight during the first 100 days than did second and third generation females but the differences were not statistically significant. This was likewise true at 200 days of age. Older rats (half grown) showed greater tolerance of the diets containing the higher percentages of propylene glycol than did young rats, but their growth rates were not equal to those of animals receiving diets containing comparable percentages of glycerol. Even when the older rats were employed, none of the test animals receiving the diets containing 50 per cent of propylene glycol survived the 20-week test period.

While only the growth responses of female rats are given (Figure 1), the growth responses for corresponding groups of male rats were of the same relative magnitude.

When young rats were fed diets containing 30 per cent of glycerol and 30 per cent of propylene glycol during alternate weeks, growth responses invariably increased during glycerol periods and were depressed during propylene glycol periods. When groups of young rats were fed purified diets containing 30 per cent of either

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[1]Where P is greater than .01 indicates a probability of less than 1 per cent that the difference is due to chance.

corn starch, dextrin, glucose or sucrose in combination with 30 per cent of glycerol or propylene glycol, growth rates were not affected by the type of carbohydrate employed, but in each instance the glycerol-fed animals made greater gains than the propylene glycol-fed animals. When rats were fed diets containing isocaloric quantities of glycerol and propylene glycol (approximating 2.5, 5.0, 7.5, 10, 20 and 30 per cent of the diet)[1] through three generations, the average weight of the females at 100 days of age was always less for the propylene glycol-fed rats than for the glycerol-fed animals (Figure 2B). In the case of both glycerol and propylene glycol-containing diets, third generation females weighed somewhat less at 100 days of age than first generation animals. However, the decrease in weight was more pronounced among the third generation propylene glycol-fed animals.

In every comparison where glycerol and propylene glycol diets were fed ad libitum to groups of rats, the glycerol diets were consumed in greatest quantities (see Figure 2A). This was true regardless of whether the alcohols were incorporated in the diets on equal percentage or equal caloric basis. When groups of young rats were permitted to choose between glycerol and propylene glycol diets during a five-week test period, the diets were consumed at the rate of approximately 20 Gm. of glycerol diet to 1 Gm. of propylene glycol diet. When the diets were changed during alternate weeks, over a 12-week period, the rats consumed approximately 25 per cent more food during the week in which the glycerol diet was fed than during the following week in which the propylene glycol diet was fed.

When starch, glycerol and propylene glycol diets were fed on the restricted basis but in equal caloric amounts (approximately 30 per cent of glycerol or propylene glycol) over an 8-week period, the rates of growth of three groups of rats were essentially the same, indicating no marked differences in the efficiency of the utilization of the three sources of energy. Where diets containing isocaloric amounts of glycerol and propylene glycol (approximately 30 per cent of each) were fed ad libitum to young growing rats, the propylene glycol animals made slightly greater gains in body weight per unit of food intake than did the glycerol-fed animals. Likewise, during the reproduction studies it was found that in four out of six possible comparisons, the young female rats receiving the propylene glycol-containing diets made greater gains in body weight per unit of food intake than did the glycerol-fed animals (see Figure 2C). Apparently these differences may be attributed to the more efficient food utilization by the slower growing animals.

Water Intake, Urine Volume, Urinary Excretion

Comparisons relative to water intake, fecal elimination and general activity were not possible at the higher levels of alcohol

[1]For composition of diets, see Table II.

intake (40, 50 and 60 per cent) owing to the high mortality among the rats receiving the propylene glycol diets. Where comparisons were possible, the glycerol-fed animals consumed less water (17 per cent), eliminated a greater number of fecal pellets and were considerably more active as indicated by the number of rotations of the wheel of the exercising cage than were comparable groups of propylene glycol-fed rats. Although there was considerable variability in the amounts of water consumed by the different animals constituting the various experimental groups, the water intakes of those animals receiving the diets containing the higher percentages of glycerol were especially large, the average daily intake being 21 ml. for the control group as compared with 62 ml. for the 60 per cent glycerol group. These increases in water intake were reflected in greater urine output. Since the animals receiving the diets containing the higher percentages of propylene glycol had died during the early days of the feeding tests, no comparison of daily urine volume was possible for these glycerol and propylene glycol groups.

An examination of the urinary excretions of representative rats from the various groups employed in the growth studies revealed that those animals receiving the high propylene glycol diets (40, 50 and 60 per cent) eliminated considerable quantities of propylene glycol in the urine. The amount eliminated seemed to depend on the amount of propylene glycol in the ingested diet and frequently ranged as high as 25 per cent of the intake. On the other hand, the amount of glycerol in the urine never exceeded 15 per cent of the amount ingested. It appears that these differences in urinary elimination may partially explain the relatively poor growth obtained with diets containing high percentages of propylene glycol.

Before death, many of the young rats subsisting on diets containing the higher concentrations of propylene glycol manifested symptoms which seemed to be characteristic of thiamine deficiency although the diets were shown to contain ample quantities of this vitamin. As a result, some of the urines collected were assayed for thiamine by the thiochrome method. The results showed that the propylene glycol-fed rats were excreting considerably larger quantities of thiamine than were the glycerol-fed rats. This was true particularly of those rats receiving diets containing 30 per cent or more of propylene glycol. It is not possible to state whether the differences were due to differences in metabolic requirement for thiamine or to an abnormal kidney function associated with propylene glycol metabolism.

Blood Glucose and Hemoglobin

The data do not show that blood glucose or the hemoglobin values bore any definite relationship to the diet consumed. All glucose and hemoglobin values were within the range of those usually reported for rats subsisting on normal diets. A second series of determinations made on the bloods of the same rats at a later stage of the experiment yielded somewhat higher hemoglobin values. This would indicate that neither glycerol nor propylene

glycol exerts any marked depressing effect on normal hemoglobin formation.

Body Composition

The composition of the diets ingested by this series of growing rats did not appear to have any effect on the water content of the body tissues, the fat content or on the iodine number of the fat. The values obtained were in accord with those obtained on the control animals and were in agreement with the values reported in the literature for normal rats.

The concentration of glycogen in the livers of glycerol-fed rats was higher than that of livers from rats receiving comparable propylene glycol diets. The concentration of liver glycogen found was somewhat higher for the glycerol fed rats than the values reported by Deuel and co-workers (11).

Pathological and Bacteriological Findings

Pathological studies[1] frequently revealed changes in the tissues of rats from certain dietary groups. These changes were found to be more characteristic and pronounced as the concentration of dietary glycerol or propylene glycol increased. No consistent pathological changes were observed in the tissues from animals which had received diets containing 7.5 per cent or less of glycerol or propylene glycol. Tissues from animals which had received diets containing 10 per cent of these substances frequently showed pathological changes. However, tissues from animals which had subsisted on diets containing 20 per cent or more of these alcohols were found to be more frequently involved and the extent of involvement was more pronounced. In general, the most marked pathological changes found in the glycerol-fed animals were found in the liver (marked hydropic and fatty degeneration of parenchymatous cells), while the kidneys were most severely involved (degeneration of tubular epithelium, accompanied by interstitial hemorrhage and edema, acute glomerular nephritis and with calcified areas in the cortex) in the propylene glycol-fed rats.

Tissues from mature animals reared and maintained on diets containing glycerol and propylene glycol (approximately 10, 20 and 30 per cent, respectively) showed essentially the same pathology as did the younger animals. While these pathological changes appeared to be somewhat more severe in the tissues from those rats which had received diets containing the higher percentages of polyhydric alcohol, tissues from second and third generation animals reared and maintained on such diets did not show an increased pathology over the tissues from the first generation animals.

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[1]We are indebted to Dr. W. T. S. Thorp, Experiment Station pathologist, for his generous cooperation during the course of the investigation.

Growing rats subsisting on purified diets exhibited no marked differences in either the type of the number of bacteria found in the intestinal tract of the glycerol or the propylene glycol-fed rats, except on those diets containing the higher concentrations of these substances. Under the latter conditions, proteolytic anaerobes and Lactobacillus acidophilus organisms were reduced to the point where difficulties were encountered in obtaining suitable cultures for study.

Reproduction and Rearing of Young

Reproduction and rearing of young were entirely unsatisfactory among all groups of animals subsisting on the purified diets, due in part, to a high degree of sterility among the animals, to weakness of females, to lack of care for the litter, and to poor growth and high mortality among the young. Reproduction and rearing of young were slightly more satisfactory among the animals receiving the glycerol diets. Attempts to improve reproduction and rearing of young by supplementing the purified diets with vitamins and minerals were without marked success. Consequently, further attempts to carry on reproduction studies with purified diets were abandoned.

Reproduction studies with diets composed of the breeding colony diet (70 per cent) and glycerol (30 per cent) or propylene glycol (30 per cent) resulted in some very striking differences. Rats which received the glycerol diet were carried through seven successive generations with a considerable degree of success while similar groups of rats receiving the propylene glycol diet failed to produce the third generation of young. The females not only failed to breed normally but when they did breed and gave birth to five litters, they were unable to feed the young satisfactorily.

Since the protein, mineral and vitamin content of the stock diet was diluted appreciably by the incorporation of 30 per cent of glycerol or 30 per cent of propylene glycol, it was impossible to state whether the poor reproduction experienced on the above diet was due to the inclusion of propylene glycol or to the diluting effect of this substance on certain essential dietary ingredients which existed in the original diet at or near threshhold levels. Consequently, a more extensive series of reproduction studies were initiated in which the diets employed contained protein, minerals and vitamins from natural sources and in which part of the energy was furnished on an isocaloric basis as glycerol or as propylene glycol (see Table II). Since diet No. 31 of this series is the breeding colony diet, the results obtained from feeding this diet are not included in this report.

As a whole, the reproduction studies with this series of diets were relatively successful in that animals from each dietary group were continued under observation through three successive generations. Figure 2D shows the effects of dietary treatment on the average age of females at the time of the birth of the first litter. These effects were obtained in spite of the fact that male and female animals were caged together from the time of wean-

ing until the pregnant females were removed from the cages (61 - 167 days). Those animals receiving the starch diet (control group) were among the first in their respective generation to produce litters. The youngest female to give birth to a litter was 78 days old and was from the third generation animals.

While no significance could be attributed to a specific generation in respect to the average age of the females at the time of dropping the first litter, the glycerol-fed females were younger at the time of the first litter than the corresponding propylene glycol-fed animals, the difference being statistically significant (P = 0.4) where the alcoholic intake was 7.5 per cent and above. All females (12 from each generation) receiving the control diet produced litters, and the average age of the 36 females at the time of their first litter was 117 days. Propylene glycol-fed rats were, on the average, somewhat older at the time of birth of the first litter than were the glycerol-fed animals. The difference was rather striking. The average age of the propylene glycol females was greater in all six comparisons, the differences being 6, 9, 18, 29, 29 and 43 days, respectively, for the six concentrations of propylene glycol employed. Here again, the effect of the diet on the age of the female at the time of the first litter was neither amplified nor decreased through three successive generations.

In view of the limited data available (Figure 3E) it is difficult to state with any degree of certainty what effect the composition of the diet had on the ability of the females to reproduce. This is especially true of those groups of animals from which one or more females died before reaching the reproduction age. All females receiving the control diet were fertile and produced one or more litters of living young. The same was true of the 18 females receiving the diet containing 5, 7.5 and 20 per cent of glycerol and 5 and 7.5 per cent of propylene glycol. One of the females (third generation) receiving the 2.5 per cent glycerol diet died giving birth to the first litter while one female (first generation) receiving the 2.5 per cent propylene glycol ration died as a result of an injury and another (third generation) was found to be sterile.

Of the 18 females originally placed on the 10 per cent glycerol diet, one died before reaching reproductive age (first generation), another died while giving birth to a litter (third generation), and a third was apparently sterile (third generation). Likewise, of the 18 females subsisting on the 10 per cent propylene glycol diet, one died while giving birth to a litter (third generation) and a second was sterile (second generation). Two of the 18 females receiving the 20 per cent propylene glycol diet (second generation) and one of the 18 females that received the 30 per cent glycerol diet (third generation animals) proved incapable of producing litters.

Nine of the 18 females placed initially on the 30 per cent propylene glycol diet failed to give birth to litters. Two of the nine unproductive females were first generation animals, two were

second generation animals and five were from the third generation. Since attempts to mate the unproductive females with other males from the respective dietary groups and finally with normal males from the breeding colony met with failure, it seemed reasonable to conclude that their unproductiveness was due to sterility. However, subsequent pathological examination of reproductive organs failed to reveal any marked abnormality.

The total number of litters produced by the three generations of females while receiving the various diets is shown in Table III. It is to be noted that the average litter production by the females receiving the various diets was not markedly different except where diets containing high concentrations of propylene glycol (20 and 30 per cent) were fed. This does not reflect the true reproductiveness of the different groups of females since it was the general practice to discard females after they had successfully weaned two or three litters, regardless of age.

The contrast in the efficiency of reproduction of the females receiving the 30 per cent glycerol diet as compared to those receiving the corresponding propylene glycol diet was particularly striking. These particular females also showed statistically significant differences (P greater than .05) between the reproductive efficiency of the first, second and third generation females. To this group of 18 females, 18 litters were born, 11 from the first generation females, 6 from the second generation females and one litter from the third generation females.

An examination of the data relative to the number of young born per litter (Figure 3F) reveals that, in general, larger litters were produced by the females receiving the glycerol diets than were produced by the females subsisting on the propylene glycol diets. In five out of six possible comparisons, the females receiving the glycerol diets produced, on an average, larger litters than were produced by females receiving the corresponding propylene glycol diet. However, the size of the litter did not appear to bear any definite relationship to the concentration of the alcohol in the diet. The average birth weight of the young from the several dietary groups ranged from 5.4 Gm. to 6.2 Gm. and did not appear to bear any consistent relationship to composition of diets fed (see Table III).

Since all litters were reduced to six young on the fifth or sixth day after birth, it is obvious that larger numbers of young were discarded from those groups producing the largest litters. Of the 689 young born to the females receiving the control diet, 28 per cent were discarded. Of those young which were produced on the 2.5, 5, 7.5, 10, 20 and 30 per cent glycerol diets, 25, 28, 24, 25, 28 and 22 per cent, respectively, were discarded. The percentage of young discarded from the litters produced by the females receiving 2.5, 5, 7.5, 10, 20 and 30 per cent propylene glycol diets were 16, 28, 14, 14, 13 and 15 per cent, respectively.

Of those young which were retained, the glycerol-fed mothers weaned a larger percentage than did the propylene glycol-fed

mothers (Figure 3G). This was found to be true in all six comparisons. The concentration of the glycerol or of the propylene glycol in the diet did not appear to have any marked influence on the female insofar as her capacity to care for the young was concerned. When the young were weaned at 21 days of age there was found to be a definite relationship between the higher concentration of the polyhydric alcohols in the various diets and the weight of the young.

Young produced by the females receiving the high glycerol and the high propylene glycol diet (30 per cent) weighed, on an average, 20 per cent less than the young from the control group or from groups receiving the diets containing the lowest percentage of the alcohols (Figure 3H, Table III). In five out of six comparisons, the progeny of glycerol-fed mothers were heavier at 21 days of age than those from propylene glycol-fed mothers, the difference being statistically significant at the 5, 7.5 and 30 per cent levels ($P = .03 - .05$.) These data indicate that the growth rate of suckling rats is affected more severely by lower concentrations of dietary glycerol and propylene glycol (20 and 30 per cent) than that of more mature animals (Figure 1).

When the progeny of third generation rats were continued on the glycerol or the propylene glycol-containing diets for three additional generations, and when similar young were transferred to the corresponding alcohol-containing diets and carried through three additional generations, and when additional young were transferred to the control diet and continued on experiment for three generations, additional data were obtained (see Figures 3 and 4, also Table III) which substantiate those obtained in the previous reproduction studies.

Female rats which had been maintained for three generations on a 10 per cent glycerol diet and then transferred to a 10 per cent propylene glycol diet and carried through three generations consumed less of the propylene glycol containing diet, grew at a slower rate, reproduced their first litter at a more advanced age, and weaned smaller progeny than did similar animals which had been maintained on the glycerol diet or which had been transferred to the control diet and continued under observation for three additional generations.

Likewise, when young female rats which had been weaned by third generation mothers subsisting on the propylene glycol-containing diets (10 and 20 per cent) were transferred to the control diet or to corresponding glycerol diets and continued under observation for three additional generations, they consumed more food and grew at a faster rate during the first 100 days, produced litters at an earlier age and weaned larger young than did comparable females continued on the propylene glycol diets for three additional generations. During this period of observation, the glyc-

erol-fed rats produced somewhat larger litters and weaned a higher percentage of the retained young than did the corresponding propylene glycol-fed rats.

Taken as a whole, the findings of these studies do not substantiate the recent report of Lakritz (12) which states: "Many toxicity tests have been conducted on propylene glycol by several laboratories; it was found to have a low systemic toxicity, in fact much lower than glycerin".

It is to be noted that the concentration of glycerol and of propylene glycol in the various diets employed in this investigation ranged from 1 to 60 per cent of the diet. It is also to be noted that no measurable effect on growth, on reproduction or on the general health of the rat was observed unless the diet contained 7.5 per cent or more of the polyhydric alcohols. The concentrations in the diet at which these alcohols were found to have a deleterious effect on the general health, the growth and the reproduction of the rat were far in excess of that which may reasonably be expected in human diets. Furthermore, it is possible that man and rat may react differently to those substances when they are ingested.

Summary

Growth and reproduction studies with rats subsisting on diets in which a portion of the carbohydrate component had been replaced by varying amounts of glycerol or propylene glycol yielded the following results:

Young rats, receiving purified diets which contained varying percentages of glycerol and propylene glycol showed marked difference in growth response. These differences were particularly marked where the diets contained 30 per cent or more of the alcohols. At the 30 per cent level of intake, the glycerol-fed animals grew at an approximately normal rate, while the propylene glycol animals grew less rapidly. Young rats lost weight and frequently died within a few days when the diets contained 40, 50 and 60 per cent of propylene glycol, whereas other young rats receiving diets containing comparable amounts of glycerol grew at a subnormal rate, but the majority of the animals survived a 20-week test period. Older rats were able to tolerate the higher intakes of propylene glycol more effectively than younger rats, but their growth response was always depressed.

When growing rats were fed glycerol-containing diets and propylene glycol-containing diets during alternate weeks, growth responses increased during glycerol periods and decreased during propylene glycol periods. The glycerol diets seemed more palatable in that they were consumed in greater quantities than were the corresponding propylene glycol diets. Without exception, the animals excreted the greatest volume of urine, the greatest percen-

tage of the thiamine intake and the greatest percentage of the alcohol intake during the weeks in which the propylene glycol-containing diets were fed. At the highest level of intake, more than 25 per cent of the ingested propylene glycol was accounted for in the urine, while in no instance was more than 1.5 per cent of the ingested glycerol accounted for.

Reproduction and subsequent rearing of young was definitely unsatisfactory with all groups of rats receiving the purified diets. However, those groups of rats receiving the glycerol-containing diets gave birth to a greater number of litters and to larger litters, and they also succeeded in weaning a higher percentage of the retained young than similar groups of rats receiving comparable propylene glycol-containing diets. Seven generations of rats were reared without particular difficulty on a diet composed of 70 parts of a breeding colony diet and 30 parts of glycerol, while other rats subsisting on a comparable diet containing 30 parts of propylene glycol failed to wean third generation young. Furthermore, six generations of rats were reared on a well-balanced diet containing 10 per cent of glycerol or 10 and 20 per cent of propylene glycol, respectively. Under comparable conditions, growth and reproduction were more satisfactory among the glycerol-fed animals.

In a more extensive investigation in which a series of diets was used which contained proteins, minerals and vitamins from natural foods and in which part of the energy was furnished as glycerol or as propylene glycol, data were obtained which show that female rats receiving the glycerol-containing diets consumed more food, grew faster, reproduced at a younger age, produced, on the average, larger litters, weaned a greater percentage of the young and weaned larger young than did other female rats receiving corresponding propylene glycol-containing diets. The effect of these diets on subsequent generations was reflected most markedly in the number of litters born, the size of the litters and in the weight of the young at weaning.

There appeared to be no marked difference in either the frequency of observation or in the intensity of the pathological conditions observed among the glycerol and the propylene glycol-fed rats, the chief difference being that the livers of the former animals were more generally adversely affected while the kidneys of the propylene glycol animals were more usually abnormal. The frequency of observations of pathological conditions and the severity of the pathology appeared to bear some relation to the concentration of the alcohols in the diet, although these differences were not always clearly defined.

It is to be noted that the concentration of glycerol and of propylene glycol in the diets employed in these growth and reproduction studies with rats were, perhaps, in most instances, greater than will ever be encountered in human diets.

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BENZOYLCHOLINESTERASE

REPORT 2: ACTIVATION OF NONSPECIFIC CHOLINESTERASE BY POLYHYDRIC ALCOHOLS*

YOSHIMI HAGIWARA

Department of Pharmacology, Showa Medical School, Tokyo

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Previously (1), I have reported on the kinetical property of benzoylcholinesterase (BzChE) and on the influence of anti-cholinesterase(anti-ChE) on BzChE using guinea pig liver, and found that BzChE is an entirely different enzyme from the nonspecific ChE enzymologically.

During the course of the previous experiments I have found that propylene glycol (P.G.), ethylene glycol (E.G.) and polyethylene glycol (PE. G.) activated nonspecific ChE, but inhibited BzChE.

This paper deals with the mechanism of their action and the enzymological characteristics of BzChE.

MATERIALS AND METHODS

1. *Enzyme preparations*

The liver of cat, chicken, rat, mouse, rabbit, dog, guinea pig, hog and human was homogenized by the same method as previously described (1).

2. *Determination of ChE*

Rates of hydrolysis were determined in duplicate by a conventional modification of Ammon's method, using the Warburg manometric technique as previously described (1). In the experiments of the mixture of substrates, the substrates were contained in the main well and the enzyme was contained in the side arm. In the experiment of the antagonistic action with inhibitors and activators the dual side arm vessels were employed, the substrate was poured into the first side arm, and the inhibitor or the activator was poured into the second side arm. The rates of hydrolysis (ChE activities) were expressed in b_{30} values which represent the amount of $CO_2\mu l$ evolved during 30 min minus the corresponding value for nonenzymic hydrolysis by 1 mg of tissue. In the

* This paper has been read before the 18th and 19th Kanto Congress of the Japanese Pharmacological Society.

experiment of polyhydric alcohols the ChE activity was corrected for acid production from the compounds by the other enzymes.

RESULTS

1. *Effect of polyhydric alcohols on ChE's of liver, brain and serum*

The effect of the organic solvents (P.G., E.G., PE. G. and acetone) on nonspecific ChE of cat liver was determined using BzCh as substrate. With the exception of acetone, the activity of nonspecific ChE was increased remarkably by the above mentioned solvents at 0.3 0.04 ml ml, especially by 0.2 ml ml of P.G. its activity was increased more than twice as much as the control (Fig. 1). When the purified enzyme was used, similarly, the activity of nonspecific ChE was increased by them. However, the activity of nonspecific ChE was decreased by acetone in both cases.

The nonspecific ChE activity of cat liver, using BuCh as substrate, was increased slightly by 0.2-0.04 ml ml of P.G., but when ACh was used as substrate, it was decreased by 0.3-0.04 ml ml of P.G. The specific ChE activity of cat brain, using ACh or MeCh as substrate, was decreased by 0.3-0.04 ml ml of P.G. (Fig. 2).

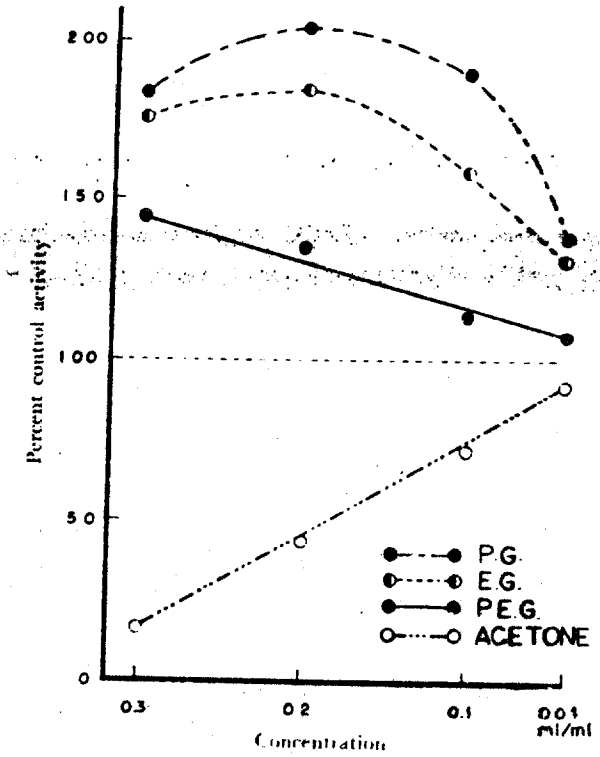

Fig. 1. Effect of organic solvents on nonspecific ChE of cat liver. (BzCh was used as substrate.)

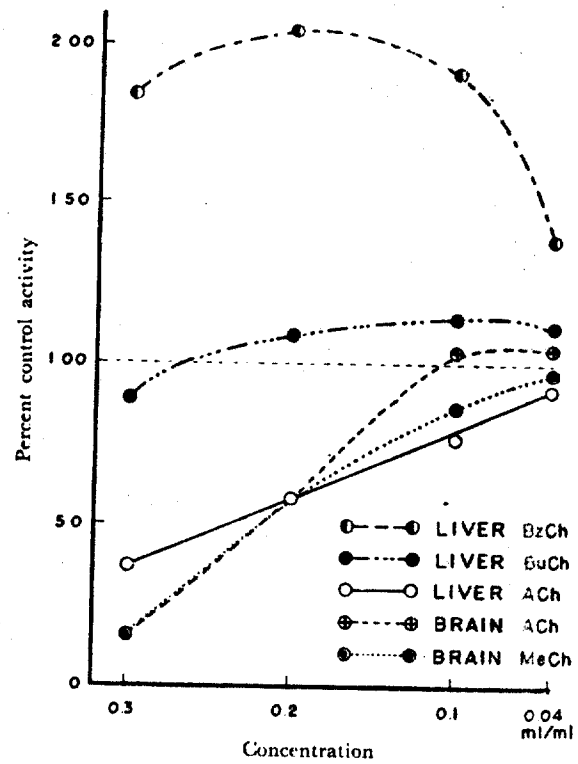

FIG. 2. Effect of 0.2 ml/ml of P.G. on nonspecific ChE of cat liver and on specific ChE of cat brain.

TABLE 1. Effect of 0.2 ml/ml of organic solvents on nonspecific ChE of cat liver and serum and on specific ChE of cat brain.

Substrate		ACh		BzCh		BuCh	MeCh
Organ		Liver	Brain	Liver	Serum	Liver	Brain
b_{50}		261.1	966.0	119.9	17.1	383.5	266.3
% Control activity	E.G.	78	83	185	135	119	91
	P.G.	60	59	205	159	109	57
	PE. G.	80	84	136	138	87	96
	Acetone	9	1	44	39	22	1

The effect of the organic solvents on ChE's of cat liver, brain and serum was summarized in Table 1. The activities of nonspecific and specific ChE, using ACh or MeCh as substrate were decreased slightly by E.G., P.G. and PE. G. The activities of nonspecific ChE of liver and serum using BzCh as substrate were increased remarkably by E.G., P.G. and PE. G., especially by E.G. and P.G. They were increased twice as much as

TABLE 2. Effect of 0.2 ml/ml of P.G. and E.G. on ChE's of liver and serum of several animals. (BzCh was used as substrate.)

	Animal	b_1	% Control activity	
			P.G.	E.G.
Liver	Cat	119.9	205	185
	Chicken	23.0	197	121
	Pig	20.8	196	118
	Dog	943.5	195	161
	Rat	39.4	166	161
	Mouse	157.5	152	111
	Human	54.4	145	202
	Rabbit	130.7	44	77
	G. Pig	628.5	29	88
Serum	Human	109.2	217	168
	Horse	74.8	165	109
	Cat	17.1	164	126

the control. The activity of nonspecific ChE of liver using BuCh as substrate was increased slightly by P.G. and E.G., but was decreased slightly by PE. G. The activities of ChE's of liver, brain and serum were decreased by acetone.

The effects of P.G. and E.G. on the activities of nonspecific ChE of liver of human, cat, dog, chicken, hog, mouse, rat and on the activities of BzChE of liver of rabbit, guinea pig, using BzCh as substrate, were summarized in Table 2. The activities of BzChE of liver of rabbit and guinea pig were decreased, while the activities of nonspecific ChE of liver and serum of the other animals were increased remarkably by 0.3 0.04 ml/ml of P.G. and E.G. (Fig. 3 and 4).

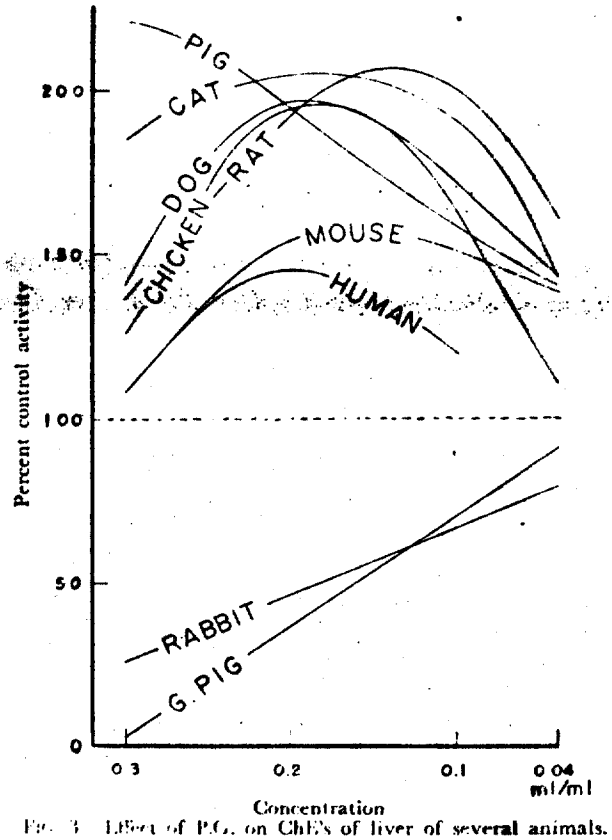

FIG. 3. Effect of P.G. on ChE's of liver of several animals. (BzCh was used as substrate.)

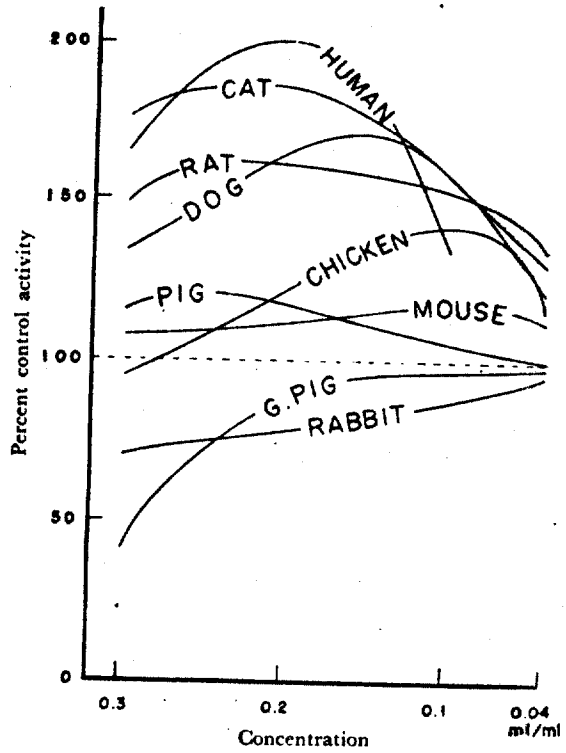

FIG. 4. Effect of E.G. on ChE's of liver of several animals. (BzCh was used as substrate.)

2. Experiment of mixture of substrates

a) The ChE activity of cat liver, using ACh and BzCh as a mixed substrate, was lower than the sum of the separate hydrolysis of ACh and BzCh, but was almost equal to the case using BzCh as a single substrate (Fig. 5). Therefore, the enzyme which hydrolysed BzCh and ACh is identical and furthermore, BzCh seemed to be preferential than ACh for nonspecific ChE.

b) The ChE activity of cat liver, using BuCh and BzCh as a mixed substrate, was remarkably lower than the sum of the separate hydrolysis of BuCh and BzCh, but was almost equal to the case using BzCh as a single substrate (Fig. 6). Therefore, the enzyme which hydrolysed BuCh and BzCh is identical and furthermore, BzCh seemed to be preferential than BuCh for nonspecific ChE.

c) The ChE activity of cat liver using BuCh and ACh as a mixed substrate, was remarkably lower than the sum of the separate hydrolysis of ACh and BuCh, but was almost equal to the case using BuCh as a single substrate (Fig. 7). Therefore, the enzyme which hydrolysed BuCh and ACh is identical and furthermore, BuCh seemed to be preferential than ACh for nonspecific ChE.

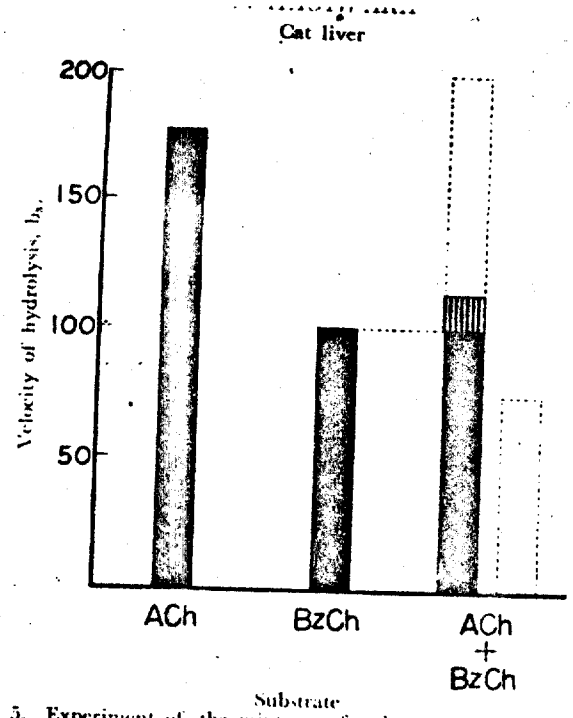

FIG. 5. Experiment of the mixture of substrates (ACh and BzCh). (Solid or hatched bars indicate an actual value of hydrolysis and open bars indicate a calculated value of hydrolysis.)

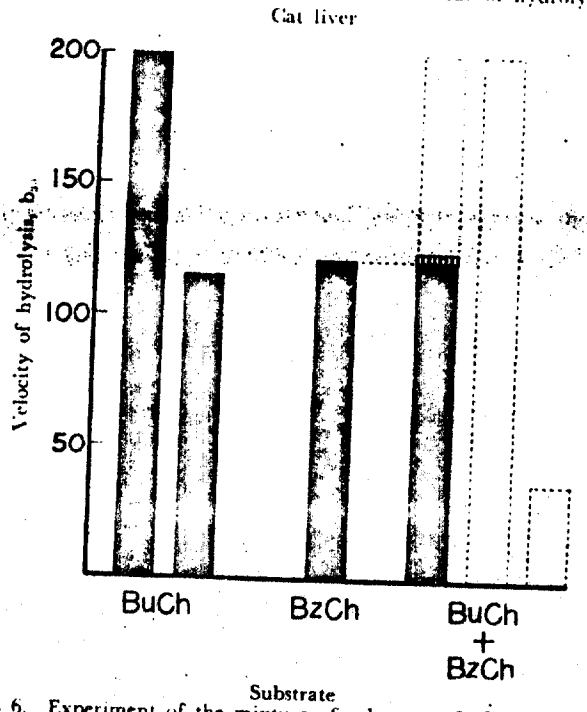

FIG. 6. Experiment of the mixture of substrates (BuCh and BzCh). (Symbols used are the same as in Fig. 5.)

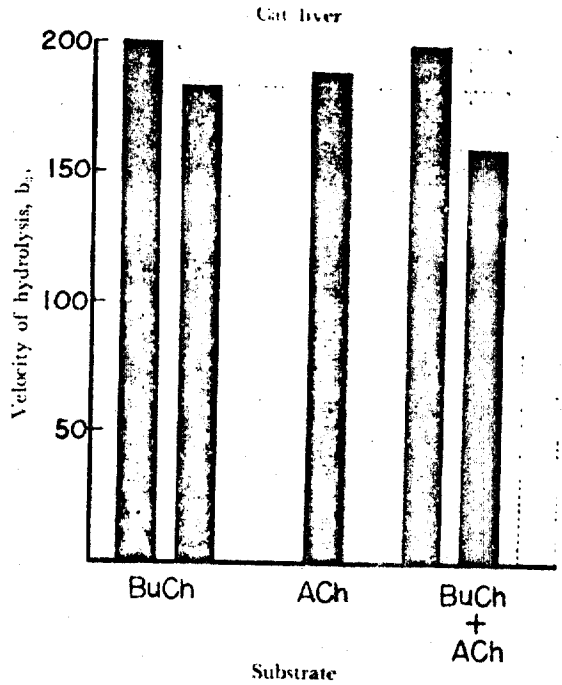

FIG. 7. Experiment of the mixture of substrates (BuCh and ACh). (Symbols used are the same as in Fig. 5.)

TABLE 3. Reversibility of P.G. action on nonspecific ChE of cat liver. (BzCh was used as substrate.)

	% Control activity	
	In absence of P.G.	In presence of P.G.
Before dilution	100	205
After dilution	100	89

TABLE 4. Effect of procedure with ether on nonspecific ChE of cat liver. (BzCh was used as substrate.)

% Control activity	
No procedure with ether	Procedure with ether
100	76

Namely, the order of the affinity, as substrate to nonspecific ChE, is BzCh, ACh.

3. Mechanism of activation of nonspecific ChE by polyhydric alcohols

a) Reversibility: The activity of nonspecific ChE in the homogenate of using BzCh as substrate, was compared before and after the dilution of two with distilled water, with and without adding 0.2 ml ml of P.G. The activit specific ChE was increased twice as much by P.G. before the dilution, but i creased 11% by P.G. after the dilution (Table 3). Therefore, this result showed has a reversible action on nonspecific ChE.

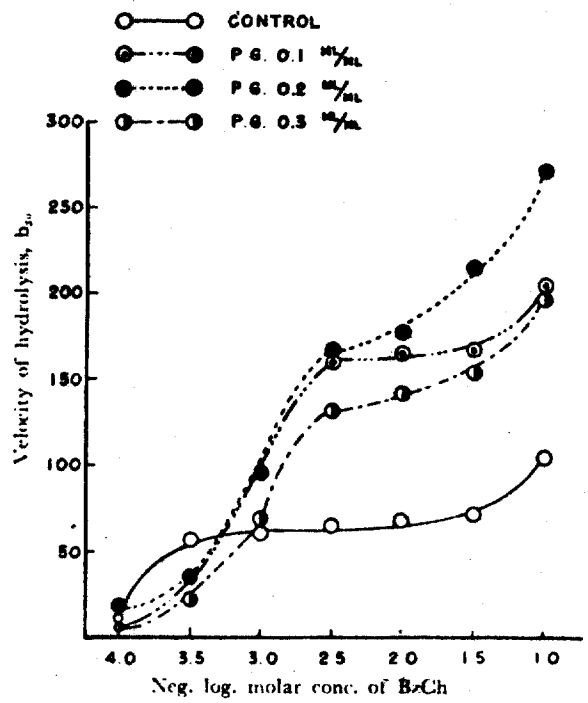

FIG. 8. Effect of P.G. on the activity PS curve of nonspecific ChE of cat liver. (BzCh was used as substrate.)

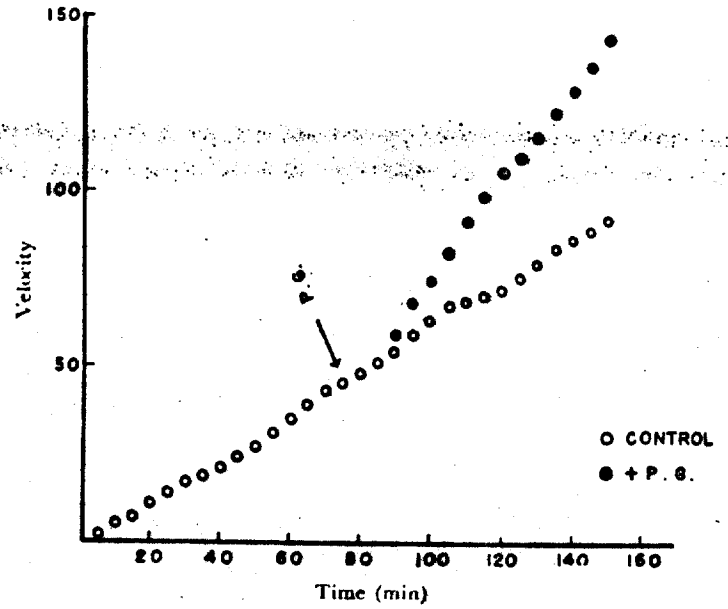

FIG. 9. Effect of 0.2 ml/ml of P.G. on nonspecific ChE of cat liver. (BzCh was used as substrate.)

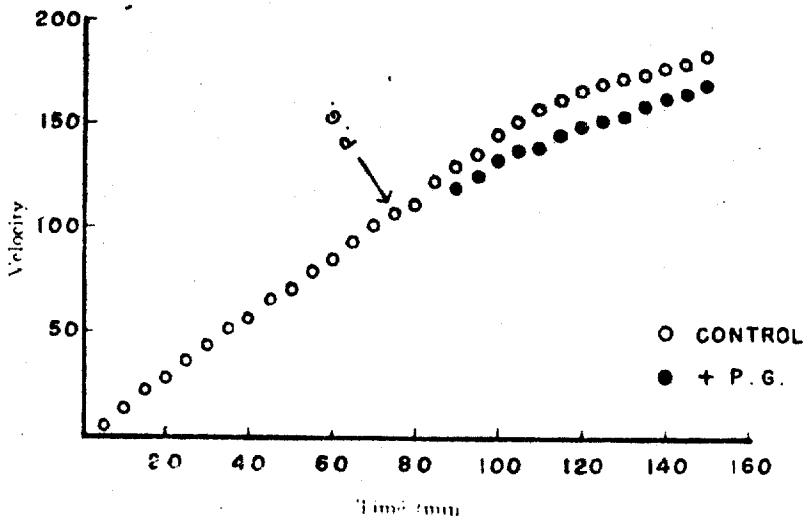

FIG. 10. Effect of 0.2 ml/ml of P.G. on nonspecific ChE of cat liver. (ACh was used as substrate.)

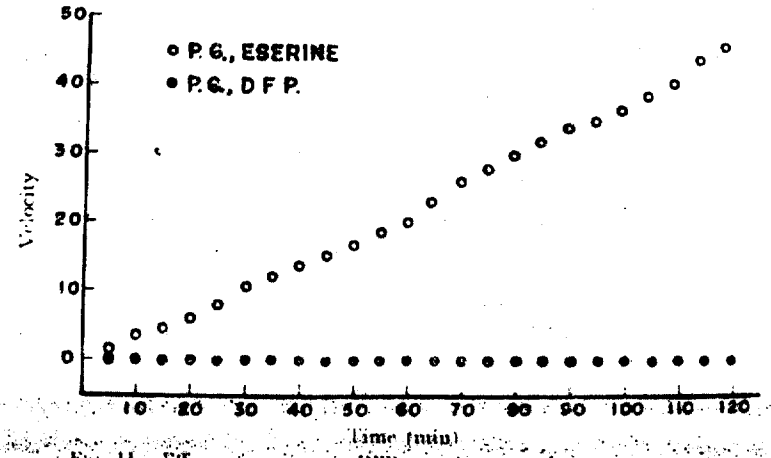

FIG. 11. Effect of eserine or DFP on the activation of nonspecific ChE of cat liver by 0.2 ml/ml of P.G. (ACh was used as substrate.)

b) Procedure of homogenate with ether: The homogenate of cat liver was mixed with ether in a separatory funnel and was shaken thoroughly, and was preserved for 24 hours in a refrigerator, and then removed ether to be used as an enzyme preparation. A quarter of activity of ChE was decreased when ether treated enzyme was used (Table 4).

c) Effect of polyhydric alcohols on the concentration of substrate. Activity-PS curve of the control showed typical S type shape and an increase of velocity of hydrolysis with the excess of substrate. The ES-reaction was activated by the administration of P.G. (0.1–0.3 ml/ml). The administration of 0.2 ml/ml P.G. caused an increase of velocity of hydrolysis by the excess of substrate.

D) Antagonistic action: When BzCh was used as substrate, the activation of the enzyme-substrate reaction was increased linearly with the elapse of time when 0.2 ml/ml of P.G. was administered (Fig. 9), however, when ACh was used as substrate, it was decreased (Fig. 10).

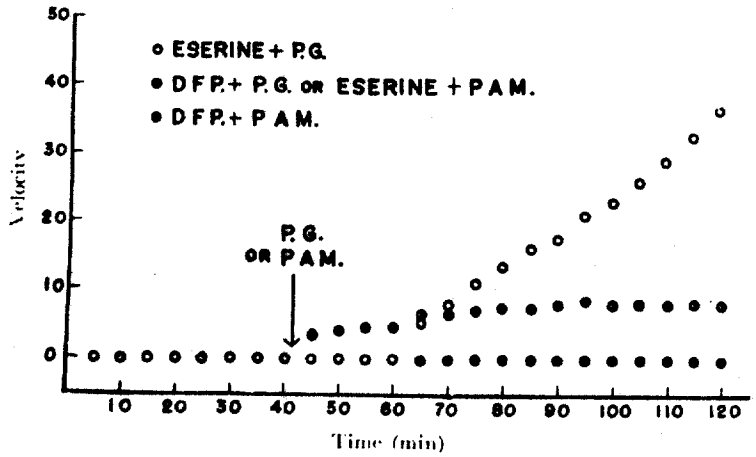

Fig. 12. Effect of P.G. or PAM on the inhibition of nonspecific ChE of cat liver by DFP or eserine. (BzCh was used as substrate.)

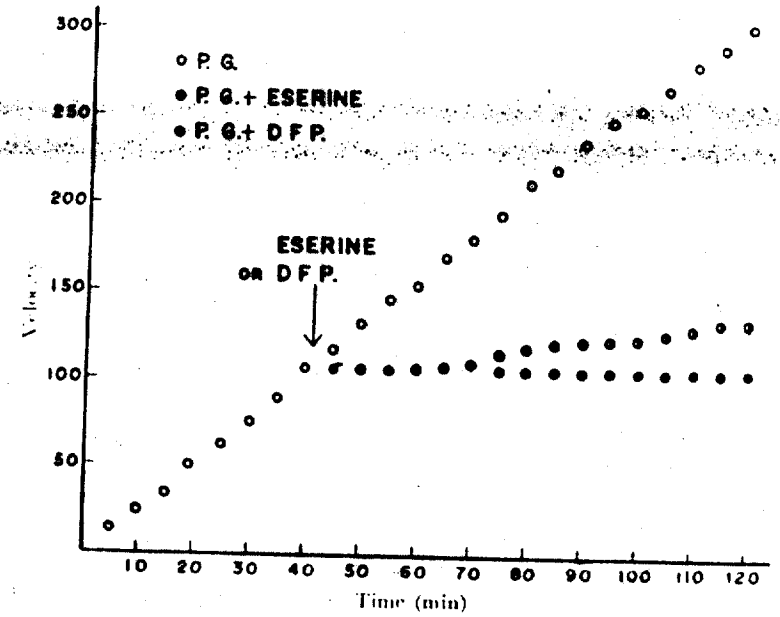

Fig. 13. Effect of eserine or DFP on the activation of nonspecific ChE of cat liver by 0.2 ml/ml of P.G. (BzCh was used as substrate.)

Eserine, which is a reversible and diisopropyl fluorophosphate (DFP), which is an irreversible inhibitor of ChE, were used as the inhibitors. The homogenate of cat liver was mixed with 0.2 ml/ml P.G. and 10^{-6}M eserine or 10^{-7}M DFP, both of which could inhibit ChE completely, then the activity of nonspecific ChE was measured with BzCh as substrate. When eserine and P.G. were mixed, the activity of ChE could be measured during the progress of reaction, but when DFP and P.G. were mixed, it could not be measured for 2 hours (Fig. 11). The administration of 0.2 ml ml of P.G. following a complete inhibition of nonspecific ChE by eserine caused a reactivation. On the other hand, the administration of 10^{-7}M 2-pyridine aldoxime methiodide (PAM) did not cause any reactivation. Similarly, the administration of P.G. following a complete inhibition of nonspecific ChE by DFP caused no reactivation, while the administration of PAM caused a slight reactivation immediately (Fig. 12). The administration of eserine following a marked activation of nonspecific ChE by P.G. (0.2 ml ml) caused a slight reactivation following a temporary inhibition. The administration of DFP following a marked activation of nonspecific ChE by P.G. caused no reactivation for more than 2 hours' observation (Fig. 13).

DISCUSSION

A considerably higher concentration of organic solvents, such as P.G., E.G. and PE. G. caused a remarkable activation of nonspecific ChE of cat liver when BzCh was used as substrate, especially 0.2 ml/ml of P.G. increased its activity twice as much as the control. Most of the powerful inhibitors of ChE we have known now are the organophosphorus compounds which are used as the potent agricultural insecticides and chemical warfare. They are not easily soluble and unstable in water, and usually used in solution or emulsion in organic solvents. It is of importance in our daily life to know the effect and the mechanism of action of organic solvents on ChE's, not only of animals, but also of human.

The activating effect of P.G. on nonspecific ChE were not unique when several different substrates were used. When ACh was used as substrate, the activity of nonspecific ChE decreased remarkably, while BuCh was used as substrate, it increased slightly. In this experiment it is necessary to prove whether the enzyme which hydrolysed BzCh, BuCh or ACh is single or multiple, since the different substrates showed a different action to P.G. By the experiment of the mixture of substrates it was proved that the enzyme in the homogenate of guinea pig was single. The different activity of P.G. on nonspecific ChE, when the different substrates were used, might be explained by the difference of susceptibility of the substrate molecule to the enzyme rather than the formation of a chemical complex. This mechanism has been proposed by Frasser (2) when he observed that mephenesin activated nonspecific ChE more strongly when BzCh was used as substrate than BuCh or ACh.

On the other hand, acetone always (3) caused an inhibition, not only to nonspecific ChE, but also to specific ChE. Therefore, acetone is not a suitable agent for anti-ChE's,

P.G. and E.G. caused the activations of nonspecific ChE of human, rat, dog, chicken, hog, mouse liver and of human, cat and horse serum, but caused the inhibitions of nonspecific ChE of rabbit and guinea pig liver when BzCh was used as substrate. This is a coincidental agreement with the Sawyer's observation (4) in which nonspecific ChE of rabbit and guinea pig liver could hydrolyse BzCh but not ACh while nonspecific ChE of other animal liver could hydrolyse BzCh and ACh equally. Furthermore, he proposed that this enzyme is an individual and named "Benzoylcholinesterase". This substantiates my previous report (1) that BzChE is an entirely different enzyme from nonspecific ChE by means of the kinetical study.

The mechanism of the activating property of P.G. on nonspecific ChE was studied. The activating property of P.G. was reversible, as butanol for the ChE (5), and unlikely to be due to the removal of the protecting substances (proteins, mucins or fats) from the enzyme, since the partial purification or the procedure with ether of the homogenate showed no significant change in its activity to the control. The negative inhibition (5) seemed also unlikely the explanation of the mechanism, since the activation was remarkable in the stage of ES-reaction, but not ESS-reaction. The activating property of P.G. was antagonized by the inhibitory property of eserine, which is reversible (6), but was not antagonized by the inhibitory property of DFP which acts to the esteratic site of ChE irreversibly (7). This may suggest that P.G. may act to the esteratic site of ChE (7) directly.

SUMMARY

1. P.G., E.G. or PE. G. caused the activations of nonspecific ChE of human, cat, chicken, dog, hog, rat and mouse liver, and of human, horse and cat serum when BzCh was used as substrate, but caused the inhibitions of BzChE of rabbit and guinea pig liver. P.G. caused the inhibition of specific ChE of cat brain.

2. Acetone caused the inhibition of nonspecific ChE of cat liver.

3. The order of the affinity, as the substrate to nonspecific ChE, is BzCh, BuCh and ACh.

4. P.G. activated ES-reaction of nonspecific ChE reversibly.

5. The activating property of P.G. might be due to the direct action on the esteratic site of ChE.

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GENERAL METABOLIC AND GLYCOGENIC ACTIONS OF PROPYLENE GLYCOL AND SOME OTHER GLYCOLS[1]

P. J. HANZLIK, A. J. LEHMAN, W. VAN WINKLE, JR. AND N. K. KENNEDY
From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California

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The comparative non-toxicity and pharmacological inertness of propylene glycol as against other glycols (1) makes it a choice solvent or vehicle for dietary and medicinal products. Being a polyalcohol with relatively low central depressant actions, and a fairly readily oxidizable agent, it could conceivably become a superior pharmaceutic and therapeutic substitute for ordinary alcohol, with certain obvious advantages over the latter. As it is closely related chemically to lactic acid, and the products of incomplete oxidation of this glycol are lactic and pyruvic acids, it could act as a glycogenic agent and thus increase the store of potential chemical energy for promoting heat and dynamic energies in the physiological economy. This would put propylene glycol into the category of carbohydrate-like foods, with possibly important actions as a carbohydrate-substitute in metabolism, body growth and weight, and muscular activity. Nothing is known of the metabolic actions of the other glycols, although they are generally more toxic and hazardous to health when consumed even in small doses.

As the glycogenic claims for propylene glycol appear worthy of confirmation or denial, we have undertaken work to test this matter and to explore various possibilities of metabolic actions of propylene glycol and to some extent other glycols, in animals and men. Comparisons were also made in certain lines with results on glycerol and dextrose in the literature. It was thought

[1] Assistance in a part of this work was given by the late Mr. P. E. Chamberlain.

that such a study would also be of value in a further estimate of the general desirability and usefulness of the glycols in dietary and medicinal products. The various subjects considered and the methods used are described as follows.

GROWTH AND BODY WEIGHT CHANGES IN RATS ON GLYCOL DIETS

Groups of 5 rats were maintained on 6 diets as follows: (1) complete stock diet (control),[2] (2) carbohydrate-free diet, (3) complete carbohydrate-substitution by an equal weight of propylene glycol (48.5 per cent), (4) three-fourths carbohydrate-substitution by propylene glycol (31 per cent), (5) one-half carbohydrate substitution by propylene glycol (24.2 per cent) and one-fourth carbohydrate-substitution by propylene glycol (12.8 per cent). The rats in all groups were approximately of the same weight, i.e., 50 grams ± 5 grams, at the start. Only 4 groups lived until the end of the study, i.e., 5½ months. Of these 4 groups, those on carbohydrate-free diet showed the most rapid growth and attained the greatest body weight. The rats on the diet containing ¼ propylene glycol and ¾ carbohydrate grew more slowly than the rats on the control diet, but their median final weights were the same. The rats on equal parts of glycol and carbohydrate grew more slowly than the preceding rats and their final weights were about 40 grams less than those on the control diet. The rats receiving the diet containing ¾ glycol and ¼ carbohydrate showed a very slight gain in weight after 3 months, i.e., 50 grams as compared to 160 grams for the controls, and all were dead after 14 weeks on this diet. The rats receiving the diet with all carbohydrate replaced by propylene glycol failed to gain weight from the start and all died within 4 weeks.

The results have been described in a previous paper (1) and need not be repeated here except to recapitulate briefly their bearing on the general metabolic phase. The stunting of growth

[2] The constituents of this diet were as follows: corn meal 68 per cent, linseed oil cake 10 per cent, dried ground alfalfa 2 per cent, powdered casein 10 per cent, lard 5 per cent, cod liver oil 3 per cent, bone ash 1.5 per cent, and sodium chloride 0.5 per cent. The composition and caloric value were as follows: protein 18.6 per cent (19.4 cal.), fat 11.8 per cent (26.9 cal.), carbohydrate 51.5 per cent (53.7 cal.), roughage 3.5 per cent, salts 3.9 per cent, and water 10.7 per cent.

and loss of body weight of the groups of rats on the propylene glycol diet as compared to the control rats was the result largely of a diminished food intake. This was due presumably to unpalatability of the medicated diets. However, when voluntary choice was eliminated and gastric administration of the glycol was forced in paired feeding experiments, the body weight and growth were maintained. The 6 pairs of rats thus used showed, without exception, that propylene glycol promoted growth and body weight more rapidly and the final body weight was greater than in the control rats. The difference in final body weight of some rats amounted to as much as 50 grams, or about 20 per cent. This difference in weight was not due to a difference in food intake, since both the glycolized and control rats received equal weights of food. However, part of the difference may have been due to the greater caloric value of propylene glycol, which yields 5.7 calories per gram as compared with 3.7 calories per gram of dextrose. Additional experiments are under way to determine whether the stimulant effects on growth and body weight of young rats can be produced by equi-caloric amounts, and even less, of propylene glycol. Many months will be required to complete satisfactorily such tedious experiments. That propylene glycol promotes stores of chemical energy is indicated by the following results on its glycogenic action.

GLYCOGENIC ACTIONS OF PROPYLENE GLYCOL AND OTHER GLYCOLS

Liver glycogen in fasting rats

The following glycols were compared: propylene, ethylene, dipropylene, and diethylene. Fasting rats weighing from 80 to 150 grams were used, the groups being of comparable body weights as much as possible. The rats were given the glycols by stomach tube, and killed by inhalation of chloroform at the end of 1 hour, and 2, 3, and 4 hours. Control rats were given equal volumes of water and killed similarly. The livers were excised immediately and dropped into boiling 40 per cent potassium hydroxide solution. Glycogen was determined according to Pflüger's method (3), the glycogen being estimated as dextrose by the method of Folin and Malmros (4). Control estimations of added

glycogen to liver and muscle showed total recoveries of from 95 to 99 per cent. Addition of glycol directly to liver did not affect the determination of added glycogen. The results on 192 fasting rats receiving the different glycols and on 48 untreated controls are shown in table 1.

It is seen that propylene glycol was the only glycol that consistently increased the content of glycogen in the liver. Doses

TABLE 1

Liver glycogen in fasting rats receiving different glycols

GLYCOL	DOSE	NUMBER OF RATS	LIVER GLYCOGEN (MGM. PER CENT)					MAXIMUM INCREASE
			Controls*	1 hour	2 hours	3 hours	4 hours	
	cc. per kgm.							mgm. per gram liver
Propylene	0.5	5	148	211	156	208	135	0.63
Propylene	1.0	5	123	333	336	330	294	2.13
Propylene	2.0	5	113	241	371	465	720	6.07
Propylene	5.0	5	241	331	425	570	852	6.11
Propylene	10.0	5	75	72	224	673	726	6.51
Propylene	15.0	5	212	359	581	699	627	4.87
Dipropylene	2.0	3	105	203	221	114	139	1.16
Dipropylene	10.0	3	119	119	96	65	173	0.59
Ethylene	2.0	3	187	132	151	118	236	0.49
Ethylene	10.0	3	166	89	70	60	84	0.77†
Diethylene	2.0	3	81	69	69	87	152	0.71
Diethylene	10.0	3	160	197	127	120	204	0.44

* The values in this column are averages from groups of 5 or 3 fasting rats without a glycol; total 48 rats. Values in all other columns under liver glycogen represent averages for the groups receiving the different glycols in different doses, groups of different rats being killed at 1 hour, 2, 3, and 4 hours; total, 192 rats.

† Represents a decrease.

of 0.5 cc. per kilogram produced no changes in glycogen or only small, irregular increases, the average increase being only 0.62 mgm. per gram liver. Definite increases occurred in 1 hour after giving 1 cc. of glycol per kilogram body weight, or 100 mgm. of propylene glycol per 100 grams rat. The average increase in glycogen after this dose was about 2.7 mgm. per gram liver. This increase was only slightly less at the end of 3 hours, but was definitely less at the end of 4 hours, or about 2 mgm. per gram

liver. Accordingly, the minimum effective glycogenic dose would be between 0.5 and 1 cc. per kilogram, or 0.75 cc. propylene glycol per kilogram rat. Doses of 2 cc. propylene glycol per kilogram produced smaller increases in the liver glycogen to the end of 2 hours, but the amounts greatly exceeded those after 1 cc. doses, at the end of 3 or 4 hours, being of the magnitude of 500 to 1000 per cent increases, the average increase per gram liver being about 6 mgm. Practically the same increases occurred after 5 and 10 cc. doses of propylene glycol per kilogram, the average increases per gram liver being 6.11 mgm. and 6.51 mgm., respectively. However, doses of 15 cc. per kilogram produced a much smaller increase in liver glycogen, i.e., an average of 4.87 mgm. per gram liver, and such high doses caused some depression of the animals. Possibly an inhibition of absorption of the glycol occurred in these rats, but this was not investigated. Such inhibitions are known for alcohol (5) and phenol (6).

Cori and Cori (7) reported that, 3 hours after feeding 117 mgm. d-sodium lactate per 100 grams rat, the average increase in liver glycogen was 14.6 mgm. per gram liver. Since it is known that glycogen may be formed directly from lactic acid, it seems probable from our results that not all the propylene glycol was converted to lactic acid and stored as glycogen. Stöhr (8) found that 100 mgm. methyl glyoxal per 100 grams rat caused an increase in liver glycogen of 4 mgm. per gram liver, and 100 mgm. pyruvic acid, an increase of 6.5 mgm. per gram. The products of incomplete combustion of propylene glycol are pyruvic and lactic acids. Catron and Lewis (9) reported that, 3 hours after feeding approximately 100 mgm. glycerol per 100 grams rat, the average increase in liver glycogen was 17.9 mgm. per gram liver. Three hours after administering about 100 mgm. dextrose per 100 grams rat, these authors found the average increase in liver glycogen to be 18.5 mgm. per gram liver. Hence, it would appear that gram for gram, propylene glycol lacks the rather high glycogenic efficiency of lactic acid, methyl glyoxal, pyruvic acid, or dextrose. On the other hand, propylene glycol is unique among the glycols thus far investigated as being the only one to produce definite increases in the important metabolite glycogen. This

confers upon it a certain food value, and also a possible therapeutic value in certain liver toxicoses, such as those of chloroform, cinchophen, etc.

Continued feeding of propylene glycol as substitute for carbohydrate

The liver glycogen was determined in rats fed diets containing propylene glycol with reduced carbohydrate during 5½ months, or about ¼ the span of life. Four groups of 4 rats each were used as follows: one on normal stock diet, one on carbohydrate-free diet, one on the diet in which one-half the carbohydrate was replaced by an equal weight of propylene glycol (25 per cent of the total diet), and one on a diet in which one-fourth the carbohydrate was substituted by an equal weight of the glycol (12.5 per cent of the total diet). The rats were killed (without fasting), when their body weights became constant, and the liver glycogen content was estimated. The results on the 17 rats used are summarized in table 2, and leave no doubt that the glycolized diets used caused definite increases in liver glycogen. The carbohydrate-free diet caused practically no change in the liver glycogen, but the rats receiving a ½ substitution of propylene glycol for the carbohydrate showed almost double the increase in glycogen as compared with rats receiving ¼ the amount of glycol. Thus, the propylene glycol substituted effectively as a glycogen former under conditions of carbohydrate deficiency. This could

TABLE 2

Liver glycogen of rats on continued administration of propylene glycol in diet

DIET	TIME	NUMBER OF RATS	MEDIAN BODY WEIGHT	AVERAGE LIVER GLYCOGEN	INCREASE OVER NORMAL
	months		*grams*	*mgm. per cent*	*mgm. per gram liver*
Normal stock	5½	5	223	694	
Carbohydrate free	5½	4	253	812	1.18
One-fourth glycol and three-fourths carbohydrate	5½	4	211	1,351	6.57
Equal parts glycol and carbohydrate	5½	4	182	1,909	12.15

certainly not be attributed to any deleterious action of glycol. On the contrary, this could be a beneficial dietary action.

Paired feeding of propylene glycol and water

Four pairs of rats were fed propylene glycol and water in the same quantities of food, according to the method of Mitchell (2). Litter mates of approximately equal weights were used. The glycolized rats received a diet containing one-fourth less carbohydrate than the controls. Propylene glycol was given the rats daily by stomach tube, the dose being 12.8 per cent of the total

TABLE 3

Liver glycogen of rats on paired feeding

GLYCOLIZED RATS*						CONTROL RATS			
Rat	Initial body weight	Final body weight	Average daily intake of propylene glycol	Liver glycogen	Difference over normal	Rat	Initial body weight	Final body weight	Liver glycogen
	grams	*grams*	*cc.*	*mgm. per cent*	*mgm. per gram liver*		*grams*	*grams*	*mgm. per cent*
1	148	316	1.68	3,085	+4.43	1A	143	258	2,642
2	157	314	1.54	3,158	−4.92	2A	138	252	3,650
5	153	277	1.42	3,230	+16.95	5A	152	240	1,535
7	110	306	1.54	2,804		7A†			

* Propylene glycol given was equal to 12.8 per cent of food eaten, or ¼ the carbohydrate content of the diet. The plus sign (+) means increase, minus sign (−) decrease.

† Died.

weight of food eaten the previous day. The control rats received an equal volume of water by stomach tube. When the glycolized rats had reached a constant weight, after about 5 months, both the controls and medicated rats were killed and the liver glycogen estimated. The results summarized in table 3 show increases in liver glycogen in 2 of the 3 pairs of rats which survived. While the second pair in the table showed a decrease in glycogen equal to the increase showed by the first pair, the increase in the third pair was so great, i.e., nearly 4 times that of the first group, that the tendency to an increase appears definite. Any increases were, of course, independently of food consumption in this experimental

arrangement. The daily doses of propylene glycol were large when translated in terms of human body weight and consumption and so it is possible that not every individual will react with an increase in liver glycogen. Smaller doses might give more consistent and greater increases in glycogen; such experiments are under way.

Glycogen of the whole body

The results just described indicate that propylene glycol definitely increases the glycogen content of the liver under different conditions. This glycogenic action occurs independently of the food intake and with diets deficient in carbohydrate. While the bulk of the body's glycogen stores are in the liver, it would be

TABLE 4

Glycogen in whole rats receiving propylene glycol

DOSE OF GLYCOL	NUMBER OF RATS	AVERAGE TOTAL GLYCOGEN AFTER 3 HOURS	INCREASE OVER CONTROLS
cc. per kgm.		*mgm. per cent body weight*	*mgm. per gram tissue*
None (controls)	13	100.7	
1	12	117.8	0.171
5	12	127.5	0.268
10	8	125.0	0.243

interesting to know whether the glycogen of the body as a whole is increased after giving propylene glycol. This was tested in 32 rats, which were given gastrically 1.5 and 10 cc. doses of propylene glycol per kilogram, body weight and their glycogen content compared with that of 13 control unmedicated rats of about the same body weight and on the same diet. The results obtained are summarized in table 4 and show definite, though small, increases in glycogen per gram of tissue. The 2 higher doses gave definitely greater increases than the smallest dose. The glycogenic action of propylene glycol is rather rapid as indicated by the fact that the increases were demonstrated 3 hours after administering the glycol. Thus, there is no doubt that propylene glycol increases not only the glycogen content in the liver but also in the tissues, in general.

RUNNING ACTIVITY OF RATS ON PROPYLENE GLYCOL

The extra glycogen formed after administration of propylene glycol could conceivably act as a potential source of dynamic energy, and result in an increase in body activity. On the other hand, propylene glycol in high doses acts as a central depressant, like alcohol, and therefore, a decrease in activity might occur, but this would depend on the dosage. Small doses might be beneficial or ineffective, and high doses depressant, on muscular activity. A test of the effects of this glycol on voluntary running activity of white rats was made for possible evidence of work performance under controlled conditions. An exceptional opportunity of doing this presented itself with a standard device tested under many known physiological conditions over many years at Stanford by Prof. R. J. Slonaker.

Using the revolving cages described by Slonaker (10), the voluntary running activity of female white rats on the stock diet containing one-fourth the carbohydrate replaced by propylene glycol was observed for 19 weeks. Their preliminary activity was recorded for from 3 weeks to 2 months before proceeding with the experiment in order to accustom the rats to the cages and to determine their running ability. The rats were kept in individual revolving cages. Then 5 rats were given the glycolized diet, and 5 other rats were kept as a control group on the stock diet. After completion of the experiment, both groups of rats continued their running on the stock diet for an after-period of 6 weeks. Daily records were kept of the number of revolutions made by the rats. A summary of the data obtained on the 10 rats used is presented in table 5.

It is seen that, after the first 5 weeks on the glycolized diet, the rats maintained a somewhat better average running activity, if anything, during the remainder of the experiment, or a total of 14 weeks. The individual variations were considerable. For instance, the activity of the individual rats fluctuated from nearly 2 to generally about 11 miles of running on successive days, or even weeks, after they became accustomed to the devices. However, the general tendency in the individual activity was similar, and there was at least no serious reduction in activity of the

glycolized rats. The small decrease in activity during the first 5 weeks was presumably the result of insufficient eating of an unpalatable diet. When the distaste was overcome, the rats ate more liberally and continued a better activity.

Since, in Slonaker's extensive experience, the volitional running activity of white rats, under these conditions, depends on good

TABLE 5

Running activity of female rats with and without propylene glycol in the diet

RAT	PRELIMINARY ACTIVITY	AVERAGE DAILY RUNNING				
		5 weeks	10 weeks	15 weeks	19 weeks	26 weeks
Control on stock diet						
	miles	*miles*	*miles*	*miles*	*miles*	*miles**
C4	4¾	7¼	7½	7¼	6	3¾
C2	7¾	9¼	7¾	5¼	5¾	3¾
B1	1¼	9¾	10¼	8¾	8¼	5¾
B3	1¼	8¾	8½	6¾	3½	4¾
B5	¾	1¾	2¼	2¼	5	4¾
Average	3¼	7¼	7¼	6	5¾	4¾
Propylene glycol in diet (¼ carbohydrate substitution)						
C5	8	5¾	1¾	2¾	1½	1¾
C3	¾	5	10¾	9¾	11	8
C1	½	8	7¼	5	3¾	1¾
B2	¾	13	11¾	9¾	10¾	8¾
B4	2¾	8¾	10¾	7¼	6¾	7¼
Average	2¾	7¼	8¾	7	6¾	5¾
Difference from controls†	−¾	−¼	+1¼	+1	+1	+¾

* After period of 6 weeks on stock diet without propylene glycol.
† The minus sign (−) means decrease and plus sign (+) increase.

physical condition and well-being, it follows that the continued daily consumption of rather large quantities of propylene glycol, i.e., equal to ¼ the carbohydrate content of the diet, and for about ⅓ the span of life, was not disturbing to the health of these animals. This is consistent with the comparatively low systemic toxicity of this glycol according to other criteria, and as compared with other glycols.

BASAL METABOLIC EFFECTS OF PROPYLENE GLYCOL, ALCOHOL AND DEXTROSE IN MEN

Three human subjects, who had previously fasted for 12 hours and were in a resting condition, took by mouth 50 cc. propylene glycol in a 50 per cent aqueous solution. B. M. R. readings were obtained before the glycol was taken and at end of ½ hour and 2 and 3 hours after the glycol. The same observations were made with 25 cc. ethyl alcohol, and 50 grams dextrose on 2 subjects each, for comparison with the propylene glycol. The results obtained are presented in table 6, and show, for the most part, small individual variations with all agents tried. Nor were the individual characteristics of the basal metabolism changed.

TABLE 6

*Effects of propylene glycol, alcohol and dextrose on basal metabolism in men**

	DEXTROSE, 50 GRAMS		ALCOHOL (25 CC.)		PROPYLENE GLYCOL (50 CC.)		
	VW	Cn	VW	Cn	VW	Cn	La
Control	−6	+20	−3	+7	0	+16	−6
½ hour	−2	+22	−8	+12	−3	+16	−5
1 hour	−2	+12	−5	+15	0	+11	−10
2 hours	−12	+20	−5	+16	0	+5	−10
3 hours	−5	+18	−5	+12	−12	0	−12

* The plus sign (+) in the table means increase, and the minus sign (−) decrease.

Therefore, the results with propylene glycol are to be regarded as essentially negative, and at least, as evidence of non-injuriousness to the physiological economy, in the doses used, which were rather generous.

PROPYLENE GLYCOL ON RESPIRATORY QUOTIENT IN RATS

Using the method of Haldane (11), the effect of propylene glycol on the respiratory quotient was observed in 13 fasting rats. First, 3 control determinations were made on each rat, and then, propylene glycol was administered by stomach tube in doses of 10 cc. per kilogram. One subsequent determination was made on each rat. As the results were essentially negative, they are

not presented in detail. Briefly, the medians of the 3 control periods were 0.69, 0.62 and 0.7, and after propylene glycol, 0.64 (range, 0.52 to 0.79). Although the respiratory quotients tended to be somewhat lower than usual, it would appear again that the propylene glycol lacked a deleterious effect, at least, as far as this criterion of physiological action was concerned.

SUMMARY AND CONCLUSIONS

1. Propylene glycol was found to possess a moderate glycogenic action, which was absent with 3 other glycols tried, namely, ethylene, diethylene, and dipropylene. The glycogenic action occurred with carbohydrate-deficient diets independently of food consumption, the increase in glycogen having been demonstrated in the liver and the whole body. The minimum effective glycogenic dose of propylene glycol in white rats was between 0.5 and 1 cc. per kilogram body weight, or about 0.75 cc. per kilogram, doses of 2.5 and 10 cc. per kilogram giving optimum effects, but 15 cc. per kilogram, less.

2. White rats tolerated as much as 24 per cent of propylene glycol in their diet, but did not grow as rapidly as control rats on a normal diet, presumably due to a lessened food intake caused by the unpalatable diet.

3. Paired feeding of white rats showed that, when the food intake of control and glycolized rats was kept equal, the rats receiving diets in which propylene glycol was substituted gram for gram for a portion of the carbohydrate grew faster and attained a greater weight than the control animals. Part of this difference might have been due to the greater number of calories ingested as propylene glycol as compared with dextrose.

4. The voluntary running activity of rats receiving large doses of propylene glycol was somewhat better than, and at least not demonstrably impaired as compared with, control groups kept on unmedicated stock diet.

5. Propylene glycol had no demonstrable effects on the basal metabolic rate in human subjects, and on the respiratory quotient in rats.

6. Accordingly, the results obtained on the glycogenic and

general metabolic actions of propylene glycol, under a variety of conditions, are consistent with the comparative non-toxicity, both acute and chronic, of this glycol as compared with several other glycols thus far investigated. This is further evidence in support of its non-injuriousness as a vehicle or solvent, if not desirability as an alcohol substitute in dietary and medicinal products.

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TOXICITY, FATE AND EXCRETION OF PROPYLENE GLYCOL AND SOME OTHER GLYCOLS

P. J. HANZLIK, H. W. NEWMAN, W. VAN WINKLE, JR., A. J. LEHMAN AND N. K. KENNEDY

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California

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The widespread interest in the usefulness of propylene glycol as a vehicle for medicinal and food products and as a possible dietary constituent prompted us to investigate further this glycol and to make comparisons with other glycols and alcohol. In this connection, it is important to investigate all aspects of toxicity and fate of the propylene glycol in the body, in order to determine all potential hazards to health before such a substance is used generally. Comparisons with other glycols are desirable because some of the other glycols have been proposed for similar uses as the propylene glycol. Previous reports on propylene glycol from this laboratory have dealt with acute and chronic toxicities (1, 2). This report amplifies the data on toxicity of this glycol by different procedures, adds evidence on its fate and excretion, and presents new data on the toxicity of other glycols. The procedures used are described with the discussions of the various results.

INTRAVENOUS TOXICITY IN DOGS

In a consideration of the toxicity of the glycols, it is desirable to make a sharp distinction between the acutely fatal dose, in which death occurs shortly after a single large dose, and the delayed fatal dose, in which death follows some time after the administration of single or divided doses. Apparently, the cause of death differs with the dosage-method; acute death being the result of central depression and circulatory collapse, and delayed

death the result of degenerative toxic changes in the liver and kidneys. Much the same situation prevails with delayed death from heavy metals such as mercury and bismuth.

It is not possible to determine the acute fatal doses of the glycols by gastric administration in dogs, as the doses are too large to be retained in the stomach. Therefore, the glycols were administered by slow intravenous infusion into a superficial leg vein, a 50 per cent solution in physiological saline solution (0.85 per cent NaCl) being given at a rate of approximately 0.5 cc. per kilogram body weight per minute. The anesthetic dose was the quantity of absolute glycol per kilogram body weight which produced abolition of the corneal reflex. Table 1 shows a comparison of average anesthetic and fatal doses for ethyl alcohol,

TABLE 1

Average fatal and anesthetic doses of glycols and alcohol according to intravenous infusion in dogs

DOG	AGENT	ANESTHETIC DOSE	FATAL DOSE
		cc. per kgm.	*cc. per kgm.*
106, 107	Ethyl alcohol	3.6	6.8
3, 24	Dipropylene glycol	5.9	11.5
4, 5, 6	Propylene glycol	21.2	25.0
1	Ethylene glycol	Convulsions at 20 cc. per kgm.	
2	Diethylene glycol	Convulsions at 20 cc. per kgm.	

and the ethylene, propylene, diethylene, and dipropylene glycols in 9 healthy dogs trained for the procedure used.

It is seen that neither ethylene nor diethylene glycol showed demonstrable anesthetic properties, and the acute fatal doses exceeded 20 cc. per kilogram. At this point, clonic convulsions of the animals made further administration of the glycol impracticable. Dipropylene glycol was less depressant than ethyl alcohol, but showed about the same relationship of anesthetic to fatal dose, while propylene glycol was less depressant still, the anesthetic dose being 80 to 90 per cent of the fatal dose.

GASTRIC ADMINISTRATION IN DOGS

Gastric administration of the glycols in divided doses yielded rather different results. Fatalities with this method of adminis-

tration were ascribable to injury of the liver and kidneys rather than to depression of the central nervous system. Indeed, those glycols which possessed the most marked depressant actions were those which could be tolerated in divided doses, while the ethylene and diethylene glycols, which possessed little if any depressant action showed a marked toxicity. Table 2 summarizes the results of gastric administration in 16 dogs.

TABLE 2

Toxicity of different glycols according to repeated gastric doses in individual dogs

DOG	GLYCOL	DOSAGE	TOTAL DOSE	RESULT
		cc. per kgm.	*cc. per kgm.*	
7	Propylene	1.5 (6)*	9	No evidence of toxicity
8	Propylene	2.0 (4)	8	No evidence of toxicity
9	Propylene	10.0 (2)	20	No evidence of toxicity
10	Ethylene	2.0 (2)	4	Ataxia; recovery
11	Ethylene	2.0 (2)	4	Convulsions; death 4 days
12	Diethylene	5.0 (4)	10	No evidence of toxicity
13	Diethylene	1.5 (6)	9	Emesis; death, 3 days
14	Diethylene	1.5 (6)	9	No evidence of toxicity
15	Diethylene	1.5 (6)	9	Emesis; death, 3 days
16	Diethylene†	1.5 (6)	9	Emesis; death, 4 days
17	Diethylene†	1.5 (6)	9	Emesis; death, 2 days
18	Diethylene	2.0 (3)	6	No evidence of toxicity
19	Dipropylene	5.0 (4)	20	No evidence of toxicity
20	Dipropylene	1.5 (6)	9	No evidence of toxicity
21	Dipropylene	2.0 (6)	12	No evidence of toxicity
22	Dipropylene	2.0 (6)	12	Emesis; recovery

* Number of doses in parentheses.
† From Eastman Kodak Company.

It is seen that, with doses of a glycol which might conceivably be ingested by man (3), the propylene and dipropylene glycols showed no evidences of toxicity, while the ethylene and diethylene glycols showed a high mortality. Two samples of diethylene glycol obtained from 2 different commercial sources showed no apparent difference in toxicity. In order to determine further the possibility of a difference in toxicity of diethylene glycol from these 2 sources, 4 cc. per kilogram were administered gastrically to 10 rats three times daily until death occurred. Five rats

given the glycol obtained from one source[1] required 48 cc. per kilogram for death while five rats receiving glycol obtained from another source[2] required only one-half the dose, or 24 cc. per kilogram. Although the total number of animals was small, there was a definite indication that one sample of diethylene glycol was decidedly more toxic than the other sample. Whether this was due to a higher actual concentration of diethylene glycol in the more toxic product, or to the presence of more toxic impurities, was not ascertained.

CONTINUED CONSUMPTION OF PROPYLENE GLYCOL WITH DIET IN RATS

Further light on the toxic action of propylene glycol was afforded by experiments in which varying amounts of propylene glycol were substituted for gravimetrically equivalent amounts of carbohydrate in the stock diet[3] of young growing rats. The results on weekly changes in body weight and growth and daily food consumption are presented as curves in figure 1.

It is seen that, when all the carbohydrate was replaced by propylene glycol (48.5 per cent of diet), the animals maintained a fairly constant weight and died in about one month. With three-fourths propylene glycol and one-fourth carbohydrate, the results were little better, but, with a half and half proportion, the rats gained weight, and the first death did not occur until 5 months after the start of the experiment. With one-fourth propylene glycol and three-fourths carbohydrate, the rats gained considerably in weight, but not quite as rapidly as the controls on the stock diet, nor was there any mortality in 20 weeks on the substitution diet. While the growth of the rats on the one-half and one-fourth proportions of propylene glycol did not quite keep pace with those on the stock diet in the beginning, later

[1] Carbide and Carbon Chemicals Corporation.
[2] Eastman Kodak Company.
[3] This whole diet contained: corn meal 68 per cent, linseed oil 10 per cent, dried ground alfalfa 2 per cent, powdered casein 10 per cent, lard 5 per cent, cod liver oil 3 per cent, bone ash 1.5 per cent, and sodium chloride 0.5 per cent. The composition was as follows: protein 11.8 per cent, carbohydrate 51.5 per cent, water 10.7 per cent, roughage 3.5 per cent, and salt 3.9 per cent.

they grew more, and the net result as to growth was not indicative of serious impairment. The food consumption was decreased somewhat in the glycol-rats, but the consumption was nevertheless considerable. The glycol consumption amounted to 30 cc. per kilogram daily for the rats on the half proportion of propylene glycol and about 8 cc. per kilogram daily for those on the one-fourth proportion. These quantities translated to human

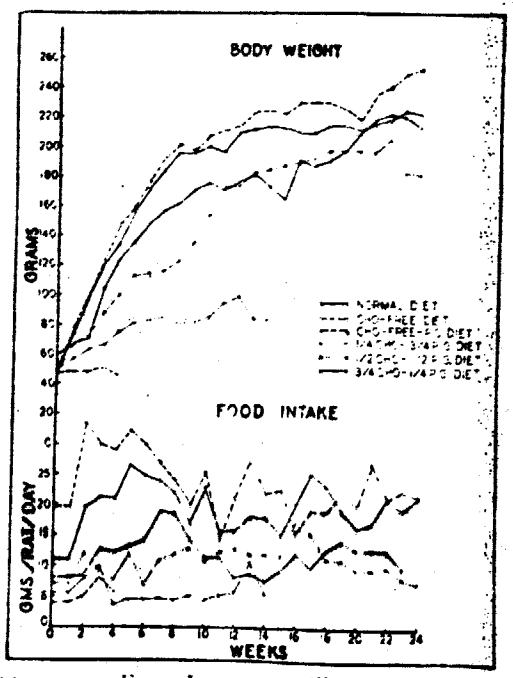

FIG. 1. BODY WEIGHT AND FOOD INTAKE OF WHITE RATS RECEIVING DIETS IN WHICH PROPYLENE GLYCOL WAS SUBSTITUTED FOR A PORTION OF THE CARBOHYDRATE

Curves show median changes in groups of 5 rats each: *CHO*, means carbohydrate, and *P.G.*, propylene glycol.

consumption are large, but it is also true, that, in practice, rather large daily quantities of propylene glycol may be given to children and adults. Such quantities, as 10 to 20 and even 40 cc. daily are said to be given to children for 1 to 9 months (4).

PAIRED FEEDING OF SUBSTITUTION DIET

The poor initial growth of the rats on the diets containing propylene glycol might have been due to their distaste for the

It is clear that the rats on propylene glycol showed a definitely greater average increase in body weight than did the controls. This certainly indicated a lack of any toxic action of the propylene glycol. The increases in body weight and growth were probably accounted for, in part at least, by the fact that the glycolized rats received a greater caloric intake than the controls. The caloric value of propylene glycol is greater than that of carbohydrate weight for weight. Additional experiments are planned to test further the effects of equi-caloric amounts, and even less, of propylene glycol under similar conditions.

PATHOLOGICAL CHANGES IN VISCERA

Dr. William Dock, of the department of pathology, examined sections from the viscera of the animals receiving the various glycols. The tissues, fixed in Bouin's fluid, were stained with hematoxylin-eosin or Sudan III. The significant changes were found to be limited to the liver and kidneys. After single intravenous fatal doses and divided gastric doses of propylene glycol, no significant changes were seen in the livers and kidneys of 2 dogs. A rat, which died after 5 months on a diet in which one-half the carbohydrate content was replaced by propylene glycol, showed moderate liver cell degeneration with some karyolysis, while the kidneys showed degenerative changes in the cells of the convoluted tubules. Two other rats on the same diet, sacrificed after an equal period, showed minimal changes, such as may be found in stock rats without medication of any kind.

The 4 dogs, which survived the repeated gastric doses of dipropylene glycol, showed minimal evidences of liver damage. In the kidneys of 2 of these dogs, there were moderate degenerative changes in the convoluted tubules, while, in the other 2 dogs, the changes were not significant.

A dog which died following repeated gastric doses of ethylene glycol showed most of the liver cells reduced to an amorphous, eosinophilic mass, while the kidneys showed tremendous necrosis of the convoluted tubules.

The 3 dogs which died after repeated doses of diethylene glycol all showed livers in which only a rare peripheral cell was

normal, the rest showing marked fatty infiltration with nuclear changes, while the kidneys showed swollen glomeruli and complete destruction of the convoluted tubules. The dogs which survived the repeated doses of diethylene glycol showed only minimal degenerative changes in the liver and kidneys. The rats which were given diethylene glycol in divided doses until death all showed changes in their livers and kidneys similar to those in the dogs succumbing to the same glycol. These changes due to diethylene glycol are similar to those described by Geiling and Cannon (6). Figure 3 illustrates typical destructive changes in the kidney and liver of a dog dead from diethylene glycol.

A B

FIG. 3. TYPICAL DEGENERATIVE CHANGES IN THE KIDNEY (A) AND LIVER (B) OF DOG 1 RECEIVING REPEATED DOSES OF DIETHYLENE GLYCOL.

Death occurred in 5 days after gastric administration of 6 doses of diethylene glycol (1.5 cc. per kilogram), total 9 cc. per kilogram.

FATE AND EXCRETION OF PROPYLENE GLYCOL

In view of the marked differences in toxicity of the closely related glycols used, it seemed advisable to investigate the mode and rate of disappearance of these agents from the body, as well as their absorption from the alimentary canal. Lehman and Newman (2) found absorption from the gastrointestinal tract to be quite rapid with small doses of propylene glycol in dogs, there being a close parallelism between the blood propylene glycol concentration after oral and intravenous administration of 2 cc. per kilogram. We found this to be true also for the ethylene.

diethylene and diethylene glycols as can be seen by the results in table 3. In man, however, this is not the case.

TABLE 3

*Blood glycol content after gastric and intravenous administrations of different glycols in dogs**

DOG	GLYCOL	ADMINISTRATION	BLOOD GLYCOL		
			2 hours	4 hours	6 hours
			mgm. per cent	*mgm. per cent*	*mgm. per cent*
103	Propylene	Intravenous	215	187	140
109	Propylene	Gastric	204	179	138
23	Ethylene	Intravenous	337	270	202
23	Ethylene	Gastric	310	283	240
24	Dipropylene	Intravenous	304	236	211
24	Dipropylene	Gastric	290	264	235
24	Diethylene	Intravenous	307	200	150
24	Diethylene	Gastric	272	209	160

* The doses used were 2 cc. per kilogram, body weight.

TABLE 4

*Rate of disappearance of propylene glycol in three human subjects**

HOURS	BLOOD			SALIVA			URINE: TOTAL AMOUNT		
	Ln	VW	Cn	Ln	VW	Cn	Ln	VW	Cn
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*grams*	*grams*	*grams*
0	0	0	0	0	0	0	0	0	0
½	174	79	47.4			112.2			
1	158	95	31.6			126.4			
2	95	111	47.4			112.2	1.86		
3	79	111	63.2			112.2			
4	111	79	63.2			94.8	7.56		5.16
6	47		15.8			94.8			
7½				95	63		6.20		6.60
8	79	16	15.8			47.4		13.10	
10							1.64		
Total							17.26	13.10	11.76

* The oral dose was 1 cc. per kilogram body weight; total for subject Ln, 75 cc., VW, 75 cc. and Cn, 68 cc. Periods in hours indicate the time after taking the glycol.

In each of 3 human subjects (table 4), who took by mouth propylene glycol in doses of 1 cc. per kg., the maximum concen-

tration in the blood was reached in about one-half hour. This same concentration persisted for about 4 hours, thus indicating delayed absorption keeping pace with the elimination of the glycol from the body. The individual differences in blood content of propylene glycol were considerable, despite the fact that the dosage was the same per kilogram body weight of each subject. With the dose used, 20 to 25 per cent of the glycol was eliminated in the urine in 10 hours. The propylene glycol content of the saliva was followed for 8 hours in 1 subject (Cn), and found to run parallel to that of the blood in the same subject, but at a level approximately 3 times as high. The salivary content of glycol in subjects Ln and Vn was obtained only once, i.e., 7½ hours after administration, and, at that time, it was of the same general order as in subject Cn. Thus it is seen that the salivary glands, as well as the kidneys, are able to concentrate propylene glycol. This indicates that propylene glycol does not possess the same diffusibility as ethyl alcohol, which is found in approximately the same concentration throughout the body.

Lehman and Newman (2) showed that as much as one-half the propylene glycol after a large dose may be recovered from the urine of dogs. Furthermore, when urinary excretion was excluded by nephrectomy, the blood concentration of propylene glycol fell slowly and at a constant rate, much as with ethyl alcohol. In this study, we have recovered approximately 30 cc. of pure propylene glycol from 850 cc. of urine excreted by a dog during the 24 hours following the gastric administration of 150 cc. propylene glycol. The glycol was recovered by fractional distillation, and identified by its refractive index (1.436) and boiling point (184°C.) and by the method of mixed boiling points (182.5°C.). The constants obtained were practically the same as those of chemically pure propylene glycol.

In order to determine if the difference in toxicity of the glycols might be due to a difference in speed of elimination, the rate of disappearance of 3 glycols from the blood stream was determined in 5 dogs. The dipropylene and diethylene glycols were administered in doses of 5 cc. per kilogram by stomach, and the propylene glycol in a dose of 8 cc. by the same route. Blood was drawn periodically from a superficial leg vein with the aid of a

syringe, after administration of the glycols. Generally, samples of about 5 cc. of blood were obtained and these were analyzed for propylene and ethylene glycols by the method of Lehman and Newman (2), and for dipropylene and diethylene glycols by the earlier method of Newman and Lehman (3). The results obtained are presented in table 5.

TABLE 5

*Diethylene glycol content of blood in dogs on repeated doses of the glycol**

DOG	RESULT	24 HOURS	48 HOURS	72 HOURS
		mgm. per cent	*mgm. per cent*	*mgm. per cent*
13	Death	53	327	273
15	Death	44	246	204
16	Death	52	108	96
17	Death	218	182	180
14	Recovery	39	34	

* The dose was 1.5 cc. per kilogram gastrically 3 times daily for 2 successive days. Periods in hours are from beginning of administration.

TABLE 6

*Rate of disappearance of the glycols from the blood-stream of dogs after oral administration**

DOG	GLYCOL	GLYCOL CONTENT OF BLOOD (MGM. PER 100 CC.)								
		Hours								
		2	4	6	8	12	16	20	24	32
108	Propylene	725	875	794	578	400			62	
19	Dipropylene	520	422	355	250		123	69		
19	Dipropylene	573	390		205					0
12	Diethylene	580	618	540	495		325	233	180	135
12	Diethylene	640	600		432				65	

* The doses used were as follows: propylene glycol, 8 cc. per kilogram, dipropylene and diethylene glycols, each 5 cc. per kilogram. Time is in hours after administration. The administration of dipropylene and diethylene glycols was repeated in the same dogs after one week's interval.

Approximately 24 hours were required for the elimination of the propylene and dipropylene glycols, while diethylene glycol required 36 hours. This difference is probably not sufficient to account for the much greater toxicity of diethylene glycol.

The striking damage to the liver and kidneys occurring in animals succumbing to the ethylene and diethylene glycols indicated that a toxic action of these glycols for these organs prob-

ably accounted for the difference. It was found that in those dogs which died from repeated doses of diethylene glycol the glycol content of the blood mounted with each successive day's dosage, and remained practically constant from the time the administration was completed until death occurred (table 6). On the other hand, dogs, which did not die, showed no tendency for accumulation of glycol in the blood, and a rather prompt disappearance of it after administration ceased. Therefore, it seems that, when the toxic action on the kidneys is such as to seriously interfere with their functional efficiency, the diethylene glycol remains in the blood stream and tissues in high concentration, and further poisons the kidneys and liver, thus setting up a vicious cycle which ends in death from renal, and probably also hepatic, insufficiency. The finding of a blood urea of 241 mgm. per cent in one dog 12 hours before death confirms this theory. However, further evidences of functional injury to these viscera are desirable. Other physiological mechanisms should be explored in this connection with a wider range of doses of the different glycols.

SUMMARY AND CONCLUSIONS

1. The acutely fatal doses of the glycols investigated were found to be rather high. The propylene and dipropylene glycols acted as depressants for the central nervous system, but not so the ethylene and diethylene glycols. With divided gastric doses, the propylene and dipropylene glycols showed no evidences of toxicity, while the same doses of the ethylene and diethylene glycols showed a high mortality.

2. Only those animals succumbing to the glycols showed marked pathological changes in the liver and kidneys, those which recovered having essentially normal viscera. The basic cause of delayed death is provisionally ascribed to a derangement of kidney function, which results in accumulation of glycol in the body, in high concentration, for a prolonged period, with resultant severe degenerative changes in the liver and kidneys. Once the avenue of elimination through the kidneys is blocked, an eventual complete degeneration of viscera seems assured.

3. Absorption of propylene glycol in men was found to be slower than in dogs, continuing for at least 4 hours, due presumably to the inhibition of intestinal absorption. The concentration in the saliva of 1 man was found to be about three times that in the blood.

4. The rate of elimination of the propylene glycol from the body was found to be about 75 per cent faster than that of the ethylene glycol, while the diethylene glycol required a 50 per cent longer period than did ethylene glycol, possibly due to renal damage.

5. Prolonged feeding of propylene glycol in large doses (25 per cent carbohydrate substitution, or 12.8 per cent of the food) in the diet to rats showed that, when the intake of glycolized food was maintained equal to that of the unmedicated controls, the rate of growth was equal or better, indicating a lack of chronic toxicity for this glycol. Propylene glycol substitution for carbohydrate greater than 25 per cent resulted in reductions of body weight and growth in glycol-fed rats, and complete substitution of glycol for carbohydrate resulted in high early mortality.

6. Thus, although very large doses of the propylene and dipropylene glycols act as central nervous depressants, these glycols are devoid of demonstrable toxicity when administered in smaller, though still large, doses for prolonged periods. While large single doses of the ethylene and diethylene glycols lack the central depressant action, repeated moderate doses of these glycols cause marked degeneration of the liver and kidneys with a fatal outcome. Accordingly, only the propylene glycol is suitable, at present, for internal or systemic use with food and medicinal products, without demonstrable hazards to health. The dipropylene glycol merits further investigation.

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L-THIOSORBITOL AND BAL AS ANTIDOTES IN PHENYLTHIOUREA POISONING

THOMAS S. HARVEY, M.D., HOWARD J. TATUM, M.D., AND SYLVIA HIMMELFARB

From Medical Division, Army Chemical Center, Edgewood Arsenal, Maryland

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Following the report of the rodenticidal action of ANTU (alpha-naphthylthiourea) and phenylthiourea by Richter (1) much interest has been aroused in these substances. Because of the wide use of these agents, accidental poisoning was anticipated and a search for a suitable antidote was instigated.

Du Bois (2) reported that sulfhydryl-containing compounds were inhibited by ANTU and suggested that an excess of available sulfhydryl radical might protect the essential sulfhydryl-containing compounds of the body.

Peters et al. (3) in England demonstrated the effectiveness of BAL (2,3-dithiolpropanol) as an antidote in arsenic poisoning. Later Gilman et al. (5) found that l-thiosorbitol was also effective in treating poisoning by sulfhydryl-binding substances. In view of this information it seemed logical to study the antidotal effectiveness of BAL and l-thiosorbitol in phenylthiourea poisoning. Since the mode of action of ANTU and phenylthiourea is apparently the same, the latter drug was used in these eperiments because of its greater relative solubility.

EXPERIMENTAL. Male rats (Wistar strain) weighing approximately 200 grams were used as the test animals. A solution of phenylthiourea (0.60%) in propylene glycol was prepared. Toxicity determinations with propylene glycol showed that as much as 7.5 ml. per Kg. of this solvent alone given intravenously was well tolerated. The average amount of this solvent injected intravenously or intraperitoneally in these experiments was 1.5 ml. per Kg. Eight milligrams of phenylthiourea per Kg. was the intravenous dose used. This dose proved to be an LD_{70} since 19 of 28 control rats died. Ten mgs. per Kg. given intraperitoneally was found to be an LD_{90}. L-thiosorbitol, which is very soluble in water, was always given intravenously in the form of a 30% or 40% aqueous solution. BAL was injected either intramuscularly or intravenously in the form of a 1.0% solution in propylene glycol. An intravenous injection of 25 mgs. of BAL per Kg. was survived by all of the 8 control rats. Previous work by Eagle (4) and others (6) has shown that doses larger than this are toxic. Fifty (50) mgs. of BAL per Kg. given in a single intramuscular dose killed 2 of 11 rats. All the intravenous injections were made into the tail veins.

RESULTS. In table 1 are presented the results of the therapeutic effect of thiosorbitol on poisoning produced by intravenously administered phenylthiourea. One gram of thiosorbitol per Kg. given intravenously immediately after the phenylthiourea increased the percentage of survival from 29.5 in the controls to 92.0 in the treated group. One and one-half grams of thiosorbitol per Kg. given intravenously immediately after the phenylthiourea raised the percentage of survival from 29.5 to 83.0. When one hour was allowed to elapse between the administration of the phenylthiourea and the intravenous dose of one gram of thiosorbitol per Kg. there was no evident antidotal effect.

Table 1 also presents data which indicate that intravenous thiosorbitol failed to protect rats poisoned intraperitoneally with an LD_{90} of phenylthiourea when a one-half hour interval elapsed between the poisoning and the treatment.

In table 2 are presented the results of the therapeutic effect of BAL on poisoning produced by intravenously administered phenylthiourea. Twenty-five (25) mgs. of BAL per Kg. given intravenously immediately after phenylthiourea resulted in a higher mortality rate than did the phenylthiourea alone. The same result was obtained when the phenylthiourea was followed immediately by 25 mgs. of BAL per Kg. intramuscularly and by a second and similar amount

TABLE 1

Treatment of phenylthiourea poisoning with intravenous thiosorbitol

TREATMENT (THIOSORBITOL)	NO. OF RATS	NO. OF SURVIVORS	PERCENTAGE OF SURVIVAL
(Intravenous phenylthiourea—LD_{70})			
1.0 gm./Kg. immediately	50	46	92
1.5 gms./Kg. immediately	12	10	83
1.0 gm./Kg. one hour after poisoning	23	7	30
(Intraperitoneal phenylthiourea—LD_{90})			
1.0 gm./Kg. one-half hour after poisoning	16	0	0

TABLE 2

Treatment of intravenous phenylthiourea poisoning (LD_{70}) with BAL

TREATMENT (BAL)	NO. OF RATS	NO. OF SURVIVORS	PERCENTAGE OF SURVIVAL
25 mgs./Kg. immediately intravenously	6	1	16
50 mgs./Kg. (25 mgs. immediately) (25 mgs. 1 hour later) intramuscularly	20	3	15
75 mgs./Kg. (25 mgs. immediately) (50 mgs. 1 hour later) intramuscularly	12	3	25

of BAL one hour later. In the third group presented in table 2 the rats received 25 mgs. of BAL per Kg. intramuscularly immediately after the phenylthiourea was administered and an additional 50 mgs. of BAL per Kg. by the same route one hour later. In this group also, the mortality rate was higher than that of the untreated controls.

DISCUSSION. The results of the experiments presented here indicate that protection ia afforded by l-thiosorbitol when given immediately following poisoning by phenylthiourea, but that if therapy is delayed for 30 minutes or longer no apparent antidotal action is evident. These findings are in agreement with those of both the British and American workers (3, 4, 5) who have investigated

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Glycerin and Glycol Substitutes.
Action on the Isolated Rat Intestine.

by

René Hazard and Charles Vaille

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Synthetic glycols that are capable of replacing glycerin in its different pharmaceutical uses have already been the subject of numerous works. We will limit ourselves to a reference to the original work of L. Launoy[1] on the general toxicity of some glycols and their local effect on intestinal mucosa. This work also contains an important bibliography on the subject.

We ourselves have recorded the effects of certain products[2] on the tonus and pendular movement of the isolated rat intestine:

Propylene glycol, $CH_2OH-CHOH-CH_3$ (bp=187°)

Diethylene glycol, $CH_2OH-CH_2-O-CH_2-CH_2OH$ (bp= 244°)

Polyglycols

Butane-diol 1-3, $CH_2OH-CH_2-CHOH-CH_2$

Diethylene of glycerin, $\begin{array}{ccc} CH_2 & -CHOH- & CH_2 \\ | & & | \\ O-C_2H_5 & & O-C_2H_5 \end{array}$

We took glycerin itself as our comparative substance, used either alone in the diluted state, or mixed with gelatin, the usual vehicle of suppositories that play a considerable role here.

We shall deal successively with: 1. the action of glycerin, 2. the action of alcohols and polyalcohols, and 3. the effect of gelatin.

Experimental Conditions

Glycerin and the other products studied were poured into 100 cc Tyrode solution, kept at 37° and oxygenated, in which the fragment of rat duodenum was immersed. They were previously diluted up to 40-50% by weight to render their distribution more uniform in the liquid. Concentrations are given in volume of the product per 100 cc Tyrode solution.

I. Glycerin. At every concentration used (1-5%) a sudden lowering of tonus was observed, sometimes followed by renewed rise, then again a drop, this time progressive.

The rhythmical contractions underwent diverse modifications according to the concentration: from 1 to 5% they were generally of considerably increased amplitude after the initial loss of tonus (Fig. 1); from 1.8 to 2.5% they were temporarily increased or decreased as the case may be (Fig. 2); from 3 to 5% they were eliminated.

II. Action of alcohols and polyglycols.

a) Propylene glycol. At every concentration used (from 1 to 5%) propylene glycol gave rise to an initial loss of tonus, somewhat less marked than in the case of glycerin. Very often a renewed increase in tonus was then observed, followed by another progressive drop.

Contractions were amplified (Fig. 3) sometimes to a considerable degree at concentrations from 1 to 3%, while at concentrations of 4% they were either increased or reduced in amplitude. High concentrations reduced them without eliminating

them, which distinguishes this glycol from glycerin and possibly constitutes an advantage over the latter.

b) Diethylene glycol. At concentration of 1 to 6.5% a loss of tonus was observed - a phenomenon noted with all the compounds studied - although less marked than with glycerin or even propylene glycol. As in the case of the latter, the initial drop was followed by signs of a return to normal, then once again a drop.

The amplitude of the rhythmical contractions was increased at concentrations of 1 to 4% (Fig. 4) and reduced at concentrations of 5 to 6.5%.

c) Polyglycol. The mixture bearing this name acts in a manner similar to the preceding products. At concentrations of 1-5% it lowers the tonus roughly to the same degree as diethylene glycol, but still stimulates contractions.

Thus on the whole a fairly similar action of roughly the same intensity is observed with glycerin, propylene glycol, diethylene glycol and polyglycols.

d) Butane-diol. From 1-5% the drop in tonus was similar to that observed with the preceding products. There was, however, a definite difference in the effect upon contractions. Only low concentrations (1%) increased the amplitude of contractions. From 2 to 4% the contractures were reduced or eliminated (Fig. 5). With a 5% concentration they were eliminated.

e) Glycerin diethylene. In this case there was also an appreciable effect, not on the tonus, which is always

considerably reduced, but on the rhythmical movements, which were always reduced at concentrations of 1 to 4%, in fact generally eliminated (Fig. 6). The inhibitory effect in this case was much stronger than in the case of glycerin.

Thus all the products studied lowered the tonus, but their effect on the contractions of the intestine varied in intensity and in direction. Propylene glycol, diethylene glycol and polyglycols were similar to glycerin, while butane-diol and above all glycerin diethylene differed by their more generally inhibiting effect.

III. Influence of gelatin.

The question arises whether gelatin, with which glycerin is usually combined in suppositories, acts merely as an ordinary vehicle, or whether it changes the effect of glycerin itself.

The isolated intestine was subjected to the action of a suppository suspended by a thread on the surface of the Tyrode solution in the reaction flask. In this manner dissolution of the mass was achieved, and its slow distribution in the liquid, that is to say a progressive effect on the intestine similar to that which takes place in humans.

The products used were incorporated in a dose of 60 g in a mass consisting of 30 g distilled water and 10 g gelatin, as in the formula for Codex glycerin suppositories. Thus 4 g suppositories were obtained, each containing roughly 2.5 g of the active product, which, when totally disoolved in the bath, gave a roughly 2.5% concentration.

Our comparison did not include glycerin diethylene, which does not yield a suppository of suitable consistency with gelatin;

nor did it include butane-diol, which produces a mass which dissolves with difficulty.

The four other products gave rise to loss of tonus, which in many cases was slight, and a considerable increase in the amplitude of contractions. The only difference was in the time of dissolution, the glycerin suppositories melting less quickly than those with a propylene glycol, polyglycol and above all a diethylene glycol base, which has a particularly soft consistency.

The most notable feature was that glycerin, which in a 2.5% aqueous solution generally increases or changes the amplitude of contractions only slightly, when mixed with gelatin at the same concentration gave rise to a considerable increase in intestinal contractions.

Yet gelatin has no effect of its own on the intestine, as can be experimentally established. Its effect is indirect: it simply intervenes in the form of a mass the slow dissolution of which allows glycerin to diffuse progressively and thus act at the low concentrations which have proved particularly effective in stimulating motility. This explains the efficacy of the mixture if we accept the fact that it is the increase in the movements of the smooth musculature far more than the lowering of the tonus which is the reason for the effectiveness of the glycerinated suppository.

Conclusions.

In low doses glycerin has a double effect on the isolated intestine: Lowering of tonus and considerable increase in peristalsis.

This stimulating effect appears to explain the effect of glycerin suppositories, for this effect is increased in the presence of gelatin, which acts by allowing progressive contact of the active product with the mucosa.

Among products used as substitutes for glycerin with its effect on the intestine, propylene glycol and diethylene glycol, the relative harmlessness of which has already been established, appear to have qualitatively the same effects as glycerin itself.

Possibly polyglycols might also be used if their composition were constant. Butane-diol and glycerin diethylene should be rejected.

Figures

1. Glycerin (1% solution).

2. Glycerin (2% solution).

1 & 2. Action of glycerin on the isolated rat intestine. Glycerin is added at ↑. Bottom left, time in seconds. Tracing reduced by 1/3.

3. Propylene glycol (2% solution)

4. Diethylene glycol (1.5% solution)

5. Butane-diol (4% solution).

6. Glycerin diethylene (2% solution)

Footnotes

1. L. Launoy, Paper presented before Paris Pharmaceutical Society, meeting of March 7, 1941. (Journ. Pharm. et. Chim., 1942 (9), 2, no.3, p. 90.

2. These products were kindly furnished by Usines de Laire.

The polyglycols are mixtures in various proportions of ordinary glycol, diethylene glycol and propylene glycol; those used by us were prepared by Etablissements Kuhlmann (boiling point = 90° to 140° under 15 mm). They consisted chiefly of diethylene glycol. According to Bouvet, ordinary glycol meets pharmaceutical requirements. (Paris Pharmaceutical Society, meeting of June 5, 1940. Journ. Pharm. et Chim., 1942, (9),2, no.3, p. 117). We have not yet tested it.

Pharmaceutical Laboratory
Trousseau Hospital
Paris.

Fig. 1. — [illegible]
(solution [illegible]

Fig. 1 et 2. — Action [illegible]
Le glycérine est ajouté en [illegible]
(T[illegible]

Fig. [illegible]
solution [illegible]

Fig. 3. — [illegible]
(solution [illegible]

Fig. [illegible]
[illegible]

Fig. 5. — *Butane-diol*
(solution à 4 %).

Fig. 6. — *Diol*[illegible]
solution à [illegible]

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Acute toxicity of radiation-sterilised propylene glycol

SIR,—Certain drugs that are subject to hydrolysis when dispensed as an aqueous solution are prepared, for pharmaceutical purposes, as a solution in propylene glycol. Such solutions may be difficult to sterilise as many of the drugs concerned are affected by heating; bacteriological filtration is tedious due to the viscous nature of the solvent and there is always a risk that the aseptic precautions necessary for the filling and sealing of the final containers may fail. Radiation sterilisation may offer a useful alternative method: it can be applied to the preparation in its sealed container.

The present investigation was stimulated by the need to sterilise a solution of di-isopropylfluorophosphonate (dyflos, DFP) in propylene glycol (1 mg/ml). The dyflos is prepared with labelled phosphorus atoms (^{32}P) and the solution is intended for parenteral use in clinical studies of blood cell turnover. Preliminary studies indicated that the potency of the dyflos itself was only very slightly affected by radiation sterilisation (Charlton, personal communication) but no information was available on the effect of radiation sterilisation on the toxicity of propylene glycol.

A sample of redistilled propylene glycol was divided into two parts, one of which was irradiated with 2·5 Mrad of gamma radiation—the dose used for sterilisation of medical equipment (Burt & Ley, 1963).

Groups of 3 male and 3 female adult SPF albino rats were given either irradiated or unirradiated propylene glycol by intraperitoneal injection at the following doses: 9·4, 11·1, 13·0, 15·3, 18·0 and 21·1 ml/kg body weight. The LD50 values were calculated from the mortality after 5 days using the method of Finney (1952). The LD50 values, with the 95% fiducial limits were for the irradiated material 13·7 (12·5–15·1) and for the control material 14·2 (12·4–16·1) ml/kg weight; differences between groups are not significant.

Irradiated or unirradiated propylene glycol was given to groups of 5 male and 5 female mice (C3H strain) by intraperitoneal injection to a dose of 5 ml/kg weight. Before injection, 1 part of propylene glycol was diluted with 2 parts of normal saline. This dose is about half the median lethal dose in mice (Lampe & Easterday, 1953) but although all mice showed signs of intoxication (lack of co-ordination of movements followed by deep narcosis and, in a few cases, convulsions), no mice died within 7 days of injection. During the week after injection with propylene glycol no differences were observed in general health, weight changes, or food and water intake between the groups receiving irradiated or unirradiated material. All the mice were killed and examined 7 days after injection; apart from evidence of inflammation of the peritoneal cavity, presumably caused by the injection, no abnormalities were observed in mice of either group.

These results show that sterilisation of propylene glycol with a dose of 2·5 Mrad of gamma radiation does not increase its acute toxicity and this information may be of interest to others who may be contemplating radiation sterilisation of other pharmaceutical preparations formulated with propylene glycol.

U.K.A.E.A. Research Group,
Isotope Research Division (A.E.R.E.),
Wantage Research Laboratory,
Berkshire.
February 11, 1965

J. R. HICKMAN

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GLYCERIN, ETHYLENE GLYCOL, PROPYLENE GLYCOL AND DIETHYLENE GLYCOL

REPORT ON FEEDING EXPERIMENTS WITH RATS

HARALD G. O. HOLCK, PH.D.
CHICAGO

The substitution of diethylene glycol for glycerin as a hygroscopic agent in industry makes it desirable to compare the toxicity of these two chemicals. Both have high boiling points, 245 and 290 C., respectively. Hence, small amounts may possibly pass unchanged with smoke from a cigaret into the body in addition to anything absorbed from the moist end of the cigaret in the mouth.

In view of the rather high toxicity of the related ethylene glycol and because of the use of propylene glycol as a solvent for iodobismitol, it was thought desirable also to include a rough comparison between commercial samples of diethylene glycol and these two related chemicals.[1]

Recent exhaustive studies [2] have shown that glycerin may constitute a large proportion of the food of rats over long periods and that smaller amounts may be added to adequate diets of growing dogs and of man without apparent harm. In fact, the rats showed normal growth and reproduction on such a diet and glycerin could be substituted for part of the food. Only with very large quantities was there diuretic action in dogs and rats. In spite of this favorable report my associates and I decided to include glycerin in our feeding experiments so as to make certain that the rats had the usual tolerance to this compound.

The recent literature dealing with the properties of the glycols is fairly extensive. A comprehensive paper by von Oettingen and Jirouch [3] deals with seven glycols, including ethylene glycol and diethylene glycol. Both caused hyaline shrinking of muscle fiber; ethylene glycol caused a slight and diethylene glycol a questionable hemolysis; methemoglobin formation was absent with ethylene glycol and uncertain with diethylene glycol. The minimal fatal dose in mice was twice as high for diethylene glycol as for ethylene glycol. Both had a slight action on the central nervous system, caused slight hyperemia and muscle rigor and had a slight antiseptic action toward yeast. Diethylene glycol was more depressing to striated muscle than ethylene glycol but had an uncertain depressive action on the intestine as compared to ethylene glycol. Neither caused edema, sensory nerve ending depression or motor nerve fiber depression. The authors concluded:

> Local irritation is comparatively small. Their application to the skin seems to be without risk. Given orally in larger doses they may produce severe gastro-enteritis and systemic symptoms. For intramuscular injection only small doses should be used, else systemic effects may arise. Subcutaneous injections should be avoided on account of local irritation. The intravenous administration would appear to be dangerous, because of the hemolytic action and of the fall of blood-pressure observed with these compounds.

Ethylene glycol was also studied extensively by Hanzlik, Seidenfeld and Johnson,[4] who used both intra-

From the Department of Physiology of the University of Chicago.
Profs. A. J. Carlson and A. B. Luckhardt gave helpful suggestions regarding this problem.

1. These two glycols were supplied by the Carbide and Carbon Chemical Corporation, South Charleston, W. Va.
2. Johnson, Victor; Carlson, A. J., and Johnson, Adelaide: Am. J. Physiol. 103: 517 (March) 1933.
3. von Oettingen, W. F., and Jirouch, E. A.: J. Pharmacol. & Exper. Therap. 42: 355 (Aug.) 1931.

muscular injections and drinking experiments.[5] In chronic feeding experiments on rats, smaller daily doses (0.7 Gm. per kilogram) stunted growth but did not injure the kidney, whereas the higher daily dose of 2.2 Gm. per kilogram caused variable degrees of oxaluria and renal calculi, verifying previous reports. As a solvent for medicinal products with intramuscular injections it was considered "comparatively innocuous." Hunt[5] reported kidney injury by oxalic acid formation when ethylene glycol was administered in the drinking water of different species of animals and cautioned against continued administration of small doses to man. In this and a subsequent paper he[6] also warned strongly against the promiscuous use of ethylene glycol in food products or as a substitute for glycerin. As to propylene glycol he found that, with a 5 per cent solution as the only liquid for drinking, rats showed normal growth and also good tolerance to it when their livers or kidneys had been injured by various poisons; Hunt suggested that propylene glycol may have a "true food value in the sense that ethyl alcohol does, and without the drug action of the latter" Wiley, Hueper and von Oettingen[7] concluded that low concentrations of ethylene glycol vapor in the air (300 mg. per thousand liters) is not toxic when intermittently given to mice or rats as judged by weight curves and histopathologic examinations, although one of ten rats and three of twenty mice died during the tests with 398 mg. per thousand liters for eight hours a day, five days a week, sixteen weeks, with no controls to show that similar mortality would occur in untreated rats and mice.

In their extensive study on bismuth excretion in rabbits and patients, Hanzlik, Mehrtens and Spaulding[8] used ethylene glycol as the solvent for iodobismitol without any untoward effects by this glycol. Seidenfeld and Hanzlik[9] found propylene glycol to be much less toxic than ethylene glycol in injection and feeding experiments as judged by animal growth and tissue appearance by microscopic examination. No demonstrable accumulation effects were seen with concentrations of from 1 to 10 per cent in the drinking water. However, propylene glycol did cause somewhat greater local irritation than ethylene glycol. Finally, Hanzlik, Mehrtens and Spaulding,[10] in a comparison of propylene glycol and diethylene glycol, concluded that iodobismitol prepared with propylene glycol is less toxic and prepared with diethylene glycol is more toxic systemically than ordinary iodobismitol prepared with ethylene glycol.

Haag and Ambrose[11] report that concentrations of 3 and 10 per cent, respectively, of diethylene glycol in the drinking water of rats proved rapidly fatal.

TOXICITY OF DIETHYLENE GLYCOL[12] IN FOOD AND WATER AND THAT OF GLYCERIN IN FOOD, AND ETHYLENE GLYCOL AND PROPYLENE GLYCOL IN WATER

The results of an eleven weeks experiment show that female rats 4½ months old on a control diet of fox-chow (Purina Mills) and water gained in weight about to the same extent as did those who had eaten fox-chow containing 20 per cent glycerin or 20 per cent water.

However, the fox-chow containing 20 per cent diethylene glycol killed five of six rats within four weeks. One kidney (found to be normal) was removed from the sixth sick rat after two weeks; on a normal diet this rat promptly recovered. When the fox-chow contained 10 per cent diethylene glycol, three of five rats died within eight weeks, but one showed normal gain in weight. From the fifth rat, which was losing weight, one kidney (found to be normal) was removed after the eighth week. On our control diet this rat recovered promptly. With fox-chow containing 5 per cent diethylene glycol, one out of six rats died after seven weeks and another after ten weeks. Of the remainder, one showed but a slight increase in body weight and the other three had made approximately normal gains.

When the drinking water contained 5 per cent diethylene glycol, the toxicity was markedly greater. All five rats died within eleven days (average duration of life, eight days). However, an equimolecular solution of commercial ethylene glycol (2.92 per cent) was even more toxic, killing all six rats within six days (average duration of life, four days). On the other hand, an equimolecular solution of commercial propylene glycol (3.58 per cent) was much less harmful than that containing the diethylene glycol. One of the six rats died after the tenth week and one had lost considerable weight at the end of the eleven weeks. The remaining rats were beginning to lose weight at the end of the experiment. Thus, the commercial propylene glycol appears to be more toxic than the highly purified substance administered to rats by Hanzlik and his associates[9] or the propylene glycol used by Hunt.[13]

Further analysis of the food and water intake showed that with fox-chow containing 20 per cent glycerin the rats ate 23 per cent less fox-chow per day than the ones fed pure fox-chow; but they ate only 11 per cent less than those who had had fox-chow with 20 per cent water as their food. The average daily consumption of glycerin was 2.7 Gm. As to water intake, no conclusion seems warranted because the rats which ate fox-chow containing 20 per cent water drank considerably more water than did the controls, indicating that there may be considerable variation in drinking habits even with animals of nearly the same size.

Considering fox-chow consumption per average hundred grams of gain in body weight during the eleven weeks, 11 per cent less of actual fox-chow was needed when the fox-chow contained 20 per cent of glycerin, indicating that glycerin was used as a food substitute.

The average daily fox-chow consumption was 6 per cent less than in the controls when the fox-chow contained 5 per cent of diethylene glycol. When it contained 10 per cent, the fox-chow consumption was 10 per cent less, or about the same as when the fox-chow had 20 per cent of glycerin. Considering again the small number of animals, these differences are not significant. However, the large increase in the average daily water intake (75 per cent) when the fox-chow contained 10 per cent of diethylene glycol may indicate an attempt to wash down the ill tasting food with water.

When the diethylene glycol proportion in the fox-chow reached 20 per cent, the average daily fox-chow consumption declined sharply to 24 per cent of the control value in these quickly dying rats. The even

5. Hunt: Indust. & Engin. Chem. 24: 361, 1932.
6. Hunt: Indust. & Engin. Chem. 24: 836, 1932.
7. Wiley, F. H.; Hueper, W. C., and von Oettingen, W. F.: J. Indust. Hyg. & Toxicol. 18: 123 (Feb.) 1936.
8. Hanzlik, P. J.; Mehrtens, H. G., and Spaulding, J. B.: Iodobismitol, Arch. Dermat. & Syph. 29: 298 (Feb.) 1934.
9. Seidenfeld, M. A., and Hanzlik, P. J.: J. Pharmacol. & Exper. Therap. 44: 109 (Jan.) 1932.
10. Hanzlik, P. J.; Mehrtens, H. G., and Spaulding, J. B.: J. Pharmacol. & Exper. Therap. 49: 500 (Nov.) 1933.
11. Haag, H. B., and Ambrose, A. M.: J. Pharmacol. & Exper. Therap. 59: 93 (Jan.) 1937.
12. This diethylene glycol was furnished by the Miner Laboratories of Chicago; it was the kind used in our earlier experiments. The Miner Laboratories also furnished the glycerin.
13. Hunt (footnotes 5 and 6).

sharper reduction in the average daily water intake to 18 per cent of normal indicates that these rats became too sick even to drink.

When the water contained 5 per cent of diethylene glycol, the reduction in average daily eating of fox-chow was even more severe than when the fox-chow had contained 20 per cent of diethylene glycol; the intake went down to 17 per cent of normal. The average daily water intake was also markedly diminished to 31 per cent of normal.

When the water contained an amount of ethylene glycol equimolecular with the 5 per cent diethylene glycol, the average daily consumption of fox-chow was nearly twice and the average daily water intake nearly three times that of the rats that had been drinking 5 per cent diethylene glycol; thus the water intake was nearly normal. Therefore, on an average, the rats took 2.7 times more ethylene glycol molecules daily than those of diethylene glycol, which may partly explain why they were dying twice as rapidly.

When an amount of propylene glycol equimolecular with the 5 per cent diethylene glycol was present in the water, the rats ate and drank approximately as much as the control rats; thus, on an average they took in three times as many propylene glycol molecules daily as when diethylene glycol had been added to the water. The latter fact further emphasizes the much greater toxicity of diethylene glycol as compared with propylene glycol.

A COMPARISON OF THE EFFECTS OF GIVING VARYING CONCENTRATIONS OF PURE DIETHYLENE GLYCOL (EASTMAN) IN THE DRINKING WATER OF YOUNG RATS

Because the toxic effects following administration of commercial diethylene glycol may possibly have been due to impurities, we added a nine weeks experiment in which pure diethylene glycol was added in varying amounts to the drinking water of rats which were from 7 to 8 weeks old; concentrations started at a level 20 per cent lower than the one that had killed all rats within eleven days. This new concentration of pure diethylene glycol was thus 4 per cent; it killed three of the five rats within nine days, but the other two survived.

On the lower concentrations of 2, 1, 0.5, 0.25 and 0.125 per cent, respectively, of diethylene glycol, no animals died. However, the average actual and percentage gains in body weight during the nine weeks was less than half of that of the controls. Not until the concentration had become as low as 0.125 per cent were these growth averages exactly up to the control values.

Looking next on the average daily amounts of fox-chow eaten, the values are 24 per cent lower than the control intake when the water contained 2 per cent of diethylene glycol. In case of the two highest concentrations (2 and 1 per cent, respectively), there was also a noticeable diminution in actual water intake to 59 and 67 per cent of normal, respectively.

With the higher concentrations of pure diethylene glycol in the water there were dark discolorations around the genitalia; these were not blood; this indicates that diethylene glycol or some darker decomposition product was being passed in the urine. Thus, some diethylene glycol was being absorbed.

In the case of the rats which died acutely from diethylene glycol poisoning, the stomach and intestine showed signs of marked local irritation and in some cases considerable blood and local injury.

Comparing the liver, kidney, lung and myocardium of the rats that had been given commercial ethylene glycol, propylene glycol, diethylene glycol or glycerin in the food or water with those of the control rats, no certain damage was demonstrable to these vital organs as judged by examinations of sections stained with scarlet red or with hematoxylin and eosin. These examinations were done by courtesy of Dr. Paul R. Cannon.

Considering the same four organs of the rats which had been given varying concentrations of the pure diethylene glycol in the drinking water, no certain damage could be seen in the liver, kidney or lung. Dr. Eleanor Humphreys, who carried out these examinations, reported an indication of a possible slight change in the myocardium with the three highest concentrations as judged by the hematoxylin and eosin staining technic, but it was slight and not present in all cases.

COMMENT

The results of these experiments place diethylene glycol between ethylene glycol and propylene glycol in toxicity. A high concentration of glycerin in the solid food (20 per cent) seemed harmless. Diethylene glycol in one fourth of this concentration in the fox-chow and in much smaller concentrations in the drinking water was distinctly harmful as judged by cases of mortality, stunted growth in the young, and impaired reproductive capacity.

Even after prolonged administration of diethylene glycol in the concentration employed, however, visible microscopic changes in the liver, kidney, lung, heart and testis were absent. Both the frozen section method and the staining with hematoxylin and eosin were used in the first four of these tissues. Although diarrhea was not observed, I believe that the absence of pathologic changes in these vital organs may indicate that local irritation probably is an important factor in the toxicity.

Because the rat in some cases is much more tolerant to poisons than man, for example, to atropine, and in other cases shows peculiar susceptibilities, for example, delayed death after slightly hypnotic doses of nostal, we do not know with certainty what the relation is with diethylene glycol. However, the report by Hunt[6] of greater susceptibility of man to ethylene glycol than the rat—comparing the per kilogram dosage—probably means that man is also more susceptible to diethylene glycol.

SUMMARY

1. In an eleven weeks experiment, younger adult female rats gained about the same in body weight as control animals when a high concentration of glycerin (20 per cent) was mixed with their solid food

2. Similar concentrations of commercial diethylene glycol killed all rats within about two weeks, and even 10 and 5 per cent proved fatal to some of the rats. Diethylene glycol is even more toxic when added to the drinking water. When the rats drank from a 5 per cent solution, the average duration of life was only eight days.

3. With the equimolecular concentration of commercial ethylene glycol (2.92 per cent) in the drinking water, the rats lived only an average of four days but actually had consumed 2.7 times as many molecules of ethylene glycol per rat daily.

4. With an equimolecular concentration of commercial propylene glycol in the water (3.58 per cent), one rat died at the tenth week, one was sick and others were beginning to lose weight at the end. The average daily

intake was three times as many molecules of propylene glycol as of diethylene glycol. This places diethylene glycol between ethylene glycol and propylene glycol in toxicity, but nearer the first one.

5. When pure diethylene glycol was added to the drinking water of 2 months old female rats, some died with the highest concentration of 4 per cent and growth was markedly stunted at this level. Even in the concentration of 0.25 per cent, some indication of impaired growth was detectable.

6. That diethylene glycol was being absorbed with the higher concentrations was indicated by discolorations around the genitalia. With high concentrations, local injury and bleeding of the stomach and intestine were frequently seen in the fatally poisoned rats.

7. In microscopic examinations of the liver, kidney, lung and myocardium the only possible change seemed to be a slight one in the myocardium with 1, 2 and 4 per cent of pure diethylene glycol, respectively, in the drinking water; but even this change was not uniformly present and hence may not be significant. Both the frozen section method and the hematoxylin and eosin staining method were employed.

8. Pregnancy did not occur when males and females were mixed and both received 0.5 per cent of pure diethylene glycol in the drinking water. There was some indication of smaller and fewer litters in females receiving 0.25 and 0.5 per cent in the drinking water when mated with frequently changed fresh stock males.

NOTES AND CORRESPONDENCE

Toxicity of Ethylene and Propylene Glycols

EDITOR'S NOTE The following letter was received in June, 1930, but the publication was delayed in the hope that further experiments would be made the author. Doctor Hunt states that he has not been able to pursue his ıdies further, and, since the subject is of considerable interest at the present time, the letter is now published.

Editor of Industrial and Engineering Chemistry:

Although comparatively little experimental work with ethylene glycol has been reported, the view has long been held that the substance is practically nonpoisonous; its use as a substitute for glycerol in some food and pharmaceutical preparations has been suggested. Recently two deaths in man have been reported [*J. Am. Med. Assocn.*, 94, 1940 (1930)] from the drinking of an antifreeze mixture consisting of ethylene glycol. Hence it seems desirable to call attention to certain undesirable and dangerous properties of this compound.

It has long been recognized that ethylene glycol, like other polyvalent alcohols, has little or no narcotic or other immediate obvious actions. It is this lack of immediate effects of even comparatively large doses which has caused a number of experimenters to overlook the inherently dangerous character of the compound.

A few years ago I performed a number of experiments with this substance upon rats, mice, guinea pigs, rabbits, cats, and dogs. The compound was administered in various ways—by intraperitoneal and intravenous injections, and by mouth. It was also added to the drinking water of the animals, and the effects of small doses, continued for several days or weeks, were observed. The compound was found to be distinctly poisonous. The toxic action was not due to the glycol itself but to one of the products of its oxidation in the body—oxalic acid. The kidneys were very severely injured by the oxalic acid, and this seemed to be the usual cause of death. Experiments on man have also shown that some of the glycol is oxidized to oxalic acid.

Ethylene glycol proved to be as poisonous as methanol, but in a different way. Of course from a practical standpoint it is a much less dangerous substance than methanol; it is so slightly volatile that there is no danger of poisonous doses being inhaled, and there is no evidence that it is absorbed from the skin. Those who are so foolish as to endeavor to quench their thirst by drinking antifreeze mixtures would probably find other means of committing suicide, but manufacturers should (and apparently do) abstain from adding ethylene glycol to any food product. Used legitimately, this compound is a far less dangerous substance than aniline, benzene, carbon disulfide, turpentine, carbon tetrachloride, and many other chemicals extensively employed in the industries.

A considerable number of experiments were performed with propylene glycol; the results were strikingly different from those obtained with the ethylene compound. I could find no indications of a poisonous action. Thus, young rats grew at the normal rate and reached maturity when the only liquid they received was a 5 per cent solution of propylene glycol: rats receiving a similar solution of ethylene glycol died in two or three days, and those receiving a 1 per cent solution frequently died within a week. The propylene compound was well tolerated by animals whose livers and kidneys had been injured by various poisons. It is possible that this glycol may have a true food value in the sense that ethyl alcohol does, and without the drug action of the latter.

REID HUNT

HARVARD MEDICAL SCHOOL
BOSTON, MASS.
June 19, 1930

From: Rev.Franc. Allerg. 6 : 200-205 (1966)

The propylene glycol allergy

By

Cl. HURIEZ, P. MARTIN and M. MENNECIER

In recent years, the glycols and their by-products became important for chemical industry, cosmetology as well as for therapautic uses as excipients of salves and as inert media of solutions.

The use of these pharmaceutic preparations was specifically turned to account by use when diagnosing some cases of propylene glycol intolerance and prompted us to extend our investigations to other glycols and to their esters.

Memo on the chemistry of the glycols. (*)

The glycols or diols are characterized by the presence in their chemical formula of two free alcohol functions.

Ethylene glycol: CH_2OH-CH_2-OH (ethylene glycol or glycol or ethane diol, or Prestone(**) is the simplest of the acylic glycols.

The predominant homologue of the ethylene glycol, the isopropylene glycol , is also known as propylenic glycol or isopropane diol: $CH_3--CHOH-CH_2OH$.

The isopropylene glycol is obtained by hydration of the propylene oxide. It can likewise result from the saponification of the propylene chloride.

The propylene glycol is the racemic isomer of two propane diols:
- the propane diol (1-3) $CH_2OH--CH_2OH$;
- and the isopropane diol (1-2) $CH_3--CHOH--CH_2OH$.

It is a colorless, sirupy, rather odourless liquid with a sweet taste, and it is hydroscopic.It can be admixed to water, alcohol, glycerine, acetone or chloroform.

Its density at 20°C is between 1,035 and 1,040.

Its freezing point is under 60°C and its boiling point is at + 188°C.

Its oxidation partially yields lactic acid.

(*) We wish to thank in particular Prof. R. MERVEILLE and Mr. DEQUIDT who guided us most helpfully when preparing these statements.
(**) American commercial name.

Its characteristics are:

- a superficial low pressure which, in dermatologic therapy, makes it highly effective as a covering medium; it has a high penetration capacity at the level of the lesions;
- slow evaporation which maintains the substance in dissolved condition for a period of time sufficient for adequate distribution and contact with the lesions;
- an outstanding solvent capacity for oragnic fats. It is likewise characterized by bactericidal action , and to a lesser extent, by fungicidal action.

Conditions of this study

In a report submitted to the French Dermatology Society in 1963 (1) investigating the effect of D.V. 511 on about one hundred leg- and eschar ulcers, one of us reported 5 cases of intolerance concerning said medication.

R.LECLERQ reported during the subsequent discussion on two of his own cases involving secondary exematous lesions with positive tests for propylene glycol within the D.V.511 compound, in about 40 of the treated leg ulcer patients.

We developed our study since that time and tested 33 leg ulcer patients on which D.V. 511 was locally used.

Said patients were tested for (reactions to) D.V. 511, 38% propylene glycol is a watery solution (corresponding to the propylene glycol concentration in the D.V. 511) and 10% propylene glycol,as well as for reactions to other components of Acerbine (benzoic acids, malic and salicylic acids , and rose water).

Most of them were subjected to complementary tests, using other glycols: ethylene glycol,polyethylene glycol and the esters of the glycols, namely: [illegible] oleate of polyglycol, [illegible] ricinoleate of polyglycol, stereate of polyglycol palmitate of isopropyl, stereate of sorbitane, for the purpose of investigating the possibility of a more extensive allergy.

Five patients who received applications of Synalar-propylene glycol should be mentioned jointly with the above 33 patients.

Results

This total of 38 patients, before treatment with remedies containing propylene glycol, responded in 17 cases, i.e. to 45%, with positive tests to said propylene glycol therapy.

From the 38 patients 16 were susceptible to a cutaneous intolerance, as indicated by the clinical progress; 12 patients showed effectively positive tests (that is, to 75%); 22 patients had not specifically attracted our attention regarding a possible intolerance and their teguments: 5 of these, however, were sensitive to propylene glycol(i.e. a ratio of 23%) (Table I).

TABLE I

NOMBRE DE CAS Number of cases	INTOLERANCE SUSPECTEE Suspected intolerance	NON SUSPECTEE Not suspected
Tests positifs Positive Tests	12	5
tests negatifs Negative "	4	17
totaux Totals	16	22

The positive criteria of the tests were unquestionable. We considered only well-defined , pruriginous reactions as positive, corresponding to quotations of 2 to 3 across (croix).

As necessary corollaries of these findings, we systematically tested 145 eczema patients with propylene glycol as "evidence"; to our knowledge, they had never been treated locally on a basis of propylene glycol. Six of them (4% of the cases) reacted positively when tested with said substance.

Remarks

The results reported by us show the remarkable frequency of allergies to propylene glycol in patients who used medication containing said diol.

A positive test ratio of 75% is not surprising in case of patients who had effectively experiences pruritus and who showed a periulcerous eczematous condition when D.V. 511 was administered. We must emphasize that our allergic patients had ulcerated legs and that only one of the patients received pulverised administrations of synalar-propylene glycol for a dermatosis which, incidentally, was no favorable indication for the use of said medium. The application of any medication whatsoever on ulcerations, on lesions when the derma is bare, always implies exposure to intolerance .

As for D.V. 511, the method of application is as important as the substance given, and our sensitized patients had frequently received ill-considered doses of the product, applied beyond the limits of the ulcer; the peripheral teguments were inadequately protected by insufficient application of a salve made of a borated mash. Neverthless, even when taking the strict precautions made possible when patients are hospitalized, we diagnosed allergies. We wish also to stress that the worst method for Acerbine application is pulverization, which (method) is the best way to galvanize the product and to spread it where it should not be.

It was more surprising for us still to find 23% of the patients reacting positively. These patients had made no specific statements on intolerance to local therapy. It is true that a number of them was checked again for said allergenic evidence one to three years after the hospitalization during which (checks) we used D.V. 511. Is the allergy at issue latent? Such a hypothesis is not excluded, while we would be extremely relucatant to prescribe further analogous applications.

Mrs. DZI. . . Martha, while hospitalized in October-November 1963, received daily applications of Acerbine on a leg ulcer, without showing intolerance, during 35 days. The ulcer was thus cicatrized.

The ulcer recurs at the end of 1964. The patient applies the same product (in pulverized form and not as a compress), for two days. Beginning on the second day, a periulcerous eczema appears; it oozes and covers the entire leg.

The patient, who is exceptionally observant, repeats in July 1965 a single powdering which results in the same discomfort; she did this for the purpose of "verification".

The above woman is subjected to vesicular tests with Acerbine and with glycol. In addition, she is sensitized to an entire series of glycols or esters (column B on Table IV).

Compared to the high sensitization percentage to propylene glycol, only 4% of our 145 control patients reacted positively to the same. It is still a considerable ratio when it is taken into consideration that these subjects apparently never had any contact with the medicinal substances containing dialcohol. How can said "unmotivated" allergy be explained?

- The patients in question suffered from eczema; we had an opportunity to test them for other allergies, and they frequently showed sensitization related to professional products or therapeutic substances;

- the substances to which they were sensitized could very well contain propylene glycol. We refer here to numerous pharmaceutic preparations, whether or not said substance is explicitly mentioned (a rare eventuality) on the package formulas, and we refer to those the manufacturer of which is content with various sibylic statements such as "hydrodispersible excipient", "specific excipient", "penetrating excipient", "vanishing cream",etc. The laconicism of these formulas has been emphasized earlier by H. THIERS (2) and we wish, as he does, that they should be corrected...

TABLE II

Current uses of propylene glycol.

1 USAGES INDUSTRIELS.
2 Agent antigel : AUTOMOBILE.
3 Solvant : PRÉPARATION DES PEINTURES, VERNIS, VARNISH, INDUSTRIE DES TEINTURES.
4 COSMÉTOLOGIE, PARFUMERIE.
5 Conservateur, émollient, émulsionnant, solvant : [illegible]
6 USAGES PHARMACEUTIQUES.
7 Véhicule pour SOLUTIONS.
8 Excipient : ÉMULSIONS DU TYPE HUILE DANS EAU.
9 Conservateur : CONSERVATION DES SIROPS.

1. Industrial uses.
2. Anti-freeze medium : automobile.
3. Solvent: preparation of paints, varnishes, solvents; dye industry.
4. Cosmetology, perfumery.
5. Preservative, emollient, emulsifier, solvent: beauty products.
6. Pharmaceutic uses
7. Vehicle for solutions.
8. Excipient: oil-type emulsions in water.
9. Preservative: preservation of syrups.

Let us likewise point out that the propylene glycol is used (Table II) in perfumery and conmetology where we find it in the rather numerous preparations of the oil-type in water.

In industry, said diol represents a good solvent for coloring materials and it is therefore used, mixed to other solvents, for the manufacture of dyes, varnishes and solvents.
It also serves as anti-freeze.

Delayed manifestaion of the allergy

The incubation delay between the first application of a substance containing propylene glycol and the manifestation of allergy symptoms is very variable. The duration of the delay varies from a few days (3 days in one case) to one or several years.

This delay is certainly a function of the frequency and duration of the contacts with the allergenic substance.

Possibilities of a wider allergic reaction range

It should be mentioned in general that the glycols are widely used in the various fields mentioned earlier.

Accordingly, the polyglycols became especially important during recent years in pharmacology and cosmetology as excipients of creams and salves (3-4). But these innumerable and highly complex polymers should not let us overlook, for example, at the other extreme od the universe formed by the glycols, the utilization of the simplest among them, i.e. of the ethylene glycol (Table III).

This presumably explains why we found in a few patients a more extensive sensitization, including other glycols and esters of glycols (Table IV).

Said Table also shows (Column H) a curious example of allergy to the oleates, ricinoleates and stearates of polyglycol, discovered while testing a patient who had been subjected to ulcer therapy with Acerbine. Neverthless, this man was not sensitized either to said medication, or to propylene glycol.

TABLE III.

Some ethylene glycol uses
(according to ROCHE, MARIN and Mrs. BACHELIER (5).

1 USAGES INDUSTRIELS.

2 Agent antigel : AUTOMOBILE.

3 Diélectrique : FABRICATION DES CONDENSATEURS ÉLECTROLYTIQUES, FABRICATION DES EXPLOSIFS.

4 Solvant et plastifiant : FABRICATION DES TEINTURES ET APPRÊT, PRÉPARATION DES VERNIS.

5 Agent refroidissant : AVIONS, TUBES A RAYONS X.
Fluide hydraulique : AMORTISSEURS.

USAGES PHARMACEUTIQUES. 6

7 Véhicule dans les préparations pharmaceutiques.

8 Conservation des sirops, extraits alimentaires.

1. Industrial uses.
2. Antofreeze medium: automobile.
3. Dielectric: manufacturing of electrolytic condensers, manufacturing of explosives.
4. Solvent and platicizer: manufacturing of dyes and priming, and preparation of varnishes.
5. Cooling agent: airplanes, X-ray tubes.
6. Pharmaceutic uses.
7. Vehicle for pharmaceutic preparations.
8. Preservation of syrups, food extracts.

TABLE IV.

Simultaneous allergies.
to certain glycols and to the esters of glycols (8 patients).

	A	B	C	D	E	F	G	H
1 Ethylène glycol								0
2 Propylène glycol								0
3 Stéarate de propylène glycol	0				0		0	0
4 Polyéthylène glycol	0	0		0		0		0
5 Oléate de polyglycol	0		0	0	0	0	0	
6 Ricinoléate de polyglycol	0	0	0	0	0	0	0	
7 Stéarate de polyglycol	0			0	0	0	0	
8 Palmitate d'isopropyle	0	0		0	0	0	0	
9 Stéarate de sorbitane	0	0	0	0	0	0	0	0

1. Ethylene glycol
2. Propylene glycol
3. Stereate of propylene glycol
4. Polyethylene glycol
5. Oleate of polyglycol
6. Ricinoleate of polyglycol
7. Stereate of polyglycol
8. Plamitate of isopropyl
9. Stereate of sorbitane

Conclusion

We believe that the attention of allergists and dermatologists should be called to the possible sensitization of the teguments to the glycols, and in particular, to propylene glycol.

Since said mediums are used in increasingly varied fields, it seems desirable to keep this possibility in mind, which however would not reflect upon the numerous beneficial properties of these substances.

(Work of the Dermatology Clinic of the Lille University, Cl. Huriez)

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THE INTRAPERITONEAL TOXICITY OF SOME GLYCOLS, GLYCOL ETHERS, GLYCOL ESTERS, AND PHTHALATES IN MICE*

LEONARD KAREL, BENJAMIN H. LANDING, 1st Lt., M.C., and THOMAS S. HARVEY

With the Technical Assistance of Patricia A. Goguss, T/4, WAC, and Arthur J. Fisk

Toxicology and Pathology Sections, Medical Division, Edgewood Arsenal, Maryland

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One of the difficulties encountered in the biological assaying of drugs which are insoluble or unstable in water is the selection of a solvent, or a suspension medium, which will not alter the chemical state of the compound under investigation, and which is non-toxic for the test animal under the experimental conditions selected. In regard to the latter, the toxicity of any solvent is a function of the animal used, the route by which the investigated substance is administered, and the quantity of solvent, per kilogram of body weight, to be used as diluent.

Since the albino mouse is one of the most readily available, and, therefore, most frequently used laboratory animals; and since, furthermore, the intraperitoneal route of drug administration is among the simplest and most expedient, particularly in preliminary studies of drugs, it was felt that an investigation of the toxicity of a number of readily obtainable solvents by intraperitoneal injection in mice would be of interest and of value to a large number of investigators.

Although the acute and chronic toxicity of various members of the glycol, glycol ether, glycol ester, and phthalate series have been investigated (1–13), no reports on the effects of the intraperitoneal administration of these substances in mice have been found in the available literature. With this in mind, toxicity studies on Carworth Farms albino mice were conducted with 16 compounds belonging to the previously mentioned series.

Experimental. Female, albino, Carworth Farms mice kept on a standard weekly diet of Gaines' dog biscuits, milk bread, meat, peanuts, cabbage or carrots, apples, oranges, and water and weighing between 18 and 27 grams, were injected intraperitoneally with one dose of the pure, undiluted, redistilled solvents under investigation and were observed for 7 days following injection.

On the basis of preliminary screening tests with each solvent, the mice were divided into 6 equal groups, each of which received a different dose, the latter being graduated on a logarithmic scale. Every dose was administered to a minimum of six mice, and each animal was injected according to body weight. In a number of instances the assays were repeated with four mice at each dosage level, the results of the second assay being combined with those of the first for purposes of calculation. Subsequent to the determination of the median lethal dose (LD_{50}) by the method of Bliss for small numbers of animals (14), as a final check, a dose corresponding to the LD_{50} for each compound was administered intraperitoneally to groups of 10 animals.

Gross and microscopic pathological studies were conducted on animals which died during the first 7 days after injection or were sacrificed at the end of the 7 day period of observation.

* An abstract of this paper appeared in Federation Proceedings 6: 312, 1947.

Since it was felt that adequate descriptions of the pathological processes following parenteral administration were available in the literature for diethylene glycol, ethylene glycol, ethyl cellosolve, and dioxane (6), these compounds were not intensively studied pathologically. Observations from the literature are, however, included in the tabulation of the data to serve as bases for comparison with those compounds reported in this study. In addition, diethylene glycol, although well covered in the literature (6), was treated as a control compound and was studied thoroughly to provide a further basis for comparison of the pathological data obtained in this study with that in the literature.

Animals for pathological study were necropsied as soon as possible after death, or immediately after being sacrificed with chloroform. Six to 10 mice were examined both grossly and microscopically (at two or more intervals) during the first 7 days after injection of each compound.

Following the gross examinations, organs were fixed in Zenker's solution, embedded in paraffin, sectioned at 5 mu, and stained with hematoxylin and eosin. Heart, lungs, liver, spleen, and kidneys were examined microscopically in all animals, and pancreas, adrenals, thymus, lymph nodes, bone marrow, sympathetic tissue, gastrointestinal tract, testis, and ovary in a smaller number.

RESULTS: *A. Median Lethal Doses, Minimum, and Maximum Mortality Data.* The median lethal millimolar doses (LD_{50}'s) in increasing order of the toxicity of the various solvents investigated were as follows: propylene glycol—127.87, diethylene glycol—91.69, ethylene glycol—90.55, triethylene glycol—54.27, dipropylene glycol—33.54, ethyl carbitol—29.14, methyl cellosolve—28.25, ethyl cellosolve—18.97, dimethyl phthalate—18.75, dibutyl phthalate—14.87, ethylene glycol monoacetate—13.93, diethyl phthalate—12.37, dioxane—8.97, glycol diacetate—8.14, butyl carbitol—5.24, and allyl diglycol carbonate—0.98. Further data, including formulas, molecular weights, and specific gravities of the compounds; LD_{50}'s in ml./kg., gm./kg., and millimoles/kg., with their respective standard errors; and regression line slopes, with their standard errors, are included in table 1. In table 2 there are enumerated for each compound, the highest doses which gave 0 per cent mortality both during the first 24 hours and during the total observation period of 7 days. In addition, this table also contains similar data for the lowest doses which produced 100 per cent mortality.

B. Pathological Changes. The pathological changes in any one organ, when present, were essentially the same for all the compounds of this series, although the relative severity of the lesions varied with the solvent and with the survival time of the animal. The observed changes were as follows:

Heart—Occasionally, cardiac dilatation was evident during the first 24 hours; there were, however, no gross changes after this time and no microscopic changes at any time.

Lungs—Varying degrees of congestion, atelectasis, edema, and hemorrhage were found. The earliest alterations observed were, usually, diffuse congestion and patchy atelectasis, which were succeeded, first, by more extensive atelectasis with patchy edema and, later, by more extensive edema with petechial hemorrhage. Massive pulmonary hemorrhage was rare, and bronchopneumonia was observed in only a few instances. In animals surviving 7 days, the pathological manifestations were decreased in severity but were of essentially the same nature.

TABLE 1

The acute intraperitoneal toxicity of some undiluted glycols, glycol ethers, glycol esters, and phthalates in C. F., female mice observed for seven days following injection

NO.	NAME OF COMPOUND (AND NUMBER OF ANIMALS USED IN ASSAY)	FORMULA	MOL. WT.	SPECIFIC GRAVITY	LD_{50} ± S.E.	LD_{50} ± S.E.	LD_{50} ± S.E.	SLOPE OF REGRESSION LINE ± S.E.	NO.
					ml./kg.	*g./kg.*	*millimoles/kg.*		
1	Propylene Glycol (60)	$CH_2OH—CHOH—CH_3$	76.094	1.040	9.36 ± 1.20	9.73 ± 1.25	127.87 ± 16.43	6.38 ± 1.48	1
2	Diethylene Glycol (38)	$(HO \cdot CH_2 \cdot CH_2)_2O$	106.120	1.118	8.70 ± 0.30	9.73 ± 0.33	91.69 ± 3.11	20.61 ± 5.94	2
3	Ethylene Glycol (60)	CH_2OH | CH_2OH	62.068	1.115	5.04 ± 1.52	5.62 ± 0.17	90.55 ± 2.71	15.42 ± 4.44	3
4	Triethylene Glycol (38)	$HOCH_2CH_2OCH_2CH_2—OCH_2CH_2OH$	150.172	1.125	7.24 ± 0.25	8.15 ± 0.28	54.27 ± 1.86	18.86 ± 5.58	4
5	Dipropylene Glycol (60)	$HOCH_2CH_2CH_2O—CH_2CH_2CH_2OH$	134.172	1.025	4.39 ± 0.51	4.50 ± 0.52	33.54 ± 3.88	5.00 ± 3.04	5
6	Ethyl Carbitol (40)	$C_2H_5OCH_2CH_2—OCH_2CH_2OH$	134.172	0.990	3.95 ± 0.21	3.91 ± 0.21	29.14 ± 1.57	8.80 ± 2.65	6
7	Methyl Cellosolve (57)	$CH_2OH—CH_2O—CH_3$	76.094	0.965	2.23 ± 0.07	2.15 ± 0.068	28.25 ± 0.89	17.01 ± 4.17	7
8	Ethyl Cellosolve (60)	$CH_2OH—CH_2O—C_2H_5$	90.120	0.936	1.83 ± 0.114	1.71 ± 0.107	18.97 ± 1.19	7.13 ± 1.80	8
9	Dimethyl Phthalate (37)	$C_6H_4(COOCH_3)_2$	194.180	1.189	3.06 ± 0.13	3.64 ± 0.21	18.75 ± 1.08	11.40 ± 5.01	9
10	Dibutyl Phthalate (60)	$C_6H_4(COOC_4H_9)_2$	278.336	1.045	3.96 ± 0.41	4.14 ± 0.43	14.87 ± 1.54	5.67 ± 1.98	10
11	Ethylene Glycol Monoacetate (36)	$HOCH_2 \cdot CH_2O \cdot CO \cdot CH_3$	104.100	1.108	1.31 ± 0.15	1.45 ± 0.17	13.93 ± 1.63	8.43 ± 4.97	11
12	Diethyl Phthalate (60)	$C_6H_4(COOC_2H_5)_2$	222.232	1.121	2.45 ± 0.10	2.75 ± 0.11	12.37 ± 0.49	17.37 ± 5.33	12
13	Dioxane (60)	$O(CH_2CH_2)_2O$	88.100	1.034	0.76 ± 0.14	0.79 ± 0.14	8.97 ± 1.59	4.64 ± 2.50	13
14	Glycol Diacetate (60)	$(CH_3CO_2CH_2)_2$	146.140	1.109	1.07 ± 0.45	1.19 ± 0.05	8.14 ± 0.34	15.40 ± 5.31	14
15	Butyl Carbitol (60)	$C_4H_9OCH_2CH_2—OCH_2CH_2OH$	162.224	0.956	0.89 ± 0.12	0.85 ± 0.11	5.24 ± 0.68	6.69 ± 2.80	15
16	Allyl Diglycol Carbonate (58)	$(CH_2CH_2OCOOCH_2—CH{=}CH_2)_2O$	274.264	1.143	0.21 ± 0.016	0.27 ± 0.018	0.984 ± 0.066	8.82 ± 2.28	16

Liver—Varying degrees of congestion were commonly seen during the first few days. Those compounds which caused lymphoid tissue damage produced similar injury to the lymphoid follicles around the larger ducts and blood vessels. Similarly, leukocytosis was accompanied by an abnormally high number of white cells in the sinusoids. In a few animals, there was pyknosis of hepatic cells during the early stages, and in a still smaller number, there was evidence of mild necrosis. No compound, however, produced the degree of liver damage reported elsewhere for dioxane and some related compounds (6), although in several cases, there was a marked increase in the number of mitotic figures visible in the liver at the end of 7 days. This effect, which was observed also

TABLE 2

Volumetric Doses Resulting in Zero and in 100 Per Cent Mortality During the First 24 Hours and During 7 Days Following Intraperitoneal Injection of the Various Solvents in Mice

COMPOUNDS IN ORDER OF INCREASING MOLAR TOXICITIES	HIGHEST DOSE WHICH GAVE 0 MORTALITY DURING 1ST 24 HOURS	HIGHEST DOSE WHICH GAVE 0 MORTALITY DURING 7 DAY PERIOD	LOWEST DOSE WHICH GAVE 100% MORTALITY DURING 1ST 24 HOURS	LOWEST DOSE WHICH GAVE 100% MORTALITY DURING 7 DAY PERIOD
	ml./kg.	*ml./kg.*	*ml./kg.*	*ml./kg.*
1. Propylene Glycol	7.37	3.69	20.84	14.70
2. Diethylene Glycol	7.38	4.94	—	—
3. Ethylene Glycol	4.49	3.63	6.16	6.16
4. Triethylene Glycol	5.34	—	—	7.98
5. Dipropylene Glycol	4.88	—	—	—
6. Ethyl Carbitol	3.61	2.93	—	—
7. Methyl Cellosolve	1.80	1.80	—	—
8. Ethyl Cellosolve	1.59	—	—	2.24
9. Dimethyl Phthalate	2.50	—	4.34	4.34
10. Dibutyl Phthalate	2.40	2.05	—	4.80
11. Ethylene Glycol Monoacetate	1.60	—	—	1.90
12. Diethyl Phthalate	1.70	1.70	3.40	3.40
13. Dioxane	1.65	—	—	1.65
14. Glycol Diacetate	0.80	0.80	—	—
15. Butyl Carbitol	1.31	—	—	1.03
16. Allyl Diglycol Carbonate	0.19	0.15	0.37	0.37

in the lymphoid tissue and gastrointestinal tract, could not be related to earlier pyknosis or necrosis of hepatic cells.

Spleen—With most of the solvents studied, splenic damage occurred very early after injection and consisted of pyknosis, degeneration, and fragmentation of the lymphocytes of the malpighian follicles, followed by phagocytosis of debris and reticulum cell proliferation. The red pulp showed congestion, lesser amounts of lymphocyte fragmentation, and occasionally an increased amount of hemosiderin. By the end of the seventh day, the acute, degenerative phenomena had decreased in severity, and active lymphocyte regeneration, with many mitotic figures, was found in many of the animals.

Kidney—Kidney damage, both glomerular and tubular, occurred with all the

compounds, although there was considerable variation in the relative degrees of damage to glomeruli and tubules among animals as well as among compounds. Protein leakage through the glomerular tufts was accompanied by varying amounts of eosinophilic protein precipitate in the capsular spaces and proximal tubules, and dilatation of the capsular spaces and proximal convoluted tubules. In some cases, the tubular damage developed as rapidly as the glomerular, but more usually it followed the glomerular changes. It consisted of mild to moderate degeneration of the epithelial cells of the proximal convoluted tubules, loops of Henle, and distal convoluted tubules, with sloughing of the cytoplasm into the lumens, vacuolation of the cytoplasm, and nuclear pyknosis. Although small protein casts, composed apparently of transuded plasma protein in some animals, and of renal epithelial cytoplasmic protein in others, were common, no evidence of crystal formation in the tubules, or of tubular obstruction by these casts was seen. Furthermore, tubular necrosis with cast extrusion, as is seen in hemoglobin kidney damage, did not occur. In several animals, small subcapsular patches of pyelonephritis were found, but this was felt to be an incidental finding, since it has been noted in many animals from the same colony sacrificed in other projects. In no instance was pyelonephritis, or interstitial infiltration, or fibrosis thought to be related to the administration of the compound. While, in some cases, the tubular changes showed significant resolution at the end of 7 days, occasionally moderate to marked epithelial flattening was observed, and, with several compounds, the glomerular protein leakage continued or even increased during this period.

Pancreas—Only one compound, allyl diglycol carbonate, caused pancreatic changes. These consisted of swelling of the nuclei of both the acinar and the islet tissues, producing large, vesicular nuclei microscopically resembling glycogen-filled liver nuclei.

Adrenals—There were no observable changes in the medullae and no evidence of necrosis or of mitotic abnormalities; the adrenals did exhibit varying degrees of lipoid depletion of the cortex.

Thymus and Lymph Nodes—These tissues showed essentially the same changes as did the splenic white pulp, with lymphocyte fragmentation followed by reticulum cell proliferation and phagocytosis of cell debris.

Bone Marrow—There were no abnormalities in the animals examined.

Sympathetic Tissue—No microscopic changes were observed in the retroperitoneal, sympathetic ganglia, or in the aortic body, which was incidentally sectioned in a few cases.

Gastrointestinal Tract—Except for edema of the mucosal stroma, and increased mucous secretion in some animals, no abnormalities of the gastrointestinal tract proper were observed during the first few days. However, the lymphoid follicles and the free cells of the wall showed degenerative changes similar to those previously described for compounds producing lymphoid damage.

Testis—In all the spermatogenic cell-layers, there was extensive pyknosis during the early stages, but no atrophy or other late effects of testicular damage were found.

Ovary—No ovarian abnormalities were observed.

Peritoneum—Fresh hemorrhage, seen in the peritoneal cavities of rare animals dying shortly after injection, was never felt to be of significant degree; microscopic evidences of perisplenitis (apparently chemical) were seen in a slightly larger number dying or sacrificed from the fifth to the seventh day. No correlation of these findings with the drug or dose given could be made, and no gross evidences of reactive peritoneal congestion or effusion were noted in any of the animals autopsied.

In table 3, for each compound investigated, there are graphically listed the relative severity of the major pathological changes observed from the first to the fourth day, inclusive, and from the fifth through the seventh day; in addition, pertinent pathological comments are included for each compound.

Discussion. That any solvent, regardless of its degree of inherent toxicity, may adversely affect the well-being of the animal to some extent is likely when it is considered that the use of a substance in pure form as a diluting medium means the introduction, into the organism, of a considerably hypertonic solution, the action of which may lower the resistance of the host and, thereby, potentiate the toxic action of the assayed material. Moreover, the production of a solvent-engendered hyperemia may result in a more rapid-than-normal drug absorption which may tend to overwhelm the detoxifying mechanisms, again apparently increasing the toxicity inherent in the drug being investigated.

In several instances, despite the fact that only redistilled compounds were used in this study, the pH of the solvents as determined with the glass electrode indicated marked deviations from neutrality towards the acid side. Check assays with neutralized solvents revealed that these deviations, probably the consequence of a low degree of hydrolysis, were not sufficiently great to alter the mortality data originally obtained.

The median lethal doses determined for all compounds were injected into groups of 10 mice which were then observed for the prescribed 7-day period. When the mortalities fell within the ranges ($P \gtrless 0.05$) calculated from the parameters of the respective dosage-mortality curves, no further toxicity data were gathered. In a few instances, when a check was not obtained, the assays were repeated. The pathological studies revealed that a small number of individuals in some groups of mice had an apparently naturally occurring leukemia which, although it may have altered the survival times of the animals, caused no significant differences in the pathological changes observed; the leukemic infiltrates, in addition, did not seem to differ, in sensitivity to the lymphotoxic action of this series of compounds, from normal lymphoid-tissue.

In general, the pathological changes observed in this study agreed with those reported in the literature, with the lungs, lymphoid tissues, and kidneys being the viscera most affected. Less liver damage was observed than has been reported for dioxane and related compounds (6); whether this is due to differences in the times at which pathological studies were done, to the different route of injection, to dietary or other factors, or to differences in the chemical structures of the compounds, is not certain, but the correlation with the literature obtained with diethylene glycol and, based on smaller numbers of animals, with ethylene glycol and dioxane, suggests that the last factor is the significant

TABLE 3

Relative severity of major pathological changes observed during seven days following the intraperitoneal injection of one lethal or sublethal dose of some glycols, glycol ethers, glycol esters, or phthalates in mice

COMPOUND	FIRST THROUGH FOURTH DAY				FIFTH THROUGH SEVENTH DAY				REF
	Lungs	Spleen and Lymphoid Tissue	Kidneys	Pathological Changes	Lungs	Spleen and Lymphoid Tissue	Kidneys	Pathological Changes	
1. Starvation Controls	–	–	–	None					
2. *Propylene Glycol*	–	–	+						(6
3. Diethylene Glycol	+	+++	+	Pulmonary congestion, hemorrhage, and edema; marked toxic reaction* in spleen and thymus; minimal renal tubular degeneration.	–	+	–	Hyperactive mitosis in spleen and GI tract; other organs negative.	
4. *Diethylene Glycol*	++	+	+++						(6)
5. *Ethylene Glycol*	–	–	+						(6)
6. Triethylene Glycol	+	+++	+	Toxic reaction in spleen and thymus; renal glomerular and tubular damage; high white count; pulmonary congestion, and atelectasis.	–	+	–	Regeneration of spleen and lymphoid tissue; no other lesions.	
7. Dipropylene Glycol	–	–	+	Slight renal tubular degeneration.	–	+	+	Hyperactive mitosis in spleen, lymphoid tissue, intestinal mucosa, and liver; renal lesions unchanged.	
8. Ethyl Carbitol	–	++	+	Toxic reaction in spleen; mild renal glomerular and tubular degeneration.	–	–	+	Continued kidney damage; lymphoid tissue normal.	
9. Methyl Cellosolve	–	++	+	Toxic reaction in spleen and lymph nodes; mild renal tubular degeneration.	–	+	++	Lymphoid regeneration; renal tubular damage continued.	
10. *Ethyl Cellosolve*	–	–	++						(6)
11. Dimethyl Phthalate	+	++	+	Pulmonary congestion and atelectasis; toxic reaction in spleen and lymph nodes; renal tubular necrosis.	–	+	–	Hyperactive mitosis in spleen, lymphoid tissue, intestine, and liver; continued renal tubular degeneration and glomerular protein leakage.	

12. Dibutyl Phthalate	++	++	+	Pulmonary congestion, atelectasis, edema, and petechial hemorrhage; toxic reaction in spleen and lymphoid tissue; minimal renal tubular degeneration.	+	+	++	Lymphoid regeneration; glomerular protein leakage and continued tubular damage; slight patchy pulmonary edema.	
13. Ethylene Glycol Monoacetate	++	+	−	Pulmonary congestion and atelectasis; congestion of viscera.	−	+	+	Hyperactive mitosis in lymphoid tissue, liver, and gastrointestinal tract; slight renal tubular degeneration, with small foci of pyelonephritis.	
14. Diethyl Phthalate	++	++	++	Pulmonary congestion, edema, and petechial hemorrhage; toxic reaction in spleen; renal tubular degeneration.	+	−	++	Continued renal tubular degeneration with flattening of the epithelium.	
15. *Dioxane*	−	−	++						(6)
16. Glycol Diacetate	++	−	+	Pulmonary congestion; moderate renal tubular degeneration.	+	−	++	Continued renal tubular damage.	
17. Butyl Carbitol	+	++	+	Pulmonary congestion and atelectasis; toxic reaction in spleen and lymphoid tissue; glomerular and tubular degeneration; edema of gastrointestinal mucosa plus pulmonary petechial hemorrhage.					
18. Allyl Diglycol Carbonate	++	+	++	Pulmonary congestion, atelectasis, and edema; toxic reaction in spleen and lymphoid tissue; congestion of viscera; renal tubular damage; swelling of pancreatic nuclei.	+	+	+	Pulmonary congestion, atelectasis, and patchy bronchopneumonia; glomerular proteinuria; healing hepatic necrosis.	

− No significant lesions, + Mild Lesions, ++ Moderate Lesions, +++ Severe Lesions.

* Toxic reaction—cf. pathology of spleen in Results, part B.

one. Similarly, hemorrhagic nephritis, and oxalate crystalluria, which have been reported to follow administration of ethylene glycol and related compounds (15), were not observed in this study.

SUMMARY

The acute, intraperitoneal median lethal doses for 16 solvents belonging to the glycol, glycol ether, glycol ester, and phthalate series were determined in Carworth Farms, female, albino mice, which were observed for 7 days following injection. The LD_{50}'s, in millimoles per kg., and the pathological changes observed with some of the solvents up to 72 hours were as follows: *propylene glycol* (127.87); *diethylene glycol* (91.69); *ethylene glycol* (90.55); *triethylene glycol* (54.27)—toxic reaction in spleen and thymus, renal glomerular and tubular damage, high white count, pulmonary congestion, and atelectasis; *dipropylene glycol* (33.54)—renal tubular degeneration; *ethyl carbitol* (29.14)—toxic reaction in spleen, renal glomerular and tubular degeneration; *methyl cellosolve* (28.25)—toxic reaction in spleen and lymph nodes, renal tubular degeneration; *ethyl cellosolve* (18.97); *dimethyl phthalate* (18.75)—pulmonary congestion and atelectasis, toxic reaction in spleen and lymph nodes, renal tubular necrosis; *dibutyl phthalate* (14.87)—pulmonary congestion, edema, and petechial hemorrhage, toxic reaction in spleen; renal tubular degeneration; *ethylene glycol monoacetate* (13.93)—pulmonary congestion and atelectasis; *diethyl phthalate* (12.37)—pulmonary congestion, edema, and petechial hemorrhage, toxic reaction in spleen, and renal tubular degeneration; *dioxane* (8.97); *glycol diacetate* (8.14)—pulmonary congestion, renal tubular degeneration; *butyl carbitol* (5.24)—pulmonary congestion and atelectasis, toxic reaction in spleen and lymphoid tissue, glomerular and tubular degeneration; *allyl diglycol carbonate* (0.98)—pulmonary congestion, atelectasis, and edema, toxic reaction in spleen and lymphoid tissue, congestion of viscera, marked renal tubular damage. All fasting controls were negative.

Additional data include formulas, molecular weights, and specific gravities of the compounds; LD_{50}'s, with their respective standard errors, in ml., kg., gm./kg., and millimoles/kg.; regression line slopes with their standard errors; highest and lowest volumetric doses resulting in 0 and in 100 per cent mortality, respectively, during the first 24 hours following intraperitoneal injection and also for the 7 day observation period; and a tabulation, with pertinent pathological comments, of the relative severity of the major pathological changes observed.

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Communications to the editors

ANTITUMOR ACTIVITY OF MONOGLYCERIDES AND OTHER ESTERS OF FATTY ACIDS

Sir:

Antitumor active monoglycerides have been isolated from the acetone extracts of the mycelia of *Sepedonium ampullosporum*[1], *Cercospora oryzae*[2] and *Coriolus unicolor*[2]. Fatty acids composition of the monoglycerides was examined and it was found that hexadecanoic, octadecenoic and octadecadienoic acids are the main components[3]. They also contain other fatty acids as the minor components.

In order to determine the active principle in the mixtures of the monoglycerides, antitumor activity *in vivo* of each monoglycerides was studied using EHRLICH ascites tumor in mice. In addition to the monoglycerides, the glycerol esters of fatty acids, other esters were examined for the activity. In this paper we report the antitumor activity of monoglycerides and other esters of fatty acids.

In this study we chose those fatty acids with even carbon numbers between ten and eighteen. For the alcoholic moieties we selected methanol, propylene glycol, sorbitol and sucrose in addition to glycerol.

A part of the monoglycerides and all the sucrose esters were synthesized in this laboratory. Monoglycerides were synthesized according to the method of DAUBERT and BALDWIN[4] except that pyridine was used instead of quinoline in the reaction mixture. For the synthesis of sucrose esters of fatty acids the method of OSIPOW *et al.*[5] was applied. Other materials were commercially available.

To examine purity of the fatty acid component of the esters each sample was subjected to methanolysis and the methyl esters obtained were analyzed with gas-

Table 1. Antitumor activity of monoglycerides

Monoglycerides	Dose (mg/mouse/day)	7 Days after implant: Tumor		Body wt. gain (g)		Survival time (days)	
Monostearin	10.0	+++	+++	+4.0	+5.3	13	15
	2.5	−	+++	+2.3	+7.8	29	22
Monoolein	6.0	−	−	+2.4	+2.9	>30	>30
	1.5	+	+++	+7.0	—	22	26
Monolinolein	6.0	+++	+++	—	—	26	24
	1.5	+++	+++	—	—	22	17
Monolinolenin	20.0	−	−	−6.8	−3.6	28	25
	5.0	+	+++	+6.0	+3.1	>30	>30
Monopalmitin	10.0	+	+	+4.7	+5.0	18	21
	2.5	−	−	+2.8	+1.6	>30	>30
Monomyristin	10.0	+	+	+4.2	+2.5	18	24
	2.5	−	+++	+5.3	+7.5	18	15
Monolaurin	10.0	−	−	+2.1	+0.5	28	>30
	2.5	−	−	+3.7	+4.4	27	24
Monocaprin	20.0	—	—	—		3	1
	5.0	−	+	+0.6	+1.9	28	22
Monocaproin	10.0	−	+++	+2.0	+5.2	18	11
	2.5	+++	+++	+7.5	+7.3	18	15
Control	0.0	+++	+++	+8.8	+9.2	15	16
		+++	+++	+9.5	+[illegible].4	13	17

The method of the assay is described in the text. Two mice were used for each dose in this experiment. The degree of the tumor growth is as follows: +++ marked growth, ++ moderate growth, + slight growth, − no growth.

liquid chromatography using a poly butanediol succinate column. The results indicated that all of the samples were 70~90 % pure for fatty acid components.

Antitumor activity *in vivo* was assayed using EHRLICH ascites tumor in mice. The ascitic tumor cells of EHRLICH carcinoma (2×10^6 cells) were implanted intraperitoneally in *ddY* mice, 5 weeks old, weighing 18 to 22 g. Saline solution of the samples was administered once daily for 5 successive days and the tumor growth and body weight gain after 7 days and life span were observed.

Antitumor activity of nine monoglycerides is shown in Table 1. When the tumor growth after 7 days was compared with that of the control mice, stearic, linoleic and caproic monoglycerides were thoroughly ineffective, whereas linolenic, oleic, palmitic, myristic and capric glycerides were somewhat effective and monolaurin completely inhibited the tumor growth at two doses tested. Most of the treated animals showed prolongation of the survival periond while control mice were observed to die within 17 days. In the cases of monolinolenin, monoolein and monopalmitin some of the mice survived more than 30 days but their tumor cure was not complete. On the other hand, monomyristin- and monolaurin-treated mice exhibited complete cure though their survival periods were more or less short. Thus, monoglycerides with carbon numbers between 12 and 16 were active against EHRLICH ascites tumors in mice. Moreover some of the unsaturated monoglycerides with 18 carbon atoms in their fatty acids, namely monoolein and monolinolenin, were also active *in vivo*.

When the antitumor activity of sucrose esters of linoleic, palmitic, myristic, lauric and capric acids was examined, palmitic, myristic and lauric esters exerted remarkable inhibition of the tumor growth with prolongation of the survival time. In the case of propylene glycol esters, oleic, palmitic and myristic esters were examined and myristic ester showed a complete cure with prolonged survival time. All of the methyl esters and sorbitol esters tested were inactive against the tumor except methyl laurate which exerted an inhibitory activity against the tumor.

The details of this study will be reported elsewhere.

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AKIKO KATO
KUNIO ANDO
SEIKICHI SUZUKI*
GAKUZO TAMURA
KEI ARIMA

Laboratory of Microbiology,
Department of Agricultural Chemistry,
the University of Tokyo
* Research Laboratories,
Chugai Pharmaceutical Co., Ltd.

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PATHOLOGIC EFFECTS OF CERTAIN GLYCOLS AND RELATED COMPOUNDS

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H. D. KESTEN, M.D.

M. G. MULINOS, M.D.

AND

LEO POMERANTZ, B.S.

NEW YORK

A number of polyhydric alcohols and their derivatives are being used in increasing quantities in many industries. They are used, for example, in the manufacture of lacquers, flavoring extracts, textiles, cosmetics, pharmaceuticals, preservatives, detergents and polishes. As part of a study of the toxicology of ethylene glycol (glycol), diethylene glycol (diglycol), propylene glycol and some of their derivatives, the organs of rats, rabbits and guinea pigs to which these substances had been administered were studied morphologically. The degenerative changes that occurred in some of these organs form the subject of this report.

TABLE 1.—*Chemical Formulas of Compounds Used**

Compound	Structural Formula
Ethylene glycol; glycol	$HO.CH_2.CH_2.OH$
Ethylene glycol diacetate	$CH_3.CO.O.CH_2.CH_2.O.CO.CH_3$
Propylene glycol	$HO.CH_2.CH.OH.CH_3$
Diethylene glycol; diglycol	$HO.CH_2.CH_2.O.CH_2.CH_2.OH$
Ethyl diethylene glycol (diglycol monoethyl ether); carbitol	$C_2H_5.O.CH_2.CH_2.O.CH_2.CH_2.OH$
Methyl diethylene glycol (diglycol monomethyl ether); methyl carbitol	$CH_3.O.CH_2.CH_2.O.CH_2.CH_2.OH$
Butyl diethylene glycol (diglycol monon-butyl ether); butyl carbitol	$C_4H_9.O.CH_2.CH_2.O.CH_2.CH_2.OH$
Diethylene dioxide (dioxane)	
Dipropylene glycol	$CH_3.OH.CH.CH_2.O.CH_2.CH.OH.CH_3$

* The compounds were of commercial grade and were manufactured by the Carbide and Carbon Chemicals Corporation.

The chemical relationship of the several glycols used is indicated in table 1.

The compounds were given by mouth to rats and guinea pigs, usually in the drinking water, and by mouth or intravenously to rabbits, for from one to two hundred and thirty-four days. The animals were fed stock laboratory diets, and they were maintained in a state of good nutrition except as noted. The tissues of animals that died during the experiments or that were killed were promptly

* From the departments of pathology and pharmacology, College of Physicians and Surgeons, Columbia University.

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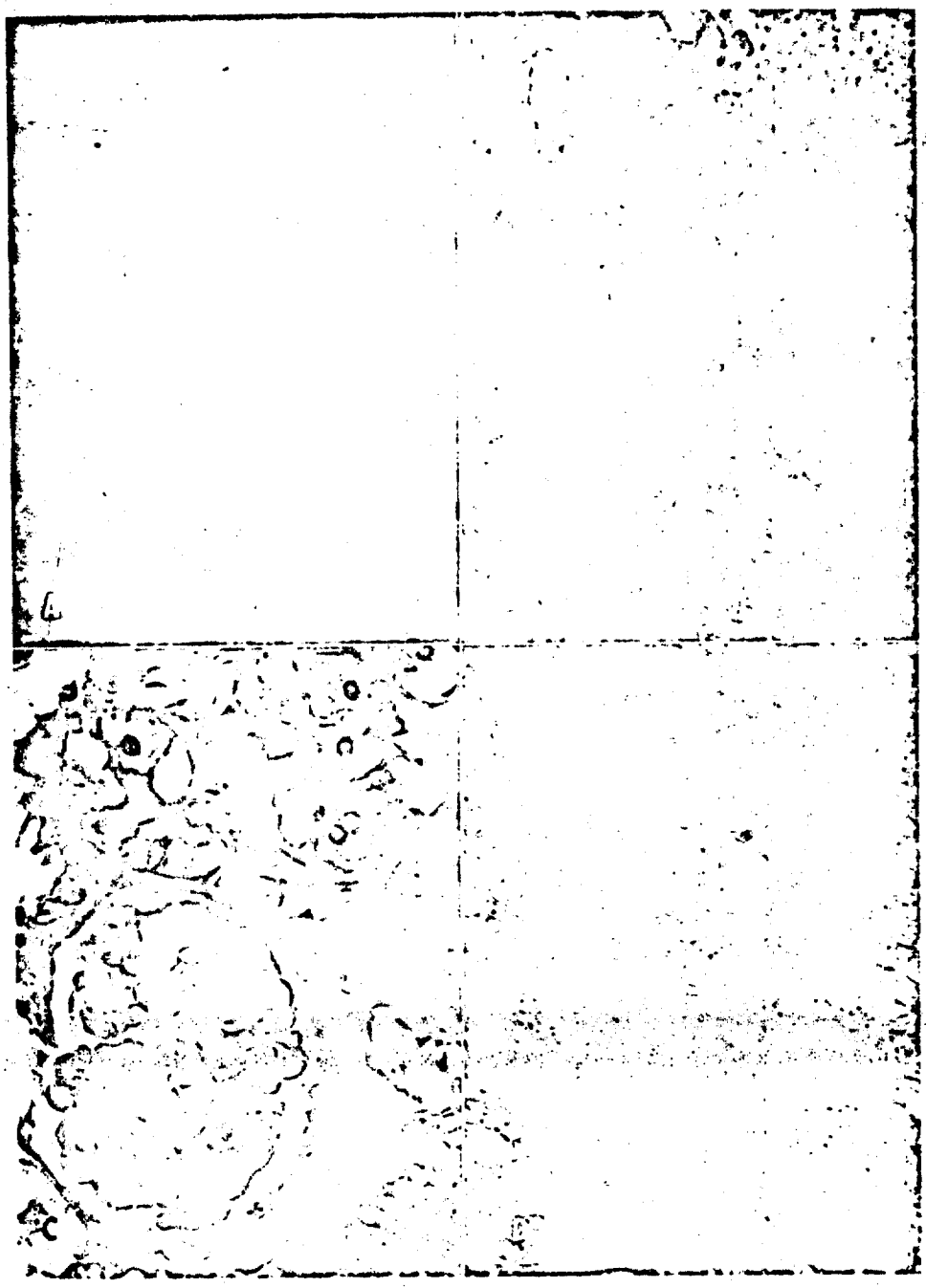

Figure 1

EXPLANATION OF FIGURE 1

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A, calcium oxalate crystals in convoluted tubules of the kidney of a rat which received ethylene glycol diacetate in a concentration of 1 per cent for one hundred and ten days and in a concentration of 3 per cent for twenty days, in the drinking water. The renal calcium amounted to 819 mg. in 100 Gm. wet weight; the blood nonprotein nitrogen, to 76.2 mg. in 100 cc.

B, hemoglobin casts in renal tubules of a rabbit given 5 cc. per kilogram of propylene glycol intravenously and killed on the ninth day. The hemoglobin content was 40 per cent (Dare); the blood nonprotein nitrogen, 69.4 mg. in 100 cc.

C, vacuolation of cells of convoluted tubules and slight leakage of protein into glomerular spaces in the kidney of a rabbit that received 2 cc. per kilogram of diethylene glycol intravenously and was killed twenty hours later.

D, hydropic degeneration of convoluted tubules and distention of glomerular spaces in the kidney of a rat that consumed diethylene glycol in a concentration of 5 per cent in the drinking water. The animal became sick and was killed at the end of seventy-two hours.

The 1,2 form of propylene glycol was administered to 39 rats in their drinking water in concentrations of from 1 to 10 per cent for from eleven to two hundred and thirty-four days with no demonstrable effect. Fifteen rabbits were given by intravenous injection single doses of from 2 to 5 cc. per kilogram of body weight. One rabbit that received a 5 cc. dose died the following night, but nothing abnormal was observed except the presence of considerable quantities of protein within the tubules of the kidneys. The other 14 rabbits were killed within from two to forty-six days after the injection. No pathologic changes were found in 10 of these.

Four rabbits which had been given intravenously 4 or 5 cc. of propylene glycol per kilogram of body weight and which were killed at the expiration of from two to twenty-three days exhibited in the kidneys evidence of the hemolytic action of the compound. This was visible grossly as reddish brown streaks and points on both the capsular and the cut surfaces. These discolorations consisted of hemoglobin-containing casts which could be found in all levels of the tubular system (fig. 1 *B*). Granular and hyaline casts were also sometimes present. Leakage of erythrocytes into glomerular spaces and convoluted tubules was sometimes seen. One of these rabbits which received a 5 cc. dose had 238 mg. of nonprotein nitrogen in 100 cc. of blood when killed forty-eight hours after the injection. In addition to all the aforementioned changes, small groups of renal convoluted tubules were necrotic, and occasional mitoses were found in adjacent remaining epithelial cells of this animal.

DIETHYLENE GLYCOL

Since the series of fatalities in October 1937 following the use of an elixir of sulfanilamide made with diethylene glycol as one of the solvents, several reports have appeared on the pathologic effects of large doses of diglycol on human beings and animals (Kesten, Mulinos and Pomerantz;[7] Holck;[6e] Cannon;[8] Ruprecht and Nelson;[9] Hagebusch;[10] Lynch;[11] Cannon and Geiling[12]). Prior to that time only two studies of the toxicity of diglycol had been published (von Oettingen and Jirouch;[13] Haag and Ambrose[14]), in neither of which were details of pathologic changes noted. Von Oettingen and Jirouch[13] mentioned

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the occurrence of acute nephrosis in rats that had been given subcutaneous injections of one or the other of the following: diethylene glycol, its ethyl ether, or carbitol; the ethyl ether of ethylene glycol, or cellosolve; cellosolve acetate; butyl cellosolve, and dioxane.

The effects of diglycol were studied as follows:

1. Seventeen rats received diglycol in 0.5 per cent concentration in the drinking water for from twenty-two to one hundred and twenty-four days. No deaths and no lesions occurred as a result of the procedure.

2. Thirty rats drank 1 per cent diglycol for from thirty-three to two hundred and seventeen days without any demonstrable effect.

3. Thirty rats received 3 per cent diglycol in the drinking water for from four to ninety-five days (approximately 3.5 cc. of diglycol per kilogram of body weight per day). Twenty-one died in from four to fifty-six days; hydropic degeneration of the renal cortex was present in 20. The livers of 13 of these contained foci of similar degeneration. The remaining 9 were killed at from fifty-one to ninety-five days. No lesions referable to the procedure were present.

4. Forty-two rats were on 5 per cent diglycol (approximately 6 cc. per kilogram of body weight per day) for from one to thirty-six days, of which 9 died in from one to six days. Hydropic degeneration had occurred in the kidneys of the 9 animals and in the livers of 5. Of the remaining 33, killed at from one to thirty-six days, similar damage of the kidneys was exhibited in 27 and degeneration of the liver in 13.

5. Six rabbits received from 1 to 4.6 cc. of diethylene glycol per kilogram of body weight in from 1 to 5 per cent concentration in the drinking water during from five to twenty-eight days. One rabbit which had consumed 1 cc. per kilogram died on the seventh day with extensive renal damage and edema of the lungs. Of 5 killed at from five to twenty-eight days, 4 presented lesions in the kidneys and 2 in the liver.

6. Thirty-five rabbits were given intravenous injections of from 1 to 2 cc. per kilogram of body weight, usually in a single dose, of full strength or 50 per cent diglycol (redistilled in a few instances). Eleven animals died in from one and a half to twenty-one days. The kidneys of 10 and the livers of 8 of them had undergone varying degrees of hydropic degeneration. The remaining 24 were killed after from one to forty-four days, and similar changes were observed in the kidneys of 15, and in the livers of 9. One instance was noted in which, following the injection of 2 cc. per kilogram of body weight, twenty hours was sufficient for the development of extensive changes in the kidneys, with a rise in the nonprotein nitrogen of the blood to 86 mg. in 100 cc. (fig. 1 *C*).

7. Five guinea pigs received from 2 to 5 cc. per kilogram of body weight of diglycol by mouth during from two to twelve days. Two died at three and five days, respectively, with lesions in the kidneys similar to those in the rats and rabbits. One showed the liver similarly involved. The remaining 3 were killed at from two to twelve days. Hydropic degeneration had occurred in the kidneys of all and in the livers of 2.

Control animals on the same stock diet without the addition of diglycol remained normal.

Animals affected by diglycol become comatose and often anuric prior to death. For several hours following intravenous injection of diglycol

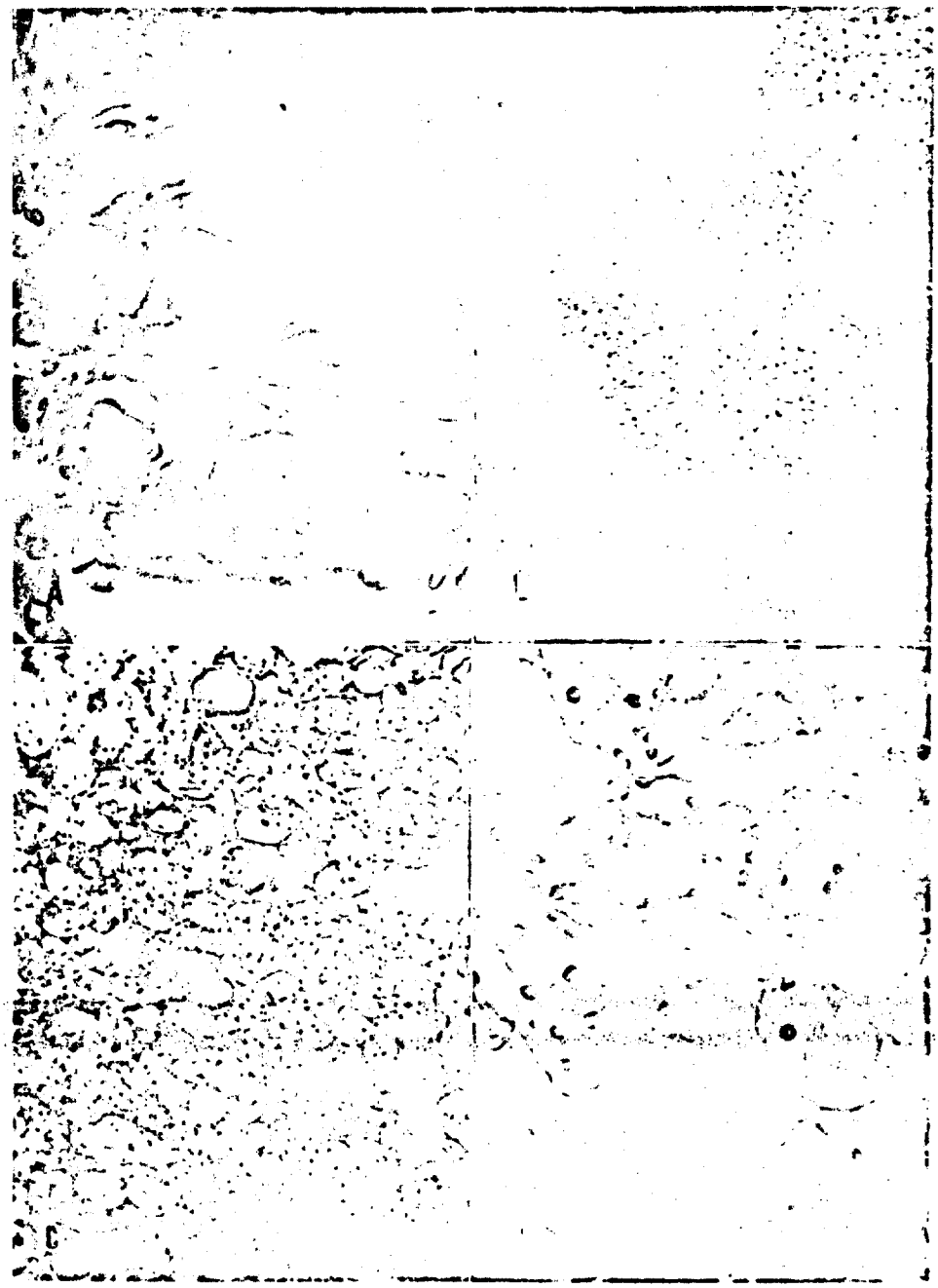

Figure 2

EXPLANATION OF FIGURE 2

A, calcification and regeneration of the convoluted tubules in the kidney of a rabbit that received 1 cc. per kilogram of diethylene glycol intravenously. The animal became sick and was killed on the ninth day. The blood nonprotein nitrogen amounted to 389 mg. in 100 cc.

B, hydropic degeneration of liver cells about efferent veins in a rabbit that received 2 cc. per kilogram of diethylene glycol intravenously. Death occurred in thirty-six hours.

C, hydropic degeneration of convoluted tubules of the kidney of a rat the drinking water of which contained 5 per cent methyl carbitol for twenty-eight days.

D, hydropic degeneration of convoluted tubules of the kidney of a rat which received butyl carbitol in a concentration of 5 per cent in the drinking water. It was killed on the eighth day. The blood nonprotein nitrogen was 162 mg. in 100 cc.

many of the rabbits pass smoky urine which reacts with benzidine. The nonprotein nitrogen of the blood in all the affected animals mounts steadily to from eight to ten times the normal amount (e. g., 389 mg. in 100 cc. in a rabbit that lived nine days after the injection of 1 cc. per kilogram of body weight).

The organs regularly affected in diglycol poisoning are the liver and kidney. The kidneys of the three species of animals used are swollen, pale yellowish tan and apparently more moist than normal. On analysis the water content is found to be increased approximately 6 per cent (to an average of 84 per cent from a normal average of 78 per cent). The cortical markings are indistinct. The medulla often appears to be congested. The outstanding histologic change is hydropic degeneration of the convoluted tubules. Every tubule may be and commonly is involved, or the change may be confined to isolated small groups of tubules. The epithelial cells swell and undergo coarse vacuolation, often with fusion of the vacuoles to a single large space that represents the entire cell save for an irregular cell membrane and a compressed pyknotic nucleus. The vacuoles are devoid of fat or glycogen, but occasionally a little finely divided fat is present in the remaining cytoplasm or in otherwise unchanged epithelial cells. Necrosis of the vacuolated cells occurs, and the tubular lumen becomes blocked by swollen cells and cell detritus. Obstruction to the outflow of urine ensues, leading to dilatation of the glomerular spaces. The glomerular tufts are often compressed and bloodless but otherwise are essentially uninvolved. Calcification of necrotic cytoplasm may set in as early as the fifth day in rabbits, and attempts at regeneration may be present, characterized by mitoses (fig. 2 *A*). Leukocytic reaction is not a feature. Changes in the medulla are confined to congestion of the capillaries and small veins and to the occurrence of many hyaline and granular casts in the collecting tubules. Occasionally a little finely divided fat is found in the epithelium of such tubules.

The liver, though less frequently involved, may be enlarged and pale, and appear moist. An increase of approximately 10 per cent in volatile matter is observed (to an average of 82 per cent from a normal average of 72 per cent). The cells about the efferent vein in each lobule, often extending well out toward the portal areas, enlarge, and the cytoplasm becomes pale. Many tiny vacuoles appear in it, which fuse together until the cell consists of a single large vacuole surrounding a compressed and pyknotic central nucleus (fig. 2 *B*). These vacuoles are also free from fat and glycogen, but in rare instances a little finely divided stainable fat is present in the other liver cells.

In a small percentage of the animals that have changes in the kidneys and liver, the epithelial cells of the outer fourth of the adrenal cortex

are swollen, pale staining and vacuolated in the same manner as the liver cells. The vacuoles do not contain stainable fat.

The lesions noted in the kidney and liver are essentially similar to those which have been described by other investigators [15] coincident with and since the appearance of our preliminary report.[7] The kidneys of animals, however, apparently do not contain the small arterial and capillary thromboses and massive cortical necrosis found in the kidneys of some of the human beings who were poisoned by large quantities of diglycol.[16]

ETHYL DIETHYLENE GLYCOL (CARBITOL)

Three ether derivatives of diethylene glycol were investigated briefly: ethyl diethylene glycol (diglycol monoethyl ether), or carbitol; methyl diethylene glycol (diglycol monomethyl ether), or methyl carbitol, and butyl diethylene glycol (diglycol mono-n-butyl ether), or butyl carbitol.

Carbitol is among the chemicals reported by von Oettingen and Jirouch [13] as producing acute nephrosis in rats. A communication from the director of a pharmaceutical laboratory to the Council on Pharmacy and Chemistry of the American Medical Association [17] stated that the equivalent of 1.67 cc. per kilogram of body weight of carbitol had been administered to rats daily for twenty days without apparent effect. One rat of the series which was put to death after sixteen days had normal kidneys and liver.

The drinking water of 6 rats was made up to 5 per cent concentration of carbitol, and the rats consumed varying amounts of it for from one to fifteen days. The intake was not over half the intake of drinking water in the preceding experiments, averaging approximately 15 cc. per day. Two rats died on the third and fifth days, respectively, and exhibited evidence of renal disease. Of the remaining 4, which were killed at the expiration of from one to fifteen days, 2, killed on the fifth day, had a nonprotein nitrogen concentration in the blood of 279 and 289 mg. in 100 cc., respectively, and exhibited lesions of the kidneys. Because of the difficulty encountered in obtaining an adequate intake of the material in the drinking water, an attempt was made to administer a solution to 2 rats by stomach tube, but this led to acute bronchitis and bronchopneumonia with death on the second and third days, respectively, without renal involvement.

A total of approximately 10 cc. of carbitol per kilogram of body weight had been consumed in the drinking water by the rat that died on the third day and that had lesions in the kidneys, and 15 cc. per kilogram by the one that died on the fifth day. However, the intake of fluid by the rats to which carbitol was offered was below normal, so that partial dehydration may have contributed to the result. Furthermore, many of the animals receiving carbitol,

15. Cannon.[8] Ruprecht and Nelson.[9] Hagebusch.[10] Lynch.[11] Cannon and Geiling.[12]

16. Cannon.[8] Ruprecht and Nelson.[9] Lynch.[11] Cannon and Geiling.[12]

17. "Carbitol," Queries and Minor Notes, J. A. M. A. **110**:1602, 1938.

methyl carbitol and butyl carbitol refused food for some time before death, and a certain degree of starvation was commonly present. Such animals not infrequently showed pathologic changes associated with inanition, such as excessive hemosiderin deposits in the spleen, small hemorrhages and erosions in the mucous membrane of the alimentary tract, and enlargement of the adrenals.[18]

The kidneys of rats receiving carbitol undergo changes identical in every respect with those seen in animals on diethylene glycol itself, i. e., extensive hydropic degeneration of the epithelium of the convoluted tubules leading to obstruction. The other viscera examined were essentially normal.

METHYL DIETHYLENE GLYCOL (METHYL CARBITOL)

No experiments on the toxicity of this compound had been carried out previously.

Nine rats were given methyl carbitol in from 3 to 5 per cent concentration in their drinking water for from eleven to sixty-four days. (Four of these had previously been on 1 per cent methyl carbitol for one hundred and ten days without apparent effect.) One animal died, at the end of sixty-four days. Except for moderate amounts of protein in the lumens of the convoluted tubules of the kidney, its organs were normal. Of the 8 that were killed at the expiration of from eleven to forty-five days, the kidneys of 3 (twenty-eight, twenty-eight and forty-five days, respectively) had been damaged.

Methyl carbitol is also not well taken in the drinking water by rats. Two attempts to administer the compound by stomach tube were followed by acute bronchitis and pneumonia.

Methyl carbitol, ingested by mouth, produces damage in the kidneys of rats analogous to that produced by diglycol (fig. 2 *C*). Somewhat larger amounts of the methyl derivative than of carbitol itself are apparently necessary to produce these effects.

BUTYL DIETHYLENE GLYCOL (BUTYL CARBITOL)

Browning[1] refers to a factory worker exposed to either butyl carbitol or butyl cellosolve or both, in whom hematuria with casts developed. No animal experiments on the effects of butyl carbitol had been carried out previously.

The compound was administered in from 3 to 5 per cent solution in the drinking water of 9 rats for from five to thirty-five days. (Two of these animals had previously been on 1 per cent butyl carbitol for one hundred and nine days without apparent effect.) Three rats died on the fifth, twenty-third and thirty-fifth days, respectively. The kidney of the rat that died on the fifth day contained an occasional small group of damaged convoluted tubules. The kidneys of 2 of the remaining rats, killed at the expiration of from eight to

18. Jackson, C. M.: Effects of Inanition and Malnutrition upon Growth and Structure, Philadelphia, P. Blakiston's Son & Co., 1925.

sixteen days, had undergone similar changes. Butyl carbitol in from 3 to 5 per cent concentration, is relatively unpalatable to rats, leading to partial dehydration and partial starvation.

The renal lesions caused by this compound are similar to those following the administration of diethylene glycol but are much less extensive with the dosage used (fig. 2 *D*).

DIETHYLENE DIOXIDE (DIOXANE)

Dioxane, the second ether of ethylene glycol, contains two ether linkages in its molecule. It is a widely used solvent, and its toxicity has been extensively studied. The literature has been summarized by Browning.[1] Dioxane is in the series of compounds causing acute nephrosis studied by von Oettingen and Jirouch.[13] Fairley, Linton and Ford-Moore[19] described peripheral necrosis of hepatic lobules and necrosis of tubular epithelium in the kidneys of guinea pigs, rats and rabbits receiving the substance, although Yant, Schrenk, Waite and Patty[20] in an earlier series of acute inhalation experiments found no lesions of these organs in guinea pigs. Following a report by Barber[21] of the death of 5 workers in the manufacture of artificial silk all of whom had central necrosis of the liver and symmetric necrosis of the renal cortex associated with vascular lesions, De Navasquez[22] reinvestigated the effects of dioxane when given intravenously and intragastrically to rabbits, cats and guinea pigs. He observed acute hydropic degeneration of the convoluted tubules in the kidneys of all the animals, and in rabbits, vacuolation of the liver cells about the efferent veins, the vacuoles being apparently filled with glycogen.

Dioxane is highly toxic to rats. Of 10 animals given the compound in 5 per cent concentration in their drinking water, 8 died in from five to twelve days. The kidneys of the 8 rats were the site of extensive degeneration, and the livers of 5 were also abnormal. The kidneys of the remaining 2 animals, killed on the sixth and eighth days, respectively, were similarly involved. One of these also showed involvement of the liver. Two rats on 1 per cent dioxane for one hundred and ten days, followed by 3 per cent for forty-one and forty-eight days, respectively, had patchy areas of degeneration in the kidneys. One also had hepatic damage.

Five per cent dioxane is relatively unpalatable to rats and was poorly taken. The estimated intake of dioxane was 1 cc. per kilogram of body weight per day, which sufficed to cause fatal renal damage in five days—an observation which emphasizes the relatively high toxicity of this compound when taken by mouth.

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19. Fairley, A.; Linton, E. C., and Ford-Moore, A. H.: J. Hyg. **34**:486, 1934.
20. Yant, W. P.; Schrenk, H. H.; Waite, C. P., and Patty, F. A.: Pub. Health Rep. **45**:2023, 1930.
21. Barber, H.: Guy's Hosp. Rep. **84**:267, 1934.
22. De Navasquez, S.: J. Hyg. **35**:540, 1936.

Two rabbits were given intravenous injections of 1.5 cc. of dioxane per kilogram of body weight in 25 per cent solution. One died two days later, and its kidneys exhibited several cortical areas of degeneration. The kidneys and liver of the second rabbit, killed on the fourth day, were markedly damaged, and the nonprotein nitrogen of the blood had risen to 242 mg. in 100 cc.

The pathologic observations in the kidneys of the animals are essentially those described by De Navasquez [22] and by Fairley, Linton and Ford-Moore.[19] They reproduce exactly the lesions due to diethylene glycol, namely, extensive hydropic degeneration of the epithelium of the convoluted tubules, every tubule being involved in most of the animals (fig. 3 *A*). High levels of blood nonprotein nitrogen ensue. Symmetric cortical necroses are not seen.

The livers of affected animals are also the site of the same lesion observed in the animals receiving diglycol, namely, swelling and hydropic degeneration of the liver cells about efferent veins (fig. 3 *B*). De Navasquez found the vacuoles in the liver cells to be filled with glycogen and was doubtful whether they could be considered pathologic. Although we were able to stain a little glycogen in some of the liver cells (by fixing thin slices in absolute alcohol immediately after death, embedding in pyroxylin, and treating with Best's carmine stain), this glycogen is finely divided or in a diffuse state and is in the cytoplasm present about the vacuoles. The vacuoles themselves remain empty looking and unstained. Fat is not present in them, nor is it present in the vacuolated cells in the kidney. A little finely divided fat is, however, occasionally seen in both the epithelial and the Kupffer cells of the liver.

DIPROPYLENE GLYCOL

The pathologic effects of dipropylene glycol, the next higher homologue of diethylene glycol, had not been studied previously.

The drinking water of 7 rats contained from 1 to 5 per cent of dipropylene glycol for from thirty-three to seventy-seven days, without detectable effect on the animals. All organs were normal. When, however, a concentration of 10 per cent was given over periods of from nine to sixty-eight days to a total of 25 rats, 7 died at the end of from ten to thirty days. The kidneys of 5 of the dead rats had become the site of lesions (as early as the tenth day in 1 rat). The remaining 18 rats were killed after from nine to sixty-eight days. The kidneys of only 4 of these had become involved (as early as the ninth day in 1).

Ten rabbits received from 2 to 4 cc. per kilogram of body weight of dipropylene glycol intravenously. Two of these rabbits died on the fourth day, and lesions had occurred in the kidneys of both. The remaining 8 were killed after from one to twenty-one days. The kidneys of 3 exhibited similar lesions (as early as the third day in a single instance). One of these also had involvement of the liver. A dose of 4 cc. per kilogram was necessary to cause renal changes and to raise the nonprotein nitrogen of the blood (to as high as 406 mg. in 100 cc. on the sixth day in 1 rat).

Lesions identical to those produced by diethylene glycol are present in animals receiving dipropylene glycol. Extensive hydropic degeneration of renal epithelium is seen, with, in some cases, an occasional hemoglobin-containing cast in the collecting tubules (fig. 3 *C*). Damage to liver parenchyma is more inconstant and more difficult to produce (fig. 3 *D*).

TABLE 2.—*Summary of Protocols*

Compound	Animals	Route of Administration	Amount	Duration, Days	Observations in Kidney (Nature of Lesion; Percentage of Animals Affected)	Observations in Liver (Hydropic Degeneration; Percentage of Animals Affected)
					Oxalate crystals	
Ethylene glycol; glycol...	30 rats	Mouth	1-5%	8-79	67	0
	11 rabbits	Vein	1-4 cc. per Kg.	1-120	36	0
Ethylene glycol diacetate	11 rats	Mouth	1-5%	7-130	73	0
					Hemoglobin casts	
Propylene glycol.....	30 rats	Mouth	1-10%	11-234	0	0
	15 rabbits	Vein	2-5 cc. per Kg.	1-46	27	0
					Hydropic degeneration	
Diethylene glycol; diglycol	47 rats	Mouth	0.5-1%	22-217	0	0
	72 rats	Mouth	3-5%	1-46	76	43
	6 rabbits	Mouth	1-5%	5-28	83	33
	35 rabbits	Vein	1-2 cc. per Kg.	1-44	71	49
	5 guinea pigs	Mouth	2-5 cc. per Kg.	2-12	100	60
Ethyl diethylene glycol (diglycol monoethyl ether); carbitol	6 rats	Mouth	5%	1-15	67	0
Methyl diethylene glycol (diglycol monomethyl ether); methyl carbitol	9 rats	Mouth	3-5%	11-64	33	0
Butyl diethylene glycol (diglycol mono-n-butyl ether); butyl carbitol	9 rats	Mouth	3-5%	3-35	33	0
Diethylene dioxide; dioxane	10 rats	Mouth	5%	3-12	100	80
	2 rabbits	Vein	1.5 cc. per Kg.	2-4	100	50
Dipropylene glycol.....	7 rats	Mouth	1-5%	35-77	0	0
	25 rats	Mouth	10%	9-68	36	0
	10 rabbits	Vein	2-4 cc. per Kg.	1-21	50	10

COMMENT

The several experiments are summarized in table 2. From them there emerge the following observations: (*a*) ethylene glycol and ethylene glycol diacetate may cause deposition of oxalates in the kidneys and mild renal insufficiency; (*b*) propylene glycol is relatively harmless, especially when given by mouth, large intravenous doses being necessary to cause hemoglobinuria and casts; (*c*) diethylene glycol, the methyl, ethyl and butyl ethers of diethylene glycol, dioxane and dipropylene glycol are all capable of causing profound degeneration of renal epithelium leading to uremia.

The members of this last group of compounds have at least one structural factor in common. The molecules of diglycol and its ethers, of dipropylene glycol and of dioxane contain an ether linkage between two glycol molecules. The cyclic compound dioxane possesses two such linkages, which may in part account for its relatively high toxicity. The molecules of ethylene glycol, ethylene glycol diacetate and propylene glycol lack this particular type of ether linkage. At the same time their respective effects on parenchymatous epithelium differ from those of the preceding compounds. It is suggested that the ether linkage between two glycol molecules may be responsible for the hydropic degeneration of the epithelium of the convoluted tubules and of the liver cords.

An outstanding feature of the effect of the compounds causing hydropic degeneration of the kidney is the extensive character of the damage wrought. In each experiment one or more of the animals was apparently unaffected by the drug, but in the injured animals the lesions were usually maximal, involving as a rule the entire cytoplasm of every cell in every convoluted tubule. Occasionally only groups of tubules were the site of degeneration, but almost invariably every cell of the segment of tubule involved was completely hydropic. Multiple small vacuoles in a given cell were uncommon. It would seem that the colloidal structure of the cytoplasm of an injured cell relatively suddenly loses its integrity and approximates a true solution.

The animals that received diglycol and its ethers, dioxane and dipropylene glycol died usually as a result of the damage to the convoluted tubules, in uremia. The carbitols and dipropylene glycol are not palatable to rats in moderate concentrations, so that some of these animals consumed less water than their minimal requirements in view of the dry nature of their diet. Partial refusal of food sometimes followed, and dehydration and starvation were probably factors in the death of certain of these animals. The cause of death of the animals that received glycol and glycol diacetate was not always obvious. In not all instances were the deposits of oxalates in the kidney sufficiently extensive to cause demonstrable obstruction of tubules and a significant rise in the nonprotein nitrogen of the blood. As suggested by Hunt [6a] a question arises as to the possible formation of toxic intermediary products.

The relative toxicity of these compounds is roughly indicated in the protocols and will be taken up in detail in a separate report. It may be emphasized, however, that appreciable amounts of the several chemicals must be given to produce demonstrable changes in the animals used. In no instance was less than 1 cc. per kilogram of body weight effective.

CONCLUSIONS

Dipropylene glycol, diethylene glycol, dioxane, carbitol, methyl carbitol and butyl carbitol when administered in adequate dosage orally or intravenously to animals cause extensive hydropic degeneration of the renal convoluted tubules, leading to uremia; less regularly, there is hydropic degeneration of the liver parenchyma. These compounds resemble each other in containing an ether linkage between glycol molecules. Ethylene glycol, ethylene glycol diacetate, and propylene glycol do not have such a structure, nor do they cause similar lesions in animals.

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COMPARISON OF THE TOXIC, HYPNOTIC AND IRRITATING PROPERTIES OF EIGHT ORGANIC SOLVENTS

ALBERT R. LATVEN AND HANS MOLITOR

From the Merck Institute of Therapeutic Research, Rahway, New Jersey

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Organic solvents are often used in the pharmacological study of other compounds not soluble in water and in consequence their own pharmacological properties must be taken into account. Despite many investigations of these[1] an accurate comparative evaluation can scarcely be made because of differences in experimental techniques and in strains of animals used. For this reason we have collected data, particularly with reference to minimum symptomatic doses, under strictly comparable conditions upon animals of the same strain, fed with a well balanced stock laboratory diet, and kept at a constant temperature of 72°F. and relative humidity of 55 per cent.

Eight solvents (see table 1) were investigated: All chemicals were pure and whenever necessary redistilled before use.

ACUTE TOXICITY

The intravenous, subcutaneous and oral toxicity was determined in white mice. Each compound was tested at six dose levels with at least four mice on each dose. A total of 950 mice were used. Results were computed according to Behrens' method (1). The mice were weighed immediately before the test within a limit of accuracy of ±0.5 gram; the doses were calculated in cubic centimeter per kilogram.

All compounds were given undiluted unless otherwise stated.

[1] The most recent and complete survey is found in the publication of the British Medical Research Council, "Toxicity of Industrial Organic Solvents," London (1937).

The animals were observed at 15-minute intervals during the first hour and at half-hour intervals during the following three hours. The acute toxicity figures were calculated from the

TABLE 1

COMPOUND	FORMULA	BOILING POINT	DENSITY
		°F.	
Alcohol, 70 per cent (vol.)	C_2H_5OH		0.890
Alcohol, 95 per cent (vol.)	C_2H_5OH	76.0–79.5	0.816
Carbitol	$CH_3CH_2OCH_2CH_2OCH_2CH_2OH$	201.9	1.025
Diacetin	$CH_3COOCH_2CH(OH)CH_2OOC\cdot CH_3$	175.6*	1.178
Ethylene glycol	$HOCH_2CH_2OH$	197.2	1.117
Ethyl lactate	$CH_3CHOHCOOCH_2CH_3$	155	1.031
Glycerol	$HOCH_2\cdot CHOH\cdot CH_2OH$	158†	1.259
Propylene glycol	$CH_3CHOHCH_2OH$	187.4	1.038
Triethylene glycol	$HOCH_2CH_2OCH_2CH_2OCH_2CH_2OH$	288.0	1.113

* At 40 mm.
† At 165 mm.

TABLE 2

	SUBCUTANEOUS					PERORAL					INTRAVENOUS				
	Toxicity			Min. sympt. dose	Max. non-sympt. dose	Toxicity			Min. sympt. dose	Max. non-sympt. dose	Toxicity			Min. sympt. dose	Max. non-sympt. dose
	LD10	LD50	LD100			LD10	LD50	LD100			LD10	LD50	LD100		
Alcohol 70 per cent	11.0	14.5	18.0	4.0	2.0	13.0	14.5	17.0	4.0	3.0	3.0	4.0	5.0	1.0	0.8
Alcohol 95 per cent	9.0	10.5	12.0	2.0	1.0	9.0	12.0	14.0	3.0	2.0	2.0	2.5	3.0	0.8	0.6
Carbitol	4.0	5.5	7.0	2.0	1.0	11.0	12.5	14.0	3.0	2.0	3.0	4.3	6.0	1.2	1.0
Diacetin	3.0	3.5	4.0	2.0	1.0	2.0	8.5	15.0	1.0	0.5	1.0	2.3	3.0	2.0	1.0
Ethylene glycol	3.0	4.5	6.0	1.0	0.8	6.0	7.5	10.0	1.0	0.8	2.0	3.0	4.0	2.0*	1.0*
														1.0†	0.8†
Ethyl lactate	2.0	2.5	3.0	1.0	0.8	1.0	2.5	4.0	0.4	0.2	0.2	0.6	1.0	0.3	0.2
Glycerol	8.0	10.0	12.0	8.0	7.0	20.0	25.0	32.0	10.0	8.0	5.0	6.0	7.0	3.0	2.0
Propylene glycol	14.0	18.5	24.0	10.0	8.0	18.0	22.0	26.0	8.0	6.0	5.0	8.0	11.0	2.0	1.0
Triethylene glycol	8.0	8.75	10.0	9.0	8.0	14.0	18.5	24.0	8.0	6.0	5.0	6.5	8.0	3.0	2.0

* Immediate.
† Late.

number of deaths during the first 24 hours. Animals which survived this period were observed for another week; deaths during this period were designated as late deaths.

Intravenous injections were made into the tail vein, subcutaneous injections under the skin of the abdomen. Oral administration was accomplished through a 16-gauge hypodermic needle, the sharp end of which had been cut off and blunted with a drop of cement. The results of the toxicity tests are summarized in table 2.

It can be seen that propylene glycol is the least toxic solvent, with the exception of glycerol of which a slightly larger dose is tolerated orally but not parenterally; the most toxic solvent is ethyl lactate.

HYPNOTIC EFFECT

In the following experiments a dose producing hypnotic symptoms in one out of four mice was regarded as minimum symptomatic. The mice were examined by placing them upon a ledge 1 cm. wide and making them walk along it; a slight dizziness was thus readily observable.

The solvents were given intravenously, subcutaneously and orally. All compounds upon intravenous injection of sufficiently large doses produced immediate symptoms, from which permanent recovery usually occurred. After ethylene glycol, however, a recurrence of symptoms was frequently noted after an apparently normal interval of from 30 minutes to 2 hours. The animals were observed in all experiments at 15 minute intervals for the first hour and every half-hour thereafter for four hours or until complete recovery had taken place. The results of these experiments are summarized in table 3.

IRRITANT PROPERTIES

The irritant properties of the organic solvents were tested by instillation into rabbits' eyes; intradermal injection into guinea pigs' abdominal skin; prolonged application to intact skin; and on the leg of reflex frogs.

(a) *Instillation in rabbit's eye*

About 0.5 cc. of each compound was instilled into one eye and kept there for one minute; the other eye served as a control.

Five tests on different rabbits were conducted with each compound. The various degrees of irritation (viz. hyperemia, edema, opacity of cornea) were observed during the first four hours and at the end of 24 hours; the regression of symptoms was observed during another week.

(b) *Intradermal injection*

One-tenth cubic centimeter of the solvent was injected intradermally into the shaved abdominal skin of guinea pigs and

TABLE 3

Irritant properties of organic solvents

COMPOUND	METHOD OF TESTING			
	Intradermal injection in guinea pigs	Frog leg reflex	Rabbit's eye	
			Edema	Hyperemia
		seconds		
Control (N/10 HCl)		0.8		
Alcohol, 70 per cent	++	0.6	+++	+++
Alcohol, 95 per cent	+++	1.1	+++	+++
Carbitol	++	0.8	++	+
Diacetin	+++	1.3	+	+
Ethylene glycol	++	2.2	+	+
Ethyl lactate	+++	0.5	+++	+++
Glycerol	+	3.6	+	+
Propylene glycol	++	2.7	++	++
Triethylene glycol	++	2.7	+	+

The results given in the above table are the average of 45 experiments in guinea pigs, 135 experiments in frogs and 90 experiments in rabbits. The signs: +, ++, +++ indicate the relative intensity of reaction.

the varying degrees and duration of irritation (viz. redness, ulceration) recorded. Observations were made during the first four hours, at the end of 24 hours, and for the subsequent week.

(c) *Reflex frog*

Legs of reflex frogs were immersed in the various solvents and the time between the first contact and the reflex retraction noted. Prior to each experiment the sensitivity of the preparation was tested by immersion in N/10 HCl. The average reaction time

of 5 immersions was in all experiments 0.8 second; the reaction time in the individual test varied between 0.6 and 1.2 second. Because of the rapid decrease of cutaneous sensitivity resulting from a prolonged contact with organic solvents only one test was performed on the same frog; fifteen frogs were therefore used for the testing of each compound and the results averaged.

(*d*) *Intact skin*

Gauze pads, 2 sq. cm. in area, were wetted with the solvent, placed on the shaved abdominal skin of rabbits, completely covered with adhesive tape and left in place for 24 hours. The results were denoted at the end of 24 hours.

Table 3 gives a summary of the various irritation tests. Although the irritating properties vary to a certain extent with the method of testing, it can be seen that in general propylene glycol and glycerol are the least and alcohol and ethyl lactate the most irritant. None of the compound caused irritation of the intact skin. The irritation resulting from other methods of testing disappeared as a rule within 24 hours, with the exception of ethyl lactate and alcohol, which caused permanent injury after instillation in the eye.

OTHER EFFECTS

All compounds depress the respiration and death is apparently due to respiratory failure, since the heart continues to beat for some time after respiration has ceased.

Some of the solvents, primarily glycerol and diacetin produce clonic convulsions.

DISCUSSION

An evaluation of the toxic and irritant properties of a number of frequently used organic solvents: propylene glycol, ethylene glycol, triethylene glycol, ethyl lactate, diacetin, carbitol, glycerol, alcohol 95 per cent and alcohol 70 per cent: was made under conditions permitting a quantitative comparison. Propylene glycol was found to be the least toxic and irritant followed by ethylene and triethylene glycol, glycerol, carbitol, and alcohol

70 per cent; the latter caused more local irritation than any of the previous compounds. Diacetin and ethyl lactate were by far the most toxic compounds and showed likewise pronounced irritant properties.

Our results indicate that of the series of organic solvents investigated propylene glycol deserves preference because of its relative freedom of toxic and irritating properties.

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THE TOXICOLOGY OF SOME GLYCOLS AND DERIVATIVES*

EDWIN P. LAUG, HERBERT O. CALVERY, HERMAN J. MORRIS AND GEOFFREY WOODARD

Division of Pharmacology, Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.

THE early studies on the pharmacology and physiology of the glycols are confined to brief studies by a very few investigators (1–7). Ethylene glycol is the only one given consideration until 1930, with the exception of the observation by Miura (6) in 1911 that propylene glycol gave rise to propylene glycol-glycuronate in the urine of dogs. Although some interest was manifest in toxicity, authors up to 1917 were interested primarily in the end products of metabolism of ethylene glycol. Pohl (1), Mayer (3, 4), Dakin (5), Miura (6) and Bachem (7) all found evidence that oxalic acid is an end product, and some of them, noting that there was kidney injury, attributed the toxicity to the formation of oxalic acid.

Since the dosage which caused definitely adverse physiological effects seemed, as a result of the previously mentioned investigations, relatively high as compared to well-known toxic substances, and since the shortage of glycerol during the war somewhat adversely affected the pharmaceutical trade, interest was aroused in the use of the glycols as a solvent and vehicle in medicine and pharmacy (7, 7a). The investigations of Page (8), of Page and Coryllos (9) and of Haag and Bond (10) in 1926 and 1927 were undoubtedly stimulated by similar considerations.

The flavoring extract and beverage manufacturers likewise became interested, primarily for economic reasons. Glycerol and alcohol were more expensive than the glycols. Toxicological investigations are mentioned below chronologically. Page (8) in 1927 reported studies of the toxicity of ethylene glycol for rats, rabbits and dogs when administered intraperitoneally and intravenously. In 1930 Hanzlik, Seidenfeld and Johnson (11) reported some comparative studies of ethylene glycol, glycerol and propylene glycol, with most of their interest confined to ethylene glycol. Local irritant action was tested on mucous surfaces (tongue and esophagus of man; conjuctiva of rabbits), on sciatic nerve of frog and in gluteal muscle of man, with negative results. There was no evidence of local irritation in rabbits, rats and guinea pigs. Acute toxicity studies were made on rabbits and rats by intramuscular and intravenous injection, and chronic studies were made on rats by repeated intramuscular injection and by inclusion in the drinking water. Also in 1930 Yant, Schrenk, Waite and Patty (12) published the results of their investi-

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gations of the effects of the vapors of dioxan on guinea pigs and human beings. Fatal exposure in guinea pigs resulted in the gross pathological findings of congestion and edema of the lungs and hyperemia of the brain. Persons exposed to 0.16 and 0.55% in the air noted irritation of the eyes and nose with lacrimation. These symptoms were marked at the higher level with the addition of a burning sensation of the throat.

von Oettingen and Jirouch (13) in 1931 reported a few observations on the toxicities of ethylene glycol, ethylene glycol mono-ethyl-ether, diethylene glycol mono-ethyl-ether, diethylene glycol, cellosolve acetate, dioxan and butyl-ethylene glycol. The acute toxicity studies were made on small numbers of mice by subcutaneous injections. These authors also made some observations on various tissues of the frog as well as antiseptic action on yeast and milk.

In 1932 Hunt (14) in two notes summarized his results and opinions on the toxicity of ethylene glycol and propylene glycol. Cognizant of the disastrous results which followed the use of methanol "which had been pronounced harmless on the basis of certain experiments on animals and also on man," he "was interested in whether somewhat similar results might occur if ethylene glycol were used in a similar way." Hunt very clearly and precisely points to the hazard which may result from the direct transfer of results from experimental animals to man. Even an occasional self-experiment is extremely dangerous of interpretation.

Seidenfeld and Hanzlik (15) also in 1932 published the first detailed investigation of propylene glycol and in 1933 Hanzlik, Mehrtens and Spaulding (16) published further information on ethylene glycol, propylene glycol and diethylene glycol. In 1934 and 1935 fifteen papers were published, several of which were stimulated primarily by the interest of cigarette manufacturers in hygroscopic agents for the retention of moisture in cigarettes (17–22). It was at this time that diethylene glycol came up for consideration. Several investigators championed its harmlessness and acclaimed its superiority (17, 18, 19), while others opposed this viewpoint (22). Fairley, Linton and Ford-Moore (23) reported that the outstanding effects in rabbits, rats, mice and guinea pigs following the administration of dioxan by inhalation, intravenous injection, feeding and application to the skin were severe lesions of the liver and kidneys. Barber (24) reported several cases of hemorrhagic nephritis and necrosis of the liver from dioxan poisoning in man. Flury and Wirth (25) studied the toxicity of some lacquer solvents. Some very interesting and unique observations were made by Lepkovsky, Ouer and Evans (26) who fed fatty acid esters of ethylene glycol, propylene glycol and diethylene glycol. The markedly injurious effects produced by the compounds of ethylene glycol and diethylene glycol on the tubular epithelium of the kidneys were in striking contrast to the normal kidneys of animals given like quantities of fatty acids as esters of propylene glycol, glycerol, ethyl alcohol, methyl alcohol and free fatty acids. This splendid investigation has been almost completely overlooked by those interested in the toxicology of

the glycols. Salter, Robb and Scharles (27) confirmed the findings of Hunt (14) that propylene glycol is converted to glycogen. de Navasquez (28) showed that after the intravenous injection of dioxan in rabbits, cats and guinea pigs, it has a selective action on the convoluted tubules which undergo acute hydropic degeneration causing intrarenal obstruction and anuria with subsequent death from uremia. Several other papers appearing during this period contributed much to our knowledge of the pharmacology of the glycols and their derivatives (29–36).

In 1936 Braun and Cartland (37) confirmed the findings of Seidenfeld and Hanzlik (15) on propylene glycol. The animals used were rats and rabbits; the methods of administration were oral, subcutaneous, intravenous and intramuscular. Fairley, Linton and Ford-Moore (38) expressed the opinion that the injury produced by dioxan is the result of tissue injury produced by diglycollic and oxalic acids. Wiley, Hueper and von Oettingen (39) studied the toxic effects of the vapors of ethylene glycol on rats and mice. Concentrations of 300 mg. per 1000 liters of air had no toxic effects. Schrenk and Yant (40) came to the conclusion that "The fatal cases of poisoning which have occurred should serve as a warning to users of all organic solvents as well as dioxan that good hygienic conditions must be maintained in order to use such compounds safely."

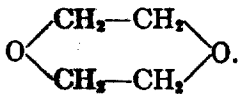
In 1937 Haag (41) and Wallace, Reinhard and Osborne (41a) reported studies of irritating and toxic properties of cigarette smoke containing the combustion products of diethylene glycol. The first investigations directed specifically to the toxicity of administered diethylene glycol appeared in this same year when Haag and Ambrose (42) published their results on the toxicity and fate of diethylene glycol. Acute toxicity studies were made on rats and rabbits by oral, intravenous, intramuscular and subcutaneous administration. Chronic effects were observed in groups of rats receiving drinking water containing 0.03, 0.1, 0.3, 1, 3, and 10% respectively of diethylene glycol. Pathological reports of the organs of animals receiving 1% and less were normal except for the spleen which indicated abnormal splenic deposits of pigment. Most of the glycol was recovered from the urine and very small amounts could be accounted for as oxalic acid.

These investigations, as well as those of Lepkovsky, Ouer and Evans (26), Hanzlik and coworkers (11, 15, 16), and Newman and Lehman (43, 44), preceded those investigations stimulated by the "Elixir of Sulfanilamide" tragedy. These latter include the reports of Geiling, Coon and Schoeffel (45) on rats, rabbits and dogs; Cannon (46), pathologic effects following the ingestion of diethylene glycol; Hagebusch (47), necropsy reports; Ruprecht and Nelson (48), clinical and pathological observations; Lynch (49), report of some cases; Poe and Witt (50); Geiling and Cannon (51), a clinical and experimental correlation of pathological effects of diethylene glycol poisoning; and Calvery and Klumpp (52), the toxicity for human beings of diethylene glycol with sulfanilamide. During this same period several other interesting and

comprehensive papers have appeared (53–63), especially with reference to propylene glycol (54, 56, 59, 60). For additional discussion of the compounds considered in this paper, the reader is referred to the chapter on the glycol group, appearing in the recent report of the British Medical Research Council (69).

In the above-mentioned publications there is considerable information concerning the toxicity of some of these compounds for human beings. Ethylene glycol has been reported to have caused seven deaths, and the amount required is estimated at around 100 cc. (14). Two other almost fatal cases were reported (64). Dioxan has proved extremely poisonous for man but the dosage level could not be estimated principally because the effect was due to the vapors (24). The same is true for ethylene glycol monomethyl ether (62). The fatal dose range for diethylene glycol was, unfortunately, recently quite well established (52). The amount of diethylene glycol in 75% solution consumed by 353 persons is known. Of these, 105 succumbed to its toxic effects. Of those who succumbed, 34 were children of 16 years of age or younger. The mean dose was 52.7 cc. with a minimum of 5 cc. and a maximum of 120 cc. For the adults, the mean dose was 98.6 cc. with a minimum of 20 cc. and a maximum of 240 cc. Of the survivors, 48 were children and the mean dose was 44.2 cc., while the minimum was 3 cc. and the maximum was 105 cc. The mean dose for the adults was 83.7 cc., while the minimum was 1 cc. and the maximum was 240 cc. For the fatal cases, the average dose was about 1 cc. per kg., which illustrates very strongly the point that one cannot neglect emphasizing the hazard and the injustice one may do by translating animal experimentation directly in terms of man. It is even dangerous to make such calculations for fear of their being misinterpreted. Propylene glycol seems to be the least toxic for man of all those related products for which we have information, and its fatal dose is not known.

In this investigation we have determined the dosage-mortality curves for rats, mice, guinea pigs and a small number of rabbits. We have also considered certain factors which might modify the toxic response of the animal. In total, 2600 animals were used to establish the precision of the results. The substances studied were: propylene glycol (CH_3—CHOH—CH_2OH); diethylene glycol (CH_2OH—CH_2—O—CH_2—CH_2OH); "carbitol" (C_2H_5—O—CH_2—O—CH_2—CH_2OH), the mono-ethyl-ether derivative of diethylene glycol; ethylene glycol (CH_2OH—CH_2OH); "cellosolve" (C_2H_5—O—CH_2—CH_2OH), the mono-ethyl-ether derivative of ethylene glycol; and dioxan



Experiments

The substances were all administered by stomach tube in single doses. The animals were housed in individual cages and fasted for about 18 hours (overnight) before the experiment. In most cases, 10 animals were used to test the toxicity at a particular dosage. The end point, of course, was death,

but was not strictly defined in time as is frequently done in other assays of this type. In general, death supervened in from 24 hours to 5 days, with greatest average mortality occurring at about 36 hours. All animals that died, particularly after 5 days, and that presented, by comparison with the mass of data, atypical responses, were carefully examined at autopsy for evidences of mechanical injury. All such animals which revealed such conditions on examination were, of course, excluded. Sexes were approximately equally distributed, and no attempt was made to differentiate this factor. There was some reason to believe that the dilution of the substances might be a factor modifying their toxicity. A large number of toxicities were, therefore, determined on a comparative basis with concentrated and 1 + 1 and 1 + 3 diluted materials. Postmortem evidence indicated that ethylene glycol monoethyl-ether especially was irritating to the gastro-intestinal tract; however, the dosage-mortality curves proved that only in the case of mice with ethylene glycol mono-ethyl-ether was there a significant increase in toxicity, when the concentrated material was compared with the 1 + 1 dilution.

The animals were selected within the following weight ranges: mice 20 ± 8 gm., rats 250 ± 75 gm., guinea pigs 300 ± 100 gm. The importance of maintaining a uniform weight was brought out in a series of studies in which considerably heavier animals were compared with the above standard groups.

The animals came from several different sources and consequently could be expected to give a heterogeneous response to the toxic substances. The experiments were conducted over a 10-month period, which fact could also be regarded as adding heterogeneity. However, none of the mortality curves made in this study were completed within a limited period, but are the composite result of mortality studies obtained throughout the extent of the experimental period. We therefore feel confident that any seasonal or strain modifications have been sufficiently varied by our method of sampling to prevent errors from these sources from affecting the results.

It is generally agreed that the estimation of LD 50, 10, or 90,* particularly the latter two, is not very satisfactory when it is required to interpolate a sigmoid curve through a number of points, some of which may be considerably in error.

The method which we have used for estimating the LD 50, as well as the regression coefficients, is not yet in general use. For complete details, the reader is referred to several papers by Bliss (65, 66). Since the method presents excellent possibilities for the evaluation of toxicity studies, however, it is believed that an example of this method as applied to our data will be valuable.

Briefly, the method is as follows: A sigmoid dosage-mortality curve is "rectified" or converted to a straight line by plotting the dose expressed in logarithms against the observed percentage mortalities expressed in probits (67) or probability units. The best-fitting provisional straight line is drawn through the transformed points and then, by the method of least

* Lethal dose killing 50, 10 or 90% of the animals.

squares, the parameters of an equation for the most probable line through these points are calculated. These parameters are: the slope, b, or regression coefficient, and the abscissa value, a, corresponding to the ordinate point "5", which is the log-dose of 50% kill. Calculations of the standard errors within the fiducial limits for odds of 19 in 20 trials give a measure of the accuracy of the determinations.

Definitions of terms used:

(1) Mortality ratio:

$$\frac{\text{No. animals killed}}{\text{No. animals used}}$$

(2) Log-dose, x: The logarithm of the dose expressed as cc./kgm.

(3) Empirical probit: The converted value of per cent mortality, which is obtained directly from a table (65).

(4) Expected probit, Y: The actual points of intersection in probits of the provisional line with ordinate Y at log-dose x. These can either be estimated by eye or calculated from the equation of the provisional line.

(5) Corrected probit, y: When the empirical probit differs from the expected value by more than 0.05, especially outside the range of 4 to 6 probits, the corrected probit is obtained from the following equation:

$$y = \left(Y + \frac{Q}{Z}\right) - q\left(\frac{1}{Z}\right)$$

where Q is the area in the "tail" of the normal probability curve, beyond the point delimited by Y; Z is the ordinate of the normal curve at the point Y; q is the observed proportion of survivors. Values for y can be read directly from tables calculated for observed values of Y and q.

(6) Weighting Coefficient w: The corrected probits, however, are often of very unequal significance for estimating the dosage-mortality curve. For this reason, each is assigned a weight, w, equal to the information which it contains. Values for w are obtained directly from tables (65) expressed in terms of the expected probit Y. Each w must be multiplied by N, the number of animals used for the particular point on the curve.

(7) Weighted mean log-dose $\bar{x}$:
Weighted mean probit $\bar{y}$:
The dosage mortality curve is computed by a modified method of least squares from the three series of numbers: (1) the logarithms of the dose x, (2) the corrected probits y, and (3) the weights w. From these operations

$$\bar{x} = \frac{S(wx)}{S(w)} \quad \text{and} \quad \bar{y} = \frac{S(wy)}{S(w)}$$

where "S( )" signifies the sum of all quantities such as are enclosed in parentheses.

(8) Weighted squares, $[wx^2]$:
Weighted products $[wxy]$:
The weighted squares and products of the deviations from the two means $\bar{x}$ and $\bar{y}$ are

$$[wx^2] = S(wx^2) - \bar{x}\, S(wx)$$
$$[wxy] = S(wxy) - \bar{y}\, S(wx)$$

where the square brackets indicate the sum of deviations from the mean.

(9) Slope, b: This is also known as the regression coefficient of the dosage-mortality curve. It is calculated as

$$b = \frac{[wxy]}{[wx^2]}, \text{ or its reciprocal } \frac{1}{b} = s$$

(10) The corrected equation, $y = \bar{y} + b(X - \bar{x})$: can be used for calculating probit kill in terms of log-dose X, or by rearrangement,

$$X = \bar{x} + s(Y - \bar{y})$$

where log-dose X is calculated in terms of probit kill. The latter equation has been used for calculating the log-dose X at 50% mortality.

(11) The standard error, s.e.: This has been calculated for the LD 50 and the regression coefficient b. By multiplying the standard error by 1.96

(assuming independence of the degrees of freedom) the fiducial limits of error for odds of 19 in 20 have been obtained.

For b, the regression coefficient

$$\text{s.e.} = \pm\sqrt{\frac{1}{[wx^2]}}$$

For log LD 50

$$\log \text{s.e.} = \pm\sqrt{s^2\left(s\frac{(5-\bar{y})^2}{[wxy]} + \frac{1}{S(w)}\right)}$$

A numerical example has been given of the calculations made for determining the equation of the curve of toxicity of ethylene glycol for rats (table 1).

In figure 1, the empirical probits were laid out as hollow circles, and the best provisional line (represented by the broken line) was drawn through them. Expected probits were then read off from this line and recorded in the tabulation. The corrected probits, obtained from tables in terms of the expected probit, are represented by the solid circles, and show how close the agreement has been. The corrected line is shown as the heavy solid line. Since the corrected line depends for its values upon the estimated values of the provisional line, it becomes obvious that unless the two lines coincide closely (the example cited here is about the outside limit), a second approximation must be made, in which the points of the first corrected line are used for calculating a new corrected line.

All of the experimental data obtained are presented in table 2. In addition, the results obtained on a different strain of rats, all raised under identical conditions and randomized with respect to weight and sex, are appended to the bottom of the table. The animals in this latter series received similar dosages of the substances at 1 + 3 dilutions, where possible, instead of 1 + 1, or concentrated. Calculations of the LD 50, based on these results, have shown in every case close agreement with the LD 50 obtained from the larger, but more heterogeneous lot of animals. This is additional evidence that, except for the case of ethylene glycol monoethyl-ether in mice, dilution does not significantly modify the dosage-mortality curve.

In order to study the dilution effect somewhat more in detail, two dosage-mortality curves were calculated for ethylene glycol: one in which it was given in concentrated form, and one in 1 + 3 dilution. Five doses for 11 animals each, spaced at equal logarithmic intervals, were used to define each curve. The rats were all from the same strain; their ages were 3 months ± 9 days, and their weights were 275 ± 25 gm. By means of the transformations already described in detail, the data presented in table 3 have been used to calculate the equations for the "rectified" dosage-mortality curves of concentrated and dilute ethylene glycol. Calculation of the standard errors of the regression coefficients and the log LD 50's, for limits of 19 in 20 determinations, shows very clearly that no significant differences between the concentrated and diluted material exist, at least for the number of animals involved in the test. Comparison also shows that the LD 50's, as well as the regression coefficients obtained here check, within the fiducial limits set, the values for

TABLE 1

CALCULATION OF THE DOSAGE-MORTALITY CURVE OF ETHYLENE GLYCOL IN RATS

DOSE	MORTALITY RATIO	LOG-DOSE x	EMPIRICAL PROBIT	EXPECTED PROBIT Y	CORRECTED PROBIT y	WEIGHT w	WEIGHT TIMES LOG-DOSE wx
cc./kg.							
3.5	0/7	0.54407	—	3.77	3.185	2.5197	1.37089
4.0	4/20	0.60206	4.1584	4.16	4.159	9.8074	5.90464
4.5	3/10	0.65321	4.4756	4.48	4.476	5.7664	3.76667
5.0	11/20	0.69897	5.1257	4.80	5.075	12.3484	8.63116
5.5	15/20	0.74036	5.6745	5.04	5.628	12.7250	9.42108
6.0	4/10	0.77815	4.7467	5.30	4.729	6.1609	4.79410
6.25	7/10	0.79588	5.5244	5.40	5.521	6.0052	4.77942
6.5	2/10	0.81291	4.1584	5.50	4.104	5.8099	4.72293
7.0	13/20	0.84510	5.3853	5.72	5.347	10.5196	8.89011
7.5	17/20	0.87506	6.0364	5.90	6.023	9.4288	8.25077

$S(w) = 81.0913$ $\qquad \bar{x} = \frac{S(wx)}{S(w)} = 0.74646$

$S(wx) = 60.53177$

$S(wy) = 408.08046$ $\qquad \bar{y} = \frac{S(wy)}{S(w)} = 5.03235$

$S(wx^2) = 45.87579$ $\qquad [wx^2] = S(wx^2) - \bar{x}S(wx) = 0.69124$

$S(wxy) = 308.27946$ $\qquad [wxy] = S(wxy) - \bar{y}S(wx) = 3.66241$

$$b = \frac{[wxy]}{[wx^2]} = 5.29831 \qquad s = \frac{[wx^2]}{[wxy]} = 0.18873$$

Corrected equation $X = \bar{x} + s(Y - \bar{y})$

when $Y = 5.0$, $X = 0.74036$ (log LD 50)

Standard error of b, the regression coefficient:

$$\text{s.e.} = \frac{1}{[wx^2]} = \pm 1.203$$

within fiducial limits corresponding to odds of 19 in 20

$1.96 \times \text{s.e.} = \pm 2.36$

Standard error of X, the log LD 50

$$\text{s.e.} = \sqrt{s^2\left(s\frac{(5-\bar{y})^2}{[wxy]} + \frac{1}{S(w)}\right)} = 0.02094$$

within fiducial limits corresponding to odds of 19 in 20

$1.96 \times \text{s.e.} = \pm 0.04106$

whence in antilogarithms

LD 50 = 5.50 cc./kg. within limits of 5.00 and 6.05 cc./kg.

ethylene glycol given in table 4 for a larger, but more heterogeneous group of rats.

In table 4 are given the constants for the equations of the "rectified" dosage-mortality curves obtained by the series of transformations from the original data of table 1. The fiducial limits for odds of 19 in 20 have been calculated for the regression coefficients as well as the LD 50's expressed as the logarithm and as the actual dose. Referring to the action of ethylene glycol mono-ethyl ether on mice, it can be seen that the LD 50's are significantly different for the concentrated and the 1 + 1 diluted substance. The difference in regression coefficients is, however, not significant, since the fiducial limits for odds of 19 in 20 overlap slightly. Two factors clearly define our knowledge of the toxicity of any of the substances examined: (1) the LD 50: This is best illustrated graphically as the abscissa or x value corresponding to the point of intersection of the "rectified" line with the y axis at probit 5. In other words, this value defines the *position* of the line; (2) the regression coefficient: This is a measure of the sensitivity* (measured,

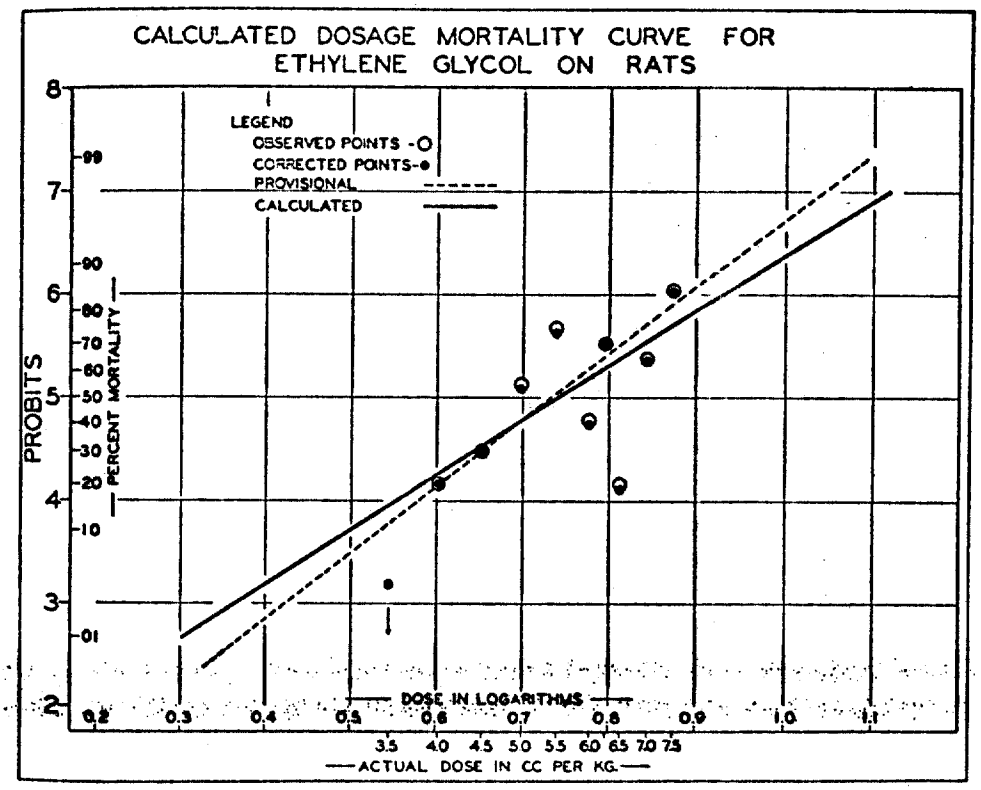


FIG. 1. Calculated dosage mortality curve for ethylene glycol on rats

* From the standpoint of the statistician, the regression coefficient is, properly speaking, a measure of the variability, since by definition s, the reciprocal of the regression coefficient, is the standard deviation of the response of a given population to a drug or poison in terms of the logarithm of the dose.

TABLE 2

THE DOSAGES AND MORTALITY RATIOS FOR ALL THE SUBSTANCES STUDIED ON GUINEA PIGS, MICE AND RATS

	Propylene glycol		Diethylene glycol		Ethylene glycol		Diethylene glycol mono-ethyl-ether		Dioxan		Ethylene glycol mono-ethyl-ether		
	Dose	Mortality ratio	Dose	Mortality ratio	Dose	Mortality ratio	Dose	Mortality ratio	Dose	Mortality ratio		Dose	Mortality ratio
	cc./kg.		cc./kg.		cc./kg.		cc./kg.		cc./kg.			cc./kg.	
Guinea pigs	15.0	0/10	5.0	1/10	5.0	2/5	2.5	0/5	3.0	0/10		2.5	1/10
			5.0	0/5			3.0	2/11					
	17.5	3/10	6.0	2/10	5.5	0/5	3.5	4/10	3.5	3/10		2.75	9/15
			7.5	4/10			4.0	5/10					
	20.0	6/10	7.5	5/10	6.0	1/10	4.0	6/10	4.0	12/20		3.0	15/20
			10.0	8/10			5.0	8/10					
	22.5	10/10	15.0	2/2	7.5	4/10	7.5	10/10	4.5	7/10		3.5	13/18
			20.0	2/2									
					8.5	7/10			5.0	10/10		4.0	10/10
					10.0	10/10							
Mice			15.0	2/10	7.5	2/10	5.0	0/14	4.5	1/10	Concentrated	3.0	0/10
	20.0	2/20	18.75	2/11	7.5	1/10	5.5	8/10	4.5	0/10		3.5	3/20
			20.0	8/40	10.0	4/10	6.0	13/30	5.0	3/10		4.0	6/10
	22.5	6/20	22.5	5/10	10.0	2/20	6.25	1/10	5.0	3/20		4.5	4/10
			25.0	14/26	11.0	4/10	6.5	7/10	5.5	10/20		5.0	7/10
	25.0	14/20	30.0	8/10	11.0	7/20	7.0	10/10	5.5	10/30		6.0	10/10
			35.0	10/10	12.5	4/10	7.5	8/18	5.75	3/10	Diluted	3.0	0/10
	30.0	9/10			12.5	12/20	8.0	17/20	5.75	5/10		3.5	2/10
					15.0	6/10	8.25	9/20	6.0	8/10		4.0	2/10
					15.0	6/10	10.0	8/10	6.0	15/20		4.5	3/10
					20.0	7/10			7.5	10/10		5.0	11/30
					20.0	9/10						5.5	6/10
												6.0	8/10
Rats—Series A	15.0	1/10	10.0	1/10	3.5	0/7	3.5	0/10	3.5	0/20		2.6	0/10
	15.0	0/10	10.0	1/10	4.0	4/20	4.0	0/20	4.0	3/20		3.0	8/10
	17.5	5/10	11.25	2/10	4.5	3/10	4.5	1/10	5.0	9/20		3.0	4/10
	17.5	3/10	12.5	10/20	5.0	11/20	5.0	4/20	6.0	14/20		3.3	5/10
	20.0	7/10	12.5	5/10	5.5	15/20	5.5	8/20	7.5	16/16		3.3	8/10
	20.0	2/10	15.0	9/20	6.0	4/10	6.0	14/20				3.5	10/30
	22.5	6/10	15.0	8/15	6.25	7/10	6.25	6/10				3.5	7/10
	22.5	4/10	17.5	5/10	6.5	2/10	6.5	10/10				3.75	7/10
	25.0	7/9	17.5	8/10	7.0	13/20	7.5	10/10				4.0	15/20
			18.75	10/10	7.5	17/20						4.0	9/10
			20.0	20/20									
Rats—Series B	17.6	1/9	10.9	0/9	4.0	2/9	5.0	2/9	4.0	0/9		3.1	3/9
	18.6	8/9	12.6	1/9	5.0	4/9	5.5	3/9	4.7	2/9		3.4	6/9
	20.0	5/9	14.1	4/9	6.3	7/9	6.0	6/9	5.6	6/9		3.7	8/9
	21.4	9/9	15.9	5/9					6.3	9/9			
	22.6	9/9	18.3	9/9									

in this case, by mortality) of a given population to a change in the dose. Graphically, sensitivity is illustrated by the *slope* of the "rectified" line. For example, referring to the effects of propylene glycol on mice, we see that this substance has a very low toxicity (LD 50 23.9 cc./kg.), but a small increment of dosage, 20%, produces a tremendous change in mortality (from 50% to 90%) because of the large regression coefficient. On the other hand, diethylene glycol-mono-ethyl-ether is very much more toxic (LD 50 6.58 cc./kg.) but because of the low regression coefficient, it requires nearly double the dose to increase the mortality from 50 to 90%. Because of the large possible variation in the regression coefficient, it becomes at once apparent why toxicities determined at any other mortality than 50% are less accurate. In order to effect accuracy of the same order, it would be necessary to use very much larger numbers of animals.

In order to reveal most effectively the relative toxicities of the six substances for each of the three species of animals, the "rectified" curves have been plotted on a graph in which the logarithm of the dose is laid off on the abscissa, on a scale sufficiently large to include the entire range of toxicities encountered. A separate Y or probit axis is given for each substance. The LD 50 can be directly interpolated from the intersection of the line with the 50% mortality or 5-probit mark.

With one exception (ethylene glycol), the descending order of toxicity to the animals is guinea pigs, rats, mice. The ascending order of toxicity for the six substances is propylene glycol, diethylene glycol, ethylene glycol, diethylene glycol mono-ethyl-

TABLE 3

EFFECT OF DILUTION ON THE TOXICITY OF ETHYLENE GLYCOL

Concentrated

DOSE	LOG DOSE	MORTALITY RATIO
cc./kg.		
3.50	0.544	2/11
3.98	0.608	2/11
4.70	0.672	4/11
5.43	0.735	5/11
6.30	0.799	6/11

Log LD 50 = 0.76819 ± 0.11143
Actual LD 50 5.86 cc./kg. within the limits of 4.54 and 7.58 in 19 out of 20 determinations
Regression Coefficient = 4.42 ± 4.04

Diluted 1 + 3

DOSE	LOG DOSE	MORTALITY RATIO
cc./kg.		
3.50	0.544	2/11
3.98	0.608	3/11
4.70	0.672	3/11
5.43	0.735	5/11
6.30	0.799	8/11

Log LD 50 = 0.72999 ± 0.0743
Actual LD 50 5.28 cc./kg. within the limits of 4.53 and 6.37 in 19 out of 20 determinations
Regression Coefficient = 5.47 ± 4.14

ether, dioxan and ethylene glycol mono-ethyl-ether.

It is to be noted that the substituted ethers, diethylene glycol mono-ethyl-ether, dioxan and ethylene glycol mono-ethyl-ether all have toxicities of approximately the same order of magnitude for the three species of animals tested. With one exception (mice, diethylene glycol mono-ethyl-ether), the regression coefficients are fairly large.

In table 5 are presented the results of experiments designed to test whether weight is a factor which might modify the response of an animal to a toxic substance. It can be seen, particularly in the case of rats and guinea pigs, but scarcely at all in mice, that diethylene glycol, ethylene

TABLE 4

Substance	Constants for equation $Y = \bar{y} + b(X - \bar{x})$				The LD 50 in cc./kg.				
	$\bar{x}$	$\bar{y}$	b	±1.96 x s.e.	Log-dose	±1.96 x s.e.	Actual dose	Limits of error: Upper	Limits of error: Lower
Guinea pigs									
Propylene Glycol	1.279	5.067	22.4	10.0	1.275	0.040	18.9	20.7	17.2
Diethylene Glycol	0.852	4.708	7.77	3.69	0.859	0.026	7.76	8.24	7.31
Ethylene Glycol	0.862	5.045	7.57	4.16	0.866	0.051	7.35	8.27	6.53
Diethylene Glycol Mono-Ethyl-Ether	0.583	4.965	8.62	3.77	0.587	0.020	3.87	4.05	3.69
Dioxan	0.597	5.104	15.5	6.4	0.590	0.024	3.90	4.12	3.69
Ethylene Glycol Mono-Ethyl-Ether	0.478	5.352	10.6	5.7	0.445	0.035	2.79	3.02	2.57
Rats									
Propylene Glycol	1.292	4.762	7.92	4.22	1.322	0.039	21.0	23.0	19.2
Diethylene Glycol	1.158	5.078	7.57	2.60	1.169	0.030	14.8	15.8	13.8
Ethylene Glycol	0.746	5.032	5.30	2.36	0.740	0.041	5.50	6.05	5.00
Diethylene Glycol Mono-Ethyl-Ether	0.749	4.993	17.2	5.4	0.743	0.016	5.54	5.73	5.33
Dioxan	0.704	4.889	11.5	3.7	0.713	0.028	5.17	5.52	4.84
Ethylene Glycol Mono-Ethyl-Ether	0.535	5.022	10.6	4.7	0.537	0.021	3.46	3.62	3.28
Mice									
Propylene Glycol	1.366	4.816	16.1	7.3	1.378	0.021	23.9	25.1	22.8
Diethylene Glycol	1.360	4.869	7.85	3.11	1.374	0.029	23.7	25.4	22.2
Ethylene Glycol	1.075	4.798	4.83	0.61	1.117	0.047	13.1	14.6	11.8
Diethylene Glycol Mono-Ethyl-Ether	0.834	5.077	4.70	2.61	0.815	0.044	6.58	7.63	5.94
Dioxan	0.742	4.817	17.6	6.8	0.752	0.013	5.66	5.84	5.49
Ethylene Glycol Mono-Ethyl-Ether:									
Concentrated	0.618	4.819	11.5	4.6	0.634	0.031	4.31	4.64	4.01
1 + 1 dilute	0.679	4.713	7.69	3.82	0.716	0.041	5.20	5.73	4.73

glycol, diethylene glycol mono-ethyl-ether and ethylene glycol mono-ethyl-ether give indications of greater toxicity for animals whose weights ranged from 37 to 108% higher than the average. Dioxan and propylene glycol, in contrast, give no clear-cut indications. It is interesting that mice heavier than the average did not react the way the rats and guinea pigs did. This may be due to the fact that the toxicity of all of the substances is lower for mice than for rats and guinea pigs.

While both "heavy" and "light" animals received the same dose per

TABLE 5

THE RELATION BETWEEN BODY WEIGHT AND THE LETHAL PROPERTIES OF THE COMPOUND

COMPOUND	"HEAVY"			"LIGHT"		
	Average weight	Dose	Mortality ratio	Average weight	Dose	Mortality ratio
Guinea pigs						
	gm.	*cc./kg.*		*gm.*	*cc./kg.*	
Propylene Glycol	—	—	—	—	—	—
Diethylene Glycol	—	—	—	—	—	—
Ethylene Glycol	558	6.0	9/9	268	6.0	1/10
Diethylene Glycol Mono-Ethyl-Ether	665	3.0	5/5	368	3.0	0/10
Dioxan	463	7.0	2/5	233	7.0	2/5
Ethylene Glycol Mono-Ethyl-Ether	540	2.75	15/16	295	2.75	3/9
Rats						
Propylene Glycol	—	—	—	—	—	—
Diethylene Glycol	396	15.0	15/15	288	15.0	10/25
Ethylene Glycol	380	4.5	7/10	214	4.5	3/10
Diethylene Glycol Mono-Ethyl-Ether	415	5.0	11/15	260	5.0	4/20
Dioxan	391	5.0	4/4	226	5.0	5/10
Ethylene Glycol Mono-Ethyl-Ether:						
Concentrated	394	3.3	1/5	233	3.3	5/10
1 + 1 dilute	365	7.0	2/8	221	7.0	5/10
Mice						
Propylene Glycol	26.8	22.5	0/10	15.6	22.5	3/10
Diethylene Glycol	29.8	20.0	5/10	16.0	20.0	2/10
	25.8	22.5	7/10	20.9	22.5	5/10
Ethylene Glycol	27.1	12.5	6/10	14.6	12.5	7/10
	30.0	11.0	6/10	22.2	11.0	1/10
Diethylene Glycol Mono-Ethyl-Ether	27.3	4.0	10/10	15.4	4.0	6/10
	27.1	7.0	10/10	20.9	7.0	10/10
Dioxan	25.9	5.75	6/10	13.4	5.75	5/10
	28.7	6.0	9/10	20.6	6.0	7/10
Ethylene Glycol Mono-Ethyl-Ether:						
Concentrated	24.1	5.0	4/10	13.7	5.0	4/10
1 + 1 dilute	24.8	7.0	10/10	19.6	7.0	10/10

kilogram in each test, the heavier animals, of course, received larger actual amounts. Two factors might serve, therefore, to increase the toxicity for the larger animal: (1) unequal distribution of the substance in body tissues (relative insolubility of the glycols in fatty tissues); this would tend to raise the level at which the substances would circulate; (2) inability of the excretory organs, chiefly the kidneys, to cope with excessive amounts of toxic materials. It is well known that the ratio of kidney to body weight decreases with the size of the animal instead of remaining constant. Thus the 400 gm. rat does not have kidneys which weigh twice as much as the 200 gm. animal. In effect, therefore, in estimating the dose on a per-kilogram basis, we have tacitly assumed that the functional tissue in an animal increases *pari passu* with its whole body weight, which actually does not occur. This has, of course, been generally recognized, since most biological assays prescribe the use of animals within certain stated weight ranges. While the experiments here cited are of somewhat preliminary nature, they nevertheless emphasize the importance of a restricted weight range.

Symptomatic Response and Pathology

Propylene glycol. Shortly after administration of large doses, the animals showed signs of loss of equilibrium, marked depression, analgesia, coma and finally death after a prolonged moribund state.

The gross observations of organs were essentially negative except for hemorrhagic areas in the small intestine. Microscopic changes in the kidney were minimal but probably relevant, with nuclear pyknosis and vacuolar degeneration of the cytoplasm. A few cortical tubules contained protein debris or loose casts. The liver showed only slight congestion and hyperemia with no fatty changes.

Diethylene glycol. The toxic syndrome in rats particularly and to some extent in rabbits, dogs, mice and guinea pigs resembled that reported for human beings. There was occasional vomiting in dogs. In rats there was early increased thirst, diuresis, ruffled coat and refusal of food. After the first 2 or 3 days there was practically complete cessation of urine excretion with heavy proteinuria. There was prostration, dyspnea, bloated appearance, coma, with pronounced lowering of body temperature for about 24 hours before death. Other animals behaved similarly except there was variation in sensitivity.

At autopsy, because of the prolonged moribund condition, there were usually some autolytic changes in the internal organs even though the autopsy was performed immediately. There was often generalized edema, hemorrhage into lungs, intestines and kidneys, and pneumonia. The liver was usually soft and fragile. The kidneys were often grossly normal in appearance but at times appeared swollen, pale and flabby. The livers and kidneys were most severely affected, and sometimes the adrenals were involved, showing extensive mitotic activity. There was extensive degeneration of the renal cortex with vacuolar (hydropic) degeneration of the convoluted tubules. In places, the degeneration of the epithelium was

so severe that nothing but the basement membranes of the tubules remained, while cellular debris, protein precipitate, and hyaline casts seemed to fill the lumen of what remained of the tubules. There was usually no clear evidence of involvement of the glomerular tufts but Bowman's space was often distended by accumulation of a protein precipitate. In some cases the vessels of the cortex were thrombosed, with possible involvement of the vessels of the medulla. Although not usually seriously affected, there may have been pronounced degeneration of the proximal tubules of the medulla with numerous hyaline casts. In animals that survived for 8 days, there was some evidence of regenerative changes in the kidney. In the liver there was widespread diffuse hydropic degeneration of the central portion of the liver lobule. This degeneration was of varying degree in different foci. In some, it proceeded to the complete disintegration of liver cells; in others, vacuoles distended the cytoplasm which retained almost normal nuclei. The lungs of most animals were essentially normal, but as mentioned above there was congestion, edema and focal interstitial pneumonia and hemorrhage in some animals. The spleen in many cases showed excessive phagocytosis of blood pigment. The heart, intestine, stomach, brain and bladder in most cases were essentially normal.

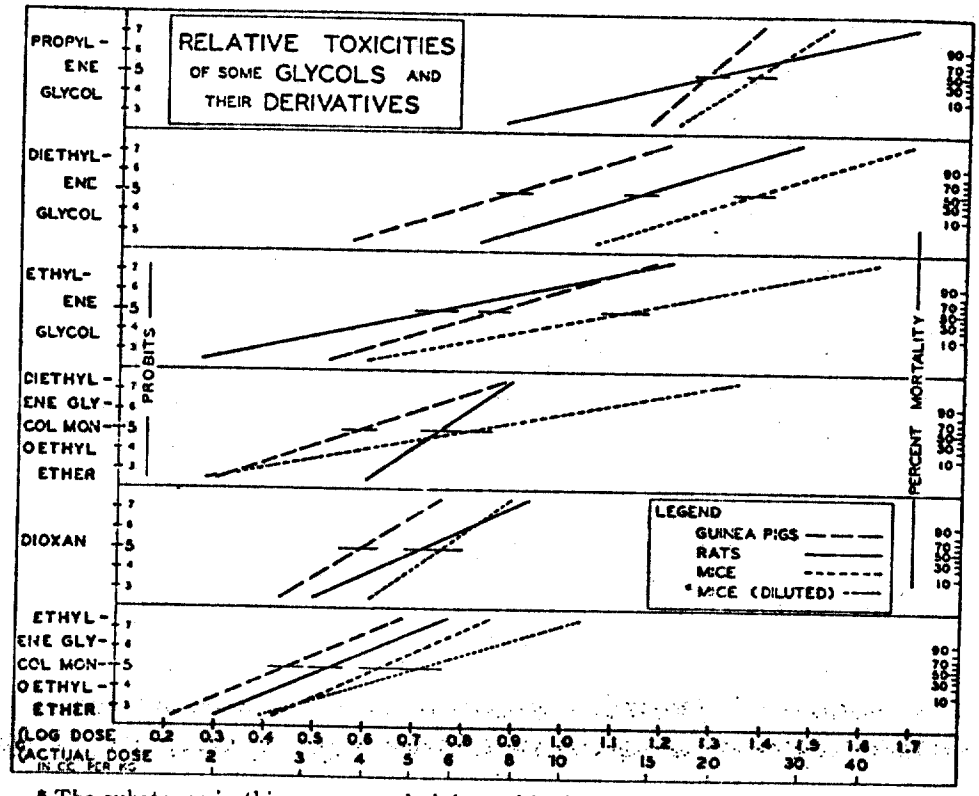
Ethylene glycol. Shortly after administration, the animals showed signs of weakness and lack of muscular coordination. This was in contradistinction to the picture following administration of diethylene glycol, where no immediate symptoms occurred. Prostration and coma were later symptoms, followed by death. At the higher doses, death occurred in from 18 to 24 hours, but, at the lower doses, was sometimes delayed for 4 to 6 days. At autopsy, the gross findings included congestion of the lungs with hemorrhage in most cases. At the higher levels of intake, the stomach usually showed hemorrhagic areas. The bladders were nearly always filled with urine that gave heavy protein tests. Microscopically there was in nearly all cases hydropic degeneration of the cells lining the cortical convoluted tubules and focal necrosis in the liver. Other organs were essentially normal.

Diethylene glycol mono-ethyl-ether. The early symptoms were very similar to those of animals receiving toxic doses of ethyl alcohol. After a period of prostration and coma, death followed within 24 to 48 hours, depending upon the size of the dose. Grossly, the organs appeared essentially normal. Microscopically, there was evidence of lobular broncho-pneumonia, focal myocarditis, focal necrosis in the liver, vacuolation of the cortical tubules with occasional tubular casts in both cortex and medulla. In some of the animals, the mitotic activity in the adrenals was striking. Other organs appeared normal.

Dioxan. Immediately after administration, some animals appeared limp and dazed, while others exhibited distinct signs of intoxication with muscular incoördination. Except for the larger doses, where these symptoms gradually verged into coma and death within 24 hours, there was often apparent recovery, with subsequent death in some cases after several

days. Gross observations showed hemorrhagic areas near the pyloric end of the stomach. The bladders were distended with urine and although there was slight proteinuria, there was no hematuria as in the case of cellosolve. The kidneys were somewhat enlarged. Other organs appeared grossly normal. In some of the animals, microscopic changes in the liver and kidneys were slight; in others, extensive changes resembling those noted for diethylene glycol occurred. All other organs examined were essentially normal.

Ethylene glycol mono-ethyl-ether. In contrast to ethylene glycol, from which it is derived, ethylene glycol mono-ethyl-ether produced no immediate symptoms whatever. In this respect it resembled diethylene glycol, even to the extent that with moderate doses, death was sometimes delayed for 4 to 6 days. With large doses, on the other hand, death usually occurred in 24 to 36 hours. Hematuria was noted in nearly all animals, and after death the bladders remained distended with bloody urine. The kidneys of some animals showed extreme tubular degeneration with almost complete necrosis of nearly all of the cortical tubules. The changes appeared to be chiefly of the hyaline droplet or colloid



* The substance in this case was administered in diluted form to mice, that is, 1 + 1.

FIG. 2. Relative toxicities of some glycols and their derivatives

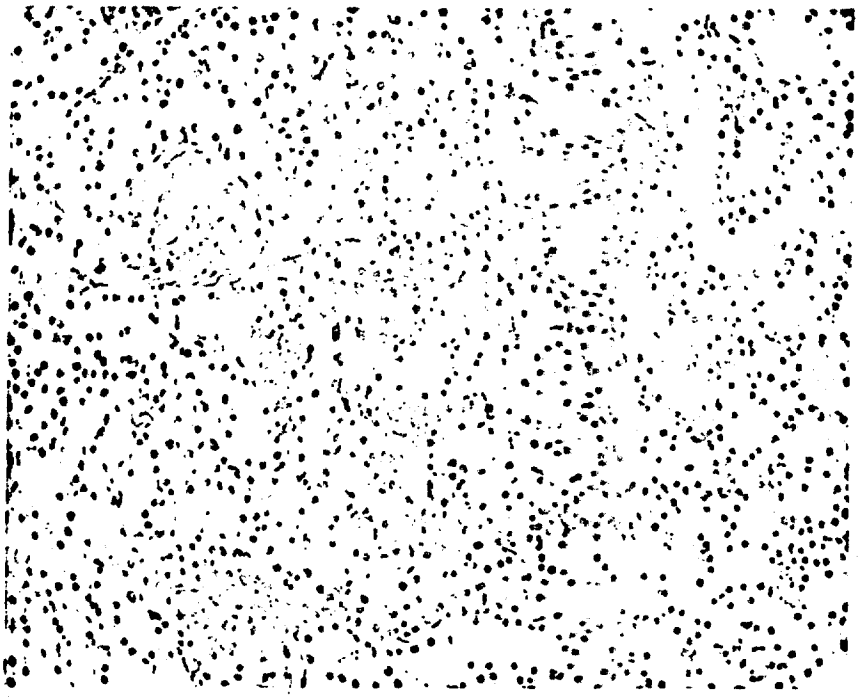


FIG. 3. Propylene glycol. Rat kidney. Normal. ×225.

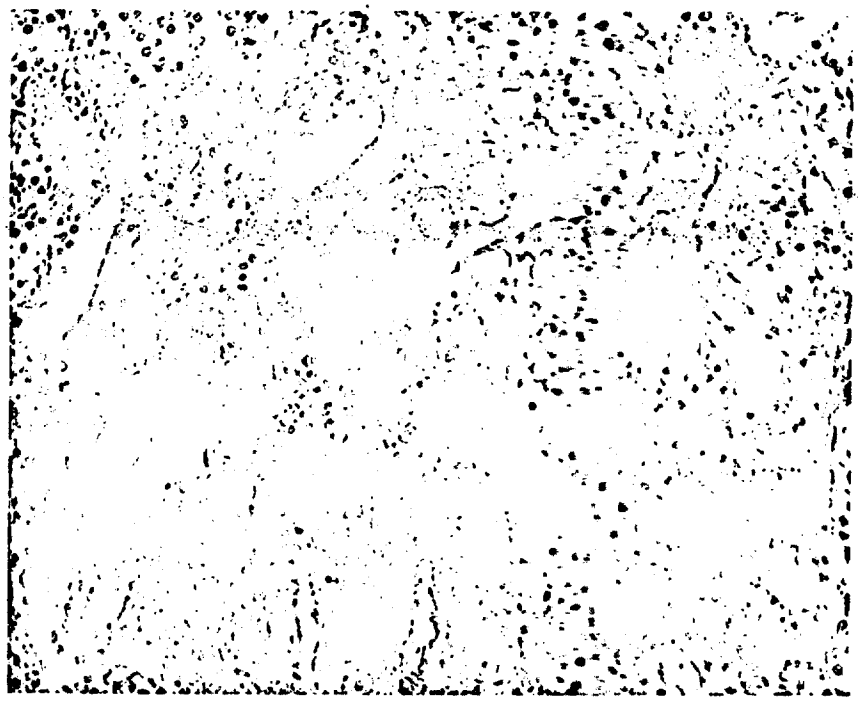


FIG. 4. Diethylene glycol. Rat kidney. Extensive necrosis of convoluted tubules (right half). ×225.

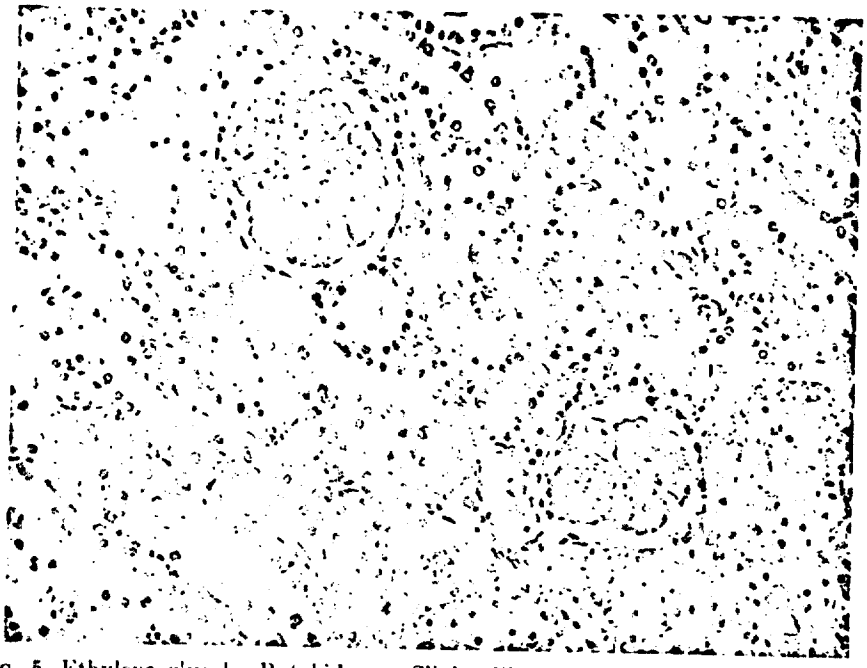


FIG. 5. Ethylene glycol. Rat kidney. Slight dilatation of convoluted tubules with proteinuria (only slight change as compared with rat kidney after diethylene glycol). ×225.

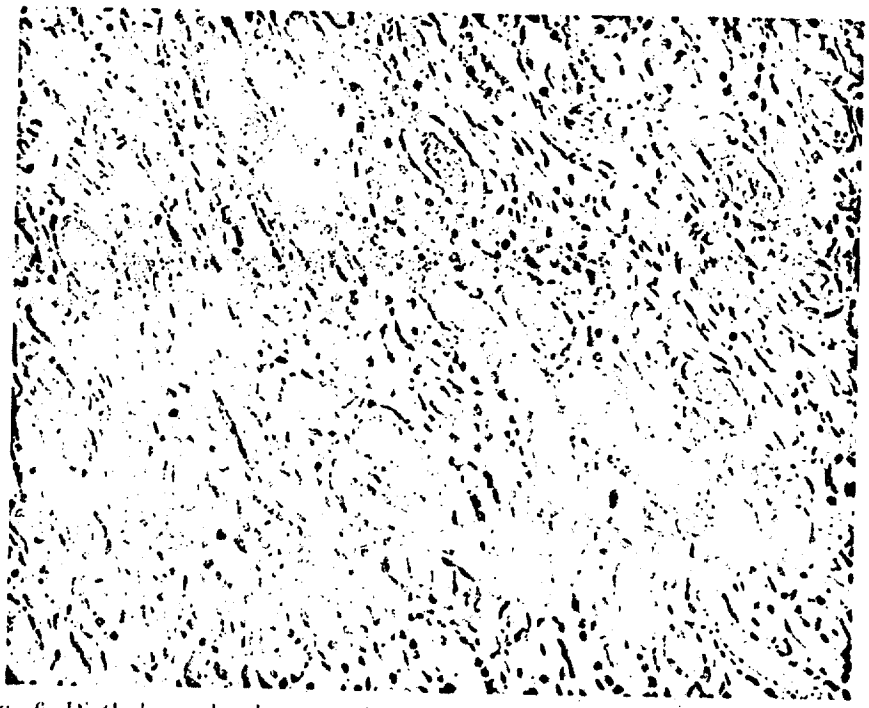


FIG. 6. Diethylene glycol mono-ethyl-ether. Rabbit kidney. Numerous granular casts in the medullary tubules. ×225.

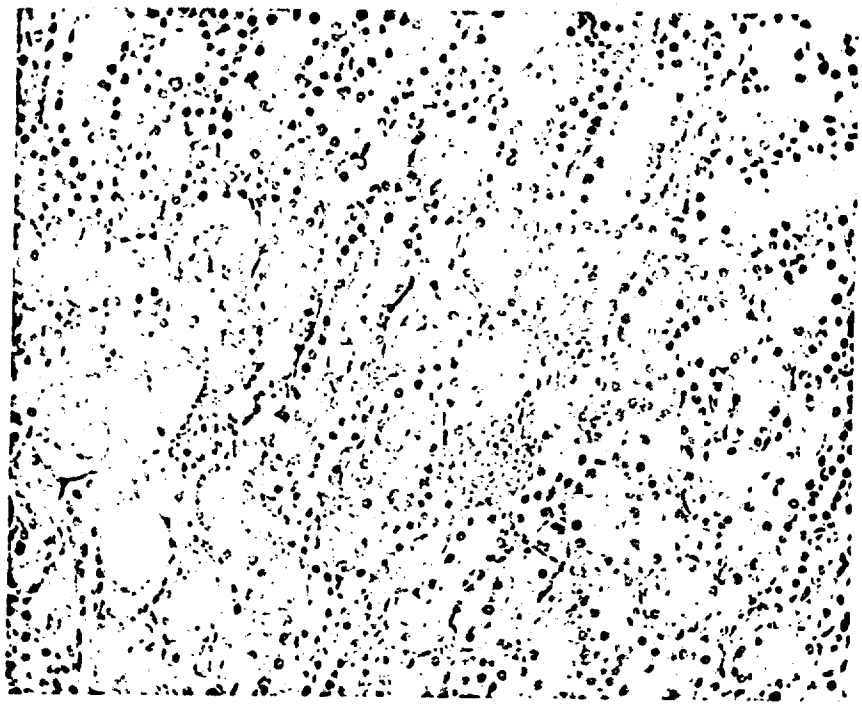


FIG. 7. Ethylene glycol mono-ethyl-ether. Rat kidney. Focal degeneration of convoluted tubules (central part of left half) and cast formation. ×225.

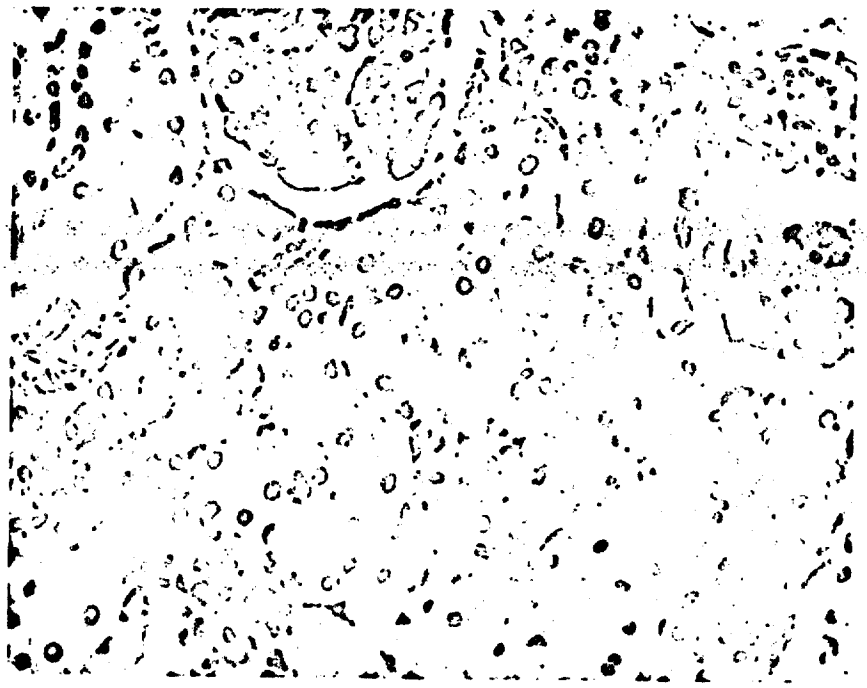


FIG. 8. Dioxan. Rat kidney. Focal degeneration of convoluted tubules (cytoplasmic degeneration with pyknosis of nuclei; note tubules in lower right hand corner). ×450.

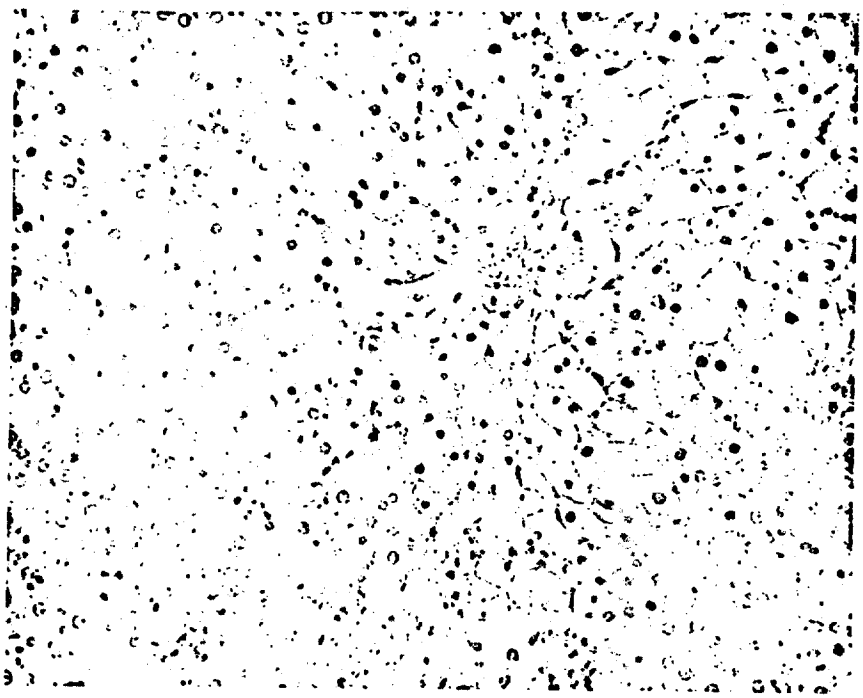


FIG. 9. Diethylene glycol. Rat liver. Hydropic degeneration of cells of center of liver lobule. ×225.

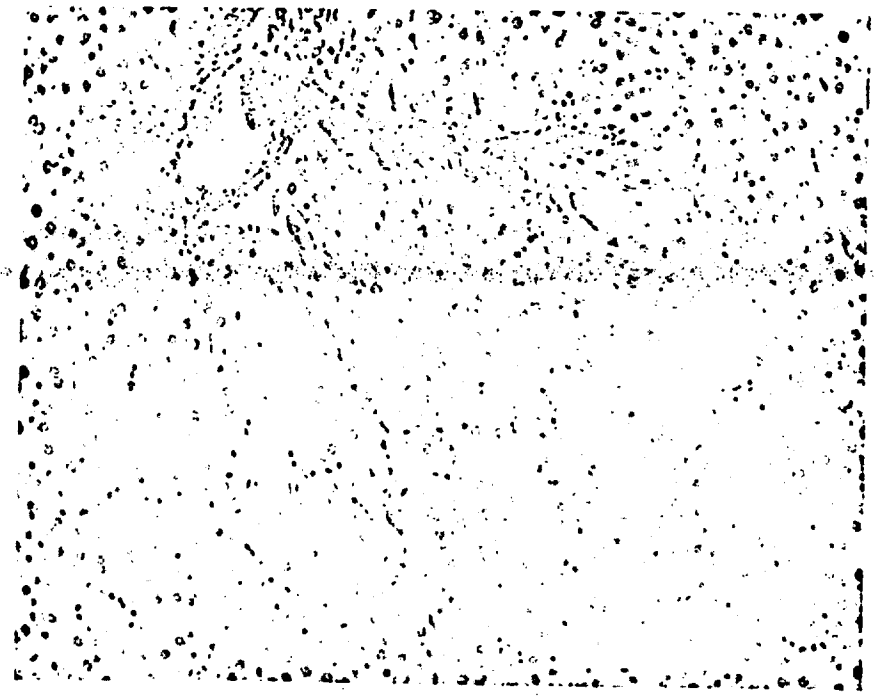


FIG. 10. Propylene glycol. Rat liver. Normal. ×225.

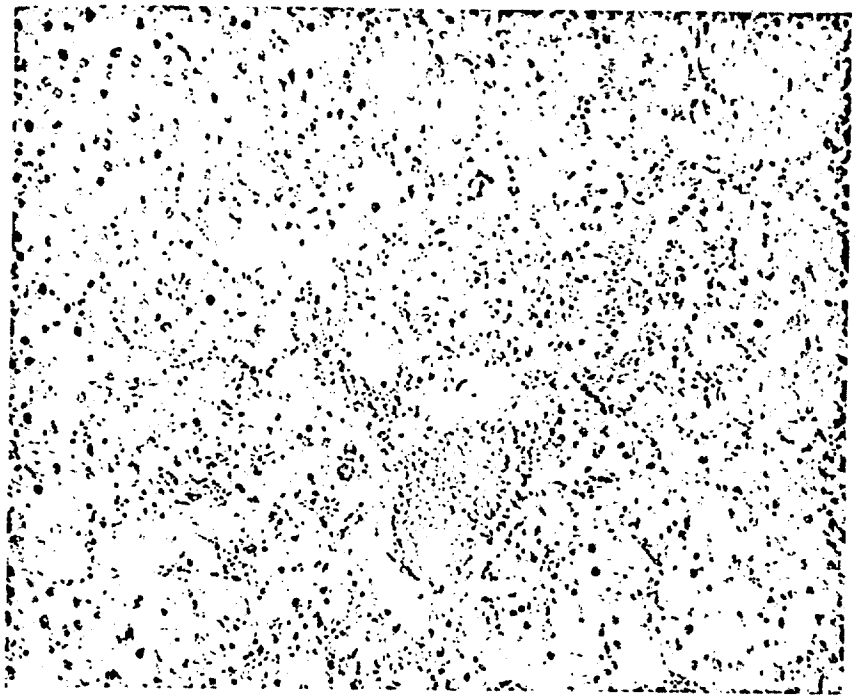


FIG. 11. Ethylene glycol. Rat liver. Degeneration of liver cells in central portion of liver lobule (less marked than in rat liver after diethylene glycol). ×225.

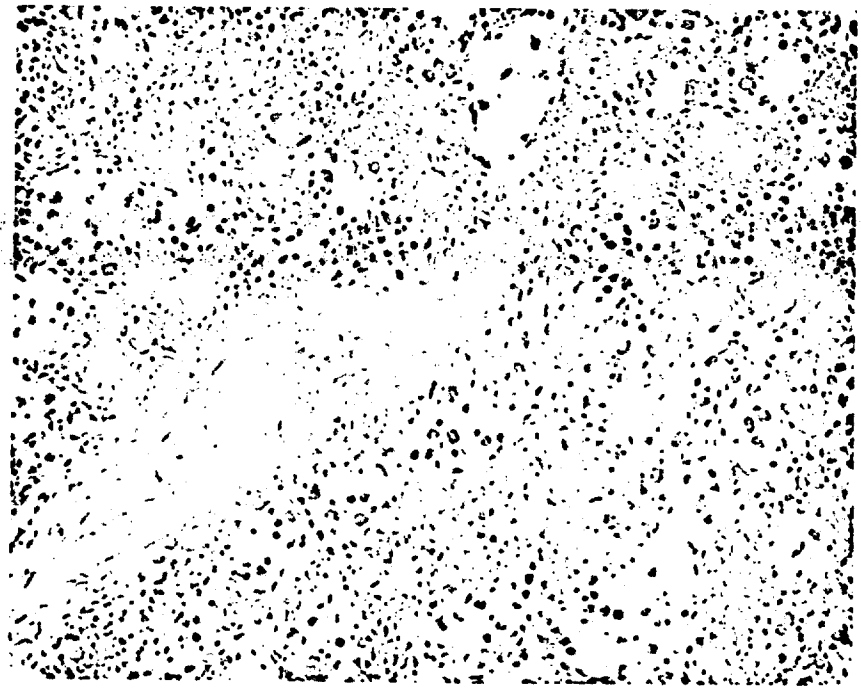


FIG. 12. Diethylene glycol mono-ethyl-ether. Rabbit spleen. Marked dilatation of sinusoids, all of which contain phagocytic cells stuffed with hemosiderin, and some of which are thrombosed (see upper right hand corner). ×225.

droplet type, rather than hydropic. About one third of the Bowman's spaces were distended and contained similar material. There was marked congestion. These extensive kidney changes were not frequent, but mild changes always occurred. Hemorrhagic areas in the stomach and intestines were seen uniformly. Liver damage was very mild as were any injuries noted in other organs.

The relative toxicities are shown in figure 2. The pathological changes noted by us and recorded here are similar to the reports in the literature where examination has been made (23, 24, 26, 28, 38, 40, 46, 47, 49, 51, 55, 60). For diethylene glycol mono-ethyl-ether and ethylene glycol mono-ethyl-ether such reports do not exist.

Discussion

A summary of the findings in the literature and of our own LD 50 after oral administration is presented in table 6. In some instances there is good agreement among the investigators, whereas in others, there are enormous variations. There are undoubtedly many causes for these variations, such as number of animals used, health and dietary condition, ages and weights of the animals, sex in some instances, form of the dose (dilute or concentrated), the number of dosage levels, and the percentage of the animals killed which is chosen to define the lethal dose. There are probably at least three of these factors principally responsible for the wide variation in the size of the dose recorded as MFD,* MLD, LD 50, or some other arbitrary designation. They are: the number of animals used, the dosage levels chosen and the percentage mortality selected as the designated MFD, MLD, etc. This dose is usually chosen more or less arbitrarily after the experiment is completed and depends on the number of animals killed at a certain point, which in turn depends on the number of animals used. As a result, there are then only two important factors, namely, the number of animals used and the dosage levels selected. We do not wish to recommend the choice of any particular point or percentage as the proper one, but we do feel very strongly that if the technic outlined in this investigation is generally followed, there will be much greater uniformity of results, and any particular percentage mortality can be read directly and accurately from the curve.

In our studies we have chosen the 50% point (LD 50) as the one for greatest consideration, but one can readily observe that the regression coefficient or slope of the curves gives almost as important information as that obtained by knowing the LD 50.

If one attempts to repeat some of the calculations, it will become at once apparent why the LD 50 point is the best one to choose on the curve, since its accuracy can be determined with a much smaller number of animals than the accuracy of any other point. For example, in order to get the same precision at 99% kill as at 50% kill, one would require 103 as against 10 animals. Yet there is reported in the literature one widely quoted investigation (13) in which the authors determined the MFD of seven different glycols and derivatives, and in which

* Minimum fatal dose, minimum lethal dose and lethal dose killing 50% of the animals.

TABLE 6

RELATIVE ORAL TOXICITIES OF SOME GLYCOLS AND DERIVATIVES FOR VARIOUS SPECIES OF ANIMALS AND MAN

LD 50 in cc. per kg. body weight (except where otherwise noted) and the total number of animals used to determine this dose.

SPECIES	PROPYLENE GLYCOL	DIETHYLENE GLYCOL	ETHYLENE GLYCOL	DIETHYLENE GLYCOL MONO-ETHYL-ETHER	DIOXAN	ETHYLENE GLYCOL MONO-ETHYL-ETHER	REFERENCE
Mouse	23.9 (70)	23.7 (117)	13.1 (150)	6.6 (152)	5.7 (160)	4.3 (conc.) (70) 5.2 (dil.) (90)	(68)
Rat	31.9 (15)						(59)
		15 (100%) (5/5) 20 (80%) (4/5)					(42)
	23.8 (?)	14.3 (?)	4.8 (?)				(42)
	21.0 (89)	14.8 (145)	5.5 (147)	5.5 (130)	5.2 (96)	3.5 (130)	(60) (68)
Guinea pig	18.9 (40)	7.8 (59)	7.4 (50)	3.9 (66)	3.9 (60)	2.8 (73)	(68)
Rabbit					2 (5)		(28)
	18 (33%) (3/9) 19 (86%) (6/7)						(37)
		4.4 (48) estimated					(37)
	14.3 (33%) (?) 17.8 (50%) (?)						(68) (60) (60)
Cat		3.3-4.7 (?) Lethal	2 (66%) (?)				(60)
					2 (5)		(28)
Dog	20 (66%) (?)						(60)
		10 (?) estimated					(45)
Man	238 persons took 32-1950 cc. over a period of 21-270 days	1(30%) (105/353) See text for discussion	100 cc. estimated lethal dose. See text				(60) (52) (14)

the total number of animals used was 30, the maximum number used for any one of these being five and in one instance only three. Although this work has never been repeated, it is not difficult to imagine that the error in the estimated MFD could be as much as 300 to 400%.

In this investigation we have also calculated the standard error of the LD 50, and from this the range of dosages, which, 19 times in 20, may be expected to cause 50% mortality. These are shown in table 4. From these figures it can be observed that there are in several instances statistically significant differences between the toxicity of the substances themselves on a given species and furthermore, that there are in most instances significant differences between species which in some cases are very marked, as with diethylene glycol, for example, where the dose for the mouse is three times that for the guinea pig. Of the three species tested, guinea pigs had the smallest LD 50's, except in the case of ethylene glycol where the LD 50 for the rat was significantly lower. Gross and microscopic examinations of the organs and tissues have not specifically aided in an explanation of this fact.

During the course of our investigation, we have also made some chronic toxicity studies and have others in progress, but these will be reported later. In order that this discussion may be more complete, it seems advisable, however, to refer to the chronic studies reported in the literature. Several investigators have given propylene glycol in drinking water in concentrations as high as 10% without noticeable effects (15, 26, 37, 54, 59, 60). One group (37) gave it by stomach tube daily, and another group (26) gave it as fatty acid esters, likewise without effect. Diethylene glycol has also been given in drinking water (42, 54, 55, 60), by stomach tube (45) and as fatty acid esters (26). In all cases the mortality was high and pathological changes marked except at very low concentrations in water. The chronic effects of ethylene glycol have likewise been observed after administration in drinking water (11, 54, 60) as fatty acid esters (26) and by inhalation of the vapors (39) with results similar to those obtained from diethylene glycol except with the vapors, where no toxic effects for rats and mice were noted for the concentrations studied. The animals were exposed to concentrations in air of about 300 mg. per 1000 liters for 8 hours per day, 5 days a week, for a period of 16 weeks. Chronic studies have not been made on the other substances.

As can be seen from table 6, other species of animals have been studied, namely, cats, dogs and rabbits. Our results for dogs and rabbits are not extensive enough to justify detailed consideration; however, with 48 rabbits and a dose range of from 1 cc. to 11.5 cc. per kilogram, we have confirmed the findings of Geiling, Coon and Schoeffel (45) that rabbits have the lowest LD 50 of the species of experimental animals studied on diethylene glycol. The kidney and liver damage was particularly striking and even more severe than that for the other species. Even the lowest dose killed some of the animals; this was approximately the average dose that killed 105 of 353 persons who

consumed the diethylene glycol as an "elixir" (52).

Geiling, Coon and Schoeffel (45) and Geiling and Cannon (51) have emphasized the importance and probable significance, for interpretation in terms of man, of the administration of divided doses during toxicity studies. In unpublished investigations of "Elixir of Sulfanilamide," we have, in general, confirmed the findings of the above investigators. It seems proper at this point to reemphasize the inadvisability of attempts to interpret experimental data on laboratory animals directly in terms of man. It is entirely too dangerous. The present investigation confirms again the wide variations that may occur between species. It should also be pointed out that acute toxicity studies, although extremely valuable, especially from a pharmaceutical standpoint, far from tell the whole story and that likewise chronic studies do not tell the whole story. For example, ethyl alcohol, glycerol and propylene glycol can be taken in quantities large enough to be acutely toxic, while small quantities are oxidized by the animal body and do not produce chronic toxicity. Again, although small doses of lead, mercury, selenium, fluorine, narcotics, etc., are acutely toxic, it is the insidious character of their chronic toxic effects that makes them even more dangerous.

In the discussion of the pathological results, it need only be emphasized that there are species differences in the histological picture presented by the tissues and that great consideration must be given to the survival period following the administration of the toxic agent. If the dose is so large or the substance so toxic that death results within about 24 hours, one can rarely expect extensive histological changes in the internal organs. In this study the most extensive changes observed were in the organs of those animals that survived for 3 to 6 days. It is undoubtedly for this reason that the animals that succumbed from propylene glycol showed relatively smaller changes than those caused by any of the other substances. Animals that received propylene glycol died within a very few hours or else survived the experimental treatment. From a clinical standpoint it seems that this failure to find pathological changes after the administration of toxic substances may be of particular significance.

Summary and Conclusions

1. The relative acute toxicities of some glycols and derivatives have been determined and the order of increasing toxicities is as follows: propylene glycol, diethylene glycol, ethylene glycol, diethylene glycol mono-ethyl-ether, dioxan and ethylene glycol mono-ethyl-ether.

2. Three species were used: rats, mice and guinea pigs. A limited number of rabbits were used on diethylene glycol. The total number of animals used was over 2600. The rabbits, in comparison with other species, were found to have the lowest LD 50 for diethylene glycol. In fact, the lowest dose that killed some rabbits was the same as the estimated average dose that killed 105 persons.

3. The regression coefficients and LD 50's, with their respective standard errors, have been calculated for each species and each substance ad-

ministered. A detailed example of the methods used in the calculations is also presented.

4. In every instance except one the LD 50's for guinea pigs were smaller than they were for the other species, while those for mice were the highest.

5. A complete pathological report is presented with photomicrographs. The most extensive histological changes were noted subsequent to the administration of diethylene glycol.

6. The LD 50's in cubic centimeters per kilogram for mice are as follows: propylene glycol 23.9, diethylene glycol 23.7, ethylene glycol 13.1, diethylene glycol mono-ethyl-ether 6.58, dioxan 5.66, ethylene glycol mono-ethyl-ether 4.31 (conc.), and 5.20 (dil.); for rats, propylene glycol 21.0, diethylene glycol 14.8, ethylene glycol 5.50, diethylene glycol mono-ethyl-ether 5.54, dioxan 5.17, ethylene glycol mono-ethyl-ether 3.46; for guinea pigs, propylene glycol 18.9, diethylene glycol 7.76, ethylene glycol 7.35, diethylene glycol mono-ethyl-ether 3.87, dioxan 3.90, ethylene glycol mono-ethyl-ether 2.79.

7. It seems that small doses of propylene glycol can be safely tolerated by the animal body since it can be oxidized and probably converted to a normal body constituent. In this respect it resembles ethyl alcohol and glycerol.

8. In larger quantities all of these substances are toxic, and, with the exception of propylene glycol, produce definitely demonstrable tissue injury.

9. The literature has been reviewed and a complete bibliography is appended.

After correlating the information in the literature with the results of these experiments, it is concluded that ethylene glycol, diethylene glycol, ethylene glycol mono-ethyl-ether, diethylene glycol mono-ethyl-ether and dioxan should be entirely omitted from food and drug preparations, and that propylene glycol should be avoided except in very small concentrations.

The microscopic pathological examinations were made by Drs. Esmond R. Long and M. B. Lurie, Henry Phipps Institute, Philadelphia, Pa., and by Dr. Eleanor Humphries, Department of Pathology, University of Chicago, Illinois.

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CONCERNING THE TOXICITY OF GLYCOLS.

By L. Launoy (1)

I. STATE OF THE QUESTION.

A. Introduction. - The study of the toxicity of glycols over the last few years had as its starting point the 93 cases of fatal intoxication occurring in America by ingestion of an elixir containing diethylene-glycol as solvent [1]. Currently, this study is based on the pharmaceutical concern of providing a replacement for glycerine.

Glycols are not unimportant. In 1930 KLAUS HANSEN [2] reported on the case of two serious intoxications in two youths saved in the nick of time after having absorbed a dose of ethylene glycol seemingly equal to 100 cm^3. The initial results obtained in animals seemed, however, encouraging, since according to POHL [3], MAYER [4], dogs and rabbits did not die as a result of glycol, even after a strong dose. This product was eliminated in the urine in the form of glycolic acid and oxalic acid, when taken orally. Hemorrhagic nephritis was determined by injecting rabbits subcutaneously. These initial papers date from 1896.

In 1917, BACHEM [5], who compared the irritating action of glycol to that of glycerol, testing isolated intestinal rabbit flexures, observed that the glycerol was more irritating than the glycol. BACHEM stated that this is a hemolytic body like glycerine and Paul MAYER [6], the same year, recognized that it could determine serious hemorrhagic nephrites. According to FRAENKEL [7], glycol was presented in Germany during World War I as a glycerine substitute under the name of Tegoglycol. For FRAENKEL, glycol is atoxic.

B. Observations on man. - In 1926 PAGE [8] along with CORYLLOS recommended glycol as an Amytal solvent. He suggested that this body could be a solvent of great physiological interest. The same year PAGE, during the course of pharmacological experiments, himself absorbed 15 cm^3 of diluted glycol with no other sensation than slight heat upon absorption. He thus repeated the experiment of BACHEM (work cited) who, in 1917, absorbed 25 g. of glycol diluted in water and 45 g. in two days with no other consequence than an increase in oxalyl urea.[9]

P.J. HANZLIK, M.A. SEIDENFELD, C.C. JOHNSON [10] made observations on themselves and on nine other subjects. Glycol absorption gave them a sensation of heat on the tongue and in the esophagus. Subcutaneous or intramuscular injection of 2 cm^3 determined a momentary burning sensation. Comparing the results obtained with the glycol and propylene glycol with these, the glycerol showed itself to be the most painful, then ethylene glycol and propylene . Yet with this latter body, the results

varied with the individual. Van ŒTTINGEN and JIROUCH [11] considering that ethylene and diethylene glycols could be interesting from the therapeutic point of view, made experiments analagous to the previous ones. They judged them to be just slightly irritating locally, but capable of provoking gastro-enteritis when absorbed through the mouth. Only small doses should be used for intramuscular injection, since injection venously seems dangerous due to the hemolytic action and the hypotension created by these compounds.

SEIDENFELD and HANZLIK [12] also observed a greater irritating action of glycerol. They recommend the use of propylene glycol instead of glycol, the former being less toxic than the latter, since, at least in animals, it would give no cumulative effect.

C. Local action studied in animals. - This study was made on the cornea of the rabbit, the sciatic nerve of the frog, the derm, the unbroken skin, the smooth intestinal muscles of the rabbit and the smooth muscles of the rat uterus, the arterial muscles, those of the peripheral vessels and the isolated heart. HANZLIK, SEIDENFELD and JOHNSON (work cited), SEIDENFELD and HANZLIK (work cited), A. LATVEN and H. MOLITOR [13] note the irritating action of glycols and of glycerol on the cornea of the rabbit. The degrees of irritation are from hyperema to oedema and to opacity of the cornea. Regression of these symptoms requires several days. Propylene glycol would be more irritating on the eye than glycerine and ethylene glycol (LATVEN and MOLITOR); with intradermic injection, glycerol would be less irritating. These latter conclusions result from our reading of Table III, incorporated in the paper of LATVEN. We do not, then, fully understand how, in their discussion, these authors were able to draw the following conclusion concerning glycols and glycerol: "Propylene glycol was found to be the least toxic and irritant followed by ethylene and triethylene glycol, glycerol, carbitol and alcohol (p. 92 in the work cited)". Glycol and diethylene glycol possess a depressing action on smooth muscle and on the uterus. The same holds true for the isolated frog heart. On the peripheral vessels, vaso-constriction preceded by vaso-dilation is noted.

Application on the sciatica of the frog provokes a decrease in excitability.

D. Experimental toxicity. - Glycols lower respiration and death seems due to the cessation of respiratory movements, the heart continuing to beat for some time when respiration has stopped. With a toxic dose, glycerol produces clonic convulsions; glycol, though, is a depressant.

Toxic doses have been researched on the rabbit, the rat, the mouse, the dog and the chicken. The following table summarizes the results obtained by different authors.

BODY	ANIMAL	ADMINISTRATION	MIN. LETHAL DOSE	LETHAL DOSE 100%	AUTHORS
Ethylene Glycol	Rabbit	i.m.	5 cm^3	6 $cm^3$25	Hanzlik
					Seidenfeld
					Johnson
		i.v.	"	4 $cm^3$5	-
		i.p.	"	9 cm^3	Page
	Rat	i.m.	3 cm^3	6 $cm^3$25	Hanzlik
					Seidenfeld
					Johnson
		i.v.	2 $cm^3$5	4 cm^3	-
		i.p.	1 cm^3	3 $cm^3$5	Page
	Dog	i.v. slow perfus.	20 cm^3	"	Hanzlik (14)
					Newman
					Van Winkle
					Lehmann
					Kennedy
	Mouse	s.c.	2 $cm^3$5	"	Oettingen
					Jirouch
		s.c.	4 $cm^3$5 (50%)	6 cm^3	Latven
					Molitor
		p. os	7.5 (50%)	10 cm^3	-
		i.v.	3 cm^3	4 cm^3	-
Diethylene Glycol	Rabbit	i.m.	3 cm^3	6 cm^3	Haag (15)
					Ambrose
		i.v.	2 cm^3	6 cm^3	-
		p. os	"	8 to 10	cited by Taeger
	Rat	s.c.	15 cm^3	20 cm^3	Haag
					Ambrose
		i.m.	7 cm^3	9 cm^3	-
		i.v.	4 cm^3	6 cm^3	-
		i.v.	5 $cm^3$8	"	Weatherby (16)
					Williams
		p. os	"	15 to 20 cm^3	Haag - Ambrose
		p. os	"	16 cm^3	cited by Taeger
		"	"	12 $cm^3$5	Poe - Witt (17)
		"	27 (50%)	"	Weatherby
				"	Williams
	Dog	i.v. slow perfus.	20 cm^3(50%)	"	Hanzlik et al.
		p. os	9 cm^3	"	cited by Taeger
		"	10 cm^3	"	Weatherby
					Williams
	Mouse	s.c.	5 cm^3	"	Oettingen
				"	Jirouch
Propylene Glycol	Rabbit	i.m.	6 cm^3	7 to 9 cm^3	Seidenfeld
					Hanzlik
		i.v.	4 cm^3	6 cm^3	-
		i.v.	"	6.5	Weatherby (18)
					Haag
	Rat	s.c.	"	22.5	-
		i.m.	"	14	results expressed
		i.v.	"	6.8	in gr.
		p. os	"	33.5	

BODY	ANIMAL	ADMINISTRATION	MIN. LETHAL DOSE	LETHAL DOSE 100%	AUTHORS
Propylene Glycol	Dog	i.v. (slow perfus.)	"	25 cm^3	Hanzlik, Newman
	Chicken	i.v. slow perfus.	"	25.7	Lehmann Newman
	Mouse	s.c.	18.5	24	
		p. os	22	26	Latven
		i.v.	8	11	Molitor

(These figures correspond to 1000 gr. of animal weight).

Comparison of the above-reported results allows us to observe that, for each author, the toxicity of the glycols studied is always ordered in the same manner - by decreasing toxicity, as follows: glycol, diethylene glycol, propylene glycol. The results of these different authors are comparable, since all give their toxic dose evaluated in volume and for one kilogram of animal weight. Lethal doses for the kilogram are obviously variable according to the type used, but this is completely unextraordinary. We must, however, point out that in the dog, the results of HANZLIK et al. lead to an almost equal estimation of the toxicity of the three glycols, when introduced venously.

E. Acute intoxication in animals. - The symptoms observed in animals with acute intoxication are renal (hemorrhagic nephritis), hepatic and cardiac lesions. This confirms the work of the first experimenters. For HANZLIK et al. (work cited), the usual symptoms, intramuscularly, are respiratory acceleration, loss of balance followed by general motor deceleration and, finally, coma and death. Venously, muscular excitation with trembling and convulsions are also observed. Such symptoms reported for ethylene glycol have also been observed with propylene glycol by SEIDENFELD and HANZLIK (work cited). These authors have also seen that moderate doses of propylene glycol provoke in particular a semi-comatose state lasting one to two days. For P.J. HANZLIK et al. (work cited), the depressive action of propylene and di-propylene glycols is an anesthetic action; ethylenes and di-ethylenes are, on the other hand, convulsion-causing.

TAEGER has also noted, in diethylene glycol, a diuretic action followed by anuresis in the final stage of intoxication. The urine eliminated after ethylene glycol (PAGE, HANZLIK), after propylene glycol (LEHMAN and NEWMAN), after glycerol may be hematuric. The phenomena of acute intoxication are observed most clearly when toxic products are administered intravenously. This is particularly true for

respiratory and circulatory phenomena. The first consist of acceleration of rhythm, increase in amplitude, then stopping of respiration. The second consist of a lowering of blood pressure, amplification of heart beats and cardiac arrest after cessation of respiration.

Among the afore-mentioned authors whose papers we have cited, some took the liberty of extrapolating for man the toxic doses obtained in different laboratory animals. Thus, HANZLIK and his colleagues (work cited, p. 394, 1931) in speaking of ethylene glycol, say: "it would seem that 77 grams (70 cm^3), might be injected intravenously without demonstrable effects in a 70 kg. man" and that(p. 393)"an intramuscular dose just causing symptoms, but resulting in ultimate recovery would be about 154 grams (140 cm^3); a fatal dose would be about 308 grams (280 cm^3)."

For propylene glycol, SEIDENFELD and HANZLIK (work cited, p. 114-115, 1932) declare, according to their results with the rabbit and the rat, that: "294 grams (280 cm^3) might be injected intravenously in a 70 kg. man without demonstrable effects." Intramuscularly, the same authors say: "transferring the results on intramuscular injection of propylene glycol in rats to an adult man of 70 kg., and assuming similar action, a dose just causing symptoms, but resulting in ultimate recovery, would be about 518 grams (490 cm^3) or more than a pound; a fatal dose would be about 1029 grams (980 cm^3) or more than 2 pounds."

We shall not discuss such reasoning; nonetheless, we feel it necessary to underline the boldness of such an extrapolation. Yet all experimenters know well that a toxicity result cannot be extrapolated even from one species to another of the same genus, especially from one order of Mammals (rodents) to another (man). Taken literally, the above extrapolations would be capable of provoking all kinds of casualties.

With these reservations well emphasized, it seems that propylene glycol is not very toxic for man, if we are to believe ALBRIGHT, BUTLER and BLOOMGERG [19], cited by HANZLIK, NEWMAN, etc. . .(work cited 14) who say that: "Such quantities, as 10 to 20 and even 40 cm^3 daily are said to be given to children for 1 to 9 months". May we add, however, that in the rat, venous injection of propylene glycol is followed by hematuria (WEATHERBY and HAAG).

CHRONIC EXPERIMENTAL INTOXICATION

1. <u>With ethylene glycol</u>. CH_2OH. CH_2OH. In 1931 HANZLIK et al. add ethylene glycol to the drinking water of their rats. In several days: 3 days for equal concentrations or concentrations greater than 10%; 5 days for 5% concentrations;

6 days for 3% concentrations and 14 days for 2% concentrations - they observed the death of the animals. Death occurred following absorption of 4 to 27 g. of glycol per kilogram of animal. After several months, the 1% concentration caused pathological changes: oxalurea, hematurea, alteration of tubuli with possible presence of crystals: phosphates, calcium carbonate and oxalate. The daily dose of 0.7 gram per kilogram for 120 to 130 days in the form of a 0.50% solution showed itself to be atoxic. HANZLIK et al. (work cited 1939) observed hepatic degeneration in a dog having taken repeated doses of glycol parenterally, the cells being reduced to an amorphous eosinophilous mass, and necrosis of the tubuli contorti in the kidneys.

That ethylene glycol was not endowed with glycogenic properties could be suspected; VELTMANN[20] had seen this in 1924 and PAGE (work cited) confirmed it.

2. Diethylene glycol. $OH.CH_2 - CH_2O.CH_2.CH_2OH$. The experiments of HAAG and AMBROSE (work cited) carried out according to the preceding technique, show that a 10% concentration of diethylene glycol given to animals proves fatal to them within eight days. Equal concentrations or concentrations less than 0.1% must be attained if no specific change is to be caused. Contrarily, with 10% glycerine survival and growth are as good as with the control animals. In 1935 LEPKOVSKI [21] et al., studying the nutritive value of the synthetic esters of natural fatty acids and of alcohols such as glycerol and the glycols under discussion here, concluded in favor of the toxic action of the esters of ethylene glycols and of the non-toxicity of those of propylene glycol. However, the esters of propylene glycol do not have the nutritive value of the esters of glycerol.

The papers of HOLCK [22], and those of GEILING, COON and SCHOEFFEL [23] on di-ethylene glycol confirm the above. HOLCK claims that chronic absorption of di-ethylene glycol, even at a concentration of 0.25%, causes rats to stop growing. WEATHERBY and WILLIAMS do not observe this phenomenon with dilutions of 2 and 3% in drinking water. HANZLIK et al., who operated on dogs, studied the histological lesions of chronic intoxication, these being the same as those reported with ethylene glycol and in conformance with the conclusions of GEILING and CANNON [24]. HANZLIK estimates, moreover, that in the dog, di-ethylene glycol requires 36 hours for elimination. The administration of repeated doses of this product, by the lesions thereby caused, gives rise to the accumulation of the poison in the blood.

3. Propylene glycol $CH_3 - CHOH - CH_2OH$. MIURA [25], according to SEIDENFELD and HANZLIK, had already studied propylene glycol on the rabbit in 1911. He found that it is eliminated in the form of a conjugate of this glycol with glycuronic acid. SEIDENFELD and HANZLIK (work cited 1932) found pyruvic and lactic acid in the intermediate metabolism of propylene glycol. Poured into the drinking water of

experimental rats, concentrations at 10% and lower, tested for 140 days, caused no harmful phenomenon. At a 10% concentration, the animals absorbed 13.2 g. of propylene glycol a day. These studies are confirmed by BRAUN and CARTLAND [26] who, however, note with the 10% concentration in drinking water, a temporary slowing down in growth for 10 days; after this period of time, normal continuation. Propylene glycol has the property of extremely rapid elimination; its speed of elimination would be 75% greater than that of ethylene glycol, whose own speed of elimination is 50% greater than that of di-ethylene glycol. Given this weak toxicity, HANZLIK made a partial substitution of propylene glycol for the hydrocarbon ration of the rat. When 25% of the hydrocarbon ration is thus substituted with propylene glycol, growth is impeded; below 25%, it is normal. Prior experiments (HANZLIK, et al. -27) on rats and those of NEWMAN et al. [28], on the isolated cat liver, show that propylene glycol is endowed with glycogenic power, while ethylene glycols are without it.

Conclusions. - It would seem to result from this bibliography that, if therapeutic use of glycols is envisaged, propylene glycol must be preferred to the ethylene glycols, by reason of its weaker toxicity, its glycogenic power and its speed of elimination.

II. PERSONAL FINDINGS

We should like to add several personal results to this bibliographic data. These results concern 1) local tolerance; 2) acute toxicity (see Addenda).

a) Local tolerance. - Our tests dealt with the action of glycols and of glycerol on the intestine: the rectum and the descending colon. The animal selected was the guinea pig. We used products that were either pure, or diluted in water to 60% or in the form of a 60% gelatinized mass. In this last case, before rectal insertion, the gelatinized mass was melted and injected at 37°. The guinea pig being maintained in dorsal decutibus, the medicine was introduced with the aid of a semi-hard rubber tube, mounted on a seringe and inserted 10 to 12 cm. into the rectum. The product is injected for 20 seconds. The tube is slowly withdrawn from the anus, kept closed between the thumb and index finger, for 5 minutes. Only males were tested.

Shortly after the anus is freed, defecation of matter found in the large intestine takes place. All of the products were active at this viewpoint; the most active being, in decreasing order of activity: glycerol, di-ethylene glycol, propylene glycol and glycol. The experiments were carried out with non-diluted products injected in equal molecular quantities.

If care is not taken to obturate the anus, defecation phenomena can be observed almost as soon as the product is inserted. In this case, of course, part of the liquid is rejected. Urinary elimination is also customarily observed, in all likelihood due to reflex-like phenomena resulting from functional intestinal excitation. This excitation itself seems to be of peripheral origin, since all the glycols tested and the glycerol have a very marked irritant action on the intestinal mucuous. Even diluted and at doses still moderate (about 0.5 cm^3 of pure product for 100 g.), often cause the expulsion of mucus sputum one-half hour to one hour after introduction into the intestine. The congestive manifestations which follow the introduction of glycols and glycerol in the intestine are sometimes such that hemorrhagic manifestations can be observed. The latter are characterized by a blood-tinged anal oozing and the expulsion of red currant jelly-like mucus. The introduction of the melted gelatinous mass is not excluded as a source of this irritating power. The condition of the intestine examined after the experimental animals were killed bears witness to these facts. It is void of feces over a length of 10 to 20 cm. beginning with the anus, the mucus is more or less red in color, at times clearly hemorrhagic according to the dose administered. Vascularization is localized in the regions reached by the liquid. Delimitation between the zone of lesion and the intact zone is always clear.

We, as several authors cited in the bibliography have, noted that glycerol is the most irritating of the bodies studied and that among the three glycols, ethylene glycol is the least irritating.

With regard to this, the sensitivity of the intestinal mucus and of the sub-mucus would not be parallel to that of the toxic coefficients on the animal <u>in toto</u>. We must add that the doses which aided us in specifying the local intestinal reaction are perfectly atoxic (at least for a single dose) on the organism in general, as we showed on the control animals. Allow us to specify that rectal injection of a comparable volume of salt water provokes in the control animals neither defecation, urination, nor elimination of mucosity. At the autopsy, the descendant colon is still filled, as it normally is, with matter arranged in chain-like fashion.

<u>General toxicity</u>. - This has been researched subcutaneously in the guinea pig and parenterally in the mouse. Let us first examine the latter and note that the density of the bodies we employed were the following:

Ethylene glycol - 1.119; di-ethylene glycol - 1.117; propylene glycol - 1.036; glycerol - 1.260.

<u>Toxicity in the mouse</u>. - The animals (1) fast for three or four hours; they are given the various products <u>per os</u>, in pure state; insertion is made by means of

a syringe mounted on a small metal tube. The injections are counted in volume; the following figures are given for one mouse, and we have noted for each mouse-dose the corresponding dose for one kilog. of animal. We establish this kilog. ration not because we attribute a particular value to it, but so that our results can be confronted with those of authors so doing.

PRODUCTS	MAXIMUM DOSES survival 100% in cm^3 (In cc. for 1,000 g.)	LETHAL DOSE 100% in cm^3
Ethlyene glycol	0.10 to 0.13 (5 to 6.5/kg)	0.26 to 0.30 (13 to 15/kg)
Di-ethylene glycol	0.20 to 0.28 (10 to 14/kg)	0.30 to 0.37 (15 to 18.5/kg)
Propylene glycol	0.30 to 0.37 (15 to 18.5/kg)	0.42 to 0.60 (21 to 30/kg)
Glycerol	0.30 to 0.37 (15 to 18.5/kg)	0.75 to 0.90 (37.5 to 45/kg)

It can be concluded from this table that parenterally, in the mouse, when basing oneself on the volumes of glycol and glycerol supported by this animal, the compounds can be classed by decreasing toxicity, in the following manner: glycol, di-ethylene glycol, propylene glycol, glycerol.

When the toxic volumes are brought back to their weights, the toxic weights 100%, for each one of these bodies will be the following for one kilogram of animal: Glycol - 14 g. 50; diethylene glycol - 16 g. 7; propylene glycol - 21 g. 7; glycerol - 47 g. 80.

We can say that a molecule of each one of these bodies will contain X-number of toxic-kg units, this toxic unit being defined by the weight of each compound which, given per os, would be 100% lethal. Still referring to the kilogram of mouse, we have:

Ethylene glycol (M.W. 62) contains 4.2 toxic units.
Di-ethylene glycol (M.W. 106) contains 6.3 toxic units.
Propylene glycol (M.W. 76) contains 3.4 toxic units.
Glycerol (M.W. 92) contains 1.9 toxic units.

These documents allow us to compare the reciprocal toxicity of each of the compounds studied according to their molecular toxicity. If we make the toxicity of glycol equal 1, that of di-ethylene glycol equals 1.5, that of propylene glycol equals 0.81 and that of glycerol equals 0.45.

This representation of the compared toxicity of glycols and glycerol (ethylene glycol being taken as a unit) does not change the order which resulted from the

comparison of the toxic volumes. We believe, however, that the system adopted by us, taking into account both the density and the molecular weight of the products studied, represents the real toxicity and not the relative toxicity.

Toxicity in the guinea pig. - We were mainly interested in the compared toxicities of the bodies injected subcutaneously. Here again, the results are given in volume and brought back to a kilog. of animal.

PRODUCTS	In cc. for 1,000 g. MAXIMUM DOSES survival 100%	SURVIVAL 50%	MINIMUM DOSES 100% dead
Ethylene glycol	10	12.5	15
Di-ethylene glycol	10	12.5	15
Propylene glycol	12.5		15
Glycerol	10		12.5

In the guinea pig, after subcutaneous injection of the above bodies, precocious convulsive disturbances are frequently observed with lethal doses of glycerol, while the glycols show, rather, a general depressing action. Propylene glycol in particular clearly retards reflexes of posture. The acute lesions manifest themselves by vasodilatation and generalized congestion, sometimes by pulmonary oedema and haematuria. There are no local lesions (necrosis, abcedation) but a melted, gelatinous mass of conjunctive tissues are found between skin and muscle at the autopsy.

According to the figures in the above table, if the volume causing death 100% is referred to, glycerol stands at the head of the lsit, the three other bodies being equal. If the weight of the products are taken to differentiate toxicity, propylene glycol would come first, followed by ethylene and di-ethylene, with glycerol at the bottom of the list. Determining as above the toxic units/kilog. guinea pig contained in one molecule, the following are found per molecule: glycol - 3.7 units; di-ethylene - 6.3 units; propylene - 4.8 units; and for glycerol - 4.8 units.

By increasing order of toxicity, glycol being 1, the bodies are thus ranked in the following manner: ethylene glycol (1); propylene glycol and glycerol (both 1.29); di-ethylene glycol (1.7).

These facts which rank glycerine as being more toxic than ethylene glycol and of equal toxicity with propylene, di-ethylene glycol maintaining first place, as in the mouse parenterally before, these facts, I say, must not surprise us. In fact, the considerable difference in toxicity of glycerol when applied parenterally, subcutaneously or intravenously has been known for a long time. In the first case, it is not toxic and considerable quantities may be absorbed or caused to be absorbed.

DUJARDIN-BEAUMETZ and AUDIGE (1879) knew that the toxicity of glycerine depended on the method of introduction. Adolf OSTWALD [29] who provided us with the opinion of DUJARDIN-BEAUMETZ and AUDIGE, adds that the non-toxicity of glycerine per os comes in all likelihood from the fact that this body is combined either in the intestinal wall or in the liver with fatty acids to form fat.

CONCLUSIONS

Di-ethylene glycol is the most toxic of the glycols which we studied; when introduced parenterally in the mouse, propylene glycol was the least toxic.

Subcutaneously, di-ethylene glycol is still first in the guinea pig, with propylene glycol second.

Parenterally, the toxicity of glycerol is clearly lower when compared to that of the three above glycols. Subcutaneously, the toxicity of glycerol approaches that of propylene glycol.

Rectally, the three glycols studied and the glycerol are clearly irritating to rectal and intestinal mucus.

Concerning the possible use of glycols intestinally, it must be recalled that the rectum, for medicine which stays in particular in the anal region, permits direct absorption into circulation through the hemorrhoidal veins. In fact, the mid and lower hemorrhoidal veins, through the intermediary of the internal and primitive iliac veins, merge with the lower vena cava, bypassing the liver.

ADDENDA TO THE BIBLIOGRAPHY

a) HANLIK and NEWMAN's assertion that propylene glycol is endowed with glycogenic power seems to justify the opinion of HUNT [30], who writes: "It is possible that this glycol is a true food, if ethynic alcohol is admitted as being one."

b) According to FAIRLEY, LINETON and FORD MOORE [31], the toxicity of ethylene glycol is due to an impurity probably analagous to dioxane. The great toxicity of dioxane can explain the ethylene glycol and di-ethylene glycol fatalities. G.C.L. BROWN [32], whose opinion we report above, judges that the administration of glycols must be done very carefully. Death by intoxication with these compounds is sudden, and the cumulative effect of these bodies can be anticipated. Thus, the problem of their therapeutic use must be extensively and carefully studied.

c) In the December, 1938 (33) issue of the Journal of the American Pharmaceutical Association, a "glycol symposium" is announced during which the question of glycols is to be discussed from the chemical, physiological, industrial and pharmaceutical points of view. I have been able to read the report of A.G. DUMEZ [34] in which the reporter discussing the question of glycols and their pharmaceutical use examines their value as solvents; he says, among other things, that propylene glycol is currently employed in place of ethylene glycol. Propylene glycol was used (ALBRIGHT [35]) as a vitamin D solvent.

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PROPYLENE GLYCOL: RATE OF METABOLISM ABSORPTION, AND EXCRETION, WITH A METHOD FOR ESTIMATION IN BODY FLUIDS

A. J. LEHMAN AND H. W. NEWMAN

From the Department of Pharmacology and Division of Neuropsychiatry, Department of Medicine, Stanford University School of Medicine, San Francisco

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The glycols have developed rapidly during the last few years as solvents for technical purposes. Their use as vehicles for medicinals, pharmaceuticals, beverages, etc., was not exploited (1, 2, 3) until ethylene and propylene glycol were brought to notice as a solvent for a bismuth product introduced for the treatment of syphilis (4). Propylene glycol, because of its low toxicity (4, 5), chemical indifference to most substances, and excellent solvent properties, which correspond more closely to water than most organic substances, has a wider range of usefulness as a solvent than any of the glycols so far investigated.

Comparatively little is known regarding the metabolic end products of propylene glycol and its general behavior in the organism. Miura (6) is the only investigator, as far as we are aware, to study its metabolism. His work was primarily concerned with the isolation of propylene glycol-monoglycuronic acid from the urine of a rabbit which had received a small dose of propylene glycol. It has been assumed (4) that the greater part of the glycol is completely burned to carbon dioxide and water. However, a considerable range of substances, including lactic and pyruvic acids, are possible as intermediary oxidation products (12). The potential effects of these products may not be a factor in the action of propylene glycol unless the glycol be taken continuously as might be the case if it were employed as a vehicle in the manufacture of beverages.

The increasing use of propylene glycol and the negative char-

acter of our knowledge of its effects suggested the need of a systematic pharmacological study covering the essential features. This report is concerned with the description of a method for the estimation of propylene glycol in body fluids, as well as observations on the absorption and excretion, diuretic effects, rate of disappearance from the blood stream, and some comparisons with ethyl alcohol. Dogs, rabbits, and chickens were used. The glycol[1] was given intravenously as 33⅓ and 50 per cent solutions in normal saline solution (0.9 per cent sodium chloride), and orally as a 50 per cent solution in water to fasting animals.

TOXICITY

The low toxicity of propylene glycol has been stressed by all investigators (4, 5, 9). Most of the acutely fatal doses were determined by injecting the undiluted glycol intramuscularly, subcutaneously, or intravenously within a very short period of time. Since large doses are necessary to produce a fatal outcome, the great distension of the tissues and the assault on the circulation, which must occur under these conditions, must be considerable factors in the toxic effects. We determined the fatal dose in chickens using slow intravenous infusion and administering 0.5 cc. of 50 per cent propylene glycol in normal saline solution per minute per kilogram body weight. By this method, the fatal dose was found to be 25.7 cc. per kilogram of propylene glycol as an average for 3 chickens. This is considerably higher than the values found by other investigators, and approximately 2.5 times the fatal dose of ethyl alcohol (10.4 cc. per kilogram) similarly determined in 4 chickens.

HEMOLYTIC ACTION

Propylene glycol, when injected intravenously as a 33⅓ per cent solution in normal saline solution produces hemoglobinuria, the blood cells apparently being destroyed osmotically in passing through the glycol at the site of injection. The hemolytic action was studied in vitro by a procedure similar to testing red cell

[1] The propylene glycol used in this study was a specially purified product kindly supplied by the Carbide and Carbon Chemicals Corporation, New York City.

fragility. One cubic centimeter of whole rabbit blood was added to 10 cc. of propylene glycol in concentrations varying from 0.2 to 50 per cent with and without sodium chloride. The tubes were inspected at the end of 3 and 24 hours. Propylene glycol in water solution produced immediate hemolysis in all concentrations. When prepared with normal saline solution (0.9 per cent sodium chloride), only those concentrations above 30 per cent produced hemolysis at the end of 3 and 24 hours. When the end concentration of sodium chloride was maintained at 0.9 per cent, complete protection for 3 hours was afforded in all tubes up to a concentration of 40 per cent propylene glycol, although partial hemolysis was present in the 40 per cent concentration at the end of 24 hours.

METHOD OF ANALYSIS

In a preliminary report (7) propylene glycol was determined in body fluids by reduction of a strongly acid solution of potassium dichromate. The accuracy of this method was influenced to a certain extent by variations in blood sugar and the concentration of the urine. Since completion of the earlier report, another oxidizing agent has been employed which was found to be more suitable, namely, sodium periodate. The method described below is an adaptation of the procedure employed by Fleury and Fatome (8) for the determination of glycerin. The essential details and steps were as follows:

Equipment. Fifty and 125 cc. Erlenmeyer flasks; 50 cc. volumetric flasks; 5 cc. pipettes; 2 50 cc. volumetric burettes; several small funnels and filter paper of suitable size; several small test-tubes of 10 x 100 mm.; ice-box; hot-air oven kept at 95°C.

Reagents. Ten per cent trichloracetic acid; powdered barium hydroxide; 95 per cent alcohol; phenolphthalein, 1 per cent in alcohol; sulphuric acid, 20 per cent by volume; sulphuric acid, N/1 (approximate); N/10 sodium hydroxide (approximate); starch suspension, 1 per cent, boiled; sodium bicarbonate, saturated solution (8 per cent); potassium iodide, 20 per cent solution.

Standard solutions. M/10 sodium periodate. Dissolve 2.94 grams in 30 cc. N/1 sulphuric acid, make up to 100 cc. with distilled water; N/10 arsenious acid. Dissolve 4.95 grams in 150 cc. distilled water with 15 grams sodium carbonate. Add 25 cc. normal acid and make up to 1000 cc. N/10 iodine. Dissolve 12.692 grams in 1,000 cc. distilled water with the aid of 18 grams potassium iodide. Each cubic centimeter of the periodate solution is the equivalent of 3.8 mgm. propylene glycol.

PREPARATION OF THE PROTEIN AND SUGAR-FREE FILTRATE FROM BLOOD

Three cubic centimeters of blood are pipetted into a 50 cc. Erlenmeyer flask and 7 cc. of 10 per cent trichloracetic acid added. Filter through a small filter and collect filtrate in a small test-tube. Five cubic centimeters of the filtrate (representing 1.5 cc. of blood) are pipetted into a 50 cc. volumetric flask containing 1 gram powdered barium hydroxide and thoroughly mixed by agitation. The mixture is allowed to stand in the ice-box for 2 hours, removed, and made up to 50 cc. with 95 per cent alcohol, then returned to the ice-box and allowed to stand for 12 to 24 hours. At the end of this period, the flask is removed, the contents centrifuged, and 40 cc. of the clear supernatant liquid transferred to a 125 cc. Erlenmeyer and the level of the fluid marked on the flask with a wax pencil. A drop of phenolphthalein solution is added and the contents of the flask made acid with 20 per cent sulphuric acid. Forty cubic centimeters distilled water are now added and the flask placed in the hot-air oven maintained at 95°C. and allowed to remain there until the volume has been reduced to the original 40 cc. The flask is then removed, the contents neutralized with N/10 sodium hydroxide, 1 drop of N/1 sulphuric acid added, and the propylene glycol titrated with periodate.

PREPARATION OF THE URINE

Five cubic centimeter samples of urine are used and treated exactly like blood except for omission of treatment with trichloracetic acid. If the urine is suspected of containing large amounts of propylene glycol, dilutions of 1:10 must be used.

TITRATION

Pipette 5 cc. of the standard periodate into the flasks containing the samples to be tested for propylene glycol and into a blank flask carried through the same procedure as the samples for propylene glycol, and place in the ice-box for 15 minutes. Remove the flasks and add to each 10 cc. of 8 per cent sodium bicarbonate solution and exactly 15 cc. of the standard arsenious acid solution and 1 cc. of 20 per cent potassium iodide solution. Let stand at room temperature for 15 minutes and titrate with the standard iodine solution after the addition of 1 cc. starch suspension. The blank value is subtracted from that for the sample taken. The calculations are made as follows:

Blood. The original volume of 3 cc. of blood is reduced by $\frac{1}{2}$, to 1.5 cc. in the precipitation of the proteins, and again reduced by $\frac{1}{5}$, i.e., to 1.2 cc. in the removal of the sugar. The calculation is then:

$$\frac{\text{cc. N/10 iodine used in sample minus cc. used in blank} \times 3.8}{1.2} = \text{mgm. per gram propylene glycol.}$$

Urine. The original volume of urine is reduced by $\frac{1}{5}$ during the process of purification, and the calculation is then:

$$\frac{\text{cc. N/10 iodine used in sample minus cc. used in blank} \times 3.8}{4} = \text{mgm. per gram propylene glycol.}$$

This result must be multiplied by the number of times the urine is diluted.

ABSORPTION

The absorption of propylene glycol was studied in 4 dogs. The glycol was given as a 50 per cent solution in warm tap water by stomach tube to fasting animals. The concentration in the blood, which reflects the rate of absorption, was followed for variable periods after doses of 2, 8, and 12 cc. per kilogram body weight. Absorption and diffusion throughout the tissues occurred promptly. The maximum concentration of blood propylene glycol was reached in 30 minutes with the 2 cc. dose (fig. 1, curve II). With the 8 and 12 cc. doses, 2 to 4 hours were required for complete absorption (fig. 1, curves V and VI). The rapidity of absorption from the gastrointestinal tract may also be shown by comparing the blood propylene glycol concentrations following gastric and intravenous administrations in the same dog (fig. 1, curves I and II). It is readily apparent that the concentrations run almost parallel, indicating prompt absorption and distribution after oral administration. Six cubic centimeters per kilogram introduced into the isolated stomach gave a blood propylene glycol of 124 mgm. per cent at the end of 3 hours indicating only slight absorption from this organ (fig. 1, curve III).

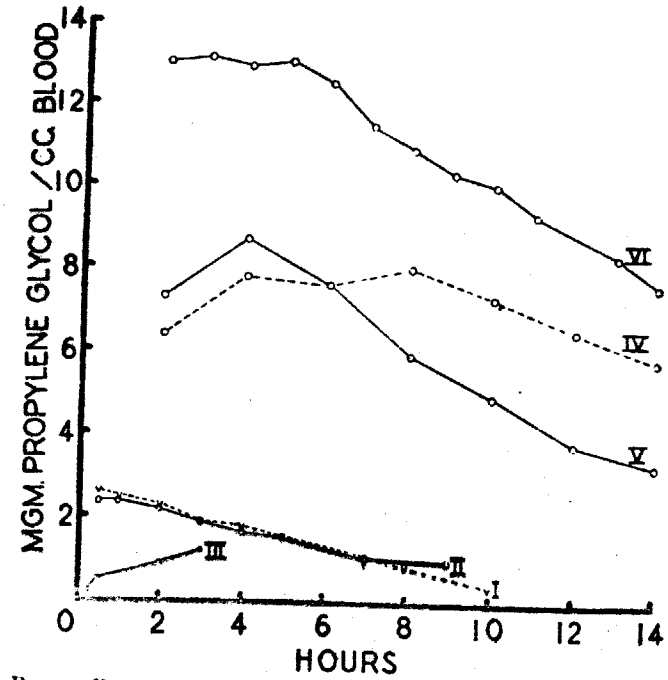

FIG. 1. BLOOD PROPYLENE GLYCOL CONCENTRATION IN DOGS FOLLOWING THE ADMINISTRATION OF PROPYLENE GLYCOL ORALLY AND INTRAVENOUSLY

Curve I, 2 cc. intravenously; curve II, 2 cc. orally; curve III, 6 cc. into isolated stomach; curve IV, 8 cc. orally with pentobarbital anesthesia; curve V, 8 cc. orally; curve VI, 12 cc. orally.

The influence of anesthesia on absorption was also studied. Eight cubic centimeters per kilogram was administered to a dog

previously anesthetized with pentobarbital, 35 mgm. per kilogram (fig. 1, curve IV). When compared with a similar dose (fig. 1, curve V) in the same dog unanesthetized, considerable delay in absorption occurred. This was probably due to a depression of gastric motility incidental to the general depression from the anesthetic. The slower disappearance of propylene glycol from the blood stream under these conditions may well be due to depression of kidney function.

RATE OF FALL OF PROPYLENE GLYCOL IN THE BLOOD

Thirteen observations were made on 3 dogs, 2 chickens, and 3 rabbits. Propylene glycol was administered orally and intravenously in dogs and intravenously in rabbits and chickens. Blood samples were obtained from a leg vein in dogs, by cardiac puncture in rabbits, and by puncture of the comb in chickens. A typical series of curves showing the rates of fall of blood propylene glycol following various doses is presented in figure 1. It is seen that the rate of fall is related to the concentration in the blood. This is in direct contrast to ethyl alcohol which falls at a constant rate regardless of the blood concentration (10, 11). The slope of the curve has a tendency to reach a plateau and fall more slowly as the concentration approaches a low level.

A number of factors may operate to remove the propylene glycol from the blood stream. The rate of combustion of the glycol may be directly proportional to the concentration. If this assumption were correct, the rate of fall in mgm. of propylene glycol per gram of blood per hour following a 4 cc. dose should be twice that following a 2 cc. dose. When this value is calculated for the various doses for the different species, this assumption does not hold. Although the rate of fall is greater with high concentrations, there is no constant relation between concentration and rate of fall. Thus some other mechanism must be responsible for the rate of disappearance of the glycol from the blood.

It is quite possible that when the kidney is faced with a high concentration of propylene glycol, the glycol may be excreted in high concentration in the urine. This was found to be the case, since it was possible to recover propylene glycol, as identified by

its refractive index and boiling point, from the urine. From 12 to 45 per cent of the total dose administered was accounted for in this way.

The part played by the kidney in the rate of fall of blood propylene glycol was demonstrated in another way. The rate of fall

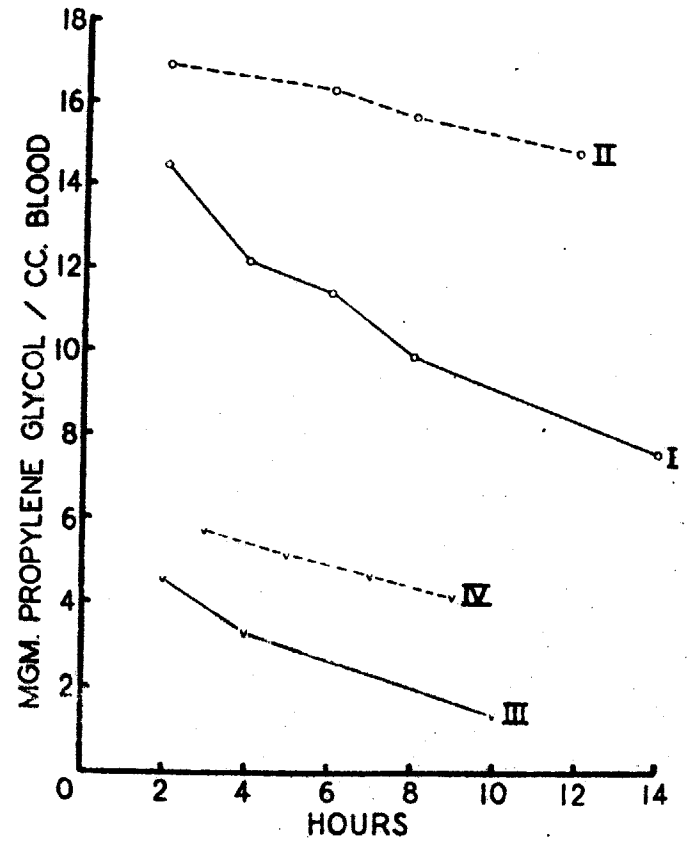

FIG. 2. RATE OF DISAPPEARANCE AND CONCENTRATION OF PROPYLENE GLYCOL IN IN THE BLOOD OF NORMAL AND NEPHRECTOMIZED DOGS

Curve I, 12 cc. orally in a normal dog, curve II, 12 cc. orally in the same dog after nephrectomy; curve III, 4 cc. intravenously in a normal dog; curve IV, 4 cc. intravenously in the same dog after nephrectomy.

was followed in 2 dogs before and after the removal of the kidneys. In figure 2, curve I shows the concentration of propylene glycol in the blood of a normal dog after receiving 12 cc. per kilogram gastrically. The highest value was reached at the end of the second hour, or 1444 mgm. per cent. This fell to 765 mgm. per

cent in 14 hours, or a fall of 679 mgm. per cent. When the kidneys were removed, the blood propylene glycol rose to 1691 mgm. per cent in 2 hours and fell to 1490 mgm. per cent in 12 hours, or a fall of only 200 mgm. per cent (curve II).

In the second dog, 4 cc. per kilogram were given intravenously to the intact animal. The blood propylene glycol attained a maximum of 457 mgm. per cent and fell to 141 mgm. per cent in 10 hours, or a total drop of 316 mgm. per cent (curve III). When the dog was nephrectomized, a much higher concentration was maintained during the time of observation, and the rate of fall was likewise less rapid. The highest value was 569 mgm. per cent which fell to 414 mgm. per cent, or a drop of 155 mgm. per cent in 9 hours (curve IV). Thus the importance of urinary excretion of propylene glycol was demonstrated.

The above experiments also throw some light on the rate of combustion of propylene glycol in the animal body. Calculating the rate of fall of the glycol in milligrams per gram of blood per hour in the nephrectomized dogs, the value for the 12 cc. gastric dose becomes 0.2 mgm. and for the 4 cc. intravenous dose, 0.26 mgm., which is a fairly close agreement. This indicates that propylene glycol behaves similarly to ethyl alcohol, and is in all probability burned at a constant rate regardless of concentration in the body. It is interesting to note that propylene glycol can reach a concentration of over 1.6 per cent in the blood and tissues when administered orally without producing hemolysis, with complete and uneventful recovery within 24 hours.

EXCRETION AND DIURETIC EFFECT

For the purpose of determining more accurately the amount of propylene glycol excreted by the kidneys, 7 experiments were made on 4 dogs. Doses of 2, 8, and 12 cc. per kilogram were administered gastrically and the urinary excretion followed for 24 to 48 hours. In 2 experiments, after administering 2 cc. doses, an average of 12 per cent of the total dose was excreted in the urine in 48 hours. With the 8 cc. dose (3 experiments), 39 per cent was found in the urine at the end of 48 hours, and 36 per cent (2 experiments) of the total administered for the 12 cc. dose, in 24 hours. The concentration of propylene glycol in the urine may reach 5 per cent with the higher doses.

A diuretic effect was observed in the same dogs. Two cubic centimeter doses produced no diuretic action, only 100 cc. of urine being excreted during a 48-hour period. Eight cubic centimeters increased the urine output by about 8 times that observed with the smaller dose under the same conditions, actually 850 cc. in 48 hours as an average of 3 experiments. Twelve cubic centimeters in one dog produced a diuresis of over 2 liters in 24 hours.

INTOXICATING EFFECT

The action of propylene glycol on the central nervous system in dogs is similar to that of ethyl alcohol, although larger doses are necessary to bring about the effects. With the higher doses, the control of muscles becomes less and less sure and the motions requiring the most coördination become affected first. The depression finally passes into somnolence. In comparing the degree of drunkenness with that produced by alcohol, it was found that the concentration of propylene glycol must approach about 3 times the concentration of alcohol necessary to produce a similar degree of narcosis. Thus about 350 mgm. per cent of alcohol will produce a similar degree of depression as is produced by 1100 mgm. per cent of propylene glycol.

CONCLUSIONS

1. Propylene glycol in body fluids may be estimated by oxidation with sodium periodate after precipitation of glucose by barium hydroxide in alcoholic solution.

2. The acute toxicity of propylene glycol by intravenous injection is less than half that of ethyl alcohol.

3. Hemoglobinuria does not occur after ingestion of large doses of propylene glycol.

4. Propylene glycol is rapidly absorbed from the gastrointestinal tract. Little absorption occurs in the stomach. After absorption, rapid distribution occurs throughout the tissues.

5. It is probable that combustion of propylene glycol in the body proceeds at a constant rate, irrespective of dosage, the more

rapid fall in blood propylene glycol at higher concentrations being accounted for by increased urinary excretion, which may amount to 45 per cent of the total dose in 24 hours.

6. The narcotic action of propylene glycol is about one-third that of ethyl alcohol.

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THE NUTRITIVE VALUE OF THE FATTY ACIDS OF LARD AND SOME OF THEIR ESTERS*

By SAMUEL LEPKOVSKY, ROY A. OUER, AND HERBERT M. EVANS

(From the Institute of Experimental Biology, University of California, Berkeley)

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The increasing importance of studies of the biochemical behavior of fats seems certain to lead to the progressive decline of the use of natural fats in nutrition research because of their complexity. The saturated fatty acids and the unsaturated fatty acids as well as the non-saponifiable matter are now known to possess different biological activities, and the natural fats must be saponified to separate these components. After saponification, the fatty acids may be fed as such, resynthesized into the glyceride, or esterified with some other alcohol such as methyl or ethyl alcohol, propylene glycol, ethylene glycol, or diethylene glycol.

Information bearing on the nutritive value of these esters as compared with fatty acids or with one another is entirely too meager. Only very recently Cox (1) has shown that the ethyl esters of lard are definitely inferior to the natural lard. The lard and its ethyl esters constituted 55 per cent of his diet. Takahashi (2) fed stearic, palmitic, lauric, and oleic acids at levels of 10 per cent and compared them with their glycerides. In all cases the glycerides were superior to the fatty acids, even when the fatty acids were fortified with the glycerol necessary for the corresponding glycerides. Why the glyceride should be superior to the fatty acids plus the glycerol when these are the compounds formed in the intestine upon hydrolysis of the glyceride remains unexplained. Evans and Lepkovsky (3) found better growth with the glyceride of stearic acid than with the ethyl ester, but the ethyl ester of palmitic acid produced somewhat better growth than the glyceride. In these

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studies the fats were fed as 25 per cent of the diet. From the foregoing work it would appear that in general the glycerol ester is superior to the fatty acids or their ethyl esters. In this study the growth produced by lard, the lard fatty acids, or their ethyl esters was reinvestigated at the levels of 25 per cent and 60 per cent of the diet. The work was extended to include the synthetic glyceride, the methyl ester, and the esters of ethylene glycol, propylene glycol, and diethylene glycol. All conditions were kept strictly constant, so that the only variable studied was the character of the fat employed.

EXPERIMENTAL

Lard fatty acids were prepared by saponification with about 30 per cent alcoholic potassium hydroxide or with 40 to 50 per cent aqueous potassium hydroxide. The fatty acids were liberated with sulfuric acid, dried, and distilled in a high vacuum at a temperature ranging from 165–190°. Some of the fatty acids distilled at as high as 230°, but biological tests seemed to show no inferiority over those distilled at temperatures under 200°.

The methyl esters were prepared by refluxing lard for 24 hours with 1.5 volumes of absolute methyl alcohol containing 2 to 2.5 per cent dry hydrochloric acid. The esters were salted out with brine, washed with water, and taken up in ether, and then dried with anhydrous sodium sulfate, the ether removed, and the methyl esters distilled at 170–185°.

The ethyl esters were prepared similarly, 2 volumes of absolute alcohol containing 7 per cent concentrated sulfuric acid being used. The glyceride and various glycol esters were prepared by heating the fatty acids and alcohol at 210–230° with dry CO_2 bubbling constantly through the mixture, washing with water, and drying.

The esters were solid at room temperature with the exception of the methyl and ethyl esters, which were liquid. All of the compounds were colorless except diethylene glycol, which was light yellow, and propylene glycol, which was light brown.

Groups of six rats 21 days old were put on the experimental diets containing 25 or 60 parts of one of the fatty compounds. The composition of the diets used is shown in Table I.

Results with 25 Per Cent Fat—The growth curves of this series are shown in Chart I. Little difference exists between the syn-

thetic glyceride, lard, free fatty acids, and their ethyl and methyl esters. The ester of ethylene glycol and the low fat diet are inferior.

Results with 60 Per Cent Fat—A second lot of the same substances was prepared and with the exception of the methyl and ethyl esters fed as 60 parts of the diet. The results of this series are shown in Chart II. At this high level of fat, the diets containing the synthetic glyceride and the lard are the best. The

TABLE I

Composition of Diets Given in Parts by Weight

	Diet No.																	
	680	681	682	683	684	685	686	687	689	690	691	694	695	696	697	716	719
Casein* (commercial)	25	35	35	35	35	35	35	35	40	40	40	40	40	40	40	40	40
Sugar "	75	46	46	46	46	46	46	46									
Salt Mixture 185 (4)	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5
Lard		25							60								
Glyceride					25							60					
Fatty acid			25							60							
" " and glycerol				25							60						
Methyl ester							25							60			
Ethyl "								25							60		
Ethylene glycol ester						25							60				
Diethylene glycol ester																	60
Propylene glycol ester																60	

These diets were supplemented by 2 drops of cod liver oil and 1 gm. of brewers' yeast given daily except Sunday. (The brewers' yeast was generously supplied through the courtesy of the president, R. M. Allen, of the Vitamin Food Company, Inc., New York.)

* The casein was generously donated by the Golden State Company, Ltd.

diets with free fatty acids, with or without the glycerol, are definitely inferior and the ester of ethylene glycol the poorest.

Another series of feeding tests at the 60 per cent level was made which consisted of the lard fatty acid esters of glycerol, ethylene glycol, propylene glycol, diethylene glycol, ethyl alcohol, and methyl alcohol. The esters of ethyl and methyl alcohol were liquid and the diets containing them were fed in small quantities in small glass dishes, and complete consumption of all the con-

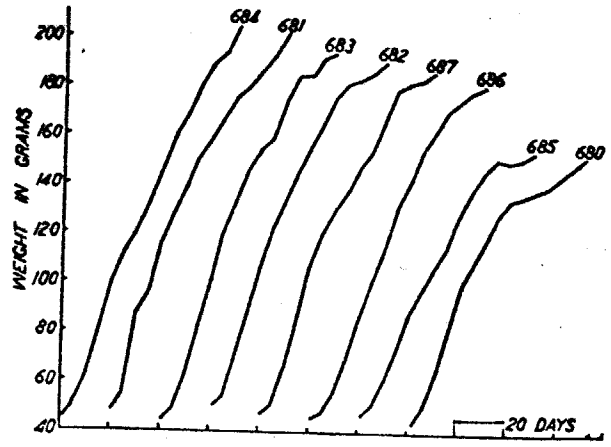

CHART I. Composite growth curves of six female rats each, showing the growth obtained when diets containing 25 per cent of lard fatty acids or their esters were fed. Diet 684, glycerol ester; Diet 681, lard; Diet 683, fatty acid and glycerol; Diet 682, fatty acids; Diet 687, ethyl esters; Diet 686, methyl esters; Diet 685, ethylene glycol esters; Diet 680, low fat controls.

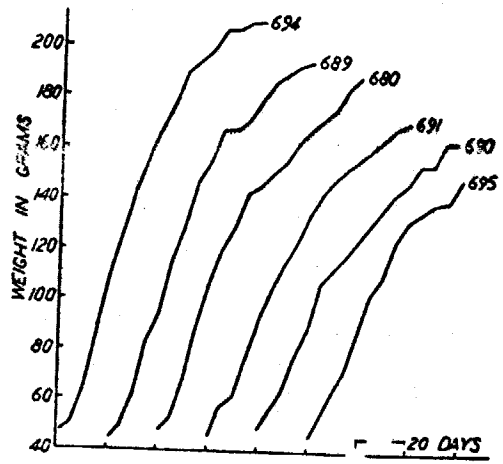

CHART II. Composite growth curves of six female rats each, showing the growth obtained when diets containing 60 per cent of lard fatty acids or their esters were fed. Diet 694, glycerol ester; Diet 689, lard; Diet 680, low fat controls; Diet 691, fatty acid and glycerol; Diet 690, fatty acids; Diet 695, ethylene glycol ester.

stituents was assured before additional diet was measured out. The results are shown in Chart III.

The glyceride (Diet 694) again proved the best, and the propylene glycol esters (Diet 716) definitely inferior. The results with the ethylene glycol esters (Diet 695) differed from those illustrated in Chart II. The mortality at the beginning was very great, five rats dying during the early part of the experiment. Six additional rats were added to the group, of which three died. The four survivors grew very poorly. The animals on the diethylene glycol

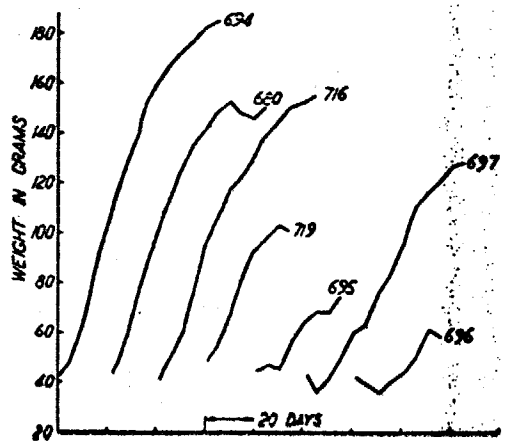

CHART III. Composite growth curves of female rats, showing the growth obtained with 60 per cent of different esters of lard fatty acids. Diet 694, glycerol ester (six rats); Diet 680, low fat controls (six rats); Diet 716, propylene glycol ester (six rats); Diet 719, diethylene glycol ester (six rats); Diet 695, ethylene glycol ester (four rats); Diet 697, ethyl ester (three rats); Diet 696, methyl ester (three rats).

esters (Diet 719) did fairly well at the beginning of the experiment, but after about 3 weeks on the diet they began to die, apparently from kidney damage. The rats receiving ethyl esters (Diet 697) did poorly at the beginning of the experiment, three out of six rats dying. Those that survived grew fairly well. The mortality among the rats receiving the methyl esters (Diet 696) was great and they never grew well at any time. The superiority of the glyceride (Diet 694) over other esters is marked.

Pathology[1]

When the experiment had been in progress 40 days, postmortem examination was made of two animals from each group. The only lesions observed grossly were in the kidneys of the animals fed with esters of ethylene glycol and diethylene glycol. These abnormal kidneys presented a striking appearance. They were definitely enlarged and considerably heavier than normal. They were milky white in color or mottled with white, the surface being studded with nodules and pits.

Microscopic sections showed marked dilatation of some of the collecting and some of the convoluted tubules. This was apparently the result of plugging of the collecting tubules by a yellowish crystalline deposit in the lumen, with a consequent hydronephrosis of the upper reaches of the tubular system. The epithelium of the collecting tubules was flattened. In the convoluted tubules the epithelial cells appeared to be normal in some portions of the sections. In other places they were clear and unstained as if the normal cytoplasm had been replaced by a substance which had dissolved out during fixation and staining, and the nuclei were flattened and pressed to one side as in fat cells. (Frozen sections stained with Sudan III failed, however, to show an excess of fat in the tubules.) In certain areas the convoluted tubules appeared to have completely degenerated and to have been replaced by a bluish substance resembling calcium or a calcium soap. In contrast to the marked abnormality of the tubules, most of the glomeruli appeared to have suffered little damage.

Microscopic sections of the kidneys of animals fed with the ester of propylene glycol did not show the characteristic lesions seen in the animals fed with esters of ethylene and diethylene glycol. No deposits were observed in the lumen of the tubules; the tubules were not noticeably dilated and the tubular epithelium appeared to be essentially normal.

[1] The authors wish to express their thanks for the cooperation of Professor Charles L. Connor of the Department of Pathology, University of California, and Professor Thomas A. Addis of the Department of Medicine, Stanford University, who kindly examined sections of the kidneys of these animals.

DISCUSSION

Of the different forms of lard fatty acids studied in this communication, that which occurs in nature, the glycerol ester, is the best, in spite of exposures to temperatures of 200–230° for 6 to 8 hours. The free lard fatty acids yield good results also, but definitely poorer than the glyceride at the 60 per cent level. No improvements resulted if free glycerol was added in amounts equal to that present in the glyceride. Since the glyceride is hydrolyzed in the intestinal tract to fatty acids and glycerol, it is difficult to understand the inferiority due to direct feeding of the glycerol and free fatty acids.

Of the other polyhydric alcohols used in esterification, the propylene glycol ester seems the best. Pharmacologically (5–7) it is apparently non-toxic, and should therefore be as good as the glyceride. This, however, was not the case. Ethylene glycol, however, is toxic (5–7), and the inferior growth and high mortality in the rats fed lard fatty acid esters of ethylene glycol may be explained on this basis. The rats fed the esters of diethylene glycol did so poorly as to suggest that the diethylene glycol is also toxic.

SUMMARY

1. Lard was saponified and its fatty acids collected, distilled, and esterified with glycerol to form "synthetic" lard; this was fully as satisfactory for the normal growth of rats as the untreated lard whether fed as 25 or 60 per cent of the diet.

2. The free fatty acids with and without glycerol additions were good sources of energy when fed as 25 per cent of the diet, but somewhat inferior to the glyceride at the 60 per cent level.

3. The methyl and ethyl esters were also good when fed as 25 per cent of the diet. When the methyl esters were fed as 60 per cent of the diet, growth was very poor. When the ethyl esters were fed as 60 per cent of the diet, there was an initial mortality but the survivors grew fairly well.

4. Of the esters of the dihydric alcohols, propylene glycol was the best. Ethylene glycol and diethylene glycol were both poor and histologically the kidneys of these animals showed grave lesions.

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Studies on Absorption and Metabolism of Propylene Glycol Distearate

C. L. Long, F. J. Domingues, V. Studer, J. R. Lowry, B. R. Zeitlin, R. R. Baldwin and R. Thiessen, Jr.

From the Research Center, General Foods Corporation, Tarrytown, New York

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Introduction

The literature is void of information on the metabolism of propylene glycol distearate.[1] With respect to other fatty acid esters of propylene glycol, Lepkovsky *et al.* (1) reported lower growth rates with rats on a 60% dietary level for 100 days as compared to glyceride esters. The above esters were prepared by saponification of lard and a resynthesis of the resulting acids after purification.

Balls and Matlock (2) studied the *in vitro* hydrolysis of fatty acid esters, including PGMS, in the presence of pancreatic enzyme. The compound was shown to be hydrolyzed to the extent of about 80% in 15 hr. at 40°C. Fodor (3) demonstrated, through *in vitro* studies on pancreatic enzyme homogenates, that propylene glycol dibutyrate, propylene glycol monobutyrate, and propylene glycol dipropionate undergo hydrolysis at a high initial velocity which decreases rapidly after a 5–8% cleavage of the ester. In this respect, it is reported that the hydrolysis rate is very similar to that of triglycerides.

The literature presents no data on the extent to which propylene glycol fatty acid esters are absorbed and digested or the metabolic fate of these esters.

The *in vivo* products of digestion, rate of uptake by the lymphatics, and mechanism of transport of PGDS are reported in a second paper in this series (4).

Metabolic studies in rats using C^{14}-labeled PGDS offer a direct means by which the course of the compound can be demonstrated within the

[1] Abbreviations used are: PGDS, propylene glycol distearate; PG, propylene glycol; PGMS, propylene glycol monostearate; and S, stearic acid.

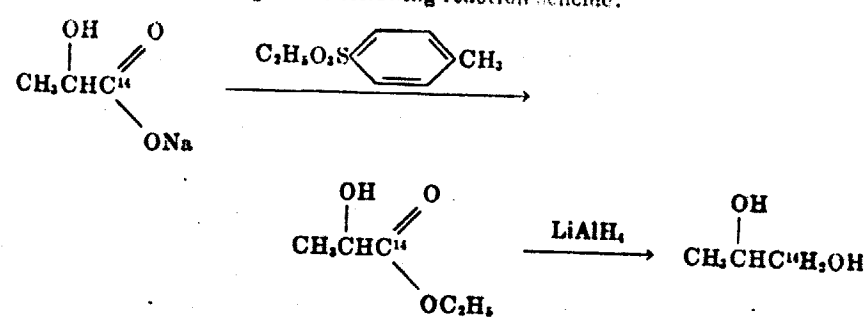
body. PGDS was synthesized as the C^{14} carboxyl-labeled stearate ester and as the 1-C^{14} propylene glycol stearate ester. The rate of uptake, course, and fate of the two fragments of the molecule were traced independently. In addition, a comparative study was made using a mixture of glyceride esters of labeled stearic acid and labeled free propylene glycol and stearic acid.

Experimental Methods

Synthesis of Radioactive Materials

1. *Propylene Glycol-1-C^{14} Distearate (PGDS*).*[2] One hundred and twenty-eight grams (0.452 mole) of stearic acid[3] including 2.75 millicuries (mc.) of stearic acid-1-C^{14}, 17.0 g. (0.223 mole) of propylene glycol, and 0.64 g. of camphor-β-sulfonic acid were reacted for 20 hr. with vigorous agitation at 150–160°C. During the reaction, nitrogen was sparged slowly through the mixture into a steam-heated condenser to remove the water of reaction and retain the propylene glycol. The fatty acid content at the end of the reaction was 7.2%. The reaction mixture was then dissolved in a minimum amount of boiling anhydrous methanol (about 14 l.) and filtered. The filtrate was allowed to crystallize overnight, and the crystals then separated. The product was crystallized three more times following this procedure. The crystals were then melted, and the methanol was removed by sparging nitrogen through the material at 100°C. under vacuum. Analysis for free fatty acid and uncombined hydroxyl groups by acetylation showed none to be present. Chromatographic studies of this material, using a solvent system capable of separating PGMS and PGDS, showed the presence of only one radioactive component (4). The over-all yield was 66%.

2. *Propylene Glycol-1-C^{14} (PG*).* Propylene glycol labeled with C^{14} in carbon-1 was prepared according to the following reaction scheme:



An amount of 37.5 g. of 60% sodium lactate (0.20 mole) was lyophilized for 8 hr.

[2] The asterisk (*) denotes the labeled portion of the molecule; the propylene glycol-1-C^{14} or the carboxyl C^{14} stearate moiety.

[3] Commercially purified stearic acid recrystallized from acetone: average molecular weight, 282.8, iodine No., 0.16.

at 2 mm. Hg pressure. Eighty-eight per cent of the water was removed. An alcohol solution of 6 mc. of sodium lactate-1-C^{14} was prepared by breaking the sealed vials containing the material, washing six times with boiling absolute ethanol, and concentrating the washings to 100 ml. This solution, along with 0.20 mole of ethyl *p*-toluenesulfonate, was added to the flask containing the dried sodium lactate, and the solution was refluxed for 5 hr. At the end of this tim 350 ml. of anhydrous ethyl ether was added through the condenser. The preci .tated salts were separated from the reaction mixture and washed six times wit' 50-ml. portions of anhydrous ethyl ether. The combined filtrates were then subjected to careful fractional distillation until the conditions of 102 mm. Hg pressure and a pot temperature of 51°C. were obtained. Two 10-ml. portions of anhydrous ethyl ether were washed through the rectifying column into the distilling flask. This solution was then added dropwise to an ethyl ether solution of lithium aluminum hydride (8.58 g. in 300 ml. of ether) under vigorous agitation at a rate such that gentle refluxing occurred in the condenser on the reaction flask. Four 25-ml. portions of ether were used to rinse the addition funnel and the transfer flask. Twenty minutes after the addition of the last washings, 120 ml. of water was added, slowly at first, to decompose the excess hydride. A rectifying column equipped with a constant-ratio distilling head was fitted onto the reaction flask, and the ether was distilled off at atmospheric pressure. An additional 200 ml. of water was added during the distillation. When all but traces of the ether were distilled over, the reaction mixture was cooled and neutralized to a pH of 7.0 with a 1:1 solution of orthophosphoric acid and water. The solution was then filtered from the copious precipitate through a coarse sintered-glass filter. The precipitate was washed with six 50-ml. portions of distilled water. The combined filtrates were then subjected to fractional vacuum distillation until practically all of the water was removed. Then 200 ml. of cyclohexane was added to the residue in the distilling flask, a Bidwell-Sterling trap and condenser attached, and the mixture was refluxed at atmospheric pressure until no further phase separation occurred in the condenser and trap. An amount of 16.5 g. of a water solution was collected in this manner over an 8-hr. period. This was carefully separated from the cyclohexane and labeled Soln. A. The rectifying column used in the above water distillation was washed with three 20-ml. portions of water into a 500-ml. flask and refluxed with cyclohexane as described above. An amount of 58.5 g. of a water solution, labeled B, was collected. Solutions A and B were analyzed for PG by periodic acid oxidation. Solution A yielded 38.5% PG, equivalent to 6.35 g. PG. Solution B yielded 5% PG, equivalent to 2.9 g. PG. This amounted to an over-all yield of about 60%. Using butanol-acetic acid-water (4:1:5) (5), resulting paper chromatograms showed only one radioactive component to be present.

While this work was in progress, a different synthesis for PG* was reported in which the starting material was radioactive carbon dioxide (6).

3. *Propylene Glycol-1-C^{14} Distearate (PG*DS)*. PGDS tagged with C^{14} in the carbon-1 of the glycol fragment was prepared as follows: A mixture of 49.1 g. (0.173 mole) of stearic acid, 57.9 g. of Soln. B (see synthesis of PG*) containing 2.9 g. of PG-1-C^{14}, and 0.06 g. of zinc chloride was subjected to fractional distillation until 53 ml. of the excess water from Soln. B was removed. Then 6.0 g. of Soln. A containing 2.3 g. of PG-1-C^{14} was added, and the reaction mixture was heated at 180°C. and 20 mm. Hg pressure for 10 hr. An air-cooled condenser was set in line between the reaction flask and a distilling head equipped with a cold-water condenser in order to return any escaping propylene glycol. The excess water and water of reaction was collected in a Dry Ice–acetone-cooled flask attached to the cold-water condenser. At the end of the reaction time, the product was cooled and crystallized three times from anhydrous methanol as described previously. After drying and clarifying, the product (19.9 g., 48% yield) analyzed for 0.08% free stearic acid and 0.0% uncombined hydroxyl groups.

4. *Mixture of Propylene Glycol-1-C^{14} Mono- and Distearates*. Amounts of 17.6 g. (0.062 mole) stearic acid[3] including 1.39 mc. stearic acid-1-C^{14}, 7.28 g. (0.096 mole) PG, and 0.035 g. stannous chloride were reacted at 170–180°C. for 5 hr. Vigorous agitation and a constant flow of nitrogen through the head space were applied during the reaction period. After cooling, the reaction mixture was dissolved in 300 ml. chloroform and washed five times with 100-ml. portions of water. The chloroform solution was dried with anhydrous sodium sulfate and then filtered. Most of the chloroform was then evaporated off on a hot plate, and the last traces were removed under vacuum. Analysis by periodic acid oxidation and acetylation indicated a composition of 68.7% PGMS*, 30.0% PGDS*, and 1.3% stearic acid. Chromatographic studies of this material, using the same method under (*a*) above showed the presence of two radioactive components.

5. *Mixture of Glycerol-1-C^{14} Mono-, Di- and Tristearates*. Amounts of 19.3 g. (0.068 mole) stearic acid including 1.39 mc. stearic acid-1-C^{14}, 2.08 g. (0.023 mole) glycerol, and 0.04 g. stannous chloride were charged into a 200-ml. flask. Nitrogen was slowly flushed through the headspace during the reaction period. The contents, under agitation, were heated at 220–230°C. for 7 hr. The reactants were cooled at the end of this time, and 2.08 g. (0.023 mole) glycerol and 0.04 g. sodium methylate were weighed into the flask. Agitation and heating at 200°C. were then continued for two more hours. The contents were allowed to cool at 90°C., and 4 drops of concentrated H_3PO_4 was added to destroy the catalysts. The reactants were then dissolved in 300 ml. chloroform. This solution was washed seven times with 150-ml. portions of hot distilled water and then dried with anhydrous sodium sulfate. It was bleached with 4 g. Magnesol at the refluxing temperature for 15 min. and then filtered. The chloroform was removed as described in the previous synthesis. Analysis by periodic acid oxidation and acetylation indicated 13.8% glycerol monostearate, 44.9% glycerol distearate, 41.2% glycerol tristearate, and 0.1% stearic acid.

Treatment of Rats

Preliminary absorption studies were made by methods outlined herein to determine whether preconditioning showed any alteration in alimentary absorption between rats fed for two weeks on a commercial dog meal[4] with and without 1% PGDS. No alteration in alimentary absorption was demonstrated.

[4] Gaines Dog Meal. Ingredients are: Meat and bone meal, corn germ meal, wheat flakes, soybean oil meal, wheat flour middlings, fish meal, fish liver and glandular meal, wheat germ meal 3%, animal fat 2%, dried skim milk 1%, brewer's dried yeast 1%, dried beet pulp 1%, alfalfa leaf meal 0.5%, iodized salt 0.5%, vitamin A feeding oil 0.5%, riboflavine feeding supplement 0.02%, D-activated plant sterol 0.006%.

Both male and female albino rats were used in these studies. Analysis of the data, however, showed no difference in absorption attributable to sex.

Charles River strain rats, weight 192 ± 20 g., were used. The animals were observed for gross manifestations of illness for a period of 2 weeks. Selected healthy animals were fasted for 16–19 hr. preceding the test but were provided with water at all times. During the experiment, they were provided with commercial dog meal[4] and water *ad libitum*.

The animals were weighed, intubated, and sacrificed with chloroform after varying time periods. The abdomens were opened immediately after sacrifice, and the entire gastrointestinal tract was removed after clamping the distal end of the descending colon and the proximal end of the esophagus. If defecation occurred during the digestion period, the feces were carefully collected and combined with the alimentary tract.

Studies on the rate and extent of absorption of PGDS were conducted using C^{14}-labeled PGDS* administered at zero time and at 1, 3, 5, and 8 hr. Four rats were used at each time interval. The absorption of all other radioactive compounds were determined only at the 3-hr. period.

Vehicle for Administration to Rats

The radioactive test compounds were prepared for administration to rats as components in a casein–sugar–water emulsion wherein the "fat" globules were dispersed by use of a Virtis high-speed homogenizer to an average diameter of 4 μ. No more than 1.0–1.5 ml. of the emulsions was administered to a rat. The average dose of radioactive compound administered was approximately 0.3 g./200-g. rat. All emulsions were prepared fresh immediately preceding gastric intubation in rats. Aliquots of the emulsion were diluted with an ethanol–chloroform (1:1) solvent mixture and combusted to determine the specific activity of the radioactive component intubated.

The test materials and methods of administration for the metabolic studies were identical to those used in the absorption study. In these experiments male rats were preconditioned to a diet of 1% PGDS in Gaines meal for 2 weeks. Two rats were given PGDS*, one rat each was given PG*DS, PG* plus nonradioactive stearic acid (equimolar amounts), PG plus S* (equimolar amounts), and a mixture of radioactive glycerol stearates. The animals were placed in a closed metabolism glass chamber, and continuous CO_2 measurements were recorded for 72 hr. Seventy-two-hour urine and feces collections were made.

At the conclusion of the 72-hr. period, animals were sacrificed with chloroform and autopsied. The dried organ weights were obtained.

Methods of Radioactive Assay

1. *Absorption Studies*. For studies on the gastrointestinal absorption rates, the tracts were dried in an oven at 100°C., pulverized in a mortar, and extracted with ethanol–chloroform (1:1). The filtrates were combined and made to volume with the extracting solvent. The gastrointestinal tract residues were saved for determination of residual radioactivity. Any residual radioactive material escaping recovery after a 12-fold extraction was considered to be incorporated and bound with the gastrointestinal tissue. Carefully measured amounts of the extract were introduced into combustion boats, and the solvent was removed by warming under an infrared lamp. The contents of the boats were then dry combusted to $C^{14}O_2$, which was collected in excess NaOH. The resulting carbonate was precipitated as the barium salt and transferred to a planchet. These were counted in an automatic "Nuclear" sample changer equipped with a "Nuclear D47" gas-flow counter. Counting total was such that maximum deviation was no greater than 1.5%. Corrections for absorption within all samples were those described by Domingues, Sarko, and Baldwin (7).

2. *Metabolism Studies*. (*a*) *Carcass and Organ Analysis*. The carcasses (total animal less specific organs) were autoclaved at 15 lb./sq. in. for 3 hr., homogenized to a smooth paste in a Waring blendor, and freeze-dried. The dried material was ball-milled for 3 days. The resulting powder was dry combusted.

The organs of the animal were weighed, dried in an oven at 100°C. overnight, and reweighed. The dry organs were pulverized in a mortar until a uniform powder was obtained. Aliquots were then removed and assayed in quadruple by dry combustion as previously described.

(*b*) *Urine and Feces Determination*. The urine was collected and made up to known volume. Duplicate 500-μl. samples were evaporated to near dryness under an infrared lamp and subsequently combusted for analyses. Seventy-two-hour feces were collected, weighed, dried at 100°C, and reweighed. The feces were ground to a fine powder in a mortar, and aliquots in quadruple were removed for combustion and subsequent assay.

(*c*) *CO_2 Assays*. A continuous record of the respired radioactive CO_2 was accomplished by a method similar to that reported by Tolbert *et al.* (8). Modifications of the above method were reported by Olsen and Mills (9). Room air was pulled through the metabolism chamber at a constant flow rate of 500 cc. min. The air was passed through Drierite and then through an ion chamber. The ion chamber was connected to a vibrating-reed electrometer and recorder which measured the rate of discharge of the chamber as a function of the amount of ionization produced by radioactive CO_2. The differential dmV/dt is calculated from the record chart and replotted versus time. The resulting differential curve is integrated to yield total millivolt change and this value is then reported as counts/min. from a previously determined calibration factor (Fig. 2).

In Vitro *Enzymic Hydrolysis Rate of Propylene Glycol Distearate*

The method of Balls and Matlock (2) was followed with some modifications. The glycerol–bile salts solution was prepared by mixing 30 g. of bile salts with 30 g. water and dissolving this in 300 ml. glycerol at 100°C.; 20.0 g. PGDS was dissolved in 217.5 g. of this solution at 90°C. The mixture was then cooled slowly with agitation to 30°C. and transferred quantitatively to a 500-ml. volumetric flask. Twenty-five milliliters of 1 *N* NH_4Cl–NH_4OH buffer and 5 ml. of a 2% $CaCl_2$ solution were added, made up to volume with water, and mixed thoroughly.

The contents of the volumetric flask were poured into a liter beaker equipped with glass stirrer, a thermometer, and pH electrodes. Then 3.04 g. steapsin (Eimer & Amend) was added. Temperature was controlled by a water bath set at 30°C., and the pH was maintained at 8.35.

Twenty-five milliliters of the emulsion was pipetted into 100 ml. of an ethanol-chloroform (1:1) mixture at time intervals of 0, 5, 30, and 45 min., and of 1, 3, and 19¼ hr., and titrated immediately with 0.1 *N* aqueous KOH.

The inflection points of the titration curves were all at pH 10.6. The per cent hydrolysis was calculated by dividing the moles of free fatty acid analyzed for by the moles initially available.

Results And Discussion

The *in vitro* study indicates that PGDS is capable of undergoing hydrolysis by steapsin to yield free fatty acid (Fig. 1). A comparative study of glycerol tristearate and PGMS was made by Balls and Matlock (2). They showed that after 15 hr. PGMS was 80% hydrolyzed, and, after 4 hr., glyceryl tristearate was 68% hydrolyzed. PGDS was found to be 60% hydrolyzed in 15 hr. and more than 80% hydrolyzed in 19 hr.

The rate of absorption of PGDS* was determined by the residual radioactivity of the gastrointestinal tract extracts and is shown in Table I. The average per cent absorption of PG*DS and S* plus PG at 3 hr. is also given in Table I. The total extent of absorption of PGDS* in the rat was found to be 33% of the administered dose in 8 hr.

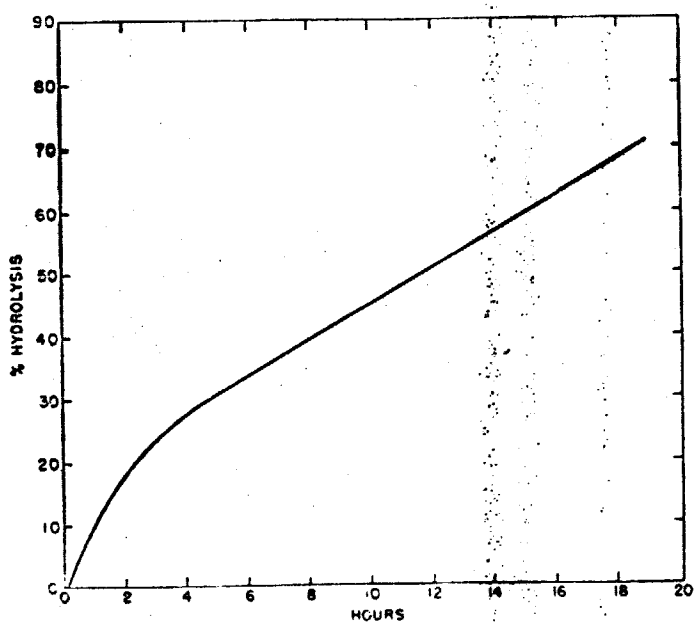


Fig. 1. *In vitro* hydrolysis of PGDS by steapsin at 30°C.

TABLE I

Intestinal Absorption of Radioactive Compounds in Rats

Component	No. of rats	Absorption time	Average fasted body weights	Average administered dose	Average recovered dose	Average per cent total dose absorbed
		hr.	*g.*	*mg.*	*mg.*	
PGDS*	2	0	182	319	313	2.0
	3	1	190	342	304	11.1
	4	3	190	301	233	22.9
	5	5	193	354	312	19.0
	4	8	188	153	102	33.3
PG*DS	3	3	181	367	275	25.0
S* + PG	4	3	202	198	133	33.0

Comparative studies using C^{14}-labeled stearic acid have demonstrated an absorption of 33% in 3 hr., which value is greater than that determined for PGDS* at this time period. Bloom *et al.* (10), using stearic acid-1-C^{14}, reported 36–54% absorption of the labeled acid in 19 hr. when administered in corn oil. This would indicate that the initial limiting factor governing PGDS* absorption is the hydrolysis rate of the ester. That the low rate for PGDS* absorption is linked to the hydrolysis of the ester molecule is also shown by comparative absorption studies undertaken at the 3-hr. digestion level using PG*DS. Here it was demonstrated that about 25% of the radioactivity is absorbed in 3 hr. After this initial 3-hr. period, the governing factor for absorption is that of the stearic acid moiety. This is evident from the results (see Table II) in which PG*DS and PG* plus S is 100% absorbed after 72 hr. as compared to 34% for PGDS* and 48% for PG plus S*. This indicates extensive hydrolysis and more rapid absorption of the PG moiety, leaving behind a slowly absorbed stearate.

The results of the metabolism studies on the radioactive components are shown in Table II.

One hundred per cent of the activity from a dose of PG* plus S and also PG*DS is absorbed in 72 hr. Rapid absorption of PG has been previously demonstrated by Salter *et al.* (11). The average percentage absorption in 5 hr. was found to be 94.0%. PG plus S* activity is absorbed only to the extent of 48% in 72 hr., generally resembling PGDS* and stearates in the extent of uptake (10, 12–15). Concomitantly, the greatest extent of fecal excretion occurred with stearate-labeled PGDS*, PG plus S*, and glyceryl stearate* mixtures.

TABLE II

Recovery of Radioactivity from C^{14}-Labeled PGDS and Related Compounds in 72 Hours

	Total per cent of dose in 72 hr.				
	PDGS*	PG*DS	Glyceryl stearate* mixture	PG + S*	PG* + S
Feces	65.8	0.2	40.6	51.8	0.5
CO_2	17.5	94.0	43.2	42.6	76.4
Urine	6.4	0.4	0.0	1.6	2.3
Organs	6.2	2.7	6.4	3.2	3.3
Carcass	10.8	2.3	8.1	4.8	16.5
Recovery	106.8	99.6	98.3	104.0	99.0
	Per cent alimentary absorption				
	34.2	99.8	59.4	48.2	99.5
	Per cent recovery of absorbed dose				
CO_2	51.3	94.8	72.8	89.0	76.4
Urine	18.9	0.4	0.0	3.4	2.3
Organs	18.5	0.9	10.7	4.2	4.5
Carcass	31.6	2.3	13.7	10.1	16.5
Recovery*	120.3	98.4	97.2	106.7	99.7

* The difference in recovery between the total dose and absorbed dose is due to the apparent per cent error in the total recovery which was compounded by assuming no error in the absorbed dose.

Approximately 6 % of the total dose or 19 % of the absorbed dose of PGDS* is excreted in the urine in 72 hr. Only 0.4 % was excreted from PG*DS, indicating the origin of urinary excretory products to be derived largely from the stearate portion of the molecule. The compounds excreted in the urine have not been identified. The glyceryl stearate* mixture gave rise to negligible radioactivity in the urine. Similar findings were obtained with PG* plus S and PG plus S*. Metabolic products of PGDS* appear to partition more toward urinary excretion than do the other compounds studied. No explanation may be offered at this time for this relatively large output of urine activity. It is indicated, however, that the metabolism of the stearic acid portion of PGDS is different from free stearic acid given in the presence of PG.

Ninety-four per cent of the absorbed radioactivity in PG*DS is uti-

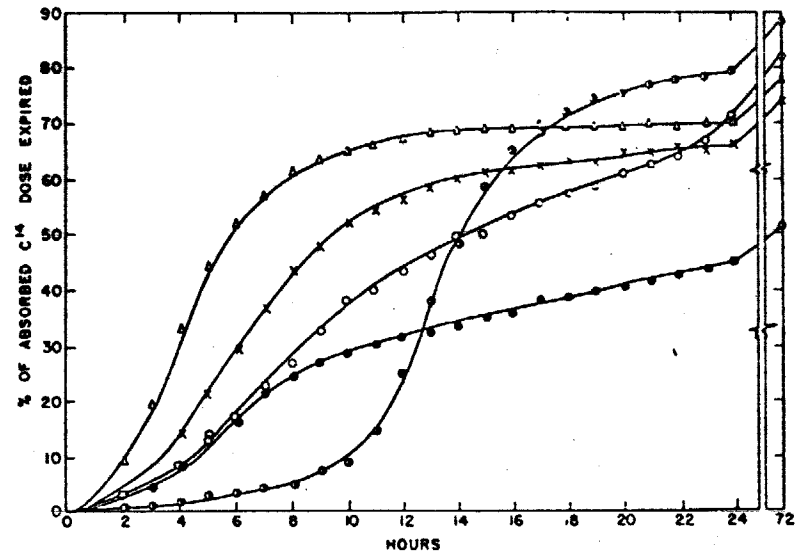


FIG. 2. Cumulative $C^{14}O_2$ excretion by rats from absorbed C^{14}-labeled PGDS and related compounds in 72 hr. △, PG* plus S; ×, glyceride stearate* mixture; ○, PG*DS; ●, PGDS*, ◑, PG + S*.

lized, as evidenced by the $C^{14}O_2$ excretion in a 72-hr. period in the nonfasting rats. The mechanism by which propylene glycol is utilized, by oxidation to lactic acid and subsequent conversion to pyruvate, has been described by several workers (16–19). Fifty-one per cent of the absorbed activity from PGDS* is metabolized to CO_2. Correspondingly, 73 % of absorbed activity of the glyceryl stearate* mixture is metabolized to CO_2.

The breath curves presented in Fig. 2 demonstrate the extent and rate of utilization of radioactive compounds studied. The rate of utilization is uncorrected for differential rates of absorption at intermediate times.

Up to 6 hr. the per cent of total absorbed C^{14} dose expired is the same for PGDS* and PG*DS. It should be pointed out that the measured absorption at 3 hr. was similar for these two compounds. After 6 hr. there is an increase in the $C^{14}O_2$ excretion from PG*DS which may be due to the more rapid absorption and turnover of free PG as compared to stearic acid.

The per cent of the absorbed radioactivity expired at the end of 72

hr. was 94% for PG*DS, 76% for PG* plus S, 72% for the glycerol stearate* mixture, and 89% for S* plus PG. Bergström *et al.* (20) have shown that the amount of CO_2 expired is directly related to the amount of labeled stearic acid absorbed, whether given as free fatty acid or incorporated into a glyceride. They were not able to demonstrate any difference in per cent of absorbed dose as $C^{14}O_2$ versus time between stearic acid and the esterified stearate at the end of 48 hr.

Inspection of Fig. 2 shows a marked difference between the free stearic acid and the glyceride mixture. This difference in the $C^{14}O_2$ output may be due to the time required for esterification of the stearic acid to the corresponding glyceride, as well as the probable absorption rate lag of the stearic acid. At the end of 72 hr., however, where the data have been corrected for absorption differences, there is a difference in the percentage of expired $C^{14}O_2$. This may indicate a difference in the metabolic rate of stearic acid and the glyceride mixture.

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Summary

The metabolism of propylene glycol distearate (PGDS) labeled with propylene glycol-1-C^{14} and carboxyl C^{14} stearic acid has been investigated. *In vitro* hydrolysis of PGDS by steapsin has been demonstrated. The extent of absorption of PG*DS, PGDS*, PG plus S* are all similar to a C^{14} carboxyl-labeled stearic acid mixture of glyceride esters. Continuous CO_2 measurements for 72 hr. have been made for the above compounds and for PG* plus S.

Results show that the propylene glycol stearate esters are hydrolized and absorbed in a manner similar to glyceride esters of stearic acid.

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An Investigation of the *in Vivo* Hydrolysis and Absorption of Propylene Glycol Distearate

C. L. Long, B. R. Zeitlin and R. Thiessen, Jr.

From the Research Center, General Foods Corporation, Tarrytown, New York

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Introduction

The preceding paper dealing with the absorption, digestion, and metabolism of propylene glycol distearate[1] demonstrated a similarity in these processes compared with the glyceride fatty acid esters (1). Extension of the study, reported herein, elucidates the *in vivo* hydrolysis and mechanism of transport of propylene glycol distearate in the rat using C^{14}-labeled compounds.

Experimental Methods

Materials

The ensuing investigation was conducted using purified 1,2-propandiol distearate (PGDS) as (*a*) 1-C^{14}-labeled 1,2-propanediol ester (PG*DS)[2] and (*b*) as stearate-C^{14} carboxyl-labeled 1,2-propanediol ester (PGDS*); PG*, PGDS*-PGMS* mixture, stearic acid* and a mixture of mono-, di-, and triglycerides of stearic acid*.

Emulsion Vehicle for Administration of Radioactive Compounds to Rats

The emulsion system is the same as described in the preceding paper (1).

The test emulsions were administered in all cases by weights. An average intubated dose was 1 ml. of emulsion or approximately 300 mg. of radioactive tracer. Replicate-measured samples were taken at the time of intubation to establish the dose administered to each animal.

[1] Abbreviations used are: PGMS, propylene glycol monostearate; PGDS, propylene glycol distearate; and PG, propylene glycol.

[2] Asterisk (*) denotes labeled portion of molecule: propylene glycol-1-C^{14} or C^{14} carboxyl stearate.

Treatment of Rats

All rats used for this study were preconditioned for 2 weeks to nonradioactive PGDS, incorporated in a commercial dog food[3] diet at a 1% level.

1. In Vivo *Gastrointestinal Study*. For the *in vivo* gastrointestinal hydrolysis study, six Charles River Wistar Strain male albino rats, each weighing approximately 200 g., were used. The rats were fasted 24 hr. preceding the test but were allowed free access to water. Three of these rats were intubated orally with a PG*DS emulsion and three with a PGDS* emulsion. The animals were sacrificed by ether anesthesia 1.5 hr. after intubation, and the entire gastrointestinal tract was removed.

2. *Intestinal Lymph Studies*. Ten male Sprague-Dawley rats, weighing 225–290 g., were deprived of food but not water for 18 hr. prior to operation. After anesthetizing the animals with ether, the intestinal lymphatics were cannulated with polyethylene tubing (2) and a gastrostomy tube was inserted. Postoperatively, the rats were placed in a special confining cage (3), and gastric infusions of 0.4% sodium chloride were made at a constant rate of 2.5 ml./hr. After 24 hr., the rats were observed for general health and lymph volume produced.

Three rats were intubated via gastric cannula with the appropriate emulsion of PGDS*, PG*DS, and glyceride stearate* mixture; two were used for the PG* plus nonradioactive stearic acid. The latter mixture was administered in the same ratio as present in PGDS.

Immediately after administration of the labeled compound to the animal, the lymph was allowed to flow into graduated centrifuge tubes. The lymph was collected at 6, 12, 24, and 48 hr. and the volumes were recorded. After each collection, 1 vol. of 95% ethanol was added to denature the protein.

Extraction Procedure for Radioactive Compounds in Gastrointestinal Tract and Lymph

The gastrointestinal tracts of rats intubated previously with the test compound were removed and homogenized in a Waring Blendor using ethanol–ether (3:1). The residue was extracted several times, and the filtrates were combined and made up to 500 ml. Aliquots were taken for radioactive counts and per cent soluble solids after evaporation and drying. Additional aliquots were dried by vacuum distillation under a nitrogen atmosphere in a water bath at 40°C. In experiments with PGDS*, the residue from the vacuum distillation was re-extracted with petroleum ether–chloroform (2:1) and dried over anhydrous Na_2SO_4. The extract was then filtered, and the filtrate was distilled as above. The solids remaining were taken up in 25 ml. chloroform. Aliquots were counted and a solids

[3] Gaines Dog Meal. Ingredients are: meat and bone meal, corn germ meal, wheat flakes, soybean oil meal, wheat flour middlings, fish meal, fish liver and glandular meal, wheat germ meal 3%, animal fat 2%, dried skim milk 1%, brewer's dried yeast 1%, dried beet pulp 1%, alfalfa leaf meal 0.5%, iodized salt 0.5%, vitamin A feeding oil 0.5%, riboflavine feeding supplement 0.02%, D-activated plant sterol 0.006%.

weight was obtained. The chloroform solution was used for separation of the radioactive compounds by the methods presented in this paper.

In experiments with PG*DS the aliquots of the filtrate taken from alimentary tract washings (ethanol–ether) were taken down to a residue by distillation as previously described. The residue, extracted with a small volume of water, formed an opalescent solution. This solution was used for the separation and identification of free PG as described below.

The lymph samples from each time collection containing 1 vol. ethanol were centrifuged. The residue was extracted four times with ethanol–ether (3:1), and all extracts were made up to 50 ml. Aliquots were vacuum distilled under nitrogen at 40°C., and the residue was extracted with petroleum ether–chloroform (2:1) and dried with Na_2SO_4. A residue was again obtained and taken up in chloroform. This solution was used for analysis of radioactive compounds in lymph.

Assay of Radioactive Samples

All samples in this study, including chromatographic fractions, were counted with a Nucleonic Corporation Radiation Counter, model RC-1, using a TCG tube. The samples were prepared for counting by plating out a 0.2-ml. aliquot on a planchet and drying under an infrared lamp. Detection of radioactive spots on paper chromatograms was made by scanning the strips using a Nuclear Instrument and Chemical Scaler equipped with a D-47 thin-window gas-flow tube.

Chromatographic Methods

The aqueous extract of the residue from the gastrointestinal tract was used for the determination of free PG. The extract was purified for paper chromatographic separation using a cellulose column. The latter was prepared by dry-packing 7.5 g. acid-washed cellulose in a 15 × 150 mm. chromatographic tube. The aqueous extract was added to the column and developed under nitrogen pressure. After two 17-ml. fractions, 1 and 2, were collected, the elution solvent was changed to chloroform–ethanol (1:1) to elute the remaining activity from the column. The two aqueous fractions, 1 and 2, from the column were turbid indicating fat contamination. Although separation of free PG from the lipides was incomplete, the effect was to concentrate free PG and to remove much of the lipide material for separation on paper.

Paper chromatographic identification of PG was made by the procedure described by Buchanan *et al.* (4) for the separation of glycols. Whatman No. 1 filter paper was used and developed by ascending chromatography with butanol–acetic acid–water (4:1:5) overnight.

Separation of all other radioactive components in the gastrointestinal tract and lymph was made by methods described below.

Borgstrom reported that phospholipides were retained on a silicic acid column when chloroform was used as the eluant (5). The neutral fat and fatty acids were eluted with this solvent, and phospholipides were removed with methanol. The performance of PGDS*–PGMS* was studied on a silicic acid column with chloroform as the eluting solvent. This mixture was capable of being quantitatively

eluted (275 ml.) in approximately the same volume reported for the removal of fat and fatty acids.

Fatty acids were separated from neutral fat on an Amberlite IRA-400 resin. Using this technique, a mixture of PGDS*–PGMS* in ether was added to an Amberlite IRA-400 resin and was quantitatively removed with ether. Fatty acids were removed from the resin using ethanol–ether–10 N HCl (50:75:10) mixture. With the removal of phospholipides and fatty acids, the separation of PGDS, PGMS, and the glyceride mixture was undertaken using kieselguhr treated with dichlorodimethylsilane. The silane vapors were allowed to remain in contact with the kieselguhr for a week. The kieselguhr was then removed and stored in a tightly stoppered bottle. Preparation of the column was similar to that described by Savary and Desnuelle (6) for the separation of mono-, di-, and tripalmitin. The developing solvent was the lower phase of cyclohexane–ethanol–water (1:3:4)

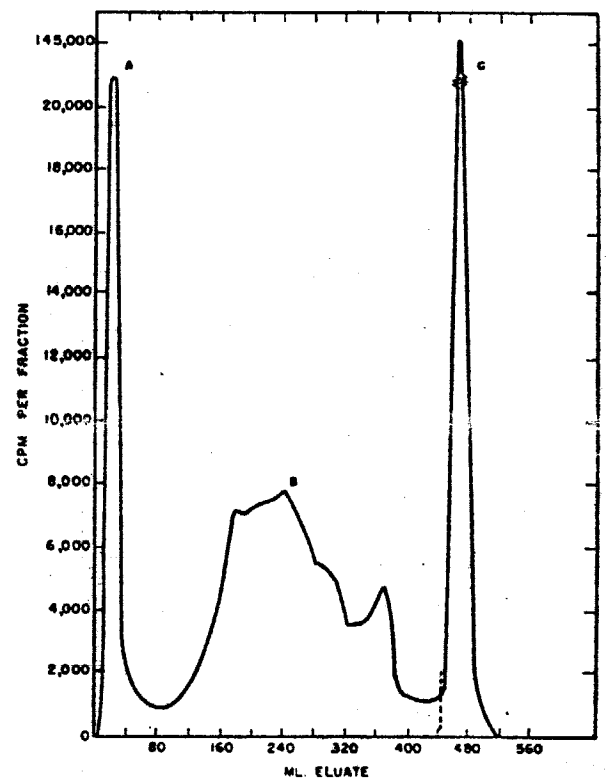

FIG. 1. Separation of mono-, di-, and triglycerides of stearic acid on a silicone-treated kieselguhr column. The dotted line indicates the change from cyclohexane–ethanol–water eluting solvent to pure cyclohexane. Peak *A* = monoglyceride; *B* = diglyceride; *C* = triglyceride.

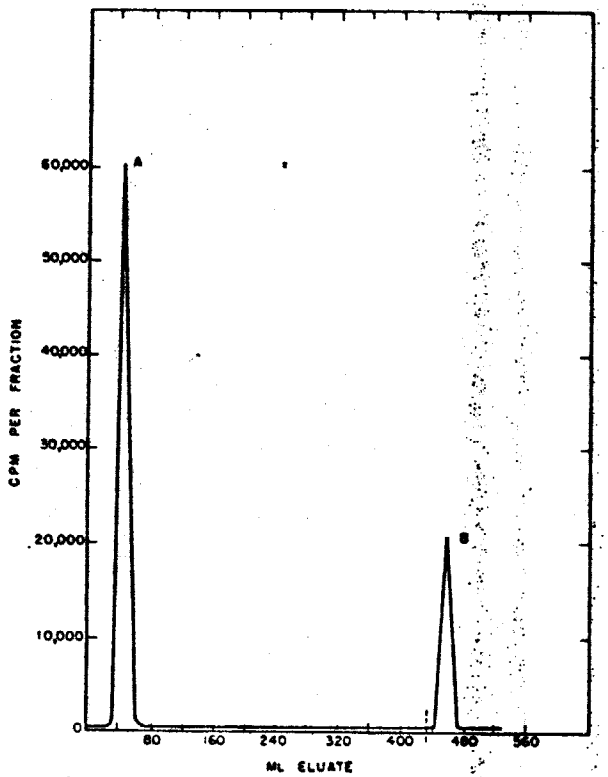

FIG. 2. Separation of 1,2-propanediol mono- and distearates (PGMS–PGDS) on a silicone-treated kieselguhr column. Dotted line indicates the change from cyclohexane–ethanol–water to pure cyclohexane. Peak *A* = PGMS; *B* = PGDS.

followed by pure cyclohexane. All column chromatography was carried out under a nitrogen atmosphere.

The performance of the radioactive mono-, di-, and tristearin was studied using the silicone-treated kieselguhr column. The results are shown in Fig. 1. Using the lower phase of cyclohexane–ethanol–water, the monoglyceride was eluted as the first peak, followed by the diglyceride. After removal of the diglyceride, the solvent was changed to pure cyclohexane which eluted the triglyceride. Similar separation was reported by Savary and Desnuelle (6) using mono-, di-, and tripalmitin.

The same technique was used to separate PGDS* and PGMS*. The results are shown in Fig. 2. The first peak is eluted in the same volume as the monostearin. The second peak is eluted only with pure cyclohexane, similar to the tristearin elution pattern. When pure PGDS* was added to the column, it was capable of being eluted only with pure cyclohexane, thus demonstrating the second peak to be the PGDS* component. PGMS* and monostearin can be separated on silicone-treated paper using heptane–80% ethanol (*v/v*). PGDS* and tristearin can be separated in a similar manner. The diglyceride has been separated as one component in the second peak from the column described above.

The peak fractions from the silicone-treated kieselguhr column were chromatographed on Whatman No. 1 paper. The paper was impregnated with a 5% (*v/v*) solution of silicone (Dow Corning 200, viscosity 100 centistokes) in cyclohexane (7). It was then blotted to remove excess solvent and dried in an oven at 110°C. for 1 hr. Concentrations of test materials ranged between 5 and 50 μg. as applied to the paper. Since the compounds were detected by scanning for radioactivity, the concentrations above gave desirable peaks with 1000–5000 counts/min. for each component.

After the strips were spotted, they were suspended in chromatographic jars lined with paper to facilitate equilibration. The solvent system used for paper chromatographic separation was heptane–80% ethanol. The lower phase was used to equilibrate the tank and the upper phase was used for developing the paper strips by the ascending technique, this requiring 8 hr.

RESULTS AND DISCUSSION

*Identification of Free PG in Alimentary Digests of PG*DS*

The original gastrointestinal tract extract from rats intubated with PG*DS, the two aqueous fractions, and the chloroform–ethanol (1:1) fraction from the cellulose column were chromatographed on Whatman paper using butanol–acetic acid–water (4:1:5) as the developing solvent. Standards consisted of radioactive PG*, PGDS*, PGMS*, stearic acid*, and the glyceride stearate* mixture. The results are shown in Table I.

The data show that pure PG has an R_f separate and distinct from the lipide components present. PGDS is also present in the original extract

TABLE I

*Paper Chromatographic Identification of 1,2-Propanediol from Alimentary Digests of Radioactive PG*DS*

Component	R_f values				
Original gut extract	0.0	0.25	0.39	0.67	0.94
Aqueous fraction 1				0.61	0.91
Aqueous fraction 2				0.67	0.94
$CHCl_3$–ethanol fractions	0.0				0.85
Pure PG				0.68	
PGDS	0.0				
PGMS					0.95
Stearic acid					0.95
Glyceride mixture	0.08				1.0

since it has an R_f value of 0.0 in this solvent. Two radioactive components, R_f 0.25 and 0.39, are present in a very small concentration; their identities are unknown. Definite proof of the presence of free PG is shown in the chromatograms obtained from aqueous fractions 1 and 2. These show two distinct components to be present: one is free PG and the other PGMS. The opalescence in the aqueous eluates was therefore due to the PGMS present.

Hydrolysis of PGDS by the rat is evident as the results show PGMS and free PG to be formed in the alimentary tract after a 1.5-hr. digestion period.

*Identification of Products in Gastrointestinal Tract after Administering PGDS**

The chloroform extract of the gastrointestinal tracts of three rats given PGDS* was added to a silicic acid column and eluted with chloroform for removal of phospholipides. No radioactive phospholipides were present since the methanol eluate gave background activity. However, nonradioactive phospholipides may be present. The chloroform eluate was prepared for the removal of fatty acids using Amberlite IRA-400 resin. The per cent fatty acids from the resin were found to be 18% of the original dose or 25% of the gastrointestinal tract contents. An aliquot from the original extract was passed through the resin column without prior treatment, and similar percentages were found to be present.

The eluate from the resin, containing neutral fat, was chromatographed on a silicone-treated kieselguhr column. Two peaks, *A* and *B*, were eluted from the column. The identity of the above peak fractions were determined by paper chromatography using heptane–80% ethanol. Standards were developed in the same tank. The results are presented in Table II.

TABLE II

Paper Chromatographic Identification of Rat Intestinal Homogenate Components from Silicone-Treated Kieselguhr Columns

Components	R_f values
Peak *A*	0.60
PGMS	0.69
Peak *B*	0.30
PGDS	0.30
Glyceride mixture	0.23 0.90

It is evident from these values that PGDS is hydrolyzed by the rat to produce PGMS and free stearic acid. These data confirm those in the previous section where free PG and PGMS were demonstrated.

The feeding of radioactive PGDS does not appear to give rise to intralumen or intestinal tissue radioactive phospholipides. Borgström (8) showed the amount of uptake of radioactivity by intestinal phospholipides to be low and remain practically constant during absorption of C^{14}-labeled fats and fatty acids.

Rate of Uptake of Radioactive PGDS and Related Compounds in the Lymphatics of Rats*

Total activity present in the lymph over 48 hr. calculated as per cent of dose for each compound is given in Table III. The per cent absorbed is expressed as an average of two or three rats. The rates of uptake are graphically represented in Fig. 3 and as cumulative rates in Fig. 4.

The rate of uptake of activity in the lymph is about the same for PGDS*, PG*DS, and the glyceride stearate mixture. The rat is capable of hydrolyzing PGDS to PGMS and free stearic acid as was demonstrated in the *in vivo* intestinal digestion study. The total extent of appearance in the lymph of the two radioactive compounds indicates that the absorption of PGDS is slightly better than the glyceride stearate mixture. The absorption of glyceride esters of fatty acids entails hydrolysis to monoglycerides, diglycerides, and free stearic acid. As these components pass through the intestinal wall, resynthesis of the triglyceride occurs. If a similar mechanism existed for PGDS*, the compound would be partially hydrolyzed in the intestine and absorbed, and PGDS* would be resynthesized in the lymph. It is also possible that the labeled stearate could combine or transesterify with glyceride esters in the intestinal mucosa or lumen.

The radioactive PG*DS rate of appearance is about the same as that

TABLE III

Total Absorption of Radioactivity in the Lymph of Rats during a 48-hr. Collection Period

Component	Rat No.	Average per cent absorbed of original dose
PGDS*	1, 2, 3	23.62 ± 1.5
PG*DS	4, 5, 6	12.39 ± 1.6
Glyceride stearate* mixture	7, 8	18.37 ± 4.5
PG* + stearic acid	9, 10	1.03 ± 0.1

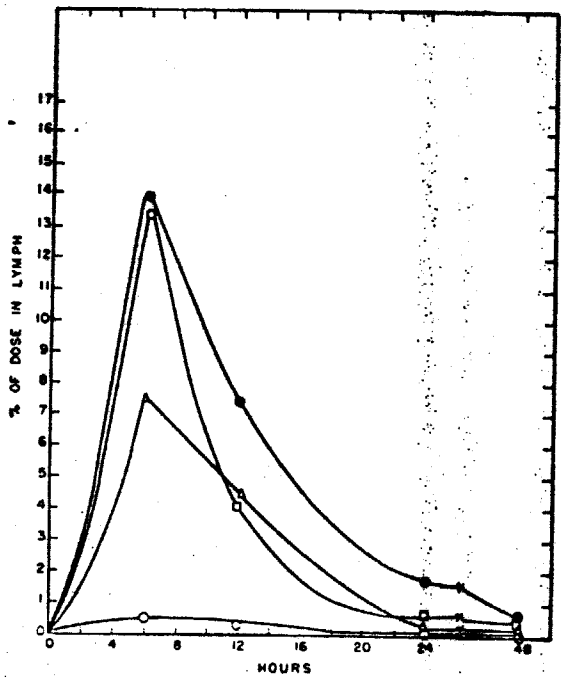

FIG. 3. Rate of appearance of radioactivity in the lymph of rats after administration of various radioactive compounds: ●, average from three PGDS* rats; □, average from the PG*DS rats; △, average from two glyceride stearate mixture rats; ○, average from two PG* plus nonradioactive stearic acid (same ratio as in PGDS).

of PGDS*, but a smaller per cent of activity is present in the lymph. Results presented in this report showed free PG as one of the hydrolysis products of PGDS in the intestine. Since less activity is found in the lymph from PG*DS, it is apparent that any free PG liberated does not go by the lymphatic system but possibly via the hepatic portal system. This would necessarily result in less activity in the lymphatics. This is in agreement with the data on absorption of free PG* plus nonradioactive stearic acid. Approximately 1% of the administered dose of PG* is present in the lymph over the 48-hr. test period.

Components Present in Lymph of Rats after Administration of PGDS and PG*DS*

The combined lymph samples from the PGDS* rats 1, 2, and 3 and PG*DS rats 4, 5, and 6 were prepared for phospholipide removal using

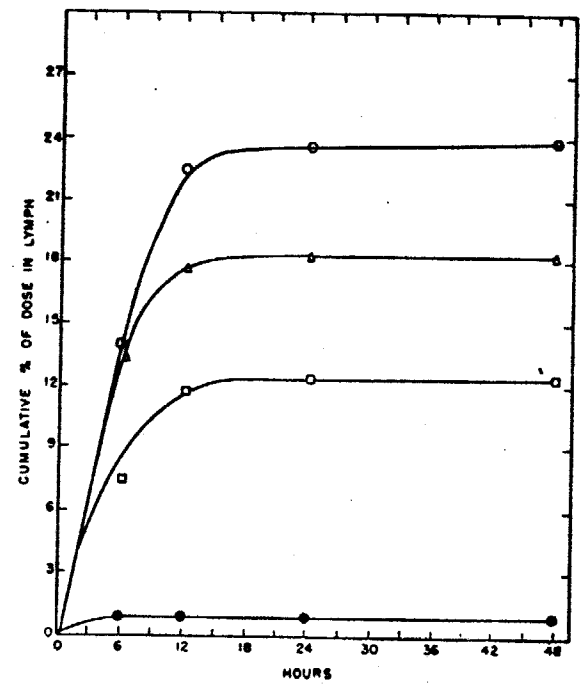

FIG. 4. Cumulative rate of appearance of radioactivity in the lymph of rats administered various radioactive compounds. ○, PGDS*; △, glyceride stearate mixture; □, PG*DS; ●, PG* plus nonradioactive stearic acid (same ratio as in PGDS).

a silicic acid column. The phospholipide fraction from the PG*DS rat lymph sample did not contain radioactivity. When the PGDS* rat lymph fraction was assayed, 6.7% radioactivity was present (calculated on the basis of lymph contents per rat.)

The sample was not assayed for its phosphorus or fatty acid content but, in accordance with the literature, this eluate may be considered the phospholipide fraction. Since PGDS* gave rise to this radioactive fraction and PG*DS did not, it is evident that hydrolysis occurred and free, labeled stearic acid was incorporated into phospholipides. Reiser *et al.* (9) reported that half of lymph phospholipides formed from ingested fat utilized the hydrolyzed fatty acids and endogenous glycerol. The other half was synthesized from absorbed glycerides. Bergström et al. (10) studied lymph fat composition of the cat after feeding fatty acids, fatty acids plus glycerides, and glycerides. In all three cases the lymph

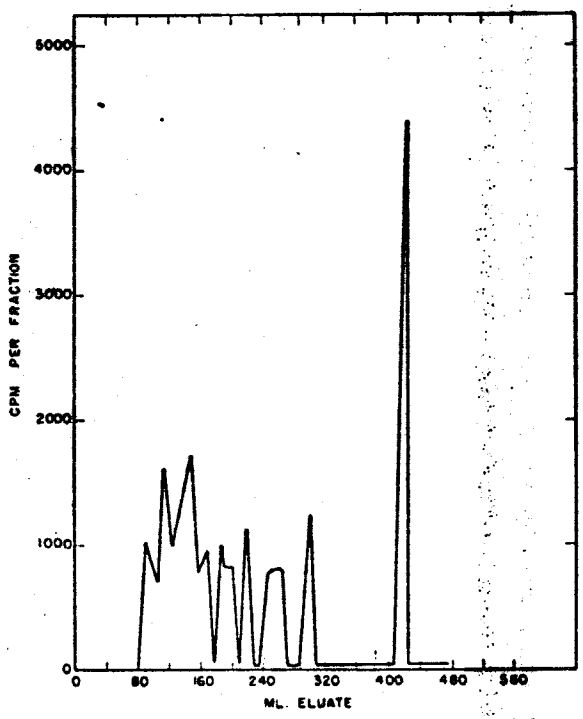

FIG. 5. Column chromatography of purified fraction of rat lymph on silicone-treated kieselguhr after administering PGDS*. Dotted line indicates the change from cyclohexane–ethanol–water to pure cyclohexane.

fat consisted of 80–90% of glycerides and 10–15% phospholipides. Similar results on rats were reported by Borgström (11). These facts are in agreement with the results found in this study.

The neutral fat and fatty acid chloroform eluate from the silicic acid columns was reduced to a residue and taken up in ether, and the fatty acids were removed using an Amberlite IRA-400 resin. The resin was void of activity, indicating both PGDS* and PG*DS rat lymph samples to be free of radioactive fatty acids. Fatty acids have been recently reported in thoracic lymph of rats by Borgström and Tryding (12) but are reported to be the original constituents of the lymph rather than derived by hydrolytic processes in the lymph.

The neutral fat eluates from each resin were chromatographed on a silicone-treated kieselguhr column. The results from the kieselguhr column for the PGDS* rat lymph sample and the PG*DS rat lymph sample

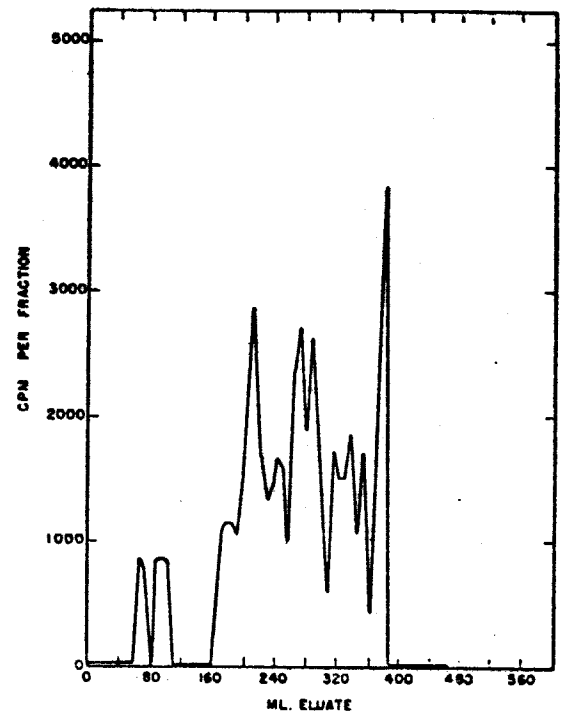

FIG. 6. Column chromatography of a purified fraction of rat lymph on silicone-treated kieselguhr after administering PG*DS. Dotted line indicates the change from cyclohexane–ethanol–water to pure cyclohexane.

are shown in Figs. 5 and 6. The elution patterns of both samples are very similar, but it appears that the column may have streaked in the development. Since pure PGDS*–PGMS* or the glyceride mixture gives good resolution, this difference may be due to interfering substances in lymph, the kieselguhr (one batch was used throughout this study), or may be real radioactive metabolites of PGDS.

A pure PGDS*–PGMS* mixture was chromatographed in the presence of rat lymph extract. The resolution of this mixture is identical to the pattern of PGMS–PGDS separation shown in Fig. 2. The PGMS* was eluted from the column in the first 40 ml. of eluate. The same solvent was continually carried through the column with no streaking of the radioactive components. PGDS* was eluted with pure cyclohexane. The elution pattern of this lymph extract–PGDS*–PGMS* mixture was identical to a column run simultaneously using pure PGDS*–

PGMS*. These facts would tend to eliminate the theory of a lymph-extractable interfering substance or to the kieselguhr itself. The remaining conclusion is that this material is a metabolite or metabolites of PGDS. In a study of the triglyceride of stearyl diacetin, Mattson reported that palmitic acid and unsaturated fatty acids are formed by the time it reaches the thoracic duct (13). PGDS may be acted on in a similar manner to produce unsaturation and shorter chain fatty acids. Recombination of these compounds to form other 1,2-propanediol fatty acid esters may occur. The performance of the kieselguhr column in separating these proposed resynthesized esters is unknown.

The most important finding is that both columns do not show the presence of a peak in the first 40 ml. of eluate. Since pure PGMS* is known to be eluted with this volume of eluate, it can be concluded that the transport of propylene glycol stearate in the lymph is not by way of the PGMS moiety. This is analogous to the mechanism for transport of triglycerides, in that monoglycerides are not present in the lymph after feeding mono-, di-, or triglycerides or free fatty acids (14).

The PGDS* rat lymph samples produced a peak in the pure cyclohexane eluate (Fig. 5), which corresponds to a column elution pattern of PGDS* or the triglyceride of stearic acid. These results suggest either PGDS or triglyceride fraction or a mixture of both is present in the lymph, since radioactive triglyceride may be formed from any liberated labeled stearic acid originating from PGDS*.

PG*DS is utilized in the same manner as PGDS*. Any activity in the lymph cannot be due to a triglyceride synthesis because of the position of the labeling in PG*DS. Since the elution pattern of PG*DS rat lymph was similar to the PGDS* lymph sample, it can be concluded that PGDS is present in rat lymph.

Acknowledgments

The authors wish to express their thanks to Dr. J. L. Bollman of the Mayo Clinic, Rochester, Minnesota, for his interest in this work and providing facilities necessary for the lymph collection and to Mr. Emery Van Hook for the intestinal cannulations of the rats. The technical assistance of Mr. R. Klose in all phases of this study is gratefully acknowledged.

Summary

1. Propylene glycol distearate, labeled with propylene glycol-1-C^{14} and with C^{14} carboxyl stearate, administered orally to rats was hydrolyzed in the gastrointestinal tract to form propylene glycol monostearate, propylene glycol, and stearic acid.

2. Cannulation of the intestinal lymphatics demonstrated the appearance of radioactivity from propylene glycol distearate in the lymph which is comparable to that appearing in the lymph after feeding glyceryl stearate esters. Only small amounts of free propylene glycol were detected in the lymph.

3. During the process of absorption, propylene glycol distearate is resynthesized from its hydrolytic products and is transported by the lymph. Free stearic acid and propylene glycol monostearate were not detected in the lymph.

4. The absorption mechanism for propylene glycol distearate is similar to the glyceryl stearate esters.

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The Injection of Chemicals into the Yolk Sac of Fertile Eggs prior to Incubation as a Toxicity Test

JOSEPH MCLAUGHLIN, JR., JEAN-PIERRE MARLIAC, M. JACQUELINE VERRETT, MARY K. MUTCHLER, AND O. GARTH FITZHUGH

Division of Pharmacology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.

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The increasingly large number of food additive chemicals introduced into the market each year has necessitated the development of rapid and reliable methods for the evaluation of their toxicity. Toxicologic studies of all these chemicals by the usual methods using animals are very difficult, and such studies sometimes give inconclusive results.

The toxicity of some chemicals, and especially of food additives, may be determined by injection of the chemical into the yolk sac of fertile eggs prior to incubation and subsequent observation of the effects of the chemical on the embryonic development of the chick. This appears to be a promising method in that it may be carried out much more economically in terms of money and space than would be possible with larger animals. Hundreds of chicken embryos may be observed in a minimum of space, and over a comparatively short period of time. The feasibility of using such large numbers is valuable also in the statistical evaluation of toxicity data.

A review of the literature shows how little work has been done in this field except in a fragmentary way on isolated cases. Most of the reports refer to injections of chemicals made after the fourth or eighth day of incubation and examination of the embryos killed before they hatch.

The earliest work that we have found in the literature was by Féré (1893). During ten years after this date he published about sixty-seven papers; a review article (Féré, 1899) contains a summary of many of his studies. His work consisted mainly of injection before incubation, but the eggs were usually opened on the third day of incubation. His interest was mainly in the teratogenic effect of chemicals.

Since 1900 other articles have appeared in the literature, but most of them concern the effects of one or two chemicals on a small number of embryos. One of the most informative papers is that of Ridgway and Karnofsky (1952) in which they report experiments on compounds containing fifty-five of the elements, mainly the metallic ones, generally injected at either the fourth or eighth day of embryonic development to study toxicity and teratogenic effects. Hamburger and Hamilton (1951) have described an elegant method for determining the stages of development of the chick embryo. More recent investigations employing the chick embryo to study the toxicity of various chemicals have been reported by Kemper (1962), Platt *et al.* (1962), and Goerttler (1962). Finally, the classic books of Romanoff and Romanoff (1949) and Romanoff (1960) contain a wealth of information on the avian embryo.

Preliminary reports of this investigation have been presented by Marliac (1962) and McLaughlin and Mutchler (1962).

EXPERIMENTAL

The fertility and hatchability of eggs and the livability of chicks are dependent on a complex interrelationship of ecological factors, among which are the genetic background and the age of the mated birds, the nutritional status and general management of the flock, and seasonal variations. In view of this the initial phase of our work, which started in 1959, was devoted to a study of the hatchability of our supply of White Leghorn eggs[1] under conditions existing in our laboratories. The data accumulated during two years for control eggs showed that the hatchability of these eggs was, in fact, consistent, reproducible, and very high. The possibility of a seasonal variation occurring in responses to compounds introduced into the eggs was also examined by repeated testing of several chemicals at all seasons of the year. No important variation was detected.

Selection of eggs. Eggs to be injected are first candled in order to discard those that are defective and to outline with a pencil the exact location of the air cell. In our laboratory 9% of 5000 eggs had to be discarded: 2% cracked, 4% with improperly calcified shells, 1.5% with a tremulous air cell, 1% with the air cell in the wrong place, and 0.5% with blood clots. After the elimination of such defective eggs, the hatch of control eggs averages 95%. A further restriction is based on the weight of the eggs: all those weighing less than 52 g or more than 63 g are rejected. After

[1] Truslow Eggs, Chestertown, Maryland.

candling, the eggs are randomized in order to avoid series of infertile eggs in any one experiment.

The initial experiment with a given chemical is for range-finding and is performed at two or more concentrations of the chemical with 10 eggs per level. On the basis of this information, 20 or more eggs are injected with the appropriate amount of the chemical.

If the chemical proves to be nontoxic, the experiment is repeated with the minimum number of eggs that will give a reliable and reproducible value for the hatchability. In the case of a toxic chemical, additional eggs are injected to determine the specific effects of the chemical. The total number of eggs used for a chemical depends upon the data obtained initially and upon the kind of information desired. Hence, data for some chemicals are based on less than one hundred eggs, whereas data for others are based on several hundred eggs.

Technique of injection. The injections of pure chemicals, chemical solutions, or suspensions are made at volumes up to 0.10 ml. When necessary, dilutions are made with solvents such as water, propylene glycol, corn oil, peanut oil, or other nontoxic solvents.

In order to avoid contamination, the injections are carried out in an Isolator Box[2] with a sterile atmosphere created by using formaldehyde vapors (produced by mixing 2 g of potassium permanganate and 50 ml of 37% formalin). During the period of a year, more than fifteen hundred noninjected eggs were exposed to formaldehyde vapors; no toxic effect was noticed. After exposure to these vapors for 30 minutes, the eggs are ready for injection.

The large end of the egg is wiped with a sterile gauze pad moistened with a 70% alcohol solution, and a hole is drilled in the shell in the center of the surface over the air cell (Fig. 1). Care must be taken not to damage the shell membrane with the point of the drill[3]; this is to avoid, if possible, contact of the air with the egg membrane. Fine particles of shell are removed with an aspirator to prevent the needle from carrying them into the yolk.

Immediately before the injection, each egg is shaken with a quick twist of the wrist. Since the germinal disc occasionally sticks to the air cell and it is possible to damage it with the needle, this movement will allow the disc to float free in the egg.

[2] Kewaunee Scientific Company, Adrian, Michigan.
[3] Burgess Vibrocrafters Inc., Grayslake, Illinois.

The needle (hypodermic, 1 inch long, either no. 22 or no. 27, depending on the viscosity of the liquid to be injected) is inserted horizontally through the air cell into the yolk (Fig. 1). Care must be taken in withdrawing the needle to avoid damaging the vitelline membrane since such damage could cause the yolk to spread out in the albumen. If the end of the needle has some yolk on it, the injection is not satisfactory. The needle should be wiped with a sterile gauze pad between each injection. As soon as the egg has been injected, the hole in the shell is covered with a small piece of Scotch tape, care being taken not to cover the entire air cell.

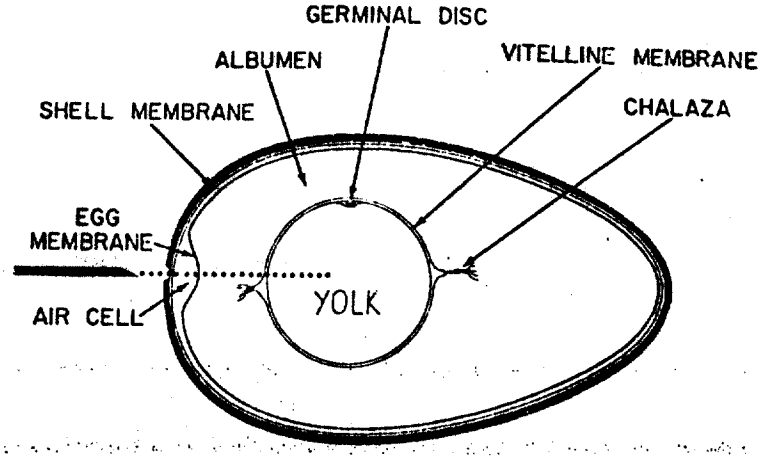


FIG. 1. Diagram of egg and position for injection.

Incubation and hatching. The injected eggs are put into the incubator trays with the large end up; the trays are placed in the incubator,[4] which automatically rotates hourly and is maintained at an optimum temperature of 38°C and a relative humidity of 60%. The eggs are candled on the fifth day of incubation and every day thereafter. Clear eggs and dead embryos are removed for examination. On the seventeenth day of incubation the fertile eggs are transferred to the hatcher[5] and kept at a temperature of 37°C until they hatch.

EVALUATION OF DATA

The injection of the chemical into the egg may produce one of four possible results: (1) the chemical is highly toxic at the level injected, and

[4] Humidaire Incubator Co., New Madison, Ohio; model no. 50, capacity 450 eggs.
[5] Brower Manufacturing Co., Quincy, Illinois.

all the embryos are killed during the first 20 hours of incubation (before the two-somite stage); (2) the chemical is toxic but allows a number of embryos to develop only up to a certain point, and some possibly even to hatch; (3) the chemical has little effect on the hatch; and (4) the chemical has no effect on the hatch or on the posthatch development of the chick ("no effect" level).

If the chemical appears to be highly toxic and all the embryos are killed, the experiment is repeated with smaller doses of the chemical until some hatch is obtained. If the chemical is toxic, but allows the embryos to develop for a longer period of time, dead embryos are examined pathologically and the chicks that do hatch are examined for eye damage, color of the feathers, weight, length of the legs, form of the beak and of the rump, hematologic changes, and condition of the internal organs (liver, kidneys, heart, gall bladder, and spleen).

It is advisable in all cases to observe the chicks for a period of a few weeks in order to detect any delayed effects. Since as much as 30% of the yolk remains at the time of hatching and is absorbed during the first 7 days thereafter, effects of a toxic chemical may first be observed at this time. There may be weight retardation, death during the first week, or the appearance of nerve damage occurring as late as 2–6 weeks after hatching.

The toxicity of a chemical is evaluated mainly from the percentage of hatch at varying dosages of the chemical as compared to noninjected (control) eggs, from a study of the embryonic development of the eggs that fail to hatch, and from a study of the appearance and development of the chicks that do hatch. However, several other factors must also be considered in this evaluation: these are specific gravity, solubility, coagulating effect, pH, and the ionic concentration of the chemical tested.

If the chemical has a high specific gravity, there is the possibility of its settling out in the bottom of the egg and thereby giving a value of apparently low toxicity.

The solubility is quite important since the availability of the chemical for utilization in the chick embryo is partially dependent on its solubility in the egg. However, since egg yolk is an emulsion, solubility problems are somewhat minimized. In the case of insoluble chemicals that are injected as suspensions, it is also necessary to consider particle size in the evaluation of toxicity data.

In order to have a toxic effect, the chemical must come in contact with the embryo either directly or indirectly through the bloodstream. Chemicals such as the lower aliphatic alcohols have a coagulating effect on the pro-

tein, and this coagulation may decrease the availability of the chemical as well as some yolk nutrients, and thereby alter the response of the embryo.

If the pH is highly acidic or basic, a pH effect differing from the true toxic effect of the chemical may be obtained due to interference with the normal acid-base equilibrium in the egg.

The ionic concentration is also important for a similar reason. The observation of increasing toxicity with increasing concentration of a chemical should be interpreted cautiously, since highly concentrated solutions may upset the physical equilibrium of the yolk by causing osmotic effects.

Finally, the introduction of a chemical into the yolk may cause a special type of toxicity because it destroys, alters, or combines with essential nutrients such as vitamins and minerals.

EXPERIMENTAL DATA

Twenty-five thousand eggs have been used in our laboratory during the past three years to test more than 100 chemicals with the following

TABLE 1
NONTOXIC CHEMICALS

Chemical	Solution injected		Per cent hatch
	Concentration	Quantity (ml)	
Water (boiled)	—	0.05	95
Propylene glycol	Undiluted	0.05	95
Corn oil	Undiluted	0.05	90
Peanut oil	Undiluted	0.05	90
Sodium chloride	0.9% in water	0.05	90
	5.0% in water	0.05	70
	5.0% in water	0.10	30
	10.0% in water	0.05	20
Dextrose	5.0% in water	0.05	90

results: (1) nontoxic chemicals injected at an appropriate level allowed the embryo to develop and to hatch as did the controls; (2) toxic chemicals produced effects at dose levels which may be compared to those producing effects in feeding experiments using animals; and (3) this technique often provided toxicologic information which had not been shown by conventional methods.

Table 1 lists the results obtained with some chemicals in common use in food and shows the dosages used and the percentages of hatched chicks.

TABLE 2
CHEMICALS WITH A HIGH ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch	Remarks
Lead acetate	10% in water	0.05	0	—
	2% in water	0.05	0	Hydrocephalus in embryos that failed to hatch
	0.5% in water	0.05	20	—
Mercuric chloride	1% in water	0.05	0	All embryos died at the beginning of incubation
Thiourea	10% in water	0.05	0	Some embryos lived up to 34 days
	5% in water	0.05	20	Hatched on the 28th day of incubation; growth retardation
Triorthocresyl phosphate	Undiluted	0.02	0	Most of the developing embryos died on the 15th day of incubation
	Undiluted	0.01	40	Hatched chicks showed growth retardation and developed paralysis 2–6 weeks after hatching
p,p'-Diaminodiphenylmethane	10% in ethanol	0.05	30	Leg damage; beak deformity (short mandible)
Sodium selenite	0.2% in water	0.05	0	—
	0.1% in water	0.05	20	—
	0.1% in water	0.02	80	—
Dibutyl-tin-dilaurate	Undiluted	0.01	0	Beak deformity
Aroclor 1242 (chlorinated polyphenyls)	Undiluted	0.025	0	—
	Undiluted	0.01	5	Beak deformity (short upper beak); edema; growth retardation

These data, supplemented by an examination of the nonviable eggs and an autopsy of the chicks that hatched, have not indicated any hazard from their use in food. However, it must be pointed out that any chemical, added at a sufficiently high concentration, may have some toxic effect. Since our data and those reported in the literature indicated safety, this phase of the study was not carried beyond a preliminary examination to ascertain that nontoxicity also was shown for these chemicals by the chick embryo technique. This is of theoretical as well as practical importance for the evaluation of any new technique to be used in toxicologic studies.

Table 2 lists our results with several chemicals which have been shown to be highly toxic to animals. In each case there is not only the low percentage of hatch at a low level of the chemical tested, but there are also congenital abnormalities and other responses that raised extremely serious questions as to the safety to the consumers of any food contaminated with these chemicals.

Lead acetate resulted in no hatch at a level of 1 mg per egg. Autopsy of the dead embryos showed extensive brain damage, as has been reported by de Franciscis and Bocalatte (1962) and by Karnofsky and Ridgway (1952).

Mercuric chloride showed no hatch even at a level of 0.5 mg per egg.

Thiourea is a known carcinogen with a basic effect on the thyroid gland. The hatch time was delayed with increasing amounts of this chemical. At a level of 5 mg per egg no chicks hatched and the embryo required 35 days to develop to the stage normally attained at 20 days. At 2.5 mg per egg some chicks hatched, but most of them had to be helped out of the shell. This effect has been reported also by Yushok (1950).

Cavanagh (1954) reported that triorthocresyl phosphate (TOCP), a well-known plasticizer for nonfood use, causes paralysis when fed to adult chickens. We observed this paralysis in some chicks which hatched from eggs injected with 10 mg of the undiluted chemical.

The compound *p,p'*-diaminodiphenylmethane has been reported by Zylberszac (1951) to cause cirrhosis of the liver in rats. We found this chemical to be extremely teratogenic at a level of 5 mg per egg; more than 90% of the chicks had a short mandible and leg damage consisting of a severe bending of the tibia and a general shortening of the bones of the leg.

Sodium selenite proved to be highly toxic. At a level of 0.1 mg per egg, no embryos developed to more than the 5-day stage.

Dibutyl-tin-dilaurate showed no hatch at a level of 10 mg per egg. The majority of the embryos, which did not live more than 15 days at this

TABLE 3

CHEMICALS WITH AN INTERMEDIATE ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch	Remarks
Acetone	Undiluted	0.05	70	
n-Butanol	Undiluted	0.01	95	* At these levels: corneal opacity, cataract, cleft palate, nerve and kidney damage
		0.02*	70	
		0.03*	20	
		0.04	0	
Carbon tetrachloride	Undiluted	0.05	40	
		0.10	0	
Diethylene glycol	Undiluted	0.05	80	
		0.10	55	
Ethyl acetate	Undiluted	0.05	35	
		0.10	15	
Ethyl alcohol	Undiluted	0.05	95	
		0.10	60	
		0.30	0	
Ethylene glycol	Undiluted	0.05	80	
		0.10	65	
Di-2-ethylhexyl phthalate	Undiluted	0.05	95	
Heptachlor	1% in propylene glycol	0.05	75	
		0.10	65	
Hydrochloric acid	4% in water	0.05	80	
Isopropanol	Undiluted	0.05	35	
		0.10	15	
Malathion	Undiluted	0.05	60	Short legs; bleaching effect on chick down
		0.10	0	
Methanol	Undiluted	0.05	90	
		0.10	65	
Styrene	5% in 80% ethanol	0.05	95	

level, had a short and/or flexed mandible; in addition, some embryos showed subcutaneous edema.

Aroclor 1242 gave no hatch at a level of 25 mg per egg. At a level of 10 mg per egg, one chick hatched out of 20 injected eggs, but died 2 days later. Some embryos, which were examined after they died, showed beak deformities (often a short upper beak), edema, and growth retardation.

Table 3 lists preliminary data on the toxicity of some chemicals which have been shown to be toxic in some degree to animals, and which may be found in some processed foods. Included in this group are various solvents, plasticizers, and insecticides. Some of the chemicals on this list require further study, including observation of the chicks until they reach maturity, before they may be classified as to low or high toxicity.

DISCUSSION

The injection of chemicals into the yolk of fertile eggs prior to incubation is a method which can be advantageously used as an element in the evaluation of the safety of food additive chemicals and drugs, and which could be used to screen new products and eventually to correlate their toxicity with that of similar products already tested.

If one considers that a chemical injected into the yolk may be compared to a substance which has the power to cross the placental barrier, this technique, in addition to being an embryonic feeding study, assumes further importance in that it is also a reproduction study. The unfortunate experiences recently suffered with chemicals that have teratogenic effects in humans, and the failure of conventional testing methods to produce this effect in animals, emphasize the urgent necessity for new methods of analysis. Preliminary work that we have done in this area has given satisfactory results (Verrett and McLaughlin, 1963).

Since this represents a system in which the chemical is in direct contact with the embryo throughout development, it is more than likely that any toxic or teratogenic effects would be readily observed. However, there is always the possibility that the chicken will not be a species susceptible to a particular compound, just as it has been shown that the other commonly used species of animals do not respond to all chemicals in a similar manner. It is also possible for the chicken to be more sensitive to a chemical than other species.

Finally, this technique may be applied also to the study of the synergistic effects of chemicals. Results of experiments in our laboratory on

the glycols. Salter, Robb and Scharles (27) confirmed the findings of Hunt (14) that propylene glycol is converted to glycogen. de Navasquez (28) showed that after the intravenous injection of dioxan in rabbits, cats and guinea pigs, it has a selective action on the convoluted tubules which undergo acute hydropic degeneration causing intrarenal obstruction and anuria with subsequent death from uremia. Several other papers appearing during this period contributed much to our knowledge of the pharmacology of the glycols and their derivatives (29-36).

In 1936 Braun and Cartland (37) confirmed the findings of Seidenfeld and Hanzlik (15) on propylene glycol. The animals used were rats and rabbits; the methods of administration were oral, subcutaneous, intravenous and intramuscular. Fairley, Linton and Ford-Moore (38) expressed the opinion that the injury produced by dioxan is the result of tissue injury produced by diglycollic and oxalic acids. Wiley, Hueper and von Oettingen (39) studied the toxic effects of the vapors of ethylene glycol on rats and mice. Concentrations of 300 mg. per 1000 liters of air had no toxic effects. Schrenk and Yant (40) came to the conclusion that "The fatal cases of poisoning which have occurred should serve as a warning to users of all organic solvents as well as dioxan that good hygienic conditions must be maintained in order to use such compounds safely."

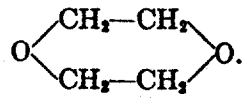
In 1937 Haag (41) and Wallace, Reinhard and Osborne (41a) reported studies of irritating and toxic properties of cigarette smoke containing the combustion products of diethylene glycol. The first investigations directed specifically to the toxicity of administered diethylene glycol appeared in this same year when Haag and Ambrose (42) published their results on the toxicity and fate of diethylene glycol. Acute toxicity studies were made on rats and rabbits by oral, intravenous, intramuscular and subcutaneous administration. Chronic effects were observed in groups of rats receiving drinking water containing 0.03, 0.1, 0.3, 1, 3, and 10% respectively of diethylene glycol. Pathological reports of the organs of animals receiving 1% and less were normal except for the spleen which indicated abnormal splenic deposits of pigment. Most of the glycol was recovered from the urine and very small amounts could be accounted for as oxalic acid.

These investigations, as well as those of Lepkovsky, Ouer and Evans (26), Hanzlik and coworkers (11, 15, 16), and Newman and Lehman (43, 44), preceded those investigations stimulated by the "Elixir of Sulfanilamide" tragedy. These latter include the reports of Geiling, Coon and Schoeffel (45) on rats, rabbits and dogs; Cannon (46), pathologic effects following the ingestion of diethylene glycol; Hagebusch (47), necropsy reports; Ruprecht and Nelson (48), clinical and pathological observations; Lynch (49), report of some cases; Poe and Witt (50); Geiling and Cannon (51), a clinical and experimental correlation of pathological effects of diethylene glycol poisoning; and Calvery and Klumpp (52), the toxicity for human beings of diethylene glycol with sulfanilamide. During this same period several other interesting and

comprehensive papers have appeared (53–63), especially with reference to propylene glycol (54, 56, 59, 60). For additional discussion of the compounds considered in this paper, the reader is referred to the chapter on the glycol group, appearing in the recent report of the British Medical Research Council (69).

In the above-mentioned publications there is considerable information concerning the toxicity of some of these compounds for human beings. Ethylene glycol has been reported to have caused seven deaths, and the amount required is estimated at around 100 cc. (14). Two other almost fatal cases were reported (64). Dioxan has proved extremely poisonous for man but the dosage level could not be estimated principally because the effect was due to the vapors (24). The same is true for ethylene glycol monomethyl ether (62). The fatal dose range for diethylene glycol was, unfortunately, recently quite well established (52). The amount of diethylene glycol in 75% solution consumed by 353 persons is known. Of these, 105 succumbed to its toxic effects. Of those who succumbed, 34 were children of 16 years of age or younger. The mean dose was 52.7 cc. with a minimum of 5 cc. and a maximum of 120 cc. For the adults, the mean dose was 98.6 cc. with a minimum of 20 cc. and a maximum of 240 cc. Of the survivors, 48 were children and the mean dose was 44.2 cc., while the minimum was 3 cc. and the maximum was 105 cc. The mean dose for the adults was 83.7 cc., while the minimum was 1 cc. and the maximum was 240 cc. For the fatal cases, the average dose was about 1 cc. per kg., which illustrates very strongly the point that one cannot neglect emphasizing the hazard and the injustice one may do by translating animal experimentation directly in terms of man. It is even dangerous to make such calculations for fear of their being misinterpreted. Propylene glycol seems to be the least toxic for man of all those related products for which we have information, and its fatal dose is not known.

In this investigation we have determined the dosage-mortality curves for rats, mice, guinea pigs and a small number of rabbits. We have also considered certain factors which might modify the toxic response of the animal. In total, 2600 animals were used to establish the precision of the results. The substances studied were: propylene glycol (CH_3—CHOH—CH_2OH); diethylene glycol (CH_2OH—CH_2—O—CH_2—CH_2OH); "carbitol" (C_2H_5—O—CH_2—O—CH_2—CH_2OH), the mono-ethyl-ether derivative of diethylene glycol; ethylene glycol (CH_2OH—CH_2OH); "cellosolve" (C_2H_5—O—CH_2—CH_2OH), the mono-ethyl-ether derivative of ethylene glycol; and dioxan



Experiments

The substances were all administered by stomach tube in single doses. The animals were housed in individual cages and fasted for about 18 hours (overnight) before the experiment. In most cases, 10 animals were used to test the toxicity at a particular dosage. The end point, of course, was death,

but was not strictly defined in time as is frequently done in other assays of this type. In general, death supervened in from 24 hours to 5 days, with greatest average mortality occurring at about 36 hours. All animals that died, particularly after 5 days, and that presented, by comparison with the mass of data, atypical responses, were carefully examined at autopsy for evidences of mechanical injury. All such animals which revealed such conditions on examination were, of course, excluded. Sexes were approximately equally distributed, and no attempt was made to differentiate this factor. There was some reason to believe that the dilution of the substances might be a factor modifying their toxicity. A large number of toxicities were, therefore, determined on a comparative basis with concentrated and 1 + 1 and 1 + 3 diluted materials. Postmortem evidence indicated that ethylene glycol monoethyl-ether especially was irritating to the gastro-intestinal tract; however, the dosage-mortality curves proved that only in the case of mice with ethylene glycol mono-ethyl-ether was there a significant increase in toxicity, when the concentrated material was compared with the 1 + 1 dilution.

The animals were selected within the following weight ranges: mice 20 ± 8 gm., rats 250 ± 75 gm., guinea pigs 300 ± 100 gm. The importance of maintaining a uniform weight was brought out in a series of studies in which considerably heavier animals were compared with the above standard groups.

The animals came from several different sources and consequently could be expected to give a heterogeneous response to the toxic substances. The experiments were conducted over a 10-month period, which fact could also be regarded as adding heterogeneity. However, none of the mortality curves made in this study were completed within a limited period, but are the composite result of mortality studies obtained throughout the extent of the experimental period. We therefore feel confident that any seasonal or strain modifications have been sufficiently varied by our method of sampling to prevent errors from these sources from affecting the results.

It is generally agreed that the estimation of LD 50, 10, or 90,* particularly the latter two, is not very satisfactory when it is required to interpolate a sigmoid curve through a number of points, some of which may be considerably in error.

The method which we have used for estimating the LD 50, as well as the regression coefficients, is not yet in general use. For complete details, the reader is referred to several papers by Bliss (65, 66). Since the method presents excellent possibilities for the evaluation of toxicity studies, however, it is believed that an example of this method as applied to our data will be valuable.

Briefly, the method is as follows: A sigmoid dosage-mortality curve is "rectified" or converted to a straight line by plotting the dose expressed in logarithms against the observed percentage mortalities expressed in probits (67) or probability units. The best-fitting provisional straight line is drawn through the transformed points and then, by the method of least

* Lethal dose killing 50, 10 or 90% of the animals.

squares, the parameters of an equation for the most probable line through these points are calculated. These parameters are: the slope, b, or regression coefficient, and the abscissa value, a, corresponding to the ordinate point "5", which is the log-dose of 50% kill. Calculations of the standard errors within the fiducial limits for odds of 19 in 20 trials give a measure of the accuracy of the determinations.

Definitions of terms used:

(1) Mortality ratio:

$$\frac{\text{No. animals killed}}{\text{No. animals used}}$$

(2) Log-dose, x: The logarithm of the dose expressed as cc./kgm.

(3) Empirical probit: The converted value of per cent mortality, which is obtained directly from a table (65).

(4) Expected probit, Y: The actual points of intersection in probits of the provisional line with ordinate Y at log-dose x. These can either be estimated by eye or calculated from the equation of the provisional line.

(5) Corrected probit, y: When the empirical probit differs from the expected value by more than 0.05, especially outside the range of 4 to 6 probits, the corrected probit is obtained from the following equation:

$$y = \left(Y + \frac{Q}{Z}\right) - q\left(\frac{1}{Z}\right)$$

where Q is the area in the "tail" of the normal probability curve, beyond the point delimited by Y; Z is the ordinate of the normal curve at the point Y; q is the observed proportion of survivors. Values for y can be read directly from tables calculated for observed values of Y and q.

(6) Weighting Coefficient w: The corrected probits, however, are often of very unequal significance for estimating the dosage-mortality curve. For this reason, each is assigned a weight, w, equal to the information which it contains. Values for w are obtained directly from tables (65) expressed in terms of the expected probit Y. Each w must be multiplied by N, the number of animals used for the particular point on the curve.

(7) Weighted mean log-dose $\bar{x}$:
Weighted mean probit $\bar{y}$:
The dosage mortality curve is computed by a modified method of least squares from the three series of numbers: (1) the logarithms of the dose x, (2) the corrected probits y, and (3) the weights w. From these operations

$$\bar{x} = \frac{S(wx)}{S(w)} \quad \text{and} \quad \bar{y} = \frac{S(wy)}{S(w)}$$

where "S( )" signifies the sum of all quantities such as are enclosed in parentheses.

(8) Weighted squares, $[wx^2]$:
Weighted products $[wxy]$:
The weighted squares and products of the deviations from the two means $\bar{x}$ and $\bar{y}$ are

$$[wx^2] = S(wx^2) - \bar{x}\, S(wx)$$
$$[wxy] = S(wxy) - \bar{y}\, S(wx)$$

where the square brackets indicate the sum of deviations from the mean.

(9) Slope, b: This is also known as the regression coefficient of the dosage-mortality curve. It is calculated as

$$b = \frac{[wxy]}{[wx^2]}, \text{ or its reciprocal } \frac{1}{b} = s$$

(10) The corrected equation, $y = \bar{y} + b(X - \bar{x})$: can be used for calculating probit kill in terms of log-dose X, or by rearrangement,

$$X = \bar{x} + s(Y - \bar{y})$$

where log-dose X is calculated in terms of probit kill. The latter equation has been used for calculating the log-dose X at 50% mortality.

(11) The standard error, s.e.: This has been calculated for the LD 50 and the regression coefficient b. By multiplying the standard error by 1.96

(assuming independence of the degrees of freedom) the fiducial limits of error for odds of 19 in 20 have been obtained.

For b, the regression coefficient

$$\text{s.e.} = \pm\sqrt{\frac{1}{[wx^2]}}$$

For log LD 50

$$\log \text{s.e.} = \pm\sqrt{s^2\left(s\frac{(5-\bar{y})^2}{[wxy]} + \frac{1}{S(w)}\right)}$$

A numerical example has been given of the calculations made for determining the equation of the curve of toxicity of ethylene glycol for rats (table 1).

In figure 1, the empirical probits were laid out as hollow circles, and the best provisional line (represented by the broken line) was drawn through them. Expected probits were then read off from this line and recorded in the tabulation. The corrected probits, obtained from tables in terms of the expected probit, are represented by the solid circles, and show how close the agreement has been. The corrected line is shown as the heavy solid line. Since the corrected line depends for its values upon the estimated values of the provisional line, it becomes obvious that unless the two lines coincide closely (the example cited here is about the outside limit), a second approximation must be made, in which the points of the first corrected line are used for calculating a new corrected line.

All of the experimental data obtained are presented in table 2. In addition, the results obtained on a different strain of rats, all raised under identical conditions and randomized with respect to weight and sex, are appended to the bottom of the table. The animals in this latter series received similar dosages of the substances at 1 + 3 dilutions, where possible, instead of 1 + 1, or concentrated. Calculations of the LD 50, based on these results, have shown in every case close agreement with the LD 50 obtained from the larger, but more heterogeneous lot of animals. This is additional evidence that, except for the case of ethylene glycol monoethyl-ether in mice, dilution does not significantly modify the dosage-mortality curve.

In order to study the dilution effect somewhat more in detail, two dosage-mortality curves were calculated for ethylene glycol: one in which it was given in concentrated form, and one in 1 + 3 dilution. Five doses for 11 animals each, spaced at equal logarithmic intervals, were used to define each curve. The rats were all from the same strain; their ages were 3 months ± 9 days, and their weights were 275 ± 25 gm. By means of the transformations already described in detail, the data presented in table 3 have been used to calculate the equations for the "rectified" dosage-mortality curves of concentrated and dilute ethylene glycol. Calculation of the standard errors of the regression coefficients and the log LD 50's, for limits of 19 in 20 determinations, shows very clearly that no significant differences between the concentrated and diluted material exist, at least for the number of animals involved in the test. Comparison also shows that the LD 50's, as well as the regression coefficients obtained here check, within the fiducial limits set, the values for

TABLE 1

CALCULATION OF THE DOSAGE-MORTALITY CURVE OF ETHYLENE GLYCOL IN RATS

DOSE	MORTALITY RATIO	LOG-DOSE x	EMPIRICAL PROBIT	EXPECTED PROBIT Y	CORRECTED PROBIT y	WEIGHT w	WEIGHT TIMES LOG-DOSE wx
cc./kg.							
3.5	0/7	0.54407	—	3.77	3.185	2.5197	1.37089
4.0	4/20	0.60206	4.1584	4.16	4.159	9.8074	5.90464
4.5	3/10	0.65321	4.4756	4.48	4.476	5.7664	3.76667
5.0	11/20	0.69897	5.1257	4.80	5.075	12.3484	8.63116
5.5	15/20	0.74036	5.6745	5.04	5.628	12.7250	9.42108
6.0	4/10	0.77815	4.7467	5.30	4.729	6.1609	4.79410
6.25	7/10	0.79588	5.5244	5.40	5.521	6.0052	4.77942
6.5	2/10	0.81291	4.1584	5.50	4.104	5.8099	4.72293
7.0	13/20	0.84510	5.3853	5.72	5.347	10.5196	8.89011
7.5	17/20	0.87506	6.0364	5.90	6.023	9.4288	8.25077

$S(w) = 81.0913$ $\qquad \bar{x} = \frac{S(wx)}{S(w)} = 0.74646$

$S(wx) = 60.53177$

$S(wy) = 408.08046$ $\qquad \bar{y} = \frac{S(wy)}{S(w)} = 5.03235$

$S(wx^2) = 45.87579$ $\qquad [wx^2] = S(wx^2) - \bar{x}S(wx) = 0.69124$

$S(wxy) = 308.27946$ $\qquad [wxy] = S(wxy) - \bar{y}S(wx) = 3.66241$

$$b = \frac{[wxy]}{[wx^2]} = 5.29831 \qquad s = \frac{[wx^2]}{[wxy]} = 0.18873$$

Corrected equation $X = \bar{x} + s(Y - \bar{y})$

when $Y = 5.0$, $X = 0.74036$ (log LD 50)

Standard error of b, the regression coefficient:

$$\text{s.e.} = \frac{1}{[wx^2]} = \pm 1.203$$

within fiducial limits corresponding to odds of 19 in 20

$1.96 \times \text{s.e.} = \pm 2.36$

Standard error of X, the log LD 50

$$\text{s.e.} = \sqrt{s^2\left(s\frac{(5 - \bar{y})^2}{[wxy]} + \frac{1}{S(w)}\right)} = 0.02094$$

within fiducial limits corresponding to odds of 19 in 20

$1.96 \times \text{s.e.} = \pm 0.04106$

whence in antilogarithms

LD 50 = 5.50 cc./kg. within limits of 5.00 and 6.05 cc./kg.

ethylene glycol given in table 4 for a larger, but more heterogeneous group of rats.

In table 4 are given the constants for the equations of the "rectified" dosage-mortality curves obtained by the series of transformations from the original data of table 1. The fiducial limits for odds of 19 in 20 have been calculated for the regression coefficients as well as the LD 50's expressed as the logarithm and as the actual dose. Referring to the action of ethylene glycol mono-ethyl ether on mice, it can be seen that the LD 50's are significantly different for the concentrated and the 1 + 1 diluted substance. The difference in regression coefficients is, however, not significant, since the fiducial limits for odds of 19 in 20 overlap slightly. Two factors clearly define our knowledge of the toxicity of any of the substances examined: (1) the LD 50: This is best illustrated graphically as the abscissa or x value corresponding to the point of intersection of the "rectified" line with the y axis at probit 5. In other words, this value defines the *position* of the line; (2) the regression coefficient: This is a measure of the sensitivity* (measured,

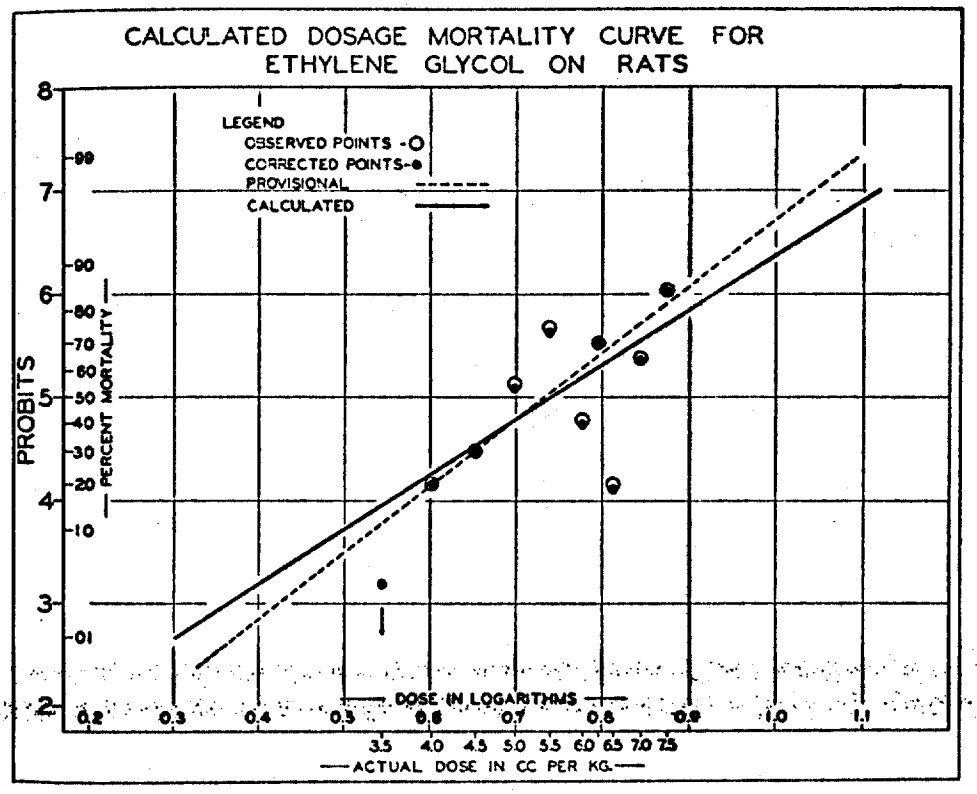


FIG. 1. Calculated dosage mortality curve for ethylene glycol on rats

* From the standpoint of the statistician, the regression coefficient is, properly speaking, a measure of the variability, since by definition s, the reciprocal of the regression coefficient, is the standard deviation of the response of a given population to a drug or poison in terms of the logarithm of the dose.

TABLE 2

THE DOSAGES AND MORTALITY RATIOS FOR ALL THE SUBSTANCES STUDIED ON GUINEA PIGS, MICE AND RATS

	Propylene glycol		Diethylene glycol		Ethylene glycol		Diethylene glycol mono-ethyl-ether		Dioxan		Ethylene glycol mono-ethyl-ether		
	Dose	Mortality ratio	Dose	Mortality ratio	Dose	Mortality ratio	Dose	Mortality ratio	Dose	Mortality ratio	Dose	Mortality ratio	
	cc./kg.		cc./kg.		cc./kg.		cc./kg.		cc./kg.		cc./kg.		
Guinea pigs	15.0	0/10	5.0	1/10	5.0	2/5	2.5	0/5	3.0	0/10	2.5	1/10	
			5.0	0/5			3.0	2/11					
	17.5	3/10	6.0	2/10	5.5	0/5	3.5	4/10	3.5	3/10	2.75	9/15	
			7.5	4/10			4.0	5/10					
	20.0	6/10	7.5	5/10	6.0	1/10	4.0	6/10	4.0	12/20	3.0	15/20	
			10.0	8/10			5.0	8/10					
	22.5	10/10	15.0	2/2	7.5	4/10	7.5	10/10	4.5	7/10	3.5	13/18	
			20.0	2/2									
					8.5	7/10			5.0	10/10	4.0	10/10	
					10.0	10/10							
Mice			15.0	2/10	7.5	2/10	5.0	0/14	4.5	1/10	3.0	0/10	Concentrated
	20.0	2/20	18.75	2/11	7.5	1/10	5.5	8/10	4.5	0/10	3.5	3/20	
			20.0	8/40	10.0	4/10	6.0	13/30	5.0	3/10	4.0	6/10	
	22.5	6/20	22.5	5/10	10.0	2/20	6.25	1/10	5.0	3/20	4.5	4/10	
			25.0	14/26	11.0	4/10	6.5	7/10	5.5	10/20	5.0	7/10	
	25.0	14/20	30.0	8/10	11.0	7/20	7.0	10/10	5.5	10/30	6.0	10/10	
			35.0	10/10	12.5	4/10	7.5	8/18	5.75	3/10	3.0	0/10	Diluted
	30.0	9/10			12.5	12/20	8.0	17/20	5.75	5/10	3.5	2/10	
					15.0	6/10	8.25	9/20	6.0	8/10	4.0	2/10	
					15.0	6/10	10.0	8/10	6.0	15/20	4.5	3/10	
					20.0	7/10			7.5	10/10	5.0	11/30	
					20.0	9/10					5.5	6/10	
											6.0	8/10	
Rats—Series A	15.0	1/10	10.0	1/10	3.5	0/7	3.5	0/10	3.5	0/20	2.6	0/10	
	15.0	0/10	10.0	1/10	4.0	4/20	4.0	0/20	4.0	3/20	3.0	8/10	
	17.5	5/10	11.25	2/10	4.5	3/10	4.5	1/10	5.0	9/20	3.0	4/10	
	17.5	3/10	12.5	10/20	5.0	11/20	5.0	4/20	6.0	14/20	3.3	5/10	
	20.0	7/10	12.5	5/10	5.5	15/20	5.5	8/20	7.5	16/16	3.3	8/10	
	20.0	2/10	15.0	9/20	6.0	4/10	6.0	14/20			3.5	10/30	
	22.5	6/10	15.0	8/15	6.25	7/10	6.25	6/10			3.5	7/10	
	22.5	4/10	17.5	5/10	6.5	2/10	6.5	10/10			3.75	7/10	
	25.0	7/9	17.5	8/10	7.0	13/20	7.5	10/10			4.0	15/20	
			18.75	10/10	7.5	17/20					4.0	9/10	
			20.0	20/20									
Rats—Series B	17.6	1/9	10.9	0/9	4.0	2/9	5.0	2/9	4.0	0/9	3.1	3/9	
	18.6	8/9	12.6	1/9	5.0	4/9	5.5	3/9	4.7	2/9	3.4	6/9	
	20.0	5/9	14.1	4/9	6.3	7/9	6.0	6/9	5.6	6/9	3.7	8/9	
	21.4	9/9	15.9	5/9					6.3	9/9			
	22.6	9/9	18.3	9/9									

in this case, by mortality) of a given population to a change in the dose. Graphically, sensitivity is illustrated by the *slope* of the "rectified" line. For example, referring to the effects of propylene glycol on mice, we see that this substance has a very low toxicity (LD 50 23.9 cc./kg.), but a small increment of dosage, 20%, produces a tremendous change in mortality (from 50% to 90%) because of the large regression coefficient. On the other hand, diethylene glycol-mono-ethyl-ether is very much more toxic (LD 50 6.58 cc./kg.) but because of the low regression coefficient, it requires nearly double the dose to increase the mortality from 50 to 90%. Because of the large possible variation in the regression coefficient, it becomes at once apparent why toxicities determined at any other mortality than 50% are less accurate. In order to effect accuracy of the same order, it would be necessary to use very much larger numbers of animals.

In order to reveal most effectively the relative toxicities of the six substances for each of the three species of animals, the "rectified" curves have been plotted on a graph in which the logarithm of the dose is laid off on the abscissa, on a scale sufficiently large to include the entire range of toxicities encountered. A separate Y or probit axis is given for each substance. The LD 50 can be directly interpolated from the intersection of the line with the 50% mortality or 5-probit mark.

With one exception (ethylene glycol), the descending order of toxicity to the animals is guinea pigs, rats, mice. The ascending order of toxicity for the six substances is propylene glycol, diethylene glycol, ethylene glycol, diethylene glycol mono-ethyl-

TABLE 3

EFFECT OF DILUTION ON THE TOXICITY OF ETHYLENE GLYCOL

Concentrated

DOSE	LOG DOSE	MORTALITY RATIO
cc./kg.		
3.50	0.544	2/11
3.98	0.608	2/11
4.70	0.672	4/11
5.43	0.735	5/11
6.30	0.799	6/11

Log LD 50 = 0.76819 ± 0.11143
Actual LD 50 5.86 cc./kg. within the limits of 4.54 and 7.58 in 19 out of 20 determinations
Regression Coefficient = 4.42 ± 4.04

Diluted 1 + 3

DOSE	LOG DOSE	MORTALITY RATIO
cc./kg.		
3.50	0.544	2/11
3.98	0.608	3/11
4.70	0.672	3/11
5.43	0.735	5/11
6.30	0.799	8/11

Log LD 50 = 0.72999 ± 0.0743
Actual LD 50 5.28 cc./kg. within the limits of 4.53 and 6.37 in 19 out of 20 determinations
Regression Coefficient = 5.47 ± 4.14

ether, dioxan and ethylene glycol mono-ethyl-ether.

It is to be noted that the substituted ethers, diethylene glycol mono-ethyl-ether, dioxan and ethylene glycol mono-ethyl-ether all have toxicities of approximately the same order of magnitude for the three species of animals tested. With one exception (mice, diethylene glycol mono-ethyl-ether), the regression coefficients are fairly large.

In table 5 are presented the results of experiments designed to test whether weight is a factor which might modify the response of an animal to a toxic substance. It can be seen, particularly in the case of rats and guinea pigs, but scarcely at all in mice, that diethylene glycol, ethylene

TABLE 4

Substance	Constants for equation $Y = \bar{y} + b(X - \bar{x})$				The LD 50 in cc./kg.				
	$\bar{x}$	$\bar{y}$	b	±1.96 x s.e.	Log-dose	±1.96 x s.e.	Actual dose	Limits of error: Upper	Limits of error: Lower
Guinea pigs									
Propylene Glycol	1.279	5.067	22.4	10.0	1.276	0.040	18.9	20.7	17.2
Diethylene Glycol	0.852	4.708	7.77	3.69	0.889	0.026	7.76	8.24	7.31
Ethylene Glycol	0.862	5.045	7.57	4.16	0.866	0.051	7.35	8.27	6.53
Diethylene Glycol Mono-Ethyl-Ether	0.583	4.965	8.62	3.77	0.587	0.020	3.87	4.05	3.69
Dioxan	0.597	5.104	15.5	6.4	0.590	0.024	3.90	4.12	3.69
Ethylene Glycol Mono-Ethyl-Ether	0.478	5.352	10.6	5.7	0.445	0.035	2.79	3.02	2.57
Rats									
Propylene Glycol	1.292	4.762	7.92	4.22	1.322	0.039	21.0	23.0	19.2
Diethylene Glycol	1.158	5.078	7.57	2.60	1.169	0.030	14.8	15.8	13.8
Ethylene Glycol	0.746	5.032	5.30	2.36	0.740	0.041	5.50	6.05	5.00
Diethylene Glycol Mono-Ethyl-Ether	0.749	4.993	17.2	5.4	0.743	0.016	5.54	5.73	5.33
Dioxan	0.704	4.889	11.5	3.7	0.713	0.028	5.17	5.52	4.84
Ethylene Glycol Mono-Ethyl-Ether	0.535	5.022	10.6	4.7	0.537	0.021	3.46	3.62	3.28
Mice									
Propylene Glycol	1.366	4.816	16.1	7.3	1.378	0.021	23.9	25.1	22.8
Diethylene Glycol	1.360	4.869	7.85	3.11	1.374	0.029	23.7	25.4	22.2
Ethylene Glycol	1.075	4.798	4.83	0.61	1.117	0.047	13.1	14.6	11.8
Diethylene Glycol Mono-Ethyl-Ether	0.834	5.077	4.70	2.61	0.818	0.044	6.58	7.63	5.94
Dioxan	0.742	4.817	17.6	6.8	0.752	0.013	5.66	5.84	5.49
Ethylene Glycol Mono-Ethyl-Ether:									
Concentrated	0.618	4.819	11.5	4.6	0.634	0.031	4.31	4.64	4.01
1 + 1 dilute	0.679	4.713	7.69	3.82	0.716	0.041	5.20	5.73	4.73

glycol, diethylene glycol mono-ethyl-ether and ethylene glycol mono-ethyl-ether give indications of greater toxicity for animals whose weights ranged from 37 to 108% higher than the average. Dioxan and propylene glycol, in contrast, give no clear-cut indications. It is interesting that mice heavier than the average did not react the way the rats and guinea pigs did. This may be due to the fact that the toxicity of all of the substances is lower for mice than for rats and guinea pigs.

While both "heavy" and "light" animals received the same dose per

TABLE 5

THE RELATION BETWEEN BODY WEIGHT AND THE LETHAL PROPERTIES OF THE COMPOUND

COMPOUND	"HEAVY"			"LIGHT"		
	Average weight	Dose	Mortality ratio	Average weight	Dose	Mortality ratio
Guinea pigs						
	gm.	*cc./kg.*		*gm.*	*cc./kg.*	
Propylene Glycol	—	—	—	—	—	—
Diethylene Glycol	—	—	—	—	—	—
Ethylene Glycol	558	6.0	9/9	268	6.0	1/10
Diethylene Glycol Mono-Ethyl-Ether	665	3.0	5/5	368	3.0	0/10
Dioxan	463	7.0	2/5	233	7.0	2/5
Ethylene Glycol Mono-Ethyl-Ether	540	2.75	15/16	295	2.75	3/9
Rats						
Propylene Glycol	—	—	—	—	—	—
Diethylene Glycol	396	15.0	15/15	288	15.0	10/25
Ethylene Glycol	380	4.5	7/10	214	4.5	3/10
Diethylene Glycol Mono-Ethyl-Ether	415	5.0	11/15	260	5.0	4/20
Dioxan	391	5.0	4/4	226	5.0	5/10
Ethylene Glycol Mono-Ethyl-Ether:						
Concentrated	394	3.3	1/5	233	3.3	5/10
1 + 1 dilute	365	7.0	2/8	221	7.0	5/10
Mice						
Propylene Glycol	26.8	22.5	0/10	15.6	22.5	3/10
Diethylene Glycol	29.8	20.0	5/10	16.0	20.0	2/10
	25.8	22.5	7/10	20.9	22.5	5/10
Ethylene Glycol	27.1	12.5	6/10	14.6	12.5	7/10
	30.0	11.0	6/10	22.2	11.0	1/10
Diethylene Glycol Mono-Ethyl-Ether	27.3	4.0	10/10	15.4	4.0	6/10
	27.1	7.0	10/10	20.9	7.0	10/10
Dioxan	25.9	5.75	6/10	13.4	5.75	5/10
	28.7	6.0	9/10	20.6	6.0	7/10
Ethylene Glycol Mono-Ethyl-Ether:						
Concentrated	24.1	5.0	4/10	13.7	5.0	4/10
1 + 1 dilute	24.8	7.0	10/10	19.6	7.0	10/10

kilogram in each test, the heavier animals, of course, received larger actual amounts. Two factors might serve, therefore, to increase the toxicity for the larger animal: (1) unequal distribution of the substance in body tissues (relative insolubility of the glycols in fatty tissues); this would tend to raise the level at which the substances would circulate; (2) inability of the excretory organs, chiefly the kidneys, to cope with excessive amounts of toxic materials. It is well known that the ratio of kidney to body weight decreases with the size of the animal instead of remaining constant. Thus the 400 gm. rat does not have kidneys which weigh twice as much as the 200 gm. animal. In effect, therefore, in estimating the dose on a per-kilogram basis, we have tacitly assumed that the functional tissue in an animal increases *pari passu* with its whole body weight, which actually does not occur. This has, of course, been generally recognized, since most biological assays prescribe the use of animals within certain stated weight ranges. While the experiments here cited are of somewhat preliminary nature, they nevertheless emphasize the importance of a restricted weight range.

Symptomatic Response and Pathology

Propylene glycol. Shortly after administration of large doses, the animals showed signs of loss of equilibrium, marked depression, analgesia, coma and finally death after a prolonged moribund state.

The gross observations of organs were essentially negative except for hemorrhagic areas in the small intestine. Microscopic changes in the kidney were minimal but probably relevant, with nuclear pyknosis and vacuolar degeneration of the cytoplasm. A few cortical tubules contained protein debris or loose casts. The liver showed only slight congestion and hyperemia with no fatty changes.

Diethylene glycol. The toxic syndrome in rats particularly and to some extent in rabbits, dogs, mice and guinea pigs resembled that reported for human beings. There was occasional vomiting in dogs. In rats there was early increased thirst, diuresis, ruffled coat and refusal of food. After the first 2 or 3 days there was practically complete cessation of urine excretion with heavy proteinuria. There was prostration, dyspnea, bloated appearance, coma, with pronounced lowering of body temperature for about 24 hours before death. Other animals behaved similarly except there was variation in sensitivity.

At autopsy, because of the prolonged moribund condition, there were usually some autolytic changes in the internal organs even though the autopsy was performed immediately. There was often generalized edema, hemorrhage into lungs, intestines and kidneys, and pneumonia. The liver was usually soft and fragile. The kidneys were often grossly normal in appearance but at times appeared swollen, pale and flabby. The livers and kidneys were most severely affected, and sometimes the adrenals were involved, showing extensive mitotic activity. There was extensive degeneration of the renal cortex with vacuolar (hydropic) degeneration of the convoluted tubules. In places, the degeneration of the epithelium was

so severe that nothing but the basement membranes of the tubules remained, while cellular debris, protein precipitate, and hyaline casts seemed to fill the lumen of what remained of the tubules. There was usually no clear evidence of involvement of the glomerular tufts but Bowman's space was often distended by accumulation of a protein precipitate. In some cases the vessels of the cortex were thrombosed, with possible involvement of the vessels of the medulla. Although not usually seriously affected, there may have been pronounced degeneration of the proximal tubules of the medulla with numerous hyaline casts. In animals that survived for 8 days, there was some evidence of regenerative changes in the kidney. In the liver there was widespread diffuse hydropic degeneration of the central portion of the liver lobule. This degeneration was of varying degree in different foci. In some, it proceeded to the complete disintegration of liver cells; in others, vacuoles distended the cytoplasm which retained almost normal nuclei. The lungs of most animals were essentially normal, but as mentioned above there was congestion, edema and focal interstitial pneumonia and hemorrhage in some animals. The spleen in many cases showed excessive phagocytosis of blood pigment. The heart, intestine, stomach, brain and bladder in most cases were essentially normal.

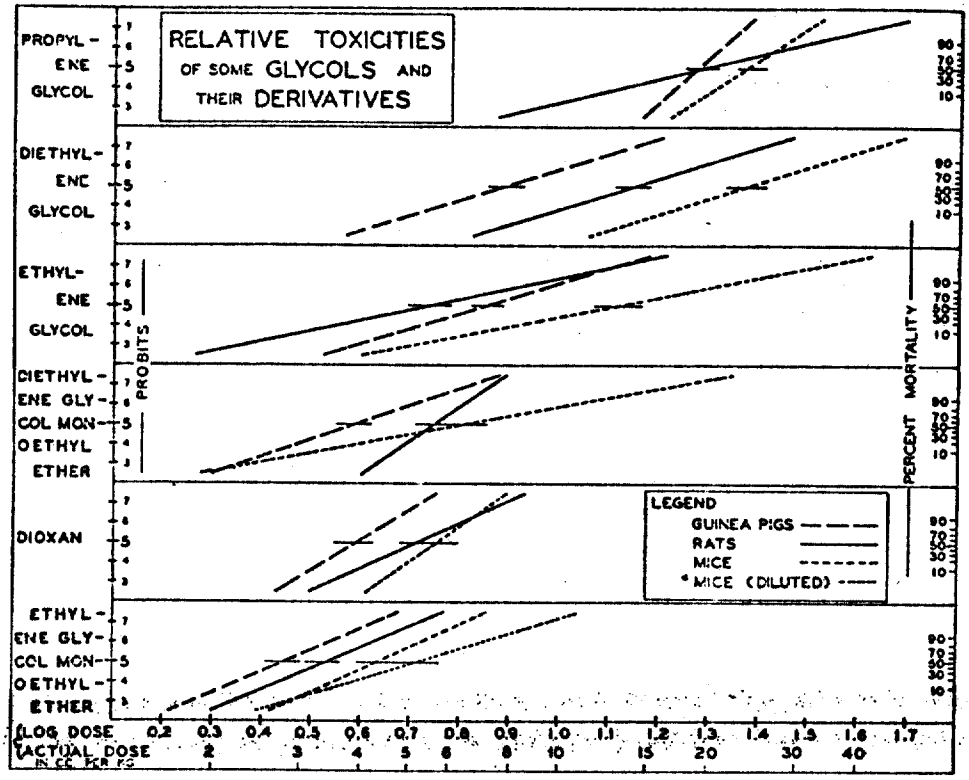
Ethylene glycol. Shortly after administration, the animals showed signs of weakness and lack of muscular coordination. This was in contradistinction to the picture following administration of diethylene glycol, where no immediate symptoms occurred. Prostration and coma were later symptoms, followed by death. At the higher doses, death occurred in from 18 to 24 hours, but, at the lower doses, was sometimes delayed for 4 to 6 days. At autopsy, the gross findings included congestion of the lungs with hemorrhage in most cases. At the higher levels of intake, the stomach usually showed hemorrhagic areas. The bladders were nearly always filled with urine that gave heavy protein tests. Microscopically there was in nearly all cases hydropic degeneration of the cells lining the cortical convoluted tubules and focal necrosis in the liver. Other organs were essentially normal.

Diethylene glycol mono-ethyl-ether. The early symptoms were very similar to those of animals receiving toxic doses of ethyl alcohol. After a period of prostration and coma, death followed within 24 to 48 hours, depending upon the size of the dose. Grossly, the organs appeared essentially normal. Microscopically, there was evidence of lobular broncho-pneumonia, focal myocarditis, focal necrosis in the liver, vacuolation of the cortical tubules with occasional tubular casts in both cortex and medulla. In some of the animals, the mitotic activity in the adrenals was striking. Other organs appeared normal.

Dioxan. Immediately after administration, some animals appeared limp and dazed, while others exhibited distinct signs of intoxication with muscular incoördination. Except for the larger doses, where these symptoms gradually verged into coma and death within 24 hours, there was often apparent recovery, with subsequent death in some cases after several

days. Gross observations showed hemorrhagic areas near the pyloric end of the stomach. The bladders were distended with urine and although there was slight proteinuria, there was no hematuria as in the case of cellosolve. The kidneys were somewhat enlarged. Other organs appeared grossly normal. In some of the animals, microscopic changes in the liver and kidneys were slight; in others, extensive changes resembling those noted for diethylene glycol occurred. All other organs examined were essentially normal.

Ethylene glycol mono-ethyl-ether. In contrast to ethylene glycol, from which it is derived, ethylene glycol mono-ethyl-ether produced no immediate symptoms whatever. In this respect it resembled diethylene glycol, even to the extent that with moderate doses, death was sometimes delayed for 4 to 6 days. With large doses, on the other hand, death usually occurred in 24 to 36 hours. Hematuria was noted in nearly all animals, and after death the bladders remained distended with bloody urine. The kidneys of some animals showed extreme tubular degeneration with almost complete necrosis of nearly all of the cortical tubules. The changes appeared to be chiefly of the hyaline droplet or colloid



* The substance in this case was administered in diluted form to mice, that is, 1 + 1.

FIG. 2. Relative toxicities of some glycols and their derivatives

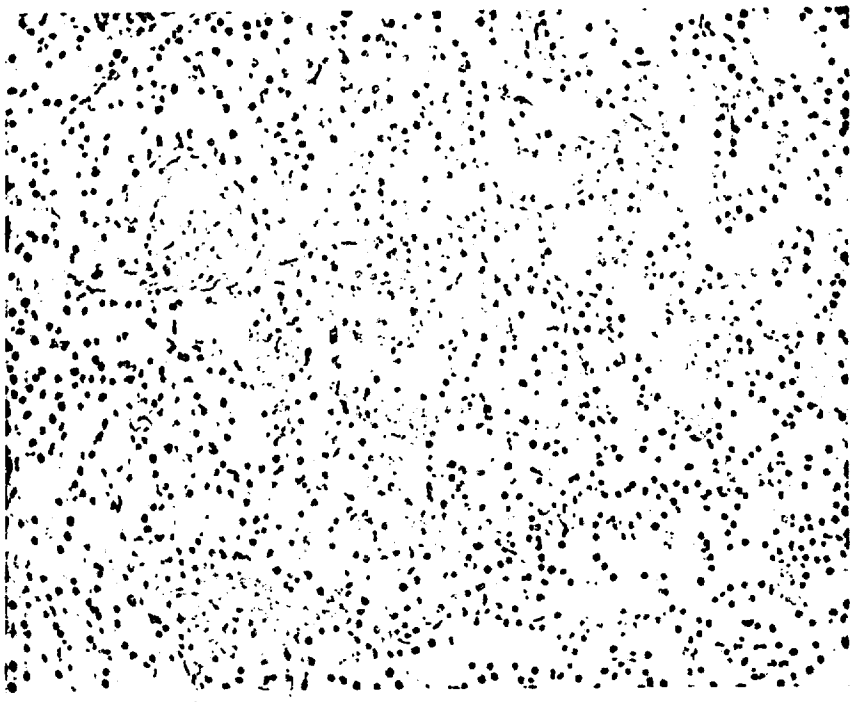


FIG. 3. Propylene glycol. Rat kidney. Normal. ×225.

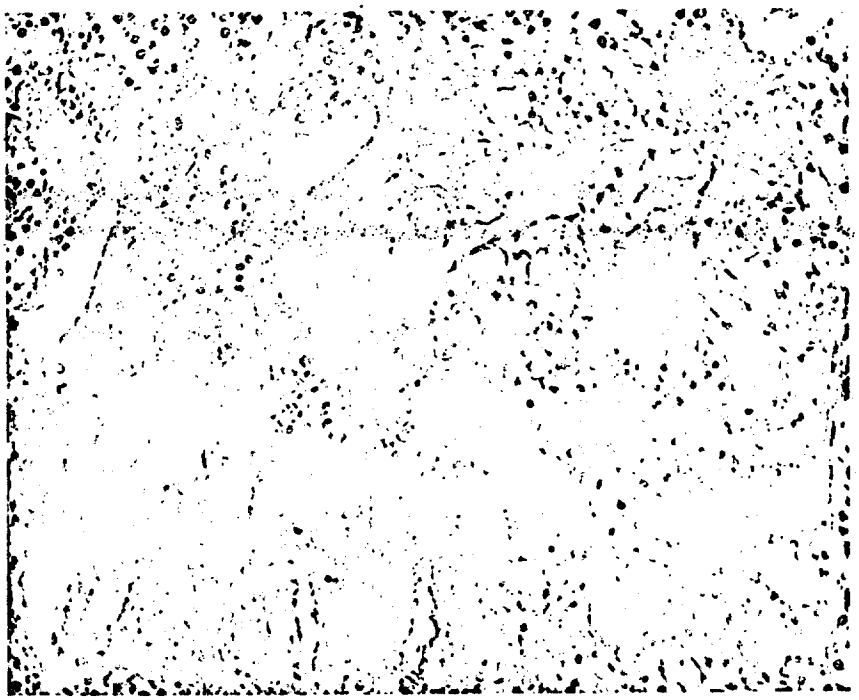


FIG. 4. Diethylene glycol. Rat kidney. Extensive necrosis of convoluted tubules (right half). ×225.

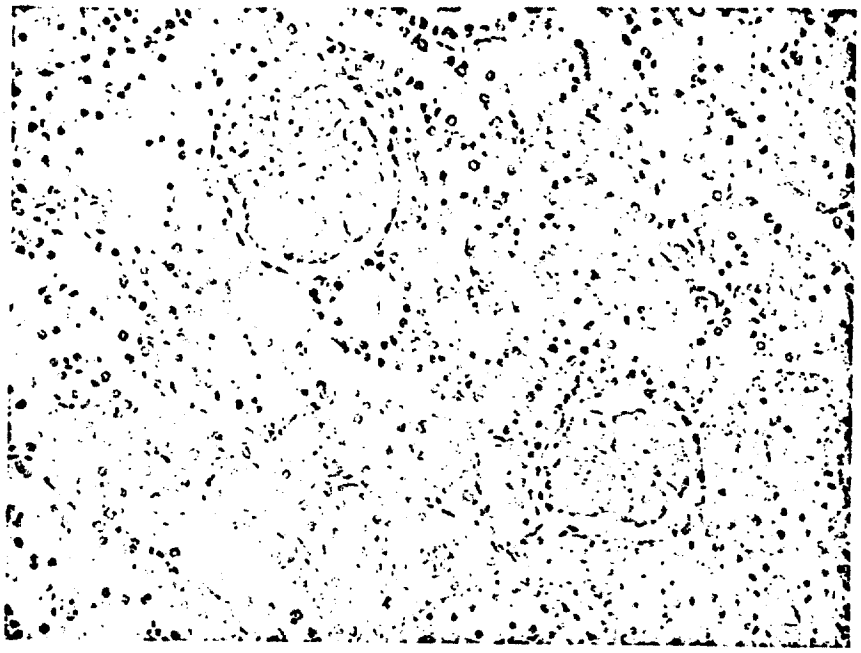


FIG. 5. Ethylene glycol. Rat kidney. Slight dilatation of convoluted tubules with proteinuria (only slight change as compared with rat kidney after diethylene glycol). ×225.

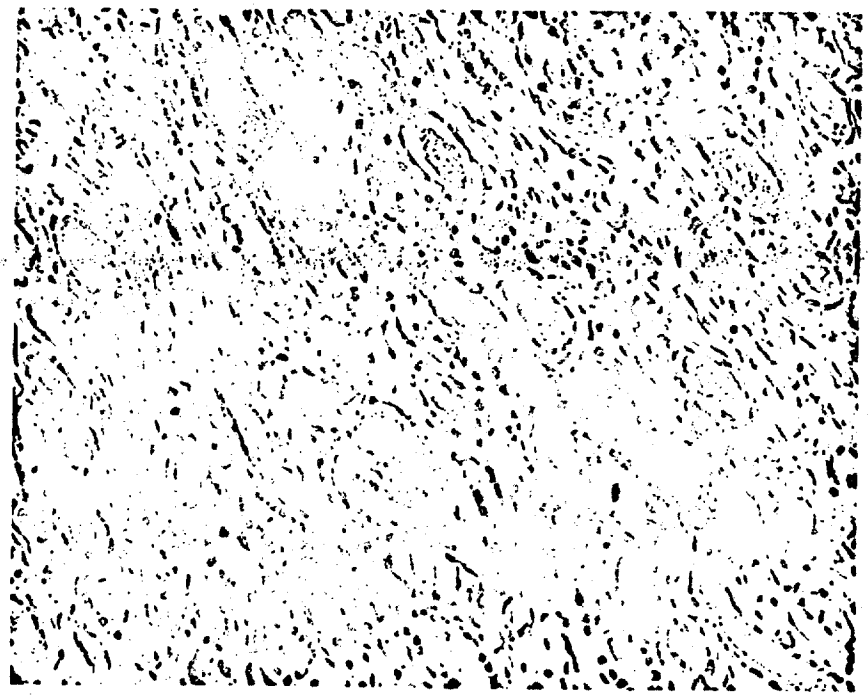


FIG. 6. Diethylene glycol mono-ethyl-ether. Rabbit kidney. Numerous granular casts in the medullary tubules. ×225.

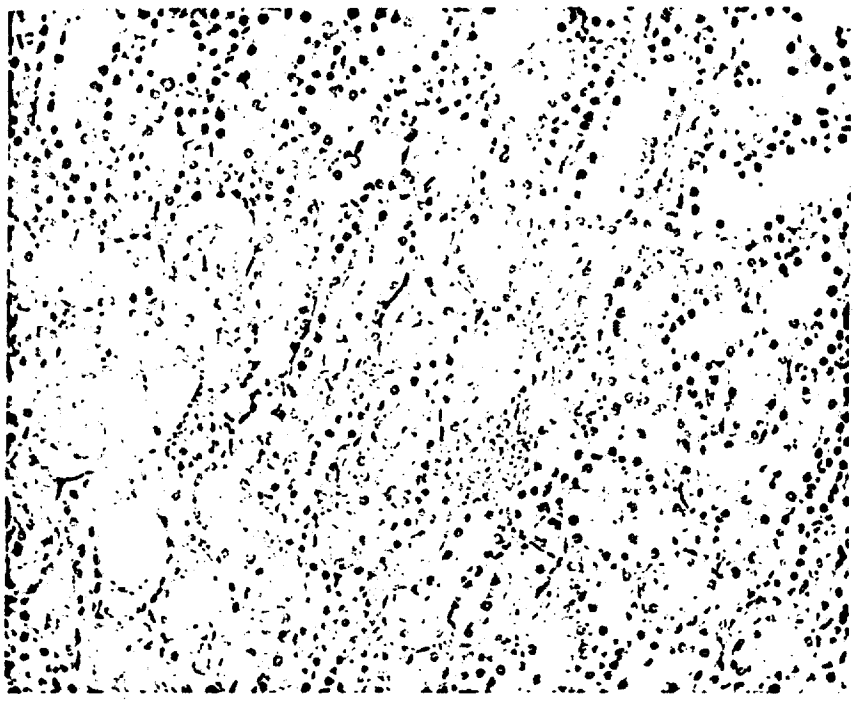


FIG. 7. Ethylene glycol mono-ethyl-ether. Rat kidney. Focal degeneration of convoluted tubules (central part of left half) and cast formation. ×225.

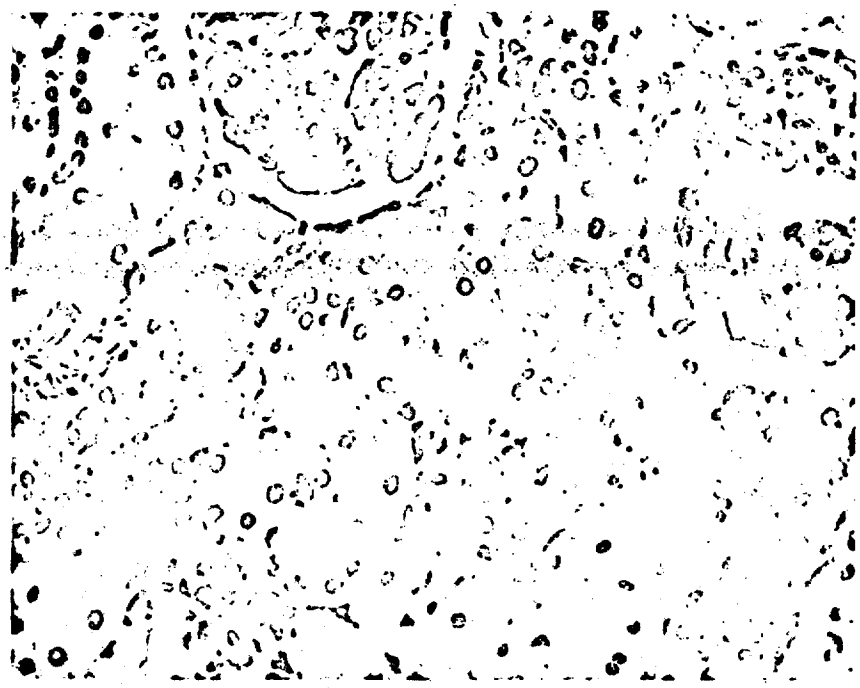


FIG. 8. Dioxan. Rat kidney. Focal degeneration of convoluted tubules (cytoplasmic degeneration with pyknosis of nuclei; note tubules in lower right hand corner). ×450.

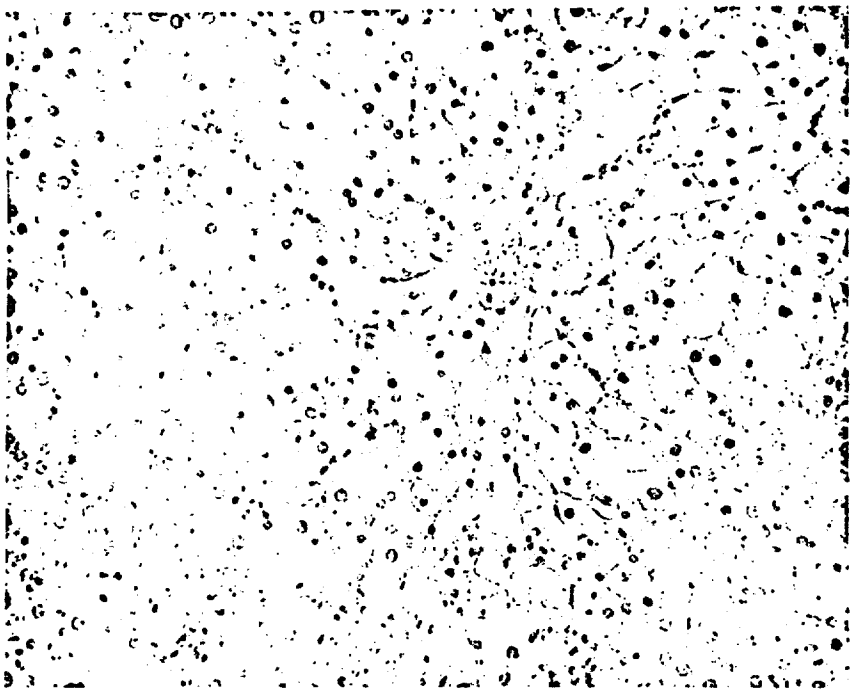


FIG. 9. Diethylene glycol. Rat liver. Hydropic degeneration of cells of center of liver lobule. ×225.

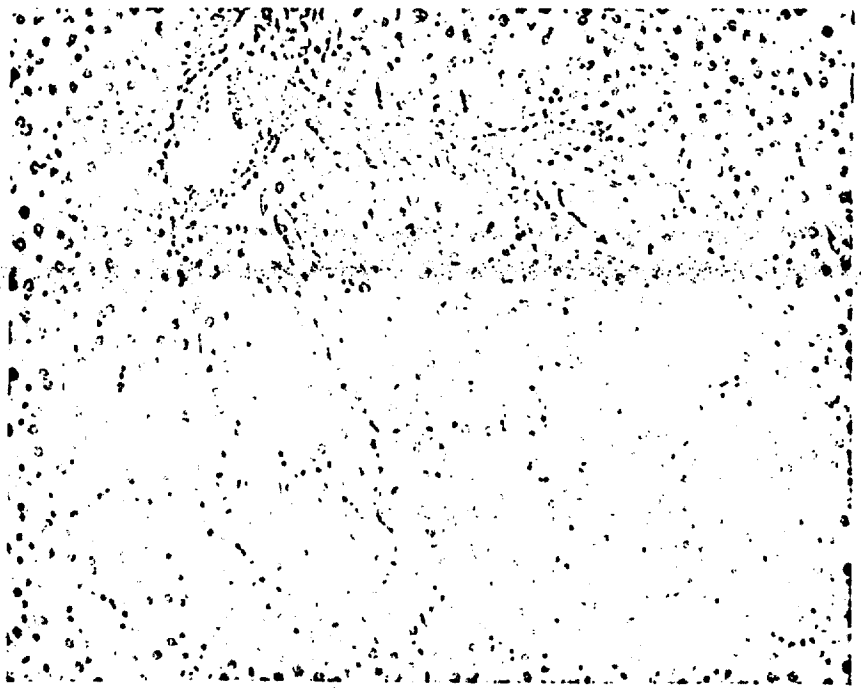


FIG. 10. Propylene glycol. Rat liver. Normal. ×225.

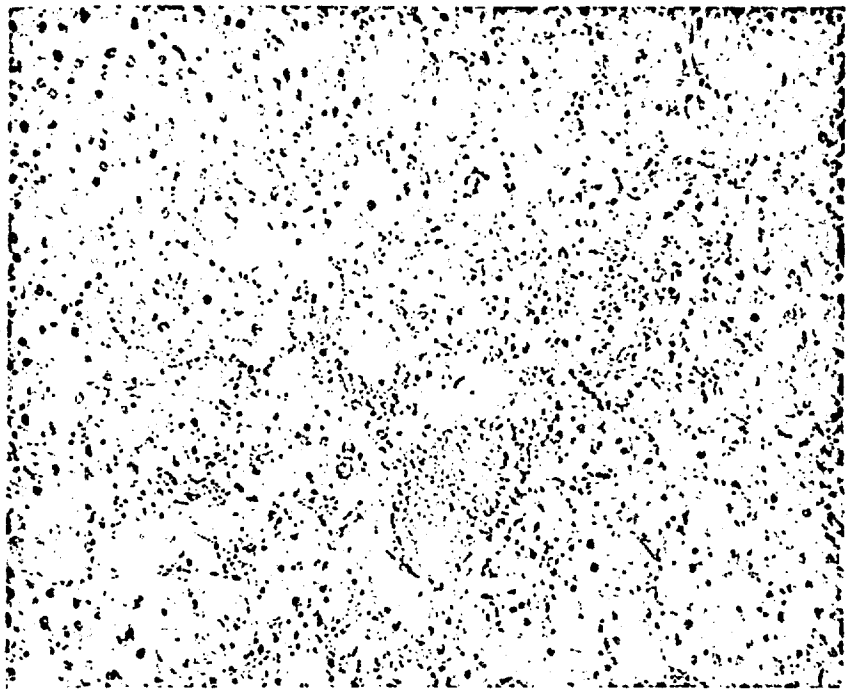


FIG. 11. Ethylene glycol. Rat liver. Degeneration of liver cells in central portion of liver lobule (less marked than in rat liver after diethylene glycol). ×225.

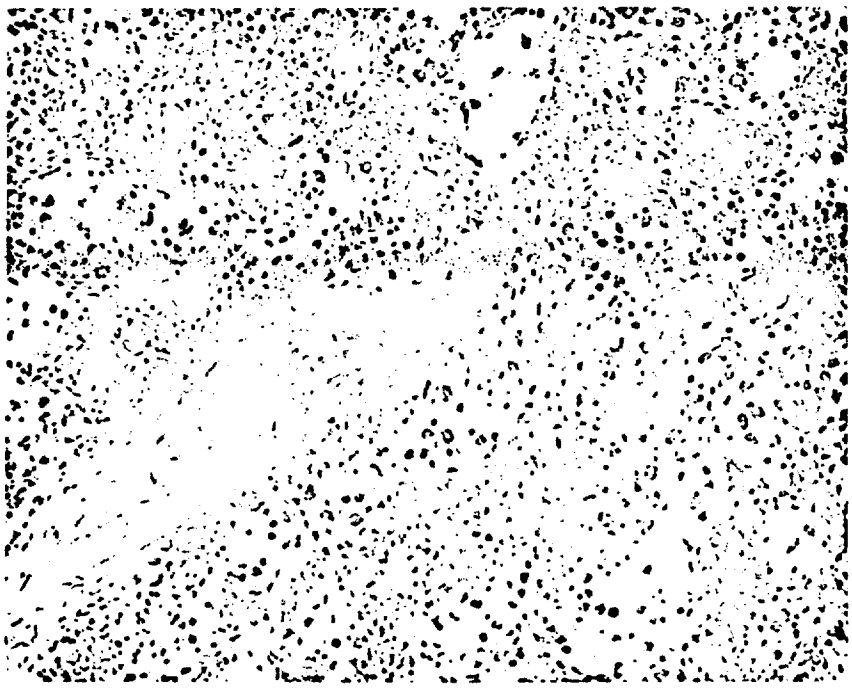


FIG. 12. Diethylene glycol mono-ethyl-ether. Rabbit spleen. Marked dilatation of sinusoids, all of which contain phagocytic cells stuffed with hemosiderin, and some of which are thrombosed (see upper right hand corner). ×225.

droplet type, rather than hydropic. About one third of the Bowman's spaces were distended and contained similar material. There was marked congestion. These extensive kidney changes were not frequent, but mild changes always occurred. Hemorrhagic areas in the stomach and intestines were seen uniformly. Liver damage was very mild as were any injuries noted in other organs.

The relative toxicities are shown in figure 2. The pathological changes noted by us and recorded here are similar to the reports in the literature where examination has been made (23, 24, 26, 28, 38, 40, 46, 47, 49, 51, 55, 60). For diethylene glycol mono-ethyl-ether and ethylene glycol mono-ethyl-ether such reports do not exist.

Discussion

A summary of the findings in the literature and of our own LD 50 after oral administration is presented in table 6. In some instances there is good agreement among the investigators, whereas in others, there are enormous variations. There are undoubtedly many causes for these variations, such as number of animals used, health and dietary condition, ages and weights of the animals, sex in some instances, form of the dose (dilute or concentrated), the number of dosage levels, and the percentage of the animals killed which is chosen to define the lethal dose. There are probably at least three of these factors principally responsible for the wide variation in the size of the dose recorded as MFD,* MLD, LD 50, or some other arbitrary designation. They are: the number of animals used, the dosage levels chosen and the percentage mortality selected as the designated MFD, MLD, etc. This dose is usually chosen more or less arbitrarily after the experiment is completed and depends on the number of animals killed at a certain point, which in turn depends on the number of animals used. As a result, there are then only two important factors, namely, the number of animals used and the dosage levels selected. We do not wish to recommend the choice of any particular point or percentage as the proper one, but we do feel very strongly that if the technic outlined in this investigation is generally followed, there will be much greater uniformity of results, and any particular percentage mortality can be read directly and accurately from the curve.

In our studies we have chosen the 50% point (LD 50) as the one for greatest consideration, but one can readily observe that the regression coefficient or slope of the curves gives almost as important information as that obtained by knowing the LD 50.

If one attempts to repeat some of the calculations, it will become at once apparent why the LD 50 point is the best one to choose on the curve, since its accuracy can be determined with a much smaller number of animals than the accuracy of any other point. For example, in order to get the same precision at 99% kill as at 50% kill, one would require 103 as against 10 animals. Yet there is reported in the literature one widely quoted investigation (13) in which the authors determined the MFD of seven different glycols and derivatives, and in which

* Minimum fatal dose, minimum lethal dose and lethal dose killing 50% of the animals.

TABLE 6

RELATIVE ORAL TOXICITIES OF SOME GLYCOLS AND DERIVATIVES FOR VARIOUS SPECIES OF ANIMALS AND MAN

LD 50 in cc. per kg. body weight (except where otherwise noted) and the total number of animals used to determine this dose.

SPECIES	PROPYLENE GLYCOL	DIETHYLENE GLYCOL	ETHYLENE GLYCOL	DIETHYLENE GLYCOL MONO-ETHYL-ETHER	DIOXAN	ETHYLENE GLYCOL MONO-ETHYL-ETHER	REFERENCE
Mouse	23.9 (70)	23.7 (117)	13.1 (150)	6.6 (152)	5.7 (160)	4.3 (conc.) (70) 5.2 (dil.) (90)	(68)
Rat	31.9 (15)						(59)
		15 (100%) (5/5) 20 (80%) (4/5)					(42)
							(42)
	23.8 (?)	14.3 (?)	4.8 (?)				(60)
	21.0 (89)	14.8 (145)	5.5 (147)	5.5 (130)	5.2 (96)	3.5 (130)	(68)
Guinea pig	18.9 (40)	7.8 (59)	7.4 (50)	3.9 (66)	3.9 (60)	2.8 (73)	(68)
Rabbit					2 (5)		(28)
	18 (33%) (3/9) 19 (86%) (6/7)						(37)
							(37)
		4.4 (48) estimated					(68)
	14.3 (33%) (?) 17.8 (50%) (?)						(60) (60)
Cat		3.3–4.7 (?) Lethal	2 (66%) (?)				(60)
					2 (5)		(28)
Dog	20 (66%) (?)						(60)
		10 (?) estimated					(45)
Man	238 persons took 32–1950 cc. over a period of 21–270 days	1(30%) (105/353) See text for discussion	100 cc. estimated lethal dose. See text				(60) (52) (14)

the total number of animals used was 30, the maximum number used for any one of these being five and in one instance only three. Although this work has never been repeated, it is not difficult to imagine that the error in the estimated MFD could be as much as 300 to 400%.

In this investigation we have also calculated the standard error of the LD 50, and from this the range of dosages, which, 19 times in 20, may be expected to cause 50% mortality. These are shown in table 4. From these figures it can be observed that there are in several instances statistically significant differences between the toxicity of the substances themselves on a given species and furthermore, that there are in most instances significant differences between species which in some cases are very marked, as with diethylene glycol, for example, where the dose for the mouse is three times that for the guinea pig. Of the three species tested, guinea pigs had the smallest LD 50's, except in the case of ethylene glycol where the LD 50 for the rat was significantly lower. Gross and microscopic examinations of the organs and tissues have not specifically aided in an explanation of this fact.

During the course of our investigation, we have also made some chronic toxicity studies and have others in progress, but these will be reported later. In order that this discussion may be more complete, it seems advisable, however, to refer to the chronic studies reported in the literature. Several investigators have given propylene glycol in drinking water in concentrations as high as 10% without noticeable effects (15, 26, 37, 54, 59, 60). One group (37) gave it by stomach tube daily, and another group (26) gave it as fatty acid esters, likewise without effect. Diethylene glycol has also been given in drinking water (42, 54, 55, 60), by stomach tube (45) and as fatty acid esters (26). In all cases the mortality was high and pathological changes marked except at very low concentrations in water. The chronic effects of ethylene glycol have likewise been observed after administration in drinking water (11, 54, 60) as fatty acid esters (26) and by inhalation of the vapors (39) with results similar to those obtained from diethylene glycol except with the vapors, where no toxic effects for rats and mice were noted for the concentrations studied. The animals were exposed to concentrations in air of about 300 mg. per 1000 liters for 8 hours per day, 5 days a week, for a period of 16 weeks. Chronic studies have not been made on the other substances.

As can be seen from table 6, other species of animals have been studied, namely, cats, dogs and rabbits. Our results for dogs and rabbits are not extensive enough to justify detailed consideration; however, with 48 rabbits and a dose range of from 1 cc. to 11.5 cc. per kilogram, we have confirmed the findings of Geiling, Coon and Schoeffel (45) that rabbits have the lowest LD 50 of the species of experimental animals studied on diethylene glycol. The kidney and liver damage was particularly striking and even more severe than that for the other species. Even the lowest dose killed some of the animals; this was approximately the average dose that killed 105 of 353 persons who

consumed the diethylene glycol as an "elixir" (52).

Geiling, Coon and Schoeffel (45) and Geiling and Cannon (51) have emphasized the importance and probable significance, for interpretation in terms of man, of the administration of divided doses during toxicity studies. In unpublished investigations of "Elixir of Sulfanilamide," we have, in general, confirmed the findings of the above investigators. It seems proper at this point to reemphasize the inadvisability of attempts to interpret experimental data on laboratory animals directly in terms of man. It is entirely too dangerous. The present investigation confirms again the wide variations that may occur between species. It should also be pointed out that acute toxicity studies, although extremely valuable, especially from a pharmaceutical standpoint, far from tell the whole story and that likewise chronic studies do not tell the whole story. For example, ethyl alcohol, glycerol and propylene glycol can be taken in quantities large enough to be acutely toxic, while small quantities are oxidized by the animal body and do not produce chronic toxicity. Again, although small doses of lead, mercury, selenium, fluorine, narcotics, etc., are acutely toxic, it is the insidious character of their chronic toxic effects that makes them even more dangerous.

In the discussion of the pathological results, it need only be emphasized that there are species differences in the histological picture presented by the tissues and that great consideration must be given to the survival period following the administration of the toxic agent. If the dose is so large or the substance so toxic that death results within about 24 hours, one can rarely expect extensive histological changes in the internal organs. In this study the most extensive changes observed were in the organs of those animals that survived for 3 to 6 days. It is undoubtedly for this reason that the animals that succumbed from propylene glycol showed relatively smaller changes than those caused by any of the other substances. Animals that received propylene glycol died within a very few hours or else survived the experimental treatment. From a clinical standpoint it seems that this failure to find pathological changes after the administration of toxic substances may be of particular significance.

Summary and Conclusions

1. The relative acute toxicities of some glycols and derivatives have been determined and the order of increasing toxicities is as follows: propylene glycol, diethylene glycol, ethylene glycol, diethylene glycol mono-ethyl-ether, dioxan and ethylene glycol mono-ethyl-ether.

2. Three species were used: rats, mice and guinea pigs. A limited number of rabbits were used on diethylene glycol. The total number of animals used was over 2600. The rabbits, in comparison with other species, were found to have the lowest LD 50 for diethylene glycol. In fact, the lowest dose that killed some rabbits was the same as the estimated average dose that killed 105 persons.

3. The regression coefficients and LD 50's, with their respective standard errors, have been calculated for each species and each substance ad-

ministered. A detailed example of the methods used in the calculations is also presented.

4. In every instance except one the LD 50's for guinea pigs were smaller than they were for the other species, while those for mice were the highest.

5. A complete pathological report is presented with photomicrographs. The most extensive histological changes were noted subsequent to the administration of diethylene glycol.

6. The LD 50's in cubic centimeters per kilogram for mice are as follows: propylene glycol 23.9, diethylene glycol 23.7, ethylene glycol 13.1, diethylene glycol mono-ethyl-ether 6.58, dioxan 5.66, ethylene glycol mono-ethyl-ether 4.31 (conc.), and 5.20 (dil.); for rats, propylene glycol 21.0, diethylene glycol 14.8, ethylene glycol 5.50, diethylene glycol mono-ethyl-ether 5.54, dioxan 5.17, ethylene glycol mono-ethyl-ether 3.46; for guinea pigs, propylene glycol 18.9, diethylene glycol 7.76, ethylene glycol 7.35, diethylene glycol mono-ethyl-ether 3.87, dioxan 3.90, ethylene glycol mono-ethyl-ether 2.79.

7. It seems that small doses of propylene glycol can be safely tolerated by the animal body since it can be oxidized and probably converted to a normal body constituent. In this respect it resembles ethyl alcohol and glycerol.

8. In larger quantities all of these substances are toxic, and, with the exception of propylene glycol, produce definitely demonstrable tissue injury.

9. The literature has been reviewed and a complete bibliography is appended.

After correlating the information in the literature with the results of these experiments, it is concluded that ethylene glycol, diethylene glycol, ethylene glycol mono-ethyl-ether, diethylene glycol mono-ethyl-ether and dioxan should be entirely omitted from food and drug preparations, and that propylene glycol should be avoided except in very small concentrations.

The microscopic pathological examinations were made by Drs. Esmond R. Long and M. B. Lurie, Henry Phipps Institute, Philadelphia, Pa., and by Dr. Eleanor Humphries, Department of Pathology, University of Chicago, Illinois.

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CONCERNING THE TOXICITY OF GLYCOLS.

By L. Launoy (1)

I. STATE OF THE QUESTION.

A. Introduction. - The study of the toxicity of glycols over the last few years had as its starting point the 93 cases of fatal intoxication occurring in America by ingestion of an elixir containing diethylene-glycol as solvent [1]. Currently, this study is based on the pharmaceutical concern of providing a replacement for glycerine.

Glycols are not unimportant. In 1930 KLAUS HANSEN [2] reported on the case of two serious intoxications in two youths saved in the nick of time after having absorbed a dose of ethylene glycol seemingly equal to 100 cm^3. The initial results obtained in animals seemed, however, encouraging, since according to POHL [3], MAYER [4], dogs and rabbits did not die as a result of glycol, even after a strong dose. This product was eliminated in the urine in the form of glycolic acid and oxalic acid, when taken orally. Hemorrhagic nephritis was determined by injecting rabbits subcutaneously. These initial papers date from 1896.

In 1917, BACHEM [5], who compared the irritating action of glycol to that of glycerol, testing isolated intestinal rabbit flexures, observed that the glycerol was more irritating than the glycol. BACHEM stated that this is a hemolytic body like glycerine and Paul MAYER [6], the same year, recognized that it could determine serious hemorrhagic nephrites. According to FRAENKEL [7], glycol was presented in Germany during World War I as a glycerine substitute under the name of Tegoglycol. For FRAENKEL, glycol is atoxic.

B. Observations on man. - In 1926 PAGE [8] along with CORYLLOS recommended glycol as an Amytal solvent. He suggested that this body could be a solvent of great physiological interest. The same year PAGE, during the course of pharmacological experiments, himself absorbed 15 cm^3 of diluted glycol with no other sensation than slight heat upon absorption. He thus repeated the experiment of BACHEM (work cited) who, in 1917, absorbed 25 g. of glycol diluted in water and 45 g. in two days with no other consequence than an increase in oxalyl urea.[9]

P.J. HANZLIK, M.A. SEIDENFELD, C.C. JOHNSON [10] made observations on themselves and on nine other subjects. Glycol absorption gave them a sensation of heat on the tongue and in the esophagus. Subcutaneous or intramuscular injection of 2 cm^3 determined a momentary burning sensation. Comparing the results obtained with the glycol and propylene glycol with these, the glycerol showed itself to be the most painful, then ethylene glycol and propylene . Yet with this latter body, the results

varied with the individual. Van ŒTTINGEN and JIROUCH [11] considering that ethylene and diethylene glycols could be interesting from the therapeutic point of view, made experiments analagous to the previous ones. They judged them to be just slightly irritating locally, but capable of provoking gastro-enteritis when absorbed through the mouth. Only small doses should be used for intramuscular injection, since injection venously seems dangerous due to the hemolytic action and the hypotension created by these compounds.

SEIDENFELD and HANZLIK [12] also observed a greater irritating action of glycerol. They recommend the use of propylene glycol instead of glycol, the former being less toxic than the latter, since, at least in animals, it would give no cumulative effect.

C. Local action studied in animals. - This study was made on the cornea of the rabbit, the sciatic nerve of the frog, the derm, the unbroken skin, the smooth intestinal muscles of the rabbit and the smooth muscles of the rat uterus, the arterial muscles, those of the peripheral vessels and the isolated heart. HANZLIK, SEIDENFELD and JOHNSON (work cited), SEIDENFELD and HANZLIK (work cited), A. LATVEN and H. MOLITOR [13] note the irritating action of glycols and of glycerol on the cornea of the rabbit. The degrees of irritation are from hyperema to oedema and to opacity of the cornea. Regression of these symptoms requires several days. Propylene glycol would be more irritating on the eye than glycerine and ethylene glycol (LATVEN and MOLITOR); with intradermic injection, glycerol would be less irritating. These latter conclusions result from our reading of Table III, incorporated in the paper of LATVEN. We do not, then, fully understand how, in their discussion, these authors were able to draw the following conclusion concerning glycols and glycerol: "Propylene glycol was found to be the least toxic and irritant followed by ethylene and triethylene glycol, glycerol, carbitol and alcohol (p. 92 in the work cited)". Glycol and diethylene glycol possess a depressing action on smooth muscle and on the uterus. The same holds true for the isolated frog heart. On the peripheral vessels, vaso-constriction preceded by vaso-dilation is noted.

Application on the sciatica of the frog provokes a decrease in excitability.

D. Experimental toxicity. - Glycols lower respiration and death seems due to the cessation of respiratory movements, the heart continuing to beat for some time when respiration has stopped. With a toxic dose, glycerol produces clonic convulsions; glycol, though, is a depressant.

Toxic doses have been researched on the rabbit, the rat, the mouse, the dog and the chicken. The following table summarizes the results obtained by different authors.

BODY	ANIMAL	ADMINISTRATION	MIN. LETHAL DOSE	LETHAL DOSE 100%	AUTHORS
Ethylene Glycol	Rabbit	i.m.	5 cm^3	6 $cm^3$25	Hanzlik Seidenfeld Johnson
		i.v.	"	4 $cm^3$5	-
		i.p.	"	9 cm^3	Page
	Rat	i.m.	3 cm^3	6 $cm^3$25	Hanzlik Seidenfeld Johnson
		i.v.	2 $cm^3$5	4 cm^3	-
		i.p.	1 cm^3	3 $cm^3$5	Page
	Dog	i.v. slow perfus.	20 cm^3	"	Hanzlik (14) Newman Van Winkle Lehmann Kennedy
	Mouse	s.c.	2 $cm^3$5	"	Oettingen Jirouch
		s.c.	4 $cm^3$5 (50%)	6 cm^3	Latven Molitor
		p. os	7.5 (50%)	10 cm^3	-
		i.v.	3 cm^3	4 cm^3	-
Diethylene Glycol	Rabbit	i.m.	3 cm^3	6 cm^3	Haag (15) Ambrose
		i.v.	2 cm^3	6 cm^3	-
		p. os	"	8 to 10	cited by Taeger
	Rat	s.c.	15 cm^3	20 cm^3	Haag Ambrose
		i.m.	7 cm^3	9 cm^3	-
		i.v.	4 cm^3	6 cm^3	-
		i.v.	5 $cm^3$8	"	Weatherby (16) Williams
		p. os	"	15 to 20 cm^3	Haag - Ambrose
		p. os	"	16 cm^3	cited by Taeger
		"	"	12 $cm^3$5	Poe - Witt (17)
		"	27 (50%)	"	Weatherby
				"	Williams
	Dog	i.v. slow perfus.	20 cm^3(50%)	"	Hanzlik et al.
		p. os	9 cm^3	"	cited by Taeger
		"	10 cm^3	"	Weatherby Williams
	Mouse	s.c.	5 cm^3	"	Oettingen
				"	Jirouch
Propylene Glycol	Rabbit	i.m.	6 cm^3	7 to 9 cm^3	Seidenfeld Hanzlik
		i.v.	4 cm^3	6 cm^3	-
		i.v.	"	6.5	Weatherby (18) Haag
	Rat	s.c.	"	22.5	-
		i.m.	"	14	results expressed in gr.
		i.v.	"	6.8	
		p. os	"	33.5	

BODY	ANIMAL	ADMINISTRATION	MIN. LETHAL DOSE	LETHAL DOSE 100%	AUTHORS
Propylene Glycol	Dog	i.v. (slow perfus.)	"	25 cm^3	Hanzlik, Newman
	Chicken	i.v. slow perfus.	"	25.7	Lehmann Newman
	Mouse	s.c.	18.5	24	
		p. os	22	26	Latven
		i.v.	8	11	Molitor

(These figures correspond to 1000 gr. of animal weight).

Comparison of the above-reported results allows us to observe that, for each author, the toxicity of the glycols studied is always ordered in the same manner - by decreasing toxicity, as follows: glycol, diethylene glycol, propylene glycol. The results of these different authors are comparable, since all give their toxic dose evaluated in volume and for one kilogram of animal weight. Lethal doses for the kilogram are obviously variable according to the type used, but this is completely unextraordinary. We must, however, point out that in the dog, the results of HANZLIK et al. lead to an almost equal estimation of the toxicity of the three glycols, when introduced venously.

E. Acute intoxication in animals. - The symptoms observed in animals with acute intoxication are renal (hemorrhagic nephritis), hepatic and cardiac lesions. This confirms the work of the first experimenters. For HANZLIK et al. (work cited), the usual symptoms, intramuscularly, are respiratory acceleration, loss of balance followed by general motor deceleration and, finally, coma and death. Venously, muscular excitation with trembling and convulsions are also observed. Such symptoms reported for ethylene glycol have also been observed with propylene glycol by SEIDENFELD and HANZLIK (work cited). These authors have also seen that moderate doses of propylene glycol provoke in particular a semi-comatose state lasting one to two days. For P.J. HANZLIK et al. (work cited), the depressive action of propylene and di-propylene glycols is an anesthetic action; ethylenes and di-ethylenes are, on the other hand, convulsion-causing.

TAEGER has also noted, in diethylene glycol, a diuretic action followed by anuresis in the final stage of intoxication. The urine eliminated after ethylene glycol (PAGE, HANZLIK), after propylene glycol (LEHMAN and NEWMAN), after glycerol may be hematuric. The phenomena of acute intoxication are observed most clearly when toxic products are administered intravenously. This is particularly true for

respiratory and circulatory phenomena. The first consist of acceleration of rhythm, increase in amplitude, then stopping of respiration. The second consist of a lowering of blood pressure, amplification of heart beats and cardiac arrest after cessation of respiration.

Among the afore-mentioned authors whose papers we have cited, some took the liberty of extrapolating for man the toxic doses obtained in different laboratory animals. Thus, HANZLIK and his colleagues (work cited, p. 394, 1931) in speaking of ethylene glycol, say: "it would seem that 77 grams (70 cm^3), might be injected intravenously without demonstrable effects in a 70 kg. man" and that(p. 393)"an intramuscular dose just causing symptoms, but resulting in ultimate recovery would be about 154 grams (140 cm^3); a fatal dose would be about 308 grams (280 cm^3)."

For propylene glycol, SEIDENFELD and HANZLIK (work cited, p. 114-115, 1932) declare, according to their results with the rabbit and the rat, that: "294 grams (280 cm^3) might be injected intravenously in a 70 kg. man without demonstrable effects." Intramuscularly, the same authors say: "transferring the results on intramuscular injection of propylene glycol in rats to an adult man of 70 kg., and assuming similar action, a dose just causing symptoms, but resulting in ultimate recovery, would be about 518 grams (490 cm^3) or more than a pound; a fatal dose would be about 1029 grams (980 cm^3) or more than 2 pounds."

We shall not discuss such reasoning; nonetheless, we feel it necessary to underline the boldness of such an extrapolation. Yet all experimenters know well that a toxicity result cannot be extrapolated even from one species to another of the same genus, especially from one order of Mammals (rodents) to another (man). Taken literally, the above extrapolations would be capable of provoking all kinds of casualties.

With these reservations well emphasized, it seems that propylene glycol is not very toxic for man, if we are to believe ALBRIGHT, BUTLER and BLOOMGERG [19], cited by HANZLIK, NEWMAN, etc. . .(work cited 14) who say that: "Such quantities, as 10 to 20 and even 40 cm^3 daily are said to be given to children for 1 to 9 months". May we add, however, that in the rat, venous injection of propylene glycol is followed by hematuria (WEATHERBY and HAAG).

CHRONIC EXPERIMENTAL INTOXICATION

1. <u>With ethylene glycol</u>. CH_2OH. CH_2OH. In 1931 HANZLIK et al. add ethylene glycol to the drinking water of their rats. In several days: 3 days for equal concentrations or concentrations greater than 10%; 5 days for 5% concentrations;

6 days for 3% concentrations and 14 days for 2% concentrations - they observed the death of the animals. Death occurred following absorption of 4 to 27 g. of glycol per kilogram of animal. After several months, the 1% concentration caused pathological changes: oxalurea, hematurea, alteration of tubuli with possible presence of crystals: phosphates, calcium carbonate and oxalate. The daily dose of 0.7 gram per kilogram for 120 to 130 days in the form of a 0.50% solution showed itself to be atoxic. HANZLIK et al. (work cited 1939) observed hepatic degeneration in a dog having taken repeated doses of glycol parenterally, the cells being reduced to an amorphous eosinophilous mass, and necrosis of the tubuli contorti in the kidneys.

That ethylene glycol was not endowed with glycogenic properties could be suspected; VELTMANN[20] had seen this in 1924 and PAGE (work cited) confirmed it.

2. Diethylene glycol. $OH.CH_2 - CH_2O.CH_2.CH_2OH$. The experiments of HAAG and AMBROSE (work cited) carried out according to the preceding technique, show that a 10% concentration of diethylene glycol given to animals proves fatal to them within eight days. Equal concentrations or concentrations less than 0.1% must be attained if no specific change is to be caused. Contrarily, with 10% glycerine survival and growth are as good as with the control animals. In 1935 LEPKOVSKI [21] et al., studying the nutritive value of the synthetic esters of natural fatty acids and of alcohols such as glycerol and the glycols under discussion here, concluded in favor of the toxic action of the esters of ethylene glycols and of the non-toxicity of those of propylene glycol. However, the esters of propylene glycol do not have the nutritive value of the esters of glycerol.

The papers of HOLCK [22], and those of GEILING, COON and SCHOEFFEL [23] on di-ethylene glycol confirm the above. HOLCK claims that chronic absorption of di-ethylene glycol, even at a concentration of 0.25%, causes rats to stop growing. WEATHERBY and WILLIAMS do not observe this phenomenon with dilutions of 2 and 3% in drinking water. HANZLIK et al., who operated on dogs, studied the histological lesions of chronic intoxication, these being the same as those reported with ethylene glycol and in conformance with the conclusions of GEILING and CANNON [24]. HANZLIK estimates, moreover, that in the dog, di-ethylene glycol requires 36 hours for elimination. The administration of repeated doses of this product, by the lesions thereby caused, gives rise to the accumulation of the poison in the blood.

3. Propylene glycol $CH_3 - CHOH - CH_2OH$. MIURA [25], according to SEIDENFELD and HANZLIK, had already studied propylene glycol on the rabbit in 1911. He found that it is eliminated in the form of a conjugate of this glycol with glycuronic acid. SEIDENFELD and HANZLIK (work cited 1932) found pyruvic and lactic acid in the intermediate metabolism of propylene glycol. Poured into the drinking water of

experimental rats, concentrations at 10% and lower, tested for 140 days, caused no harmful phenomenon. At a 10% concentration, the animals absorbed 13.2 g. of propylene glycol a day. These studies are confirmed by BRAUN and CARTLAND [26] who, however, note with the 10% concentration in drinking water, a temporary slowing down in growth for 10 days; after this period of time, normal continuation. Propylene glycol has the property of extremely rapid elimination; its speed of elimination would be 75% greater than that of ethylene glycol, whose own speed of elimination is 50% greater than that of di-ethylene glycol. Given this weak toxicity, HANZLIK made a partial substitution of propylene glycol for the hydrocarbon ration of the rat. When 25% of the hydrocarbon ration is thus substituted with propylene glycol, growth is impeded; below 25%, it is normal. Prior experiments (HANZLIK, et al. -27) on rats and those of NEWMAN et al. [28], on the isolated cat liver, show that propylene glycol is endowed with glycogenic power, while ethylene glycols are without it.

Conclusions. - It would seem to result from this bibliography that, if therapeutic use of glycols is envisaged, propylene glycol must be preferred to the ethylene glycols, by reason of its weaker toxicity, its glycogenic power and its speed of elimination.

II. PERSONAL FINDINGS

We should like to add several personal results to this bibliographic data. These results concern 1) local tolerance; 2) acute toxicity (see Addenda).

a) Local tolerance. - Our tests dealt with the action of glycols and of glycerol on the intestine: the rectum and the descending colon. The animal selected was the guinea pig. We used products that were either pure, or diluted in water to 60% or in the form of a 60% gelatinized mass. In this last case, before rectal insertion, the gelatinized mass was melted and injected at 37°. The guinea pig being maintained in dorsal decutibus, the medicine was introduced with the aid of a semi-hard rubber tube, mounted on a seringe and inserted 10 to 12 cm. into the rectum. The product is injected for 20 seconds. The tube is slowly withdrawn from the anus, kept closed between the thumb and index finger, for 5 minutes. Only males were tested.

Shortly after the anus is freed, defecation of matter found in the large intestine takes place. All of the products were active at this viewpoint; the most active being, in decreasing order of activity: glycerol, di-ethylene glycol, propylene glycol and glycol. The experiments were carried out with non-diluted products injected in equal molecular quantities.

If care is not taken to obturate the anus, defecation phenomena can be observed almost as soon as the product is inserted. In this case, of course, part of the liquid is rejected. Urinary elimination is also customarily observed, in all likelihood due to reflex-like phenomena resulting from functional intestinal excitation. This excitation itself seems to be of peripheral origin, since all the glycols tested and the glycerol have a very marked irritant action on the intestinal mucuous. Even diluted and at doses still moderate (about 0.5 cm^3 of pure product for 100 g.), often cause the expulsion of mucus sputum one-half hour to one hour after introduction into the intestine. The congestive manifestations which follow the introduction of glycols and glycerol in the intestine are sometimes such that hemorrhagic manifestations can be observed. The latter are characterized by a blood-tinged anal oozing and the expulsion of red currant jelly-like mucus. The introduction of the melted gelatinous mass is not excluded as a source of this irritating power. The condition of the intestine examined after the experimental animals were killed bears witness to these facts. It is void of feces over a length of 10 to 20 cm. beginning with the anus, the mucus is more or less red in color, at times clearly hemorrhagic according to the dose administered. Vascularization is localized in the regions reached by the liquid. Delimitation between the zone of lesion and the intact zone is always clear.

We, as several authors cited in the bibliography have, noted that glycerol is the most irritating of the bodies studied and that among the three glycols, ethylene glycol is the least irritating.

With regard to this, the sensitivity of the intestinal mucus and of the sub-mucus would not be parallel to that of the toxic coefficients on the animal *in toto*. We must add that the doses which aided us in specifying the local intestinal reaction are perfectly atoxic (at least for a single dose) on the organism in general, as we showed on the control animals. Allow us to specify that rectal injection of a comparable volume of salt water provokes in the control animals neither defecation, urination, nor elimination of mucosity. At the autopsy, the descendant colon is still filled, as it normally is, with matter arranged in chain-like fashion.

General toxicity. - This has been researched subcutaneously in the guinea pig and parenterally in the mouse. Let us first examine the latter and note that the density of the bodies we employed were the following:

Ethylene glycol - 1.119; di-ethylene glycol - 1.117; propylene glycol - 1.036; glycerol - 1.260.

Toxicity in the mouse. - The animals (1) fast for three or four hours; they are given the various products *per os*, in pure state; insertion is made by means of

a syringe mounted on a small metal tube. The injections are counted in volume; the following figures are given for one mouse, and we have noted for each mouse-dose the corresponding dose for one kilog. of animal. We establish this kilog. ration not because we attribute a particular value to it, but so that our results can be confronted with those of authors so doing.

PRODUCTS	MAXIMUM DOSES survival 100% in cm^3 (In cc. for 1,000 g.)	LETHAL DOSE 100% in cm^3
Ethlyene glycol	0.10 to 0.13 (5 to 6.5/kg)	0.26 to 0.30 (13 to 15/kg)
Di-ethylene glycol	0.20 to 0.28 (10 to 14/kg)	0.30 to 0.37 (15 to 18.5/kg)
Propylene glycol	0.30 to 0.37 (15 to 18.5/kg)	0.42 to 0.60 (21 to 30/kg)
Glycerol	0.30 to 0.37 (15 to 18.5/kg)	0.75 to 0.90 (37.5 to 45/kg)

It can be concluded from this table that parenterally, in the mouse, when basing oneself on the volumes of glycol and glycerol supported by this animal, the compounds can be classed by decreasing toxicity, in the following manner: glycol, di-ethylene glycol, propylene glycol, glycerol.

When the toxic volumes are brought back to their weights, the toxic weights 100%, for each one of these bodies will be the following for one kilogram of animal: Glycol - 14 g. 50; diethylene glycol - 16 g. 7; propylene glycol - 21 g. 7; glycerol - 47 g. 80.

We can say that a molecule of each one of these bodies will contain X-number of toxic-kg units, this toxic unit being defined by the weight of each compound which, given per os, would be 100% lethal. Still referring to the kilogram of mouse, we have:

Ethylene glycol (M.W. 62) contains 4.2 toxic units.
Di-ethylene glycol (M.W. 106) contains 6.3 toxic units.
Propylene glycol (M.W. 76) contains 3.4 toxic units.
Glycerol (M.W. 92) contains 1.9 toxic units.

These documents allow us to compare the reciprocal toxicity of each of the compounds studied according to their molecular toxicity. If we make the toxicity of glycol equal 1, that of di-ethylene glycol equals 1.5, that of propylene glycol equals 0.81 and that of glycerol equals 0.45.

This representation of the compared toxicity of glycols and glycerol (ethylene glycol being taken as a unit) does not change the order which resulted from the

comparison of the toxic volumes. We believe, however, that the system adopted by us, taking into account both the density and the molecular weight of the products studied, represents the real toxicity and not the relative toxicity.

Toxicity in the guinea pig. - We were mainly interested in the compared toxicities of the bodies injected subcutaneously. Here again, the results are given in volume and brought back to a kilog. of animal.

PRODUCTS	In cc. for 1,000 g. MAXIMUM DOSES survival 100%	SURVIVAL 50%	MINIMUM DOSES 100% dead
Ethylene glycol	10	12.5	15
Di-ethylene glycol	10	12.5	15
Propylene glycol	12.5		15
Glycerol	10		12.5

In the guinea pig, after subcutaneous injection of the above bodies, precocious convulsive disturbances are frequently observed with lethal doses of glycerol, while the glycols show, rather, a general depressing action. Propylene glycol in particular clearly retards reflexes of posture. The acute lesions manifest themselves by vaso-dilatation and generalized congestion, sometimes by pulmonary oedema and haematuria. There are no local lesions (necrosis, abcedation) but a melted, gelatinous mass of conjunctive tissues are found between skin and muscle at the autopsy.

According to the figures in the above table, if the volume causing death 100% is referred to, glycerol stands at the head of the lsit, the three other bodies being equal. If the weight of the products are taken to differentiate toxicity, propylene glycol would come first, followed by ethylene and di-ethylene, with glycerol at the bottom of the list. Determining as above the toxic units/kilog. guinea pig contained in one molecule, the following are found per molecule: glycol - 3.7 units; di-ethylene - 6.3 units; propylene - 4.8 units; and for glycerol - 4.8 units.

By increasing order of toxicity, glycol being 1, the bodies are thus ranked in the following manner: ethylene glycol (1); propylene glycol and glycerol (both 1.29); di-ethylene glycol (1.7).

These facts which rank glycerine as being more toxic than ethylene glycol and of equal toxicity with propylene, di-ethylene glycol maintaining first place, as in the mouse parenterally before, these facts, I say, must not surprise us. In fact, the considerable difference in toxicity of glycerol when applied parenterally, sub-cutaneously or intravenously has been known for a long time. In the first case, it is not toxic and considerable quantities may be absorbed or caused to be absorbed.

DUJARDIN-BEAUMETZ and AUDIGE (1879) knew that the toxicity of glycerine depended on the method of introduction. Adolf OSTWALD [29] who provided us with the opinion of DUJARDIN-BEAUMETZ and AUDIGE, adds that the non-toxicity of glycerine per os comes in all likelihood from the fact that this body is combined either in the intestinal wall or in the liver with fatty acids to form fat.

CONCLUSIONS

Di-ethylene glycol is the most toxic of the glycols which we studied; when introduced parenterally in the mouse, propylene glycol was the least toxic.

Subcutaneously, di-ethylene glycol is still first in the guinea pig, with propylene glycol second.

Parenterally, the toxicity of glycerol is clearly lower when compared to that of the three above glycols. Subcutaneously, the toxicity of glycerol approaches that of propylene glycol.

Rectally, the three glycols studied and the glycerol are clearly irritating to rectal and intestinal mucus.

Concerning the possible use of glycols intestinally, it must be recalled that the rectum, for medicine which stays in particular in the anal region, permits direct absorption into circulation through the hemorrhoidal veins. In fact, the mid and lower hemorrhoidal veins, through the intermediary of the internal and primitive iliac veins, merge with the lower vena cava, bypassing the liver.

ADDENDA TO THE BIBLIOGRAPHY

a) HANLIK and NEWMAN's assertion that propylene glycol is endowed with glycogenic power seems to justify the opinion of HUNT [30], who writes: "It is possible that this glycol is a true food, if ethynic alcohol is admitted as being one."

b) According to FAIRLEY, LINETON and FORD MOORE [31], the toxicity of ethylene glycol is due to an impurity probably analagous to dioxane. The great toxicity of dioxane can explain the ethylene glycol and di-ethylene glycol fatalities. G.C.L. BROWN [32], whose opinion we report above, judges that the administration of glycols must be done very carefully. Death by intoxication with these compounds is sudden, and the cumulative effect of these bodies can be anticipated. Thus, the problem of their therapeutic use must be extensively and carefully studied.

c) In the December, 1938 (33) issue of the Journal of the American Pharmaceutical Association, a "glycol symposium" is announced during which the question of glycols is to be discussed from the chemical, physiological, industrial and pharmaceutical points of view. I have been able to read the report of A.G. DUMEZ [34] in which the reporter discussing the question of glycols and their pharmaceutical use examines their value as solvents; he says, among other things, that propylene glycol is currently employed in place of ethylene glycol. Propylene glycol was used (ALBRIGHT [35]) as a vitamin D solvent.

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PROPYLENE GLYCOL: RATE OF METABOLISM ABSORPTION, AND EXCRETION, WITH A METHOD FOR ESTIMATION IN BODY FLUIDS

A. J. LEHMAN AND H. W. NEWMAN

From the Department of Pharmacology and Division of Neuropsychiatry, Department of Medicine, Stanford University School of Medicine, San Francisco

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The glycols have developed rapidly during the last few years as solvents for technical purposes. Their use as vehicles for medicinals, pharmaceuticals, beverages, etc., was not exploited (1, 2, 3) until ethylene and propylene glycol were brought to notice as a solvent for a bismuth product introduced for the treatment of syphilis (4). Propylene glycol, because of its low toxicity (4, 5), chemical indifference to most substances, and excellent solvent properties, which correspond more closely to water than most organic substances, has a wider range of usefulness as a solvent than any of the glycols so far investigated.

Comparatively little is known regarding the metabolic end products of propylene glycol and its general behavior in the organism. Miura (6) is the only investigator, as far as we are aware, to study its metabolism. His work was primarily concerned with the isolation of propylene glycol-monoglycuronic acid from the urine of a rabbit which had received a small dose of propylene glycol. It has been assumed (4) that the greater part of the glycol is completely burned to carbon dioxide and water. However, a considerable range of substances, including lactic and pyruvic acids, are possible as intermediary oxidation products (12). The potential effects of these products may not be a factor in the action of propylene glycol unless the glycol be taken continuously as might be the case if it were employed as a vehicle in the manufacture of beverages.

The increasing use of propylene glycol and the negative char-

acter of our knowledge of its effects suggested the need of a systematic pharmacological study covering the essential features. This report is concerned with the description of a method for the estimation of propylene glycol in body fluids, as well as observations on the absorption and excretion, diuretic effects, rate of disappearance from the blood stream, and some comparisons with ethyl alcohol. Dogs, rabbits, and chickens were used. The glycol[1] was given intravenously as $33\frac{1}{3}$ and 50 per cent solutions in normal saline solution (0.9 per cent sodium chloride), and orally as a 50 per cent solution in water to fasting animals.

TOXICITY

The low toxicity of propylene glycol has been stressed by all investigators (4, 5, 9). Most of the acutely fatal doses were determined by injecting the undiluted glycol intramuscularly, subcutaneously, or intravenously within a very short period of time. Since large doses are necessary to produce a fatal outcome, the great distension of the tissues and the assault on the circulation, which must occur under these conditions, must be considerable factors in the toxic effects. We determined the fatal dose in chickens using slow intravenous infusion and administering 0.5 cc. of 50 per cent propylene glycol in normal saline solution per minute per kilogram body weight. By this method, the fatal dose was found to be 25.7 cc. per kilogram of propylene glycol as an average for 3 chickens. This is considerably higher than the values found by other investigators, and approximately 2.5 times the fatal dose of ethyl alcohol (10.4 cc. per kilogram) similarly determined in 4 chickens.

HEMOLYTIC ACTION

Propylene glycol, when injected intravenously as a $33\frac{1}{3}$ per cent solution in normal saline solution produces hemoglobinuria, the blood cells apparently being destroyed osmotically in passing through the glycol at the site of injection. The hemolytic action was studied in vitro by a procedure similar to testing red cell

[1] The propylene glycol used in this study was a specially purified product kindly supplied by the Carbide and Carbon Chemicals Corporation, New York City.

fragility. One cubic centimeter of whole rabbit blood was added to 10 cc. of propylene glycol in concentrations varying from 0.2 to 50 per cent with and without sodium chloride. The tubes were inspected at the end of 3 and 24 hours. Propylene glycol in water solution produced immediate hemolysis in all concentrations. When prepared with normal saline solution (0.9 per cent sodium chloride), only those concentrations above 30 per cent produced hemolysis at the end of 3 and 24 hours. When the end concentration of sodium chloride was maintained at 0.9 per cent, complete protection for 3 hours was afforded in all tubes up to a concentration of 40 per cent propylene glycol, although partial hemolysis was present in the 40 per cent concentration at the end of 24 hours.

METHOD OF ANALYSIS

In a preliminary report (7) propylene glycol was determined in body fluids by reduction of a strongly acid solution of potassium dichromate. The accuracy of this method was influenced to a certain extent by variations in blood sugar and the concentration of the urine. Since completion of the earlier report, another oxidizing agent has been employed which was found to be more suitable, namely, sodium periodate. The method described below is an adaptation of the procedure employed by Fleury and Fatome (8) for the determination of glycerin. The essential details and steps were as follows:

Equipment. Fifty and 125 cc. Erlenmeyer flasks; 50 cc. volumetric flasks; 5 cc. pipettes; 2 50 cc. volumetric burettes; several small funnels and filter paper of suitable size; several small test-tubes of 10 x 100 mm.; ice-box; hot-air oven kept at 95°C.

Reagents. Ten per cent trichloracetic acid; powdered barium hydroxide; 95 per cent alcohol; phenolphthalein, 1 per cent in alcohol; sulphuric acid, 20 per cent by volume; sulphuric acid, N/1 (approximate); N/10 sodium hydroxide (approximate); starch suspension, 1 per cent, boiled; sodium bicarbonate, saturated solution (8 per cent); potassium iodide, 20 per cent solution.

Standard solutions. M/10 sodium periodate. Dissolve 2.94 grams in 30 cc. N/1 sulphuric acid, make up to 100 cc. with distilled water; N/10 arsenious acid. Dissolve 4.95 grams in 150 cc. distilled water with 15 grams sodium carbonate. Add 25 cc. normal acid and make up to 1000 cc. N/10 iodine. Dissolve 12.692 grams in 1,000 cc. distilled water with the aid of 18 grams potassium iodide. Each cubic centimeter of the periodate solution is the equivalent of 3.8 mgm. propylene glycol.

PREPARATION OF THE PROTEIN AND SUGAR-FREE FILTRATE FROM BLOOD

Three cubic centimeters of blood are pipetted into a 50 cc. Erlenmeyer flask and 7 cc. of 10 per cent trichloracetic acid added. Filter through a small filter and collect filtrate in a small test-tube. Five cubic centimeters of the filtrate (representing 1.5 cc. of blood) are pipetted into a 50 cc. volumetric flask containing 1 gram powdered barium hydroxide and thoroughly mixed by agitation. The mixture is allowed to stand in the ice-box for 2 hours, removed, and made up to 50 cc. with 95 per cent alcohol, then returned to the ice-box and allowed to stand for 12 to 24 hours. At the end of this period, the flask is removed, the contents centrifuged, and 40 cc. of the clear supernatant liquid transferred to a 125 cc. Erlenmeyer and the level of the fluid marked on the flask with a wax pencil. A drop of phenolphthalein solution is added and the contents of the flask made acid with 20 per cent sulphuric acid. Forty cubic centimeters distilled water are now added and the flask placed in the hot-air oven maintained at 95°C. and allowed to remain there until the volume has been reduced to the original 40 cc. The flask is then removed, the contents neutralized with N/10 sodium hydroxide, 1 drop of N/1 sulphuric acid added, and the propylene glycol titrated with periodate.

PREPARATION OF THE URINE

Five cubic centimeter samples of urine are used and treated exactly like blood except for omission of treatment with trichloracetic acid. If the urine is suspected of containing large amounts of propylene glycol, dilutions of 1:10 must be used.

TITRATION

Pipette 5 cc. of the standard periodate into the flasks containing the samples to be tested for propylene glycol and into a blank flask carried through the same procedure as the samples for propylene glycol, and place in the ice-box for 15 minutes. Remove the flasks and add to each 10 cc. of 8 per cent sodium bicarbonate solution and exactly 15 cc. of the standard arsenious acid solution and 1 cc. of 20 per cent potassium iodide solution. Let stand at room temperature for 15 minutes and titrate with the standard iodine solution after the addition of 1 cc. starch suspension. The blank value is subtracted from that for the sample taken. The calculations are made as follows:

Blood. The original volume of 3 cc. of blood is reduced by $\frac{1}{2}$, to 1.5 cc. in the precipitation of the proteins, and again reduced by $\frac{1}{5}$, i.e., to 1.2 cc. in the removal of the sugar. The calculation is then:

$$\frac{\text{cc. N/10 iodine used in sample minus cc. used in blank} \times 3.8}{1.2} = \text{mgm. per gram propylene glycol.}$$

Urine. The original volume of urine is reduced by $\frac{1}{5}$ during the process of purification, and the calculation is then:

$$\frac{\text{cc. N/10 iodine used in sample minus cc. used in blank} \times 3.8}{4} = \text{mgm. per gram propylene glycol.}$$

This result must be multiplied by the number of times the urine is diluted.

ABSORPTION

The absorption of propylene glycol was studied in 4 dogs. The glycol was given as a 50 per cent solution in warm tap water by stomach tube to fasting animals. The concentration in the blood, which reflects the rate of absorption, was followed for variable periods after doses of 2, 8, and 12 cc. per kilogram body weight. Absorption and diffusion throughout the tissues occurred promptly. The maximum concentration of blood propylene glycol was reached in 30 minutes with the 2 cc. dose (fig. 1, curve II). With the 8 and 12 cc. doses, 2 to 4 hours were required for complete absorption (fig. 1, curves V and VI). The rapidity of absorption from the gastrointestinal tract may also be shown by comparing the blood propylene glycol concentrations following gastric and intravenous administrations in the same dog (fig. 1, curves I and II). It is readily apparent that the concentrations run almost parallel, indicating prompt absorption and distribution after oral administration. Six cubic centimeters per kilogram introduced into the isolated stomach gave a blood propylene glycol of 124 mgm. per cent at the end of 3 hours indicating only slight absorption from this organ (fig. 1, curve III).

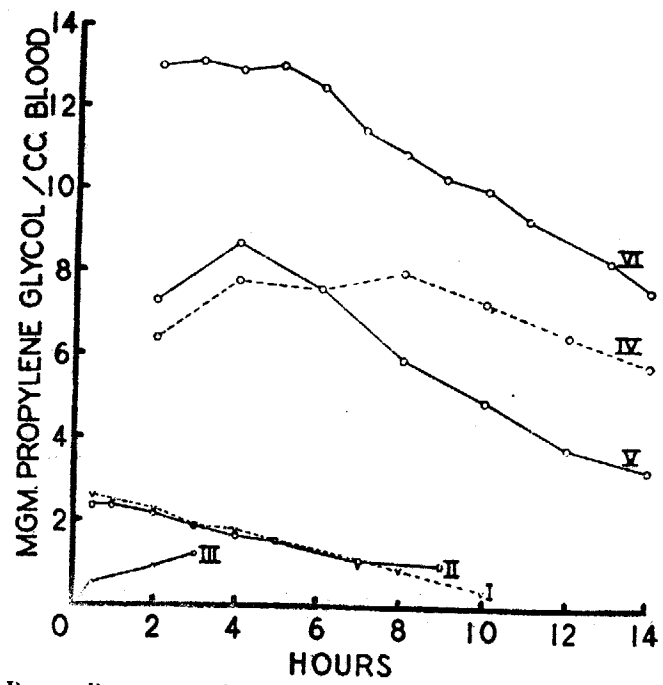

FIG. 1. BLOOD PROPYLENE GLYCOL CONCENTRATION IN DOGS FOLLOWING THE ADMINISTRATION OF PROPYLENE GLYCOL ORALLY AND INTRAVENOUSLY

Curve I, 2 cc. intravenously; curve II, 2 cc. orally; curve III, 6 cc. into isolated stomach; curve IV, 8 cc. orally with pentobarbital anesthesia; curve V, 8 cc. orally; curve VI, 12 cc. orally.

The influence of anesthesia on absorption was also studied. Eight cubic centimeters per kilogram was administered to a dog

previously anesthetized with pentobarbital, 35 mgm. per kilogram (fig. 1, curve IV). When compared with a similar dose (fig. 1, curve V) in the same dog unanesthetized, considerable delay in absorption occurred. This was probably due to a depression of gastric motility incidental to the general depression from the anesthetic. The slower disappearance of propylene glycol from the blood stream under these conditions may well be due to depression of kidney function.

RATE OF FALL OF PROPYLENE GLYCOL IN THE BLOOD

Thirteen observations were made on 3 dogs, 2 chickens, and 3 rabbits. Propylene glycol was administered orally and intravenously in dogs and intravenously in rabbits and chickens. Blood samples were obtained from a leg vein in dogs, by cardiac puncture in rabbits, and by puncture of the comb in chickens. A typical series of curves showing the rates of fall of blood propylene glycol following various doses is presented in figure 1. It is seen that the rate of fall is related to the concentration in the blood. This is in direct contrast to ethyl alcohol which falls at a constant rate regardless of the blood concentration (10, 11). The slope of the curve has a tendency to reach a plateau and fall more slowly as the concentration approaches a low level.

A number of factors may operate to remove the propylene glycol from the blood stream. The rate of combustion of the glycol may be directly proportional to the concentration. If this assumption were correct, the rate of fall in mgm. of propylene glycol per gram of blood per hour following a 4 cc. dose should be twice that following a 2 cc. dose. When this value is calculated for the various doses for the different species, this assumption does not hold. Although the rate of fall is greater with high concentrations, there is no constant relation between concentration and rate of fall. Thus some other mechanism must be responsible for the rate of disappearance of the glycol from the blood.

It is quite possible that when the kidney is faced with a high concentration of propylene glycol, the glycol may be excreted in high concentration in the urine. This was found to be the case, since it was possible to recover propylene glycol, as identified by

its refractive index and boiling point, from the urine. From 12 to 45 per cent of the total dose administered was accounted for in this way.

The part played by the kidney in the rate of fall of blood propylene glycol was demonstrated in another way. The rate of fall

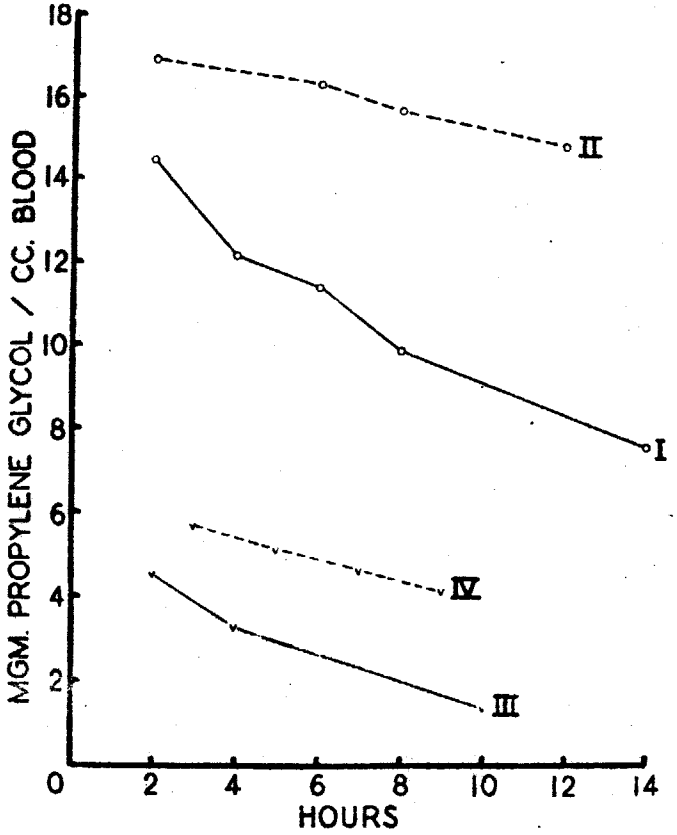

FIG. 2. RATE OF DISAPPEARANCE AND CONCENTRATION OF PROPYLENE GLYCOL IN IN THE BLOOD OF NORMAL AND NEPHRECTOMIZED DOGS

Curve I, 12 cc. orally in a normal dog, curve II, 12 cc. orally in the same dog after nephrectomy; curve III, 4 cc. intravenously in a normal dog; curve IV, 4 cc. intravenously in the same dog after nephrectomy.

was followed in 2 dogs before and after the removal of the kidneys. In figure 2, curve I shows the concentration of propylene glycol in the blood of a normal dog after receiving 12 cc. per kilogram gastrically. The highest value was reached at the end of the second hour, or 1444 mgm. per cent. This fell to 765 mgm. per

cent in 14 hours, or a fall of 679 mgm. per cent. When the kidneys were removed, the blood propylene glycol rose to 1691 mgm. per cent in 2 hours and fell to 1490 mgm. per cent in 12 hours, or a fall of only 200 mgm. per cent (curve II).

In the second dog, 4 cc. per kilogram were given intravenously to the intact animal. The blood propylene glycol attained a maximum of 457 mgm. per cent and fell to 141 mgm. per cent in 10 hours, or a total drop of 316 mgm. per cent (curve III). When the dog was nephrectomized, a much higher concentration was maintained during the time of observation, and the rate of fall was likewise less rapid. The highest value was 569 mgm. per cent which fell to 414 mgm. per cent, or a drop of 155 mgm. per cent in 9 hours (curve IV). Thus the importance of urinary excretion of propylene glycol was demonstrated.

The above experiments also throw some light on the rate of combustion of propylene glycol in the animal body. Calculating the rate of fall of the glycol in milligrams per gram of blood per hour in the nephrectomized dogs, the value for the 12 cc. gastric dose becomes 0.2 mgm. and for the 4 cc. intravenous dose, 0.26 mgm., which is a fairly close agreement. This indicates that propylene glycol behaves similarly to ethyl alcohol, and is in all probability burned at a constant rate regardless of concentration in the body. It is interesting to note that propylene glycol can reach a concentration of over 1.6 per cent in the blood and tissues when administered orally without producing hemolysis, with complete and uneventful recovery within 24 hours.

EXCRETION AND DIURETIC EFFECT

For the purpose of determining more accurately the amount of propylene glycol excreted by the kidneys, 7 experiments were made on 4 dogs. Doses of 2, 8, and 12 cc. per kilogram were administered gastrically and the urinary excretion followed for 24 to 48 hours. In 2 experiments, after administering 2 cc. doses, an average of 12 per cent of the total dose was excreted in the urine in 48 hours. With the 8 cc. dose (3 experiments), 39 per cent was found in the urine at the end of 48 hours, and 36 per cent (2 experiments) of the total administered for the 12 cc. dose, in 24 hours. The concentration of propylene glycol in the urine may reach 5 per cent with the higher doses.

A diuretic effect was observed in the same dogs. Two cubic centimeter doses produced no diuretic action, only 100 cc. of urine being excreted during a 48-hour period. Eight cubic centimeters increased the urine output by about 8 times that observed with the smaller dose under the same conditions, actually 850 cc. in 48 hours as an average of 3 experiments. Twelve cubic centimeters in one dog produced a diuresis of over 2 liters in 24 hours.

INTOXICATING EFFECT

The action of propylene glycol on the central nervous system in dogs is similar to that of ethyl alcohol, although larger doses are necessary to bring about the effects. With the higher doses, the control of muscles becomes less and less sure and the motions requiring the most coördination become affected first. The depression finally passes into somnolence. In comparing the degree of drunkenness with that produced by alcohol, it was found that the concentration of propylene glycol must approach about 3 times the concentration of alcohol necessary to produce a similar degree of narcosis. Thus about 350 mgm. per cent of alcohol will produce a similar degree of depression as is produced by 1100 mgm. per cent of propylene glycol.

CONCLUSIONS

1. Propylene glycol in body fluids may be estimated by oxidation with sodium periodate after precipitation of glucose by barium hydroxide in alcoholic solution.

2. The acute toxicity of propylene glycol by intravenous injection is less than half that of ethyl alcohol.

3. Hemoglobinuria does not occur after ingestion of large doses of propylene glycol.

4. Propylene glycol is rapidly absorbed from the gastrointestinal tract. Little absorption occurs in the stomach. After absorption, rapid distribution occurs throughout the tissues.

5. It is probable that combustion of propylene glycol in the body proceeds at a constant rate, irrespective of dosage, the more

rapid fall in blood propylene glycol at higher concentrations being accounted for by increased urinary excretion, which may amount to 45 per cent of the total dose in 24 hours.

6. The narcotic action of propylene glycol is about one-third that of ethyl alcohol.

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THE NUTRITIVE VALUE OF THE FATTY ACIDS OF LARD AND SOME OF THEIR ESTERS*

By SAMUEL LEPKOVSKY, ROY A. OUER, AND HERBERT M. EVANS

(From the Institute of Experimental Biology, University of California, Berkeley)

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The increasing importance of studies of the biochemical behavior of fats seems certain to lead to the progressive decline of the use of natural fats in nutrition research because of their complexity. The saturated fatty acids and the unsaturated fatty acids as well as the non-saponifiable matter are now known to possess different biological activities, and the natural fats must be saponified to separate these components. After saponification, the fatty acids may be fed as such, resynthesized into the glyceride, or esterified with some other alcohol such as methyl or ethyl alcohol, propylene glycol, ethylene glycol, or diethylene glycol.

Information bearing on the nutritive value of these esters as compared with fatty acids or with one another is entirely too meager. Only very recently Cox (1) has shown that the ethyl esters of lard are definitely inferior to the natural lard. The lard and its ethyl esters constituted 55 per cent of his diet. Takahashi (2) fed stearic, palmitic, lauric, and oleic acids at levels of 10 per cent and compared them with their glycerides. In all cases the glycerides were superior to the fatty acids, even when the fatty acids were fortified with the glycerol necessary for the corresponding glycerides. Why the glyceride should be superior to the fatty acids plus the glycerol when these are the compounds formed in the intestine upon hydrolysis of the glyceride remains unexplained. Evans and Lepkovsky (3) found better growth with the glyceride of stearic acid than with the ethyl ester, but the ethyl ester of palmitic acid produced somewhat better growth than the glyceride. In these

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studies the fats were fed as 25 per cent of the diet. From the foregoing work it would appear that in general the glycerol ester is superior to the fatty acids or their ethyl esters. In this study the growth produced by lard, the lard fatty acids, or their ethyl esters was reinvestigated at the levels of 25 per cent and 60 per cent of the diet. The work was extended to include the synthetic glyceride, the methyl ester, and the esters of ethylene glycol, propylene glycol, and diethylene glycol. All conditions were kept strictly constant, so that the only variable studied was the character of the fat employed.

EXPERIMENTAL

Lard fatty acids were prepared by saponification with about 30 per cent alcoholic potassium hydroxide or with 40 to 50 per cent aqueous potassium hydroxide. The fatty acids were liberated with sulfuric acid, dried, and distilled in a high vacuum at a temperature ranging from 165–190°. Some of the fatty acids distilled at as high as 230°, but biological tests seemed to show no inferiority over those distilled at temperatures under 200°.

The methyl esters were prepared by refluxing lard for 24 hours with 1.5 volumes of absolute methyl alcohol containing 2 to 2.5 per cent dry hydrochloric acid. The esters were salted out with brine, washed with water, and taken up in ether, and then dried with anhydrous sodium sulfate, the ether removed, and the methyl esters distilled at 170–185°.

The ethyl esters were prepared similarly, 2 volumes of absolute alcohol containing 7 per cent concentrated sulfuric acid being used. The glyceride and various glycol esters were prepared by heating the fatty acids and alcohol at 210–230° with dry CO_2 bubbling constantly through the mixture, washing with water, and drying.

The esters were solid at room temperature with the exception of the methyl and ethyl esters, which were liquid. All of the compounds were colorless except diethylene glycol, which was light yellow, and propylene glycol, which was light brown.

Groups of six rats 21 days old were put on the experimental diets containing 25 or 60 parts of one of the fatty compounds. The composition of the diets used is shown in Table I.

Results with 25 Per Cent Fat—The growth curves of this series are shown in Chart I. Little difference exists between the syn-

thetic glyceride, lard, free fatty acids, and their ethyl and methyl esters. The ester of ethylene glycol and the low fat diet are inferior.

Results with 60 Per Cent Fat—A second lot of the same substances was prepared and with the exception of the methyl and ethyl esters fed as 60 parts of the diet. The results of this series are shown in Chart II. At this high level of fat, the diets containing the synthetic glyceride and the lard are the best. The

TABLE I

Composition of Diets Given in Parts by Weight

	Diet No.																	
	680	681	682	683	684	685	686	687	689	690	691	694	695	696	697	716	719
Casein* (commercial)	25	35	35	35	35	35	35	35	40	40	40	40	40	40	40	40	40
Sugar "	75	46	46	46	46	46	46	46									
Salt Mixture 185 (4)	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5
Lard		25							60								
Glyceride					25							60					
Fatty acid			25							60							
" " and glycerol				25							60						
Methyl ester							25							60			
Ethyl "								25							60		
Ethylene glycol ester						25							60				
Diethylene glycol ester																	60
Propylene glycol ester																60	

These diets were supplemented by 2 drops of cod liver oil and 1 gm. of brewers' yeast given daily except Sunday. (The brewers' yeast was generously supplied through the courtesy of the president, R. M. Allen, of the Vitamin Food Company, Inc., New York.)

* The casein was generously donated by the Golden State Company, Ltd.

diets with free fatty acids, with or without the glycerol, are definitely inferior and the ester of ethylene glycol the poorest.

Another series of feeding tests at the 60 per cent level was made which consisted of the lard fatty acid esters of glycerol, ethylene glycol, propylene glycol, diethylene glycol, ethyl alcohol, and methyl alcohol. The esters of ethyl and methyl alcohol were liquid and the diets containing them were fed in small quantities in small glass dishes, and complete consumption of all the con-

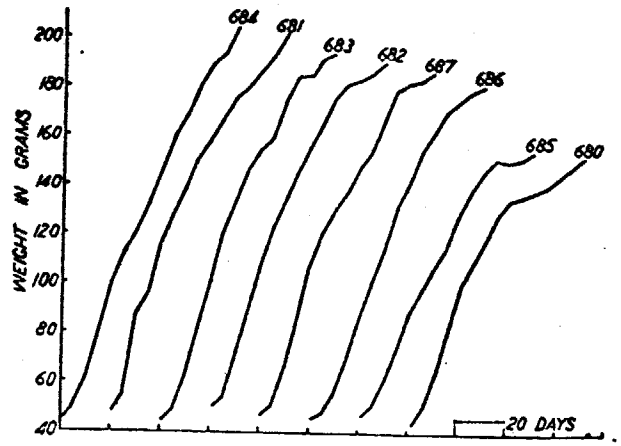

CHART I. Composite growth curves of six female rats each, showing the growth obtained when diets containing 25 per cent of lard fatty acids or their esters were fed. Diet 684, glycerol ester; Diet 681, lard; Diet 683, fatty acid and glycerol; Diet 682, fatty acids; Diet 687, ethyl esters; Diet 686, methyl esters; Diet 685, ethylene glycol esters; Diet 680, low fat controls.

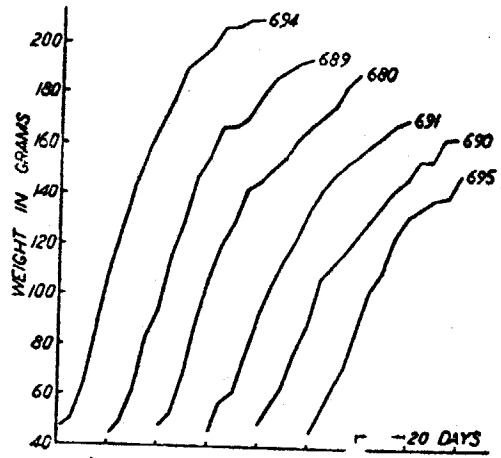

CHART II. Composite growth curves of six female rats each, showing the growth obtained when diets containing 60 per cent of lard fatty acids or their esters were fed. Diet 694, glycerol ester; Diet 689, lard; Diet 680, low fat controls; Diet 691, fatty acid and glycerol; Diet 690, fatty acids; Diet 695, ethylene glycol ester.

stituents was assured before additional diet was measured out. The results are shown in Chart III.

The glyceride (Diet 694) again proved the best, and the propylene glycol esters (Diet 716) definitely inferior. The results with the ethylene glycol esters (Diet 695) differed from those illustrated in Chart II. The mortality at the beginning was very great, five rats dying during the early part of the experiment. Six additional rats were added to the group, of which three died. The four survivors grew very poorly. The animals on the diethylene glycol

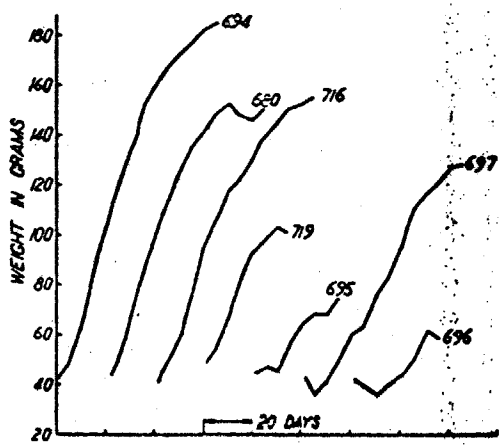

CHART III. Composite growth curves of female rats, showing the growth obtained with 60 per cent of different esters of lard fatty acids. Diet 694, glycerol ester (six rats); Diet 680, low fat controls (six rats); Diet 716, propylene glycol ester (six rats); Diet 719, diethylene glycol ester (six rats); Diet 695, ethylene glycol ester (four rats); Diet 697, ethyl ester (three rats); Diet 696, methyl ester (three rats).

esters (Diet 719) did fairly well at the beginning of the experiment, but after about 3 weeks on the diet they began to die, apparently from kidney damage. The rats receiving ethyl esters (Diet 697) did poorly at the beginning of the experiment, three out of six rats dying. Those that survived grew fairly well. The mortality among the rats receiving the methyl esters (Diet 696) was great and they never grew well at any time. The superiority of the glyceride (Diet 694) over other esters is marked.

Pathology[1]

When the experiment had been in progress 40 days, postmortem examination was made of two animals from each group. The only lesions observed grossly were in the kidneys of the animals fed with esters of ethylene glycol and diethylene glycol. These abnormal kidneys presented a striking appearance. They were definitely enlarged and considerably heavier than normal. They were milky white in color or mottled with white, the surface being studded with nodules and pits.

Microscopic sections showed marked dilatation of some of the collecting and some of the convoluted tubules. This was apparently the result of plugging of the collecting tubules by a yellowish crystalline deposit in the lumen, with a consequent hydronephrosis of the upper reaches of the tubular system. The epithelium of the collecting tubules was flattened. In the convoluted tubules the epithelial cells appeared to be normal in some portions of the sections. In other places they were clear and unstained as if the normal cytoplasm had been replaced by a substance which had dissolved out during fixation and staining, and the nuclei were flattened and pressed to one side as in fat cells. (Frozen sections stained with Sudan III failed, however, to show an excess of fat in the tubules.) In certain areas the convoluted tubules appeared to have completely degenerated and to have been replaced by a bluish substance resembling calcium or a calcium soap. In contrast to the marked abnormality of the tubules, most of the glomeruli appeared to have suffered little damage.

Microscopic sections of the kidneys of animals fed with the ester of propylene glycol did not show the characteristic lesions seen in the animals fed with esters of ethylene and diethylene glycol. No deposits were observed in the lumen of the tubules; the tubules were not noticeably dilated and the tubular epithelium appeared to be essentially normal.

[1] The authors wish to express their thanks for the cooperation of Professor Charles L. Connor of the Department of Pathology, University of California, and Professor Thomas A. Addis of the Department of Medicine, Stanford University, who kindly examined sections of the kidneys of these animals.

DISCUSSION

Of the different forms of lard fatty acids studied in this communication, that which occurs in nature, the glycerol ester, is the best, in spite of exposures to temperatures of 200–230° for 6 to 8 hours. The free lard fatty acids yield good results also, but definitely poorer than the glyceride at the 60 per cent level. No improvements resulted if free glycerol was added in amounts equal to that present in the glyceride. Since the glyceride is hydrolyzed in the intestinal tract to fatty acids and glycerol, it is difficult to understand the inferiority due to direct feeding of the glycerol and free fatty acids.

Of the other polyhydric alcohols used in esterification, the propylene glycol ester seems the best. Pharmacologically (5–7) it is apparently non-toxic, and should therefore be as good as the glyceride. This, however, was not the case. Ethylene glycol, however, is toxic (5–7), and the inferior growth and high mortality in the rats fed lard fatty acid esters of ethylene glycol may be explained on this basis. The rats fed the esters of diethylene glycol did so poorly as to suggest that the diethylene glycol is also toxic.

SUMMARY

1. Lard was saponified and its fatty acids collected, distilled, and esterified with glycerol to form "synthetic" lard; this was fully as satisfactory for the normal growth of rats as the untreated lard whether fed as 25 or 60 per cent of the diet.

2. The free fatty acids with and without glycerol additions were good sources of energy when fed as 25 per cent of the diet, but somewhat inferior to the glyceride at the 60 per cent level.

3. The methyl and ethyl esters were also good when fed as 25 per cent of the diet. When the methyl esters were fed as 60 per cent of the diet, growth was very poor. When the ethyl esters were fed as 60 per cent of the diet, there was an initial mortality but the survivors grew fairly well.

4. Of the esters of the dihydric alcohols, prepylene glycol was the best. Ethylene glycol and diethylene glycol were both poor and histologically the kidneys of these animals showed grave lesions.

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Studies on Absorption and Metabolism of Propylene Glycol Distearate

C. L. Long, F. J. Domingues, V. Studer, J. R. Lowry, B. R. Zeitlin, R. R. Baldwin and R. Thiessen, Jr.

From the Research Center, General Foods Corporation, Tarrytown, New York

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Introduction

The literature is void of information on the metabolism of propylene glycol distearate.[1] With respect to other fatty acid esters of propylene glycol, Lepkovsky *et al.* (1) reported lower growth rates with rats on a 60% dietary level for 100 days as compared to glyceride esters. The above esters were prepared by saponification of lard and a resynthesis of the resulting acids after purification.

Balls and Matlock (2) studied the *in vitro* hydrolysis of fatty acid esters, including PGMS, in the presence of pancreatic enzyme. The compound was shown to be hydrolyzed to the extent of about 80% in 15 hr. at 40°C. Fodor (3) demonstrated, through *in vitro* studies on pancreatic enzyme homogenates, that propylene glycol dibutyrate, propylene glycol monobutyrate, and propylene glycol dipropionate undergo hydrolysis at a high initial velocity which decreases rapidly after a 5–8% cleavage of the ester. In this respect, it is reported that the hydrolysis rate is very similar to that of triglycerides.

The literature presents no data on the extent to which propylene glycol fatty acid esters are absorbed and digested or the metabolic fate of these esters.

The *in vivo* products of digestion, rate of uptake by the lymphatics, and mechanism of transport of PGDS are reported in a second paper in this series (4).

Metabolic studies in rats using C^{14}-labeled PGDS offer a direct means by which the course of the compound can be demonstrated within the

[1] Abbreviations used are: PGDS, propylene glycol distearate; PG, propylene glycol; PGMS, propylene glycol monostearate; and S, stearic acid.

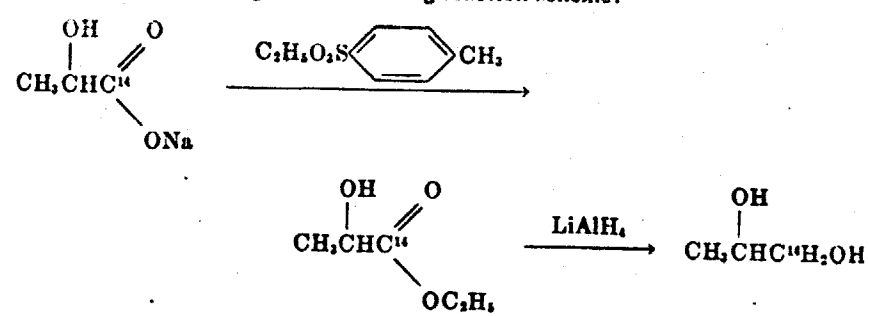
body. PGDS was synthesized as the C^{14} carboxyl-labeled stearate ester and as the 1-C^{14} propylene glycol stearate ester. The rate of uptake, course, and fate of the two fragments of the molecule were traced independently. In addition, a comparative study was made using a mixture of glyceride esters of labeled stearic acid and labeled free propylene glycol and stearic acid.

Experimental Methods

Synthesis of Radioactive Materials

1. *Propylene Glycol-1-C^{14} Distearate (PGDS*).*[2] One hundred and twenty-eight grams (0.452 mole) of stearic acid[3] including 2.75 millicuries (mc.) of stearic acid-1-C^{14}, 17.0 g. (0.223 mole) of propylene glycol, and 0.64 g. of camphor-β-sulfonic acid were reacted for 20 hr. with vigorous agitation at 150–160°C. During the reaction, nitrogen was sparged slowly through the mixture into a steam-heated condenser to remove the water of reaction and retain the propylene glycol. The fatty acid content at the end of the reaction was 7.2%. The reaction mixture was then dissolved in a minimum amount of boiling anhydrous methanol (about 14 l.) and filtered. The filtrate was allowed to crystallize overnight, and the crystals then separated. The product was crystallized three more times following this procedure. The crystals were then melted, and the methanol was removed by sparging nitrogen through the material at 100°C. under vacuum. Analysis for free fatty acid and uncombined hydroxyl groups by acetylation showed none to be present. Chromatographic studies of this material, using a solvent system capable of separating PGMS and PGDS, showed the presence of only one radioactive component (4). The over-all yield was 66%.

2. *Propylene Glycol-1-C^{14} (PG*).* Propylene glycol labeled with C^{14} in carbon-1 was prepared according to the following reaction scheme:



An amount of 37.5 g. of 60% sodium lactate (0.20 mole) was lyophilized for 8 hr.

[2] The asterisk (*) denotes the labeled portion of the molecule; the propylene glycol-1-C^{14} or the carboxyl C^{14} stearate moiety.

[3] Commercially purified stearic acid recrystallized from acetone: average molecular weight, 282.8, iodine No., 0.16.

at 2 mm. Hg pressure. Eighty-eight per cent of the water was removed. An alcohol solution of 6 mc. of sodium lactate-1-C^{14} was prepared by breaking the sealed vials containing the material, washing six times with boiling absolute ethanol, and concentrating the washings to 100 ml. This solution, along with 0.20 mole of ethyl *p*-toluenesulfonate, was added to the flask containing the dried sodium lactate, and the solution was refluxed for 5 hr. At the end of this tim 350 ml. of anhydrous ethyl ether was added through the condenser. The preci .tated salts were separated from the reaction mixture and washed six times wit' 50-ml. portions of anhydrous ethyl ether. The combined filtrates were then subjected to careful fractional distillation until the conditions of 102 mm. Hg pressure and a pot temperature of 51°C. were obtained. Two 10-ml. portions of anhydrous ethyl ether were washed through the rectifying column into the distilling flask. This solution was then added dropwise to an ethyl ether solution of lithium aluminum hydride (8.58 g. in 300 ml. of ether) under vigorous agitation at a rate such that gentle refluxing occurred in the condenser on the reaction flask. Four 25-ml. portions of ether were used to rinse the addition funnel and the transfer flask. Twenty minutes after the addition of the last washings, 120 ml. of water was added, slowly at first, to decompose the excess hydride. A rectifying column equipped with a constant-ratio distilling head was fitted onto the reaction flask, and the ether was distilled off at atmospheric pressure. An additional 200 ml. of water was added during the distillation. When all but traces of the ether were distilled over, the reaction mixture was cooled and neutralized to a pH of 7.0 with a 1:1 solution of orthophosphoric acid and water. The solution was then filtered from the copious precipitate through a coarse sintered-glass filter. The precipitate was washed with six 50-ml. portions of distilled water. The combined filtrates were then subjected to fractional vacuum distillation until practically all of the water was removed. Then 200 ml. of cyclohexane was added to the residue in the distilling flask, a Bidwell-Sterling trap and condenser attached, and the mixture was refluxed at atmospheric pressure until no further phase separation occurred in the condenser and trap. An amount of 16.5 g. of a water solution was collected in this manner over an 8-hr. period. This was carefully separated from the cyclohexane and labeled Soln. A. The rectifying column used in the above water distillation was washed with three 20-ml. portions of water into a 500-ml. flask and refluxed with cyclohexane as described above. An amount of 58.5 g. of a water solution, labeled B, was collected. Solutions A and B were analyzed for PG by periodic acid oxidation. Solution A yielded 38.5% PG, equivalent to 6.35 g. PG. Solution B yielded 5% PG, equivalent to 2.9 g. PG. This amounted to an over-all yield of about 60%. Using butanol–acetic acid–water (4:1:5) (5), resulting paper chromatograms showed only one radioactive component to be present.

While this work was in progress, a different synthesis for PG* was reported in which the starting material was radioactive carbon dioxide (6).

3. *Propylene Glycol-1-C^{14} Distearate (PG*DS)*. PGDS tagged with C^{14} in the carbon-1 of the glycol fragment was prepared as follows: A mixture of 49.1 g. (0.173 mole) of stearic acid, 57.9 g. of Soln. B (see synthesis of PG*) containing 2.9 g. of PG-1-C^{14}, and 0.06 g. of zinc chloride was subjected to fractional distillation until 53 ml. of the excess water from Soln. B was removed. Then 6.0 g. of Soln. A containing 2.3 g. of PG-1-C^{14} was added, and the reaction mixture was heated at 180°C. and 20 mm. Hg pressure for 10 hr. An air-cooled condenser was set in line between the reaction flask and a distilling head equipped with a cold-water condenser in order to return any escaping propylene glycol. The excess water and water of reaction was collected in a Dry Ice–acetone-cooled flask attached to the cold-water condenser. At the end of the reaction time, the product was cooled and crystallized three times from anhydrous methanol as described previously. After drying and clarifying, the product (19.9 g., 48% yield) analyzed for 0.08% free stearic acid and 0.0% uncombined hydroxyl groups.

4. *Mixture of Propylene Glycol-1-C^{14} Mono- and Distearates*. Amounts of 17.6 g. (0.062 mole) stearic acid[3] including 1.39 mc. stearic acid-1-C^{14}, 7.28 g. (0.096 mole) PG, and 0.035 g. stannous chloride were reacted at 170–180°C. for 5 hr. Vigorous agitation and a constant flow of nitrogen through the head space were applied during the reaction period. After cooling, the reaction mixture was dissolved in 300 ml. chloroform and washed five times with 100-ml. portions of water. The chloroform solution was dried with anhydrous sodium sulfate and then filtered. Most of the chloroform was then evaporated off on a hot plate, and the last traces were removed under vacuum. Analysis by periodic acid oxidation and acetylation indicated a composition of 68.7% PGMS*, 30.0% PGDS*, and 1.3% stearic acid. Chromatographic studies of this material, using the same method under (*a*) above showed the presence of two radioactive components.

5. *Mixture of Glycerol-1-C^{14} Mono-, Di- and Tristearates*. Amounts of 19.3 g. (0.068 mole) stearic acid including 1.39 mc. stearic acid-1-C^{14}, 2.08 g. (0.023 mole) glycerol, and 0.04 g. stannous chloride were charged into a 200-ml. flask. Nitrogen was slowly flushed through the headspace during the reaction period. The contents, under agitation, were heated at 220–230°C. for 7 hr. The reactants were cooled at the end of this time, and 2.08 g. (0.023 mole) glycerol and 0.04 g. sodium methylate were weighed into the flask. Agitation and heating at 200°C. were then continued for two more hours. The contents were allowed to cool at 90°C., and 4 drops of concentrated H_3PO_4 was added to destroy the catalysts. The reactants were then dissolved in 300 ml. chloroform. This solution was washed seven times with 150-ml. portions of hot distilled water and then dried with anhydrous sodium sulfate. It was bleached with 4 g. Magnesol at the refluxing temperature for 15 min. and then filtered. The chloroform was removed as described in the previous synthesis. Analysis by periodic acid oxidation and acetylation indicated 13.8% glycerol monostearate, 44.9% glycerol distearate, 41.2% glycerol tristearate, and 0.1% stearic acid.

Treatment of Rats

Preliminary absorption studies were made by methods outlined herein to determine whether preconditioning showed any alteration in alimentary absorption between rats fed for two weeks on a commercial dog meal[4] with and without 1% PGDS. No alteration in alimentary absorption was demonstrated.

[4] Gaines Dog Meal. Ingredients are: Meat and bone meal, corn germ meal, wheat flakes, soybean oil meal, wheat flour middlings, fish meal, fish liver and glandular meal, wheat germ meal 3%, animal fat 2%, dried skim milk 1%, brewer's dried yeast 1%, dried beet pulp 1%, alfalfa leaf meal 0.5%, iodized salt 0.5%, vitamin A feeding oil 0.5%, riboflavine feeding supplement 0.02%, D-activated plant sterol 0.006%.

Both male and female albino rats were used in these studies. Analysis of the data, however, showed no difference in absorption attributable to sex.

Charles River strain rats, weight 192 ± 20 g., were used. The animals were observed for gross manifestations of illness for a period of 2 weeks. Selected healthy animals were fasted for 16–19 hr. preceding the test but were provided with water at all times. During the experiment, they were provided with commercial dog meal[4] and water *ad libitum*.

The animals were weighed, intubated, and sacrificed with chloroform after varying time periods. The abdomens were opened immediately after sacrifice, and the entire gastrointestinal tract was removed after clamping the distal end of the descending colon and the proximal end of the esophagus. If defecation occurred during the digestion period, the feces were carefully collected and combined with the alimentary tract.

Studies on the rate and extent of absorption of PGDS were conducted using C^{14}-labeled PGDS* administered at zero time and at 1, 3, 5, and 8 hr. Four rats were used at each time interval. The absorption of all other radioactive compounds were determined only at the 3-hr. period.

Vehicle for Administration to Rats

The radioactive test compounds were prepared for administration to rats as components in a casein–sugar–water emulsion wherein the "fat" globules were dispersed by use of a Virtis high-speed homogenizer to an average diameter of 4 μ. No more than 1.0–1.5 ml. of the emulsions was administered to a rat. The average dose of radioactive compound administered was approximately 0.3 g./200-g. rat. All emulsions were prepared fresh immediately preceding gastric intubation in rats. Aliquots of the emulsion were diluted with an ethanol–chloroform (1:1) solvent mixture and combusted to determine the specific activity of the radioactive component intubated.

The test materials and methods of administration for the metabolic studies were identical to those used in the absorption study. In these experiments male rats were preconditioned to a diet of 1% PGDS in Gaines meal for 2 weeks. Two rats were given PGDS*, one rat each was given PG*DS, PG* plus nonradioactive stearic acid (equimolar amounts), PG plus S* (equimolar amounts), and a mixture of radioactive glycerol stearates. The animals were placed in a closed metabolism glass chamber, and continuous CO_2 measurements were recorded for 72 hr. Seventy-two-hour urine and feces collections were made.

At the conclusion of the 72-hr. period, animals were sacrificed with chloroform and autopsied. The dried organ weights were obtained.

Methods of Radioactive Assay

1. *Absorption Studies*. For studies on the gastrointestinal absorption rates, the tracts were dried in an oven at 100°C., pulverized in a mortar, and extracted with ethanol–chloroform (1:1). The filtrates were combined and made to volume with the extracting solvent. The gastrointestinal tract residues were saved for determination of residual radioactivity. Any residual radioactive material escaping recovery after a 12-fold extraction was considered to be incorporated and bound with the gastrointestinal tissue. Carefully measured amounts of the extract were introduced into combustion boats, and the solvent was removed by warming under an infrared lamp. The contents of the boats were then dry combusted to $C^{14}O_2$, which was collected in excess NaOH. The resulting carbonate was precipitated as the barium salt and transferred to a planchet. These were counted in an automatic "Nuclear" sample changer equipped with a "Nuclear D47" gas-flow counter. Counting total was such that maximum deviation was no greater than 1.5%. Corrections for absorption within all samples were those described by Domingues, Sarko, and Baldwin (7).

2. *Metabolism Studies*. (*a*) *Carcass and Organ Analysis*. The carcasses (total animal less specific organs) were autoclaved at 15 lb./sq. in. for 3 hr., homogenized to a smooth paste in a Waring blendor, and freeze-dried. The dried material was ball-milled for 3 days. The resulting powder was dry combusted.

The organs of the animal were weighed, dried in an oven at 100°C. overnight, and reweighed. The dry organs were pulverized in a mortar until a uniform powder was obtained. Aliquots were then removed and assayed in quadruple by dry combustion as previously described.

(*b*) *Urine and Feces Determination*. The urine was collected and made up to known volume. Duplicate 500-μl. samples were evaporated to near dryness under an infrared lamp and subsequently combusted for analyses. Seventy-two-hour feces were collected, weighed, dried at 100°C, and reweighed. The feces were ground to a fine powder in a mortar, and aliquots in quadruple were removed for combustion and subsequent assay.

(*c*) *CO_2 Assays*. A continuous record of the respired radioactive CO_2 was accomplished by a method similar to that reported by Tolbert *et al.* (8). Modifications of the above method were reported by Olsen and Mills (9). Room air was pulled through the metabolism chamber at a constant flow rate of 500 cc. min. The air was passed through Drierite and then through an ion chamber. The ion chamber was connected to a vibrating-reed electrometer and recorder which measured the rate of discharge of the chamber as a function of the amount of ionization produced by radioactive CO_2. The differential dmV/dt is calculated from the record chart and replotted versus time. The resulting differential curve is integrated to yield total millivolt change and this value is then reported as counts/min. from a previously determined calibration factor (Fig. 2).

In Vitro *Enzymic Hydrolysis Rate of Propylene Glycol Distearate*

The method of Balls and Matlock (2) was followed with some modifications. The glycerol–bile salts solution was prepared by mixing 30 g. of bile salts with 30 g. water and dissolving this in 300 ml. glycerol at 100°C.; 20.0 g. PGDS was dissolved in 217.5 g. of this solution at 90°C. The mixture was then cooled slowly with agitation to 30°C. and transferred quantitatively to a 500-ml. volumetric flask. Twenty-five milliliters of 1 *N* NH_4Cl–NH_4OH buffer and 5 ml. of a 2% $CaCl_2$ solution were added, made up to volume with water, and mixed thoroughly.

The contents of the volumetric flask were poured into a liter beaker equipped with glass stirrer, a thermometer, and pH electrodes. Then 3.04 g. steapsin (Eimer & Amend) was added. Temperature was controlled by a water bath set at 30°C., and the pH was maintained at 8.35.

Twenty-five milliliters of the emulsion was pipetted into 100 ml. of an ethanol-chloroform (1:1) mixture at time intervals of 0, 5, 30, and 45 min., and of 1, 3, and 19¼ hr., and titrated immediately with 0.1 *N* aqueous KOH.

The inflection points of the titration curves were all at pH 10.6. The per cent hydrolysis was calculated by dividing the moles of free fatty acid analyzed for by the moles initially available.

Results And Discussion

The *in vitro* study indicates that PGDS is capable of undergoing hydrolysis by steapsin to yield free fatty acid (Fig. 1). A comparative study of glycerol tristearate and PGMS was made by Balls and Matlock (2). They showed that after 15 hr. PGMS was 80% hydrolyzed, and, after 4 hr., glyceryl tristearate was 68% hydrolyzed. PGDS was found to be 60% hydrolyzed in 15 hr. and more than 80% hydrolyzed in 19 hr.

The rate of absorption of PGDS* was determined by the residual radioactivity of the gastrointestinal tract extracts and is shown in Table I. The average per cent absorption of PG*DS and S* plus PG at 3 hr. is also given in Table I. The total extent of absorption of PGDS* in the rat was found to be 33% of the administered dose in 8 hr.

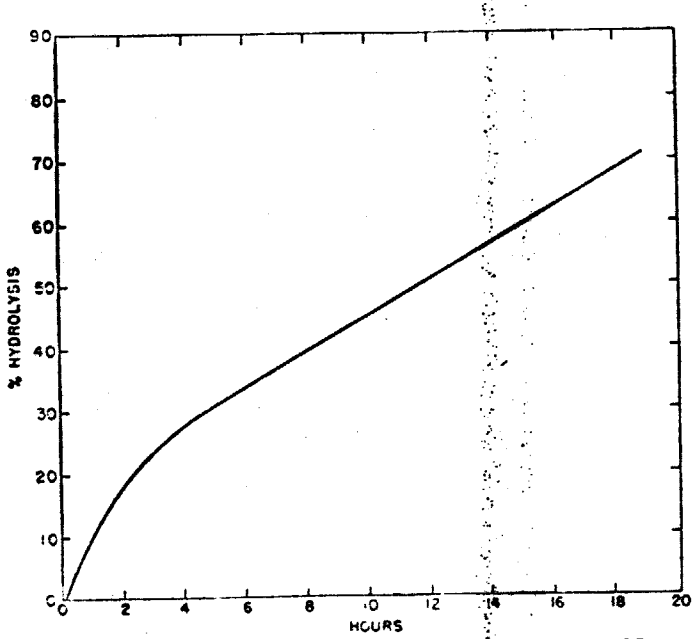


Fig. 1. *In vitro* hydrolysis of PGDS by steapsin at 30°C.

TABLE I

Intestinal Absorption of Radioactive Compounds in Rats

Component	No. of rats	Absorption time	Average fasted body weights	Average administered dose	Average recovered dose	Average per cent total dose absorbed
		hr.	*g.*	*mg.*	*mg.*	
PGDS*	2	0	182	319	313	2.0
	3	1	190	342	304	11.1
	4	3	190	301	233	22.9
	5	5	193	354	312	19.0
	4	8	188	153	102	33.3
PG*DS	3	3	181	367	275	25.0
S* + PG	4	3	202	198	133	33.0

Comparative studies using C^{14}-labeled stearic acid have demonstrated an absorption of 33% in 3 hr., which value is greater than that determined for PGDS* at this time period. Bloom *et al.* (10), using stearic acid-1-C^{14}, reported 36–54% absorption of the labeled acid in 19 hr. when administered in corn oil. This would indicate that the initial limiting factor governing PGDS* absorption is the hydrolysis rate of the ester. That the low rate for PGDS* absorption is linked to the hydrolysis of the ester molecule is also shown by comparative absorption studies undertaken at the 3-hr. digestion level using PG*DS. Here it was demonstrated that about 25% of the radioactivity is absorbed in 3 hr. After this initial 3-hr. period, the governing factor for absorption is that of the stearic acid moiety. This is evident from the results (see Table II) in which PG*DS and PG* plus S is 100% absorbed after 72 hr. as compared to 34% for PGDS* and 48% for PG plus S*. This indicates extensive hydrolysis and more rapid absorption of the PG moiety, leaving behind a slowly absorbed stearate.

The results of the metabolism studies on the radioactive components are shown in Table II.

One hundred per cent of the activity from a dose of PG* plus S and also PG*DS is absorbed in 72 hr. Rapid absorption of PG has been previously demonstrated by Salter *et al.* (11). The average percentage absorption in 5 hr. was found to be 94.0%. PG plus S* activity is absorbed only to the extent of 48% in 72 hr., generally resembling PGDS* and stearates in the extent of uptake (10, 12–15). Concomitantly, the greatest extent of fecal excretion occurred with stearate-labeled PGDS*, PG plus S*, and glyceryl stearate* mixtures.

TABLE II

Recovery of Radioactivity from C^{14}-Labeled PGDS and Related Compounds in 72 Hours

	PDGS*	PG*DS	Glyceryl stearate* mixture	PG + S*	PG* + S
	Total per cent of dose in 72 hr.				
Feces	65.8	0.2	40.6	51.8	0.5
CO_2	17.5	94.0	43.2	42.6	76.4
Urine	6.4	0.4	0.0	1.6	2.3
Organs	6.2	2.7	6.4	3.2	3.3
Carcass	10.8	2.3	8.1	4.8	16.5
Recovery	106.8	99.6	98.3	104.0	99.0
	Per cent alimentary absorption				
	34.2	99.8	59.4	48.2	99.5
	Per cent recovery of absorbed dose				
CO_2	51.3	94.8	72.8	89.0	76.4
Urine	18.9	0.4	0.0	3.4	2.3
Organs	18.5	0.9	10.7	4.2	4.5
Carcass	31.6	2.3	13.7	10.1	16.5
Recovery[a]	120.3	98.4	97.2	106.7	99.7

[a] The difference in recovery between the total dose and absorbed dose is due to the apparent per cent error in the total recovery which was compounded by assuming no error in the absorbed dose.

Approximately 6 % of the total dose or 19 % of the absorbed dose of PGDS* is excreted in the urine in 72 hr. Only 0.4 % was excreted from PG*DS, indicating the origin of urinary excretory products to be derived largely from the stearate portion of the molecule. The compounds excreted in the urine have not been identified. The glyceryl stearate* mixture gave rise to negligible radioactivity in the urine. Similar findings were obtained with PG* plus S and PG plus S*. Metabolic products of PGDS* appear to partition more toward urinary excretion than do the other compounds studied. No explanation may be offered at this time for this relatively large output of urine activity. It is indicated, however, that the metabolism of the stearic acid portion of PGDS is different from free stearic acid given in the presence of PG.

Ninety-four per cent of the absorbed radioactivity in PG*DS is uti-

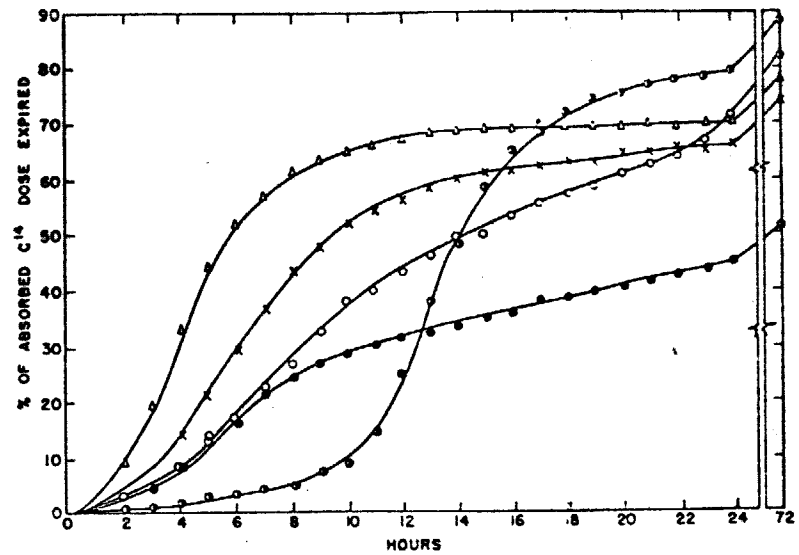


FIG. 2. Cumulative $C^{14}O_2$ excretion by rats from absorbed C^{14}-labeled PGDS and related compounds in 72 hr. △, PG* plus S; ×, glyceride stearate* mixture; ○, PG*DS; ●, PGDS*, ◑, PG + S*.

lized, as evidenced by the $C^{14}O_2$ excretion in a 72-hr. period in the nonfasting rats. The mechanism by which propylene glycol is utilized, by oxidation to lactic acid and subsequent conversion to pyruvate, has been described by several workers (16–19). Fifty-one per cent of the absorbed activity from PGDS* is metabolized to CO_2. Correspondingly, 73 % of absorbed activity of the glyceryl stearate* mixture is metabolized to CO_2.

The breath curves presented in Fig. 2 demonstrate the extent and rate of utilization of radioactive compounds studied. The rate of utilization is uncorrected for differential rates of absorption at intermediate times.

Up to 6 hr. the per cent of total absorbed C^{14} dose expired is the same for PGDS* and PG*DS. It should be pointed out that the measured absorption at 3 hr. was similar for these two compounds. After 6 hr. there is an increase in the $C^{14}O_2$ excretion from PG*DS which may be due to the more rapid absorption and turnover of free PG as compared to stearic acid.

The per cent of the absorbed radioactivity expired at the end of 7

hr. was 94% for PG*DS, 76% for PG* plus S, 72% for the glycerol stearate* mixture, and 89% for S* plus PG. Bergström *et al.* (20) have shown that the amount of CO_2 expired is directly related to the amount of labeled stearic acid absorbed, whether given as free fatty acid or incorporated into a glyceride. They were not able to demonstrate any difference in per cent of absorbed dose as $C^{14}O_2$ versus time between stearic acid and the esterified stearate at the end of 48 hr.

Inspection of Fig. 2 shows a marked difference between the free stearic acid and the glyceride mixture. This difference in the $C^{14}O_2$ output may be due to the time required for esterification of the stearic acid to the corresponding glyceride, as well as the probable absorption rate lag of the stearic acid. At the end of 72 hr., however, where the data have been corrected for absorption differences, there is a difference in the percentage of expired $C^{14}O_2$. This may indicate a difference in the metabolic rate of stearic acid and the glyceride mixture.

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The authors wish to acknowledge the invaluable technical assistance rendered by Mrs. H. Goodman and Messrs. K. J. Gildner, J. F. Mezzino, S. J. Mulé, R. J. Tarantino, and R. Soukup.

Summary

The metabolism of propylene glycol distearate (PGDS) labeled with propylene glycol-1-C^{14} and carboxyl C^{14} stearic acid has been investigated. *In vitro* hydrolysis of PGDS by steapsin has been demonstrated. The extent of absorption of PG*DS, PGDS*, PG plus S* are all similar to a C^{14} carboxyl-labeled stearic acid mixture of glyceride esters. Continuous CO_2 measurements for 72 hr. have been made for the above compounds and for PG* plus S.

Results show that the propylene glycol stearate esters are hydrolized and absorbed in a manner similar to glyceride esters of stearic acid.

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An Investigation of the *in Vivo* Hydrolysis and Absorption of Propylene Glycol Distearate

C. L. Long, B. R. Zeitlin and R. Thiessen, Jr.

From the Research Center, General Foods Corporation, Tarrytown, New York

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Introduction

The preceding paper dealing with the absorption, digestion, and metabolism of propylene glycol distearate[1] demonstrated a similarity in these processes compared with the glyceride fatty acid esters (1). Extension of the study, reported herein, elucidates the *in vivo* hydrolysis and mechanism of transport of propylene glycol distearate in the rat using C^{14}-labeled compounds.

Experimental Methods

Materials

The ensuing investigation was conducted using purified 1,2-propandiol distearate (PGDS) as (*a*) 1-C^{14}-labeled 1,2-propanediol ester (PG*DS)[2] and (*b*) as stearate-C^{14} carboxyl-labeled 1,2-propanediol ester (PGDS*); PG*, PGDS*-PGMS* mixture, stearic acid* and a mixture of mono-, di-, and triglycerides of stearic acid*.

Emulsion Vehicle for Administration of Radioactive Compounds to Rats

The emulsion system is the same as described in the preceding paper (1).

The test emulsions were administered in all cases by weights. An average intubated dose was 1 ml. of emulsion or approximately 300 mg. of radioactive tracer. Replicate-measured samples were taken at the time of intubation to establish the dose administered to each animal.

[1] Abbreviations used are: PGMS, propylene glycol monostearate; PGDS, propylene glycol distearate; and PG, propylene glycol.

[2] Asterisk (*) denotes labeled portion of molecule: propylene glycol-1-C^{14} or C^{14} carboxyl stearate.

Treatment of Rats

All rats used for this study were preconditioned for 2 weeks to nonradioactive PGDS, incorporated in a commercial dog food[3] diet at a 1% level.

1. In Vivo *Gastrointestinal Study*. For the *in vivo* gastrointestinal hydrolysis study, six Charles River Wistar Strain male albino rats, each weighing approximately 200 g., were used. The rats were fasted 24 hr. preceding the test but were allowed free access to water. Three of these rats were intubated orally with a PG*DS emulsion and three with a PGDS* emulsion. The animals were sacrificed by ether anesthesia 1.5 hr. after intubation, and the entire gastrointestinal tract was removed.

2. *Intestinal Lymph Studies*. Ten male Sprague-Dawley rats, weighing 225–290 g., were deprived of food but not water for 18 hr. prior to operation. After anesthetizing the animals with ether, the intestinal lymphatics were cannulated with polyethylene tubing (2) and a gastrostomy tube was inserted. Postoperatively, the rats were placed in a special confining cage (3), and gastric infusions of 0.4% sodium chloride were made at a constant rate of 2.5 ml./hr. After 24 hr., the rats were observed for general health and lymph volume produced.

Three rats were intubated via gastric cannula with the appropriate emulsion of PGDS*, PG*DS, and glyceride stearate* mixture; two were used for the PG* plus nonradioactive stearic acid. The latter mixture was administered in the same ratio as present in PGDS.

Immediately after administration of the labeled compound to the animal, the lymph was allowed to flow into graduated centrifuge tubes. The lymph was collected at 6, 12, 24, and 48 hr. and the volumes were recorded. After each collection, 1 vol. of 95% ethanol was added to denature the protein.

Extraction Procedure for Radioactive Compounds in Gastrointestinal Tract and Lymph

The gastrointestinal tracts of rats intubated previously with the test compound were removed and homogenized in a Waring Blendor using ethanol–ether (3:1). The residue was extracted several times, and the filtrates were combined and made up to 500 ml. Aliquots were taken for radioactive counts and per cent soluble solids after evaporation and drying. Additional aliquots were dried by vacuum distillation under a nitrogen atmosphere in a water bath at 40°C. In experiments with PGDS*, the residue from the vacuum distillation was re-extracted with petroleum ether-chloroform (2:1) and dried over anhydrous Na_2SO_4. The extract was then filtered, and the filtrate was distilled as above. The solids remaining were taken up in 25 ml. chloroform. Aliquots were counted and a solids

[3] Gaines Dog Meal. Ingredients are: meat and bone meal, corn germ meal, wheat flakes, soybean oil meal, wheat flour middlings, fish meal, fish liver and glandular meal, wheat germ meal 3%, animal fat 2%, dried skim milk 1%, brewer's dried yeast 1%, dried beet pulp 1%, alfalfa leaf meal 0.5%, iodized salt 0.5%, vitamin A feeding oil 0.5%, riboflavine feeding supplement 0.02%, D-activated plant sterol 0.006%.

weight was obtained. The chloroform solution was used for separation of the radioactive compounds by the methods presented in this paper.

In experiments with PG*DS the aliquots of the filtrate taken from alimentary tract washings (ethanol–ether) were taken down to a residue by distillation as previously described. The residue, extracted with a small volume of water, formed an opalescent solution. This solution was used for the separation and identification of free PG as described below.

The lymph samples from each time collection containing 1 vol. ethanol were centrifuged. The residue was extracted four times with ethanol–ether (3:1), and all extracts were made up to 50 ml. Aliquots were vacuum distilled under nitrogen at 40°C., and the residue was extracted with petroleum ether–chloroform (2:1) and dried with Na_2SO_4. A residue was again obtained and taken up in chloroform. This solution was used for analysis of radioactive compounds in lymph.

Assay of Radioactive Samples

All samples in this study, including chromatographic fractions, were counted with a Nucleonic Corporation Radiation Counter, model RC-1, using a TCG tube. The samples were prepared for counting by plating out a 0.2-ml. aliquot on a planchet and drying under an infrared lamp. Detection of radioactive spots on paper chromatograms was made by scanning the strips using a Nuclear Instrument and Chemical Scaler equipped with a D-47 thin-window gas-flow tube.

Chromatographic Methods

The aqueous extract of the residue from the gastrointestinal tract was used for the determination of free PG. The extract was purified for paper chromatographic separation using a cellulose column. The latter was prepared by dry-packing 7.5 g. acid-washed cellulose in a 15 × 150 mm. chromatographic tube. The aqueous extract was added to the column and developed under nitrogen pressure. After two 17-ml. fractions, 1 and 2, were collected, the elution solvent was changed to chloroform–ethanol (1:1) to elute the remaining activity from the column. The two aqueous fractions, 1 and 2, from the column were turbid indicating fat contamination. Although separation of free PG from the lipides was incomplete, the effect was to concentrate free PG and to remove much of the lipide material for separation on paper.

Paper chromatographic identification of PG was made by the procedure described by Buchanan *et al.* (4) for the separation of glycols. Whatman No. 1 filter paper was used and developed by ascending chromatography with butanol–acetic acid–water (4:1:5) overnight.

Separation of all other radioactive components in the gastrointestinal tract and lymph was made by methods described below.

Borgstrom reported that phospholipides were retained on a silicic acid column when chloroform was used as the eluant (5). The neutral fat and fatty acids were eluted with this solvent, and phospholipides were removed with methanol. The performance of PGDS*–PGMS* was studied on a silicic acid column with chloroform as the eluting solvent. This mixture was capable of being quantitatively

eluted (275 ml.) in approximately the same volume reported for the removal of fat and fatty acids.

Fatty acids were separated from neutral fat on an Amberlite IRA-400 resin. Using this technique, a mixture of PGDS*–PGMS* in ether was added to an Amberlite IRA-400 resin and was quantitatively removed with ether. Fatty acids were removed from the resin using ethanol–ether–10 *N* HCl (50:75:10) mixture. With the removal of phospholipides and fatty acids, the separation of PGDS, PGMS, and the glyceride mixture was undertaken using kieselguhr treated with dichlorodimethylsilane. The silane vapors were allowed to remain in contact with the kieselguhr for a week. The kieselguhr was then removed and stored in a tightly stoppered bottle. Preparation of the column was similar to that described by Savary and Desnuelle (6) for the separation of mono-, di-, and tripalmitin. The developing solvent was the lower phase of cyclohexane–ethanol–water (1:3:4)

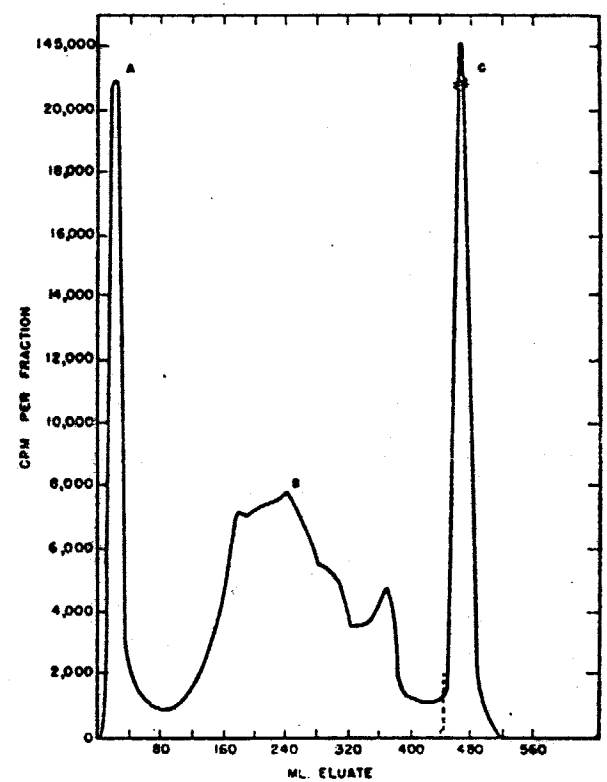

FIG. 1. Separation of mono-, di-, and triglycerides of stearic acid on a silicone-treated kieselguhr column. The dotted line indicates the change from cyclohexane–ethanol–water eluting solvent to pure cyclohexane. Peak *A* = monoglyceride; *B* = diglyceride; *C* = triglyceride.

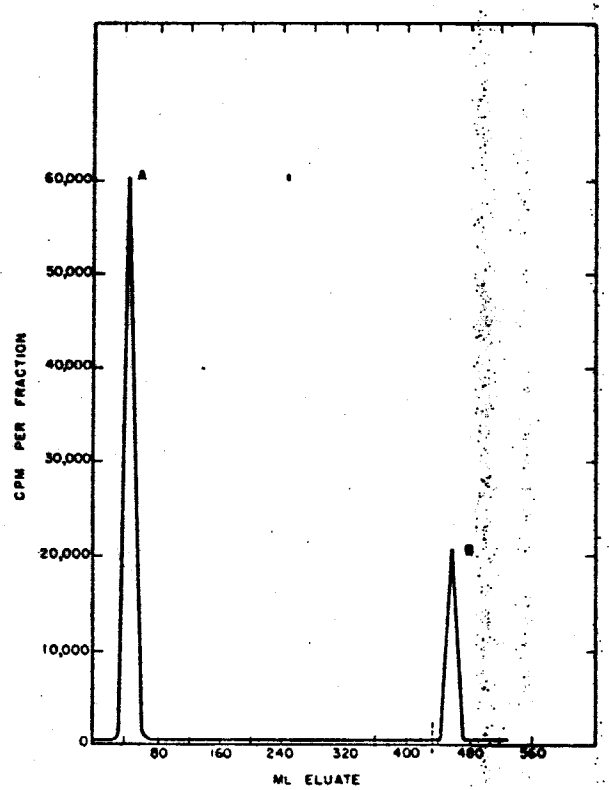

FIG. 2. Separation of 1,2-propanediol mono- and distearates (PGMS–PGDS) on a silicone-treated kieselguhr column. Dotted line indicates the change from cyclohexane–ethanol–water to pure cyclohexane. Peak A = PGMS; B = PGDS.

followed by pure cyclohexane. All column chromatography was carried out under a nitrogen atmosphere.

The performance of the radioactive mono-, di-, and tristearin was studied using the silicone-treated kieselguhr column. The results are shown in Fig. 1. Using the lower phase of cyclohexane–ethanol–water, the monoglyceride was eluted as the first peak, followed by the diglyceride. After removal of the diglyceride, the solvent was changed to pure cyclohexane which eluted the triglyceride. Similar separation was reported by Savary and Desnuelle (6) using mono-, di-, and tripalmitin.

The same technique was used to separate PGDS* and PGMS*. The results are shown in Fig. 2. The first peak is eluted in the same volume as the monostearin. The second peak is eluted only with pure cyclohexane, similar to the tristearin elution pattern. When pure PGDS* was added to the column, it was capable of being eluted only with pure cyclohexane, thus demonstrating the second peak to be the PGDS* component. PGMS* and monostearin can be separated on silicone-treated paper using heptane–80% ethanol (v/v). PGDS* and tristearin can be separated in a similar manner. The diglyceride has been separated as one component in the second peak from the column described above.

The peak fractions from the silicone-treated kieselguhr column were chromatographed on Whatman No. 1 paper. The paper was impregnated with a 5% (v/v) solution of silicone (Dow Corning 200, viscosity 100 centistokes) in cyclohexane (7). It was then blotted to remove excess solvent and dried in an oven at 110°C. for 1 hr. Concentrations of test materials ranged between 5 and 50 μg. as applied to the paper. Since the compounds were detected by scanning for radioactivity, the concentrations above gave desirable peaks with 1000–5000 counts/min. for each component.

After the strips were spotted, they were suspended in chromatographic jars lined with paper to facilitate equilibration. The solvent system used for paper chromatographic separation was heptane–80% ethanol. The lower phase was used to equilibrate the tank and the upper phase was used for developing the paper strips by the ascending technique, this requiring 8 hr.

Results and Discussion

*Identification of Free PG in Alimentary Digests of PG*DS*

The original gastrointestinal tract extract from rats intubated with PG*DS, the two aqueous fractions, and the chloroform–ethanol (1:1) fraction from the cellulose column were chromatographed on Whatman paper using butanol–acetic acid–water (4:1:5) as the developing solvent. Standards consisted of radioactive PG*, PGDS*, PGMS*, stearic acid*, and the glyceride stearate* mixture. The results are shown in Table I.

The data show that pure PG has an R_f separate and distinct from the lipide components present. PGDS is also present in the original extract

TABLE I

*Paper Chromatographic Identification of 1,2-Propanediol from Alimentary Digests of Radioactive PG*DS*

Component	R_f values				
Original gut extract	0.0	0.25	0.39	0.67	0.94
Aqueous fraction 1				0.61	0.91
Aqueous fraction 2				0.67	0.94
$CHCl_3$–ethanol fractions	0.0				0.85
Pure PG				0.68	
PGDS	0.0				
PGMS					0.95
Stearic acid					0.95
Glyceride mixture	0.08				1.0

since it has an R_f value of 0.0 in this solvent. Two radioactive components, R_f 0.25 and 0.39, are present in a very small concentration; their identities are unknown. Definite proof of the presence of free PG is shown in the chromatograms obtained from aqueous fractions 1 and 2. These show two distinct components to be present: one is free PG and the other PGMS. The opalescence in the aqueous eluates was therefore due to the PGMS present.

Hydrolysis of PGDS by the rat is evident as the results show PGMS and free PG to be formed in the alimentary tract after a 1.5-hr. digestion period.

*Identification of Products in Gastrointestinal Tract after Administering PGDS**

The chloroform extract of the gastrointestinal tracts of three rats given PGDS* was added to a silicic acid column and eluted with chloroform for removal of phospholipides. No radioactive phospholipides were present since the methanol eluate gave background activity. However, nonradioactive phospholipides may be present. The chloroform eluate was prepared for the removal of fatty acids using Amberlite IRA-400 resin. The per cent fatty acids from the resin were found to be 18% of the original dose or 25% of the gastrointestinal tract contents. An aliquot from the original extract was passed through the resin column without prior treatment, and similar percentages were found to be present.

The eluate from the resin, containing neutral fat, was chromatographed on a silicone-treated kieselguhr column. Two peaks, *A* and *B*, were eluted from the column. The identity of the above peak fractions were determined by paper chromatography using heptane–80% ethanol. Standards were developed in the same tank. The results are presented in Table II.

TABLE II

Paper Chromatographic Identification of Rat Intestinal Homogenate Components from Silicone-Treated Kieselguhr Columns

Components	R_f values
Peak *A*	0.60
PGMS	0.69
Peak *B*	0.30
PGDS	0.30
Glyceride mixture	0.23 0.90

It is evident from these values that PGDS is hydrolyzed by the rat to produce PGMS and free stearic acid. These data confirm those in the previous section where free PG and PGMS were demonstrated.

The feeding of radioactive PGDS does not appear to give rise to intralumen or intestinal tissue radioactive phospholipides. Borgström (8) showed the amount of uptake of radioactivity by intestinal phospholipides to be low and remain practically constant during absorption of C^{14}-labeled fats and fatty acids.

Rate of Uptake of Radioactive PGDS and Related Compounds in the Lymphatics of Rats*

Total activity present in the lymph over 48 hr. calculated as per cent of dose for each compound is given in Table III. The per cent absorbed is expressed as an average of two or three rats. The rates of uptake are graphically represented in Fig. 3 and as cumulative rates in Fig. 4.

The rate of uptake of activity in the lymph is about the same for PGDS*, PG*DS, and the glyceride stearate mixture. The rat is capable of hydrolyzing PGDS to PGMS and free stearic acid as was demonstrated in the *in vivo* intestinal digestion study. The total extent of appearance in the lymph of the two radioactive compounds indicates that the absorption of PGDS is slightly better than the glyceride stearate mixture. The absorption of glyceride esters of fatty acids entails hydrolysis to monoglycerides, diglycerides, and free stearic acid. As these components pass through the intestinal wall, resynthesis of the triglyceride occurs. If a similar mechanism existed for PGDS*, the compound would be partially hydrolyzed in the intestine and absorbed, and PGDS* would be resynthesized in the lymph. It is also possible that the labeled stearate could combine or transesterify with glyceride esters in the intestinal mucosa or lumen.

The radioactive PG*DS rate of appearance is about the same as that

TABLE III

Total Absorption of Radioactivity in the Lymph of Rats during a 48-hr. Collection Period

Component	Rat No.	Average per cent absorbed of original dose
PGDS*	1, 2, 3	23.62 ± 1.5
PG*DS	4, 5, 6	12.39 ± 1.6
Glyceride stearate* mixture	7, 8	18.37 ± 4.5
PG* + stearic acid	9, 10	1.03 ± 0.1

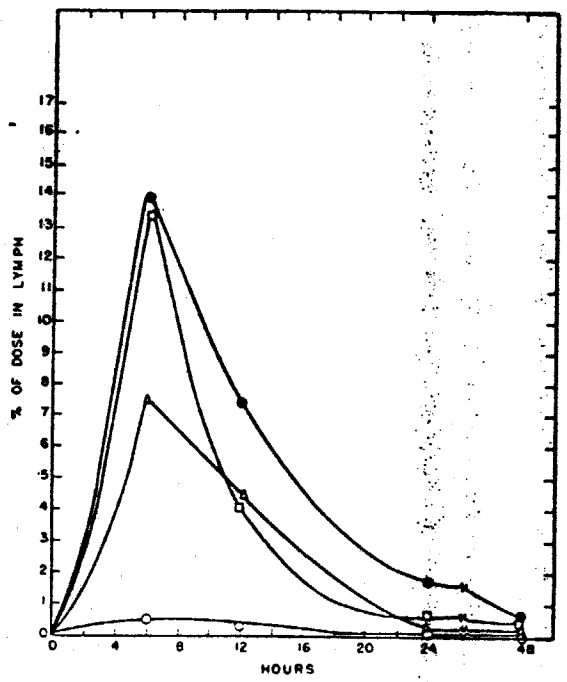

FIG. 3. Rate of appearance of radioactivity in the lymph of rats after administration of various radioactive compounds: ●, average from three PGDS* rats; □, average from the PG*DS rats; △, average from two glyceride stearate mixture rats; ○, average from two PG* plus nonradioactive stearic acid (same ratio as in PGDS).

of PGDS*, but a smaller per cent of activity is present in the lymph. Results presented in this report showed free PG as one of the hydrolysis products of PGDS in the intestine. Since less activity is found in the lymph from PG*DS, it is apparent that any free PG liberated does not go by the lymphatic system but possibly via the hepatic portal system. This would necessarily result in less activity in the lymphatics. This is in agreement with the data on absorption of free PG* plus nonradioactive stearic acid. Approximately 1% of the administered dose of PG* is present in the lymph over the 48-hr. test period.

Components Present in Lymph of Rats after Administration of PGDS and PG*DS*.*

The combined lymph samples from the PGDS* rats 1, 2, and 3 and PG*DS rats 4, 5, and 6 were prepared for phospholipide removal using

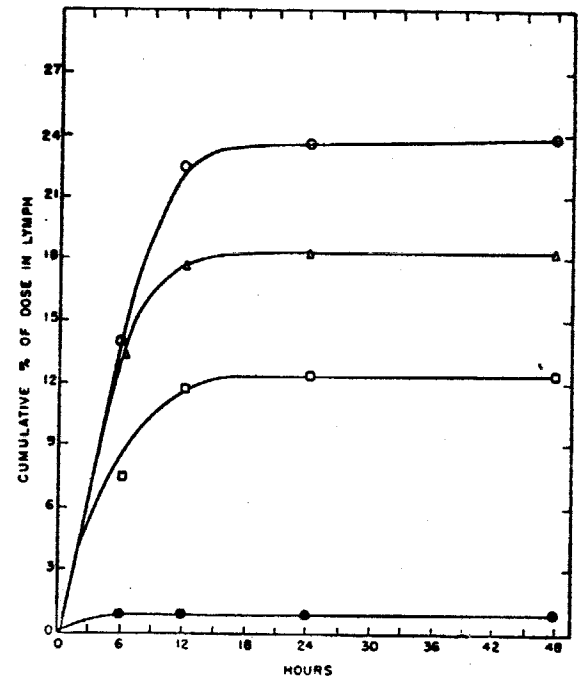

FIG. 4. Cumulative rate of appearance of radioactivity in the lymph of rats administered various radioactive compounds. ○, PGDS*; △, glyceride stearate mixture; □, PG*DS; ●, PG* plus nonradioactive stearic acid (same ratio as in PGDS).

a silicic acid column. The phospholipide fraction from the PG*DS rat lymph sample did not contain radioactivity. When the PGDS* rat lymph fraction was assayed, 6.7% radioactivity was present (calculated on the basis of lymph contents per rat.)

The sample was not assayed for its phosphorus or fatty acid content but, in accordance with the literature, this eluate may be considered the phospholipide fraction. Since PGDS* gave rise to this radioactive fraction and PG*DS did not, it is evident that hydrolysis occurred and free, labeled stearic acid was incorporated into phospholipides. Reiser *et al.* (9) reported that half of lymph phospholipides formed from ingested fat utilized the hydrolyzed fatty acids and endogenous glycerol. The other half was synthesized from absorbed glycerides. Bergström et al. (10) studied lymph fat composition of the cat after feeding fatty acids, fatty acids plus glycerides, and glycerides. In all three cases the lymph

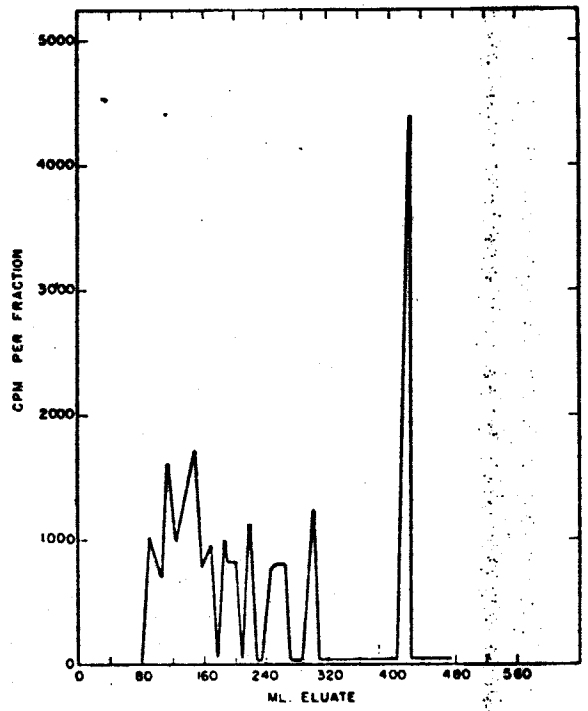

FIG. 5. Column chromatography of purified fraction of rat lymph on silicone-treated kieselguhr after administering PGDS*. Dotted line indicates the change from cyclohexane–ethanol–water to pure cyclohexane.

fat consisted of 80–90% of glycerides and 10–15% phospholipides. Similar results on rats were reported by Borgström (11). These facts are in agreement with the results found in this study.

The neutral fat and fatty acid chloroform eluate from the silicic acid columns was reduced to a residue and taken up in ether, and the fatty acids were removed using an Amberlite IRA-400 resin. The resin was void of activity, indicating both PGDS* and PG*DS rat lymph samples to be free of radioactive fatty acids. Fatty acids have been recently reported in thoracic lymph of rats by Borgström and Tryding (12) but are reported to be the original constituents of the lymph rather than derived by hydrolytic processes in the lymph.

The neutral fat eluates from each resin were chromatographed on a silicone-treated kieselguhr column. The results from the kieselguhr column for the PGDS* rat lymph sample and the PG*DS rat lymph sample

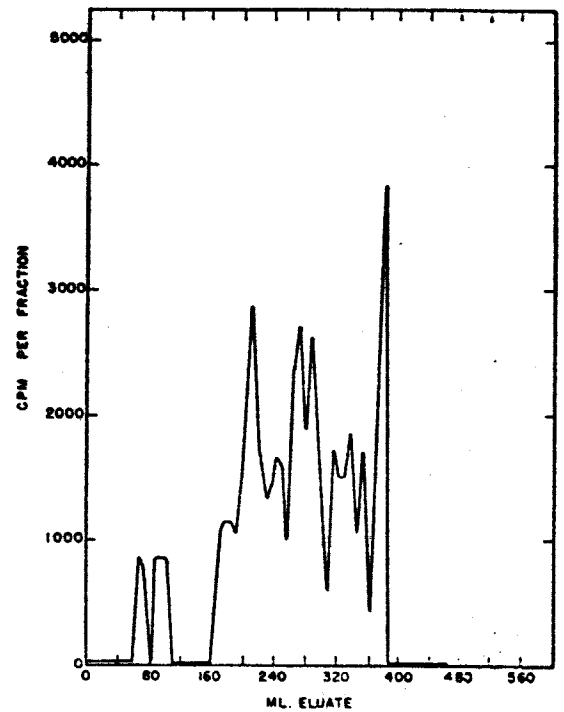

FIG. 6. Column chromatography of a purified fraction of rat lymph on silicone-treated kieselguhr after administering PG*DS. Dotted line indicates the change from cyclohexane–ethanol–water to pure cyclohexane.

are shown in Figs. 5 and 6. The elution patterns of both samples are very similar, but it appears that the column may have streaked in the development. Since pure PGDS*–PGMS* or the glyceride mixture gives good resolution, this difference may be due to interfering substances in lymph, the kieselguhr (one batch was used throughout this study), or may be real radioactive metabolites of PGDS.

A pure PGDS*–PGMS* mixture was chromatographed in the presence of rat lymph extract. The resolution of this mixture is identical to the pattern of PGMS–PGDS separation shown in Fig. 2. The PGMS* was eluted from the column in the first 40 ml. of eluate. The same solvent was continually carried through the column with no streaking of the radioactive components. PGDS* was eluted with pure cyclohexane. The elution pattern of this lymph extract–PGDS*–PGMS* mixture was identical to a column run simultaneously using pure PGDS*–

PGMS*. These facts would tend to eliminate the theory of a lymph-extractable interfering substance or to the kieselguhr itself. The remaining conclusion is that this material is a metabolite or metabolites of PGDS. In a study of the triglyceride of stearyl diacetin, Mattson reported that palmitic acid and unsaturated fatty acids are formed by the time it reaches the thoracic duct (13). PGDS may be acted on in a similar manner to produce unsaturation and shorter chain fatty acids. Recombination of these compounds to form other 1,2-propanediol fatty acid esters may occur. The performance of the kieselguhr column in separating these proposed resynthesized esters is unknown.

The most important finding is that both columns do not show the presence of a peak in the first 40 ml. of eluate. Since pure PGMS* is known to be eluted with this volume of eluate, it can be concluded that the transport of propylene glycol stearate in the lymph is not by way of the PGMS moiety. This is analogous to the mechanism for transport of triglycerides, in that monoglycerides are not present in the lymph after feeding mono-, di-, or triglycerides or free fatty acids (14).

The PGDS* rat lymph samples produced a peak in the pure cyclohexane eluate (Fig. 5), which corresponds to a column elution pattern of PGDS* or the triglyceride of stearic acid. These results suggest either PGDS or triglyceride fraction or a mixture of both is present in the lymph, since radioactive triglyceride may be formed from any liberated labeled stearic acid originating from PGDS*.

PG*DS is utilized in the same manner as PGDS*. Any activity in the lymph cannot be due to a triglyceride synthesis because of the position of the labeling in PG*DS. Since the elution pattern of PG*DS rat lymph was similar to the PGDS* lymph sample, it can be concluded that PGDS is present in rat lymph.

Acknowledgments

The authors wish to express their thanks to Dr. J. L. Bollman of the Mayo Clinic, Rochester, Minnesota, for his interest in this work and providing facilities necessary for the lymph collection and to Mr. Emery Van Hook for the intestinal cannulations of the rats. The technical assistance of Mr. R. Klose in all phases of this study is gratefully acknowledged.

Summary

1. Propylene glycol distearate, labeled with propylene glycol-1-C^{14} and with C^{14} carboxyl stearate, administered orally to rats was hydrolyzed in the gastrointestinal tract to form propylene glycol monostearate, propylene glycol, and stearic acid.

2. Cannulation of the intestinal lymphatics demonstrated the appearance of radioactivity from propylene glycol distearate in the lymph which is comparable to that appearing in the lymph after feeding glyceryl stearate esters. Only small amounts of free propylene glycol were detected in the lymph.

3. During the process of absorption, propylene glycol distearate is resynthesized from its hydrolytic products and is transported by the lymph. Free stearic acid and propylene glycol monostearate were not detected in the lymph.

4. The absorption mechanism for propylene glycol distearate is similar to the glyceryl stearate esters.

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The Injection of Chemicals into the Yolk Sac of Fertile Eggs prior to Incubation as a Toxicity Test

JOSEPH MCLAUGHLIN, JR., JEAN-PIERRE MARLIAC, M. JACQUELINE VERRETT, MARY K. MUTCHLER, AND O. GARTH FITZHUGH

Division of Pharmacology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington 25, D. C.

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The increasingly large number of food additive chemicals introduced into the market each year has necessitated the development of rapid and reliable methods for the evaluation of their toxicity. Toxicologic studies of all these chemicals by the usual methods using animals are very difficult, and such studies sometimes give inconclusive results.

The toxicity of some chemicals, and especially of food additives, may be determined by injection of the chemical into the yolk sac of fertile eggs prior to incubation and subsequent observation of the effects of the chemical on the embryonic development of the chick. This appears to be a promising method in that it may be carried out much more economically in terms of money and space than would be possible with larger animals. Hundreds of chicken embryos may be observed in a minimum of space, and over a comparatively short period of time. The feasibility of using such large numbers is valuable also in the statistical evaluation of toxicity data.

A review of the literature shows how little work has been done in this field except in a fragmentary way on isolated cases. Most of the reports refer to injections of chemicals made after the fourth or eighth day of incubation and examination of the embryos killed before they hatch.

The earliest work that we have found in the literature was by Féré (1893). During ten years after this date he published about sixty-seven papers; a review article (Féré, 1899) contains a summary of many of his studies. His work consisted mainly of injection before incubation, but the eggs were usually opened on the third day of incubation. His interest was mainly in the teratogenic effect of chemicals.

Since 1900 other articles have appeared in the literature, but most of them concern the effects of one or two chemicals on a small number of embryos. One of the most informative papers is that of Ridgway and Karnofsky (1952) in which they report experiments on compounds containing fifty-five of the elements, mainly the metallic ones, generally injected at either the fourth or eighth day of embryonic development to study toxicity and teratogenic effects. Hamburger and Hamilton (1951) have described an elegant method for determining the stages of development of the chick embryo. More recent investigations employing the chick embryo to study the toxicity of various chemicals have been reported by Kemper (1962), Platt *et al.* (1962), and Goerttler (1962). Finally, the classic books of Romanoff and Romanoff (1949) and Romanoff (1960) contain a wealth of information on the avian embryo.

Preliminary reports of this investigation have been presented by Marliac (1962) and McLaughlin and Mutchler (1962).

EXPERIMENTAL

The fertility and hatchability of eggs and the livability of chicks are dependent on a complex interrelationship of ecological factors, among which are the genetic background and the age of the mated birds, the nutritional status and general management of the flock, and seasonal variations. In view of this the initial phase of our work, which started in 1959, was devoted to a study of the hatchability of our supply of White Leghorn eggs[1] under conditions existing in our laboratories. The data accumulated during two years for control eggs showed that the hatchability of these eggs was, in fact, consistent, reproducible, and very high. The possibility of a seasonal variation occurring in responses to compounds introduced into the eggs was also examined by repeated testing of several chemicals at all seasons of the year. No important variation was detected.

Selection of eggs. Eggs to be injected are first candled in order to discard those that are defective and to outline with a pencil the exact location of the air cell. In our laboratory 9% of 5000 eggs had to be discarded: 2% cracked, 4% with improperly calcified shells, 1.5% with a tremulous air cell, 1% with the air cell in the wrong place, and 0.5% with blood clots. After the elimination of such defective eggs, the hatch of control eggs averages 95%. A further restriction is based on the weight of the eggs: all those weighing less than 52 g or more than 63 g are rejected. After

[1] Truslow Eggs, Chestertown, Maryland.

candling, the eggs are randomized in order to avoid series of infertile eggs in any one experiment.

The initial experiment with a given chemical is for range-finding and is performed at two or more concentrations of the chemical with 10 eggs per level. On the basis of this information, 20 or more eggs are injected with the appropriate amount of the chemical.

If the chemical proves to be nontoxic, the experiment is repeated with the minimum number of eggs that will give a reliable and reproducible value for the hatchability. In the case of a toxic chemical, additional eggs are injected to determine the specific effects of the chemical. The total number of eggs used for a chemical depends upon the data obtained initially and upon the kind of information desired. Hence, data for some chemicals are based on less than one hundred eggs, whereas data for others are based on several hundred eggs.

Technique of injection. The injections of pure chemicals, chemical solutions, or suspensions are made at volumes up to 0.10 ml. When necessary, dilutions are made with solvents such as water, propylene glycol, corn oil, peanut oil, or other nontoxic solvents.

In order to avoid contamination, the injections are carried out in an Isolator Box[2] with a sterile atmosphere created by using formaldehyde vapors (produced by mixing 2 g of potassium permanganate and 50 ml of 37% formalin). During the period of a year, more than fifteen hundred noninjected eggs were exposed to formaldehyde vapors; no toxic effect was noticed. After exposure to these vapors for 30 minutes, the eggs are ready for injection.

The large end of the egg is wiped with a sterile gauze pad moistened with a 70% alcohol solution, and a hole is drilled in the shell in the center of the surface over the air cell (Fig. 1). Care must be taken not to damage the shell membrane with the point of the drill[3]; this is to avoid, if possible, contact of the air with the egg membrane. Fine particles of shell are removed with an aspirator to prevent the needle from carrying them into the yolk.

Immediately before the injection, each egg is shaken with a quick twist of the wrist. Since the germinal disc occasionally sticks to the air cell and it is possible to damage it with the needle, this movement will allow the disc to float free in the egg.

[2] Kewaunee Scientific Company, Adrian, Michigan.
[3] Burgess Vibrocrafters Inc., Grayslake, Illinois.

The needle (hypodermic, 1 inch long, either no. 22 or no. 27, depending on the viscosity of the liquid to be injected) is inserted horizontally through the air cell into the yolk (Fig. 1). Care must be taken in withdrawing the needle to avoid damaging the vitelline membrane since such damage could cause the yolk to spread out in the albumen. If the end of the needle has some yolk on it, the injection is not satisfactory. The needle should be wiped with a sterile gauze pad between each injection. As soon as the egg has been injected, the hole in the shell is covered with a small piece of Scotch tape, care being taken not to cover the entire air cell.

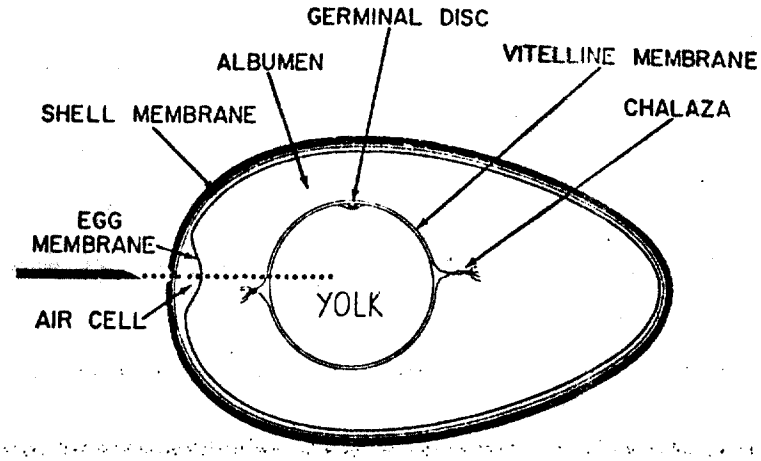

FIG. 1. Diagram of egg and position for injection.

Incubation and hatching. The injected eggs are put into the incubator trays with the large end up; the trays are placed in the incubator,[4] which automatically rotates hourly and is maintained at an optimum temperature of 38°C and a relative humidity of 60%. The eggs are candled on the fifth day of incubation and every day thereafter. Clear eggs and dead embryos are removed for examination. On the seventeenth day of incubation the fertile eggs are transferred to the hatcher[5] and kept at a temperature of 37°C until they hatch.

EVALUATION OF DATA

The injection of the chemical into the egg may produce one of four possible results: (1) the chemical is highly toxic at the level injected, and

[4] Humidaire Incubator Co., New Madison, Ohio; model no. 50, capacity 450 eggs.
[5] Brower Manufacturing Co., Quincy, Illinois.

all the embryos are killed during the first 20 hours of incubation (before the two-somite stage); (2) the chemical is toxic but allows a number of embryos to develop only up to a certain point, and some possibly even to hatch; (3) the chemical has little effect on the hatch; and (4) the chemical has no effect on the hatch or on the posthatch development of the chick ("no effect" level).

If the chemical appears to be highly toxic and all the embryos are killed, the experiment is repeated with smaller doses of the chemical until some hatch is obtained. If the chemical is toxic, but allows the embryos to develop for a longer period of time, dead embryos are examined pathologically and the chicks that do hatch are examined for eye damage, color of the feathers, weight, length of the legs, form of the beak and of the rump, hematologic changes, and condition of the internal organs (liver, kidneys, heart, gall bladder, and spleen).

It is advisable in all cases to observe the chicks for a period of a few weeks in order to detect any delayed effects. Since as much as 30% of the yolk remains at the time of hatching and is absorbed during the first 7 days thereafter, effects of a toxic chemical may first be observed at this time. There may be weight retardation, death during the first week, or the appearance of nerve damage occurring as late as 2–6 weeks after hatching.

The toxicity of a chemical is evaluated mainly from the percentage of hatch at varying dosages of the chemical as compared to noninjected (control) eggs, from a study of the embryonic development of the eggs that fail to hatch, and from a study of the appearance and development of the chicks that do hatch. However, several other factors must also be considered in this evaluation: these are specific gravity, solubility, coagulating effect, pH, and the ionic concentration of the chemical tested.

If the chemical has a high specific gravity, there is the possibility of its settling out in the bottom of the egg and thereby giving a value of apparently low toxicity.

The solubility is quite important since the availability of the chemical for utilization in the chick embryo is partially dependent on its solubility in the egg. However, since egg yolk is an emulsion, solubility problems are somewhat minimized. In the case of insoluble chemicals that are injected as suspensions, it is also necessary to consider particle size in the evaluation of toxicity data.

In order to have a toxic effect, the chemical must come in contact with the embryo either directly or indirectly through the bloodstream. Chemicals such as the lower aliphatic alcohols have a coagulating effect on the pro-

tein, and this coagulation may decrease the availability of the chemical as well as some yolk nutrients, and thereby alter the response of the embryo.

If the pH is highly acidic or basic, a pH effect differing from the true toxic effect of the chemical may be obtained due to interference with the normal acid-base equilibrium in the egg.

The ionic concentration is also important for a similar reason. The observation of increasing toxicity with increasing concentration of a chemical should be interpreted cautiously, since highly concentrated solutions may upset the physical equilibrium of the yolk by causing osmotic effects.

Finally, the introduction of a chemical into the yolk may cause a special type of toxicity because it destroys, alters, or combines with essential nutrients such as vitamins and minerals.

EXPERIMENTAL DATA

Twenty-five thousand eggs have been used in our laboratory during the past three years to test more than 100 chemicals with the following

TABLE 1
NONTOXIC CHEMICALS

Chemical	Solution injected		Per cent hatch
	Concentration	Quantity (ml)	
Water (boiled)	—	0.05	95
Propylene glycol	Undiluted	0.05	95
Corn oil	Undiluted	0.05	90
Peanut oil	Undiluted	0.05	90
Sodium chloride	0.9% in water	0.05	90
	5.0% in water	0.05	70
	5.0% in water	0.10	30
	10.0% in water	0.05	20
Dextrose	5.0% in water	0.05	90

results: (1) nontoxic chemicals injected at an appropriate level allowed the embryo to develop and to hatch as did the controls; (2) toxic chemicals produced effects at dose levels which may be compared to those producing effects in feeding experiments using animals; and (3) this technique often provided toxicologic information which had not been shown by conventional methods.

Table 1 lists the results obtained with some chemicals in common use in food and shows the dosages used and the percentages of hatched chicks.

TABLE 2
CHEMICALS WITH A HIGH ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch	Remarks
Lead acetate	10% in water	0.05	0	—
	2% in water	0.05	0	Hydrocephalus in embryos that failed to hatch
	0.5% in water	0.05	20	—
Mercuric chloride	1% in water	0.05	0	All embryos died at the beginning of incubation
Thiourea	10% in water	0.05	0	Some embryos lived up to 34 days
	5% in water	0.05	20	Hatched on the 28th day of incubation; growth retardation
Triorthocresyl phosphate	Undiluted	0.02	0	Most of the developing embryos died on the 15th day of incubation
	Undiluted	0.01	40	Hatched chicks showed growth retardation and developed paralysis 2–6 weeks after hatching
p,p'-Diaminodiphenylmethane	10% in ethanol	0.05	30	Leg damage; beak deformity (short mandible)
Sodium selenite	0.2% in water	0.05	0	—
	0.1% in water	0.05	20	—
	0.1% in water	0.02	80	—
Dibutyl-tin-dilaurate	Undiluted	0.01	0	Beak deformity
Aroclor 1242 (chlorinated polyphenyls)	Undiluted	0.025	0	—
	Undiluted	0.01	5	Beak deformity (short upper beak); edema; growth retardation

These data, supplemented by an examination of the nonviable eggs and an autopsy of the chicks that hatched, have not indicated any hazard from their use in food. However, it must be pointed out that any chemical, added at a sufficiently high concentration, may have some toxic effect. Since our data and those reported in the literature indicated safety, this phase of the study was not carried beyond a preliminary examination to ascertain that nontoxicity also was shown for these chemicals by the chick embryo technique. This is of theoretical as well as practical importance for the evaluation of any new technique to be used in toxicologic studies.

Table 2 lists our results with several chemicals which have been shown to be highly toxic to animals. In each case there is not only the low percentage of hatch at a low level of the chemical tested, but there are also congenital abnormalities and other responses that raised extremely serious questions as to the safety to the consumers of any food contaminated with these chemicals.

Lead acetate resulted in no hatch at a level of 1 mg per egg. Autopsy of the dead embryos showed extensive brain damage, as has been reported by de Franciscis and Bocalatte (1962) and by Karnofsky and Ridgway (1952).

Mercuric chloride showed no hatch even at a level of 0.5 mg per egg.

Thiourea is a known carcinogen with a basic effect on the thyroid gland. The hatch time was delayed with increasing amounts of this chemical. At a level of 5 mg per egg no chicks hatched and the embryo required 35 days to develop to the stage normally attained at 20 days. At 2.5 mg per egg some chicks hatched, but most of them had to be helped out of the shell. This effect has been reported also by Yushok (1950).

Cavanagh (1954) reported that triorthocresyl phosphate (TOCP), a well-known plasticizer for nonfood use, causes paralysis when fed to adult chickens. We observed this paralysis in some chicks which hatched from eggs injected with 10 mg of the undiluted chemical.

The compound *p,p'*-diaminodiphenylmethane has been reported by Zylberszac (1951) to cause cirrhosis of the liver in rats. We found this chemical to be extremely teratogenic at a level of 5 mg per egg; more than 90% of the chicks had a short mandible and leg damage consisting of a severe bending of the tibia and a general shortening of the bones of the leg.

Sodium selenite proved to be highly toxic. At a level of 0.1 mg per egg, no embryos developed to more than the 5-day stage.

Dibutyl-tin-dilaurate showed no hatch at a level of 10 mg per egg. The majority of the embryos, which did not live more than 15 days at this

TABLE 3

CHEMICALS WITH AN INTERMEDIATE ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch	Remarks
Acetone	Undiluted	0.05	70	
n-Butanol	Undiluted	0.01	95	* At these levels: corneal opacity, cataract, cleft palate, nerve and kidney damage
		0.02*	70	
		0.03*	20	
		0.04	0	
Carbon tetrachloride	Undiluted	0.05	40	
		0.10	0	
Diethylene glycol	Undiluted	0.05	80	
		0.10	55	
Ethyl acetate	Undiluted	0.05	35	
		0.10	15	
Ethyl alcohol	Undiluted	0.05	95	
		0.10	60	
		0.30	0	
Ethylene glycol	Undiluted	0.05	80	
		0.10	65	
Di-2-ethylhexyl phthalate	Undiluted	0.05	95	
Heptachlor	1% in propylene glycol	0.05	75	
		0.10	65	
Hydrochloric acid	4% in water	0.05	80	
Isopropanol	Undiluted	0.05	35	
		0.10	15	
Malathion	Undiluted	0.05	60	Short legs; bleaching effect on chick down
		0.10	0	
Methanol	Undiluted	0.05	90	
		0.10	65	
Styrene	5% in 80% ethanol	0.05	95	

level, had a short and/or flexed mandible; in addition, some embryos showed subcutaneous edema.

Aroclor 1242 gave no hatch at a level of 25 mg per egg. At a level of 10 mg per egg, one chick hatched out of 20 injected eggs, but died 2 days later. Some embryos, which were examined after they died, showed beak deformities (often a short upper beak), edema, and growth retardation.

Table 3 lists preliminary data on the toxicity of some chemicals which have been shown to be toxic in some degree to animals, and which may be found in some processed foods. Included in this group are various solvents, plasticizers, and insecticides. Some of the chemicals on this list require further study, including observation of the chicks until they reach maturity, before they may be classified as to low or high toxicity.

DISCUSSION

The injection of chemicals into the yolk of fertile eggs prior to incubation is a method which can be advantageously used as an element in the evaluation of the safety of food additive chemicals and drugs, and which could be used to screen new products and eventually to correlate their toxicity with that of similar products already tested.

If one considers that a chemical injected into the yolk may be compared to a substance which has the power to cross the placental barrier, this technique, in addition to being an embryonic feeding study, assumes further importance in that it is also a reproduction study. The unfortunate experiences recently suffered with chemicals that have teratogenic effects in humans, and the failure of conventional testing methods to produce this effect in animals, emphasize the urgent necessity for new methods of analysis. Preliminary work that we have done in this area has given satisfactory results (Verrett and McLaughlin, 1963).

Since this represents a system in which the chemical is in direct contact with the embryo throughout development, it is more than likely that any toxic or teratogenic effects would be readily observed. However, there is always the possibility that the chicken will not be a species susceptible to a particular compound, just as it has been shown that the other commonly used species of animals do not respond to all chemicals in a similar manner. It is also possible for the chicken to be more sensitive to a chemical than other species.

Finally, this technique may be applied also to the study of the synergistic effects of chemicals. Results of experiments in our laboratory on

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the potentiation of a few pesticides have been very encouraging (Marliac and Mutchler, 1963).

SUMMARY

An evaluation of toxicity by injection of the chemical into the yolk sac of fertile eggs prior to incubation gave the following results:

Water, propylene glycol, corn oil, peanut oil, isotonic saline solution, and isotonic glucose solution showed no toxicity or a very low order of toxicity.

Mercuric chloride, lead acetate, selenium, triorthocresyl phosphate, *p,p'*-diamino-diphenylmethane, thiourea, Aroclor 1242, and dibutyl-tin-dilaurate showed a high order of toxicity and/or teratogenic effects at certain levels.

Acetone, methanol, ethanol, *n*-butanol, diethylene glycol, ethylene glycol, isopropanol, di-2-ethylhexyl phthalate, hydrochloric acid, carbon tetrachloride, ethyl acetate, malathion, heptachlor, and styrene showed an intermediate order of toxicity.

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OBSERVATIONS ON THE CHRONIC TOXICITIES OF PROPYLENE GLYCOL, ETHYLENE GLYCOL, DIETHYLENE GLYCOL, ETHYLENE GLYCOL MONO-ETHYL-ETHER, AND DIETHYLENE GLYCOL MONO-ETHYL-ETHER

HERMAN J. MORRIS, ARTHUR A. NELSON, AND HERBERT O. CALVERY

From the Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.

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In a previous publication from this laboratory (1) an extensive investigation of the acute toxicity of some glycols and derivatives was reported. Microscopic observations of tissue changes caused by acute doses were described and a bibliography of the literature dealing with the pharmacological and toxicological properties of the glycols which had been reported up to that time was appended. At that time we stated that chronic toxicity studies of some of the glycols and their derivatives were in progress. The results of these studies are the subject of the present report. As is nearly always the case with investigations of this nature, it is obvious that further work is indicated on these same compounds and others of this series, but the results are of such a nature that it seems advisable to present them at this time for the information of others who are working in this field.

Propylene glycol has already been investigated to some extent from the subacute and chronic standpoint (2, 3, 4, 5, 6, 7, 8, 9, 10). In most instances the propylene glycol was administered in the drinking water in concentrations as high as 10 per cent. One group administered it daily by stomach tube, another group fed it as fatty acid esters mixed in the diet, and still another group fed propylene glycol as a substitute for part or all of the carbohydrate of the stock diet. In the chronic toxicity studies of diethylene glycol it has been given in drinking water (5, 7, 11, 12, 13), by stomach tube (14), and as fatty acid esters (3). The chronic effects of ethylene glycol have been observed after administration in drinking water (5, 7, 15), as fatty acid esters (3), and by inhalation of the vapors (16). In all the investigations of propylene glycol no noticeable effects of a deleterious nature have been noted except in those cases where large acute doses were administered (1). In all the cases where diethylene glycol has been studied the mortality has been high and pathological changes marked except when it was administered in very low concentrations in drinking water. In the case of ethylene glycol the results have been similar to those obtained from diethylene glycol, except where the animals were exposed to the vapors, in which case no toxic effects for rats and mice were noted for the concentrations studied. The only other investigations of the chronic toxicity of glycols that have been reported are those of trimethylene glycol (17), and of triethylene glycol (18).

EXPERIMENTAL PROCEDURE. Whereas the acute toxicity studies previously reported (1) were made on several species of animals, the chronic studies herein reported are limited to a single species, namely the albino rat from our own colony of an inbred strain. The breeding colony and the experimental animals were all housed in quarters which are kept at constant temperature and humidity. The animals for these experiments were selected in groups of 4 litter mates of the same sex and placed on experiment at 3 weeks of age. Three of the 4 litter mates were each placed on diets containing one of the substances under investigation. The fourth, serving as a control, received the basic diet only. Twenty litters were used in any one experiment which means that 20 animals were used on each experimental substance and that each animal had 3 litter mates, 2 of which were each on a different experimental substance, and 1 on the basic diet. By this means the effects of each of 3 substances could be compared on litter mate animals as well as with the litter mate control. The 3 glycols were compared in one series of 20 litters whereas the mono-ethyl-ethers of ethylene glycol and diethylene glycol were studied as a part of another series. The third substance of this latter series was not of a similar nature to the glycols and is not being reported at this time. The 20 animals on each of the 3 glycols were divided into 2 groups of 10 each, 1 group being used at each of the 2 levels at which these substances were studied. There were 6 males and 4 females in any one group of 10 animals. The substances being investigated were incorporated in the basic diet which has been described elsewhere (19). Ethylene glycol was fed at levels of 1 per cent and 2 per cent and the diethylene glycol was fed at equimolecular concentrations, or 1.71 per cent and 3.42 per cent. The propylene glycol was fed at levels of 2.45 per cent and 4.9 per cent, the lower concentration being the molecular equivalent of the 2 per cent ethylene glycol and the higher percentage twice this quantity. The ethylene glycol mono-ethyl-ether and the diethylene glycol mono-ethyl-ether were fed at levels of 1.45 and 2.16 per cent respectively, which are equivalent in molecular concentration to the 1 per cent ethylene glycol. These two substances were fed only at the one level. Litter mate male animals were placed on each of these and on the basic diet in groups of 20 animals on each diet. All animals were placed in individual cages and given free access to their respective diets and water. The weights and food consumption were determined at weekly intervals.

RESULTS. *Growth rate and food consumption.* When the weekly growth rates and weekly food consumptions were plotted for all of the experimental and control animals and the data analyzed for the first 55 weeks of the experiment the differences between the control and experimental animals were not statistically significant.

Survival. Statistical tests cannot be applied to the mean survival time since all animals were sacrificed at the conclusion of 24 months and it is not known how much longer they might have lived.

The number of animals surviving 2 years does not give a true picture because in the case of the rats receiving 2 per cent ethylene glycol the deaths are distributed over both years while in the control groups there were very few deaths until well into the second year. Accordingly the number of rats surviving the first year of the experiment was taken as a criterion of relative toxicity. Only in the case of the 2 per cent level of ethylene glycol is there a significant difference from the mortality of the control group. Seven of the 10 experimental animals died whereas all of the litter mate controls survived the first year. The chances are less than 2 in 1000 ($P = .0016$) that the consumption of 2 per cent ethylene glycol has not influenced the mortality of rats in the first year of the experiment. Gross and microscopic examinations of

the tissues of those animals which failed to survive provided no obvious explanation for this greater mortality.

Pathological changes. Inasmuch as a pathologist was not available until near the end of the experiment only 50 of the 100 experimental rats were microscopically examined. Therefore the 50 experimental animals studied are to a certain degree selected, since they are essentially those surviving the longest. There was also some selection of the ethylene glycol animals because those with stones in the bladder were always examined and the others sometimes discarded. Only 10 of the 40 litter mate controls were examined because of limited facilities and because of the examinations of large numbers of other control animals which had been fed the same basic diet. Microscopic sections of the lung, heart, liver, spleen, kidney, adrenal, and testis were made routinely; pancreas, stomach, intestines and lymph nodes were sectioned in about half of the animals, and other organs were occasionally sectioned.

Two outstanding lesions were observed. These were the production of laminated mulberry bladder stones from 0.8 to 1.7 cm. in diameter in 8 male rats given ethylene glycol and diethylene glycol (fig. 1), and testicular enlargement, edema and tubular atrophy in fully two-thirds of the animals that had received ethylene glycol mono-ethyl-ether (fig. 2). The latter lesion has not to our knowledge been reported in rats under any condition and bladder stones such as those seen in our rats have not been mentioned in previous reports on the chronic toxicity of the glycols. Table 1 briefly summarizes the pathological lesions found in the various groups of rats that were examined microscopically.

The testicular lesions were more often bilateral than unilateral. They consisted of marked interstitial edema and marked tubular atrophy, with the weight of one testis without its epididymis increased to as much as 7 grams instead of the normal 2 grams. The group of rats on ethylene glycol mono-ethyl-ether was outstanding in this respect, and the group on diethylene glycol mono-ethyl-ether was intermediate between this and the other groups. We have not yet been able to determine the pathogenesis of the lesions.

Large urinary calculi were grossly observed in a total of 6 animals fed ethylene glycol; 4 of these received one per cent ethylene glycol, the others 2 per cent. The difference in the number of stones observed in two groups of animals may probably be explained by the longer survival time of those animals receiving one per cent ethylene glycol with the diet. Large calculi were likewise observed grossly in 3 animals given diethylene glycol, each bladder containing 2 stones of approximately the same size; in the animals that received ethylene glycol several small stones frequently accompanied a single large one. Marked chronic cystitis accompanied the bladder stones to a greater degree in the ethylene glycol group than in the diethylene glycol group. Small colorless crystalline concretions, often very numerous, were found in the kidney tubules of 7 of our animals; the microscopic appearance and in one case crystallographic examination showed that calcium oxalate was the chief component. These concretions occurred in rats both with and

without bladder stones. Somewhat similar concretions have been found previously (20, 21).

Other chronic renal lesions consisted of tubular atrophy with less glomerular atrophy; tubular casts, usually hyaline, and slight degrees of lymphocytic infiltration and fibrosis. In the group of animals receiving ethylene glycol, because of the presence of bladder stones and renal oxalate concretions, the renal lesions were more severe than in the other groups. The chronic hepatic

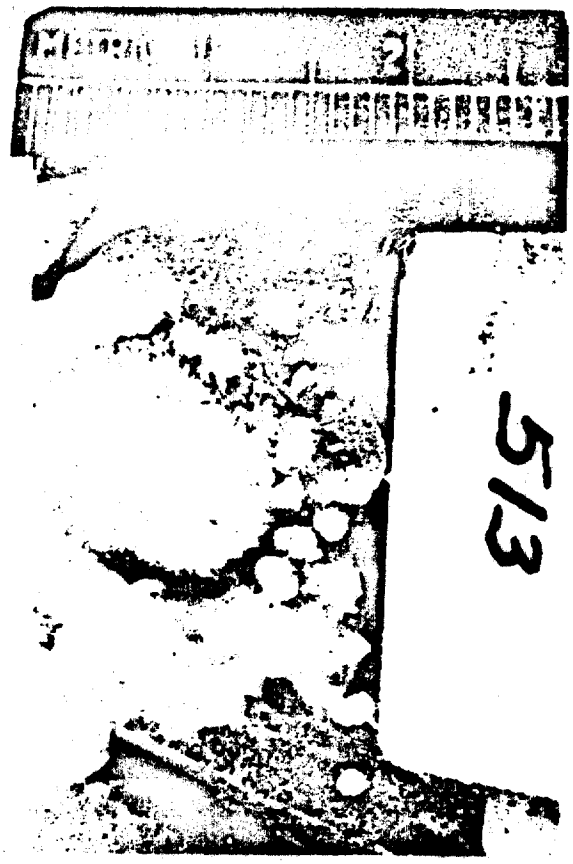

FIG. 1. ONE LARGE AND SEVERAL SMALLER BLADDER STONES REPRESENTATIVE OF THOSE FOUND IN SEVERAL OF THE ANIMALS RECEIVING ETHYLENE GLYCOL AND DIETHYLENE GLYCOL. THE LARGE STONE ALMOST FILLS THE OPENED AND THICKENED BLADDER

lesions consisted of diffuse or centrolobular atrophy, bile duct proliferation and fatty degeneration. In each of these organs more than one of the conditions mentioned were often present. The lungs, heart, spleen, lymph nodes, pancreas, stomach, intestines, and adrenals of the rats on the various diets containing added experimental substances showed no lesions beyond those in the control animals.

Chemical analysis of the urinary concretions. One of the 2 stones found in

the bladder of an animal that received diethylene glycol, and stones or portions of stones from 3 of the animals receiving ethylene glycol, were chemically analyzed. The principal constituents are shown in table 2. When the stone from the animal receiving diethylene glycol was rinsed with water it was found to be soft and it began to crumble. On further agitation with water about 40 per cent of the entire stone was readily separated from the inner portion, which proved to be very hard and compact. The 2 portions of this

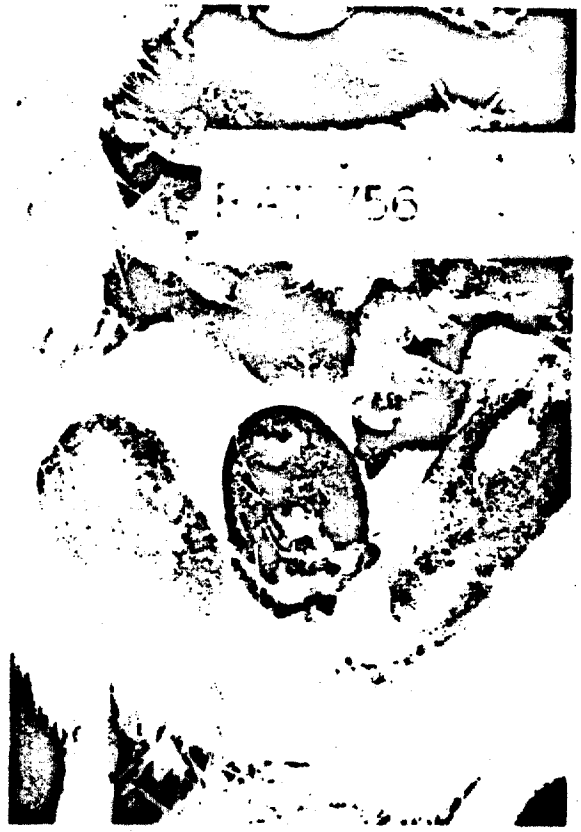

FIG. 2. TESTES OF RATS SHOWING LESIONS REPRESENTATIVE OF THOSE COMMONLY OBSERVED IN ANIMALS RECEIVING ETHYLENE GLYCOL MONO-ETHYL-ETHER, AND OCCASIONALLY IN OTHER ANIMALS. THE STRUCTURE BETWEEN THE LARGE EDEMATOUS TESTES IS THE DISTENDED URINARY BLADDER

stone were separately analyzed. All stones were analyzed for calcium, oxalate, phosphate, and carbonate ions. In those cases in which equivalent quantities of oxalate and calcium were found in the same stone the values have been reported as calcium oxalate. This was true for all of the stones produced by the ingestion of ethylene glycol and also for the inner portion of the stone from the animal receiving diethylene glycol. Tests were made for the presence of other constituents in these stones but since they were found only in traces they are not included in the table. Measurable quantities of phos-

phates were found only in the outer portion of the stone from the animal receiving diethylene glycol and in this material the oxalate and calcium contents were very low. As may be seen from table 2, not all of the constituents of the stones have been accounted for and we were unable to identify the remaining percentage. The stones were dried at a temperature of 200 to 250°C. for several hours before being analyzed and hence should have lost the water of hydration which usually is present in calcium oxalate. The relatively

TABLE 1

SUBSTANCE	BLADDER STONES	OXALATE CONCRETIONS IN KIDNEYS	ENLARGED EDEMATOUS TESTES*	CHRONIC KIDNEY DAMAGE*	CHRONIC LIVER DAMAGE*
Ethylene glycol	6†	6	None	Marked	Slight
Diethylene glycol	3		None	Slight	Slight
Propylene glycol			None	None	Slight
Ethylene glycol mono-ethyl-ether			Two-thirds of animals	Slight	None
Diethylene glycol mono-ethyl-ether		1	Few	None	Slight

* Beyond the number or degree in control animals.

† One of these was a 5 mm. ureteral stone, in an animal receiving 2 per cent ethylene glycol in the diet.

TABLE 2

Composition of stones found in the bladders of rats which had ingested ethylene glycol or diethylene glycol

SUBSTANCE	DRY WEIGHT	CALCIUM OXALATE
	mgm.	*per cent*
Ethylene glycol	35.2	82.5
Ethylene glycol	10.3	81.2
Ethylene glycol	21.0	80.4
Diethylene glycol* (inner portion)	52.5	92.9

* The outer portion of this stone weighed 30.9 mgm. and contained 11 per cent of calcium, 7.3 per cent of oxalate ion, and 75.4 per cent of phosphate ion.

constant value of approximately 80 per cent of calcium oxalate in the stones indicates a rather uniform character of formation.

DISCUSSION AND SUMMARY

The results of this investigation illustrate clearly the advantage of the long-time chronic toxicity study. The results obtained by continuing the experiment for two years that would not have been noted if it had been discontinued at the end of one year were the occurrence of urinary calculi in each of the series of the animals receiving ethylene glycol and diethylene glycol. It is possible also that the microscopic lesions and testicular enlargement observed would not have been as distinctly different between the experimental

and control animals if the experiment had been continued for only one year. As we have emphasized elsewhere (22) chronic toxicity studies should be continued over long periods of time especially in the case of those substances that are to be included in the diet of man or are to be administered as therapeutic agents or applied as cosmetics repeatedly over long periods of time.

Although the actual number of urinary calculi found in this series of animals is small the significance of their occurrence is increased by the fact that over a thousand additional animals from our colony have been fed the same basic diet for control and experimental studies and in no case have such stones been found. The higher incidence of stones at the lower level of intake of ethylene glycol has already been referred to. Stones were found only in male animals, 6 in the ethylene glycol series and 3 in the diethylene glycol series. Although the number of male animals used predominates, the chances are less than 5 in 1000 ($P = 0.0018$) that the sex of the animal does not influence the occurrence of stones. The presence of urinary calculi composed primarily of calcium oxalate following the ingestion of diethylene glycol is almost conclusive proof that in the rat the ether linkage of diethylene glycol is broken and the end products so formed are converted to oxalic acid. The only alternative explanation, which is not very plausible, is that in these particular animals stones were formed from the very small amount of oxalic acid present in the basic diet.

Fully two-thirds of the animals on ethylene glycol mono-ethyl-ether showed marked testicular enlargement, edema and tubular atrophy. This condition occurred less frequently in the group on diethylene glycol mono-ethyl-ether and only occasionally in the other experimental groups and in the control groups. Chronic kidney damage was marked and renal concretions of calcium oxalate frequent only in the case of those animals receiving ethylene glycol. The group of animals receiving propylene glycol differed only very slightly from the controls.

Some of the important questions left unanswered by this investigation are obvious, namely, do other species respond in similar manner, and what are the lowest levels that can be ingested without the occurrence of stones and without effects on the vital organs, such as the liver, kidney, and reproductive organs? Until these questions have been answered the safety of the repeated use in any quantity of those substances producing such effects has not been established.

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(22) Submitted for publication in Am. Jour. Public Health.

Toxicology Studies

COMPANY CODE [illegible]; FPC No. 0164

PROPYLENE GLYCOL

This is a summary of single exposure studies on animals. The data indicate the relative degree of hazard in handling the product. Increasing degrees of hazard are expressed by these terms: slight, moderate, definite, serious. It must be remembered that results of experiments on animals cannot be numerically translated to probable human response.

The National Research Council defines toxicity as the capacity of a substance to produce injury. Hazard is the probability that injury will result from the handling or use of the substance in the quantity, frequency and manner proposed.

Toxicity is only one factor important in determining the degree of hazard in handling a chemical or in a proposed use. Physical properties of the chemical together with extent and frequency of exposure are equally important.

The term LD_{50} has been adopted as a uniform expression of single dose toxicity for comparing one chemical with another. It refers to that quantity of chemical which kills 50 per cent of exposed animals. For further uniformity, quantities are expressed in grams or milliliters of chemical per kilogram of animal body weight.

Single skin penetration refers to a covered 24-hour skin contact with the liquid chemical.

Single inhalation refers to continuously breathing a certain concentration of chemical vapors for a specified period of time.

Primary irritation refers to the skin response following uncovered skin contact. A covered contact can be expected to have a more severe effect.

Eye injury refers to surface damage produced by contact of the eye with the chemical.

Legal responsibility is assumed only for the fact that all studies reported here, and all opinions, are those of qualified experts.

PROPYLENE GLYCOL

Single oral dose in rats: Slight hazard.

LD50 - 26.3 grams per kilogram body weight.

This indicates a very low order of toxicity. Ethanol, for comparative purposes, has an LD50 of 13.6 grams per kilogram, while glycerine is quantitatively similar with an LD50 of 27.5 grams per kilogram.

Single skin penetration in rabbits: Slight hazard.

LD50 - in excess of 20 milliliters per kilogram body weight.

This figure indicates that the chemical does not penetrate the skin in harmful amounts.

Single inhalation by rats: Slight hazard.

Vapor pressure: 0.07 mm. Hg at 20° C.

Breathing a mist generated from the chemical held at a temperature of 170° C. killed one of six animals after eight hours of exposure. Vapors in a state of saturation in air at room temperature killed no animals exposed for eight hours.

Skin irritation: Slight hazard.

The undiluted chemical caused no irritation on the tender skin of the rabbit belly.

For Further Information Write To:
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UNION CARBIDE CORPORATION

(over)

Skin sensitization:

Animal studies and repeated insult tests on human volunteers did not demonstrate development of sensitivity.

Eye injury: Slight hazard.

Flooding the rabbit eye with an excess of the chemical caused a reaction no more severe than moderate inflammation.

Single parenteral dose data:

Intraperitoneal: undiluted chemical
LD_{50} - 16.25 milliliters per kilogram body weight.

Intravenous: undiluted chemical
LD_{50} - 12.3 milliliters per kilogram body weight.

Intravenous: 35 per cent concentration in saline
LD_{50} - 18.7 grams per kilogram body weight.

6/29/59

From: Arch. Exp.Path. Pharmak. (Archives for Experimental Pathology and Pharmacology, 46 : 133 (901).

VII.

Publications of the Pharmacological Institute of the German University at Prague.

II. Series, No. 12.

On glycuronic acid pairing in substances of the aliphatic series.

By Dr. Otto NEUBAUER

Former assistant at the Institute.

While numerous researchers studied the question concerning the physiological effect of organic compounds as a function of their chemical composition during recent decades, comparatively few more specific studies are available on the correlation between the chemical composition and destiny of chemical substances in the animal body. Whatever is known concerning said correlation is restricted to an admittedly large number of occasional single findings.

As for the principal groups of the compounds with open carbon chain, i.e. the substances of the aliphatic series, in particular: the hydrocarbons, alcohols, ketones, aldehydes and acids--none of them has so far been systematically tested regarding its behavior in the animal body; only the destiny of the initial chain links of said series were subjected to numerous tests. The tertiary alcohols constitute an exception; THIERFELDER and MEING[1] demonstrated regarding the latter that they are paired with glycuronic acid in the organism of the rabbit, but not in the organism of dog and man. The authors could not demonstrate any similar pairing for the secondary and primary alcohols; a generally accepted suppostion today seems to indicate that said substances, insofar as they are not again excreted partially unchanged, are burned up in the organism like ethyl-alcohols, and transformed into carbon dioxide and water. The adelhydes have been investigated regarding their two lowest (chain) links only.[1] Concerning ketones: L. SCHWARZ[2] specified that they leave the body again in a partially undecomposed condition ; it became evident that the component which is eliminated undecomposed is proportionately smaller as the number of methyl groups in the ketone decreases.

1.) THIERFELDER & MEING, Journal for Physiologic Chemistry, Vol. IX. (1885), page 511.

(For Footnotes 1 - 6 see Page 2 of this translation).

It is reasonable to assume here as well that the not recovered ketone component was oxidized in the body.

The destiny of the monobasic fatty acids is better known; a complete oxidation in the animal body undoubtedly occurs concerning the low links of this series (with the exception of formic acid),as well as for high links having 16 to 20 carbon atoms. the behavior of the dibasic and tribasic fatty acids, hydroxy acids adelhyde acids and ketone acids, on the other hand, is known only in correlation with single links of these series.

A laboratory finding indicated that the feeding of ethyl alcohol, i.e. of a mixture of two primary alcohols,results in the appearance of paired glycuronic acid in the urine, which contradicts the views of THIERFELD and MERING (l.c.); this prompted a systematic investigation of the substances of the aliphatic series regarding their capacity to pair with glycuronic acid within the organism.
This synthesis was ascertained for a large number of cycloid compounds and in particular, for the aromatic compounds ; however, only a small number of substances in the aliphatic series is known today; they belong to two groups, namely:
1) The tertiary alcohols (tertiary butyl alcohol, tertiary amyl alcohol, Pinakon)[3];
2) Alcohols substituting chlorine (triethylchloride alcohol, trybutylchloride alcohol[4], adelhydes (chloralhydrate, butylchloralhydrate[5] and ketones(dichloracetone)[6].

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3) THIERFELDER and MERING,l.c.
4) KULZ, Biology Journal, Vol. XX.(1884), p. 157.
5) MUSCULUS and MERING, Report of the German Chemistry Assoc., Vol. VIII (1875), pp. 640,662.
6) SUNDVIK, Animal Chemistry, Yearly Report, Vol. XVI.(1886), p.76.

The results of my tests on the occurence of glycuronic acid pairing in aliphatic alcohols, ketones and aldehydes, as well as in certain other substances, are reported below.

The following methods were used for obtaining evidence of paired glycuronic acids:[1)]

1. Testing of left-sided urine polarization which reverts to counterclockwise rotation when treated with thinned sulfuric acid in the sealed tube at 100°, or which (left handed polarization) is at least considerably reduced by said treatment . Regrettably, after precipitation with acid the color of the solution is frequently so dark, that the testing of its optical behavior does not longer seem feasible.

2. Testing of behavior towards FEHLING solution. As known, the lack of reduction of the native urine and the occurence of a reduction after boiling with thinned mineral acids are characteristic here. Admittedly, certain paired glycuronic acids reduce already before precipitation. In single cases, the reduction capacity of the native urine presupposes the simultaneous presence of glucose; in such cases, the glucose is at first removed by fermentation with yeast.

3. The Orcin-test of TOLLENS, as initially used by P. MAYER[2)] to obtain evidence of paired glycuronic acid; the test result is negative in case of paired acid; it is positive for free glycuronic acid, i.e. after precipitation. As suggested by SALKOWSKI, iced vinegar (Eisessig) was added to clear the solution which had become cloudy; the agitating of the formed pigment with amyl alcohol was omitted because the commercially available amyl alcohol frequently contains impurities which might lead to misinterpretations.

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1) See NEUBAUER and VOGEL, Urine Analysis. 10.edition. Revised by HUPPERT. P. 194.
2) P. MAYER, Berlin Clinical Weekly Review, Vol. XXXVI. (1899), p. 590.

4) <u>The precipitability through lead salts</u>. None of the known glycuronic acids is precipitated by neutral plumbic acid; on the other hand, some of them are precipitated by lead acetate, and all of them (are precipitated) by lead acetate + ammonia. Precipitation with lead acetate is mostly incomplete.

Rabbits and dogs were used for the tests. Unless indicated otherwise, the substances were administered with an esophagus probe; the entire quantity was fed to them at once, or it was supplied in several doses. Apart from the above, the animals received their customary food: mixed feed for dogs, oats for rabbits; with this method, the regular urine reaction is acid.

The urine excreted 12 to 24 hours after the administration of the respective substances was precipitated with neutral plumbic acid . The filtrate was tested regarding its polarization (rotation) capacity; whenever counterclockwise rotation could be established, the urine was collected until the latter vanished; the collected urine was in some cases used immediately for the implementation of the other tests; in most cases, the urine was precipitated with lead acetate and ammonia. The obtained deposits were rinsed with water, decomposed with hydrogen sulfide; the excess hydrogen sulfide was eliminated by letting air pass through; the filtrates were neutralized, condensed over the water bath and subjected to the glycuronic acid tests.

Before each test the urine was tested regarding its inactivity and its behavior relatively to the <u>Fehling</u> solution. Animals the urine of which showed a definite counterclockwise rotation (rotations up to -10' are not infrequently observed even in rabbits which appear to be perfectly normal), were excluded from the tests. Quite minor left-oriented polarizations, up tp -4', were disregarded.

I. Secondary alcohols of the aliphatic series.

TABLE I.

Secondary alcohols. Tests with rabbits.

TABELLE I.

Secundäre Alkohole. Versuche an Kaninchen.

1 Gereichte Substanz	2 Verabreichte Menge in g	3 Gewicht des Thieres in g	4 Harnmenge in ccm	5 Drehung: 6 des nativen Harns[1]	7 des auf 100 ccm gebrachten Harns[2]	8 nach der Spaltung	9 Reduction: 10 vor d. Spaltung	11 n. d. Spaltung	12 Orcinprobe: 13 vor d. Spaltung	14 n. d. Spaltung	15 Die linksdrehende Substanz ist fällbar durch
Isopropylalkohol[3] $CH_3-CHOH-CH_3$	3,4	950	108	−44,5′	−49′	−	θ	+	θ	+	Bleiessig + Ammoniak 17
Methyl-propyl-carbinol[4] $CH_3-CHOH-C_3H_7$	1,6	1640	123	−54′	−66,5′	16	θ	+	θ	+	—
Methyl-hexyl-carbinol (sec. Caprylalkohol) $CH_3-CHOH-C_6H_{13}$	1,6	1590	107	−42′	−45′	schwach rechts	θ	+	θ	+	Bleiessig 18

1. Administered substance
2. Supplied quantity in g
3. Weight of the animal in g
4. Urine volume in ccm
5. Polarization (rotation)
   6. of the native urine[1)]
   7. of the urine reduced to 100 ccm[2)]
   8. after precipitation[a)]
9. Reduction
   10. before precipitation
   11. after precipitation
12. Orcin test
   13. before precipitation
   14. after precipitation
15. The substance, polarized (rotating to) the left is precipitable with . .
16. slightly to the right
17. Lead acetate + ammonia
18. Lead acetate

---

1) Obtained with computation from the polarization of the urine which was precipitated with a concentrated solution of acetate of lead and filtered; the stated values refer to an observation tube with a length of 1 dm.

2) Computed from the figures of the previous column.

3) Acetone-free preparation (no precipitate with acetate of phenylhydrazine.)

4) Resulting from the decomposition of its carbamic acid ester through of soda lye; the substance in question is traded by the Bayer & Co factory under the name Hedonal as a soporific; said factory kindly made the substance available for our uses.

a) Translator's note: Spaltung = precipitation, separation, decompositio

TABLE II.

TABELLE II.

Secundäre Alkohole. Versuche an Hunden.

1 Gereichte Substanz	Verabreichte Menge in g 2	Gewicht des Thieres in g 3	Harnmenge in ccm 4	Drehung 5: des nativen Harns 6	des auf 100 ccm gebrachten Harns 7	nach der Fällung 8	Reduction 9: vor d. Spaltung 10	n. d. Spaltung 11	Orcinprobe 12: vor d. Spaltung 13	n. d. Spaltung 14	Die linksdrehende Substanz ist fällbar durch 15
Methyl-propyl-carbinol	4,1 subcutan in 2 Dosen 16	3020	225	—26′	—59′	—	θ	+	—	—	Bleiessig 17
Methyl-hexyl-carbinol	4,1	7700	197	—17,5′	—34,5′	—	θ	+	+	+	Bleiessig + Ammoniak 18

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
   6) of the native urine
   7) of the urine reduced to 100 ccm
   8) after precipitation
9) Reduction
   10) before precipitation
   11) after precipitation
12) Orcin test
   13) before precipitation
   14) after precipitation
15) The substance, polarized (rotating to) the left is precipitable with . .
16) 4.1 subcutaneously in 2 doses
17) Lead acetate
18) Lead acetate + ammonia

The reported tests show that the secondary alcohols of the aliphatic series are paired with the glycuronic acid in the animal body; in the organism of the dog a much smaller part of the alcohol undergoes said pairing, as compared to the (process) in the rabbit[1]. A minimal polarization was shown by the urine after feeding the initial link of the series: isopropylalcohol. But even with the latter, the fact of a partial synthesis with glycuronic acid cannot be doubted. So far, this synthesis was observed only in case of a single secondary alcohol, i.e. menthol[2] which however does not belong to the aliphatic series; it belongs to the group of hydro-aromatic substances.

1) According to THIERFELDER and MERING (l.c.) tertiary alcohols synthesize with glycuronic acid only in the rabbit organism, but not in the dog organism
2) PELLACINI, Arch. of Exp. Pathol. & Pharmacol. Vol.XVII(1883),p.369.

II. Primary alcohols of the aliphatic series

To date nothing had become known in case of these substances either--apart from the chlorine-substituting alcohols--concerning the occurrence of a pairing with glycuronic acid.

TABLE III.
Primary alcohols. Tests with rabbits.
(Pages 138-139 of original text) (next page)

1) Administered substance.
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
   6) of the native urine
   7) of the urine reduced to 100 ccm
   8) after precipitation
9) Reduction
   10) before precipitation
   11) after precipitation
12) Orcin test
   12) before precipitation
   13) after precipitation
15) The substance, polarized (rotating), to the left is precipitable with ..
16) Modification polarized (rotating) to the left[6]
17) Inactive modification
18) Doses
19) right
20) inactive
21) slightly to the right
22) weak +
23) strong +
24) Lead acetate

FOOTNOTES, TABLE III:

1) Purest preparation from KAHLBAUM, Berlin.
2) The tests were implemented with a special purified alcohol: absolute alcohol, in which no fusel oil could be detected with the customary tests, was distilled; the first and last third of the distillate was discarded; the intermediate third was once more treated in the same way.
3) Preparation from KAHLBAUM, Berlin.
4) Original preparation from LINNEMANN (boiling point: 116,88°), which I obtained through courtesy of Prof. Guido GOLDSCHMIDT.
5) Free from active amylalcohol; prepared from inactive amylsulfate of barium.
6) Preparation from SCHUCHART, Görlitz; in the 1-dm tube, it shows a counter-clockwise polarization (rotation) of -3°39'.
7) Preparation from SCHUCHART, Görlitz; according to the business firm's notification, prepared by heating active amylalcohol with alkali.
8) Preparation from SCHIMMEL & Co., Leipzig.

Tabelle II.

Primäre Alkohol

Versuche an Kaninchen.

1 Gereichte Substanz	2 Verabreichte Menge in g	3 Gewicht des Thieres in g	4 Harnmenge in ccm	6 Drehung des negativen Harns	5 Drehung des auf 100 ccm gebrachten Harns	8 Drehung nach der Spaltung	9 Reduction vor der Spaltung	11 Reduction nach der Spaltung	13 Orcinprobe vor der Spaltung	14 Orcinprobe nach der Spaltung	15 Die linksdrehende Substanz ist fällbar durch
Methylalkohol [1]) CH_4O CH_3OH	9,5 in 2 Dosen	1080	209	−3′	−6,5′	—	⊖	⊖	⊖	⊖	—
Aethylalkohol [2]) C_2H_6O $CH_3.CH_2.OH$	9,5	1710	107	−21,5′	−23′	rechts	⊖	+	⊖	+	Bleiessig
Propylalkohol [3]) C_3H_8O $CH_3.CH_2.CH_2OH$	5,5	1050	62	−25′	−17,5′	rechts	⊖	+	⊖	+	—
	6,5 in 2 Dosen	1390	133	−17′	−22,5′	—	⊖	+	⊖	+	—
Norm. Butylalkohol [4]) $C_4H_{10}O$ $CH_3.CH_2.CH_2.CH_2OH$	1,6	1640	25	−23′	−5,5′	—	—	+	?	schwach +	—
Isobutylalkohol [3]) $C_4H_{10}O$ $(CH_3)_2CH-CH_2OH$	3,2 in 2 Dosen	1980	65	−30′	−19,5	inactiv	⊖	+	schwach +	+	—
Amylalkohol [5]) $C_5H_{12}O$ (Isobutylcarbinol) $(CH_3)_2CH-CH_2-CH_2OH$	1,6	890	64	−26′	−16,5′	—	schwach +	stark +	⊖	+	Bleiessig
Activer Amylalkohol $C_5H_{12}O$: $(CH_3OH)(CH_3)CH-CH_2-CH_3$	—	—	—	—	—	—	—	—	—	—	—
a) Linksdrehende Modification [6])	3,2 in 2 Dosen	1460	35	−60′	−21′	rechts	⊖	+	⊖	+	Bleiessig
b) Inactive Modification [7])	3,2 in 2 Dosen	1380	78	−45,5′	−35,5′	rechts	⊖	+	schwach +	stark +	Bleiessig
Octylalkohol [3]) $C_8H_{18}O$ $CH_3-(CH_2)_6-CH_2OH$	4,2 in 2 Dosen	1480	68	−15′	−10′	—	⊖	+	schwach +	schwach +	—
Cetylalkohol $C_{16}H_{37}O$ $CH_3-(CH_2)_{14}-CH_2.OH$	5,0	1070	61	⊖	⊖	—	⊖	⊖	⊖	⊖	—
Geraniol $C_{10}H_{18}O$ [8]) $(CH_3)_2C=CH-CH_2-CH_2-C(CH_3)=CH-CH_2OH$	1,8	1240	215	−12,5′	−27′	schwach rechts	⊖	+	⊖	+	—

1) Reinstes Präparat von Kahlbaum, Berlin.

2) Die Versuche wurden mit einem besonders gereinigten Alkohol angestellt: absolut[illegible] onnte, wurde destillirt; das erste und das letzte Drittel des Destillates wurde verworfe[illegible] [illegible]lkohol, in welchem mit den gebräuchlichen Proben kein Fuselöl nachgewiesen werden [illegible]as mittlere Drittel noch einmal in gleicher Weise behandelt.

3) Präparat von Kahlbaum, Berlin.

4) Originalpräparat von Linnemann (Sdp. 116,55°), das ich der Güte des Herrn Pr[illegible] [illegible]nido Goldschmidt verdanke.

5) Frei von activem Amylalkohol; dargestellt aus inactivem amylschwefelsaurem Baryu[illegible] −3° 39′.

6) Präparat von Schuchardt, Görlitz; zeigt im 1 dm-Rohr eine Linksdrehung v[illegible] [illegible]rhitzen von activem Amylalkohol mit Alkali.

7) Präparat von Schuchardt, Görlitz; laut Mittheilung der Firma dargestellt durc[illegible]

8) Präparat von [illegible]

TABLE IV.
Primary alcohols. Tests with dogs
(Page 140 of original text)

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
    6) of the native urine
    7) of the urine reduced to 100 ccm
    8) after precipitation
9) Reduction
    10) before precipitation
    11) after precipitation
12) Orcin test
    13) before precipitation
    14) after precipitation
15) The substance, polarized (rotating) to the left, is precipitable with. .
16) Remarks
17) Standard butylalcohol
18) Active amylalcohol a) modification with counterclockwise polarization
19) Inactive modification
20) . . . subcutaneous, in 2 doses
21) right
22) slightly to the right
23) strong
24) Lead acetate
25) " "
26) Before fermentation
27) After fermentation (the urine contains glucose during 4 days: 4,4+6,7+2,0+2,9 = 16,0 g).
28) Fasting animal (3-day fasting before the administration of the alcohol).
29) Before fermentation.
30) After fermentation (the urine contains 0,9 g glucose).

Tabelle IV.
Primäre Alkohole. Versuche an Hunden.

Gereichte Substanz	Verabreichte Menge in g	Gewicht des Thieres in g	Harnmenge in ccm	Drehung: des nativen Harns	Drehung: des auf 100 ccm gebrachten Harns	Drehung: nach der Spaltung	Reduction: vor der Spaltung	Reduction: nach der Spaltung	Oreinprobe: vor der Spaltung	Oreinprobe: nach der Spaltung	Die linksdrehende Substanz ist fällbar durch	Bemerkungen
Aethylalkohol	25,5	6400	91	−17,5′	−16′	—	0	schwach	—	schwach +	—	—
	19,8	5650	265 +83	−3,5′ −35′	−38,5′	—	schwach +	stark +	0	+		—
Norm. Butylalkohol	4,0 subcutan in 2 Dosen	5650	72	−26′	−19′	—	0	+	0	+	Bleiessig	—
Isobutylalkohol	8,0 subcutan in 2 Dosen	5380	71	−51′	−71,5	—	0	+	0	+	—	—
	8,0 subcutan in 2 Dosen	6200	403	+3′ −31,5′	+11,7′ −127′	—	+ 0	— +	— —	— —	— —	Vor dem Vergähren Nach dem Vergähren (Der Harn enthält 4 Tage lang Traubenzucker: 4,4 + 6,7 + 2,0 + 2,9 = 16,0 g.)
	6,4 subcutan in 2 Dosen	4850	120	−56,5′	−68′	—	0	+	—	—	—	Hungerthier (3 tägige Carenz vor Verabreichung d. Alkohols)
Activer Amylalkohol a) linksdrehende Modification	4,0	6700	170	−12′ −28,5′	−20,5′ −48′	—	+ 0	— +	— —	— +	Bleiessig	Vor dem Vergähren Nach dem Vergähren (Der Harn enthält 0,9 g Traubenzucker)
	4,0 subcutan	6700	299	−17′	−50,5′	rechts	—	—	—	—	Bleiessig	
b) inactive Modification	4,0 subcutan in 2 Dosen	7050	210	−11,5′	−24,5′	schwach rechts	0	+	0	+	Bleiessig	—

Accordingly, the primary alcohols are likewise paired with glycuronic acid; in this case, however, the synthesis occurs to a conisderably lesser extent than in case of the tertiary and secondary alcohols, which might be the reason why said fact was completely overlooked until now, while the destiny of ethyl alcohol in particular has already been the subject of numerous investigations.

Methyl alcohol represents an exception among primary alcohols: even after the feeding of large volumes it was not possible to establish positively that glycuronic acid is present in the urine. However, this coincides completely with the specific chemical and physiological [1] behavior of the methane-derivatives.

The behavior of ethyl alcohol possibly deserves special attention.

The work of BIZ, BODLANDER and STRASSMANN established that only a small part, 5 to 10 percent, of the administered ethyl alcohol is excreted unchanged by the kidneys and lungs; the rest, i.e. 90 to 95 percent, was therefore completely oxidized in the organism. The available investigations seem to prove the fact that even part of this remnant, although a quite minor part, is not oxidized, but leaves the body paired with glycuronic acid. The volume of said part proved to be quite constant in rabbits when equal doses of alcohol were supplied. Accordingly, the left-hand polarization (rotation) computed on the basis of a 100 ccm urine volume after the administration of 7,9 g alcohol amounted for two animals of approximately similar size to -27' and -23' respectively; after the administration of 9,5 g, it amounted to -23' and -27' respectively. The simultaneous administration of glucose did not result in any substantial increase of the component part bound to glycuronic acid. The first of the two rabbits received after a few days 20 g glucose per os, 1½ hours later it again was supplied with 9,5 g alcohol; the polarization of the urine, computed once more an the basis of the 100 ccm volume, amounted to -24,5'. In dogs, however, individual differences seem to be important. Urine polarization read at -28,5' in some animals after the administration of 19,8 g alcohol, while only traces of paired glycuronic acid could be detected in other (dogs) under identical conditions.

1) POHL, Arch. f. Exper. Pathol. and Pharmac., Vol . XXXI (1893), p. 281.

Part of the lead acetate-ammonia precipitate decomposed by hydrogen sulfide from the urine of the ethyl alcohol-rabbits was distilled after having been treated with thinned sulfuric acid. Ethyl alcohol could be detected in the distillate; at least, it showed a iodoform reaction, and after oxidation with potassium bicarbonate and repated distillation, positive adelhyde reactions resulted; they had previously been negative; similar reactions resulted with sulfurous fuchsin acid, with an ammoniacal silver solution ; the LEWIN reactions were also similar .[1)]

After the administration of octyalcohol only traces of paired glycuronic acid were excreted. None could be detected in the urine of rabbits which had received cetyalcohol subcutabeously or per os. Therefore the high primary alcohols, like methyl alcohol, show a behavior which differs from (the behavior of) the other alcohols.

The polarization (rotation) of the urine is comparatively extensive, especially in dogs, after feeding them isobutylalcohol and active amylalcohol. The presence of a branched carbon chain in these substances could possibly be involved here.

According to its chemical properties, geraniol represents a transition to the terpenes, since ring closing occurs easily within the molecule.

III. Polyatomic alcohols of the aliphatic series.

Paired glycuronic acid was found in the urine after the administration of the diatomic primary-secondary alcohol γ - propyleneglycol; on the other hand, none was found after feeding with the triatomic alcohol glycerine.

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1) LEWIN, Report of the German Chemistry Association, Vol. XXXII., (1899), p. 3388.

TABLE V
Polyatomic alcohols. Tests with rabbits.

TABELLE V.
Mehratomige Alkohole. Versuche an Kaninchen.

1 Gereichte Substanz	Verabreichte Menge in g 2	Gewicht des Thieres in g 3	Harnmenge in ccm 4	Drehung 5 des nativen Harnes	Drehung 6 auf 100 ccm gebrachten Harnes	Drehung 7 nach der Spaltung 8	Reduction 9 vor der Spaltung 10	Reduction 9 nach der Spaltung 11	Orcinprobe 12 vor der Spaltung 13	Orcinprobe 12 nach der Spaltung 14
α-Propylenglycol $C_3H_8O_2$ $CH_3-CHOH-CH_2OH$	6,7	1040	154	−26,5′	−41′	keine deutl. Drehung 15	−	+	schwach + 16	+
Glycerin $C_3H_8O_3$ $CH_2OH-CHOH-CH_2OH$	19,0	1260	196	−4′	−		⊖	⊖	−	−

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
   6) of the native urine
   7) of the urine reduced to 100 ccm
   8) after precipitation
9) Reduction
   10) before precipitation
   11) after precipitation
12) Orcin test
   13) before precipitation
   14) after precipitation
15) No well-defined polarization
16) weak

IV. The ketones and aldehydes of the aliphatic series

After finding that the capacity of pairing with glycuronic acid in the organism is a near-general characteristic of the aliphatic alcohols, it was reasonable to assume that said characteristic could be utilized to detect how other substances fared in the animal body; all compounds of the aliphatic series which convert into the corresponding alcohols within the organism must cause the excretion of paired glycuronic acids. At first, it seemed of interest to test the behavior in the animal body of the next oxidation products of the alcohols, i.e. of the ketones (as the next oxidation stage of secondary alcohols) and of the aldehydes (as next oxidation stage of the primary alcohols).

It was a priori not unlikely that said substances are reduced into alcohols and subsequently, of course, are excreted, paired with glycuronic acid. An example of a similar behavior has been known for a long time: the chlorine-substituting aldehydes (chloral, butylchloral) convert in the body into the corresponding chlorine-substituting primary alcohols (trychlorethylalcohol, trychlorbutyl-alcohol); subsequently, paired glycuronic acids identical with those evident after the feeding of chlorine-substituting alcohols, appear in the urine as final products. Tests were undertaken from this viewpoint concerning the destiny of the ketones and aldehydes.

For the ketones the test results did in fact confirm the expected parallelims with the behavior of the secondary alcohols: the urine polarization (rotation) in rabbits was likewise more extensive here, as compared to the process in dogs (with the exception of Pinakolin) ; it was more extensive for the higher series than for the initial (chain) links. The last-mentioned fact is easily comprehensible since according to the tests of L SCHWARZ mentioned earlier (l.c) the component which is excreted again unchanged in case of the lowest link, i.e. acetone, is the largest; it is already smaller in case of methylethylketone, and still smaller for ketones with five carbon atoms. This behavior might partially be related to the fact that the volatility of the substances decreases as the atomic weight increases. Furthermore, according to L SCHWARZ, a different oxidation energy of the organism for the single links of the series must be considered, whereby the number of the existing methyl groups is of specific importance. (See Tables VI and VII).

Evidence had still to be provided to show that the ketones are subject to synthesis not only as such, but that they are first reduced to secondary alcohols in the body; these are first paired with glycuronic acid; if this is the case, then the acids paired during precipitation would have to be present--not as ketones--but as secondary alcohols, representing precipitation products.

TABLE VI

Ketones. Tests with rabbits
(Page 144-145 of original text) (next page)

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
   6) of the native urine
   7) of the urine reduced to 100 ccm
   8) after precipitation
9) Reduction
   10) before precipitation
   11) after precipitation
12) Orcin test
   13) before precipitation
   14) after precipitation
15) The substance, polarized (rotating) to the left, is precipitable with.
16) Remarks
17) in 2 doses
18) in 3 doses
19) polarized (rotating) to the left
20) no well-defined polarization
21) slightly to the left
22) slightly to the right
23) weak
24) Lead acetate
24 - 25) Lead acetate + ammonia
26) Animal succumbed 2¼ hours after administration
27) Before fermentation
28) After fermentation (the urine contains 0,2 g glucose).

1) Preparation from E. MERCK, Darmstadt, resulting from the decomposition of acetone-sodium bisulfide.
2) Preparation from SCHUCHARDT
3) Preparation from KAHLBAUM.

Tabelle VI.

Ketone. Versuche an Kaninchen.

Gereichte Substanz	Verabreichte Menge in g	Gewicht des Thieres in g	Harnmenge in ccm	Drehung des nativen Harns	Drehung des auf 100 ccm gebrachten Harns	Drehung nach der Spaltung	Reduction vor der Spaltung	Reduction nach der Spaltung	Oreinprobe vor der Spaltung	Oreinprobe nach der Spaltung	Die linksdrehende Substanz ist fällbar durch	Bemerkungen
Aceton [1]) C_3H_6O $CH_3-CO-CH_3$	4,0	1150	76	−26′	−19,5′	—	⊖	+	⊖	+	Bleiessig	—
Methyl-äthyl-keton C_4H_8O [2]) $CH_3-CO-CH_2.CH_3$	1,6	1670	68	−44,5′	−30,5′	—	⊖	+	—	—	Bleiessig + Ammoniak	—
Methyl-propyl-keton $C_5H_{10}O$ [3]) $CH_3-CO-CH_2.CH_2.CH_3$	2,3 in 2 Dosen	1640	69	−143′	−98,5′	keine deutliche Drehung	⊖	+	⊖	+	Bleiessig	—
Methyl-isopropyl-keton $C_5H_{10}O$ [3]) $CH_3-CO-CH(CH_3)_2$	2,4 in 2 Dosen	1750	140	−80,5′	−122,5′	—	⊖	+	—	—	—	—
Diäthylketon $C_5H_{10}O$ [3]) $CH_3CH_2-CO-CH_2.CH_3$	1,6	1560	78	−76′	−59,5′	keine deutliche Drehung	⊖	+	⊖	+	Bleiessig + Ammoniak	—
Methyl-butyl-keton $C_6H_{12}O$ [3]) $CH_3-CO-CH_2.CH_2.CH_2CH_3$	1,6	1700	83	−75,5′	−62,5′	schwach rechts	⊖	+	⊖	+	—	—
Methyl-tertiärbutyl-keton $C_6H_{12}O$ [3]) (Pinakolin) $CH_3-CO-C(CH_3)_3$	1,6	1590	90	−84′	−76′	rechts	⊖	+	schwach +	+	Bleiessig	—
Äthyl-propyl-keton $C_6H_{12}O$ [3]) $CH_3-CH_2-CO-CH_2.CH_2CH_3$	1,6	1570	—	linksdrehend	—	—	⊖	+	⊖	+	—	Thier 2½ h. n. d. Darreichung zu Grunde gegangen
Äthyl-isopropyl-keton $C_6H_{12}O$ [3]) $CH_3-CH_2-CO-CH(CH_3)_2$	1,2	1410	59	−86′	−51′	schwach links	⊖	+	—	—	—	—
Methyl-hexyl-keton $C_8H_{16}O$ [3]) $CH_3-CO-C_6H_{13}$	1,6	1190	136	−34,5′	−47′	schwach rechts	⊖	+	⊖	+	—	—
Methyl-isobutylen-keton [3]) (Mesityloxyd) $C_6H_{10}O$ $CH_3-CO-CH=C(CH_3)_2$	2,7 in 3 Dosen	2030	142	−34′	−48′	—	schwach +	+	⊖	+	—	—
Acetyl-aceton $C_5H_8O_2$ $CH_3-CO-CH_2-CO-CH_3$ [2])	1,9	1690	118	−39′ −44,5′	−46′ −52,5′	— —	+ ⊖	— +	— ⊖	— +	— —	Vor d. Verfahren Nach d. Verfahren (Der Harn enthält 0,5% Traubenzucker.

1) Präparat von E. Merck, Darmstadt, dargestellt durch Zersetzungen von Aceton-Natriumbisulfit.
2) Präparat von Schuchardt.
3) Präparat von Kahlbaum.

TABLE VII

Ketones. Tests with dogs.

TABELLE VII.

Ketone. Versuche an Hunden.

Gewichte Substanz	Verabreichte Menge in g	Gewicht des Thieres in g	Harnmenge in ccm	Drehung des nativen Harns	Drehung des auf 100 ccm gebrachten Harns	Drehung nach der Spaltung	Reduction vor der Spaltung	Reduction nach der Spaltung	Orcinprobe vor der Spaltung	Orcinprobe nach der Spaltung	Die linksdrehende Substanz ist fällbar durch
Aceton	32,0	6900	160	—7′	—11,5′	—	⊖	schwach + 19	⊖	⊖	—
Methyl-isopropyl-keton	2,4	9750	115	—21′	—16′	—	⊖	+	—	—	—
Diäthylketon	16 4,2 subcutan	6000	114	—4′	—4,5′	—	⊖	schwach +	—	schwach +	—
Methyl-tertiärbutyl-keton (Pinakolin)	2,4	7800	313	—62,5′	—195,5′	schwach links	⊖	+	—	+	Bleiessig + Ammoniak 20
Aethyl-propyl-keton	16 2,4 subcutan	5280	90	—32,5′	—29′	keine deutl. Drehung	⊖	+	⊖	+	Bleiessig + Ammoniak

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
   6) of the native urine
   7) of the urine reduced to 100 ccm
   8) after precipitation
9) Reduction
   10) before precipitation
   11) after precipitation
12) Orcin test
   13) before precipitation
   14) after precipitation
15) The substance, polarized (rotating) to the left, is precipitable with .
16) subcutaneous
17) slightly to the left
18) no well-defined polarization
19) weak
20) Lead acetate + ammonia

(Text continued from p. 146 of original)

This could in fact be demonstrated as follows: after precipitation with sulfuric acid, the separation product is distilled; the distillate yielded no ketone reaction; when it was subsequently oxidized with potassium bichromate and sulfuric acid and distilled once more, the ketone tests[1] in the distillate were positive. The same method was applied to acetone, ethylisopropylketone and methylhexyketone. The reactions of the separation product of the paired glycuronic acid which appear in the urine after the administration of ethylisopropylketone are shown as an example.

	Vor der Oxydation	Nach der Oxydation
Nitrophenylhydrazin	θ	Sofort starker krystallinischer Niederschlag.
Legal'sche Probe	θ	+
Ammoniakalische Silberlösung . .	θ	Keine deutliche Reaction.

1) Besonders bewährte sich dabei das von Bamberger (Ber. d. Deutsch. chem. Gesellsch. Bd. XXXII. 1899. S. 1906) zum Nachweis von Aldehyden und Ketonen empfohlene p-Nitrophenylhydrazin.

1) Before oxidation
2) After oxidation
3) . . . (illegible name) test
4) Ammoniacal silver solution
5) Immediate strong crystalline precipitate
6) No well-defined reaction.

---

1) The p-nitrophenylhydrazine recommended by BAMBERGER (Report of the German Chemistry Assoc., Vol. XXXII, 1899, p. 1906) for the determination of aldehydes and ketones proved to be especially efficient thereby.

Besides the listed tests, STOCK's ketone reaction test[1] was applied to the separation product ; it was likewise positive only after oxidation.

<u>This seems to confirm the fact for the entire aliphatic ketone group that said ketones are at least partially subjected to reduction in the animal body</u>.

It is especially remarkable that <u>acetone</u> is also partially converted in the body into a paired glycuronic acid; this is true at least regarding rabbits; no evidence of paired acid could be obtained in dogs. It remains to be seen how humans behave in this respect: whether, specifically in case of a pathologic acetone excretion, a part of the acetone likewise appears in the urine in the form of paired isopropylalcohol-glycuronic acid. This has still to be investigated. Information supplied by P. MAYER[2] in this context is neverthless noteworthy: he was able to detect a considerable quantity of paired glycuronic acid in a case of grave diabetes, involving a copious excretion of acetone. The author, however, attempted to explain this diagnosis differently. According to the above statements, the acetone is reduced to isopropylalcohol in the organism before pairing. ALBERTONI[3] reported on the other hand that the supplied isopropylalcohol is partially oxidized into acetone within the body; therefore the reaction at issue proceeds in two opposite directions and represents a "<u>reversible</u>" reaction. Similar reversible chemical processes in the animal organism are known; I refer hereby to the synthesis and decomposition of glycogen, to the synthesis and separation of the hippuric acid in the dog[4], and I furthermore refer to the oxidation of arsenious acid into arsenic acid in the animal body[5].

The test with acetylacetone showed that the behavior of the diketones is similar to (the behavior of) the simple ketones.

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1) A. STOCK, Dissertation, Berlin, 1899.
2) P. MAYER, Berlin Clinical Weekly Review, Vol. XXXVI.(1899), p.590.
3) ALBERTONI, Medical Chemistry and Pharmacology Review, I., p.413
4) SCHMIEDBERG, Arch. for Exp. Pathol. and Pharmac., Vol. XVI (1881), p.379
MINKOWSKI, same publ., Vol. XVII(1883), p. 445.
5) See the works of BINZ and SCHULZ, Arch. for Exp. Pathol. & Pharmac., Volumes XI, XIII, XIV, XV, XXXVI, and XVIII.

TABLE VIII
Aldehydes. Tests with rabbits.
(Page 148 of original text)

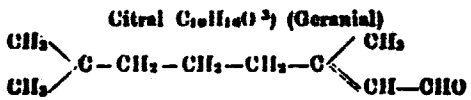
1. Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
    6) of the native urine
    7) of the urine reduced to 100 ccm
    8) after precipitation
9) Reduction
    10) before precipitation
    11) after precipitation
12) Orcin test
    13) before precipitation
    14) after precipitation
15) Remarks
16) in . . . doses
17) No well-defined polarization (rotation)
18) weak
19) The animal succumbed after 60 hours.

---

1) Preparation from SCHUCHARDT.
2) Preparation from KÖNIG & Co., Leipzig.
3) Preparation from KAHLBAUM.

Tabelle VIII.

Aldehyde. Versuche an Kaninchen.

Gereichte Substanz	Verabreichte Menge in g	Gewicht des Thieres in g	Harnmenge in ccm	Drehung: des nativen Harns	Drehung: des auf 100 ccm gebrachten Harns	Drehung: nach der Spaltung	Reduction: vor der Spaltung	Reduction: nach der Spaltung	Orcinprobe: vor der Spaltung	Orcinprobe: nach der Spaltung	Bemerkungen
Isobutyraldehyd [1]) C_4H_8O	3,2 in 4 Dosen	1390	67	—6′	—4′	—	Θ	schwach +	Θ	?	Thier nach 60 h. zu Grunde gegangen.
Isovaleraldehyd [1]) $C_5H_{10}O$	2,4 in 2 Dosen	1240	55	—15′	—8,5′	—	Θ	Θ	Θ	schwach +	—
Heptylaldehyd [2]) $C_7H_{14}O$ (Oenanthol) $CH_3—(CH_2)_5—CHO$	4,9 in 4 Dosen	1610	73	—7′	—5′	—	Θ	Θ	Θ	?	—
Citral $C_{10}H_{16}O$ [3]) (Geranial) 	1,8	1790	97	—42′	—41′	keine deutliche Drehung	+	+	+	+	—

1) Präparat von Schuchardt.
2) Präparat von König und Co., Leipzig.
3) Präparat von Kahlbaum.

 (Text cont. from p.147)

It could a priori be expected concerning the aldehydes that after their administration only minor quantities of paired glycuronic acid would be present in the urine, since after the feeding of primary alcohols the urines already showed a comparatively minor counterclockwise polarization (rotation). The adelhydes, which are more volatile, can be excreted more easily unchanged by the lungs; furthermore, only small doses of the same can be administered because of a higher degree of toxicity. It cannot be surprising therefore that tests with isobutylaldehyde, isovaleraldehyde and oenanthol yielded questionable results. On the other hand, the presence of paired glycuronic acid[1] could be confirmed after the administration of the heavy volatile aldehyde of geraniol, Citral (Geranial), which admittedly is closely associated with the terpenes.

VI. Unsaturated hydrocarbons of the aliphatic series

These hydrocarbons can be obtained from tertiary and secondary alcohols by the separation of 1 mol. H_2O; in reverse, they are once more converted into the corresponding alcohols when treated with sulfuric acid and boiled with water; the conversion takes place through the absorption of 1 mol. H_2O and the dissolution of the double bond.

TABLE IX.
Hydrocarbons with double bond. Tests with rabbits.

Gereichte Substanz	Verabreichte Menge in g	Gewicht des Thieres in g	Harnmenge in ccm	Drehung des nativen Harns	Drehung des auf 100 ccm gebrachten Harns	Drehung nach der Spaltung	Reduction vor der Spaltung	Reduction nach der Spaltung	Orcinprobe vor der Spaltung	Orcinprobe nach der Spaltung
Trimethyläthylen[2] (Pental) C_5H_{10} CH_3 CH_3 >C=CH—CH_3	2,5 in zwei Dosen	1050	24	—33,5'	—8'	—	θ	+	—	—
Octylen[3] (Caprylen) C_8H_{16} wahrscheinlich $C_{17}H_{14}$=CH_2	5,7 in drei Dosen	1180	181	—42,5'	—77'	—	θ	+	θ	+

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization (rotation)
6) of the native urine
7) of the urine reduced to 100 ccm
8) before precipitation
9) Reduction
10) before precipitaion
11) after precipitation
12) Orcin test
13) before precipitation
14) after precipitation
15) . . in two doses
16) . . . in three doses

(for FOOTNOTES 1 to 3 see page 19 of translation)

To decide the question whether said dissolution of the double bond could eventually take place within the animal body as well, the testing of the urine for paired glycuronic acid proved to be useful, since the alcohols which form during the process had to undergo such a synthesis.

Paired glycuronic acids could in fact be detected. It should be pointed out concerning trimethylethylene that it evaporates with great ease through the lungs as a result of its considerable volatility (it boils already at 37°, i.e. at normal body temperature); therefore no large quantities of paired acid can à priori be expected after its administration.

FOOTNOTES, page 149 of original text; p. 18 of translation:

1) The investigations under consideration were already terminated when the report of HILDEBRANDT was published (Arch. of Exper. Pathol. and Pharmac., Vol. XLV, 1900, p.110) which (report) likewise proves the presence of paired glycuronic acids, besides ofther conversion products, after the administration of Geraniol and Citral to rabbits.
2) Preparation from KAHLBAUM, Berlin, boiling point between 36 and 37°.
3) Preparation from KAHLBAUM, Berlin.

Following tests concerning the destiny of the alcohols, ketones adelhydes and of the unsaturated hydrocarbons of the aliphatic series, several other substances with ring-shaped carbon bond were likewise subjected to investigation. The major part of the compounds known to date as capable of pairing with glycuronic acid belong to the aromatic series.

However, these are almost exclusively substances which convert into phenols within the organism. According to our findings regarding the aliphatic series, aromatic alcohols, ketones and adelhydes presumably perform said pairing as well; sporadic data on the point at issue are already available in literature. Accordingly, the o-nitrobenzylalcohol[1] which forms in the organism through the oxidation of the supplied o-nitrotoluol, appears in the urine in the form of a paired glycuronic acid. According to MERING[2], benzaldehyde causes a left-handed polarization of the urine. Acetophenon, which is converted into benzoic acid in the animal body according to the investigations of NENCKI[3], yields--according to a test carried out by SUNDVIK (l.c) in a dog--a minor quantity of paired glycuronic acid, besides benzoic acid; it remains quentionable thereby, however, whether the pairing could have occurred in a secondary alcohol group formed through the reduction of the ketone group, or whether it could have occurred in a phenol group originating from the oxidation of a hydrocarbon of the benzol ring. Hoping to obtain a much more complete synthesis in rabbits, I undertook a rabbit test.

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1) JAFFÉ, Journal for Physiol. Chemistry, Vol. II (1878), p.47.
2) MERING, Journal for Physiol. Chemistry, Vol. VI. (1882),p. 494
3) NENCKI, Journal for Chemistry in Practice, Vol. XVIII, N.F. (1878),p.288.

TABLE X.

Aromatic substances. Tests with rabbits.

TABELLE X.

Aromatische Substanzen. Versuche an Kaninchen.

Gereichte Substanz	Verabreichte Menge in g	Gewicht des Thieres in g	Harnmenge in ccm	Drehung des nativen Harns	Drehung des auf 100 ccm gebrachten Harns	Drehung nach der Spaltung	Reduction vor der Spaltung	Reduction nach der Spaltung	Orcinprobe vor der Spaltung	Orcinprobe nach der Spaltung	Die linksdrehende Substanz ist fällbar durch
Acetophenon[1]) C_8H_8O $C_6H_5—CO-CH_3$	1,0	1090	56	—111′	—95,5′	rechts	—	+	—	+	—
Aethylbenzol[2]) C_8H_{10} $C_6H_5—CH_2-CH_3$	0,96	960	65	—54′	—35′	—	—	—	—	—	—
	4,3	1820	295	—114′	—340′	rechts	Θ	+	Θ	+	Bleiessig
Styrol[2]) C_8H_8 $C_6H_5—CH=CH_2$	3,7	1410	144	—30,5′	—44′	—	Θ	?	Θ	+	—

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization
   6) of the native urine
   7) of the urine reduced to 100 ccm
   8) after precipitation
9) Reduction
   10) before precipitation
   11) after precipitation
12) Orcin test
   13) before precipitation
   14) after precipitation
15) The substance, polarized to the left, is precipitable with . .
16) right
17) Lead acetate

The polarization to the left of the urine after the administration of acetophenon is in fact considerable. To obtain evidence confirming that the pairing took place through the intermediary of a secondary alcohol group and not through the intermediary of a phenol group, a procedure similar to the procedure used for the ketones of the aliphatic series was followed.

1) Preparation from KAHLBAUM.
2) Preparation from SCHUCHARDT.

	Destillat nach Spaltung mit verdünnter Schwefelsäure[3])	Destillat, mit Chromsäure oxydirt
Geruch	jasminähnlich (verschieden von dem Geruch des Acetophenons)	nach Acetophenon
Phenylhydrazin	θ	krystallinischer Niederschlag
Legal'sche Probe . . .	θ	blauviolette Färbung
Fuchsin-schweflige Säure .	θ	θ
Eisenchlorid	θ	θ

1) Distillate after precipitation with thinned sulfuric acid[3])
2) Distillate oxidized with chromic acid
3) Smell
4) Similar to jasmine (differs from the smell of acetophenon)
5) after acetophenon
6) Crystalline precipitate, color: blue-violet
7) LEGAL test
8) Sulfuric fuchsin acid
9) Ferric chloride

Accordingly, the acetophenon was reduced to the corresponding secondary alcohol (methylphenylcarbinol) and the latter was then synthesized.

The fact that acetophenon or the alcohol (methylphenylcarbinol) originating from the latter, enters the urine of rabbits as paired glycuronic acid, could be evaluated as follows: according to the tests of NENCKI and GIACOSA[4]) with dogs, ethylbenzene, $C_6H_5-CH_2-CH_3$, is oxidized into benzoic acid; both authors then raise the question whether the oxidation attacks the primary carbon atom (i.e. the CH_3-group) first, or whether the secondary carbon atom (i.e. the CH_2-group) is attacked first. They decided to adopt the last-mentioned supposition as correct, since the acetophenon $C_6H_5-CO-CH_3$ which would occur as intermediate product in this case, is likewise oxidized by the organism (according to tests with dogs) into benzoic acid, while the phenylacetic acid $C_6H_5-CH_2--COOH$, which would form through oxidation at the primary carbon atom, is not attacked by the animal body, but reappears in the urine instead. Therefore NENCKI and GIACOSA lay down the rule that "the attack of the oxidized oxygen always hits (either the benzene nucleus or) the carbon atom linked to the nucleus".

3) To eliminate the unchanged acetophenon, the urine was first agitated with ether.

4) NENCKI and GIACOSA, Journal for Physiol. Chemistry, Vol. IV (1880), p. 325.

In case of a rabbit which excretes the oxidation products of the acetylbenzene(oxidized at the secondary carbon), the methylphenylcarbinol and the acetophenon to a larger extent in the form of paired glycuronic acid , the feeding of ethylbenzene must result in the appearance of paired glycuronic acid in the urine, as it does after the administration of acetophenon-- provided that NENCKI's and GIACOSA's rule is correct. The urine did in fact contain ample quantities of paired glycuronic acid (see Table X), the decomposition product of which displayed the expected behavior. It was therefore possible to obtain direct evidence confirming the rule detected indirectly by NENCKI and GIACOSA in the dog; said direct evidence was obtained by finding the product oxidized at the secondary carbon atom.

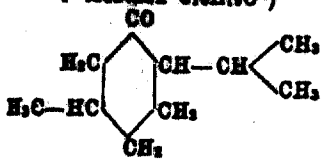
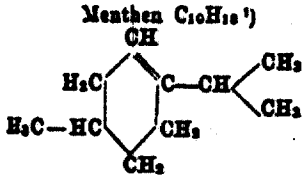
It could be taken into consideration as well that the unsaturated hydrocarbon Styrol $C_6H_5-CH = CH_2$ converts in the organism through water absorption and dissolution of the double bond into methylpehnylcarbinol. This reaction, however, certainly occurs only to a negligible extent, for the following reasons: after the administration of 0,9 g styrol to a rabbit, the urine was optically inactive; after the feeding of 3,7 g the glycuronic acid tests were positive, but the polarization to the left was comparatively minor as compared to the polarization after the administration of acetophenon.

In the hydro-aromatic series

the fact of the glycuronic acid pairing is confirmed by two substances, apart from the camphor type (substances): it is confirmed by the hydrocarbon Pinen (oil of turpentine) and by the secondary alcohol Menthol. It had now to be investigated whether the above-mentioned parallelism found concerning the destiny of the ketones and the secondary alcohols is valid for the hydro-aromatic series as well. For this purpose Menthon , which is the ketone corresponding to Menthol, was tested. A test was likewise carried out with the unsaturated hydrocarbon Menthen, which originates from Menthol through the separation of 1 mol.H_2O while a double bond forms.

TABLE XI.
Hydro-aromatic substances. Tests with rabbits.

Hydroaromatische Substanzen. Versuche an Kaninchen.

Gereichte Substanz	Verabreichte Menge in g	Gewicht des Tieres in g	Harnmenge in ccm	Drehung des nativen Harns	Drehung des auf 100 ccm gebrachten Harns	Drehung nach der Spaltung	Reduktion v. d. Spaltung	Reduktion n. d. Spaltung	Orcinprobe v. d. Spaltung	Orcinprobe n. d. Spaltung	Die Hinzufügung der Substanz ist fällbar
l-Menthon $C_{10}H_{18}O$ [1]) 	4,5 in zwei Dosen	1640	102	−64′	−65,5′	schwach rechts 7	+	+	θ	+	Bleiessig 18
Menthen $C_{10}H_{18}$ [1]) 	1,6	1970	17	−89,5′ [2])	−15′	schwach rechts 7	+	+	θ	+	

1) Administered substance
2) Supplied quantity in g
3) Weight of the animal in g
4) Urine volume in ccm
5) Polarization
   6) of the native urine
   7) of the urine reduced to 100 ccm
   8) after precipitation
9) Reduction
   10) before precipitation
   11) after precipitation
12) Orcin test
   13) before precipitation
   14) after precipitation
15) The substance, polarized to the left, is precipitable with . .
16) . . in two doses
17) slightly to the right
18) Lead acetate.

It follows that conditions here are identical with those in the aliphatic series.

The intensive raspberry-red coloring of the Menthon-urine was conspicuous; it continued to intensify when left standing accesible to air[3]); the spectroscopic investigation showed a streak of yellowish-green to green coloring in the urine (λ 551 - λ 496).

Preparation from SCHIMMEL and Co., Leipzig.

2) Prior to the determination of the optical rotation the urine was repeatedly agitated with ether, so as to eliminate the Menthen which transferred unchanged

3) The faintly colored filtrate obtained after precipitation of the urine with lead acetate reverted likewise to a dark red color when left stading for a prolonged period of time.

Brief reference shall additionally be made to the occurence of glucose besides the paired glycuronic acids. I observed the same in sporadic cases in dogs as well as in rabbits, occasionally after feeding them ethylalcohol, isobutylalcohol, amylalcohol polarized to the right, and acetylacetone. The conditions and causes of this curious phenomenon, defined by NAUNYN[1] as "levorotatory dextrosuria"are still completely unknown. The acetoglycosuria described by RUSCHHAUPT[2] could perhaps be classified as such.

When I summarize the obtained results briefly, the following rule ensues: almost all alcohols, the ketones , certain unsaturated carbohydrates and some aldehydes convert--at least to some extent--into paired glycuronic acids in the animal body, specifically in the rabbit organism. The above rule applies to the aliphatic series as well as to the cyclic compounds. Admittedly, the rule is subject to certain exceptions, such as concerning methylalcohol, the high primary alcohols and the polyatomic alcohols of the aliphatic series and possibly also the primary aromatic alcohols which have not yet been subjected to thorough testing.

Since normal urine contains only minimal quantities of paired glycuronic acids, and since these substantially consist of phenol-glucoronic acids[3], the conclusion is reached on the basis of the above rule that the investigated alcohols and ketones do not occur in larger quantities as intermediate products during normal animal metabolism.

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1) NAUNYN, "Diabetes melitus". Vienna, 1898.
2) RUSCHHAUPT, Archives of Experimental Pathology and Pharmacology, Vol. XLIV(1900), p. 127.
3) P. MAYER and NEUBERG, Journal for Physiol. Chemistry, Vol.XXIX. (1900), p. 526.

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35: 601, 1937

9068 P

Propylene Glycol: Rate of Disappearance from the Blood Stream.

H. W. NEWMAN AND A. J. LEHMAN.

From the Department of Medicine, Division of Neuropsychiatry, and Department of Pharmacology, Stanford University School of Medicine, San Francisco.

The increasing use of propylene glycol as a solvent for various pharmaceutical preparations[1] and coloring extracts,[2] and its possible use as a food, indicates the need for a better understanding of the actions of this alcohol. The known production of oxalic acid in the metabolism of ethylene glycol[3, 4] has been responsible for the substitution of propylene glycol for the former, since oxalic acid is not a possible metabolite of the latter.[5] The properties, general actions, and acute and chronic toxicities of propylene glycol have been investigated by Seidenfeld and Hanzlik[5]; toxicity was unusually low.

The concentration of propylene glycol in the blood was determined by treating the protein-free filtrate with a solution of potassium dichromate in strong sulphuric acid, and estimating the amount of reduced dichromate iodometrically. From this value was subtracted the amount of oxidizable material normally present in the blood, a variable which remains relatively constant in fasting animals. In urine, the glycol was estimated directly by using an aliquot portion

1 Brown, C. L. M., *Quart. J. Pharm. Pharmacol.*, 1935, 8, 390.

2 Rae, J., *Pharm. J.*, 1935, 135, 539.

3 Hunt, R., *Ind. Eng. Chem.*, 1932, 24, 361.

4 Hanzlik, P. J., Seidenfeld, M. A., and Johnson, C. C., *J. Pharm. Exp. Therap.*, 1930, 41, 387.

5 Seidenfeld, M. A., and Hanzlik, P. J., *J. Pharm. Exp. Therap.*, 1932, 44, 109.

of a 1:100 dilution, and subtracting the blank value for normal urine from the final result.

The concentration of propylene glycol in the bloods of 2 dogs and 10 rabbits was followed for variable periods after giving doses from 1 to 12 cc. per kilogram body weight, intravenously or orally; the dogs were used repeatedly.

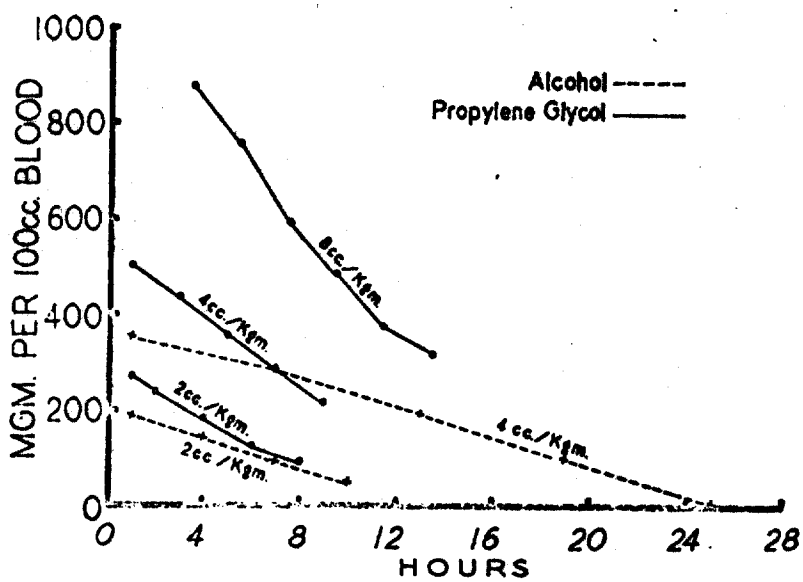

FIG. 1.
The rate of disappearance of propylene glycol from blood of dogs as compared with alcohol.

Figure 1 shows the rate of disappearance of propylene glycol from the blood of dogs following the administration of 2 cc. and 4 cc. per kilo intravenously, and 8 cc. gastrically; the intravenous doses are compared with similar doses of ethyl alcohol.

The rate of disappearance of propylene glycol from the blood is proportional to its concentration in the body, as contrasted with alcohol, which disappears at a constant rate.[6] This difference may possibly be accounted for by excretion of the propylene glycol by the kidneys, since it was possible to recover one-half the amount injected from the urine.

The concentration of propylene glycol in the blood after gastric administration indicates rapid absorption of the compound. Once it has entered the blood stream, it escapes rapidly and apparently diffuses throughout the body.

[6] Newman, H. W., and Cutting, W. C., *J. Pharm. Exp. Therap.*, 1936, 57, 358.

The depressant effect is much less than that of alcohol. About 1100 mg. % of propylene glycol in the blood was required to attain the same degree of narcosis produced by a blood-alcohol concentration of 350 mg. %. The results on rabbits were essentially similar to those on dogs.

J. Pharm. Exp. Therap.
68: 194-200, 1940

COMPARATIVE EFFECTS OF PROPYLENE GLYCOL, OTHER GLYCOLS, AND ALCOHOL ON THE LIVER DIRECTLY

H. W. NEWMAN, W. VAN WINKLE, JR., N. K. KENNEDY, AND M. C. MORTON

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, Calif.

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The toxicity of propylene glycol is the lowest of 5 glycols studied to date and this is the only glycol capable of a glycogenic action in intact animals (1, 2). Since the liver is an important organ for detoxication of poisons and also for glycogenic action and would be the first appendage to be affected by propylene glycol, when administered orally, it was deemed important to determine the effect of this glycol on the liver directly. This was done by comparing the effects of propylene glycol with those of ethyl alcohol and some other glycols on the isolated cat's liver. Briefly, the results obtained confirm the uniqueness of propylene glycol among other glycols and alcohol in being least directly injurious for the liver as it does not interfere with the glycogenic (metabolic) function of this organ.

METHODS

A total number of 72 cats was used, of which 48 were donors of perfusion-blood, and 24 were used for the livers. Anesthesia was produced with sodium pentobarbital, 35 mgm. per kilogram, or seconal, 25 mgm. per kilogram, given intraperitoneally. Food was withdrawn from all cats for 48 hours before each experiment, which required 3 cats. Two cats were exsanguinated and the blood was defibrinated, pooled, diluted 25 per cent with physiologic salt solution (0.85 per cent NaCl) and used within 1 hour for the perfusion fluid. One cat was used for the liver perfusion, the mechanical arrangement being essentially the same as that of Nielsen (3), and the perfusion-procedure, that of Lundsgaard, Nielsen and Orskov (4). The rate of blood flow through the liver was about 60 cc. per minute, the total volume of perfusion fluid, about 350 cc., and the perfusion pressure, between 10 and 25 cm. of water. The number of pulsations in the perfusion system was 60 per minute. The isolated liver was left *in situ* enclosed in the carcass which was kept warm at 38°C. with an electrical warming pad. In this way, the liver was kept in good condition for relatively long periods.

The timing of the perfusion and removing of samples of blood from the perfusion system and of liver for various analyses, were as follows:

0 minutes: perfusion started.
15 minutes: arterial blood for oxygen and carbon dioxide; venous blood for oxygen, carbon dioxide, lactic acid and sugar.
30 minutes: venous blood for lactic acid and sugar; piece of liver for glycogen.
31 minutes: glycol added to perfusion fluid.
45 minutes: venous blood for glycol-content.
75 minutes: venous blood for oxygen, carbon dioxide, lactic acid, sugar and glycol; arterial blood for oxygen and carbon dioxide.
105 minutes: venous blood for lactic acid, sugar and glycol.
135 minutes: venous blood for glycol; piece of liver for glycogen.

Blood oxygen and carbon dioxide were determined according to the manometric method of Van Slyke and Neill (5) and blood lactic acid according to Avery and Hastings (6). The lactic acid analyses were occasionally checked by Friedeman and Kendall's method (7) and found to agree within 3 mgm. per cent. Blood sugar was estimated according to Folin and Malmros' method (8), glycogen according to Pfluger's method (9), propylene glycol and ethylene glycol according to Lehman and Newman (10), dipropylene glycol and diethylene glycol, according to Newman and Lehman (11), and alcohol, according to Newman (12).

Three livers were perfused as controls without added glycol or alcohol. Five livers were perfused with propylene glycol in high concentrations corresponding to those toxic in intact animals, i.e., from 500 to 600 mgm. per cent in the perfusion-blood. Three livers were perfused with propylene glycol in low or relatively non-toxic concentrations, i.e., from 100 to 200 mgm. per cent. In 3 livers, zinc-crystalline insulin was added with the high concentrations of propylene glycol in amounts sufficient to make one unit for each 100 cc. of perfusion fluid. The object of this was to see if the reputed stimulant (catalytic) effect of insulin on the hepato-glycogenic action of dextrose could be demonstrated with propylene glycol. For comparison with the effects of propylene glycol, 3 livers were perfused with ethyl alcohol and dipropylene glycol and 2 each with the ethylene and diethylene glycols. The results are presented graphically in figures 1 and 2, the curves indicating average changes in the metabolites and the glycols.

PROPYLENE GLYCOL

It is seen that, whereas in control unpoisoned livers the oxygen consumption decreased only 8 per cent, the livers perfused with propylene glycol showed a drop of about 45 per cent in oxygen consumption during a similar period of about 105 minutes (fig. 1). The same relative decrease also occurred in the carbon dioxide production. This decrease in oxygen consumption suggested a depression or poisoning of the liver by the propylene glycol. However, continued administration of propylene glycol to dogs and rats, in doses sufficient to produce blood concentrations similar to those of the perfused livers, produced no demonstrable pathological changes in the intact liver (2).

3. Ethylene, diethylene and dipropylene glycols were not utilized by the perfused liver, but depressed the oxygen consumption and increased the lactic acid production, dipropylene glycol being the least toxic in these respects.

4. Ethyl alcohol was metabolized directly by the perfused liver, but depressed its functional activity as indicated by a decrease in oxygen consumption; blood lactic acid was increased and liver glycogen decreased.

5. Thus the direct action of propylene glycol on the isolated liver again stands apart from the actions of ethyl alcohol and the other glycols tested, and to the effect that it is the least injurious and objectionable of all these solvents.

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From the Pharmacology Institute of the Westphalian Wilhelms-University at Münster, Westphalia.
(Director: Prof. and Dr. of Medicine, Dr. of Philosophy A. LOESER)

On the glycogen-forming effect of the glycols.

By Klaus OPITZ

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While the glycogen-forming and carbohydrate-substituent effect of 1,2 propanediol (1,2 prolylene glycol) has been known for some time and was repeatedly investigated (HUNT, SALTER and collaborators, HANZLIK and collaborators, NEUMAN and collaborators, WINKLE, LOESER and OPITZ), the question whether and to what extent other aliphatic glycols are involved in the carbohydrate metabolism has not yet been adequately answered. Diethylene glycol and 1,3 propanediol allegedly do not have a glycol-forming effect in the rat (HANZLIK and collaborators, WINKLE). SCHLÜSSEL investigated the utilization of polyalcohols in nutrition. In case of rats the addition to the food of 1,2 butanediol or 1,3 butanediol respectively, resulted in increased food consumption and intensified weight increase; the calorie consumption per gram of weight increase was less after the intake of fodder containing glycol. Said "low consumption effect" is allegedly connected with the narcotic properties of the alcohols. The glycol-forming effect of the butanediols was not investigated.

Tests with frog larvae showed that not only the warm-blooded organisms can utilize the glycols. 1,3 butanediol as well as glycerine promoted the weight increase and metamorphose of tadpoles (GROSSKINSKY and STÜRMER), and the addition of 1,2 propanediol or 1,3 butanediol respectively, to the rearing medium caused an increased glycogen deposit (LOESER and OPITZ).

It became evident, during several tests related to the formation of glucose and glycogen <u>in vitro</u> from multivalent alcohols that glucose, but not 1,3 propanediol or 1,3 butanediol, can form in rat liver sections from 1,2 propanediol and 1,2,4 butanediol, as well as from 1,2,3 propanetriol (glycerine).

The assumption was therefore stated that only those bi- and trivalent alcohols can be used in the liver of warm-blooded animals for the development of carbohydrates the carbon chain of which (alcohols) includes three or more C-atoms, and which show at least two adjacent hydroxyl groups, one of the latter being terminal (OPITZ). Whether said supposition is correct should have been shown by tests during which the effect of several glycols on the glycol content of fasting rats was investigated. The following substances were used: ethanediol[1], diethylene glycol[2], 1,2 propanediol[3], 1,3 propanediol[4], 1,2 butanediol[2], 1,3 butanediol[2], 1,4 butanediol[5], and 2,3 butanediol[6]. The glycol-forming effect of glycol aldehyde[2] (Fp. 95 to 98°), an oxidation and metabolism product of ethanediol, as well as its origin were additionally investigated.

Three of the listed compounds are of biological importance, namely: 1,2 propanediol, 2,3 butanediol and glycogen aldehyde.

As a phosphoric acid (1,2 propanediol - 1 - phosphate = PDP), 1,2 propanediol is a physiological component of numerous animal organs and could be isolated from tumor tissue (RUDNEY). PDP is oxidized by the α-glycerophosphate hydrogenesis (MÜLLER and collaborators). Its position in the intermediate metabolism was investigated by RUDNEY, HUGGINS and MILLER.

2,3 butanediol is formed in the organism from pyruvate and from acetoin, and can, in reverse, release acetoin again. This reaction is introduced by the 2,3 butanediol-dehydrasis, which was first isolated by AUBERT and GAVARD (see ST ECKER and HAMALY; [illegible]). In vitro, the 2,3 butanediol can increase the breathing of tissue homogenates (DAWSON and MULLIN).

Finally, glycogen is a metabolism product which attracted increased attention since it became known that an active enzyme complex of this compound is involved in the conversion of the pentoses into hexoses, by participating in the trans-ketolase reaction. When trans-ketolase is present, various keto-sugars and oxy-pyruvates form an "activated glycol aldehyde" which condenses into new keto-sugars with an acceptoral dehyde. Free glycol aldehyde, however, does not occur during said reaction, which is of specific importance during the glucose reduction over the pentose-phosphate cycle (LANG).

Furthermore, glycolaldehyde is supposed to be an intermediate product during the biosynthesis of the riboses, during the conversion of ethanolamine into glycoroll, as well as during the formation of acetate from ethanolamine or pentose (for literature, see FRIEDMANN and collaborators). BREUSCH found in 1943 a specific glycol dehydrogenesis, unknown earlier, in the cat liver. In the body of rats, glycolaldehyde 2.C^{11} is speedily transformed into glycoroll and into CO_2; part of the latter, however, is converted into formic acid and oxalic acid, as well as into glycogen. The above-mentioned glycogen formation from glycolaldehyde is thought to take an indirect course over glycoroll, serin and triose (FRIEDMANN and collaborators). In view of the specific importance of glycolaldehyde for the intermediate metabolism, it is noteworthy that it could so far not be isoled from biological material--apart from the tests conducted with microorganisms (GOEPFERT and NORD; KAUSHAL and WALKER). This might be due to the fact that glycolaldehyde is "utilized" as fast as it forms (from ethanediol), and therefore evades interception procedures (SUMNER and MYRBÄCK).

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1. E. MERCK, Darmstadt. 2. Chemical Plant, Hüls. 3. Hoechst Dye Plants.
4. Heyl & Co., Berlin and Hildesheim.
5. Baden Aniline and Soda Manufacturing Plant, Ludwigshafen a. Rhine.
6. Amcel Co., Inc., New York.

Methodology

1. Investigation of the glycogen-forming effect. Male rats with a body weight of 150 to 250 g were kept in single cages for several weeks; they received standard dry feed[1] and tap water--both ad libidum; light and temperature were maintained at uniform levels. After having been deprived of fodder for 48 hours, the animals were given the substance to be investigated as a watery solution in the morning (10 to 20 cm^3/kg), orally administered; (only glycolaldehyde was intraperitoneally injected). Simultaneously, the control animals were orally fed with 10 or 20 cm^2/kg per body weight respectively, of a physiological saline solution, or they were given the above intraperitoneally, in case of tests with glycolaldehyde. The animals were killed 3 hours later and the immediately removed liver was placed into a previously weighed container, together with hot potassium hydroxide (30%). This procedure required not more than 30 seconds. After having weighed the containers once more, the liver glycogen was isolated according to STAUDINGER and polimetrically determined according to the modification indicated by BARREITHER and RÜCKEL. The glycogen content was always specified in milligrams per 100 g liver (live weight).

2. Investigation of the formation of glycogen from ethanediol. After fasting for 48 hours, male rats (250 to 330 g) were intraperitoneally given 4,5 cm^3/kg ethanediol in the form of a watery solution (45 vol-%). 2 hours later the animals were killed in the cooling chamber and the liver was immersed immediately into liquid nitrogen. The frozen organ was pulverized and dispelled in a few cubic centimeters of a trichloracetic acid (20%).--For the purpose of determining the glycolaldehyde in the blood of the rats as well, 2 cm^2 of blood was collected from the rats, pre-treated as stated above, by means of heart puncture; protein was removed from this blood according to FOLIN WU. -- The determination of the glycolaldehyde in the protein-free filtrates was implemented according to

DISCHE and SCHWARZFELD. The course of the calibration curve within a concentration zone of 0,1 to 2,0 mg% was linear, so that 1γ glycolaldehyde was still demonstrable.

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1) Hard cakes made of a mixture of 44,2% corn meal, 33,2% oat flakes, 8,2% dry yeats, 11,1% skim milk powder, 2,2% cod liver oil and 1,1% salt, according to McCOLLUM and SIMMONDS (34,6 g of sodium, 69,4 g. prim. sodium phosphate, 190,8 g.sec. potassium phosphate, 108,0 g prim.calcium phosphate, 23,6 g ferricitrate, 260,0 g calciumlactate and 52,2 g magnesium sulfate.

Results

The examination of the substances which influence the glycogen content of the liver is difficult on account of the considerable physiological fluctuations. Therefore test conditions were selected which guarantee at least an approximately uniform glycogen content. The values for the control animals on Tables 1 and 2 indicate that the above is possible to a limited extent only. The substances to be tested were administered in equimolar doses--usually 50 m.mol/kg--to permit comparison of their effects as speedily as possible. 1,4 butanediol, however, had to be administered in smaller doses on account of its comparatively high toxicity. In general, all glycols, in quantities as indicated, were tolerated without toxic symtoms. A minor narcotic effect occured only sporadically.

The results compiled in Table 1 show, despite the considerable divergence of single values, that from the glycols under investigation, only 1,2 propanediol, 1,2 butanediol and ethanediol have a glycol-forming effect. Diethylene glycol, 1,3 propanediol and 2,3 butanediol did not affect the glycogen content of the liver, while 1,3 butanediol and 1,4 butanediol reduced said glycol content.

Table 1. Glycol content of the liver of fasting rats, 3 hours after the oral administration of various glycols in a watery solution or of a physiological saline solution respectively.

Substanz 1	Dosis pro kg K. G. 2		Anzahl der Tiere 5	Leberglykogen (Mittelwerte in mg-%, ± mittlere Abweichung) 6
	mMol 3	cm³ wäßrige Lösung 4		
7 physiol. Kochsalzlösung		20	11	135 ± 82
8 1,2-Propandiol	100	20	12	739 ± 304
9 1,2-Butandiol	100	20	13	358 ± 212
10 physiol. Kochsalzlösung		10	26	138 ± 92
11 Äthandiol	50	10	12	255 ± 153
12 Diäthylenglykol	50	10	7	183 ± 122
13 1,2-Propandiol	50	10	14	779 ± 368
1,3-Propandiol	50	10	11	116 ± 92
1,2-Butandiol	50	10	17	283 ± 125
14 1,3-Butandiol	50	10	12	71 ± 39
2,3-Butandiol	50	10	16	105 ± 56
1,4-Butandiol	10	10	11	77 ± 51

The boldface average values as compared to the corresponding control values are considerably higher ($P < 0.05$, t-Distribution, acc. to Student or Fisher, respectively.)

1. Substance
2. Dose pr kg of body weight.
3. ~~in mol~~ mMol
4. mc³ watery solution
5. Number of animals
6. Liver glycogen (average value in%, ± average deviation)
7. Physiological saline solution
8. 1,2 propanediol
9. 1,2 butanediol
10. Physiological saline solution
11. ethanediol
12. diethylenediol
13. propanediol
14. butanediol

The chemical structure of the three glycogen-forming groups: 1,2 propanediol, 1,2 butanediol and ethanediol--is characterized by two adjacent hydroxyl groups, one of which is in end (terminal) position. The above-mentioned hypothesis is confirmed in this context. The finding according to which ethanediol also belongs to the glycol-forming bivalent alcohols is surprising at first because this compound is not included among those regarded as sugar-forming (see FLASCHENTRAGER and LEHNARTZ). During our own tests with rat liver sections the formation of sugar from ethanediol could in fact be demonstrated. However, the glycogen-forming effect of ethanediol is explainable when taking into consideration that the conversion of glycogen into carbohydrates is possible only indirectly, over various intermediate substances. As known, ethanediol is oxidized in the organism, to the stage of oxalic acid, whereby the steps to be passed could be glycolaldehyde, glycolic acid, glyoxal and glycoxilic acid. Large quantities of glycolic acid and oxalic acid were found, for example, in the urine of rabbits which had received ethanediol per os; (MAYER). Since, according to data in literature, the first, admittedly not yet isolated, conversion product of ethanediol, i.e. glycolaldehyde, is sugar-forming, while the old concept contending that the three glycolaldehyde molecules can unite to form a single hexosemolecule, is obviously not correct, the question arises whether the increased glycogen content of the liver found after the administration of ethanediol originates from the formation of glycogen from glycolaldehyde.

To clarify the above question, it was investigated 1.) whether glycolaldehyde as such does in fact increase the glycol content of the liver, and 2.) whether the formation of glycolaldehyde from ethanediol can be detected in the organism.

After the oral administration of 25 m.mol/kg of glycolaldehyde (2.5 molar watery solution) the glycol content in the liver of 4 out of 6 test animals had increased considerably: to 547, 243, 648 and 792 mg %. Toxic symptoms were not present in these animals.

Table 2. Glycogen content of the liver (in mg %) of fasting rats, 3 hours after the intraperitoneal adminsitration of 3 m mol Glycolaldehyde in the form of a watery solution.

Volume: 10 cm^2 (test animals) and 10 cm^2 respectively, of a saline solution (control animals) per kilogram of body weight.

Versuchstiere ♂	Kontrolltiere ♂
168	147
444	133
304	52
101	43
370	128
250	53
109	189
210	50
276	184
339	69
868	193
654	28
175	146
302	183
418	24
206	194
183	–
299 mg-%	111 mg-%

($P < 0.01$).

1. Test animals. 2. Control animals.

A deep narcosis occurred in the rest of the animals; liver glycogen content: 134 and 29 mg-% respectively. This variable behavior of the test animals could be connected with the non-uniform resorption conditions. During a second test series the aldehyde was therefore interperitoneally injected (see Table 2). This dose (5 m.mol/kg) of glycolaldehyde was well-tolerated, almost without exceptions, under the specified conditions, and caused an increase of the liver glycogen volume in the majority of the test animals. It becomes evident, when comparing the average values, that the 5 m.mol/kg glycoldehyde has a stronger glycogen-forming effect than the 50 m.mol/kg ethanediol.

The second question, namely: whether the formation of glycolaldehyde can be ascertained in vivo, caused considerable difficulties. These difficulties were due to the fact that the glycolaldehyde generated from ethanediol obviously undergoes a further and speedy oxidation in the organism; therefore merely traces of the same are present in each case. The highly sensitive method of DISCHE and BORENFREUND is adequate for the determination of minor quantities. With said method, 30,57 and 93 γ -% of glycolaldehyde could be detected in the liver tissue of three test animals which had intraperitoneally received 4,5 cm^3/kg ethanediol (45 vol-%, watery solution). On the other hand, not even traces of glycolaldehyde were found in the liver of the control animals. Tests intended to demonstrate glycolaldehyde in the blood as well after the administration of ethanediol, were unsuccessful, probably because the qunatities of the substance under investigation were too small.

It can be assumed, on the basis of the above results, that the increase of the glycogen volume in the liver of fasting rats after the oral administration of ethanediol is in fact due to the conversion of ethanediol into glycolaldehyde. This finding is to a certain extent a counterpart of the observations made by KAUSHAL and WALKER who were able to demonstrate that microorganisms (acetobacter acetigenum) can form cellulose from ethanediol, whereby glycolaldehyde could be determined as an intermediate product. In both cases live cells produce a polysaccharide--glycogen or cellulose respectively--from ethanediol.

The question: which intermediate products provide the glycol-forming effect when other glycols such as 1,2 propanediol and 1,2 butanediol are involved, cannot be answered here.

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Propylene Glycol as an Energy Source for Poultry[1]

J. N. Persons, B. L. Damron, P. W. Waldroup[2] and R. H. Harms
Florida Agricultural Experiment Station, Gainesville, Florida 32601

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THE use of propylene glycol as a source of energy in animal feeds was reviewed by Bayley *et al.* (1967). These workers also reported data which indicated that up to eight percent of propylene glycol could replace an equivalent amount of corn starch, but at the 16 percent level the propylene glycol depressed the growth rate of the birds.

Four experiments reported herein were conducted to study the use of propylene glycol as an energy source for chicks and laying hens.

PROCEDURE

Chick experiments: The control diet was formulated to contain 23 percent protein with 2970 kilocalories of metabolizable energy per kilogram of diet (chick diet, Table 1). Builder's sand was used as a non-nutritive filler to allow for substitution of propylene glycol without changing the ratio of corn and soybean meal; thus, the protein and amino acid levels were equal in all diets.

Two series of substitutions were compared. In the first series, propylene glycol replaced corn oil and sand iso-calorically. In the second series, propylene glycol replaced sand; thus the energy content of the feed was increased. The energy levels of the diets containing 2.5 and 5.0 percent propylene glycol were 3115 and 3260 kilocalories per kilogram, respectively. This resulted in six experimental diets. Energy values of 5792, 3476, and 8470 kilocalories of metabolizable energy per kilogram were used for propylene glycol, corn, and corn oil, respectively.

Table 1.—*Composition of diets*

Ingredients	Chick	Hen
Yellow Corn	42.00	69.6
Soybean Meal (50% protein)	38.10	19.0
Alfalfa Meal (17% protein)	—	2.5
Defluorinated Phosphate (18% P & 32% Ca)	1.62	1.95
Ground Limestone	0.80	5.90
Iodized Salt	0.40	0.25
Micro-ingredients[1]	0.50	0.50
Variables[2]	16.58	——
Calcium Propionate	——	0.30

[1] Supplied per kg. of feed: 6,600 I.U. vitamin A, 2,200 I.C.U. vitamin D_3, 11 mg. menadione sodium bisulfite, 4.4 mg. riboflavin, 13.2 mg. calcium pantothenate, 39.6 mg. niacin, 500 mg. choline chloride, 22 mcg. vitamin B_{12}, 125 mg. ethoxyquin, 20 mg. iron, 2 mg. copper, 200 mcg. cobalt, 1.1 mg. iodine, 100 mcg. zinc, and 175 mg. manganese.

[2] Contained corn oil, propylene glycol and/or builder's sand.

In experiment 1, each diet was fed to 10 pens of 10 broiler-type chicks in battery brooders from one day to three weeks of age. Feed and water were given *ad libitum*. One-half of the pens contained male chicks and the other half contained female chicks. Body weights were obtained at three weeks of age and feed efficiencies were calculated at that time.

In experiment 2, each diet was fed to 120 broiler chicks in 6 floor pens. Each pen contained 10 males and 10 females. Each pen contained peanut hulls as litter, one automatic water fount, one tube-type feeder and one infra-red bulb as a heat source. Body weights were obtained at three weeks of age and feed efficiency was calculated.

The third experiment was conducted to confirm the growth depressing effect from

[1] Florida Agr. Exp. Sta., Journal Series No. 2714.

[2] Present address: Dept. of Animal Science, University of Arkansas, Fayetteville, Ark.

feeding a level of propylene glycol. Only the control diet and the diet containing 10 percent propylene glycol were fed. Three pens, each containing five male and five female broiler-type chicks, were fed each diet. They were grown from one day to three weeks of age in battery brooders.

Hen experiment: Miller (1964) reported that beef cattle could be fed up to five percent propylene glycol without decreasing dry matter intake. Therefore, this experiment was conducted in order to determine whether this material could be added to hen feeds without producing a change in feed intake due to an alteration in the dietary energy content.

The basal diet used in this experiment contained 16.1 percent protein and 2914 kilocalories of metabolizable energy per kilogram (hen diet, Table 1). Four other experimental diets were formulated by adding either 2.5 or 5.0 percent propylene glycol, or 1.2 or 2.2 percent corn oil. The diets containing the low and high levels of these materials contained 2959 and 3009 kilocalories of metabolizable energy per kilogram, respectively. These materials were substituted for corn, and the level of soybean meal was increased to compensate for the change in protein content. Egg production-type hens in their tenth month of lay were used for the experiment, which was started in July. Six replicates of five individually caged hens were fed each of the five experimental diets for two 28-day periods. Weekly feed consumption was obtained on a group basis; however, since the week × treatment interaction was not significant, weekly intakes are not presented. Daily egg production for the experimental period was recorded and reported on a hen-day basis. All hens were individually weighed at the beginning and end of the experiment and weight gains calculated.

Statements of probability in this paper are based on Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

Chick experiments: In the first two experiments, body weight of chicks was significantly depressed when 5 percent propylene glycol was added to the diet (Table 2). In the first experiment only the 10 percent level of propylene glycol depressed feed efficiency; however, in experiment 2, the 5 percent level also lowered feed efficiency.

In experiment 1, the mortality was significantly greater in groups receiving the diet containing 10 percent propylene glycol.

TABLE 2.—*Body weight and feed efficiency of three-week old chicks when fed diets containing various levels of propylene glycol*

Diets	Body wts.[4]		Feed efficiency[4]		Mortality[4]	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
	(gms.)		(gms. feed/gm. gain)		(%)	
Control[1]	376[a]	345[a]	1.45[b]	1.63[bc]	1[a]	0
Control+2.5% PG[1]	376[a]	341[a]	1.45[b]	1.64[bc]	1[a]	0.9
Control+5.0% PG[1]	360[b]	321[b]	1.51[b]	1.75[a]	1[a]	0
Control+10.0% PG[1]	266[d]	322[b]	1.73[a]	1.71[ab]	12[b]	0.9
Control+2.5% PG[2]	359[b]	326[b]	1.42[b]	1.73[a]	2[a]	0.9
Control+5.0% PG[3]	331[c]	325[b]	1.47[b]	1.58[c]	1[a]	0

[1] Contains 2,970 Calories of metabolizable energy/kg.
[2] Contains 3,115 Calories of metabolizable energy/kg.
[3] Contains 3,260 Calories of metabolizable energy/kg.
[4] Means with different superscripts are significantly different according to Duncan's multiple range test (1955).
PG = propylene glycol.

However, the second experiment had no mortality differences.

In the third experiment the diet containing 10 percent propylene glycol significantly depressed growth rate (328 *vs.* 231 grams) and feed efficiency (1.64 *vs.* 1.98 grams feed/gram gain), but had no effect on mortality.

The data from the first two experiments indicate that a level of 2.5 percent propylene glycol can be used in the diet of growing chicks without adversely affecting body weight or feed utilization. However, levels of 5 percent or more depressed both body weight and feed utilization. The finding that low levels of propylene glycol can be tolerated by chicks agrees with the report by Bayley *et al.* (1967). However, these workers found that levels as high as 8 percent of the diet did not depress chick performance. This does not agree with the data in this paper where much lower levels depressed performance.

Hen experiment: The increased dietary energy from the addition of propylene glycol and corn oil resulted in decreased feed consumption (Table 3). This reduction was a result of an attempt by the hens to adjust to meet their energy requirement. However, the hens receiving the diets containing 5.0 percent propylene glycol or corn oil did not consume as much energy as those hens receiving the two other diets. A portion of this difference may be attributed to a lowered rate of production which decreased the energy requirement.

Feed intake of all hens was lower than normally expected (Table 3). This may be partially explained by the high temperature; however, the loss of body weight would indicate that the hens were not consuming sufficient energy to maintain their weight. The hens did not appear to differentiate between the energy from propylene glycol and that of corn oil. This is in contrast to results with beef cattle which did not compensate for the additional energy by decreasing dry matter intake (Miller, 1964).

TABLE 3.—*Feed and calories consumed per hen per day, weight gain and egg production with hens fed five different diets*

Diet	Feed consumed per hen day (gm.)	Calories per hen day[1]	Weight gained (gm.)	Egg prod. (hen day)[1] (%)
Control	78.3[a]	228[a]	−96[a]	59.6[a]
+2.5% PG[2]	77.9[a]	231[a]	−64[a]	59.8[a]
+5.0% PG	72.8[b]	219[b]	−9[b]	46.5[b]
+1.2% CO[3]	71.9[b]	213[b]	+62[a]	42.9[b]
+2.2% CO	71.5[b]	215[b]	−88[a]	48.2[b]

[1] Means with different superscripts are significantly different according to Duncan's multiple range test.
[2] PG = Propylene glycol.
[3] CO = Corn oil.

SUMMARY

Four experiments, three with chicks and one with laying hens, were conducted to study the use of propylene glycol as an energy source. Both chicks and laying hens appeared to utilize and tolerate a level of 2.5 percent. However, higher levels depressed performance of chicks. Chickens appear to adjust feed intake due to the change of energy content resulting from the addition of propylene glycol, which is in contrast to results obtained from feeding it to beef cattle (Miller, 1964).

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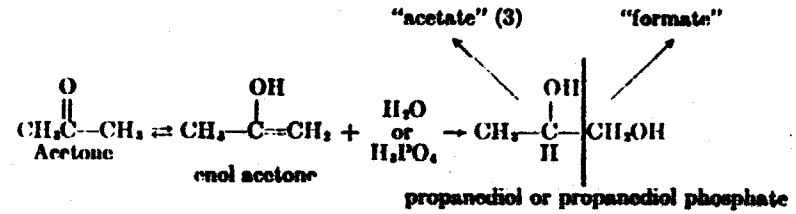
The Metabolism of 1,2-Propanediol [1]

The metabolic significance of propanediol phosphate which has been isolated from mammalian tissues (1) is uncertain. The unphosphorylated compound *dl*-propanediol is well metabolized, and is glycogenic although less efficient in this respect than lactic acid, pyruvic acid, or pyruvaldehyde (2).

The possibility that propanediol is an intermediate in acetone metabolism according to the following series of hypothetical reactions is being investigated.



Sakami (4) has demonstrated that the acetone methyl carbon is transformed into "formate"[2] by showing its incorporation into the serine β-carbon, and choline methyl groups. According to the above hypothesis the C^{14} of C-1-labeled propanediol should also be incorporated into these groups.

TABLE I

Incorporation of Isotope from Propanediol into Various Metabolites

Compound isolated	Isotopic concentration, counts/min./mg. of carbon			
	Methyl carbon of choline	3,4-Carbon of glucose, or COOH carbon of serine	2,5-Carbon of glucose, or α-carbon of serine	1,6-Carbon of glucose, or β-carbon of serine
Choline	62	—	—	—
Glycogen		145	48	68
Serine		14	3	166

Six fasted (48 hr.) male rats weighing from 80 to 100 g. each were given 2 ml. of 2.5 *M* glycine by stomach tube, and 0.25 ml. of 0.3 *M* *dl*-propanediol intraperitoneally per 100 g. The dose of propanediol which was labeled in the C-1 position with C^{14} (92,500 counts/min./mg. carbon) was repeated at the end of the 3rd, 6th, and 10th hr. The animals were sacrificed after 14 hr. The choline of the abdominal viscera and the liver serine and glycogen were isolated and degraded.[3] The results shown in Table I are

[1] This investigation has been assisted in part by a grant from the American Cancer Society recommended by the Committee on Growth of the National Research Council. The C^{14} was obtained on allocation from the U. S. Atomic Energy Commission.

[2] "Acetate" and "formate" denotes the compound itself or a metabolite thereof.

[3] Synthesis, isolation, and degradation procedures will be published shortly.

consistent with a role for propanediol as an intermediate of acetone metabolism. The C^{14} of the hydroxymethyl carbon of propanediol like that of methyl-labeled acetone, was incorporated into the serine β-carbon, and choline methyl groups, and the 2,5- and 1,6-carbons of the glycogen with higher labeling in the 1,6-positions. The relatively high activity of the 3,4-carbons of the glycogen suggests that some propanediol is being converted directly to lactic acid, thus accounting for its glycogenic activity.

The possibility that propanediol or its phosphate ester is an intermediate in acetone metabolism is being further investigated.

Acknowledgments

The author acknowledges with thanks the interest and advice of Dr. W. Sakami, and the technical assistance of Jean Lafayo.

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Department of Biochemistry,
Western Reserve University Medical School,
Cleveland 6, Ohio
Harry Rudney[4]

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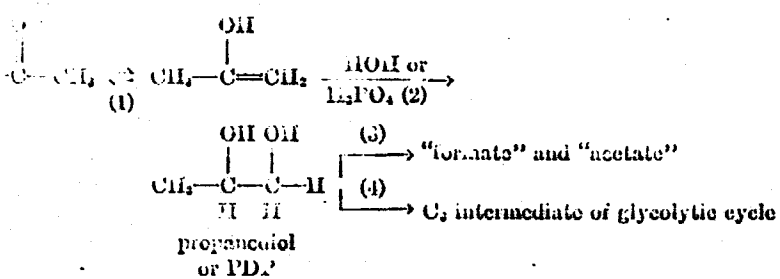
PROPANEDIOL PHOSPHATE AS A POSSIBLE INTERMEDIATE IN THE METABOLISM OF ACETONE*

By HARRY RUDNEY

(From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland, Ohio)

(Received for publication, November [illegible], 1953)

Acetone and propanediol have been found to undergo analogous reactions in [illegible] plant tissues (5) in that both compounds can be split to C_1 and [illegible] (6, 7) and converted to a C_3 intermediate of the glycolytic cycle [illegible]. The possibility was therefore considered that propanediol or PDP [illegible] one of the intermediates in the breakdown of acetone according to the following series of hypothetical over-all reactions.



[illegible] was tested by feeding propanediol-1-C^{14} to rats. It was [illegible] that the β-carbon of serine and the methyl carbon of choline were [illegible], indicating that the hydroxymethyl carbon of propanediol was converted to a 1-carbon unit which entered the formate pool (1). The distribution of isotope in liver glycogen also indicated the possibility that propanediol was being converted directly to a 3-carbon precursor of the glycolytic cycle.[2]

[illegible] was supported in part by grants from the Life Insurance Medical [illegible] the Elizabeth Severance Prentiss Fund of Western Reserve University and the American Cancer Society. Part of the work was completed during [illegible] a predoctoral fellowship from the National Institutes of Health, 1949–[illegible] of the data in this paper has been taken from a thesis presented to the [illegible] School of Western Reserve University in partial fulfilment of the requirements for the degree of Doctor of Philosophy. Preliminary reports of part of this [illegible] appeared (1-5). The radiocarbon used in this work was obtained on allocation from the United States Atomic Energy Commission.

[illegible] following abbreviations are used in this paper: 1,2-propanediol-1-phosphate = PDP; trichloroacetic acid = TCA; adenosine triphosphate = ATP.

[2] When these results were reported, specific activity determinations of the respiratory CO_2 were not included. It is pertinent to mention here that the specific activity

The present paper deals with the conversion of acetone to propanediol phosphate. This was tested by administering carbonyl-labeled acetone to rats and isolating PDP. It was found that PDP was labeled solely in carbon 2, indicating that acetone could be converted to PDP. The experiments and additional findings on the metabolism of PDP and propanediol in relation to the conversion of acetone *in vitro* to a 3-carbon unit of the glycolytic cycle are presented.

Methods

Methods for the isolation and the synthesis of PDP and propanediol are presented in the preceding paper (11).

Experiments in Vivo.—Labeled acetone (0.3 M) was administered subcutaneously to a group of rats. After the specified time the animals were sacrificed under ether anesthesia, and the livers were treated as described previously (11). The glycogen obtained from the 50 per cent alcohol precipitation of the TCA filtrate was purified by precipitation from LiCl solution (12) and degraded by the method of Wood *et al.* (10).

Experiments in Vitro.—The liver was minced with four razor blades mounted 0 mm. apart, until a consistent paste was obtained. This usually required 1 minute. The pooled pastes were suspended in Krebs' Ringer phosphate medium, 20 ml. per 10 gm. of paste. 20 ml. of suspension were placed in large Warburg flasks of 125 ml. capacity. Labeled substrates were then added, and the suspension was incubated for 2 hours at 38° with 100 per cent O_2 unless otherwise indicated. Respiratory carbon dioxide was collected in alkali-soaked papers placed in the center well. 3 ml. of [illegible] N sulfuric acid were added from a side arm to stop the reaction and release bound carbon dioxide from the medium. The carbon dioxide was released from the papers with acid and was counted as barium carbonate. When PDP and glycogen were to be isolated from pooled liver suspension, 5 ml. of 40 per cent TCA were placed in the side arm and the TCA filtrate was handled as described previously (11). After the proteins had been precipitated, the carriers were added. The suspension was centrifuged, and the supernatant fluid was taken up in ether-washed Celite (Johns-Manville No. 535) (2 gm. per ml.) and extracted with ether 12 hours. The ether extract was steam-distilled to obtain formic and other volatile acids. The formate was oxidized to carbon dioxide by the specific mercuric oxide

of the 3,4-carbons of glycogen (445 c.p.m. per mg. of C) was 82 per cent of the high specific activity of the respiratory CO_2 (545 c.p.m. per mg. of C) determined hourly throughout the experiment. Generally, when $C^{14}O_2$ is administered to a fasted rat the specific activity of the 3,4 carbons of glycogen is about 16 per cent that of the respiratory CO_2 (10). It seems likely that the high activity in the 3,4-carbons was not due to CO_2 fixation, but arose from the hydroxymethyl carbon of propanediol itself.

[illegible] (13). Lactic acid was isolated from the non-volatile fraction by [illegible]tography on Celite with butanol and chloroform as solvents (14), [illegible] by the ion exchange separation of Busch *et al.* (15).

When propanediol was isolated, the sulfuric acid filtrate was neutralized [illegible] treated with 0.5 ml. of 10 per cent metaphosphoric acid, to insure com[illegible] freedom from the presence of proteins which interfere in the subse[illegible] ion exchanger manipulations. The acid filtrate was passed succes[illegible]ly through a column of a cation exchanger (IR-120, H^+ form) and an [illegible] exchanger (IRA-400, OH^- form). This treatment removed all the [illegible]ions and anions, leaving a filtrate containing neutral compounds and [illegible]panediol. Later it was found that the ether extract of the sulfuric acid [illegible]trate was free of protein and contained all the propanediol. The de[illegible]ed extract was concentrated *in vacuo* to 2.0 ml., placed on a Celite [illegible] and purified and degraded as described previously (11). Dowex 50 [illegible] Dowex 1 served equally well as deionizing agents. The anion fraction [illegible] was held on the IRA-400 or Dowex 1 column was obtained by pass[illegible] NaOH through the column and was steam-distilled. Formate in the [illegible]illate was degraded to carbon dioxide and the lactate in the residue was [illegible]fied by chromatography as described above and degraded (10).

The carbon dioxide from the various degradations was plated as disks of [illegible]um carbonate of finite thickness, or was counted directly in a gas [illegible]ter by the procedure of Van Slyke *et al.* (16).

Carbonyl and methyl C^{14}-labeled acetone were synthesized as described [illegible] Sakami (6) from carboxyl- and methyl-labeled acetic acid. Cholesterol [illegible] isolated as the digitonide (17). Propanediol-1-C^{14} was prepared by [illegible]duction of $CH_2CHOH—C^{14}OOH$ (11). $C^{14}H_2CHOHCOOH$ was ob[illegible]ed as a gift from Dr. H. Saz.

Results

Experiments with Labeled Acetone in Vivo—Thirty fed albino rats of mixed [illegible] weighing 200 to 250 gm., were injected subcutaneously with 1 ml. of [illegible] acetone per 100 gm. of body weight. The acetone-2-C^{14} possessed a [illegible] activity of 4×10^5 c.p.m. per mM. After 12 hours the animals [illegible] sacrificed, and the livers were treated as outlined under "Methods." [illegible] glycogen was isolated and degraded. PDP was isolated after the [illegible] of PDP-P^{32} to the TCA fraction. It was subjected to enzymatic [illegible]ysis, carrier propanediol was added, and the propanediol was puri[illegible] paper chromatography as described in the preceding paper (11). [illegible] containing the propanediol was cut lengthwise into two strips, [illegible] and the propanediol from each strip was degraded. As is [illegible] the activity of the propanediol from the strips was similar, [illegible] that the [illegible] was pure. In addition, in the

periodate degradation, formaldehyde and acetaldehyde agreed within [illegible] experimental error. It is noted that the PDP is labeled solely in carbon [illegible] in agreement with the idea that the acetone carbon chain has been con[illegible]verted to PDP. The pattern of labeling found in the carbons of glucose from the glycogen is similar to that found by Sakami and Lafaye when carbonyl-labeled acetone (8) or α-labeled lactate or pyruvate (18) was administered to fasted rats; namely, isotope was distributed throughout all the carbons of glucose with a higher concentration of isotope in the 2,5-carbons than in the 1,6-carbons. The fact that the 1 and 3 carbons of PDP are unlabeled indicates that PDP is not being formed to any great extent from any of the intermediates of the glycolytic cycle. Thus if acetone is converted to glycogen via PDP, the randomization occurs subsequent to the formation of PDP.

TABLE I

Distribution of Isotope in PDP and Glucose after Administration of Acetone-2-[illegible]

Strip No.	Propanediol phosphate			Glucose		
	Carbon 1	Carbon 2	Carbon 3	Carbons 3,4	Carbons 2,5	Carbons 1,6
1	0	6400	0	1475	1485	1175
2	0	6650	0			

Specific activity is expressed as counts per minute per millimole of carbon.

Experiments with Labeled Acetone in Vitro—In considering the results obtained with carbonyl-labeled acetone, the possibility arose that PDP might have become labeled solely in carbon 2 by virtue of a condensation of a C_2 unit with a C_1 unit. More conclusive evidence on this point could be derived from the use of methyl-labeled acetone. If the PDP obtained from such an experiment had equal labeling in carbons 1 and 3 and none in carbon 2, the possibility that PDP was formed by $C_2 + C_1$ condensation would be unlikely, since equality could occur only if the dilution of the C_2 and C_1 units were the same.

C^{14}-methyl-labeled acetone was incubated with a liver mince, and at the end of the incubation period any remaining isotopic acetone was diluted by the addition of non-isotopic acetone. PDP-P^{32} was added as a tracer and carrier and isolated as described in the preceding paper. The concentration of PDP in the incubated liver mince was so low that there was [illegible] significant dilution of the PDP-P^{32}, and thus the actual original C^{14} specific activity of PDP could not be calculated. However, the isolated propanediol contained C^{14}, and it was possible to degrade it and measure the C^{14} activity on a gas counter. The results appear in Table II, Experiment [illegible]

although no radioactivity was observed in the center carbon, an activity of 10 per cent of that found in carbons 1 and 3 would not have been detected. The actual specific activity of the carbons of the original PDP could have

TABLE II

Distribution of Isotope from C^{14}-Methyl-Labeled Acetone into Various Metabolites

Experiment No.	Respiratory CO_2	Formate	Glycogen	Propanediol			PDP			Lactic acid		
				C_3	C_2	C_1	C_3	C_2	C_1	C_3	C_2	C_1
1	24,000 (2.3)	3200 (0.2)	0*				139†	0*	121†	42,000	3880 (0.27)	42,000
2	18,600 (1.85)	2800 (0.23)		283†	(0.033)	345†				9,700	1860 (0.33)	9,700

The figures in parentheses represent per cent of total counts added as substrate; other values are in counts per minute per millimole of carbon.

Experiment 1—That liver was minced and suspended in Krebs' Ringer-phosphate and incubated as described under "Methods." 30 μc of acetone, specific activity 3 × 10⁷ c.p.m. per mM of methyl carbon, were added to each flask containing 20 gm. of liver suspension. The deproteinized filtrates were pooled until a total of 140 gm. of liver had been processed and glycogen and PDP were isolated. Formate, lactate, and carbon dioxide were isolated from a portion of the liver suspension (100 gm. of liver) which was incubated in the presence of a pool of formate (0.1 mM per flask). After incubation 1 mM of formate was added as carrier. For further details see "Methods."

Experiment 2—40 gm. of rat liver were minced and suspended in Krebs' Ringer-phosphate solution. The acetone concentration and specific activity were the same as for Experiment 1. Deproteinization was performed with sulfuric and then metaphosphoric acid, 1 mM of formate, and 1.32 mM of propanediol were added as carrier, and the filtrate was passed successively through a column of 180 gm. of IR-120 in the H^+ form and 400 gm. of IRA-400 in the OH^- form. Propanediol was isolated from the deionized filtrate, while lactate and formate were isolated from the anion fraction.

*0 indicates that a trace of radioactivity was detected, but it was not considered significant; *i.e.*, 1 to 2 c.p.m. above background.

† These figures are values obtained with the gas counter and do not take into consideration the dilution factors. A discussion of the dilution factors involved in the individual cases is given in the text.

is at least 300 times higher when all the dilution factors based on the amounts of carrier PDP and propanediol added are considered. Labeling of the 3 position is somewhat higher than in the 1 position, but, because of the low activity, the significance is questionable. The important point is that the pattern of labeling duplicates within experimental error that of the methyl-labeled acetone.

It seemed probable that the decrease in the level of PDP during the

mincing and incubation might have occurred because of phosphatase action and, if this were the case, the free propanediol from such a hydrolytic action would reflect the labeling of PDP.

Five propanediol (1.32 mM) was added to the liver mince as carrier at acetone incubation, isolated (11), and found to be labeled (Experiment 2, Table II). Degradation showed that the tracer appeared only in carbons 1 and 3. In this case labeling in position 1 was somewhat higher than in position 3, but the difference probably is not significant. The distribution of the isotope in the PDP and free propanediol is in agreement with that having arisen from acetone either directly or via some closely related derivative. This experiment indicates the occurrence of free propanediol in an animal tissue preparation. The glycogen isolated from Experiment 1 was unlabeled, but the lactic acid contained the highest isotope concentration in the carboxyl and β-carbons, thereby offering a more direct demonstration and confirmation of Sakami's finding that acetone is converted to a precursor of the glycolytic cycle.

Since it was not possible in either experiment to determine the specific activity of the PDP or propanediol, direct information on the possibility of its being a precursor of the lactate was not obtained. A rough approximation of the original specific activity of the propanediol was attempted by determining the free propanediol in tissue treated similarly to that in the experiment. 0.568 mM of propanediol-1-C^{14} was added as carrier to 40 gm. of liver mince incubated under similar conditions so that the endogenous PDP might be hydrolyzed. The propanediol was then isolated. The ratio of specific activities in carbon 1 was 1.180, and hence the amount of free propanediol present in the liver suspension after incubation was 0.107 mM. In Experiment 2 the dilution factor becomes (1.32 + 0.107)/0.107 = 13.3 and the corresponding counts in carbons 1 and 3 would be 3840 and 4660 c.p.m. per mM of C. The calculated values for propanediol are somewhat less than half those for lactate. These values are approximations and it is not considered that they are evidence negating the rôle of propanediol or PDP as an intermediate in the conversion of acetone to lactate. To obtain direct evidence on the intermediate rôle of these compounds, time and specific activity curves would be necessary.

At present the technical problem of determining the specific activity of PDP and propanediol makes this very difficult to accomplish. It is clear that acetone does yield propanediol and PDP, and it is shown in Table III that propanediol yields lactate. Thus, although the intermediate rôle of propanediol and PDP in the reaction acetone → lactate is not established, it remains a good possibility.

The possibility remained that the propanediol and PDP phosphate could acquire the label by reduction of lactic acid. 43 μM of lactate-3-C^{14} with

[illegible] c.p.m. per mM were added to 25 gm. of liver mince. After incu- [illegible] 2 hours at [illegible] under O_2, the mince was deproteinized, propane- [illegible] mM) was added as carrier, and the propanediol and lactic acid

TABLE III

Incorporation of Isotope from 1,2-Propanediol-1-C^{14} into Various Metabolites

Substrate	Substances isolated						
	Formate		Lactate				Cholesterol
	C.p.m. per mM × 10^3	Per cent incorporation†	COOH, c.p.m. per mM	α, c.p.m. per mM‡	β, c.p.m. per mM‡	Per cent incorporation*	
Propanediol-1-C^{14}	2.20	0.43	1.15 × 10^5	0	0	0.44	0§
Propanediol-1-C^{14}							0§
Methyl C^{14}-acetone							525
Propanediol-1-C^{14} (heated slices)						0.04	

Experiment 1—5 gm. of rat liver slices were pooled, and 2.5 gm. were placed in [illegible] two flasks. Each flask contained [illegible] mM of propanediol-1-C^{14} with a [illegible] activity of [illegible] × 10^6 c.p.m. per mM in [illegible] ml. of Ursbal Ringer-bicarbonate [illegible] gassed with 95 per cent O_2 and 5 per cent CO_2. Incubation time, [illegible] hours [illegible]. The slices were centrifuged, and cholesterol was isolated as the digitonide. [illegible] medium was acidified with H_2SO_4, reduced in volume, taken up in the Celite [illegible] per ml.), and extracted with ether overnight. Formate and lactate were re- [illegible] from the ether extract as outlined under "Methods."

Experiment 2—[illegible] gm. of slices were pooled, and 2.5 gm. were put in each of four [illegible]. Two flasks contained [illegible] mM of propanediol-1-C^{14} with [illegible] × 10^6 c.p.m. per [illegible] contained [illegible] mM of acetone, specific activity [illegible] × 10^6 c.p.m. per mM of [illegible] carbon.

Experiment 3—Same conditions as in Experiment 1, except that the slices were [illegible] for [illegible] minutes.

[illegible] carrier added.

[illegible] incorporation = (total counts recovered in compound isolated)/(total [illegible] as substrate) × [illegible].

[illegible] by difference between specific activities of total carbon and COOH

[illegible] count above background; this is not considered significant.

[illegible] per minute from 12.[illegible] mg. of cholesterol digitonide.

[illegible] and degraded. No radioactivity could be detected in any of [illegible] of propanediol, while lactic acid had the following activity, [illegible] per minute per millimole of carbon: carboxyl carbon [illegible]. These results show that under the [illegible] conditions propanediol does not arise from lactic acid. They [illegible] the conclusion reached from the experiment *in vivo* that PDP does

not arise to any major extent from a 3-carbon intermediate of the glycolytic cycle.

Metabolism of Propanediol—The results in Tables I and II show that acetone is converted to both propanediol and PDP and to lactate and glycogen. It has previously been established that propanediol is converted to glycogen *in vivo*. To obtain further data on the possible rôle of propanediol in the postulated conversions, some studies with propanediol-1-C^{14} were undertaken with rat liver slices *in vitro*. As shown in Table III, propanediol-1-C^{14} was converted to lactate labeled solely in the carboxyl group. Formate was also labeled. The foregoing experiments, therefore, present evidence for Reactions 3 and 4; namely, that propanediol can be split to a 1-carbon unit and in addition can be converted to a 3-carbon precursor of the glycolytic cycle.

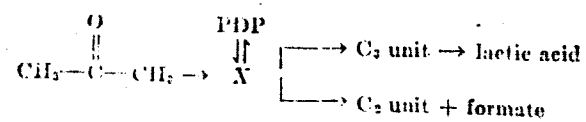
Since propanediol-1-C^{14} gave rise to lactic acid labeled solely in the carboxyl group, it seems unlikely that propanediol was dehydrated to acetone. If such a reaction occurred, incorporation of isotope in the α and β positions of lactic acid would be expected, in addition to the labeling of the carboxyl group. This suggests that Reaction 2 does not simply involve the addition of water or phosphoric acid to acetone as written, but that it is more complex and irreversible. This might be the case if some endergonic intermediate reaction were involved.

Price and Rittenberg (15) have considered the possibility that, in addition to acetate, a 3-carbon oxidation product of acetone is involved in the conversion of acetone to cholesterol. It was of interest, therefore, to determine whether propanediol could be converted to cholesterol. Under conditions in which acetone is readily converted to cholesterol, propanediol was not incorporated to any extent (Experiment 2, Table III).

DISCUSSION

The experimental results demonstrate that acetone is converted to PDP and propanediol and that the isotope distribution is in conformity with that expected via Reactions 2, 3, and 4. It was not possible, however, in the present work to obtain specific activity data to establish the actual precursor sequence.

The results could also be interpreted in another manner; namely, that another compound, *X*, might be the direct intermediate and that PDP and propanediol appear to be an intermediate by virtue of being formed from *X* as outlined in the accompanying scheme. According to this concept



PDP would be on a side path in the series of reactions whereby acetone is [illegible]. The present results do not provide information on this possibility. It could be acetol phosphate. Miller *et al.* (25) have recently shown that PDP can be oxidized via glycerol phosphate dehydrogenase and diphosphopyridine nucleotide to a compound which is presumably acetol phosphate. Levine and Trampitsch have suggested that acetol is an intermediate in the oxidation of acetone by a dipthoid mold organism (21). Acetol is readily reduced by yeast to propanediol (22). Although propanediol is glycogenic when administered to fasted rats (6), acetol, under similar conditions, has little or no effect on the level of liver glycogen (23). Acetol, however, does disappear rapidly from the blood when injected (24). If acetol or its phosphate ester were a primary intermediate in acetone metabolism, one would expect some of it to be converted to glycogen. It is possible that, under the conditions of these experiments, acetol is rapidly split to formate and "acetate," thereby leading to no net increase in glycogen. At the present stage of the work, it is not possible to decide whether acetol or propanediol, or one of their phosphate esters, is the first intermediate in the catabolism of acetone.

Another point requiring clarification is the relationship between PDP and free propanediol. Throughout this paper the two compounds have been considered equivalent, but this may not be the case. For example, free propanediol may yield "acetate" and formate and the phosphorylated derivative may yield the glycolytic precursors.

A major question only partly answered by the present work deals with the significance of the presence of PDP in animal tissues. How does it originate and what is its metabolism other than that indicated in the present work? It has been shown (25) that PDP is rapidly metabolized in vivo. Apparently the phosphate group enters the ATP fraction prior to transfer to hexose phosphate esters. In addition some evidence has also been obtained that the ester may play a role in sterol metabolism. Neuberg and Sarfatti showed that, in addition to acetol, methylglyoxal and lactic aldehyde could be reduced to propanediol by yeast, while glyceraldehyde and dihydroxyacetone, on the other hand, were not. The studies of Price (27) suggest a relationship, since methylglyoxal reduced the incorporation of C^{14} from acetone into cholesterol, whereas pyruvate was without effect.

On the other hand, pyruvate can be reduced to PDP in fluoride-poisoned yeast or animal (26). PDP as such was not isolated and purified in these experiments, and the possibility of a contaminant has not been [illegible]. Under the conditions adopted in these experiments with liver [illegible] and in the experiments in vivo, this conversion does not appear to [illegible] to any great extent; at least lactate was not converted to propanediol. This does not rule out the possibility that under conditions of fluoride

poisoning the pathways might not shunt towards the reduction of pyruvate as proposed by Groth *et al.*

The author is deeply grateful to Dr. Warwick Sakami and Dr. [illegible] Wood for their advice and generous help. He is also indebted to Dr. Eskin Huff for his assistance with the synthesis of C^{14}-methyl-labeled acetone and for the isolation and degradation of one of the lactic acid samples.

SUMMARY

1. C^{14}-carbonyl-labeled acetone was administered to rats in vivo; methyl-labeled acetone was added in vitro to preparations of rat liver; and the distribution of isotope in formate, lactate, propanediol, and propanediol phosphate was determined.

2. Propanediol-1-C^{14} was added to a liver slice preparation. It was found that propanediol is split to give a 1-carbon unit containing the isotope and that the rest is converted to lactic acid. No incorporation into cholesterol was detected.

3. Under the conditions adopted in these experiments, there is no conversion of lactic acid to propanediol.

4. The possible role of propanediol and propanediol phosphate as intermediates in the catabolism of acetone to acetate and formate and the conversion of acetone to a 3-carbon unit entering the glycolytic cycle are considered.

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LIVER GLYCOGEN FROM DERIVATIVES OF GLUCOSE

W. T. SALTER, P. D. ROBB AND F. H. SCHARLES
Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Boston

FOUR FIGURES

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Many investigators have studied the ability of the liver to store various foodstuffs in the form of glycogen. The conversion of naturally occurring hexoses (like galactose and fructose) into liver glycogen has been well described (Cori, '26; and Cori and Cori, '27). This type of chemical transformation has even been suggested as a test of liver function (Banks, Sprague and Snell, '33). Nevertheless, the versatility of this chemical synthetic capacity of the hepatic cells has not as yet been completely described in its qualitative aspects.

Drury and Salter ('34) administered a series of glucose derivatives to rabbits whose livers had been removed. They found that substitution of even a single chemical group in the hexose molecule usually rendered the sugar useless for such animals. When given to normal animals (mice), however, many of these carbohydrates were stored as liver glycogen. This paper records liver glycogen formation by six carbohydrates for which data in the extant literature are scanty or lacking. For purposes of comparative standards, this paper also describes the glycogen content of livers in fasting mice when a single feeding of glucose is administered.

The dose of glucose used is varied in successive experiments, so that a considerable range of nutrition is covered. Glycogen storage can be demonstrated in the liver soon after

the sugar is administered; but as fasting continues, the glycogen disappears. The rate of absorption, and the height of liver glycogen depend upon the amount of glucose administered. Four sets of data are presented, representing four levels of carbohydrate storage. It is hoped that the data may serve as standard curves of reference for the study of carbohydrate turnover in mice.

EXPERIMENTAL PROCEDURE

Young, mature mice of both sexes were used for these experiments. The stock was fed bread, hemp and millet seed, crackers, cabbage, milk, and cod liver oil. Prior to the experiment, they were fasted for 48 hours and then fed through stomach tube a standard amount of sugar in solution. (The selection of standard amounts is described below.) The use of a small glass tube, suitably molded, attached to a tuberculin syringe, permits accurate delivery of liquid. The animals were allowed water ad libitum. They were kept in individual fruit jars in a thermostat controlled animal room at 70° F. At various intervals after feeding, groups of animals were sacrificed.

The animals were stunned, carotid arteries cut, and allowed to bleed for half a minute. The liver was then removed as rapidly as possible and dropped into a tared tube containing 1.5 cc. of 60 per cent warm potassium hydroxide. Each tube was stoppered with a glass bulb, to the undersurface of which a stirring rod was attached. The tissue was speedily disintegrated. The tube was then cooled and weighed again. Ligatures were next placed about the esophagus and rectum and the gastro-intestinal tract removed to a beaker. The contents of the gut were then washed out with several successive portions of hot water, and collected in a volumetric flask. In this manner, unabsorbed sugar could be determined.

CHEMICAL METHODS

The chemical procedures followed were essentially those described by Cori ('25 a). Glycogen was precipitated after

3 hours' digestion (in aqueous potassium hydroxide at 100° C.) by adding water and alcohol. Two methods were employed in the final estimation of the sugar produced by acid-hydrolysis of the glycogen. The colorimetric method of Folin ('29) proved as accurate as the iodometric titration method of Somogyi, modified from Shaffer-Hartman (Shaffer and Somogyi, '33), but the latter conveniently obviated preliminary determinations of glucose for approximate concentration.

The washings from the gut were deproteinized with colloidal iron as described by Cori ('25 b). The filtrates were then analyzed for reducing substance. Control, fasted animals yielded negligible values by this procedure.

The accuracy of the experimental procedure was controlled by several parallel experimental manipulations. Delivery from the syringe was much more constant than the biological reaction of the animals. The intestines and stomachs of fasted animals yielded negligible reducing values. Livers of 48-hour fasted mice contained (apparently) 0.07 per cent of glycogen which was allowed as a blank. The accuracy of the glycogen determination itself was tested by preparing glycogen from mouse livers by the method of Pflüger, modified by Cori (personal communication), and then analyzing known amounts, both separately and when added to fasting liver tissue. The recovery of added glycogen was 95 ± 3 per cent of theoretical.

INGESTION LEVEL

All the results here reported concern the fate of carbohydrate solutions delivered into the stomach. The maximum amount which can be administered in this fashion depends, first, upon the capacity of the stomach and, secondly, upon the tolerance of the gut to irritating hypertonic solutions. Mice weighing about 25 gm. were selected, and solutions so made up that about ½ cc. of solution was given. The actual amount of sugar to be administered was determined by the weight of each animal. Regurgitation and diarrhea were

carefully looked for, and such animals excluded. Even in the absence of diarrhea, the intestine may be markedly distended by fluid soon after feeding a large amount of sugar.

It was found that the gut tolerates more of glucose than of most other sugars. Accordingly, glucose feeding was conducted at several distinct levels (maximal, two intermediate, and low) so that the effect of other sugars could be compared at similar ingestion levels.

SPECIAL CARBOHYDRATES STUDIED

Of the special carbohydrate substances used, five were hexose derivatives. These included sorbitol, glucosamine, gluconic acid, and the two glucosides of methyl and ethyl alcohol, respectively. The chemical configuration of these or similar compounds is given by Herring, Irvine and Macleod ('24), as well as by Armstrong ('24). The sorbitol was purchased from Pfanstiehl Company. The gluconate (calcium salt) was generously contributed by Sandoz Chemical Company. The other hexose derivatives were prepared in this laboratory: e.g., α-ethyl d-glucoside.

In addition, a glycerol derivative, propylene glycol (propane-diol 1, 2) was also tested. This propylene glycol was obtained from the National Carbon Company through the courtesy of Dr. Reid Hunt.

EXPERIMENTAL DATA FOR GLUCOSE

Table 1 shows both the change in liver glycogen concentration and in the fraction of the glucose fed which was recovered as glycogen. Two sets of data are given for the maximal dosage curve, one obtained during the winter months and the other in the summer. No significant difference is evident. For the latter curve, pedigreed inbred black mice were employed as well as stock white mice. They showed no obvious difference in behavior.

The maximal level is presented in duplicate. This last level represents the maximum amount of glucose which the gastrointestinal tract would tolerate consistently.

TABLE 1

Recovery of liver glycogen in mice after feeding glucose in various doses

		Time after feeding	Number of mice: Used	Discarded	Died	Average glycogen content of liver	Average fraction of ingested glucose as liver glycogen
		Hours				*Per cent*	*Per cent*
Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse		4½	6	1		0.09	4.7
		6	3			0.12	5.2
		8	6			0.13	6.4
		13½	8	1		0.14	6.9
Molecular glucose—equivalent: 0.2 gm. per 100 gm. of mouse		4	9			0.85	22.3
		6½	7			0.75	17.8
		12½	6			0.48	11.7
		24½	7			0.08	2.3
Molecular glucose—equivalent: 0.5 gm. per 100 gm. of mouse		1	5			1.20	10.9
		2	5			2.00	20.3
		3	4	1		2.49	22.3
		4	8			1.64	17.0
		6	16	1		1.11	11.8
		10	12	1		0.69	6.8
		15	8	2		1.16	11.5
		20	9	2		0.51	4.6
Molecular glucose—equivalent: 0.7 gm. per 100 gm. of mouse	Summer curve	4¼	12			2.77	17.7
		4½	6			2.21	16.4
		4¾	9			2.40	14.4
		6	4			4.39	26.5
		10	5			2.70	19.0
		18	7			0.96	5.9
		24	5			0.29	1.5
	Winter curve	¼	2			0.83	7.8
		½	3	1		0.85	7.1
		1	14			1.17	10.8
		2	10			1.93	14.6
		3	12			2.85	20.3
		4	13			2.49	19.1
		5	11	1		3.00	24.2
		6	7			2.71	19.8
		7	5			2.42	19.9
		11	4			2.44	18.1
		13	4			2.13	16.2
		15	4			2.03	12.4
		16	4			1.78	11.3
		17	4			1.44	8.6
		20	6	2		1.30	9.7
		24	7	2	2	0.85	4.7
		30	5			0.47	2.8
		40	3		3	0.88	5.5
		48	4	1		0.14	0.8
		Blank	10			0.07	

The rate of intestinal absorption for the highest level is given in table 2. These data for mice, obtained by a method similar to Cori's ('26) procedure in rats, show a somewhat different trend in relation to time from the results obtained on rats.

Figure 1 shows that the postprandial content of glycogen in the liver becomes progressively higher as increasing

TABLE 2

Intestinal absorption in mice after feeding glucose and derivatives of glucose

	TIME AFTER FEEDING	NUMBER OF MICE	AVERAGE ABSORPTION PERCENTAGE OF CARBOHYDRATE FED
	Hours		*Per cent*
Glucose	¼	3	24.6
	½	4	59.2
	1	6	78.8
	2	2	91.4
	3	4	95.7
	4	5	96.5
	5	3	96.9
Propylene glycol	½	3	77.8
	1	3	61.6
	1½	3	74.7
	2	6	81.3
	3	8	84.7
	5	3	93.7
	7	6	93.6
Glucosamine	2	3	45.4
	5	4	80.6
Ethyl glucoside	2	3	46.1
	5	1	58.2

amounts of glucose are administered. The limiting value is fixed, not by the liver, but by gastro-intestinal tolerance to a single large feeding. That is, up to the point where diarrhea interferes, liver glycogen concentration is greater when larger amounts of glucose are fed.

By way of contrast, the fraction of fed glucose which the liver stores remains nearly the same despite wide variation in the actual amount fed. This is not true when a very small

amount of sugar is fed; but applies over a considerable range of dosage. Figure 2 shows that the total glycogen stored in the previously fasted liver is roughly proportional to the amount of glucose fed, provided that the dose of sugar is a significant portion of the maximal dose which is tolerated.

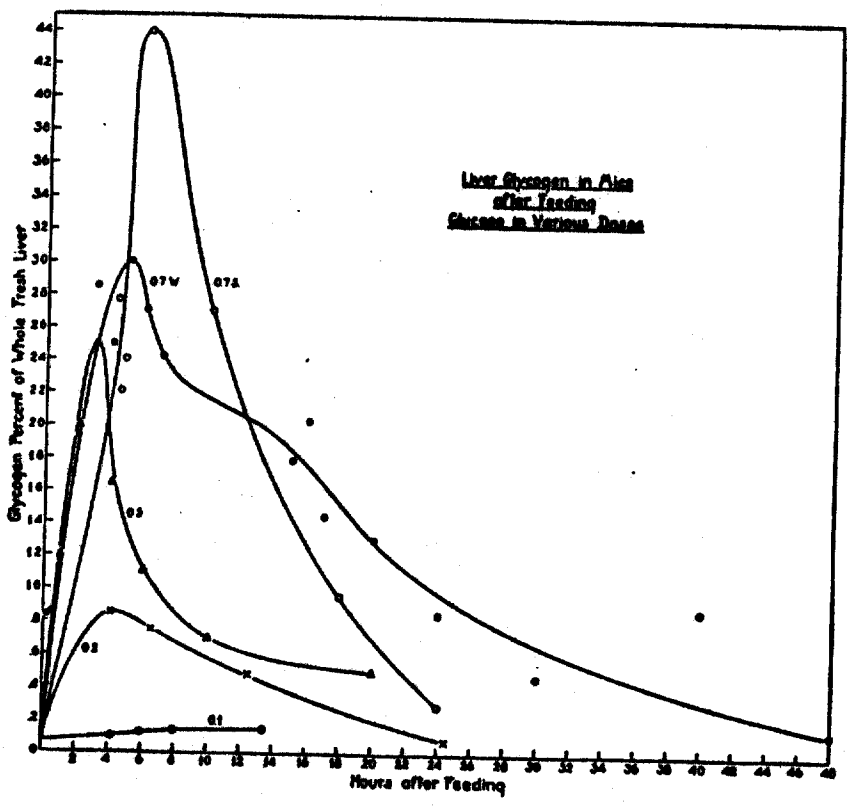

Fig. 1 The figures attached to each curve indicate the grams of glucose fed per 100 gm. of mouse. For 0.7 gm., both a winter and a summer curve are given.

EXPERIMENTAL DATA FOR SPECIAL CARBOHYDRATES

The results of the liver glycogen determinations after feeding derivatives of glucose are presented in table 3. Results are calculated in terms of total glycogen recovered, as well as in terms of the per cent of glycogen in the liver. The figures are plotted in figure 3 for comparison with the (similarly fed) molecular equivalents of glucose, given in figures 1 and 2.

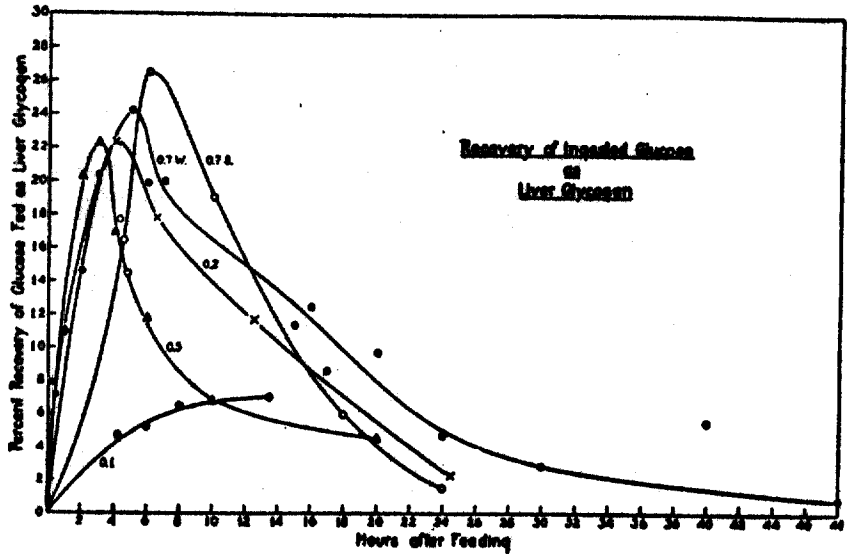

Fig. 2 The symbols attached to each curve have the same significance as in figure 1.

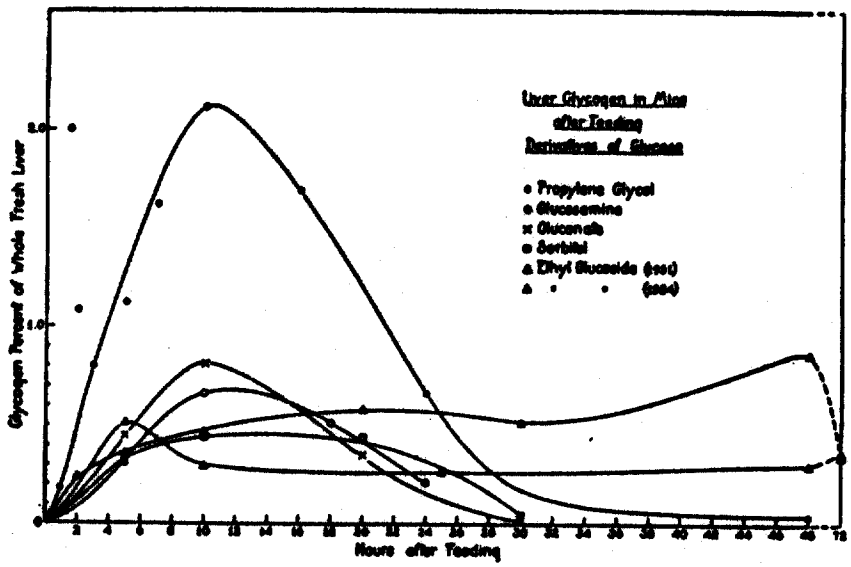

Fig. 3 The amounts of each carbohydrate fed are given in table 3. For ethyl glucoside, data are plotted as obtained in two separate years (1931 and 1934).

TABLE 3

Recovery of liver glycogen in mice after feeding derivatives of glucose

		Time after feeding	Number of mice: Used	Number of mice: Discarded	Number of mice: Died	Average glycogen content of liver	Average fraction of ingested carbohydrates
		Hours				*Per cent*	*Per cent*
Propylene glycol feeding	Molecular glucose—equivalent: 0.75 gm. per 100 gm. of mouse	1	3			0.18	1.2
		1½	3			2.00	13.1
		2	9			1.08	7.8
		3	10			1.80	5.4
		5	8			1.12	9.6
		7	7			1.62	10.9
		10	4			2.11	15.4
		16	2			1.69	11.5
		24	8	1	1	0.66	4.4
		30	2			0.02	0.2
		48	2		6	0.04	0.1
Glucosamine feeding	Molecular glucose—equivalent: 0.370 gm. per 100 gm. of mouse	2	4				4.4
		5	6			0.32	4.6
		10	4			0.66	9.1
		18	3			0.51	7.0
		24	6	1		0.21	2.7
Gluconate feeding	Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse	5	4			0.45	23.9
		10	4			0.81	47.5
		20	6			0.35	18.7
		30	2		3	0.01	0.1
Sorbitol feeding	Molecular glucose—equivalent: 0.1 gm. per 100 gm. of mouse	5	4			0.33	22.0
		10	2			0.44	25.2
		20	3			0.44	22.2
		30	2			0.05	2.2
Ethyl glucoside feeding (1931)	Molecular glucose—equivalent: 0.23 gm. per 100 gm. of mouse	2	5			0.24	3.1
		5	7			0.36	9.0
		10	3			0.46	13.4
		20	5			0.58	13.1
		30	4			0.51	11.9
		48	4			0.86	16.7
		72	2			0.35	8.7
Ethyl glucoside feeding (1934)	Molecular glucose—equivalent: 0.217 gm. per 100 gm. of mouse	5	4			0.51	13.5
		10	3		2	0.29	7.6
		25	7			0.26	3.3
		48	8		13	0.30	5.3
		72	3		5	0.36	7.0

Figure 4 shows the percentage recovery (in the liver) of the carbohydrate fed. This figure, like figure 3, describes increases in liver glycogen; but relates it to the amount of the carbohydrate fed, rather than to the weight of fresh liver in which it was stored.

Data are also presented in table 2 to illustrate the rate of absorption as evidenced by analysis of intestinal contents.

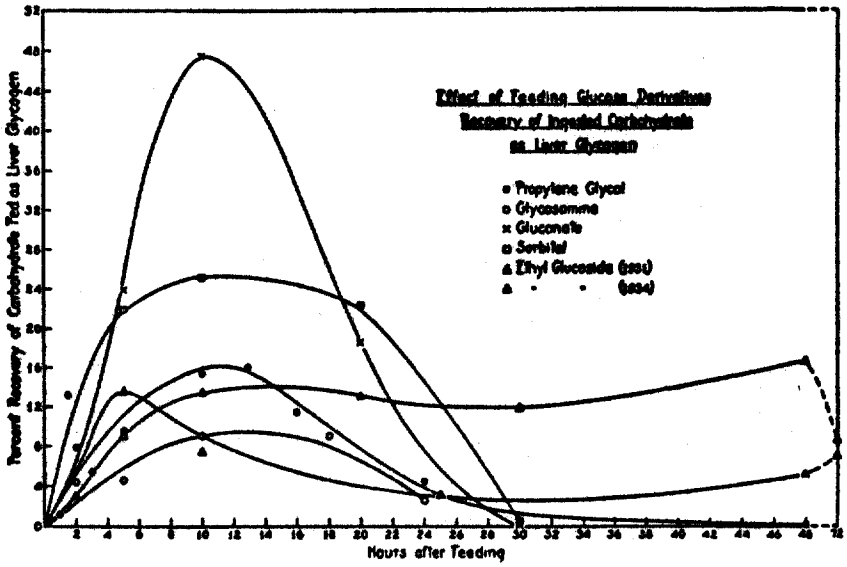

Fig. 4 These curves correspond to the data in figure 3, recalculated.

In the case of propylene glycol, the analytical method was the chromate oxidation described by Strebinger and Streit ('24) for the analysis of glycerine. The conversion factor was determined by analysis of known amounts of propylene glycol. This method is crude, because there was a large blank due to naturally occurring oxidizable matter in the intestine. The results are significant, therefore, only in indicating roughly how soon absorption was approximately finished.

DISCUSSION

It should be emphasized that these results apply only to the history of a single carbohydrate meal administered to

fasting animals. The highest liver glycogen contents, here reported, do not attain the levels reported in the literature for continued high ingestion of carbohydrates. As might be expected, the concentration of glycogen in the liver rises higher when the glucose meal is larger.

One might expect a certain amount of glucose to be immediately burned. Indeed, our results with a very small glucose meal indicate very little storage in the liver. Above this level, however, it is not obvious why the fraction selected for deposition in the liver should remain a constant aliquot of the glucose fed.

Because of the limit imposed by gastro-intestinal tolerance, none of the substances here tested could be used in amounts as high as the maximal glucose ingestion. Nor is the storage of any of them better than that of glucose; indeed, two failed of as complete absorption as the glucose equivalent. Nevertheless, all showed undoubted evidence of glycogen storage, and its subsequent glycogenolysis as fasting proceeded. How much sugar was directly or indirectly utilized by the tissues at large was not determined in this work. It is believed, however, that the paying out of stored glycogen as fasting continued is good evidence that these substances are available as foods.

One word of caution is necessary as to the interpretation of the results. These substances were given to intact animals. The effect of intestinal action is, therefore, merged in the net effect produced. That sucrose, given parenterally, acts as a foreign substance is well recognized. It is conceivable that some of these compounds would not be utilized by an animal whose gastro-intestinal tract had been removed, even though the liver remained intact.

The effect of methyl glucoside is interesting. Its toxicity is apparent because the animals died an hour or two after feeding. Nevertheless, glycogen storage within the liver was indubitable a short time before death, as the analyses showed. The lethal effect is presumably due to progressive liberation of methyl alcohol. Similarly, the results obtained with ethyl

glucoside were erratic, presumably because of the profound anaesthetic effect produced during the first day after feeding.

No attempt has been made in this paper to review the literature pertinent to these compounds, which have not been extensively studied as sources of energy for mammals. Drury and Salter ('34) have given references to a few such investigations, most of which have been concerned with the relief of insulin hypoglycaemia, or with organ perfusions.

SUMMARY

When the fasted mouse is fed a single large amount of appropriate carbohydrate, much of this is stored in the liver as glycogen. In the case of glucose, the maximal amount stored is roughly proportional to the amount fed, despite wide variation in the actual concentration of liver glycogen. As fasting proceeds, the stored glycogen is released from the liver. Standard curves are presented which illustrate this process in mice at various levels of glucose feeding.

For comparison, the storage and discharge of liver glycogen was followed after the ingestion of sugar derivatives in equivalent amounts. Five simple derivatives of glucose (sorbitol, gluconate, glucosamine, and the glucosides of methyl and ethyl alcohol) were studied, and one triose derivative (propylene glycol). All increased liver glycogen.

The results are compared with the effect produced by the molecular equivalent in glucose.

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J. Pharmacol. Exp. Therap.
44(1): 109-121, 1932

THE GENERAL PROPERTIES, ACTIONS AND TOXICITY OF PROPYVENE GLYCOL

M. A. SEIDENFELD AND P. J. HANZLIK

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California

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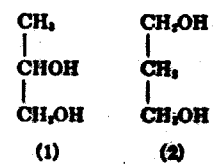
Propylene glycol has commanded little or no interest in chemistry and medicine, although it was described seventy-two years ago by C. Wurtz, who was also the first to prepare ethylene glycol (1). According to Lawrie (2), "although the product is obtainable commercially, it has not so far found a very wide market;" hence, he devotes little space to it in his monograph. Miura (3), in 1911, reported experiments on rabbits with the ethylene and propylene glycols, and glycerol, the object of which was to determine the end products of their metabolism in the urine and their general behavior in the organism. Miura found that subcutaneous and oral administrations of propylene glycol caused the excretion of propylene glycol-monoglycuronic acid, a conjugation product of propylene glycol and glycuronic acid. Miura used only one rabbit, which was given at different times a total subcutaneous dose of 10 cc. of propylene glycol in 40 cc. of water; in one experiment on the same animal he administered gastrically a total of 25 cc. propylene glycol mixed with 75 cc. of water. This latter mixture was administered in 2 divided doses of 80 and 20 cc., within a twenty-four-hour period: no toxic symptoms were observed. This is about the extent of the information on the actions of propylene glycol in the literature.

We became interested in propylene glycol as a possible substitute for ethylene glycol, which is being used as a solvent and vehicle for a bismuth product introduced for the treatment of syphilis and neurosyphilis. Although the harmful potentialities of ethylene glycol existed only when used in very high and toxic,

or at least extra-therapeutic, doses, yet if these could be completely eliminated by the use of propylene glycol the latter might be preferred. The objection to ethylene glycol is based on the possibility of cumulative toxicity from oxalate-formation, which, however, appears to be largely theoretical so far as scope of usage in medicine is concerned; this matter was investigated by us and the result on actions an toxicity have been published (4). On the other hand, there is no possibility of the formation of oxalate in the oxidation of propylene glycol, which can yield pyruvic and lactic acids as intermediary products. As a matter of fact, both the ethylene and propylene glycols are for the most part completely oxidized in the body to carbon dioxide and water. In excessive doses, continuously administered, the potential effects of the intermediary products of oxidation of both these glycols exist, and probably any effects of lactic and pyruvic acids would be less objectionable than those of oxalic acid. However, it could not be known if such a difference could be claimed in favor of propylene glycol until the matter was investigated. Therefore, we undertook a comprehensive study of acute and chronic toxicity of propylene glycol along the same general lines as in the previous study of ethylene glycol (4), and compared the results with the two glycols as much as possible.

PHYSICAL AND CHEMICAL PROPERTIES

Propylene glycol is the next higher homolog of ethylene glycol. It is a dihydric alcohol, which has two isomeric forms with the following formulas:



The usual form is the first, which is reputed to be less active than the trimethylene, or second form, and was the type used by us.[1]

[1] Generous supplies of a specially purified product for our work were supplied by the Carbide and Carbon Chemicals Corporation, South Charleston, West Virginia.

Pure propylene glycol is a colorless, odorless liquid with a mildly acrid and sweetish taste. Its specific gravity is 1.051 at 0°C.; hence, it has about the same density as water, but is about as viscous as ethylene glycol. It has a rather high degree of affinity for water. Propylene glycol is freely miscible with water, glycerol, methyl and ethyl alcohols, acetone, ether, chloroform, and ethyl acetate, but it is insoluble in carbon tetrachloride, carbon disulphide, and benzene. In general, propylene glycol has all the excellent solvent properties of ethylene glycol. It was observed that turbid or slightly discolored samples would give a precipitate on the addition of sodium iodobismuthite (Na_3BiI_6) to it, but this was not true for perfectly clear and pure samples. The cause of this phenomenon was not investigated, but the reaction would not be permissible with propylene glycol in case the latter should be used as a solvent and vehicle for the iodobismuthite, and therefore, only those samples of propylene glycol which failed to give such precipitation were used in our work.

LOCAL IRRITANT ACTIONS

On the tongue, propylene glycol caused a temporary burning sensation without apparent injury to this organ or the oral mucosa. The taste appears to be more acrid than, but not as sweet as, glycerol or ethylene glycol. It spreads easily. A preliminary comparison of the irritant properties, intramuscularly, of glycol, propylene glycol, and glycerol in 3 human subjects has been previously reported (4). Six additional subjects were given equal doses (2 cc.) intramuscularly of ethylene glycol and propylene glycol in opposite gluteal regions. One-half of all the 9 subjects complained of more pain and burning in the areas injected with propylene glycol than those injected with ethylene glycol. The 3 subjects receiving both glycols and glycerol complained of most irritation from glycerol. In all cases, the irritant action was fleeting, lasting about five to ten minutes and there were no after-effects locally and systemically. In animals, the propylene glycol seemed to cause more irritation than ethylene glycol or glycerol immediately after intramuscular and hypodermic injections, i.e., there was generally more restlessness and limping

or lifting of an extremity after the propylene glycol. Trial on a much greater number of individuals than we could possibly obtain would be necessary to settle the point definitely. If it should prove to be more irritating locally this might preclude its usefulness as an injection vehicle despite its lower systemic toxicity.

Whole propylene glycol and 50 per cent and 2.5 (isotonic) per cent solutions were applied to the conjunctivae of 6 rabbits. Only the undiluted compound caused a definite chemosis, which lasted for several hours. The 50 per cent solution caused an increased lachrymation, but no chemosis, while the isotonic solution had no demonstrable effect. Therefore, the chemosis of the whole propylene glycol was probably due to hypertonicity. In general, these results were similar to those with ethylene glycol (4).

On the whole, therefore, the propylene and ethylene glycols would be about equally irritating to mucosae, but, when injected intramuscularly, the propylene glycol appears at least equally as irritating as, and possibly more irritating than, ethylene glycol. Although the apparently greater irritant action is fleeting, it remains to be seen if propylene glycol could be used advantageously over ethylene glycol as a vehicle for injection; its use might be favored by a lower systemic toxicity.

ACUTE TOXICITY

This was studied in rabbits and white rats which received the whole propylene glycol intravenously and intramuscularly. Five to 15 rats were used for each dose, the larger numbers being used with border-line doses. Three rabbits were used for all doses tested. The results on mortality percentages are summarized in table 1.

There were no symptoms after intramuscular injection in rats and rabbits until doses above 6.3 to 7.4 grams per kilogram were reached. With doses above these limits the symptoms consisted of increased respiratory rate, loss of equilibrium, followed by depression, and after the highest doses, coma and death. It is interesting to note that the moderately high doses (10 to 12 cc.) caused in rats a semi-comatose condition which lasted from

rats was about 13 per cent less, and in rabbits, 31 per cent greater, than the fatal intramuscular dose. In general, the only difference in symptoms from intramuscular injections was in those animals receiving fatal doses intravenously. The latter frequently caused marked muscular tremors in the posterior extremities. Some animals with these symptoms died within two or three minutes, others were comatose and again became active; death, however, occurred within twenty-four hours. Occasionally, an animal receiving a sublethal dose became comatose, but recovered after twenty-four hours. According to these results, 294 grams (280 cc.) might be injected intravenously in a 70-kgm. man without demonstrable effects. The possible ineffective dose of ethylene glycol for an adult was estimated as 73.5 grams (70 cc.), according to which the propylene glycol would be only about one-fourth as toxic as the ethylene glycol. There is no known indication for propylene glycol intravenously in man, but such large amounts would be far in excess of those which might be used by any method of administration.

Effects on arterial blood pressure, pulse and respiration were observed after intravenous injections of propylene glycol in 3 cats ranging in weight from 2.5 to 3.6 kgm. Injection of the whole propylene glycol in divided doses of 5 cc. each, using short intervals, caused temporary reduction in blood pressure. When longer intervals were used between injections, recovery of the pressure occurred, but each injection was followed by a new and lower level, amounting to about 15 to 20 mm. Hg below the previous level. With this method, from 45 to 50 cc. of the glycol could be injected before death occurred. The heart beat regularly and more strongly and the respiration increased in depth, but became slower after the propylene glycol. After 6 or 7 cc. had been injected, there were marked general muscular twitching, straightening out of the tail, and marked extension of the limbs. In one cat a steady injection of the propylene glycol caused death after a total dose of 17 cc. (4.5 or 4.7 grams per kilogram); the blood pressure fell steadily to the zero level. Obviously, the steady rapid injection required much less glycol to cause death, since slow and interrupted administrations required about 17.9

grams (17.0 cc.) per kilogram or 3½ times as much. The results of intravenous injections were not further analyzed, owing to the very high doses required for the effects and the undesirability of the intravenous use of glycol for practical purposes. Presumably, the effects were due mainly, if not entirely, to the hypertonicity of the propylene glycol. More important than all this would be the effects of prolonged administration of smaller quantities of the compound.

CONTINUED DRINKING OF PROPYLENE GLYCOL

The cumulative effects of propylene glycol might be reflected in growth and body-weight changes after drinking of the compound during long periods. Such effects might occur from the pyruvic and lactic acids, the intermediary products of oxidation of the propylene glycol. A possible deleterious effect might be vascular damage from the pyruvate, which has been employed for the purpose by Kapp and Mancke (5). On the other hand, both the pyruvate and lactate, when formed in small quantities, would be oxidized and not occur as such in the body. The matter was tested on white rats.

Seven groups containing 5 rats each of about 50 grams body weight were placed in cages and given free access to the modified whole artificial diet of Steenbock.[2] One group was given water to drink as control and the other groups were given 50, 25, 10, 5, 2 and 1 per cent solutions of propylene glycol in water. The consumption of food, propylene glycol, and body weight were recorded thrice weekly during the first month, and weekly thereafter. The rats receiving water and the 10, 5, 2 and 1 per cent solutions of propylene glycol were kept on these régimes for a total of 140 days, equal to about one-eighth of their life-span. Then each group of rats was placed in cages and their urines were collected, centrifugalized, and examined microscopically. After this was done, the animals were killed and autopsied and their kidneys, hearts, spleens and livers were examined histologically

[2] This diet contains: Feed corn-meal, 68 parts; linseed oil cake-meal, 10 parts, dried ground alfalfa, 2 parts; powdered casein, 10 parts; lard, 5 parts; cod liver oil, 3 parts; bone ash, 1.5 parts and sodium chloride, 0.5 part.

by Drs. William Ophüls and D. A. Wood of the department of pathology of this university.

The results were as follows: The majority of rats receiving 50 per cent propylene glycol died at the end of the ninth day, and those receiving 25 per cent propylene glycol died at the end of the sixth day. In both groups, the daily fluid-intake per rat was less than 0.5 cc. and the rats showed an average loss of 10 to 20 grams in body weight. The amount of propylene glycol ingested per kilogram was 3.5 cc. (3.7 grams) in the group drinking 50 per cent, and 2.1 cc. in the group drinking 25 per cent solutions. These amounts were very much less than the intramuscular or intravenous fatal doses. Presumably, death of these animals was due not so much to the actual amounts of the glycol ingested as to starvation and dehydration resulting from a distaste for the solutions. Since the rats were found dead unexpectedly, their urines and viscera were not examined. Rats drinking concentrations of 10 per cent and less of propylene glycol remained well and lived to the end of the twenty-week period of the experiments. The results on these rats as to changes in average body weight and consumption of propylene glycol and food are presented in figure 1.

It is seen that the growth and body weight of the rats were not demonstrably affected by the propylene glycol in all the concentrations used. The small differences, both increases and decreases in body weight, from the controls represented small variations. The intake of fluid was fairly uniform in all the rats drinking the different concentrations. The rats on 10 per cent propylene glycol took an average daily amount of about 13.2 grams of absolute propylene glycol per kilogram of rat; those on 5 per cent took an average daily amount of 7.7 grams per kilogram; those on 2 per cent took an average daily amount of 3.68 grams per kilogram and those on 1 per cent took an average daily amount of 1.6 gram per kilogram. The results are in contrast with those on rats drinking ethylene glycol (4) which caused definite stunting of growth after continuous drinking of 0.5 and 1 per cent solutions, that is, after one-twentieth to one-tenth the highest ineffective concentration (10 per cent) of propylene glycol used.

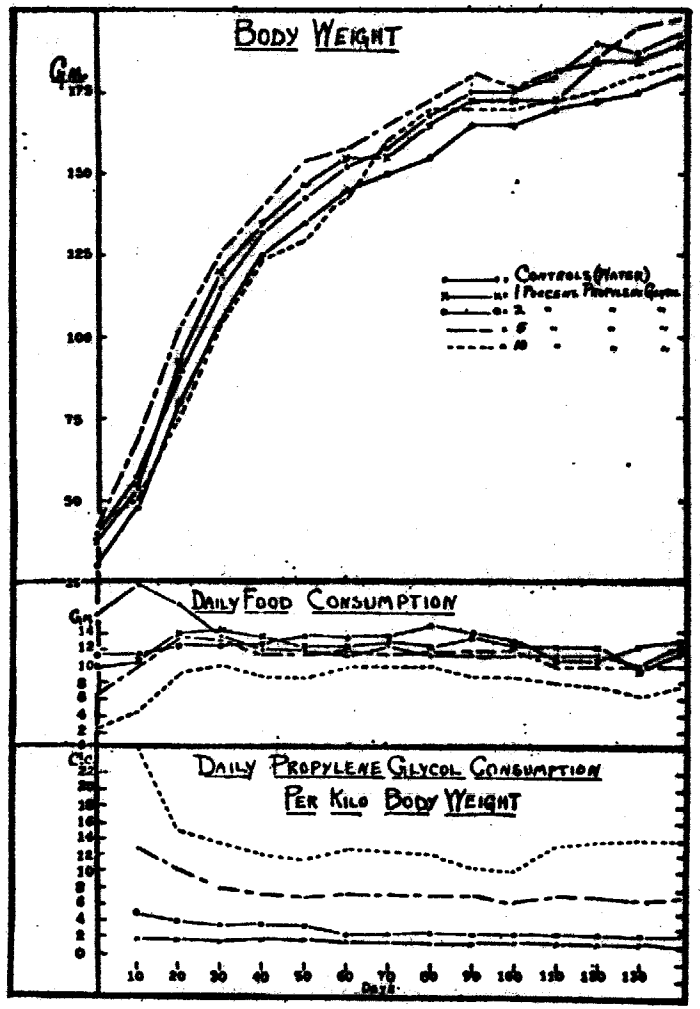


FIG. 1. EFFECTS OF CONTINUED DRINKING OF DIFFERENT CONCENTRATIONS OF PROPYLENE GLYCOL BY WHITE RATS

The intake of absolute propylene glycol varied directly with the concentrations. Since 13.2 grams per kilogram daily of propylene glycol showed no demonstrable effects and 2.2 grams per kilogram of ethylene glycol showed definite stunting of growth, it would appear that ethylene glycol is at least 6 times as injurious as propylene glycol when drunk continuously. This, in general, corroborates the comparatively low toxicity according to acute fatal doses.

The amounts of propylene glycol drunk were very large, amounting to the daily use of about $\frac{9}{16}$, $\frac{1}{2}$, 1 and $\frac{1}{8}$, in descending order of concentration, the M.F.D. of propylene glycol intramuscularly in the same species, during about one-eighth the normal life-span. These quantities for a 70-kgm. man would be the equivalent of the daily ingestion of 924 grams, (over 1$\frac{1}{2}$ pounds), 537 grams (over 1 pound), 258 grams (over $\frac{1}{2}$ pound), and 110 grams (over 3 ounces), respectively, of propylene glycol during a period of about eight or nine years. These would be enormous quantities of the propylene glycol to take at once and to tolerate for long periods; they are, in fact, unthinkable for man. Yet, in rats, their equivalents caused practically no demonstrable pathological changes in the viscera examined. The only suggestion of tissue abnormality was in the kidneys, where occasional vacuolization of the epithelial cells of the proximal conconvoluted tubules was found, but this was also present in the control rats drinking water. The absence of renal morphological injury was corroborated by the absence of abnormal urinary constituents, i.e., increases in albuminuria, cells and casts, in all the rats. The spleens and hearts were entirely free of pathological changes; and so were the livers, except those of the group drinking the 10 per cent propylene glycol. The livers of the latter rats showed a moderate degree of vacuolization of the central lobular cells, but the livers of the control rats on water were not markedly different. There were no evidences of vascular damage in any of the viscera. It would appear that any pyruvate, which might have been present as the result of drinking the propylene glycol, did not cause vascular injury in the sense of Kopp and Mancke (5).

Accordingly, the long continued drinking of propylene glycol in large quantities appears to be innocuous, since cumulative effects, functional and morphological, are not demonstrable in the organism as a whole.

CONCLUSIONS

1. The general properties, irritant and toxic actions of propylene glycol are discussed and compared with ethylene glycol from the standpoint of its usefulness, and its substitution for ethylene glycol, as a vehicle and solvent for pharmaceutical preparations in medicine.

2. Propylene glycol caused a moderate, though fleeting, local irritation of mucosae (tongue of man, conjunctiva of rabbits), and in the muscles and subcutaneous tissues of rats, rabbits and men, the irritation being probably somewhat more marked than that of ethylene glycol and glycerol. This action is due to hypertonicity.

3. In rats and rabbits, the symptoms of toxicity were characterized by general motor depression, increased respiratory rate, followed by tremors, coma and death after the largest doses. Intravenously, the M.F.D. for the majority of white rats was found to be about 16.8 grams per kilogram and for the majority of rabbits, 5.25 grams per kilogram. Intramuscularly, the M.F.D. for the majority of white rats was about 14.7 grams per kilogram and for the majority of rabbits, 7.5 grams per kilogram.

4. The continued drinking of 1, 2, 5 and 10 per cent solutions in water of propylene glycol by rats during about one-eighth of the normal span of life caused no demonstrable effects on growth and body weight. Practically no pathological changes in the kidneys, heart, spleen and liver were demonstrated, even after the enormous daily quantities of 13.3 gram absolute propylene glycol per kilogram body weight, or the equivalent of 931 grams ($1\frac{1}{3}$ pound) daily for a 70 kgm. man.

5. As a solvent and vehicle, propylene glycol offers generally all the advantages of ethylene glycol, and at the same time, it is much less toxic and causes no demonstrable cumulative effects, at least in animals. It remains to be demonstrated on a much

larger scale if the apparently somewhat greater local irritation of the propylene glycol might outweigh the apparent advantages of low systemic toxicity in its general use as a vehicle and solvent in medicine and pharmacy.

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THE SINGLE DOSE TOXICITY OF SOME GLYCOLS AND DERIVATIVES*

HENRY F. SMYTH, JR., JANE SEATON AND LOUISE FISCHER
Mellon Institute, Pittsburgh, Pa.

LAUG and his colleagues (1) have reviewed the toxicological literature on the glycols and have presented experimental results obtained with six members of the group. Their paper and more recent articles are considered briefly toward the end of this contribution.

In this investigation we have determined the dosage-mortality curves of 60 glycols and glycol derivatives for albino rats, and of most of these compounds for guinea pigs as well. The data are regarded as preliminary information, to be supplemented by results from a more thorough study of those chemicals which prove to be pharmacologically, technically, and economically desirable. Over 5400 animals were used to obtain the facts presented here, and one prescribed method was followed throughout.

EXPERIMENTS

Most of the substances examined were of the usual commercial grades, manufactured in quantity, and meeting definite physical and chemical specifications, but not chemically pure. Some others were specially prepared on a laboratory scale or carefully purified from commercial products. The tables to follow indicate the grade of each material used.

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The substances were all administered by stomach tube in single doses. The greatest concentration employed was 50%; actually 1 ml. of solution contained 0.50 gm. of active compound to facilitate calculations in terms of grams of material. More dilute solutions of the more toxic samples were administered to give volumes large enough to be measured conveniently, and the more irritating materials were also diluted further to reduce local action in the digestive tract.

The diluent was always water. A few materials were insufficiently soluble to be fed in solution and were handled as temporary dispersions in 1% aqueous sodium sulfate of heptadecanol (trademarked "Tergitol" penetrant 7). It will be shown elsewhere that the LD50 of this penetrant for rats is 5.7 gm./kg., or the LD50 of a 1% solution would be not less than 570 ml./kg. It seems probable that the dispersing agent did not contribute detectably to the toxicity of the material being fed. One product was a solid and was administered as a suspension in a 1% gum tragacanth mucilage.

The largest dose which the animal stomachs could safely hold at one time was just over 10% of the body weight, equivalent to 50 gm./kg. when a substance was given as a 50% solution.

In most cases 10 animals were used

to determine the toxicity of a particular dosage, and enough dosages were administered to include those at which no animal died and those at which all died. Most deaths occurred within the first 2 days, but all deaths within 14 days of administration of the dose were considered in calculating the LD50. Any victim whose death was inconsistent with the indications from others receiving the same substance was autopsied, and if found to be infected it was not included in the calculations. Tissues were not studied microscopically because this step was beyond the scope of the preliminary investigation planned.

The animals received an adequate diet of Purina chows, supplemented by fresh vegetables, and water was freely available. The diet was given in the afternoon, and the doses administered the following morning in most cases.

The data were calculated by the method of probits, described by Bliss (2, 3) and now becoming familiar. No attempt was made to use adequate animals to obtain extreme precision in the LD50 figures, for the use to which such information is put seldom justifies extreme accuracy. The precision is indicated by the range of 95% probability listed in the tables.

All materials were given to albino male Wistar strain rats, weighing 90–120 gm. when dosed. Guinea pigs of about the same stage of development were used, that is, 250–300 gm., but both sexes were dosed because of the difficulty of purchasing pigs of only one sex.

Diethylene glycol was administered also to some female Wistar rats and to males of two hybrid strains.

All animals were obtained from commercial breeders, and the source for rats did not change during the 3 years this study was in progress.

Toxicity Data

The results appear in table 1 in the form of LD50 figures, together with the range of 95% probability of the LD50 ($\pm 1.96\sigma$) and the slope of the dosage-mortality line when plotted by the probit method. This slope has a high uncertainty inasmuch as equal numbers of animals were given each dosage, instead of dosing larger numbers as extremes of mortality were approached.

The most marked deviation from the literature was early seen to be the LD50 of diethylene glycol. We found it to be 20.76 gm./kg. for rats, whereas Laug and his group reported 14.8 gm./kg. 15 samples were examined in an attempt to explain this discrepancy, and the information obtained is shown in table 2. In some cases only 20 rats received a sample, and LD50's were estimated by the use of the mortality curve for the first sample.

Sample 16 listed in table 2 is believed (4) to be the identical one with which Laug and his fellows (1) obtained the LD50 of 14.8 gm./kg. It is seen that here was the least toxic sample of diethylene glycol found, when tested under the conditions of our study. This sample was fed to 3 strains of male rats and to females of the Wistar strain. We did not use the Osborne and Mendel strain which those workers dosed, but our Wistar strain rats were from the same breeder as those they identify as Series B (4), and which did not give mortalities significantly different from those of the former strain. We found the

TABLE 1

LD50, Limits of LD50 with a Certainty of 95% (Both as gm./kg. Body Weight) and Slope of Dose-Mortality Curve

(Commercial Grades Dissolved in Water Unless Noted Otherwise)

Material[1]	Dilution	Rats				Guinea Pigs			
		LD50	Limits		Slope	LD50	Limits		Slope
			Lower	Upper			Lower	Upper	
	%								
Glycols									
Ethylene glycol	50	8.54	7.31	9.99	5.71	6.61	5.06	8.63	5.02
Diethylene glycol	50	20.76	19.51	22.10	14.57	13.21	11.64	14.99	9.79
Triethylene glycol	50	22.06	19.38	25.13	17.21	14.66	13.75	15.70	18.72
Tetraethylene glycol	50	32.77	28.22	36.34	10.69				
Hexaethylene glycol	50	31.64	28.63	34.98	11.85	19.55	14.54	26.37	4.80
Nonaethylene glycol[2]	50	37.41	34.21	40.99	15.46				
Polyethylene glycol, approx. mol. wt. 1250	50	51.31	45.77	57.52	17.20				
Polyethylene glycol, approx. mol. wt. 3600[3]	50	>50.00				>50.00			
Propylene glycol	50	26.38	24.50	28.39	13.38	18.35	16.94	19.87	13.33
1-3 Butylene glycol[2]	50	18.61	17.43	19.88	22.78	11.46	10.29	12.77	12.22
Esters									
Ethylene glycol diformate[2]	5	1.51	1.28	1.78	5.45	0.39	0.27	0.58	2.61
Ethylene glycol monoacetate	50	8.25	7.97	8.63	24.97	3.80	3.35	4.30	8.70
Ethylene glycol diacetate	50	6.86	6.64	7.08	30.33	4.94	4.55	5.35	14.47
Triethylene glycol di (2-ethyl butyrate)[2]	50	8.42	7.17	9.89	6.57	3.13	2.74	3.57	8.02
Triethylene glycol di (2-ethyl hexoate)[2]	50	31.37	25.41	37.26	7.31	20.55	13.53	31.30	2.79
Propylene glycol monoacetate	50	17.93	15.72	20.45	7.60				
Propylene glycol diacetate	50	13.53	11.00	16.64	4.78	3.42	3.01	3.90	7.64
Ethers									
Ethylene oxide	1	0.33	0.29	0.36	7.83	0.27	0.19	0.38	3.54
Propylene oxide	5	1.14	1.05	1.23	16.04	0.69	0.58	0.85	6.44
Dioxane	50	7.12	6.38	8.05	9.18	3.15	2.72	3.65	6.08
Ethylene glycol monomethyl ether	50	2.46	2.13	2.84	5.90	0.95	0.84	1.08	9.22

[1] Trade marked names are not tabulated. Ethers of ethylene glycol bear the trade mark "Cellosolve," and those of diethylene glycol "Carbitol." Polyethylene glycols of high molecular weight carry the trade mark "Carbowax."

[2] Laboratory preparation or specially purified commercial product.

[3] Dispersed temporarily in 1% aqueous "Tergitol" penetrant 7 (sodium sulfate of heptadecyl alcohols).

[4] Suspended in 1% gum tragacanth mucilage.

[5] 50 gm./kg. killed none of 10 rats: 30 gm./kg. killed 1 of 10 guinea pigs: 40 gm./kg. killed 2 of 9.

TABLE 1—*Continued*

MATERIAL[1]	MAX. CONC. FED	RATS				GUINEA PIGS			
		LD50	Limits		Slope	LD50	Limits		Slope
			Lower	Upper			Lower	Upper	
	%								
ETHERS—*Continued*									
Ethylene glycol monoethyl ether	50	3.00	2.51	3.59	6.16	1.40	1.22	1.60	7.75
Ethylene glycol diethyl ether	10	4.39	3.65	5.31	9.00	2.44	2.22	2.69	9.19
Ethylene glycol monobutyl ether	10	1.48	1.15	1.91	3.62	1.20	0.96	1.50	3.92
Ethylene glycol monophenyl ether	2	1.26	1.12	1.42	11.84				
Ethylene glycol monobenzyl ether	2	1.19	1.01	1.39	6.30				
Diethylene glycol monomethyl ether	50	9.21	7.90	10.71	7.07	4.16	3.53	4.89	6.60
Diethylene glycol monoethyl ether	50	9.74	9.01	10.30	26.88	4.97	4.00	6.24	4.31
Diethylene glycol monoethyl ether[2]	50	8.69	7.25	10.41	5.16	3.67	3.14	4.26	7.42
Diethylene glycol diethyl ether	10	4.97	4.75	5.24	30.44	1.85	1.60	2.02	6.12
Diethylene glycol monobutyl ether	50	6.56	5.47	7.86	5.65	2.00	1.72	2.31	8.85
Tetraethylene glycol dimethyl ether	50	5.14	4.53	5.83	7.86				
Propylene glycol monomethyl ether, alpha[2]	50	7.51	6.45	8.62	7.70				
Propylene glycol monomethyl ether, beta[2]	50	5.71	4.94	6.64	8.35				
Propylene glycol monoethyl ether, alpha[2]	50	7.11	6.79	7.43	11.22				
Propylene glycol monoethyl ether, beta[2]	50	7.00	5.95	8.23	8.21				
Dipropylene glycol monoethyl ether, mixed isomers	50	9.10	7.55	10.96	4.04				
ETHER-ESTERS									
Ethylene glycol monomethyl ether acetate	10	3.93	3.29	4.69	5.54	1.25	1.08	1.45	5.71
Ethylene glycol monomethyl ether oleate[2]	50	11.21	7.13	17.59	8.12				
Ethylene glycol monomethyl ether acetyl ricinoleate[2]	50	15.07	10.35	21.93	4.82	12.43	8.80	17.56	2.92
Ethylene glycol monoethyl ether acetate[2]	50	5.10	4.60	5.66	11.52	1.91	1.72	2.13	10.37

TABLE 1—*Concluded*

MATERIAL[1]	MAX. CONC. FED	RATS				GUINEA PIGS			
		LD50	Limits		Slope	LD50	Limits		Slope
			Lower	Upper			Lower	Upper	
	%								
ETHER-ESTERS—*Continued*									
Di(ethylene glycol monobutyl ether) phthalate[2]	50	9.70	7.91	12.00	4.57				
Diethylene glycol monomethyl ether acetate	50	11.96	10.68	13.44	10.00	3.46	2.76	4.32	5.38
Diethylene glycol monoethyl ether acetate	50	11.00	10.40	11.59	21.21	3.93	3.49	4.31	12.74
Diethylene glycol monobutyl ether acetate[2]	50	11.92	10.88	13.10	14.26	2.34	1.90	2.88	4.59
CHLOR-COMPOUNDS									
Ethylene chlorhydrin	1	0.095	0.067	0.117	3.12	0.11	0.077	0.164	9.07
Triethylene glycol dichloride[2]	2	0.25	0.20	0.32	6.04	0.12	0.095	0.154	5.06
Propylene chlorhydrin	5	0.22	0.19	0.27	4.48	0.72	0.62	0.83	6.93
NITROGEN COMPOUNDS									
Ethylene imine[2]	0.2	0.015	0.0054	0.042	2.99				
Propylene imine[2]	0.2	0.019	0.0046	0.079	2.18				
Ethylene diamine	20	1.16	0.98	1.37	7.34	0.47	0.40	0.54	6.91
Morpholine hydrochloride[2]	20	4.58	4.06	5.16	10.23				
Phenyl morpholine[4]	2	0.93	0.87	1.01	20.92				
Monoethanolamine	20	10.20	9.22	11.31	11.92				
Diethanolamine	20	12.76	11.61	13.02	13.67				
Tetraethanol ammonium hydroxide	4	2.25	1.88	2.69	6.29	3.51	3.22	3.82	14.23
Phenyl diethanolamine	1	0.98	0.91	1.05	24.28				
Isopropanolamines, mixed[6]	50	5.24	4.52	6.05	6.20	1.52	1.35	1.71	9.07
Triisopropanolamine	50	6.50	5.62	7.54	6.84	1.58	1.08	2.32	8.16
MISCELLANEOUS									
Glycolic acid	5	1.95	1.36	2.93	8.52	1.92	1.73	2.14	9.86
Glyoxal[2]	10	2.02	1.63	2.52	4.68	0.76	0.55	1.04	2.89
Thiodiethylene glycol	10	6.61	6.10	7.16	15.19	3.96	3.44	4.56	8.92
RELATED NON-GLYCOLS									
Glycerol[2]	50	27.50	23.95	31.61	8.90	7.75	6.03	9.95	3.97
Methanol[2]	50	12.88	11.44	14.40	8.53				
Ethyl alcohol[2]	50	13.66	11.17	16.71	4.57	5.56	4.82	6.41	6.14
Ammonium hydroxide (calculated as NH_3)[2]	1	0.35	0.32	0.40	8.40				
Lactic acid[2]	5	3.73	3.02	4.61	4.04	1.81	1.69	1.93	15.70
Formaldehyde	2	0.80	0.73	0.87	12.44	0.26	0.22	0.30	6.93

[6] 12% mono-, 44% di-, 44% triisopropanolamine.

TABLE 2

DOSES OF VARIOUS DIETHYLENE GLYCOL SAMPLES, AS 50% SOLUTIONS, AND LD50'S FOR RATS ESTIMATED THEREFROM

SAMPLE	STRAIN OF RATS	SEX	DOSAGE	MORTALITY RATIO	CALCULATED BY METHOD OF PROBITS				LD50 FROM LINE[1]
					LD50	Range		Slope	
						Lower	Upper		
			gm./kg.						
1. Commercial, shipped 1937	Wistar	Male	10	0/4	20.76	19.51	22.10	14.57	
			15	1/14					
			20	2/10					
			21	4/10					
			22.5	8/10					
			25	8/8					
16. Commercial, shipped 1937	Wistar	Male	22	0/4	32.40	30.18	34.79	20.46	
			27.5	0/4					
			30	4/10					
			35	4/5					
16. Commercial, shipped 1937	Wistar	Female	8	0/10	12.57	11.31	13.95	11.46	
			10	0/10					
			12.6	6/10					
			15.8	9/10					
			20	8/10					
16. Commercial, shipped 1937	Hybrid A	Male	12.6	0/10					21.1
			15.8	0/10					
			22	5/10					
16. Commercial, shipped 1937	Hybrid B	Male	15.8	2/10					20.2
			22	4/10					
2. Commercial, shipped 1939	Wistar	Male	22	1/10					26.7
			25	4/10					
15. Commercial, redistilled 1931	Wistar	Male	22	1/10					26.6
			27.5	6/10					
3. Commercial, redistilled by Eastman Kodak Co.	Wistar	Male	15	0/10	22.80	19.81	26.29	8.80	
			21	5/10					
			30	8/10					
4. Commercial, redistilled 1938	Wistar	Male	10	0/4	16.00	14.38	17.81	8.23	
			15	4/15					
			16	7/10					
			18	6/15					
			21	9/10					

[1] Estimated from probit vs. log-dosage plot by drawing line parallel to that for sample No. 1, and taking 0/10 mortality as a 5% death rate.

TABLE 2—*Concluded*

SAMPLE	STRAIN OF RATS	SEX	DOSAGE	MORTALITY RATIO	CALCULATED BY METHOD OF PROBITS				LD50 FROM LINE[1]
					LD50	Range		Slope	
						Lower	Upper		
			gm./kg.						
5. Molecular still product	Wistar	Male	22 25 27.5	1/10 4/10 7/10	25.78	24.17	27.56	18.68	
6. Cut 1 of 20 in fractional dist'n	Wistar	Male	15 18 22	6/10 7/10 10/10	15.90	14.35	17.61	13.45	
7. Cut 10 of 20 in fractional dist'n	Wistar	Male	22 27.5	5/10 6/10					24.5
8. Cut 19 of 20 in fractional dist'n	Wistar	Male	22 27.5	5/10 7/10					23.9
9. Cut 1 of 20 in vacuum dist'n	Wistar	Male	12 18 22	1/10 8/10 8/9	15.63	13.71	17.88	10.31	
10. Cut 2 of 20 in vacuum dist'n	Wistar	Male	22 27.5	1/10 9/10					27.8
11. Cut 5 of 20 in vacuum dist'n	Wistar	Male	22 27.5	1/10 4/10					27.8
12. Cut 10 of 20 in vacuum dist'n	Wistar	Male	27.5	6/10					26.6
13. Cut 19 of 20 in vacuum dist'n	Wistar	Male	22 27.5	1/10 4/10					27.8
14. Cut 20 of 20 in vacuum dist'n	Wistar	Male	22 27.5	1/10 1/10					29.2

Wistar strain males more resistant than males of the 2 hybrid strains. The higher toxicity of diethylene glycol for female Wistar rats was a great surprise. The rats used by Laug's group were about half females, and it is thought that this proportion may be the chief cause of the conflict between our data and theirs. Weatherby and Williams (5) also dosed male Wistar rats with diethylene glycol; they observed a toxicity lower than others have reported with mixed sexes (27 cc./kg.) but did not attempt to explain their deviation.

Animal weight is another possible difference which was not investigated for this glycol. Laug and his col-

leagues used rats of 250 ± 75 gm.; we used 105 ± 15 gm. This difference in weight does not seem important because Weatherby and Williams's (3) male rats were 200 ± 25 gm. and their results are closer to ours than to Laug's. The two results are surprisingly close together if calculated in terms of grams per rat. The respective LD50's in these terms are 3.38 and 3.7, with Laug's data showing slightly more diethylene glycol needed to kill one of his rats than we needed to kill one of ours. Laug's article gives some discussion of this point, showing that heavier rats and guinea pigs are more susceptible to some glycol derivatives, but the differences he brings out are not so great as between his data and ours.

Table 2 also includes results obtained on cuts from a careful fractional distillation of commercial diethylene glycol, indicating that only a small part of the toxicity can be charged to an impurity. This impurity, which boils at a lower temperature than the main constituent, is probably ethylene glycol.

In the course of the work represented by table 2 it was found that the toxicity of diethylene glycol or of its fractions did not vary in proportion to the content of acetals, chlorides or sulfur compounds, all of which are hypothetically possible impurities.

Symptoms and Gross Pathology

Fatal or near fatal doses of the glycols and their esters produced no narcosis but varying degrees of sluggish depressed functioning. The ethers, ether-acetates, and chlor-compounds caused narcosis but in most cases only at the LD50 or above. The nitrogen compounds were primarily irritant depressants without narcotic action. Thiodiethylene glycol resembled the glycols, while glycolic acid and glyoxal were like the nitrogen compounds in symptoms.

All the doses caused some degree of digestive tract irritation. The primary action of all the samples, as revealed by gross pathology, was upon the kidneys, rarely proceeding as far as bloody urine and free blood beneath the capsule from the largest dosages. The liver was affected less, but bile was often orange or reddish. The glycol esters have an action quite independent of their glycol content, for they produced livers of a bright eosin color.

Death from the ethers and some of the glycols was delayed about a week; and if only deaths within the first 2 days had been considered, the LD50's reported would usually have been much higher.

Related Non-Glycols

Table 1 includes 6 materials which are not glycol derivatives whose toxicity was determined by the same methods. Some comparisons with these compounds are pharmacologically justified, but the data must be used with caution. It appears correct to say that in single doses, glyoxal is 40% as toxic as formaldehyde, that glycolic acid is twice as toxic as lactic acid, that ethylene diamine is 33% as toxic as ammonia, and that propylene glycol is about as toxic as glycerol, or one-half as toxic as ethyl alcohol. Other comparisons with these non-glycols are probably

unjustified because of differences in mode of action.

Comparisons with Other Workers

Table 3 lists our results together with some findings recently reported by others. No attempt has been made to include all the literature in this table; it is restricted to work by comparable methods.

Diethylene glycol has been adequately discussed. It is possible that sex and weight differences may go a long way toward explaining other discrepancies in the data. We have felt that it was more important to assemble results on a large number of compounds obtained by exactly the same method than it was to attempt to confirm the already disagreeing data in the literature.

TABLE 3

Some Comparisons of Results of This Study with Those of Others

LD50 of this study in gm./kg. of various dilutions, of others in cc./kg. undiluted. Parentheses give reference number

Material	Rats	Guinea pigs	Mice
Ethylene glycol	8.54 5.50 (1)	6.61 7.35 (1)	7.5 (7) 13.1 (1)
Diethylene glycol	20.76 14.8 (1) 27 (5)	13.21 7.76 (1)	23.7 (1)
Triethylene glycol	22.06	14.66	18.5 (7)
Propylene glycol	26.38 21.0 (1) 33.5 (6)	18.35 18.8 (1)	22.0 (7) 23.9 (1)
Dioxane	7.12 5.17 (1)	3.15 3.90 (1)	
Ethylene glycol mono ethyl ether	3.00 3.46 (1)	1.40 2.79 (1)	4.31 (1)
Diethylene glycol mono ethyl ether	9.74 5.54 (1)	4.97 3.87 (1)	6.58 (1) 12.5 (7)

Conclusions

This paper, presented as preliminary information obtained upon small animals, bears only upon single-dose toxicity, not upon the effects of repeated administration.

In the ethylene glycol series, as molecular weight increases, acute toxicity by mouth goes down.

Esters of glycols may be more or less toxic than the glycol, depending on the acid radical, but some at least have an action of their own different from that of the glycol.

Glycol ethers have a greater acute toxicity than the glycol, varying with the other alcohol involved. They possess narcotic action. The alpha ethers of propylene glycol appear to be less toxic than the beta ethers.

Esters of glycol ethers have a lower acute toxicity than the glycol ethers.

Chlorine derivatives have a high acute toxicity.

Nitrogen derivatives vary enormously among themselves, the oxygenated ones usually being less toxic than those without oxygen.

Summary

The single dose toxicities of 60 glycols and glycol derivatives to male albino rats and to guinea pigs of mixed sexes are presented. All are expressed in terms of LD50 in gm./kg., calculated by the method of probits and obtained by the same rigidly defined experimental procedures.

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Effect of Feeding Polyhydric Alcohols on Tissue Lipids and the Resistance of Rats to Extreme Cold

G. S. STOEWSAND, H. A. DYMSZA, S. M. SWIFT, M. A. MEHLMAN[1] AND D. G. THERRIAULT

Nutrition Branch, Food Division, U. S. Army Natick Laboratories and U. S. Army Research Institute of Environmental Medicine, Natick, Massachusetts

ABSTRACT Three polyhydric alcohols, propylene glycol (PG), 1,3-butanediol (BD), and glycerol are being investigated as synthetic energy-yielding food compounds. These polyols were fed to growing rats within 2 environments in order to assess their relative calorie potential for growth, and to study comparative responses on epididymal adipose tissue and liver lipids. In addition, resistance to extreme cold (−20°) was also measured. Weight gain was lower in rats fed BD or PG diets for 4 weeks, but was similar to that of controls when growth was calculated on a per unit food or calorie intake basis. Epididymal adipose tissue was lower in the groups fed BD and PG. Liver lipids and liver cholesterol increased in the rats fed PG. A lowered resistance to intense cold was exhibited in both BD and PG groups, which may indicate that the lowered fat stores of these animals were a causative factor in this response. Liver cholesterol was reduced by moderate cold exposure (5°) only in rats fed PG. The rats fed the glycerol diets did not show any of these metabolic changes observed in the groups fed BD or PG.

Compounds that can be chemically synthesized or derived from natural products, such as petroleum, and algae, offer a future potential as food sources. Our laboratories recently reported that the utilization of 1,3-butanediol, as a source of dietary energy in the rat, results in lowered epididymal adipose tissue lipids and a slight increase in liver and plasma neutral lipids (1, 2).

The purpose of the present paper is to describe the effects of feeding diets containing different non-toxic polyhydric alcohols on growth, liver lipids, liver cholesterol and epididymal adipose tissue of rats exposed to temperate (25°) or cold (5°) environments. In addition, resistance to intense cold (−20°) was measured by comparing the survival rates of rats fed these compounds.

EXPERIMENTAL METHODS

Male weanling albino rats of 40 to 60 g each, of the CFE Carworth strain, were divided equally into the following 4 dietary treatments: 1) 30% fat (control); 2) 30% fat + 20% 1,3-butanediol (BD); 3) 30% fat + 20% propylene glycol; and 4) 30% fat + 20% glycerol. They were housed in individual cages and supplied with water and diets ad libitum.

These 4 diets were fed to: 1) 10 rats per each dietary treatment maintained for 4 weeks at 25°; 2) 10 rats per each dietary treatment maintained for 4 weeks at 5°; and 3) 18 rats per each dietary treatment maintained for 5 weeks at 25°, followed by exposure to −20° until each group had 9 surviving rats (50% survival).

The composition of the 30% fat diet, and the 30% fat diet with 20% BD[2] substituted isocalorically for carbohydrates has been reported (1), except that agar and additional water (agar-gel-type diet) was not added to the diets in the present study. By rat-bioassay studies of Dymsza et al.[3] have shown that BD contains 5 to 6 kcal/g of net energy. Diets containing 20% propylene glycol and 20% glycerol had all other dietary ingredients identical with the BD diet. Propylene glycol has a gross energy value of 5.8 kcal/g (3). Hanzlik et al. (4, 5) have shown that increased growth was observed in rats fed

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[1] Present address: Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin.

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[3] Dymsza, H. A., S. A. Miller and A. M. Browning 1963 Utilization of 1,3-butanediol as a synthetic source of dietary energy. Proc. 6th International Congress of Nutrition, E. and S. Livingstone, Lts., Edinburgh, p. 498 (abstract).

diets in which propylene glycol was substituted for dextrose on a weight basis, with equalized food intake to the control rats. It was estimated, therefore, that this glycol contains 4 to 5 kcal/g of metabolizable energy. The metabolizable energy supplied by glycerol was estimated as 4.1 kcal/g by Brambila and Hill (6) in the growing chicken, and this value was assumed in the present study.

After 4 weeks, 5 rats in each of the dietary treatments at 25° and 5° were killed. Liver was immediately excised, weighed, and total lipids extracted with chloroform-methanol (2:1). Non-lipid components were separated from the chloroform-methanol extract by means of partition chromatography on a Sephadex G-25 column as described by Therriault and Poe (7). Total lipid was determined gravimetrically after evaporation of the solvent under a stream of nitrogen at reduced pressure. Liver cholesterol was determined by a modified fluorometric procedure of Albers and Lowry (8). Both epididymal fat pads were quantitatively excised from each animal and weighed. The pads were dried in a vacuum oven at 50° to constant weight.

Statistical methods used in this study are described in Steel and Torrie (9). Regression analysis on the survival rates of the rats at −20° was performed with the aid of a G.E. 225 Computer.

RESULTS AND DISCUSSION

The average weight gain, food intake, calculated metabolizable energy intake (ME), food and calorie efficiencies of rats fed the 4 diets for 4 weeks at 25° and 5° are presented in table 1. The food and calorie efficiencies of the rats consuming the diets with polyhydric alcohols are similar to the efficiencies of rats fed the 30% fat control diet at 25° or 5°. All animals maintained in the cold had food efficiency values about one-half that of rats in the temperate environment. This increased level of food consumption with smaller body weight gains reflects the increased calorie demands of the animal in cold acclimation (10). The calorie intake of the rats fed either the BD or propylene glycol diets was lower than that of the controls or glycerol-fed rats at 25°. However, at 5° the calorie intake of the rats fed propylene

TABLE 1

Average weight gain, food consumption, metabolizable energy intake (calculated), food and calorie efficiency of rats fed different polyols for 4 weeks at 2 environmental temperatures

	Control [1]		BD		PG		Gly	
	25°	5°	25°	5°	25°	5°	25°	5°
Weight gain, g	143±10 [2]	116±3	118±3	81±6	137±6	102±10	160±5	116±6
Food intake, g	318±16	394±9	257±6	326±7	290±9	406±15	342±12	407±12
Food efficiency [3]	45	29	46	25	47	25	47	28
ME [4] intake, kcal/rat	1827	2009	1285	1630	1392	1949	1607	1913
Calorie efficiency [5]	8.8	5.8	9.2	5.0	9.8	5.2	10.0	6.1

[1] Control indicates 30% fat diet; BD indicates 30% fat + 20% 1,3-butanediol diet; PG indicates 30% fat + 20% propylene glycol diet; Gly indicates 30% fat + 20% glycerol diet.
[2] Mean ± SE.
[3] As g gain/g food intake × 100.
[4] Metabolizable energy (calculated).
[5] As g gain/kcal intake × 100.

glycol was similar to that of the controls and of the animals fed glycerol. At 5° the BD-fed rats still had a relatively lowered calorie intake as compared with the other groups at this environment.

The epididymal adipose tissue weight, liver lipids, and liver cholesterol of rats maintained at the temperate and cold environments for 4 weeks are presented in table 2. In view of the excellent correlation between fresh weight of adipose tissue and extractable lipid content (11) it was felt that the epididymal adipose tissue weight could be used as a measure of alterations in adipose tissue lipids. The epididymal fat pads from rats maintained at 25° had a water content of 7 to 13%, whereas rats maintained at 5° had a water content of 10 to 23%. Boshart et al. (11) had observed that the water content of epididymal fat tissue increases with decreasing adipose tissue weight. In order to eliminate any variation due to water composition, we measured the dry weight of the epididymal fat pads. It was found that 93 to 97% of the dry weight was lipid consisting mainly of triglycerides.

All animals had reduced adipose tissue pads when exposed to the cold; however, rats fed the glycols, BD and propylene glycol, had significantly lower ($P < 0.05$) adipose tissue weight as compared with controls or glycerol-fed rats at both environments. In view of the lowered food intake in the rats fed BD and propylene glycol at 25° as compared with the control or glycerol-fed groups, the decreased epididymal adipose tissue weights may be merely a reflection of the lowered intake of calories. This does not appear to be the case since the calculated calorie efficiency (table 1) of all groups, within each of the 2 environments, was similar and the quantity of epididymal adipose tissue is presented in table 2 on a unit body weight basis. In addition, the calculated calorie consumption of the control, glycerol, and propylene glycol groups was similar in the 5° environment, yet the adipose tissue per 100 g body weight of the rats fed propylene glycol was still very much lower. This reduction in adipose tissue in the animals fed glycols may, therefore, indicate that there is an increased lipolysis of adipose tissue triglycerides with increased fatty acid mobilization, since previous investigations from these laboratories have shown that BD-fed rats have elevated plasma nonesterified fatty acids (2). Marsh and Whereat (12) observed that plasma nonesterified fatty acids represent the form in which fatty acids are mobilized from the fat depots.

Liver lipids and liver cholesterol were significantly higher ($P < 0.05$) in rats fed propylene glycol at 25° than in any of the other treatments. Cold reduced both the lipid and cholesterol content of the livers of rats fed propylene glycol. Treadwell et al. (13, 14) and Sellers and You (15, 16) observed that cold either decreases or prevents fatty liver in rats fed choline-deficient diets. Wood (17) has reported recently that cold had little or no effect on liver and other tissue cholesterol levels provided the diet was adequate in choline and inositol. In the present study, cold had no effect on the liver cholesterol levels of the controls, or the animals fed BD or glycerol.

Although the quantity of total liver lipids of rats fed BD at 25° was slightly higher than the liver lipids of the controls, analysis of variance showed no significant differences ($P > 0.05$) which is in agreement with our previous report (2). However, in the cold, the liver lipids of the BD-fed rats, as well as the propylene glycol group, was significantly increased ($P < 0.05$) as compared with the controls in the same environment.

When the rats were fed the 4 diets for 5 weeks and placed in the intense cold, the number of hours 50% of each group survived is illustrated by negative regression lines in figure 1. The rats consuming the glycols (BD or propylene glycol) withstood the cold only about one-half as long as the rats fed the control or glycerol diet. An analysis of variance of these data with a comparison of slopes (b values) of each line with the control group showed that the survival rate was significantly decreased ($P < 0.05$) when rats were fed the BD or propylene glycol diet. Feeding glycerol produced a survival rate similar to that of the controls. The amount of epididymal adipose tissue of rats at either 25° or 5° (table 2) appears to be correlated with the resistance of the animals to the ex-

TABLE 2

Epididymal adipose tissue weight, liver lipids, and liver cholesterol of rats fed different polyols for 4 weeks at 2 environmental temperatures

	Control [1]		BD		PG		Glycerol	
	25°	5°	25°	5°	25°	5°	25°	5°
Epididymal adipose tissue,[2] g/100 g body wt	1.27 ± 0.11 [a,3]	0.71 ± 0.06 [b]	0.84 ± 0.02 [b]	0.41 ± 0.04 [c]	0.89 ± 0.05 [b]	0.49 ± 0.04 [c]	1.38 ± 0.12 [a]	0.78 ± 0.05 [b]
Liver lipids, mg/g liver	68.19 ± 6.20 [bc]	52.01 ± 2.33 [d]	76.01 ± 5.21 [b]	68.43 ± 2.82 [bc]	99.50 ± 3.45 [a]	73.50 ± 3.41 [bc]	60.19 ± 5.36 [cd]	62.79 ± 2.00 [cd]
Liver cholesterol mg/g liver	2.38 ± 0.20 [cd]	2.28 ± 0.07 [cd]	2.84 ± 0.26 [c]	2.88 ± 0.08 [c]	6.63 ± 0.41 [a]	3.52 ± 0.20 [b]	2.42 ± 0.09 [cd]	2.03 ± 0.13 [d]

[1] Control indicates 30% fat diet; BD indicates 30% fat + 20% 1,3-butanediol diet; PG indicates 30% fat + 20% propylene glycol diet; glycerol indicates 30% fat + 20% glycerol diet.

[2] Dry weight.

[3] Mean ± SE. Means having a common letter in superscript are not significantly different ($P > 0.05$)

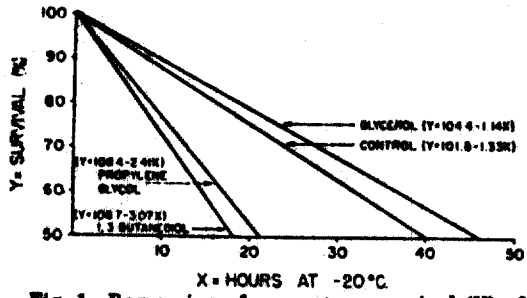

Fig. 1 Regression of percentage survival (Y) of rats fed diets containing specific polyhydric alcohols on number of hours (X) exposed to −20°. Diets were fed for 5 weeks prior to exposure.

treme cold. It is postulated that the quantity of epididymal adipose tissue is a good indication of the animal's resistance to intense cold. LeBlanc (18) observed that increased dietary lipids permitted higher sustained body temperatures of rats exposed to severe cold. Increases in dietary lipids have been shown in our laboratories to increase quantities of epididymal adipose tissue lipids in the rat (1,2).

Skin resistance and electromyogram measurements [4] were taken in rats fed the control 30% fat diet and BD at 25° and 5°. Normal increased skin resistance and shivering occurred in both groups of rats placed in the cold, indicating that rats consuming BD can initially respond to a lowered temperature.

From these studies it appears that feeding relatively large amounts of specific non-toxic glycols as a source of calories decreases the quantity of adipose tissue per gram of body weight which could be a causative factor in decreasing the resistance of the rat to intense cold. The mechanism whereby propylene glycol produces elevated liver lipids and liver cholesterol is unknown.

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[4] Grass Model 7 Polygraph, Grass Instruments, Quincy, Massachusetts.

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Synthetic Sources of Calories in the Diets of Rats and Dogs

G. S. Stoewsand and *H. A. Dymsza*, Nutrition Branch, Food Division, U.S. Army Natick Laboratories, Natick, Massachusetts

Introduction

Compounds that can be used as sources of dietary energy which could be synthesized in the laboratory may be necessary at a future time for austere situations such as long term space travels, life in uninhabitable regions or in areas of the world that become agriculturally depleted due to a variety of causes.

Glycols and other polyhydric alcohols as a source of utilizable calories were first proposed by Schlüssel [5]. Dymsza et al. [2] at the 6th Nutrition Congress showed that the glycol, 1,3-butanediol (BD) contains between 5 to 6 calories of net energy in the growing rat. Miller et al. [4] showed that 1,3-butanediol is metabolized via carbohydrate pathways.

Previous studies by Stoewsand et al. [6] and Mehlman et al. [3] have shown that feeding BD has an effect on lipid stores and perhaps lipid metabolism in the rat. Experiments were conducted to further investigate lipid interactions with feeding polyhydric alcohols in both the rat and dog.

Experimental Studies

Rat Experiment -- Male, weanling, Carworth strain albino rats were fed diets containing 30 % fat and either 20 % 1,3-butanediol (BD), 20 % propylene glycol (PG) or 20 % glycerol *ad libitum* for 4 weeks. These diets are presented in Table I.

The 4 week weight gain, food intake, and calculated calorie intake is presented in Table II. In the rats fed the glycols (BD or propylene glycol), there were lowered weight gains with lowered food and energy intake; however, as observed in Table III, the food and calorie efficiency of the rats consuming the glycols were similar to both the control and the glycerol fed groups.

At the end of 4 weeks, the rats were killed and both epididymal fat pads were quantitatively excised from each animal and weighed after vacuum drying. Livers were analyzed for total lipids by the method of Therriault and Poe [7] and cholesterol by the method

Table I. Experimental Diets

	Basal %	1,3-Butanediol %	Propylene Glycol %	Glycerol %
Casein	22.0	22.0	22.0	22.0
Glucose Monohydrate	13.0	3.0	3.0	3.0
Sucrose	13.0	3.0	3.0	3.0
Dextrin	13.0	3.0	3.0	3.0
Lard	22.5	22.5	22.5	22.5
Corn Oil	7.5	7.5	7.5	7.5
Cellulose	3.6	13.6	13.6	13.6
Minerals	4.0	4.0	4.0	4.0
Vitamins	1.0	1.0	1.0	1.0
Choline Chloride (50 %)	0.4	0.4	0.4	0.4
1,3-Butanediol (BD)	—	20.0	—	—
Propylene Glycol (PG)	—	—	20.0	—
Glycerol	—	—	—	20.0
kcals / g	5.1	5.0	4.8	4.7

Table II. Average weight gain, food consumption and ME intake (calculated) of rats fed 3 polyols for 4 weeks

	Basal	BD [2]	PG [3]	Glycerol
Weight gain (g)	143 ± 10 [1]	118 ± 3	137 ± 6	160 ± 5
Food intake (g)	319 ± 16	257 ± 6	290 ± 9	342 ± 12
ME intake (kcal/rat)	1627	1285	1392	1607

[1]) Mean ± SE.
[2]) 1,3-Butanediol.
[3]) Propylene Glycol.

Table III. Average food and caloric efficiency of rats fed 3 polyols for 4 weeks

	Basal	BD	PG	Glycerol
Food efficiency [1])	45	46	47	47
Calorie efficiency [2])	8.8	9.2	9.8	10.0

[1]) g gain/g food intake x 100.
[2]) g gain/kcal intake x 100.

of Albers and Lowry [1]. As seen in Table IV, the epididymal adipose tissue as expressed by g/100 g body weight is significantly ($P<0.05$) lower in the rats fed the glycols (BD or PG) than the controls or glycerol fed rats. Total liver lipids and liver cholesterol were significantly higher ($P<0.05$) in the animals fed PG.

Table IV. Average epididymal adipose tissue weights, liver lipids and liver cholesterol of rats fed 3 polyols for 4 weeks

	Basal	BD	PG	Glycerol
Epididymal adipose tissue [1]) (g/100 g body wt.)	1.29 ± 0.11[a2]	0.84 ± 0.02[b]	0.89 ± 0.05[b]	1.38 ± 0.12[a]
Liver lipids (mg/g liver)	68.19 ± 6.20[ab]	76.01 ± 5.21[b]	99.50 ± 3.45[c]	60.19 ± 5.36[a]
Liver cholesterol (mg/g liver)	2.38 ± 0.20[a]	2.48 ± 0.26[a]	6.63 ± 0.41[b]	2.42 ± 0.09[a]

[1]) Dry weight.
[2]) Mean ± SE. Means having a common letter in superscript are not significantly ($P > 0.05$) different.

Dog Experiment – In order to asses BD as an energy yielding food source during muscular activity, studies were initiated with 6 adult, male Beagles that were trained for treadmill running.
The 30 % fat basal diet (Table I) was fed to all dogs in one meal per day, initially at 100 kcal/kg body weight, but adjusted so that initial body weight was maintained. After a 2 week feeding period, 3 of the dogs were placed on the 30 % fat and 20 % BD diet (Table I). The 3 remaining dogs continued eating the same basal diet. The endurance capacity of the dogs was measured on treadmills 24 hours after eating their daily meal at four specified times. Figure 1 shows a series of 4 horizontal bar graphs for each individ-

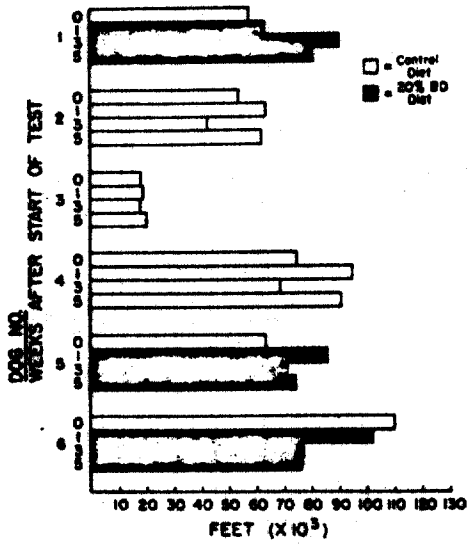

Fig. 1
Endurance capacity of adult beagles consuming 1,3 butanediol.

ual dog. The first bar graph for each dog represents 0 time or baseline performance of individual dogs fed the basal diet for 2 weeks. The bottom 3 bar graphs for each dog represents the performance at 1, 3, and 5 weeks after feeding the BD diet to dogs No. 1, 5, and 6. Dogs No. 1, 2, 3, 4, and 5 all increased their treadmill performance at the 5th.

week. However, the performance of dog No. 6 decreased from his baseline, but the 77,000 feet he ran was still comparatively high for the group. Also, heart worms were later found in dog No. 6 which could have decreased his activity after his initial performance.

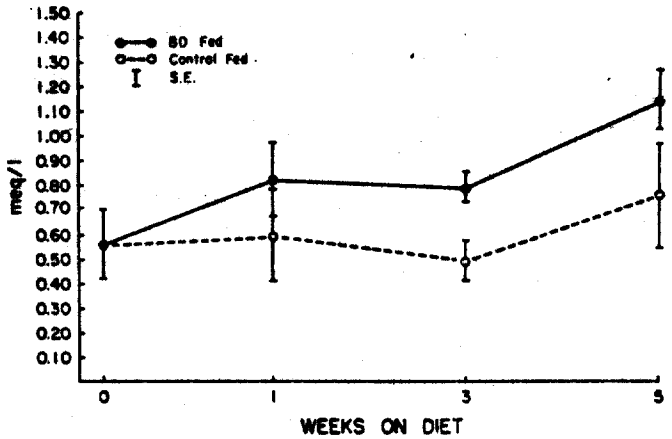

Fig. 2 Plasma free fatty acids (FFA) of dogs consuming 1,3-butanediol.

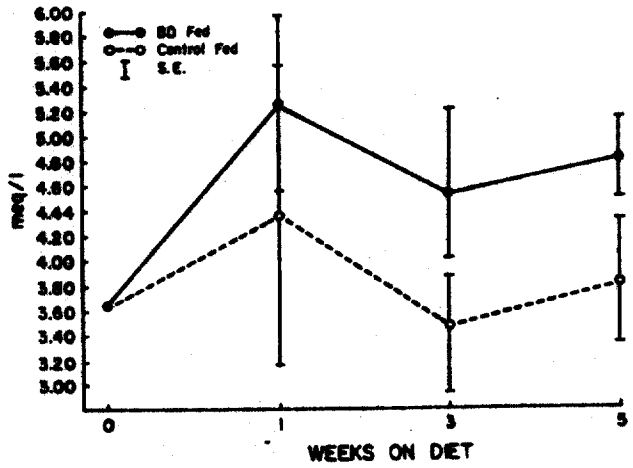

Fig. 3
Plasma free fatty acids (FFA) of dogs consuming 1,3-butanediol after exercise.

Plasma free fatty acids (FFA) increased in the growing rat fed BD as Mehlman et al. [3] reported, therefore, plasma FFA were measured in the 6 dogs before and after their treadmill exercise. The plasma FFA of dogs just before exercise started is shown in Fig. 2. The dogs fed the 30 % fat diet for 2 weeks (0 time) had about 0.57 meq l FFA in their plasma. Feeding BD for 1 week increased plasma FFA which remained at the same proportionally higher level than the controls for the 5 weeks on diet. Fig. 3 shows plasma FFA of dogs after exhaustive running. The baseline performance at 0 weeks shows a 6-fold increase of plasma FFA due to the exercise. Again, the FFA in BD fed dogs after exercise was still proportionally higher through the 5 week period on the diet.

The dogs fed the 20 % BD diet were then placed on a diet whereby all of the carbohydrates of the basal diet were replaced by 1,3-butanediol. A definite incoordination of the dogs was observed 2 to 4 hours after eating. Additional studies indicate that when this glycol is injected into the dog at 6 ml/kg a depression of the central nervous system occurs, similar to ethanol, as seen by electroencephalograms in Fig. 4.

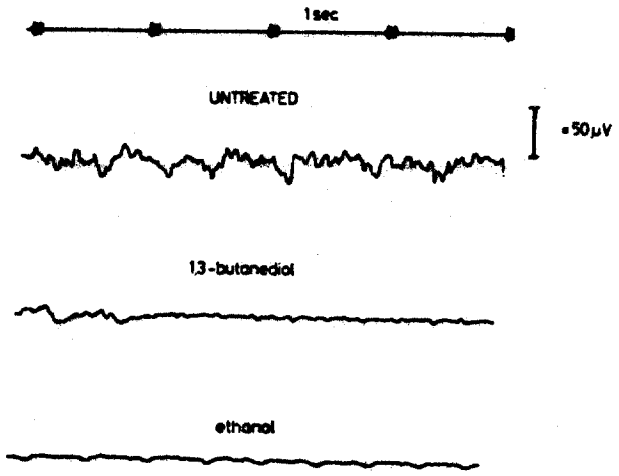

Fig. 4
Electroencephalograms of dogs injected with either 1,3-butanediol or ethanol. Measurements were made with a Grass Model 7 polygraph.

Conclusions

These studies show that feeding glycols as a source of dietary calories to 2 species have definite effects on adipose tissue, liver lipids, plasma free fatty acids, and on the central nervous system. These compounds may have limited use *per se* as a practical component of specific rations but may be indicative perhaps of model compounds, and a starting point for research and development of synthetic sources of calories. These investigations do point out, however, that continued studies with glycols, and other food energy yielding derivatives with hydroxyl groups should encompass its possible influence on lipid metabolism, and its potential effect on the central nervous system at various dietary levels.

Summary

Two glycols, 1,3-butanediol and propylene glycol, can be used as a synthetic source of food calories in the diets of rats and dogs. These compounds, however, produce quantitative lipid changes in adipose tissue, blood and liver. Also, 1,3-butanediol administered to the dog in relatively high levels can produce a central nervous system depression.
Further nutritional investigations with glycols and other polyhydric alcohols may produce useful food sources with relatively high caloric content, but possible changes in lipid metabolism and/or action on the central nervous system must not be overlooked.

In conducting the research described in this report, the investigator(s) adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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RANGE-FINDING TOXICITY TESTS ON PROPYLENE GLYCOL IN THE RAT*

JOAN F. THOMAS, RAYMOND KESEL, AND HAROLD C. HODGE

Department of Pharmacology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

TWO considerations underlay our proposal to use propylene glycol as a solvent in toxicity tests: 1) Certain water-insoluble materials are soluble in it, and 2) it has low toxicity.

TABLE 1

ACUTE TOXICITY OF PROPYLENE GLYCOL IN RATS

DOSE		AV. BODY WT.	MORTALITY (24 HRS.)
A. Intraperitoneal Administration (approx. LD50—13 cc./kg.)			
(cc.)	(cc./kg.)		
1.0	3.4	292	0/5
2.0	6.7	303	0/5
2.5	12.0	210	3/5
3.0	16.8	179	4/5
5.0	34.6	144	5/5
B. Intramuscular Administration (approx. LD50—20 cc./kg.)			
4.0	14.5	276†	0/5
5.0	17.8	292†	3/5
6.0	27.2	296†	5/5
7.0	23.3	303†	3/5
8.0	36.9	215	5/5
C. Subcutaneous Administration (approx. LD50—28 cc./kg.)			
2.0	10.0	200	0/5
4.0	22.7	190	0/5
5.0	28.4	222	2/5
6.0	32.9	208	5/5
8.0	38.6	208	5/5
D. Oral Administration (approx. LD50—28 cc./kg.)			
3.0	18.2	167	0/5
4.0	27.7	147	4/5
5.0	31.6	164	3/5
8.0	50.4	161	5/5
10.0	57.2	178	5/5

† Males; others were female rats.

Range-finding toxicity tests were carried out on adult albino male and female rats. The propylene glycol was administered intraperitoneally, intramuscularly, subcutaneously, and orally (the latter rats had been fasted for 24 hours) to groups of 5 rats each at the dosage levels indicated in table 1. The 24-hour mortality was recorded. Approximate LD50's were determined graphically by plotting the data on probit-logarithm dosage paper. The LD50's were found to be of the following orders of magnitude: intraperitoneally, 13 cc./kg.; intramuscularly, 20 cc./kg.; subcutaneously, 28 cc./kg.; orally, 28 cc./kg.

TABLE 2

SUMMARY OF WORK DONE ON ACUTE TOXICITY OF PROPYLENE GLYCOL IN RATS

ROUTE	DOSE KILLING NONE OR FEW	DOSE KILLING ALL OR MOST	REFERENCE
	(cc./kg.)	(cc./kg.)	
Intravenous	8	25	(1)
	6*	12	(3)
Subcutaneous	18	24**	(2)
	20	30	(3)
	22	32	This report
Intramuscular	7	15	(1)
	14	16‡	(2)
	12	18	(3)
	14	27	This report
Intraperitoneal	7	30	This report
Oral	15	20	(2)
	25	35§	(3)
	18	50	This report

** 1/10
‡ 4/5
§ 3/5

In table 2 the data of this report and more or less comparable data taken from the literature are presented not as LD50 values but in terms of the lethal range, i.e., the dose that killed none or few versus the dose that killed all or most. The data exhibit marked consistency. The lethal dose is least when the propylene glycol is administered intravenously; the intramuscular dose is somewhat less than the subcutaneous; the intraperitoneal dose appears to be comparable to these doses. Somewhat larger doses are required orally.

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QUANTITATIVE GASTROINTESTINAL ABSORPTION AND RENAL EXCRETION OF PROPYLENE GLYCOL

W. VAN WINKLE, JR.

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California

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Propylene glycol is practically a non-toxic solvent or vehicle and preservative for medicinal and food products, and possesses a definite glycogenic action. These collective properties are not shared by other glycols thus far investigated, or by ethyl alcohol (1, 2, 3). These desirable properties have resulted in an extensive and increasing utility in the pharmaceutical and food industries and in medicine for drugs, chemicals, vitamins, and hormones for internal use, and recently it has been proposed as an antiseptic aerosol (9). Oral administration of propylene glycol has frequently resulted in such rapid, spectacular absorption that it was thought desirable to obtain more data according to more direct criteria of quantitative absorption and excretion than the indirect criteria used heretofore. It appeared especially desirable to determine how the absorption from the alimentary canal compared with that of ethyl alcohol, which obeys fairly definite laws. This was deemed essential for a further and more complete understanding of this glycol before its general use in pharmaceutical products and foods is officially approved. This report presents the results of extensive studies of the quantitative aspects of gastrointestinal absorption and of renal excretion of this glycol.

Most of the data on absorption were obtained on cats, comparisons with rats and rabbits being made where indicated. Differences between species will be pointed out where such differences occurred. In general, ligated intestinal loops were employed for the determination of absorption, variable amounts and concentrations of propylene glycol being injected directly into the loop.

ABSORPTION

Absorption according to anatomical site of the gastrointestinal tract

A total of 48 cats, 72 rats and 16 rabbits was used for this purpose. In some experiments loops of intestine 15 cm. long were ligated, in others the entire stomach or colon was ligated and the absorption from these regions determined. Ten cc. of 10 per cent propylene glycol per kilo of body weight were injected into the loops. At the end of the absorption period each loop

was removed and washed with 150 cc. of distilled water. The volume of washings was made up to 200 cc. and the propylene glycol determined in an aliquot portion according to the method of Lehman and Newman (4). The results on cats are shown in figure 1 as curves of average absorption from different anatomical regions of the alimentary canal. The results on rabbits and rats were tabulated, but the tabulations are omitted to save space. For one thing, the absorption in rabbits and cats was the same, and therefore, figure 1 suffices to illustrate the actual, quantitative results in both species.

Rats showed a somewhat more rapid absorption of the glycol than cats and rabbits, but the quantitative relations were similar. For instance, absorption from the jejunum was complete within 20 to 30 minutes in rats, but was only 91 per cent in cats and rabbits at the end of 1 hour. All animals showed

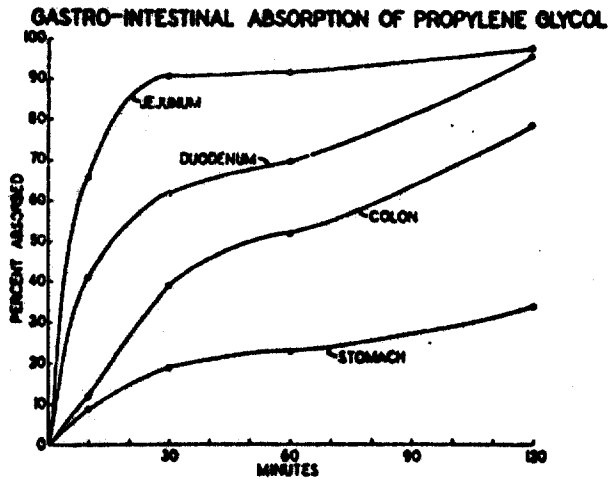

FIG. 1. GASTROINTESTINAL ABSORPTION OF PROPYLENE GLYCOL ACCORDING TO ANATOMICAL SITE

absorption of this glycol from both the stomach and colon, in agreement with the absorption of ethyl alcohol reported by Hanzlik and Collins (5). No difference between the absorption of propylene glycol from the jejunum and ileum could be detected and the results were lumped together.

Effect of concentration on absorption

Ten cats were used. Four equal loops of jejunum were tied off and the glycol introduced in concentrations of 5, 10, 50 and 100 per cent. Care was taken to keep the absolute quantity of the glycol the same in each loop. In half the cats the absorption period was 15 minutes, in the others one hour. The results obtained are shown in table 1.

It is seen that the lower concentrations of propylene glycol were more rapidly absorbed than the higher, the difference between the per cent absorbed

from a 10 per cent concentration and a 100 per cent concentration being more than twice the variation between the absorption of the same concentration in different animals. This is different from phenol and alcohol where concentration made no difference in absorption. At the end of one hour all concentrations were about equally absorbed.

Effect of area of absorbing surface on intestinal absorption

Ten cats were used. Two loops of jejunum, one 15 cm. and one 75 cm. long, were tied off. Equal quantities of 10 per cent propylene glycol were injected into each loop. In half the cats the absorption period was 15 minutes, in the others one hour. The average per cent absorption from the shorter loops (smaller area) was 83.7 per cent in 15 minutes and 91 per cent in one hour. The results were almost the same for the intestinal loops 5 times longer, i.e., averages of 82.8 per cent in 15 minutes and of 92.8 per cent in one hour. Accordingly, and within the limits used, the length of the intestinal loop,

TABLE 1

Effect of concentration of propylene glycol on absorption from the jejunum

CONCENTRATION OF GLYCOL USED	AMOUNT ABSORBED IN 15 MINUTES	AMOUNT ABSORBED IN 1 HOUR
per cent	*per cent*	*per cent*
5	66.4	91.5
10	73.3	95.2
50	58.3	88.2
100	49.4	90.7

and hence the area of intestinal mucosa in contact with the glycol, had no demonstrable effect on the quantity and the rapidity of the absorption of glycol. This was also true for alcohol (5).

Inhibition of absorption

The results on the effects of concentration of propylene glycol on absorption suggested that the higher concentrations might cause an inhibition of absorption. Inhibition of absorption has been shown for alcohol, phenol and iodide (5, 6, 7). Experiments similar to those used for alcohol were made with propylene glycol. In each of 5 cats, 4 equal loops of jejunum were tied off. Five cc. of 10 per cent propylene glycol per kilogram body weight were injected into the first loop. After 15 minutes a blood sample was taken from the mesenteric vein of this loop and the loop excised. The second loop was then injected with the same quantity of propylene glycol, and immediately 2.5 cc. of 50 per cent propylene glycol per kilogram were injected rapidly into the saphenous vein. At the end of another 15 minutes, a blood sample was taken, as before, and the loop excised. The third loop was injected

next with propylene glycol, as before, and a slow intravenous infusion of 50 per cent propylene glycol begun. After 15 minutes, a blood sample was taken from a mesenteric vein and the infusion stopped. Both the third and fourth loops were excised, the latter loop being a control on possible re-excretion of propylene glycol into the intestine. The results are shown in table 2.

It is seen that, with a rising concentration of propylene glycol in the intestinal venous blood, there was a decrease in rate of absorption of the glycol from the lumen of the same intestine. The saturation of the blood might be responsible for the inhibition of absorption. However, the effect might be due to a decreased blood flow through the intestine resulting either from a local damaging effect produced by the handling of the loops, to the operation involved in removing portions of the gastrointestinal tract, or to the intravenous injection of a presumably hypertonic solution resulting in a fall of blood pressure, splanchnic pooling, and decreased blood flow through the intestine.

To test this, a second series of loops was used exactly similar to those

TABLE 2

*Increasing mesenteric blood concentration of propylene glycol on absorption of the glycol from loops of small intestine**

	LOOP 1	LOOP 2	LOOP 3	LOOP 4
Glycol absorbed (per cent)	85.4	78.5	70.4	0
Mesenteric blood concentration of glycol (mgm. per cent)	345	869	1321	

* The loops were injected with glycol and removed in the order numbered in the table, Loop 1 being first, and Loop 4, last.

described above but substituting another substance for the intravenous injection. It was thought desirable to use some naturally occuring body constituent in an equivalent osmotic concentration for this injection. In order to choose such a substance, it was first necessary to determine how propylene glycol behaved osmotically. This was tested simply by determining the concentration necessary to produce hemolysis in cat's blood. To a series of tubes containing 5 cc. quantities of various concentrations of propylene glycol in distilled water, or in 0.9 per cent sodium chloride, 0.02 cc. of cat's blood was added. The mixture was shaken, allowed to stand for 40 minutes, centrifuged, and the hemoglobin estimated in the supernatant liquid with a photoelectric colorimeter.

Propylene glycol was found to hemolyze blood in all concentrations in distilled water. However, in 0.9 per cent saline solution, hemolysis did not commence until the concentration of propylene glycol reached 38 per cent, and was complete at 45 per cent. Hence propylene glycol acted much like urea in its osmotic behavior. For this reason a 40 per cent solution of urea

was used as the control injection fluid. A 40 per cent aqueous urea solution is the molecular equivalent of a 50 per cent aqueous propylene glycol solution. The results with the urea solution used intravenously, instead of the propylene glycol solution, are presented in table 3.

It is seen that the mesenteric blood glycol concentrations did not rise above those found without the injection of urea (table 2). Whereas the blood concentration of propylene glycol was increased four-fold and the rate of absorption from the intestine decreased 15 per cent, without the use of urea (table 2), the blood concentration of propylene glycol remained low and the absorption rate was decreased only 3 per cent, when urea was used (table 3). Therefore these results suggested an inhibition of absorption. This inhibition was most probably related to a high blood concentration of propylene glycol rather than to the effects of operative procedure, intravenous injection, or change of blood flow resulting from these procedures. This is true because the two sets of experiments were identical as to operative and injection

TABLE 3

*Effect of intravenous injection of 40 per cent urea solution on absorption of 50 per cent propylene glycol from loops of small intestine**

	LOOP 1	LOOP 2	LOOP 3	LOOP 4
Glycol absorbed (per cent)	72.3	67.4	60.5	0
Mesenteric blood concentration of glycol (mgm. per cent)	231	314	342	

* The loops were used in the same way as in Table 2.

procedures, the only difference being in the solution used for injection. These results do not rule out the possibility that the inhibition of absorption might have resulted from a slowing of the circulation caused by the presence of a high blood concentration of propylene glycol. The blood pressure was not recorded, but judging from the results of previous experiments (4) we believe that it was probably lowered somewhat.

A further test of this explanation of the inhibition of absorption was made on 10 cats in which the operative procedure was less extensive. Following a rapid intravenous injection of 2.5 cc. of 50 per cent propylene glycol per kilogram, a loop of jejunum of 15 cm. length was ligated and 5 cc. of 10 per cent propylene glycol per kilogram placed into the loop. After 15 minutes, a sample of blood was removed from a mesenteric vein and the loop excised. The average blood concentration of glycol was 1032 mgm. per cent, the average absorption of glycol 56 per cent. This was the smallest absorption ever demonstrated for a 15-minute absorption period, which supports the explanation that an inhibition of absorption resulted from a high concentration of propylene glycol in the blood stream. The physical factor in the absorption

was important in that an evenness of the glycol concentration on the opposite sides of the absorbing surface tended to limit or arrest the absorption.

Effect of variations in absolute amount of glycol in intestine on absorption

In order to determine whether the absolute quantity of propylene glycol in a loop of intestine altered the rate of absorption, the following experiment was performed: In each of 5 cats, 3 loops of jejunum 15 cm. in length were ligated. Into the first loop 0.5 cc. of 50 per cent propylene glycol per kilogram was introduced, into the second loop 1.0 cc. per kilogram, and into the third loop 2.0 cc. per kilogram. After 15 minutes the loops were excised and the residual propylene glycol determined. The results are presented in table 4.

It is seen that, although the actual quantity of absolute propylene glycol absorbed increased as the amount of glycol in the intestine increased, i.e., 0.4 cc., 0.69 cc. and 1.14 cc., the percentage absorbed decreased. This was consistent with an inhibition of absorption described in the previous section.

TABLE 4

Effect of different quantities of 50 per cent propylene glycol on absorption from intestine

	LOOP 1	LOOP 2	LOOP 3
Quantity of glycol introduced (cc. per kgm.)	0.5	1.0	2.0
Average absolute amount of glycol absorbed (cc.)	0.4	0.69	1.14
Average per cent absorbed	79.5	68.8	57.0

Effect of iodoacetic acid and chloral on the intestinal absorption

It is claimed by Verzár and co-workers (8) that iodoacetic acid specifically inhibits the absorption of dextrose, galactose, glycerol, fats, lipoids, and certain other substances, reducing their rate of absorption as compared with pentose sugars and other substances which are absorbed by "non-specific" mechanisms. Since propylene glycol is absorbed with great rapidity, like dextrose and glycerol, it was of interest to determine whether iodoacetic acid decreased the absorption rate of this polyalcohol. Since iodoacetic acid must be given in very large doses for any inhibitory effects, the inhibition might be due to a general non-specific depression. Therefore the tests with this acid were controlled with large doses of chloral, a well known general and protoplasmic depressant. The dose of iodoacetic acid used was 100 mgm. per kilogram, that of chloral 300 mgm. per kilogram, both being injected subcutaneously. In all, 8 cats were used. The results were negative throughout, no difference in absorption being demonstrable between the poisoned animals (both iodoacetic and chloral) and the unpoisoned controls. We conclude that the absorption of propylene glycol was not mediated through some specific mechanism which might be inhibited by iodoacetic acid.

DISTRIBUTION OF PROPYLENE GLYCOL IN THE CIRCULATION DURING ABSORPTION FROM THE GASTROINTESTINAL TRACT

The concentration of propylene glycol in the portal and venous bloods (right heart) was determined in 48 cats during absorption of propylene glycol from different regions of the gastrointestinal tract. The doses and method of administration of the glycol used have been described in the first section of this report. The average results are presented in figure 2.

It is seen that there were considerable variations in the blood concentration of the glycol during absorption from the stomach and colon. This was not

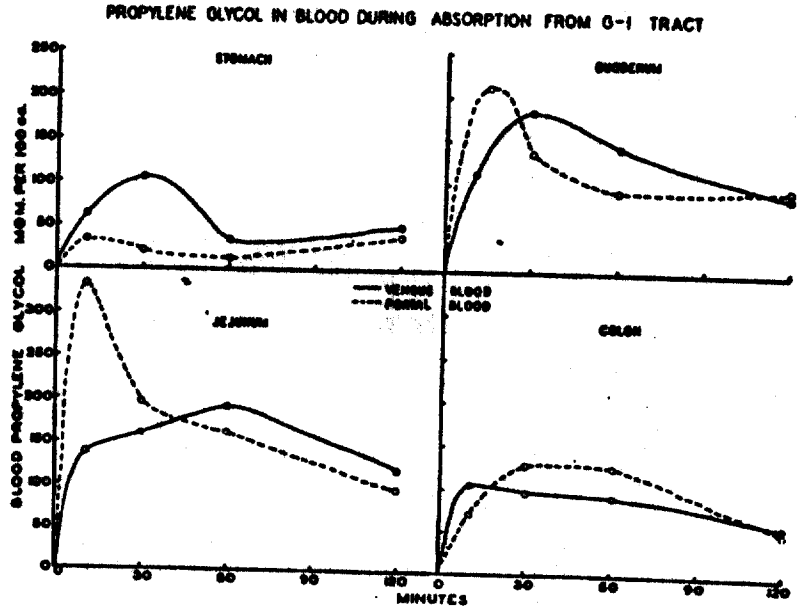

FIG. 2. BLOOD CONTENT OF GLYCOL DURING ABSORPTION OF PROPYLENE GLYCOL FROM DIFFERENT REGIONS OF THE GASTROINTESTINAL TRACT

surprising, since there are two possible venous paths from these organs, one to the general venous system directly, another to the portal system. In general the results indicated a higher concentration in the caval system than in the portal, this difference becoming less as absorption was completed. The reverse was true for the jejunum and duodenum, the portal concentration being initially much higher than the caval, and later the caval being slightly higher than the portal. In general the distribution of propylene glycol in the blood stream was found to be about as might be expected anatomically and physiologically.

RENAL EXCRETION OF PROPYLENE GLYCOL

Five rats were placed in individual cages suspended above funnels in such a way that the urine could be collected quantitatively, free from feces. Food

and water were allowed freely. After a control period of 8 or 9 days, during which fluid intake and fluid output were recorded daily, the drinking water was changed to a solution of propylene glycol, the concentrations used being 5, 10, 15 and 20 per cent. Daily records were made of fluid intake and output,

TABLE 5

Fluid balance in rats drinking different concentrations of propylene glycol

RAT	CHANGE IN* MEAN FLUID INTAKE	CHANGE IN* MEAN FLUID OUTPUT	BODY WEIGHT CHANGE DURING GLYCOL PERIOD	BODY WEIGHT CHANGE IN THE FIRST 24 HOURS ON WATER†
		5 per cent propylene glycol		
	cc. per diem	*cc. per diem*	*grams*	*grams*
1	−7 ±2.01	−2.7 ±0.37	−4	0
2	−3 ±1.10	−1.3 ±0.69	+7	+1
3	−1 ±1.61	−0.2 ±0.18	+5	+2
4	0	+0.1 ±0.50	+2	+1
5	−2 ±1.76	+0.2 ±0.25	+1	−1
		10 per cent propylene glycol		
1	0	+1.2 ±0.92	+3	+1
2	+9 ±1.45	+6.7 ±1.49	−5	+3
3	−1 ±2.08	+0.7 ±0.56	+2	+0
4	+6 ±2.11	+6.1 ±2.09	−3	+2
5	0	+2.9 ±0.70	−5	+4
		15 per cent propylene glycol		
1	3 ±1.83	+4.4 ±1.46	−28	+20
2	+1 ±2.55	+10.7 ±3.40	−39	+23
3	+3 ±1.50	+4.8 ±0.66	−17	+15
4	−1 ±2.56	+3.1 ±1.27	−29	+5
5	+3 ±1.14	+6.0 ±1.94	−41	+23
		20 per cent propylene glycol		
1	−6 ±1.46	+2.7 ±0.79	−65	+36
2	−9 ±1.58	+2.6 ±1.68	−64	+35
3	−6 ±1.76	+3.4 ±0.87	−50	+35
4	−16 ±2.76	0	−97	+44

* Mean change ± standard error of mean.

† Change in body weight during first 24 hours after glycol period was terminated; − = decrease, + = increase.

and daily analyses of urine for glycol content. After one week on the glycol the solution was replaced by water and the observations continued. When this second control on fluid balance was obtained the rats were given another concentration of propylene glycol and the observations repeated. The results obtained are presented in table 5.

It is seen that when the rats drank a concentration of 5 per cent propylene glycol they showed little, if any, change in fluid balance. On 10 or 15 per cent propylene glycol the fluid intake was only slightly increased. However, the fluid output was uniformly increased in all these rats and the net result was a dehydrating action, especially with 15 per cent propylene glycol. On the 20 per cent concentration the disagreeable taste of the mixture reduced the fluid intake markedly, but at the same time the fluid output was increased. As a result the animals lost nearly one-third their body weight in 1 week. That this was largely due to loss of water was shown by the fact that one-half this weight loss was regained within 24 hours when water was substituted for the glycol solution, and 90 per cent was regained within 48 hours. The relationship between fluid intake and output is indicated by the summary in table 6, which also shows the urinary excretion of propylene glycol.

TABLE 6

Renal excretion of propylene glycol in rats

CONCENTRATION OF GLYCOL IN DRINKING WATER	RATIO: INTAKE TO OUTPUT		CONCENTRATION OF GLYCOL IN URINE
	Fluid	Glycol	
per cent			*per cent*
0	4.1	—	—
5	3.6	4.5	3.6
10	2.7	3.1	8.4
15	2.2	3.1	10.6
20	1.9	3.5	10.0

This summary shows that, as the concentration of propylene glycol in the drinking water increased, the ratio between the fluid intake and output decreased. However, the ratio between glycol intake and output remained quite constant, indicating that, regardless of the concentration of glycol drunk, the kidneys excreted approximately one-third the amount ingested. The remaining two-thirds of the glycol must have been metabolized by the body. Apparently the rats, under the conditions used, were unable to concentrate the glycol beyond 10 per cent in the urine. No hematuria occurred.

SUMMARY AND CONCLUSIONS

1. Propylene glycol was found to be readily absorbed from all anatomical regions of the gastrointestinal tract of rabbits, rats, and cats, i.e., from the stomach, duodenum, jejunum and colon, the absorption being particularly rapid from the jejunum.

2. The following quantitative characteristics of the intestinal absorption of this glycol were demonstrated: absorption was more rapid with low concentrations than high; rate of absorption was not affected by variations in

the area of the intestinal surface; inhibition of absorption occurred during high blood concentrations of the glycol; rate of absorption decreased as the absolute amount of glycol in a given region increased; iodoacetic acid and chloral had no demonstrable effects on the absorption, thus differing from dextrose, glycerol, fats and lipoids; during absorption, large differences in concentration of the glycol occurred in different parts of the venous circulation of the gastrointestinal tract.

3. Propylene glycol in distilled water hemolyzed blood in all concentrations, but, in 0.9 per cent sodium chloride, hemolysis did not occur until the concentration reached 38 per cent, and was complete at 45 per cent.

4. A marked dehydrating action occurred in rats drinking concentrations above 5 per cent of this glycol, the ratio of intake to output remaining constant in rats drinking freely various concentrations.

5. Approximately one-third the administered propylene glycol was excreted by the kidneys, the remaining two-thirds being metabolized in the body.

6. The concentration of propylene glycol in the urine did not exceed 10 per cent, after the drinking of highest tolerated doses by rats.

7. In general, the quantitative gastrointestinal absorption of propylene glycol agreed in several respects with that demonstrated by others previously for alcohol, phenol and iodide, except that absorption of propylene glycol was more rapid and complete.

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COMPARATIVE EFFECTS OF PROPYLENE GLYCOL AND SOME OTHER AGENTS ON OXYGEN CONSUMPTION OF THE ORGANISM*

W. VAN WINKLE, JR., M.D., SAN FRANCISCO, CALIF.

THE voluntary running activity of white rats receiving propylene glycol was found previously to be increased somewhat.[1] These rats were not depressed, and they stored more glycogen than normal animals. The oxygen consumption of isolated liver perfused with propylene glycol was decreased, and the organ stored glycogen simultaneously.[2] These results are related to the possible food value of propylene glycol, which has a definite glycogenic action. But some increase in general activity should theoretically increase oxygen consumption or leave it unchanged. Therefore, it was thought desirable to investigate further the metabolic actions of this glycol, using oxygen consumption as the criterion. This report presents the results obtained.

METHOD

The apparatus used was of the closed-circuit type, a unit consisting of a Hempel desiccator acting as a rat chamber, which was connected by glass tubing and a three-way stopcock to a large glass cylinder of 4,500 c.c. capacity and

*From the Department of Pharmacology, Stanford University School of Medicine, San Francisco.

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graduated at 10 c.c. intervals. Soda lime and calcium chloride were put in the desiccator to absorb carbon dioxide and water. The large cylinder was filled with pure oxygen. As a rat used oxygen, water was allowed to siphon into the large cylinder from an 18-liter reservoir, thus equalizing the pressure inside the system with that of the atmosphere. The amount of water siphoned over was a measure of the oxygen consumed, after appropriate corrections for changes in temperature and barometric pressure. Six such units were operated at once.

The rats were observed for seven hours in this metabolic arrangement. No rat was used for more than four consecutive days. Usually 2 runs with the use of glycol or other agents were made, alternated with 2 control runs. The oxygen consumption was expressed as cubic centimeters of oxygen for 100 sq. cm. of body surface per minute. Since the metabolism varied with the environmental temperature, the control observations on each rat were plotted against the environmental temperature and the line through these points calculated by the method of least squares. The point at which this line crossed the 22°-axis was chosen to represent the average control result. Direct comparison of the results on controls and with different agents was made by moving the experimental points parallel to the control line until the 22°-axis was reached. The values so obtained were averaged, and the averages are summarized in Table I.

The nutritional state of the animals and the environmental temperature were as close to those of previous feeding and activity experiments as possible.[1] The animals were fed the usual stock diet of this department and were not fasted prior to the determination of oxygen consumption. The environmental temperature was not held constant but was carefully observed, and the mean values of oxygen consumption were corrected for the variations in temperature.

The doses of propylene glycol and other agents used and methods of administration were as follows: propylene glycol, 5 c.c. and 10 c.c. per kilogram, gastrically; benzedrine, 2 mg. per kilogram, subcutaneously; pentobarbital, 30 mg. per kilogram, subcutaneously; and chloral hydrate, 250 mg. per kilogram, intraperitoneally. The other agents, besides glycol, were used as a further check on the results with the glycol. Benzedrine, which is a general excitant, was expected to increase the oxygen consumption, and pentobarbital and chloral, depressants, were expected to decrease it. However, only the benzedrine acted according to expectations. A total of 505 determinations on 19 rats was made. The duration of each observation was seven hours. The other agents were compared in the same rats receiving the glycol.

CONTROL RESULTS

A total of 256 control observations on 19 rats showed an average oxygen consumption of 1.363 c.c. per 100 sq. cm. per minute at 22° C. (Table I). This value had a variability of less than 3 per cent.

GLYCOL AND OTHER AGENTS

Propylene glycol, in both doses used, depressed the oxygen consumption significantly, i.e., down to an average of 1.142 c.c. from the control average of 1.363 c.c. Benzedrine raised the oxygen consumption in 14 rats and increased

Table I

Oxygen Consumption of Rats Receiving Propylene Glycol and Other Agents

Rat	Control			Propylene Glycol				Benzedrine		Pentobarbital		Chloral	
				10 c.c. per kg.		5 c.c. per kg.							
	Determinations	O_2*	S.E.†	Determinations	O_2*	Determinations	O_2*	Determinations	O_2*	Determinations	O_2*	Determinations	O_2*
1	8	1.302	±0.009	4	1.138			4	1.974				
2	9	1.285	±0.016	4	1.147			4	1.593				
3	7	1.431	±0.032	3	1.303			3	1.735				
4	9	1.327	±0.029	5	1.158			3	1.668				
5	22	1.315	±0.026	5	1.269	5	1.132	4	1.585	5	1.273		
6	6	1.324	±0.047	3	1.229			3	1.661				
7	18	1.249	±0.030	5	1.241	5	1.470	5	1.476	5	1.483		
8	19	1.275	±0.034	5	1.112	4	1.209	5	1.597	4	1.163		
9	19	1.252	±0.035	5	1.017	5	1.183	5	1.448	5	1.263		
10	15	1.300	±0.036	5	1.103	2	1.071	5	1.525	2	1.220		
11	19	1.457	±0.039	5	1.065	5	1.309	5	1.670	5	1.343		
12	20	1.348	±0.029	5	1.205	5	1.329	5	1.629	5	1.243		
13	21	1.426	±0.041			5	1.437			5	1.152	5	1.363
14	6	1.290	±0.086	4	0.917			2	1.377				
15	7	1.435	±0.038	4	1.099			3	1.560				
16	19	1.452	±0.036			5	1.355			5	1.475	5	1.400
20	11	1.242	±0.029			5	1.125			5	1.074		
21	11	1.352	±0.045			5	1.325			4	1.629		
22	12	1.650	±0.068			5	1.531			5	1.519		
	258	1.363		62	1.142	56	1.289	56	1.607	55	1.319	10	1.381

Oxygen consumption = average c.c. oxygen consumed per 100 sq. cm. of body surface per minute at 22° C.
Standard error of mean.

the activity, i.e., increased general excitability but did not cause tremors; the oxygen consumption was raised to 1.607 c.c. Interestingly enough, pentobarbital decreased the oxygen consumption only slightly in 12 rats. This was interesting, since the animals which received propylene glycol behaved in the same way as did the unmedicated controls and were not narcotized, whereas those receiving pentobarbital were markedly depressed and, in fact, comatose, although the reflexes could be elicited. Recovery occurred at the end of a seven-hour period. Therefore, the depression of oxygen consumption caused by propylene glycol was not due to a decrease in general activity. This confirmed the observations on the isolated perfused liver and explained why the rats, although showing no depression of running activity, were able to store energy (liver glycogen) better than control rats. The burning of propylene glycol requires less oxygen to release a given amount of energy than do most other foodstuffs (except fat).

The results in 2 rats with chloral, which markedly depressed these animals and caused coma with loss of reflexes, were also interesting in that the oxygen consumption was practically the same as that of the controls, contrary to expectations. However, the results with the agents, other than glycol, are of value in showing that the depressant effects of propylene glycol on oxygen consumption are peculiar to this agent, since all other agents tried, except benzedrine, compared favorably with the controls. The explanation of this effect of propylene glycol is not at hand, although the results in the whole animal are consistent with a similar depression of oxygen consumption of the isolated liver perfused with this glycol. This storage of glycogen in the isolated liver, as well as in whole animals, indicates storage of energy for which there is less need of oxygen, despite a fair general activity of the animals.

CONCLUSIONS

1. Propylene glycol was found to depress the oxygen consumption of white rats, this depression not being due to a decrease in activity, since the depressions of pentobarbital and chloral in the same rats did not significantly decrease the oxygen consumption.

2. These results on whole animals are in agreement with previous results on isolated liver perfused with propylene glycol, which also showed a decrease in oxygen consumption.

3. The doses of propylene glycol used were beyond those likely to be taken as solvent or vehicle in food or medicinal agents, but the depression of oxygen consumption could occur with very large or toxic doses. This result is essentially of scientific importance, because the use of propylene glycol has a wide margin of safety.

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[illegible] OF RATS FED PROPYLENE [illegible] OTHER GLYCOLS

[illegible] and [illegible] KENNEDY

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California

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There is no doubt that propylene glycol stands out in sharp contrast with a number of other glycols and ethyl alcohol as to comparative non-toxicity and exclusive glycogenic actions (1, 2). These differences are reflected in other physiological actions. For instance, voluntary running activity of rats given propylene glycol remains unimpaired (2). However, the other glycols have not been tested according to this criterion. Therefore, this report presents results on the comparative running activity of other rats receiving 3 other glycols besides propylene glycol for comparison. The outcome was again favorable to propylene glycol.

METHODS

Slonaker's revolving cages were used with 30 female white rats in the same way as in a previous report (2). A portion of the carbohydrate in the stock diet[1] eaten voluntarily by the rats was replaced by one of the glycols to be tested for 15 weeks. The preliminary running activity of all rats was recorded for from 3 weeks to 2 months before administering the glycol-diet in order to accustom the rats to the cages and to determine their individual running ability. The rats were then divided into 6 groups of 5 rats each. One group each received propylene glycol, 12 per cent; ethylene glycol, 12 per cent; diethylene glycol, 12 per cent; and dipropylene glycol, 12 per cent, in the diet. Since ethylene glycol caused a 100 per cent mortality within 5 weeks, another group of 5 rats was given 6 per cent ethylene glycol in their diet. At the end of 15 weeks of glycol-feeding, all the rats were again given the control diet without glycol for another 2 weeks, when the experiment was stopped. The rats were kept in individual revolving cages and daily records kept of the number of revolutions. The average results presented graphically in figure 1 speak for themselves.

[1] The whole stock diet contained: cornmeal, 68 per cent; linseed oil, 10 per cent; dried ground alfalfa, 2 per cent; powdered casein, 10 per cent; [illegible] 5 per cent; cod liver oil, 3 per cent; bone ash, 1.5 per cent; and sodium chloride [illegible]. The composition was as follows: protein, 11.5 per cent; carbohydrate [illegible] per cent; roughage, 5.5 per cent; and salt, [illegible] per cent.

RESULTS

Propylene glycol proved to be the least toxic of the glycols studied, as the rats showed an increased activity, if anything, as compared with the controls. The greater activity lasted nearly throughout the entire period of glycol feeding, i.e., for 12 out of 15 weeks. The greatest activity occurred during the first 6 or 7 weeks of glycol feeding. The increased activity is not surprising, since propylene glycol can furnish about 1.5 times as much energy per gram as does dextrose. The carbohydrate in the diet was replaced by an equal weight of propylene glycol, and therefore, the rats received a larger number of calories per gram of diet than the controls.

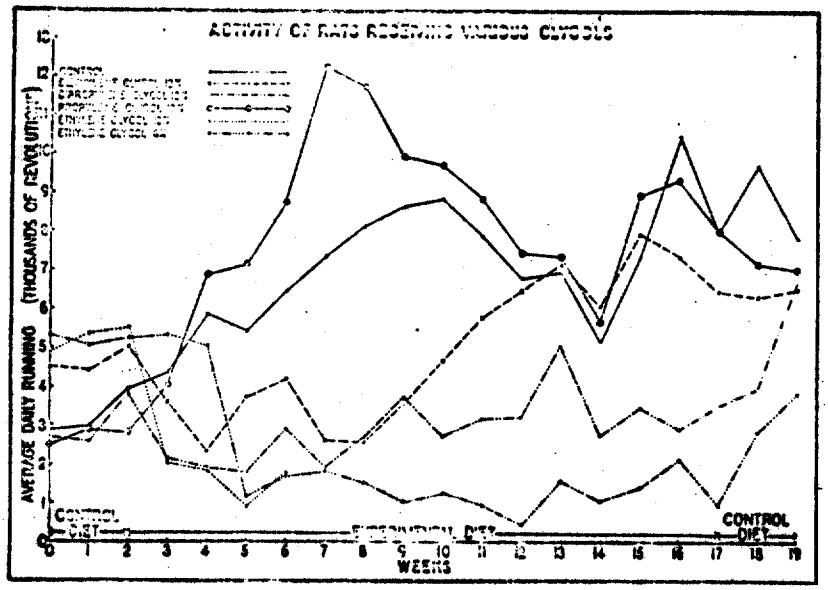

FIG. 1. VOLUNTARY RUNNING ACTIVITY OF WHITE RATS RECEIVING PROPYLENE GLYCOL AND OTHER GLYCOLS

The experimental diet contained different strengths of the glycols

On the other hand, the other 3 glycols, in the same or smaller concentrations in the same diet, depressed the running activity of the rats, but especially ethylene glycol. Diethylene glycol was interesting in that it reduced the activity for about 3 weeks, and then the activity recovered to that of the controls at the end of 15 weeks. Since the voluntary running activity of white rats, under these conditions, depends upon good physical condition, it follows that the daily consumption of large quantities (about 1.2 grams) of propylene glycol for nearly one-third the span of life is not injurious to health. In terms of human consumption such quantities would far exceed the amounts [illegible] which might be used as a vehicle or solvent [illegible]

CONCLUSIONS

1. Propylene glycol, when substituted for one-fourth the carbohydrate in diet (12 per cent of total diet), increased, if anything, the voluntary running activity of white rats, while the ethylene, diethylene and dipropylene glycols, in equal or smaller amounts, depressed the running activity under the same conditions.

2. Ethylene glycol was the most toxic. Recovery occurred from the initial depression of diethylene glycol, but not with dipropylene glycol.

3. These results are again consistent with other actions of propylene glycol, and to the effect that this is the least toxic or injurious of the glycols thus far investigated.

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FURTHER RESULTS OF CONTINUED ADMINISTRATION OF PROPYLENE GLYCOL

W. VAN WINKLE, JR., AND H. W. NEWMAN
Department of Pharmacology, Stanford University School of Medicine, San Francisco, California

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Propylene glycol continues to be of increasing interest and importance to those concerned with certain food and pharmaceutical products. It is being proposed as a substitute for alcohol and some polyalcohols in flavoring extracts which are used in the baking industry, in domestic cooking, in soda-fountain syrups, etc. It is used as a solvent and preservative for vitamins. It has a definite glycogenic action and can act as a carbohydrate substitute with corresponding effects on metabolism, body growth, and muscular activity. Before these and other uses are generally accepted and officially approved, however, further investigations of the usefulness and limitations of propylene glycol are desirable.

Previous reports on propylene glycol from this laboratory—Hanzlik, Newman, Van Winkle, Lehman, and Kennedy (1939); Seidenfeld and Hanzlik (1932); Lehman and Newman (1937); Hanzlik, Lehman, Van Winkle, and Kennedy (1939); and Van Winkle and Kennedy (1940)—have demonstrated a comparatively low toxicity for this glycol as compared with a number of other available glycols. This was indicated by the results of single and continued administration of propylene glycol in different animals. The doses for fatal effect were large and without demonstrable gross pathological changes in important organs. Beneficial, if anything, were a glycogenic action, a satisfactory substitution for carbohydrate in the diet, and a sustained, or promotion of, voluntary running activity caused by the propylene glycol.

Since propylene glycol may be an ingredient of medicinal and dietary products, which are taken by mouth, it appeared desirable to investigate the possible effects of this glycol on the functional efficiency and the histology of the liver and kidneys. This would appear especially desirable with continued administration of the glycol for long periods. Moreover, the results of liver perfusion, previously reported by Newman, Van Winkle, Kennedy, and Morton (1940), indicated that propylene glycol caused a decrease in oxygen consumption with an increase in lactic acid and glycogen of the liver. These changes did not clearly distinguish between an impairment of

TABLE 1

Propylene Glycol on Liver and Kidney Function in Dogs

Dog No.	Days	Body weight	Liver function: Rose bengal[1] in blood				Galactose[2] in 2-hr. urine	Uric acid[3] in 2-hr. urine	Kidney function: P.S.P.[4] excretion in 2 hrs.	Glycol in 100 c.c. blood
			1 min.	5 min.	15 min.	30 min.				
		kg.	*pct.*	*pct.*	*pct.*	*Control*	*gm.*	*mgm.*	*pct.*	*mgm.*
1	0	15.0	100	60	30	0	1.16	26.6	52	0
	14	.....	100	75	40	0	1.09	19.9	47	.....
	18	14.2	.....	Began drinking 5-per cent propylene glycol				.....	.....	.....
	49	14.9	100	80	15	0	1.34	53.5	68	117
	92	.....	100	81	47	37	1.29	23.4	70	.....
	121	16.3	100	75	29	0	0.17	11.2	60	.....
	243	17.7	100	75	30	0	1.05	24.7	61	363
2	0	21.0	100	61	17.8	*Control* 3.5	0.46	13.7	58	0
	10	.....	100	34	17	0	1.20	20.8	48	.....
	20	20.7	.....	Began drinking 5-per cent propylene glycol				.....	.....	.....
	122	21.0	100	42	8.3	0	0.52	15.6	65	340
	161	21.7	100	50	12.5	0	0.75	20.9	68	275
3	0	6.2	.....	.....	.....	*Control*	0.68	7.31	58	0
	6	.....	.....	Began drinking 5-per cent propylene glycol				.....	.....	.....
	34	6.8	.....	.....	.....	.....	0.90	7.0	57	.....
	65	7.0	.....	.....	.....	.....	0.85	8.0	.....	.....
	168	7.5	.....	.....	.....	.....	0.60	10.0	61	293
4	0	16.0	100	80	50	*Control* 0	0.34	28.1	70	0
	14	15.6	.....	.....	.....	.....	1.40	41.0	78	.....
	29	15.0	100	100	33	0	0.50	6.3	76	.....
	30	.....	.....	Began drinking 5-per cent propylene glycol				.....	.....	.....
	57	15.0	.....	.....	.....	.....	1.10	10.4	74	109
	86	.....	100	71	60	10	1.19	17.3	.....	.....
	181	16.9	.....	.....	.....	.....	1.15	14.3	62	.....
	218	18.7	100	54	25	0	1.43	16.2	73	353

[1] 3 c.c. per kg. body weight of a one-per cent solution in six-per cent dextrose injected intravenously. [2] Five grams of galactose given by stomach tube. [3] One gram of uric acid given by stomach tube. [4] Phenolsulfonphthalein.

function of and a beneficial action on the liver. Besides the results of continued absorption (liver) and continued elimination (kidney), it was desirable to obtain further information on the relationship of glycogenic action of this glycol to growth and also on serum calcium, which has been claimed to be increased by Hofbauer (1933). This report presents the results obtained on these various phases of the continued administration of propylene glycol in animals.

LIVER AND KIDNEY FUNCTION IN DOGS DRINKING PROPYLENE GLYCOL

Two groups of four dogs each were used. The first group, consisting of female dogs, drank five per cent propylene glycol for from five to nine months and received no other fluid. They had access to the glycol mixture for one hour twice daily, thus insuring a higher blood glycol level than if the dogs drank small quantities of the glycol mixture at frequent intervals. The solid diet consisted of Purina Dog Chow. The functional tests were made during a control period before glycol, at irregular intervals when the dogs drank glycol, and at the end of the drinking period. Then the animals were sacrificed and histological examinations of the tissues were made. Three tests of liver function were made: galactose excretion, Bauer (1906) and Schulte (1937); uric acid excretion, Schulte (1937), Bollman, Mann, and Magath (1925), and Bollman and Mann (1932); and the rose bengal content of blood, Koch, Lee, and Tainter (1935). Kidney function was tested by the ability of the dogs to excrete phenolsulfonphthalein in a two-hour test period.

It is seen from the results for this group of dogs (Table 1) that the propylene glycol caused no significant changes in functional efficiency of the liver and kidneys, according to the different tests made. The small differences from the control values, in all tests, fell within the range of normal variations and of experimental error. The quantities of propylene glycol drunk were rather large, the average daily intake during the drinking period for each dog being 5.1 c.c. per kilogram of body weight. The average glycol content of the blood was also high, 325 mgm. per cent, this being the average peak concentration attained twice daily. All the dogs remained in good health, having gained in body weight from one to two kilograms during the drinking periods. Histological examination showed no pathological changes in the livers and kidneys. Accordingly, the noninjuriousness of propylene glycol to functional efficiency and morphology of the liver and kidneys and to the general health of these dogs was consistent with its known comparative systemic nontoxicity in different animals.

An attempt was made to keep a second group of dogs on 10-per cent propylene glycol, but this concentration was found to be too hypertonic as it caused the dogs to increase their fluid intake threefold in an attempt to combat the obvious dehydration. This increased intake of 10-per cent glycol resulted in the production of coma, and the dogs died from the marked central depression or from a subsequent pneumonia. The blood-glycol concentration in these dogs averaged 800 mgm. per cent. To combat the dehydration, a group of four male dogs were allowed to drink 600 c.c. daily of 10-per cent propylene glycol each, the glycol being given in the morning. In the evening the dogs were allowed free access to water for one hour. On this regimen the dogs remained in good health and their average daily intake of propylene glycol amounted to 4.5 c.c. per kilogram of body weight. These dogs were maintained on this regimen for five to six months. Only the changes in renal function were observed in this group, as indicated by the blood urea at intervals throughout the drinking period. The control values ranged from 14 to 24 mgm. per cent, and after drinking glycol for five to six months, from 12 to 33 mgm. per cent. The average blood-glycol concentration in these dogs was 493 mgm. per cent, which was the average peak concentration attained daily. These negative results after drinking 10-per cent propylene glycol confirmed the noninjuriousness of this glycol for renal functional efficiency.

EQUI-CALORIC PAIRED FEEDING OF PROPYLENE GLYCOL

It was shown previously in paired feeding of rats with propylene glycol substituted for a portion of the carbohydrate that the glycol-treated rats grew better and had more liver glycogen than their litter-mate controls—Hanzlik, Newman, Van Winkle, Lehman, and Kennedy (1939) and Hanzlik, Lehman, Van Winkle, and Kennedy (1939). These results indicated that the increase in growth might have been due to the excess energy furnished by the glycol as compared with carbohydrate. Therefore, the data of these experiments have been extended, substituting the glycol in equi-caloric amounts for the carbohydrate.

Five pairs of rats were fed propylene glycol and water, the dose of propylene glycol being such that the total caloric intake of each rat of a pair was the same each day. Litter-mates of approximately equal weight were used. The diet of the glycolized rats contained one-fourth less carbohydrate than the controls, the caloric equivalent of this portion of the diet being substituted by propylene glycol given by stomach tube daily. The control rats received an equal quantity of water by stomach tube. The results obtained (Table 2)

confirm those previously obtained with rats on an uncontrolled caloric intake (see references given on page 512).

There was a definite tendency for the rats on propylene glycol to grow more rapidly and attain greater body weights than their litter-mate controls. Most striking were the differences in liver glycogen, the glycolized rats having two to seven times as much liver glycogen per gram of liver as their unmedicated controls. This cannot be accounted for on the basis of differences in food or caloric intake, since these were kept the same for each pair of rats. Two possibilities are suggested which might account for these results:

TABLE 2

Changes in Growth, Body Weight, and Liver Glycogen of Rats in Paired Feeding of Propylene Glycol

Glycolized rats [1]							Control rats			
Rat No.	Days	Initial body weight	Final body weight	Total glycol intake	Liver glycogen	Difference over control [2]	Rat No.	Initial body weight	Final body weight	Liver glycogen
		gm.	*gm.*	*c.c.*	*mgm. in 100 gm.*	*mgm. per gm. liver*		*gm.*	*gm.*	*mgm. in 100 gm.*
1	127	63	236	89.4	2,365	+11.90	1A	63	176	1,175
2	155	61	175	99.0	3,560	+31.33	2A	66	167	427
3	163	71	253	123.8	3,825	+32.37	3A	74	220	588
4	132	68	225	89.2	1,478	+10.08	4A	63	200	470
5	154	50	286	130.7	1,536	+10.81	5A	60	262	455

[1] Propylene glycol was substituted for one-fourth the caloric content of the carbohydrate in diet, each rat in a pair receiving the same daily calorie intake. [2] The plus sign in this column means "increase."

(1) Propylene glycol might be a more readily available source of energy than the carbohydrate of the diet; and (2) the glycol might depress the animals enough to decrease their energy utilization and thus account for the difference. Of these two possibilities, we are inclined to favor the second, despite the fact that propylene glycol does not decrease the voluntary running activity of rats, Van Winkle and Kennedy (1940). Under the latter conditions, periodic central nervous depression with a small decrease in general muscle tonus are conceivable, although this was not determined at the time and no measurements were made of oxygen consumption. However, propylene glycol depresses the oxygen consumption of the isolated liver, according to Newman, Van Winkle, Kennedy, and Morton (1940), and it is conceivable that this may be true for the entire animal in a degree sufficient to result in an increase in liver glycogen.

EFFECT OF PROPYLENE GLYCOL AND OTHER GLYCOLS ON SERUM CALCIUM

Hofbauer (1933) claims that propylene glycol causes an increase, and ethylene glycol a decrease, in serum calcium. Since the serum

calcium may fluctuate over a range of five or six mgm. per cent, according to Borne and Campbell (1932), and it is extremely difficult to alter the serum calcium beyond this range even by administration of calcium salts or by depriving an animal of calcium, Peters and Van Slyke (1932), the changes, often amounting to as much as 70 per cent, reported by Hofbauer appear incredible. If true, such changes in calcium would be of extreme importance and might be against the use of propylene glycol as a medicinal vehicle. Therefore, it appeared desirable to confirm or refute Hofbauer's claims.

A total of 31 cats was used. After obtaining a blood sample by heart puncture, the glycols were administered by intramuscular injection. After that, samples of blood were taken at four and 24

TABLE 3

Effect of Various Glycols on Serum Calcium of Cats

Glycol	Dose—c.c. per kg.	Number of cats	Serum calcium (average mgm. per cent)		
			Before glycol	After glycol	
				4 hours	24 hours
Physiologic saline solution (control)	5	6	11.0	10.0	9.8
Propylene	5	6	10.2	9.7	9.4
Dipropylene	5	4	10.8	10.8	10.7
Ethylene	2	7	9.9	10.1	8.0 [1]
Diethylene	2	4	11.1	10.6	11.1
Trimethylene	5	4	11.5	11.3	11.3

[1] Two of seven cats showed very low serum calcium at the end of 24 hours, i.e., 3.4 and 6.3 mgm. per cent.

hours. The calcium was determined by the method of Halverson and Bergeim (1917).

With the possible exception of ethylene glycol, none of the glycols tried altered the average serum-calcium concentration significantly (Table 3). The slight drop in serum calcium after some of the glycols falls within the range of experimental error, and also possible small changes in blood volume as indicated by simultaneous hemoglobin changes in some cats. These hemoglobin changes generally paralleled the changes in serum calcium. Two of seven cats receiving ethylene glycol showed very low serum calcium at the end of 24 hours, i.e., reduction of about one-third and one-half the control values. These animals were moribund. Histological examination of the kidneys of these two cats and of two other cats, which received ethylene glycol, but without changes in serum calcium, showed crystals in the lumens of the convoluted tubules of all cats. These crystals resembled those of calcium oxalate previously de-

scribed by Hanzlik, Seidenfeld, and Johnson (1931) in rats receiving ethylene glycol. Dille (1934) has reported that this glycol had no effect on the serum calcium either in acute or chronic intoxication of rabbits, which is in agreement with the results in the majority of animals used.

To test the effect of prolonged administration of propylene glycol on the serum calcium, calcium was determined at intervals in the serum of dogs used in renal and hepatic functional tests on continued propylene glycol medication. However, no significant changes in serum calcium were demonstrable in eight such dogs. Therefore, we were unable to confirm Hofbauer's claims. The comparative noninjuriousness of propylene glycol was again indicated by an absence of disturbances of systemic calcium and of renal calculi, as compared with ethylene glycol, at least.

SUMMARY AND CONCLUSIONS

Propylene glycol, when given in five- and 10-per cent concentrations in the drinking water of dogs for from five to nine months, causes no demonstrable inpairment of liver function as indicated by quantitative excretions of galactose and uric acid and by the rose bengal content of blood; and caused no impairment of kidney function as indicated by phenolsulfonphthalein excretion and changes in blood urea. No pathological tissue changes were demonstrable in these viscera.

Paired feeding of rats in which the diet of the tested rats contained one-fourth the carbohydrate substituted by the caloric equivalent in propylene glycol showed that the glycolized rats grew better than their litter-mate controls and had two to seven times as much glycogen per gram of liver as the controls.

No alteration in serum calcium of cats and dogs could be detected following the administration of large doses of propylene glycol and some other glycols, except ethylene glycol which lowered the serum calcium in only two of seven cats and caused renal calculi in four cats. The results with propylene glycol refute a positive claim in the literature.

These results taken together further confirm the comparative noninjuriousness to health and important organs of propylene glycol as compared with other available glycols.

There is no good reason to believe that the internal use of propylene glycol, in such quantities as would likely be present in food, beverages, vitamin, and medicinal products, would present individual or public-health hazards.

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Evaluation of Propylene Glycol as an Energy Source in Broiler Diets

P. W. WALDROUP AND T. E. BOWEN
Department of Animal Sciences, University of Arkansas, Fayetteville, Arkansas 72701

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WITHIN the past few years nutritionists have become interested in obtaining synthetic sources of nutrients such as protein from organisms grown on petroleum. Propylene glycol, a compound which results from the cracking of propane, may be a possible synthetic energy source in animal diets. Propylene glycol (1-2-dihydroxy propane) is unique among the glycols because its low toxicity permits it to be taken internally.

Hanzlik *et al.* (1931) compared the intramuscular irritant properties of propylene glycol and found that it caused a temporary burning sensation when placed on the tongue of human subjects. However, there was no apparent injury to this organ or to the oral mucosa. The irritant action was fleeting, lasting 5 to 10 minutes, and there were no after effects locally or systemically.

Seidenfeld and Hanzlik (1932) fed up to 10% solutions of propylene glycol in water to rats during about one-eighth of their normal span of life with no demonstrable effects on growth and few pathological changes in the kidneys, hearts, spleen or liver, even though daily quantities of 13.3 grams per kilogram of body weight were consumed. Similar results were reported by Weatherly and Hoag (1938).

Braun and Cartland (1936) administered oral dosages of up to 5 c.c. per kilogram of body weight to young rabbits for a 50-day period with no adverse effect on growth rate. In studies with rabbits and dogs, propylene glycol was quickly absorbed from the intestinal tract (Newman and Lehman, 1939) and slightly absorbed from the stomach (Lehman and Newman, 1937). Hanzlik *et al.* (1939a) reported that the absorption of propylene glycol from the human gastrointestinal tract was less rapid than in dogs, due presumably to the inhibition of intestinal absorption. Hanzlik *et al.* (1939b) fed large doses of propylene glycol to rats (25% carbohydrate substitution or 12.8% of the diet) and found that when the intake of the glycol diet was maintained equal to that of untreated controls, the rate of growth was equal or better on the glycol diet. Propylene glycol substitution for more than 25% of the carbohydrate resulted in reduction in body weight gains with complete substitution of propylene glycol for carbohydrate resulting in high mortality. These workers reported that propylene glycol possessed a moderate glycogenic action. Propylene glycol has been found to be effective in the treatment of bovine ketosis (Maplesden, 1954) because of its glycogenic properties.

So far as can be determined, the only study dealing with the feeding value of propylene glycol for chicks is the report of Bayley *et al.* (1967).[1] These workers found that up to 8% propylene glycol could replace an equivalent amount of corn starch in the diet of White Leghorn chicks. A level of 16% propylene glycol depressed the growth of the chicks to subnormal levels.

Experiments have been conducted at this station to study the possible value of propylene glycol as an ingredient in broiler diets. If this product could be efficiently utilized by chicken, it could provide a synthetic energy source to alleviate demands on cereal grains.

MATERIALS AND METHODS

Experiment 1. Propylene glycol[2] was mixed into broiler diets at levels of 0, 2.5, 5.0 and 10.0%. All diets were calculated to contain 23% protein. The composition of the diets is given in Table 1. Dietary adjustments were made by manipulation of the corn oil, sand and propylene glycol content of the diet so that the amino acid composition of the diet was unchanged. No data was available regarding the metabolizable energy value of the propylene glycol when these studies were initiated. Therefore, the gross energy value (5793 kcal./kg.) was used in calculation of the diets. If this product is highly digested and metabolized then this would result in minimal differences in energy content of the diet. Bayley *et al.* (1967) have since reported that they were

[1] During the time that this manuscript has been in publication, a report has appeared by Persia *et al.* (1968) regarding the use of propylene glycol in poultry feeds. These workers found that chicks and hens could utilize and tolerate a level of 2.5% but that higher levels depressed performance of chicks.

[2] Sirlene, containing 99.5% propylene glycol. Produced by Dow Chemical Co., Midland, Michigan.

unable to determine the metabolizable energy content of propylene glycol as it was volatile during freeze-drying.

Each of the four experimental diets was fed to two pens of 10 day-old broiler chicks each, equally divided as to sex. The chicks were randomly assigned to compartments in electrically heated battery brooders with raised wire floors. The experimental diets and tap water were provided on an *ad libitum* basis. The same randomization procedure and number of chicks per pen was followed in each trial.

At 21 days of age the chicks were individually weighed and group feed consumption determined. The data were subjected to the analysis of variance as outlined by Steel and Torrie (1960) with significant differences between treatment means determined by the multiple range test of Duncan (1955). All statements of probability are based on the results of these analyses.

Experiment 2. The purpose of this experiment was to further study the feeding value of propylene glycol and to determine if adverse effects resulting from feeding high levels could be overcome by starting the chicks on the experimental diets at 7 days of age rather than at day-old.

The same dietary treatments examined in the first experiment were studied in this trial. Three pens of chicks were placed on each diet at day-old. An additional 3 pens were fed the basal diet to 7 days of age and then placed on the experimental diet to determine if starting the chicks on the diets at a later age would influence their acceptance of the diet and subsequent performance.

The experiment was terminated at 28 days and individual body weight gains and group feed consumption determined.

Experiment 3. This trial was conducted to further evaluate propylene glycol as a feed ingredient and to attempt to establish a metabolizable energy value in relation to corn oil, which has a known metabolizable energy value of 8800 kcal./kg. (Matterson *et al.*, 1965). A basal diet (Diet 5, Table 1) was prepared and aliquots taken for the experimental diets. Corn oil was added to four diets at levels of 1.3, 2.6, 3.9 and 5.2 percent of the diet while propylene glycol was added to four additional diets at levels of 1.9, 3.8, 5.7, and 7.6 percent. Differences in the amounts added were made up by the addition of sand. These levels were calculated to provide 110, 220, 330 and 440 M.E. kcal./kg. of diet from the corn oil and a similar amount of gross energy from the propylene glycol. Two additional diets were fed in which 110 and 220 gross and metabolizable energy kcal./kg. were each supplied by propylene glycol and corn oil, respectively. Each of the 10 experimental diets was fed to 6 pens from day-old to 28 days of age.

TABLE 1.—*Composition of diets*

Ingredient	Percent of diet[1]				
	1	2	3	4	5
Yellow corn	42.00	42.00	42.00	42.00	48.98
Soybean meal (50% protein)	38.10	38.10	38.10	38.10	40.00
Corn oil	7.04	5.33	3.62	0.20	—
Propylene glycol	—	2.50	5.00	10.00	—
Sand	9.44	8.65	7.86	6.28	—
Defluorinated phosphate	1.62	1.62	1.62	1.62	—
Dicalcium phosphate	—	—	—	—	1.62
Ground limestone	0.80	0.80	0.80	0.80	0.80
Iodized salt	0.40	0.40	0.40	0.40	0.40
Microingredients[2]	0.50	0.50	0.50	0.50	0.50
Trace mineral mix[3]	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	92.40
Calculated analysis					
% Protein	23.00	23.00	23.00	23.00	24.61
% Calcium	1.00	1.00	1.00	1.00	0.84
% Phosphorus	0.70	0.70	0.70	0.70	0.70

[1] Diets 1–4 used in experiments 1 and 2. Diet 5 used in experiment 3 only.

[2] Supplied per kg. of diet: 6600 I.U. vit. A; 2200 I.C.U. vit. D_3; 22 I.U. vit. E; 1.1 mg. vit. K; 6.6 mg. riboflavin; 17.6 mg. pantothenic acid; 44 mg. niacin; 1870 mg. choline; 11 mcg. vit. B_{12}; 0.0125% ethoxyquin.

[3] Supplied 100 p.p.m. iron, 100 p.p.m. zinc, 100 p.p.m. manganese, 10 p.p.m. copper, 1 p.p.m. cobalt, 3 p.p.m. iodine.

RESULTS AND DISCUSSION

Experiment 1. Chicks fed the control diet with no propylene glycol had the greatest body weights at 21 days of age (Table 2).

TABLE 2.—*Effect of propylene glycol on body weight, feed consumption and feed utilization of broiler chicks (experiment 1)*

% Propylene glycol	0–21 day body weight gain (grams)[1]	0–21 day feed consumption (grams)	Feed gain[1]	Mortality
0.0	341[a]	520	1.52[a]	4/20
2.5	338[a]	502	1.40[a]	1/20
5.0	319[a]	510	1.60[ab]	1/20
10.0	249[b]	444	1.78[b]	1/20

1. Means having the same superscript do not differ significantly (P = .05).

Chicks fed the diets containing 2.5 and 5.0% propylene glycol, while weighing less, did not differ significantly from those fed the control diet. Chicks fed the diet with 10.0% propylene glycol had significantly lower body weight gains than those fed the other experimental diets.

Chicks fed the diet with 10% propylene glycol consumed 9% less feed than those fed the control diet. However, there was no statistically significant difference noted between any of the groups. The small number of treatment and replicate groups imposed a difficulty in achieving statistical differences. However, the differences in feed:gain ratios between the chicks fed the diet with 10% propylene glycol and those fed either the control diet or the diet with 2.5% propylene glycol were statistically significant.

There were no significant differences in mortality noted among any of the four treatments. It was observed, however, that the chicks fed the higher levels of propylene glycol had a high incidence of toe and hock deformities. The toe deformity, reported in detail in an earlier communication (Bowen and Waldroup, 1968), was characterized by the toes turning outward at each joint of the toe. The hock disorder manifested itself as a perosis-like condition. These findings indicate some effect of the propylene glycol on nutrient absorption or on bone and cartilage formation. Similar toe defects were noted by Rutter *et al.* (1953) on a diet high in lactose.

The feces of chicks fed the diets with propylene glycol had a strong, penetrating, sweetish smell. Diarrhea was present in the chicks fed the diets containing 5.0 and 10.0% propylene glycol.

Experiment 2. The body weight gains of the chicks in this trial declined as the level of propylene glycol in the diet increased (Table 3). Chicks fed a diet containing 5.0% propylene glycol had significantly lower gains than those fed the control diet. A further increase to 10.0% of the diet resulted in a significant depression in weight gains.

There were no significant differences noted in weight gains that could be attributed to the age at which the chicks were placed on the experimental diets. Chicks started on treatment at 7 days of age tended to be smaller than those started at day of age, however these differences were not statistically significant.

In contrast to the first experiment, there was no reduction in feed consumption as the level of propylene glycol in the diet increased; in fact, chicks fed the diets containing propylene glycol consumed more feed than did those receiving the control diet. The feed:gain ratio reflected the differences in body weight gains with significant increases resulting from feeding the diets with 5.0 or 10.0 percent propylene glycol.

Mortality was not adversely affected by any of the experimental treatments. It was again observed that the chicks fed the higher levels of propylene glycol had a high incidence of hock and toe deformities. The unusual fecal odor and diarrhea resulting from feeding the high level of propylene glycol was again observed.

Experiment 3. Levels of 1.90, 3.80 and

TABLE 3.—*Effect of age at starting time on response of broiler chicks to different levels of propylene glycol (experiment 2).*

% Propylene glycol	Starting age (days)	0–28 day body weight gain (grams)[1]	0–28 day feed cons. (grams)	Feed:Gain[1]	Mortality
0.0	1	517	796	1.57	0/30
	7	496	804	1.62	2/30
	Ave.	506[a]	800	1.59[a]	2/60
2.5	1	503	873	1.74	0/30
	7	479	868	1.81	0/30
	Ave.	491[ab]	871	1.77[ab]	0/60
5.0	1	471	852	1.81	1/30
	7	480	921	1.92	2/30
	Ave.	476[b]	886	1.86[b]	3/60
10.0	1	388	830	2.13	2/30
	7	382	813	2.13	1/30
	Ave.	385[c]	822	2.13[c]	3/60

[1] Means having the same superscript do not differ significantly (P = .05).

5.70% propylene glycol in broiler diets had no adverse effects on body weight gains, feed utilization, or mortality. These levels were calculated to provide 110, 220, and 330 gross energy kcal./kg. of diet (Table 4). A level of 7.60% propylene glycol (440 G. E. kcal./kg.) resulted in a significant depression in body weight gains and increase in the feed:gain ratio.

The addition of corn oil at levels calculated to provide 110, 220, 330 and 440 M. E. kcal./kg. resulted in no depression in weight gains or feed utilization as the level in the diet increased. Thus, the adverse effect from the high level of propylene gylcol is apparently not due to an excess of calories from a non-carbohydrate source.

It was obvious from comparison of the feed utilization data that the gross energy from the propylene glycol was not utilized as well as the metabolizeable energy from corn oil. A comparison at 110 kcal./kg. revealed a difference in feed utilization of 0.05 grams of feed per gram of gain. At 220

TABLE 4.—*Performance of chicks fed different amounts of corn oil and propylene glycol*

Treatment	% Propylene glycol	% Corn oil	0–28 day Weight gain (grams)[1]	0–38 day Feed/chick (grams)	Feed:gain[1]	Mortality
1	1.90	—	481[ab]	785	1.61[a]	0/60
2	3.80	—	451[bc]	781	1.73[ab]	0/60
3	5.70	—	474[ab]	808	1.71[ab]	1/60
4	7.60	—	433[c]	788	1.81[b]	0/60
5	—	1.30	496[a]	778	1.56[a]	0/60
6	—	2.60	497[a]	791	1.59[a]	0/60
7	—	3.90	492[a]	781	1.58[a]	0/60
8	—	5.20	483[ab]	746	1.55[a]	0/60
9	1.90	1.30	458[abc]	784	1.71[ab]	0/60
10	3.80	2.60	469[abc]	761	1.62[ab]	0/60

[1] Means having the same superscripts do not differ significantly (P = .05).

kcal./kg. there was a difference of 0.14 grams of feed per gram of gain. However, at 330 kcal./kg. there was a difference of only 0.13 grams of feed per gram of gain. Thus, while there was apparently a marked loss of the energy from propylene glycol during digestion no attempt was made in these studies to estimate a metabolizable energy value for this compound.

The combinations of corn oil and propylene glycol added to the diet in amounts calculated to supply 110 and 220 kcal./kg. (M.E. for corn oil, G.E. for propylene glycol) supported body weight gains and feed utilization as well as a like amount from either source alone.

The results of these trials indicate that levels of propylene glycol in excess of 5.0% of the diet result in a significant depression in performance. Levels of 7.6 and 10.0% were sufficient to cause a deleterious effect on body weight gains and feed utilization. These results are not in complete agreement with the report of Bayley *et al.* (1967) who found that a level of 8% propylene glycol did not impair performance. However, it should be noted that these workers used a White Leghorn chick rather than the faster growing broiler chick used in the studies reported herein.

It is concluded that propylene glycol can be utilized as an energy source in broiler diets at levels not exceeding 5%. However, information is needed regarding its metabolizeable energy value before its economic worth can be fully evaluated.

SUMMARY

Three experiments have been conducted to evaluate the nutritional worth of propylene glycol as an energy source in broiler diets. This compound, which is a by-product of petroleum manufacturing, can be utilized without adverse effects at levels not exceeding 5% of the diet. Higher levels resulted in depressed body weight gains, reduced efficiency of feed utilization, diarrhea, and the development of deformed toes.

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American Pharmaceutical Association

TOXICITY OF PROPYLENE GLYCOL.*

BY J. H. WRATHERBY,[1] PH.D., AND H. B. HAAG,[1] M.D.

INTRODUCTION.

During the past several years suggestions have been made that propylene glycol is a suitable solvent for a variety of purposes, pharmaceutical and otherwise. As a result of this interest several papers (1, 2) have appeared in which attention was directed particularly to toxicological studies on this substance. In connection with another investigation involving the use of propylene glycol it was considered advisable to extend some of these studies for the purpose of completing our own data on this subject. Some of the results to be described confirm those of other authors; and some are believed to be new observations not previously described in the literature. The propylene glycol used in these studies was the alpha form (1, 2 propane diol) obtained from the Carbide and Carbon Chemicals Corporation.

Seidenfeld and Hanzlik (1) reported acute fatal doses for propylene glycol when given intramuscularly and intravenously to white rats and rabbits. They also investigated the effects in growing rats of the continued drinking of water containing various amounts of propylene glycol. The intramuscular fatal doses were found to be about 14 Gm. per Kg. body weight for rats, and about 7 Gm. per Kg. for rabbits. The intravenous fatal doses were found to be about 16 Gm. per Kg. for rats, and about 5 Gm. per Kg. for rabbits. Practically no effects were observed in rats from drinking water containing less than 10% propylene glycol; but with higher concentrations the fluid intake was greatly restricted, and the animals lived only a few days. No definite microscopic changes were observed in organs of the animals sacrificed at the end of the experimental period of 140 days. It is interesting to note that these authors found the intravenous fatal dose for rats to be greater than the intramuscular fatal dose, a relationship rarely observed. This is particularly significant since the intravenous fatal dose for another rodent (rabbit) was found to be considerably less than the intramuscular fatal dose, which is the usual relationship.

Braun and Cartland (2) investigated the acute toxicity of propylene glycol for rats by subcutaneous, intramuscular and intravenous injections, and obtained results which were in satisfactory agreement with those reported by Seidenfeld and

* Presented before Scientific Section, A. PH. A., New York meeting, 1937.

[1] Medical College of Viriginia, Dept. of Pharmacology.

Hanzlik. In addition Braun and Cartland investigated the acute toxicities on oral administration to rabbits.

EXPERIMENTAL METHODS AND RESULTS.

Acute Toxicity.—In the acute experiments propylene glycol was administered intravenously to rabbits; and orally, subcutaneously, intramuscularly and intravenously to white rats. All animals were adults, but otherwise unselected as to age, source, weight or sex, and were maintained on standard diets (Purina). The animals given propylene glycol orally received no food for twenty-four hours prior to the experiment.

The results of the acute toxicity experiments are shown in Table I. The Average Fatal Dose is taken as the quantity required to kill 50% of the animals. This figure was arrived at by plotting dose against per cent mortality, and then deriving the fatal dose for 50% of the animals from the curve so constructed. In Table II is shown a summary of the data published by various authors to whom references are made. These figures also represent doses fatal to 50% of the animals, and were derived from data published by the original authors. In many instances the number of animals used for each dose was so small as to make the accuracy of such a procedure questionable. One author used only four mice in the entire series. However, since there is no consis-

TABLE I.—TOXICITY OF PROPYLENE GLYCOL.

Dose (per Kg. Body Wt.). Cc.	Gm.	Number Animals.	Died.	Lived.	% Mortality
		* Oral Administration: Rats.			
25	26.0	5	0	5	00
30	31.2	5	2	3	40
35	36.4	5	3	2	60
		Subcutaneous Administration: Rats.			
20	20.8	5	1	4	20
25	26.0	5	4	1	80
30	31.2	5	5	0	100
		Intramuscular Administration: Rats.			
12	12.48	5	0	5	00
14	14.56	5	3	2	60
16	16.64	5	4	1	80
18	18.72	5	5	0	100
		Intravenous Administration: Rats.			
6	6.24	10	1	9	10
7	7.28	5	4	1	80
8	8.32	10	8	2	80
9	9.36	5	5	0	100
10	10.40	5	4	1	80
12	12.48	5	5	0	100
		Intravenous Administration: Rabbits.			
5	5.2	3	0	3	00
6	6.24	5	1	4	20
7	7.28	5	4	1	80
8	8.32	5	5	0	100

* Larger doses were impractical due to limited capacity of rat's stomach.

TABLE II.

Animal.	Method of Administration.	Propylene, Gm./Kg.	Diethylene, Gm./Kg.	Ethylene, Gm./Kg.
Mouse	Subcutaneous	...	...	5.6 (3)
Rat	Oral	33.5 (6)	16.8 (4)	...
Rat	Subcutaneous	22 (2)	15.7 (4)	...
		22.5 (6)	...	...
Rat	Intramuscular	14 (1)	7.87 (4)	4.2 (5)
		15.5 (2)	...	...
		14 (6)	...	...
Rat	Intravenous	16 (1)	4.75 (4)	...
		18.9 (2)	...	...
		6.8 (6)	...	...
Rabbit	Oral	19.3 (2)	...	...
Rabbit	Intramuscular	7 (1)	3.9 (4)	6 (5)
Rabbit	Intravenous	5 (1)	2.25 (4)	4.7 (5)
		6.5 (6)	...	...

Numbers in parentheses refer to papers from which these data were obtained. The data represent Average Lethal Doses (50% mortality) calculated from data published by the various authors.

tency in the per cent mortality taken by various authors as indicating Average Fatal Dose, it is believed that this treatment of the data gives results which are more nearly comparable. The intravenous fatal dose for rabbits, and the intramuscular fatal dose for rats agree satisfactorily with those reported by Seidenfeld and Hanzlik, but the intravenous fatal dose for rats was found to be far less than that reported by these authors. Also, results obtained from subcutaneous and intramuscular injections in rats were in excellent agreement with those reported by Braun and Cartland, but again there was a marked discrepancy in the intravenous fatal doses for rats. When this discrepancy first became apparent it was thought that perhaps diet, age or some other uncontrolled factor may have been responsible, but the experiment has since been repeated on two different occasions on animals from other sources of supply. Essentially the same results were obtained on all three groups of rats, those from two groups being included in Table I.

During the early attempts at injections into veins of the tails of rats it was observed several times that the needle passed completely through the vein and into loose tissue surrounding the vein. Then, upon aspiration with the syringe, a droplet of blood was drawn into the syringe, giving a false indication of the position of the needle. This droplet of blood was the result of leakage from the punctured vein, and was not obtained directly from the lumen of the vein. Upon injecting the contents of the syringe the vein was obliterated by external pressure of the injected material on the vein. This material passed toward the body through tissues surrounding the vein, and ultimately most of it accumulated in muscular and subcutaneous tissues at the base of the tail. Such injections gave many of the appearances of intravenous injections without actually having been intravenous. It was found that such accidents could be obviated almost entirely by first placing the rat's tail in water at a temperature of about forty degrees Centigrade for one or two minutes before attempting the injection. This caused dilatation of the veins so that the needle could be introduced quite easily, and with little danger of failure.

Neither Seidenfeld and Hanzlik nor Braun and Cartland mentioned the veins selected by them for injections. Concerning these injections Braun and Cartland state: "The intravenous injection of undiluted propylene glycol produced rapid obliteration of the veins making administration very difficult. At doses of 16 to 19 cc. per Kg. intravenously, all rats showed marked muscular tremors and later became comatose." The authors of this communication did not find the administration of propylene glycol difficult after previous dilatation of the vein as mentioned above, nor were the veins rapidly obliterated. With sub-lethal doses, the veins were seen to refill with blood just about as promptly after withdrawal of the needle as if saline or glucose solutions had been injected. Following fatal doses death occurred within a short time in most instances, although with barely fatal doses the animals sometimes lived for ten or fifteen minutes, exceptionally one lived as long as several hours.

The data presented by Seidenfeld and Hanzlik in their Table I suggests that some such accident as previously described may have happened in many of their injections. An increase in dose from 9 cc. to 12 cc. per Kg. was accompanied by a drop in fatalities from 30% to 20%, and a further increase in dose to 15 cc. per Kg. was accompanied by an increase in fatalities to only 40%. To increase the number of fatalities from 30% to 70% it was found necessary to increase the dose from 9 cc. to 18 cc. per Kg. One would predict just such a relationship between dose and number of fatalities if approximately 30% to 40% of the injections actually were made into veins. Then, upon approaching the intramuscular fatal dose (variously reported between 14 and 15.5 Gm. per Kg.) the mortality rate would naturally approach that for the intramuscular method of administration. The fact that the intravenous fatal dose was found by Seidenfeld and Hanzlik actually to exceed the intramuscular fatal dose may have been due to retention of part of the injected material in the tissues of the tail, from which absorption was exceedingly slow because of obliteration of the vein.

Symptoms following the administration of fatal or near fatal doses of propylene glycol were found to be essentially as described by Seidenfeld and Hanzlik and Braun and Cartland. The fatal period varied from a minimum of two or three minutes following intravenous injections to a

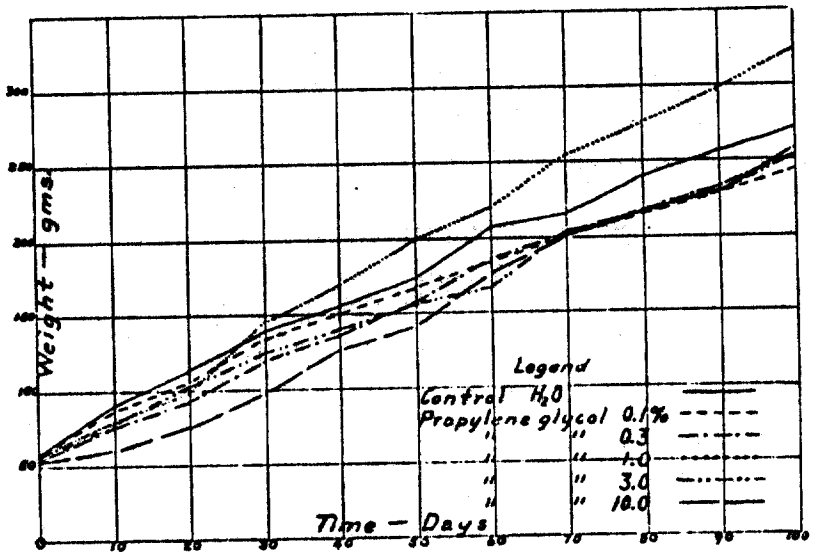

Fig. 1.—The effect on the growth of white rats of adding various concentrations of propylene glycol to the drinking water.

maximum of about two days following oral administrations. Muscular tremors and mild clonic convulsions occurred frequently. The animals became comatose more or less rapidly depending on the method of administration. Hematuria was observed frequently, especially after intravenous injections.

Chronic Toxicity.—The chronic toxicity of propylene glycol was investigated by administering the substance to growing rats by way of their drinking water. Male rats at weaning age (about 55 Gm. average weight) were used in groups of five animals each. Propylene glycol was added to the drinking water in concentrations of 0.1%, 0.3%, 1.0%, 3.0% and 10.0% by weight. A control group received distilled water instead of propylene glycol solution. All animals received the diet recommended by Osborn and Mendel. Food and drink were allowed ad libitum. Weights of the animals and consumption of food and drink were recorded at five-day intervals for the duration of the experiment—100 days. At the end of this period all surviving animals were sacrificed, and all internal organs were prepared for microscopic examination. During the first ten days of the experimental period those animals which received 10.0% propylene glycol increased in weight less rapidly than the remainder of the animals, and appeared to be in poor physical condition. However, very soon thereafter, those animals recovered from their poor start, and began to increase in weight at a rate about equal to that of the control group. Two animals which received

10.0% propylene glycol and one which received 1.0% died before the termination of the experiment. Growth curves for the six groups of animals are shown in Fig. 1. No significant differences were noted in consumption of food and drink by the various groups. It is worthy of note that those animals which received 10.0% propylene glycol solution consumed from 9.6 to 15.8 Gm. per Kg. per day, whereas, the acute oral fatal dose was found to be 33.5 Gm. per Kg.

Microscopic examination of organs from these animals was made by Doctor G. Z. Williams, Associate Professor of Pathology of this institution. In no way were the organs from animals which had received propylene glycol found to differ from those of the control group, which had received distilled water. These results substantiate those reported by Seidenfeld and Hanzlik from a similar experiment.

Hemolysis.—The frequent occurrence of hematuria following administration of sub-lethal doses of propylene glycol to rats, and the previous mention of this action by Von Oettingen and Jirouch (3), prompted a study of this phenomenon *in vitro*. Isotonic solutions of diethylene glycol and propylene glycol were prepared (0.278 molar). These solutions were mixed with varying quantities of 0.9% NaCl solution so that a series of mixtures resulted which contained isotonic glycol and isotonic saline in the proportions of 9 to 1, 8 to 2, 7 to 3, 6 to 4, and so on. A separate series of solutions was prepared by diluting isotonic saline with distilled water in the same manner. These last solutions were used for ascertaining the fragility of the red corpuscles, and whether or not the blood used was normal in this respect. One drop of human blood was added to each tube, which contained 5 cc. of one of the solutions prepared as described above. The results are shown in Table III. Three such experiments were performed, each with blood from a different individual.

TABLE III.—HEMOLYSIS FROM DIETHYLENE GLYCOL AND PROPYLENE GLYCOL.

Tube No.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Dilution	0	9/10	8/10	7/10	6/10	5/10	4/10	3/10	2/10	1/10
Molar conc.	0.278	0.250	0.222	0.195	0.167	0.139	0.111	0.083	0.056	0.028
Diethylene	xxxx	xxxx	xxxx	xxxx	xxxx	x	0	0	0	0
Propylene	xxxx	xxxx	xxxx	xxxx	xxxx	x	0	0	0	0
Saline	0	0	0	0	0	x	0	0	0	0
Conc. saline	0.9%	0.81%	0.72%	0.63%	0.54%	0.45%	0.36%	0.27%	0.18%	0.09%

xxxx indicates complete hemolysis. 0 indicates no hemolysis.

Fragility of corpuscles was normal in each instance, and there was no significant variation in the effects of the glycols. The line of demarcation between hemolysis and non-hemolysis was very definite. Methemoglobin could not be detected in any of the tubes by means of a small hand-spectroscope.

NOTE: Since the above experiments were completed, Lehman and Newman (7) reported results from somewhat similar experiments. These authors found that if propylene glycol solutions were prepared with a final concentration of 0.9% NaCl, then no hemolysis occurred with glycol concentrations of 30% or less; but that if distilled water were used as a diluent, hemolysis occurred in all dilutions. Thus, 0.9% NaCl seems to have afforded marked protection against hemolysis. The explanation for this apparent protection probably lies in the fact that certain substances penetrate the red cell membrane so rapidly that they exert no appreciable osmotic effect on the cell. Therefore, aqueous solutions of propylene glycol behave essentially as distilled water, and produce rapid and complete hemolysis; whereas, the presence of 0.9% NaCl provides an approximately isotonic medium in the presence of less than 30% glycol. This explanation was offered for the same type of phenomenon observed by Johnson, Carlson and Johnson (8) in connection with the hemolytic action of glycerine. These latter authors also stated that a similar situation exists with respect to aqueous solutions of urea. On the basis of data presented in Table III, it appears that dilution of 0.9% NaCl solution with 0.278 molar solutions of either propylene or diethylene glycol was equivalent to dilution with distilled water in so far as production of hemolysis was concerned. These results are therefore in accord with the observations of Lehman and Newman and Johnson, Carlson and Johnson.

SUMMARY.

1. In rats the acute Average Fatal Dose of propylene glycol was found to be 33.5 Gm. per Kg. orally, 22.5 Gm. subcutaneously, 14 Gm. intramuscularly and 6.8 Gm. intravenously. These figures are in agreement with those reported by other authors, with the exception of the intravenous fatal dose, which is somewhat less than half that reported by other authors. Reasons are given for believing the lower figure to be correct for the intravenous fatal dose.

2. In rabbits the acute Average Fatal Dose on intravenous administration was found to be 6.5 Gm. per Kg.

3. The chronic toxicity of propylene glycol was studied by the administration of the material to growing rats by way of their drinking water. Concentrations of 3% or less caused no appreciable change in rate of growth. A concentration of 10% caused a temporary slowing in rate of growth which lasted for about ten days. Rate of growth after this initial slowing became essentially normal. No significant changes were found on microscopic examination of organs of these animals sacrificed at the end of the experimental period of 100 days.

4. Hematuria was observed following the intravenous administration of sublethal doses of propylene glycol to rats. Hemolysis was produced *in vitro* by both diethylene glycol and propylene glycol in concentrations greater than 0.14 molar. This *in-vitro* action seems to be due to the dilution of isosmotic NaCl solution with the osmotically inactive glycol solution, rather than to a specific hemolytic action of these glycols in the dilutions used.

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Results of Feeding Propylene Glycol in the Diet to Dogs for Two Years. [Food additives]

C. S. WEIL, M. D. WOODSIDE, H. F. SMYTH, JR. and C. P. CARPENTER

Mellon Institute, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213, USA

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Abstract—Groups of five male and five female beagle dogs were fed diets containing propylene glycol or dextrose, the latter as equicalorific controls, for 2 yr. Dosage levels used were 5·0 and 2·0 g propylene glycol/kg and 6·35 and 2·54 g dextrose/kg, and in addition a control group received neither compound. The criteria of effect included mortality, body-weight change, diet utilization and water consumption, micropathology, liver, kidney and spleen weights, and measurements of blood, urine and biochemical parameters.

None of the criteria measured were significantly altered at 2 g propylene glycol/kg. The higher dose levels of propylene glycol and dextrose were maintained by the inclusion of approximately 20 and 25%, respectively, in the diets of the dogs. As both compounds provide calories available for conversion to adipose tissue, both groups gained somewhat more weight than the controls during the first part of the study. Propylene glycol, in high concentrations, increases the rate of erythrocyte haemolysis. When it was given at the 5-g/kg level, haemoglobin and haematocrit values and the total erythrocyte count were slightly low, while the incidence of anisocytosis, poikilocytes and reticulocytes was increased, findings indicative of erythrocyte destruction with accelerated replacement from the bone marrow. Even at a propylene-glycol concentration of 20% of the diet, this effect was not sufficient to result in any changes that appeared to be irreversible and there was no evidence of damage to bone marrow or spleen. The majority of parameters relevant to effects on the liver were similar in all dosage groups. In dogs consuming 5 g propylene glycol/kg there was a slight increase in total bilirubin.

It is concluded that propylene glycol fed to dogs at a concentration of approximately 8% in the diet (2 g/kg/day) is utilized as a carbohydrate energy source without any adverse effects.

INTRODUCTION

The acceptance of the safety of propylene glycol for many food-additive uses was based upon demonstrations by Hanzlik, Lehman, Van Winkle & Kennedy (1939), by Van Winkle & Kennedy (1940), by Newman, Van Winkle, Kennedy & Morton (1940) and by Van Winkle (1942) that this solvent can replace part of the carbohydrate in the diet as an energy source. This basic demonstration of compatibility with the metabolism of the body was supplemented by the indirect evidence of Morris, Nelson & Calvery (1942), who reported that rats eating diets containing 2·45 or 4·9% propylene glycol for 2 yr were not harmed beyond "slight chronic liver damage" (not described further), and by two clinical reports (Abrams & Bauer, 1938; Albright, Butler & Bloomberg, 1937) concerning the prolonged use of the glycol as a solvent for vitamins given in the treatment of patients suffering from vitamin deficiencies.

No 2-yr feeding of any species other than rats has been reported. Briefer periods of feeding (Guerrant, Shitlock, Wolff & Dutcher, 1947; Bornmann, 1954 & 1955) and other

experimental studies (Stoewsand, Dymsza, Swift, Mehlman & Therriault, 1966; Rudney, 1950; Gessner, Parke & Williams, 1960) cast no doubt on the safety of current uses of the glycol as a food additive. Nevertheless, at present, considerations of the safety of food additives lean heavily upon the indirect evidence of prolonged feeding of experimental animals, in preference to the assurance given by long, but of course unmonitored, human consumption. The present report on the feeding of dogs for 2 yr on a diet containing propylene glycol should help to fill what may appear to be a gap in the evidence of the safety of this solvent for use as a food additive.

EXPERIMENTAL

Materials. The propylene glycol USP used in the study was a product of the Dow Chemical Co., Midland, Mich. In addition to meeting the Food Chemicals Codex specification, it also met all requirements of USP XVII and the Toilet Goods Association. The dextrose (*d*-glucose) used was Clintose brand, "A" granulation.

Diets. In studies of the long-term toxicity of a chemical that has no calorific value, the appropriate control is a group of animals receiving diet containing none of the test chemical. It has long been known, however, that propylene glycol is utilized in nutrition as a carbohydrate. Hence, a diet containing high levels of propylene glycol is nutritionally very different from a diet containing none, and the only proper control is a diet containing a carbohydrate in an amount nutritionally equivalent to the propylene glycol given to the treated groups. Dextrose was used for this purpose in the present study, and nutritional equivalence was estimated by the growth of weanling rats during 1 wk by the method of Rice, Warner, Mone & Poling (1957).

Groups of five weanling rats each received a basal diet of 5 g/rat/day either with no supplement or with supplements of propylene glycol or dextrose equivalent to 5–20% of the total diets. Weight changes recorded in animals given 15 or 20% propylene glycol indicated that this material had an adverse effect at these dosage levels with such nutritionally restricted diets. On the basis of the results with the 5 and 10% levels, and the assumption of 3·7 cal./g for dextrose, the relative biological calorific value for propylene glycol was estimated to be 4·7 cal./g. Hence, a dosage level of 6·35 g dextrose/kg (group H) was equivalent to 5 g propylene glycol/kg (group A), and 2·54 g dextrose/kg (group E) was the equicalorific control for 2 g propylene glycol/kg (group C). Group B was used as a negative control, being given the basal diet with neither propylene glycol nor dextrose added.

The principal ingredient of the dry basal diet for all the dogs was Friskies Mix, a dog-food meal produced by Carnation Company, Los Angeles, Cal. Preliminary feeding studies with the high levels of propylene glycol to be used in the 2-yr study demonstrated the necessity of providing a mask for the taste of the glycol to ensure acceptance of the test diets by the dogs. Therefore, the following mix was freshly prepared each day for each group of five dogs, including the controls: 10 cups dry diet (with appropriate amount of propylene glycol or dextrose); 1 cup ground cooked liver; 3 cups liver broth; 6 cups water.

Test procedure. Initially, each group consisted of five male and five female beagle dogs. The dogs were 10–14 months of age when the treatment was started. Freshly prepared diet and water were provided *ad lib.* Water was available in glass, half-gallon bottles, fitted with adjustable-flow ball watering tubes and attached to the cages with Sure-lock bottle holders, Ring-seal bottle stoppers being used to prevent leaking. The tubes, holders and stoppers

were products of Atco Manufacturing Company, Napa, Cal. Water and diet consumption were recorded each day.

The propylene glycol and dextrose concentrations in the diets (X%) were adjusted weekly for each group of five male or five female dogs to approximate the pre-determined dosage levels (K g/kg), using the mean body weights (W) and mean diet consumptions (G) for the group, according to the following formula:

$$X = 100 \frac{KW}{G}$$

The appropriate diets were presented to the dogs every day. The dogs were weighed at weekly intervals. Before the start of treatment and after 6, 12 and 23 months on test, haematology, urine analyses and studies of blood biochemistry were carried out. The parameters studied were the total erythrocyte count, total and differential leucocyte counts, haemoglobin, haematocrit, erythrocyte fragility (initial and complete haemolysis), urine volume and specific gravity, urine pH and micro-examination, serum alkaline phosphatase, total bilirubin, bromsulphthalein retention (5 mg/kg, 15 min after dose), blood-glucose level, serum propylene-glycol level, serum glutamic–oxalacetic and glutamic–pyruvic transaminase activities and blood urea-nitrogen. Some of these parameters were also studied at intermediate stages to determine whether differences that had been found were reproducible.

During the final month of the study or when the dogs were killed for histopathological study of organ sections, the following criteria of effect were also assessed: anisocytosis and poikilocytosis, nucleated-erythrocyte count, reticulocyte count, direct and indirect bilirubin estimations (Van den Bergh), liver glycogen, metabolic rate of liver slices using glucose and propylene glycol as substrates, total liver lipids, liver triglycerides, water content of the liver (from constant weight of 1 g dried in a vacuum oven at 60°C and 21 mm Hg) and weights of liver, kidney and spleen. The organs examined, grossly and micropathologically, were liver, kidney, lung, heart, diaphragm, spleen, adrenal, thyroid, parathyroid, salivary and maxillary glands, tongue, lymph nodes, gall bladder, oesophagus, stomach, duodenum, pancreas, ileum, jejunum, colon, urinary bladder, testicle, epididymis, prostate, uterus, ovary, skin, eye, brain, pituitary and bone marrow.

Statistical evaluation. For the majority of the quantitative measurements all five groups were intercompared by Bartlett's test for homogeneity of variance. If the variances were homogeneous, F for analysis of variance was determined. When F was significant, the Duncan multiple range test was used to denote intergroup differences.

When the variances were heterogeneous, the groups were compared, in pairs, by the F test. If the probability for the F test was greater than 0·05, the Student's t test was used to denote the significance of differences between the means. When P was less than 0·05 for the F test, the Cochran t test was used to compare the means.

The frequency of occurrence of abnormalities was intercompared by the use of tables compiled by Finney (1963) for testing significance in a 2 × 2 contingency table.

For growth effects the individual weight of each dog was adjusted to the gain in weight over its weight on the day treatment was started. These mean weight gains were statistically intercompared, as were change-data for various other criteria, such as bilirubin and erythrocytes. Where appropriate, the non-parametric test of comparison of sum of ranks was used.

RESULTS

Mortality

Only one dog died and one was killed for examination during the course of the study. One was a female fed 2·54 g dextrose/kg for 81 wk, while the other was a male fed 2·0 g propylene glycol/kg for 85 wk. Both, therefore, were from the lower dosage levels. The former had a ventricular thrombus while the latter had suppurative endocarditis and myocarditis. Neither of these conditions was deemed to be related to treatment.

Body-weight change

The mean changes in body weight, calculated from the immediate pretreatment weight of each dog, are presented in Fig. 1 for the males and in Fig. 2 for the females. At each of the

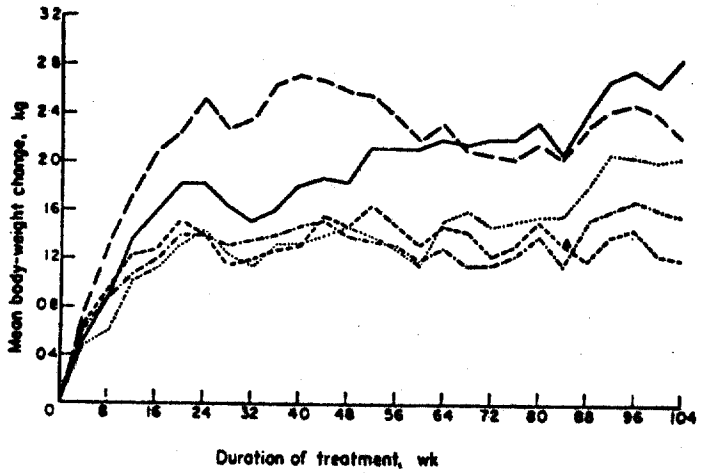

FIG. 1. Mean changes in body weight of male dogs fed diets containing propylene glycol (group A, 5 g/kg ———; group C, 2 g/kg - - - -) or dextrose (group H, 6·35 g/kg — —; group E, 2·54 g/kg -·-·-·-) compared with those of control group B (· · · · · ·) fed the basic diet. Dog 9048 (▲) from group C was killed at wk 85.

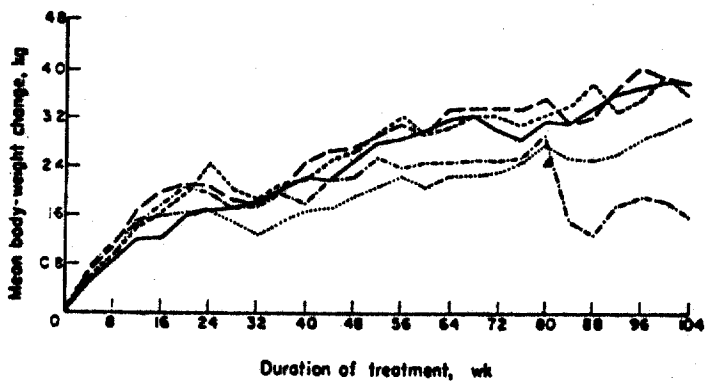

FIG. 2. Mean changes in body weight of female dogs fed diets containing propylene glycol (group A, 5 g/kg ———; group C, 2 g/kg - - - -) or dextrose (group H, 6·35 g/kg — —; group E, 2·54 g/kg -·-·-·-) compared with those of control group B (· · · · · ·) fed the basic diet. Dog 9042 (▲) from group E died at wk 81.

27 intervals at which statistical comparisons were made during the course of the 2-yr feeding study, the mean body weights of the dogs on propylene-glycol diets, groups A and C, were comparable to those of the control group B. For the first half-year, the males of group H (6·35 g dextrose/kg) showed a significantly greater increase in weight than did those of control group B; at only one period, wk 8, was group H significantly higher than its equicalorific control group A. From Fig. 1, it is apparent that the male dogs in both the groups on a high calorific intake (A and H) gained more weight than did the males in the other three groups. For the second year of treatment both groups showed practically equal changes in body weight.

Dietary levels, dosage and intake of food and water

The median percentages of propylene glycol or dextrose added to the diets of the dogs are listed in Table 1. The percentages of the materials in the diets were increased fairly rapidly

Table 1. *Levels of propylene glycol and dextrose added to the basic diet and consumption figures for test and control diets over the 2-yr period*

Group	Intended dose (g/kg/day)	Test compound in diet (median %)		Diet consumed (g/dog/day)			
				Males		Females	
		Males	Females	Median	Range	Median	Range
A	PG: 5	19·4	21·0	259	238–271	232	216–250
H	D: 6·35	24·1	26·1	273	252–301	258	218–286
C	PG: 2	7·4	8·9	260	244–269	233	214–259
E	D: 2·54	9·0	9·8	266	248–282	240	211–270
B	0 (control)	—	—	265	248–278	260	234–278

PG = propylene glycol D = dextrose

The values given were derived from the monthly means for each group.

during the first 6 months, to maintain a constant dosage, the increases being relatively slower during the remainder of the study. It should be noted that high percentages of propylene glycol and dextrose were necessary to attain the dosage goals of 5 and 6·35 g/kg, respectively. Median values of approximately 20% for propylene glycol and of 25% for dextrose were required. The dosages attained were within ± 2% of the goals for the males and ± 5% for the females.

The medians and ranges of the monthly computed diet-consumption data are also shown in Table 1. The primary difference, if any, was during the first year of treatment. As the dogs gained most weight during the first 6 months of treatment and as the only gross indication of possible diet-consumption differences was also apparent during this period, diet utilization was calculated for the first and for the second 3-month periods as well as on the combined data for the first 6 months. For each dog, the body-weight change (in g) was divided by the diet consumed (in g) and this ratio was multiplied by 100. The mean data are presented in Table 2. The only significant differences were the early increases in weight gain per unit of diet consumed for the males in groups A and H, given the high levels of propylene glycol and dextrose. As the diets of these dogs contained more calories than the diet of the group B controls, this was not unexpected.

Table 2. *Mean diet utilization during the first 6 months of the test in dogs given diets containing propylene glycol or dextrose*

Group	Dosage (g/kg/day)	Mean diet utilization† in Males: First 3 months	Males: Second 3 months	Males: First 6 months	Females: First 3 months	Females: Second 3 months	Females: First 6 months
A	PG: 5	5·88*	1·69	3·79	5·94	3·45	4·70
H	D: 6·35	6·93**	2·31	4·62	7·26	1·12	4·19
C	PG: 2	5·16	0·52	2·84	6·36	4·59	5·47
E	D: 2·54	4·67	1·16	2·92	4·16	0·80	2·48
B	0 (control)	3·84	1·60	2·72	6·20	1·11	3·65

PG = propylene glycol D = dextrose

† $\frac{\text{g weight change}}{\text{g diet consumed}} \times 100$

Significant differences are marked by asterisks: *A > B[b] and **H > B[b], E[a] ([a]0·05 > P > 0·01; [b]0·01 > P > 0·001).

During the first year of the study, and particularly during the first 2 months that water consumption was measured, the dogs given propylene glycol drank less water than did their controls. While the consumption by these dogs was also numerically lower than that of their equicalorific dextrose controls during this period, in general, the differences were not statistically significant. A summary of the medians and ranges of the monthly means of water consumption for the entire 2 yr is given in Table 3. The overall median quantities of

Table 3. *Water consumption over the 2-yr test period by dogs fed diets containing propylene glycol or dextrose*

Group	Dosage (g/kg/day)	Water consumption (ml/day) by Males: Median	Males: Range	Females: Median	Females: Range
A	PG: 5	360	224–450	306	162–448
H	D: 6·35	343	227–445	276	232–378
C	PG: 2	403	246–492	265	191–335
E	D: 2·54	444	308–495	334	264–459
B	0 (control)	365	258–468	376	288–434

PG = propylene glycol D = dextrose

The values given were derived from the monthly means for each group recorded throughout the test period.

water consumed by the male dogs given propylene glycol were equivalent to the value for the control group B and to those of the equicalorific dextrose groups. For the females, the median amount of water consumed was numerically lower in all four treated groups than in group B.

Haematology, urinalysis biochemistry and organ weights

Findings in both sexes of the groups given propylene glycol were comparable with those in the equicalorific and other controls at each interval measured in respect of differential leucocyte counts, erythrocyte fragility, urinary pH and micro-examination, alkaline phosphatase, bromsulphthalein retention, liver glycogen, blood glucose, total liver lipids, metabolic rate of liver slices using propylene glycol, activities of serum glutamic–oxalacetic and glutamic–pyruvic transaminases, percentage of water in the liver and liver weights, *per se* and relative to body weight.

In a few parameters, there were statistically significant changes that were not dose-related and were not deemed indicative of adverse effect. These were sporadically lower total leucocyte counts in males given propylene glycol at 2 but not at 5 g/kg, elevated blood-urea nitrogen in females given 6·35 g dextrose/kg (after 6 months of treatment only), a lower level of liver triglyceride in females given propylene glycol at 2 but not at 5 g/kg, and slightly increased kidney weights *per se* in females given 5 g propylene glycol/kg. Kidney weight to body weight ratios were all statistically equivalent and no kidney micropathology was seen.

In certain parameters, the group given the higher dosage level of propylene glycol (5 g/kg; group A) differed significantly from the equicalorific control group H and/or from the negative control group B. Means or total values for these are presented in Table 4.

The mean total erythrocyte count as well as the haemoglobin and haematocrit values of group A (5 g propylene glycol/kg) were numerically lower in both sexes than those of group H (6·35 g dextrose/kg) and of group B (the negative controls) at 6 and 12 months and the decreases were statistically significant after 23 and 23·5 months of treatment, both on the *per se* and the change from pretreatment level bases. Furthermore, the percentages of nucleated erythrocytes were higher in group A, compared with groups H and B at 23 months, as was the mean reticulocyte count and the degree of anisocytosis and poikilocytosis. These changes were usually present to a greater degree in the females than in the males.

Total bilirubin was numerically increased in the group A males at 6 and 24 months and was numerically and/or statistically higher in the group A females, when compared with groups H and B at 6, 8, 12, 13, 23 and 24 months of treatment. Van den Bergh direct and indirect bilirubin values were normal near the termination of the 2-yr period of treatment.

Urine output of the females in group A was significantly raised at 6 and 12 months as was that for the males at 23 months. As previously discussed, the water intake of the dogs in this group during the first year of treatment was somewhat depressed. Furthermore, at intervals throughout the study, the specific gravity of the urine was relatively lower in dogs of group A.

Serum propylene-glycol levels were, of course, higher in the dogs given 5 or 2 g propylene glycol/kg than in those that received no propylene glycol in the diet. The highest recorded individual value, measured 24 hr after the daily dose of glycol, was equivalent to 0·2% (v/v), but all other individual values were below 0·1% (v/v). In an additional experiment on naïve, previously untreated females, plasma levels were determined as a function of time after a single dose of 5 g propylene glycol/kg. Peak values were reached between 6 and 8 hr after treatment, levels at 12 hr being approximately half those at 8 hr. The maximum value reached was 0·56% propylene glycol.

The only significant changes in glucose metabolic rates, when glucose was used as substrate, were found in the males of the propylene-glycol groups A and C using the lipid[^{14}C]

Table 4. *Mean or total values for parameters showing statistically significant differences between groups*

Time (months)		Values in males of group					Values in females of group				
		A (5 g PG/kg)	H (6.35 g D/kg)	C (2 g PG/kg)	E (2·54 g D/kg)	B (control)	A (5 g PG/kg)	H (6·35 g D/kg)	C (2 g PG/kg)	E (2·54 g D/kg)	B (control)
					Total erythrocytes (10^6/mm³)						
0		7·33	6·92	7·25	7·01	7·23	7·96	7·63	7·79	7·54	7·24
6		6·97	8·13[1]	7·01	7·42	7·50	6·84	8·00	7·26	7·91	7·62
12		6·68	7·44	7·19	7·94	7·28	7·07	7·31	7·61	7·89	7·43
23		6·64[a]	7·53	7·08	7·47	7·48	7·08[10,d]	8·52	7·87	7·78	8·07
23·5		6·40[f]	7·46	6·53	7·39	7·47	6·25[11,e]	7·66	6·98	7·67	7·21
					Haemoglobin (g/100 ml)						
0		15·0	14·6	14·6	14·3	15·1	17·2	15·6	16·3	15·9	15·2
6		16·7	19·1[1]	16·7	17·7	18·0	16·3	18·3	17·3	18·5	17·7
12		17·1	18·8	18·1	18·6	18·2	17·5	18·2	18·6	19·3	18·8
23		16·8	18·7	17·4	18·6	18·8	17·8	20·1	19·2	18·9	20·1
23·5		15·7[h]	18·1	16·3	17·8	17·8	15·4[g]	18·5	16·8	18·3	18·5
					Haematocrit (%)						
0		45·9	44·6	43·6	42·9	46·1	50·4	46·1	48·1	48·3	45·5
6		47·4	52·4[3]	46·1	47·9	49·1	46·3	50·0	48·6	49·4	48·3
12		46·7	50·0	48·1	49·4	48·1	47·9	46·9	49·9	50·9	49·9
23		45·5	49·7	46·4	49·2	49·6	47·6[j]	53·7	51·3	49·4	51·8
23·5		43·2	48·1	42·0	47·7	47·6	42·7[e]	48·8	46·3	48·0	47·3
					Nucleated erythrocytes (% of erythrocytes)						
23·5		4·4	2·6	2·8	2·8	3·6	8·0[m]	1·6	3·0	2·0	1·8
					Reticulocytes (% of erythrocytes)						
24		2·08	1·44	1·55	1·32	1·80	3·36[9]	1·44	2·04	1·50	1·32
					Anisocytosis (no. of dogs)						
23·5	Normal...	0[j]	0	4	5	5	0[k]	4	4	4	5
	Slight...	2	5	0	0	0	3	1	1	0	0
	Moderate...	3	0	0	0	0	2	0	0	0	0
					Poikilocytosis (no. of dogs)						
23·5	Normal...	0[j]	0	4	5	5	0[m]	4	4	4	5
	Slight...	2	5	0	0	0	3	1	1	0	0
	Moderate...	3	0	0	0	0	2	0	0	0	0
					Total bilirubin (mg/100 ml)						
0		0·30	0·29	0·28	0·29	0·26	0·39	0·36	0·37	0·29	0·36
6		0·74[8]	0·41	0·40	0·35	0·43	0·51[4]	0·35	0·34	0·33	0·29
8		0·28	0·17	0·21	0·26	0·19	0·36	0·31	0·28	0·25	0·24
12		0·34	0·26	0·29	0·30	0·19	0·48[5]	0·23	0·32	0·31	0·32
13		—	—	—	—	—	0·46[6]	0·25	0·27	0·34	0·29
23		0·40	0·28	0·31	0·33	0·29	0·55[7]	0·36	0·42	0·39	0·34
24		0·41[12]	0·26	0·28	0·25	0·23	0·41	0·31	0·34	0·30	0·29
					Urine output (ml)						
0		325	326	346	413	295	212	259	196	276	353
6		202	94[13]	125	254	132	235[16]	110	139	82[18]	173
12		274	177	214	234	174	393[17]	215	241	209	295
23		277[14]	201	148	270[15]	117	217	158	190	158	208
					Water consumption (ml)						
1		222	227	246	308	322	162[19]	283	204[20]	312	370
2		240	251	291	366	366	169[19]	279	198[21]	327	382
6		304	330	374	446	334	224	272	222	310	346
12		410	367	438	493	468	373	310	315	459	434
23		389	415	422	437	356	448	330	302	358	411
					Specific gravity of urine						
0		1·029	1·026	1·028	1·020	1·034	1·037	1·031	1·045	1·034	1·028
6		1·029[22]	1·053	1·043	1·036[23]	1·056	1·029[17]	1·044	1·047	1·047	1·035
12		1·028	1·037	1·038	1·035	1·043	1·023	1·035	1·036	1·037	1·030
23		1·025[19]	1·029	1·045	1·030	1·051	1·033	1·041	1·038	1·044	1·039
					Metabolic rate liver slice, glucose substrate, lipid[^{14}C] (× 10^2)						
24		24·6[24]	69·4	44·2[25]	100·6	65·8	37·6	64·6	36·0	41·8	68·8

PG = propylene glycol D = dextrose

Values differing from those of other groups are marked by superscripts, as follows (probability being indicated by $^{a}0·05 > P > 0·01$; $^{b}0·01 > P > 0·001$; $^{c}P < 0·001$):

per se Basis

[1]H > A[b], C[b]
[2]H > A[a], C[b], E[a]
[3]A < H[a], C[a], E[a], B[a]
[4]A < H[b], C[b], E[b], B[a]
[5]A < H[c], E[b], B[b]
[6]A < H[b], C[a], B[a]
[7]A < H[c], E[c], B[b]
[8]A < H[b], E[b], B[b]
[9]A < H[b], C[a], E[b], B[b]
[10]A < H[b], B[a]
[11]A < H[b], E[c]
[12]A < H[b], C[b], E[c], B[c]
[13]H < B[a]
[14]A < C[a], B[b]
[15]E < B[a]
[16]A < H[b], C[a], E[b]
[17]A < H[a], C[a], E[b]
[18]E < B[a]
[19]A < B[b]
[20]C < B[a]
[21]C < E[a], B[b]
[22]A < H[a], C[a], B[a]
[23]E < H[a], B[a]
[24]A < H[a], E[c], B[a]
[25]C < E[a]

Change from pre-dose basis

[d]A < H[b], B[b]
[e]A < H[c], E[a], B[a]
[f]A < H[a], E[a]
[g]A < H[b], E[b], B[b]
[h]A < H[b], E[b]
[i]A < H[b], C[b], B[b]

By chi square

[j]A ≠ B[a]; A ~ H; H = C
[k]A ≠ H[a], B[a]; H ~ B

By sum of ranks

Table 5. *Summary of pathological findings in liver, kidney, spleen and bone marrow*

			Findings in males* given					Findings in females* given				
			Propylene glycol (g/kg/day)			Dextrose (g/kg/day)		Propylene glycol (g/kg)			Dextrose (g/kg)	
			5·0	2·0	0	6·35	2·54	5·0	2·0	0	6·35	2·54
Organ and finding	Examination	Total no. of dogs examined...	5	4	5	5	5	5	5	5	5	4
Liver												
Adhesions to diaphragm	G		0	0	0	0	0	0	1	0	0	0
Lymphoid accumulations	M		0	1	0	0	0	0	0	0	0	0
Round-cell accumulations	M		1	0	0	0	0	2	1	1	0	0
Bile-duct proliferation, slight	M		1	0	2	1	0	2	1	1	0	0
Haemosiderosis	M		0	0	0	0	0	1	0	0	0	0
Glycogen, infiltration of hepatic cord cells	M		0	0	0	0	0	1	0	0	0	0
Gomori stain for iron, negative reaction	M		5	4	5	5	5	5	5	5	5	4
Collagen increase in blood vessels	M		0	0	0	0	1	0	0	0	0	0
Kidney												
Surface scarring	G		0	0	0	0	1	0	0	0	0	0
Rough surface	G		0	0	0	0	0	0	1	0	0	0
Difference in size	G		0	0	0	0	0	0	1	0	0	0
Old infarct-like area	M		0	0	0	0	1	0	1	0	0	0
Mineral spicules, medulla	M		3	0	1	4	2	1	1	1	2	3
Interstitial reaction	M		0	0	0	0	1	0	0	0	0	0
Spleen												
Rough surface	G		0	0	0	0	0	2	0	0	0	0
Enlarged	G		0	0	0	0	0	0	1	0	0	0
Haemorrhagic infarct	G		0	3	2	0	0	0	0	1	0	3
Siderotic plaques	G		3	4	2	2	3	2	2	1	3	2
Haemorrhagic infarct	M		0	3	2	0	1	1	0	1	0	1
Siderotic plaques	M		3	1	3	3	2	1	1	1	2	0
Blood vessels staining with haematoidin	M		0	1	0	0	0	0	0	0	0	0
Congestion	M		0	0	0	0	0	0	1	0	0	0
Haemosiderosis	M		0	0	0	0	0	0	1	0	1	1
Reticulo-endothelial hyperplasia	M		0	0	1	0	0	0	0	0	0	0
Gomori stain for iron:												
positive reaction	M		2	1	0	2	3	2	1	1	2	1
negative reaction	M		3	3	5	3	2	3	4	4	3	3
Bone marrow												
Fatty	G		0	1	1	1	2	0	0	2	0	1
Fatty	M		0	1	0	0	0	0	0	0	0	0
Few megakaryocytes	M		0	2	0	0	0	0	0	0	0	0
Numerous megakaryocytes	M		2	0	0	0	0	1	3	0	0	0
Normally active	M		4	3	0	3	3	2	2	2	4	1
Moderately active	M		1	1	3	0	1	3	3	1	1	1
Slightly or moderately inactive	M		0	0	1	1	0	0	0	1	0	1
Markedly inactive	M		0	0	1	1	1	0	0	1	0	1
Haemosiderosis	M		2	1	0	0	0	0	0	0	0	0
Bacteria	M		0	1	0	0	0	0	0	0	0	0

measurement. Here, the values for group A males were significantly lower than those for groups H and B, and those for the males on 2 g propylene glycol/kg were lower than those for the equicalorific dextrose counterpart, group E.

Pathology

Although numerous sporadic lesions (Table 5) were observed in the tissues of dogs treated with propylene glycol or dextrose, similar lesions were found in the controls and, as the findings were not dosage-related they were not considered to be the result of treatment. The pathologist also considered that the slight increase in bone-marrow activity in group A females was physiological (not pathological).

DISCUSSION

Although the diets of groups A (5 g propylene glycol/kg) and H (6·35 g dextrose/kg) were isocalorific, the calories in the diet were not necessarily equally available for conversion to adipose tissue. The smaller propylene glycol molecule may pass from the blood through the kidneys to the urine more rapidly and in larger amounts than the larger dextrose molecule. Thus, more of the dextrose calories could be available for conversion to adipose tissue and this could account for differences in the rate of weight gain in the early weeks of the study.

When oxidized in the body, 1 g propylene glycol yields 0·95 g water and 1 g dextrose yields 0·60 g water. Group A received approximately 50 g propylene glycol/day, group H 66 g dextrose, group C 20 g propylene glycol and group E 24 g dextrose in excess of the carbohydrate in the basic diet received by group B. Water generated from oxidation of these added intakes of carbohydrate is adequate to explain the observations of low water intake, the discrepancies between water intake and urine volumes, and the low specific gravity of some urine samples. These are, therefore, not deleterious effects.

In group A (5 g propylene glycol/kg/day), an effect upon erythrocytes was evident in comparison with the nutritionally equivalent group H. Haemoglobin, haematocrit and total erythrocyte count were slightly low while increases were seen in anisocytosis, poikilocytes and reticulocytes. These changes were indicative of some erythrocyte destruction with replacement from the bone marrow. The dietary concentration of propylene glycol necessary to produce a dosage level of 5 g/kg/day averaged about 20%, while for the 2 g/kg group it approximated 8%. All of the dose-related significant haematological findings were consistent with an increased rate of erythrocyte haemolysis associated with the high concentrations of propylene glycol in the blood. Even at a propylene-glycol concentration of 20% in the diet, this effect was not sufficient to result in any changes that appeared to be irreversible and there was no evidence of damage to bone marrow or spleen. These haematological changes were not evident in dogs of group C, which received 2 g propylene glycol/kg/day.

There was a statistically significant reduction in the rate of lipid formation from glucose by liver slices in the male dogs of group A compared with their dextrose controls. No other dose-related, statistically significant effects referable to the liver were seen. Specifically there were no effects on bromsulphthalein retention, serum alkaline phosphatase, serum glutamic-oxalacetic and glutamic-pyruvic transaminases, amounts of glycogen, lipids and water in the liver or microscopic histopathology.

No evidence of the uncharacterized "slight chronic liver damage", which Morris *et al.* (1942) reported in rats receiving up to 4·9% propylene glycol in the diet, was seen in dogs

consuming 2 g/kg/day (7·4–8·9% in the diet) for 2 yr; nor was any other effect seen. In dogs consuming 5 g/kg/day (20% in the diet) there was no more than a slight increase in total bilirubin, and a decrease in erythrocytes accompanied by immature circulating cells attributable to increased fragility from the transient presence of the glycol in the blood plasma after each meal. Therefore, it is concluded that propylene glycol fed at a concentration of approximately 8% in the diet (2 g/kg/day) is utilized as a carbohydrate energy source without any adverse effects.

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Incorporation de propylène-glycol au régime alimentaire des chiens: Résultats d'un essai de deux ans

Résumé—Deux groupes de chiens bigles, cinq mâles et cinq femelles, ont été soumis pendant deux ans à un régime alimentaire contenant du propylène-glycol ou du dextrose, ce dernier comme témoin équicalorique. Le propylène-glycol était dosé à 5,0 et à 2,0 g/kg et le dextrose à 6,35 et à 2,54 g/kg. Un autre groupe faisait office de témoin et n'a reçu aucun de ces deux produits. Les effets des différents régimes ont été évalués d'après la mortalité, la croissance pondérale, l'assimilation des aliments et la consommation d'eau, la micropathologie, le poids du foie, des reins et de la rate et les paramètres hématologiques, urologiques et biochimiques.

La dose de 2 g de propylène-glycol n'a influé significativement sur aucun de ces critères. Les dosages plus élevés de propylène-glycol et de dextrose ont été maintenus par l'introduction de respectivement environ 20 et 25% dans le régime des chiens. Les deux produits fournissant des calories susceptibles de transformation en tissu adipeux, les deux groupes en cause ont gagné un peu plus de poids que les animaux témoins pendant la première phase de l'expérience. Aux hautes concentrations, le propylène-glycol fait augmenter le taux d'hémolyse des érythrocytes. Au régime de 5 g de propylène-glycol/kg, le taux d'hémoglobine, l'hématocrite et la numération globulaire ont donné des valeurs plutôt basses, tandis qu'augmentait la fréquence de l'anisocytose, des poïkilocytes et des réticulocytes. Ces phénomènes indiquent une destruction des érythrocytes et leur remplacement accéléré par la moelle osseuse. Cet effet n'était pas suffisamment prononcé, même quand la dose de propylène-glycol était portée à 20% du régime, pour provoquer des altérations d'apparence irréversible. Aucun indice de lésions de la moelle osseuse ou de la rate n'a été relevé. La plupart des paramètres relatifs aux effets sur le foie ont été similaires à tous les dosages. La bilirubine totale a légèrement augmenté chez les chiens qui recevaient 5 g de propylène-glycol/kg.

L'article conclut que le propylène-glycol consommé par les chiens à raison de 8% du régime (2 g/kg/jour) est utilisé comme source d'énergie glucidique, et cela sans effets néfastes.

Ergebnisse der Verfütterung von Propylenglycol im Futter von Hunden auf die Dauer von zwei Jahren

Zusammenfassung—Gruppen von fünf männlichen und fünf weiblichen Beaglehunden erhielten zwei Jahre lang Futter, das Propylenglycol oder Dextrose enthielt, die letztere als kaloriengleiche Kontrolle. Die verwendeten Konzentrationen waren 5,0 und 2,0 g Propylenglycol/kg und 6,35 und 2,54 g Dextrose/kg, und ausserdem erhielt eine Kontrollgruppe keine der beiden Verbindungen. Zu den Wirkungskriterien gehörten Mortalität, Körpergewichtsveränderung, Nahrungsmittelverwertung und Wasserverbrauch, Mikropathologie, Leber-, Nieren- und Milzgewichte und Messungen von Blut-, Urin- und biochemischen Parametern.

Keines der gemessenen Kriterien änderte sich signifikant bei 2 g Propylenglycol/kg. Die höheren Dosierungen von Propylenglycol und Dextrose wurden durch Zugabe von etwa 20 bzw. 25% zum Futter der Hunde erhalten. Da beide Verbindungen Kalorien liefern, die zur Umwandlung in Fettgewebe zur Verfügung stehen, nahm das Körpergewicht beider Gruppen während des ersten Teils des Versuchs etwas stärker zu als bei den Kontrolltieren. Propylenglycol erhöht in hohen Konzentrationen die Geschwindigkeit der Erythrocytenhämolyse. Als es mit der Konzentration 5 g/kg verfüttert wurde, waren der Hämoglobin- und Hämatokritwert und die Erythrocytengesamtzahl etwas niedrig, während die Häufigkeit von Anisocytosis, Poikilocyten und Reticulocyten erhöht war, Befunde, die auf Erythrocytenzerstörung mit beschleunigter Neubildung im Knochenmark schliessen lassen. Selbst bei einer Propylenglycolkonzentration von 20% im Futter war dieser Effekt noch nicht ausreichend, um irreversibel erscheinende Veränderungen zu bewirken, und nichts liess auf Schädigung von Knochenmark oder Milz schliessen. Die Mehrzahl der Parameter, die mit Auswirkungen auf die Leber zusammenhängen, war bei allen Dosierungsgruppen ähnlich. Bei Hunden, die 5 g Propylenglycol/kg erhielten, war eine leichte Erhöhung des Gesamtbilirubins vorhanden.

Es ergibt sich der Schluss, dass Propylenglycol bei der Verfütterung an Hunde in einer Konzentration von etwa 8% im Futter (2 g/kg/Tag) als Kohlenhydrat-Energiequelle ohne nachteilige Wirkungen verwertet wird.

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Pharmacotoxic Evaluation of Nine Vehicles Administered Intraperitoneally to Mice

ELMER G. WORTHLEY AND C. DONALD SCHOTT
(*Natural Products Office, Medical Research Laboratories, Edgewood Arsenal, Maryland*)

Choosing a vehicle for resuspension of ethanol extracts is a bothersome problem faced by the Natural Products Laboratory. Resuspended ethanol extracts are not injected intravenously because a true solution is not usually obtained; hence, fatal occlusion of pulmonary arteries by masses of suspended material could result. Pulmonary capillaries cannot pass the larger particles; *i.e.*, those 20 μ or larger in diameter (7). As a result, all ethanol extracts are administered intraperitoneally when the extract fails to go into solution upon resuspension in isotonic saline.

Any active principle(s) that may be present in a crude extract is probably already so diluted that any "overt symtoms" displayed by mice may not occur until a dose as high as 150 mg/kg is reached. Usually, crude extract material must not be concentrated in excess of 100 mg/ml because it may clog the syringe. The vehicle (diluent) should have a high ED_0 (highest dose at which no effects are seen in any of the test population) because, if it causes a reaction in the test animal, it may be impossible to distinguish a symptom caused by the crude extract from that caused by a pharmacologically active solvent.

The purpose of the present study was to determine the LD_0 (highest dose at which no lethalities occur in a test population) and ED_0 for intraperitoneal injections into mice of the following nine solvents: (1) 100% ethanol, (2) 100% polyethylene glycol-200, (3) 100% propylene glycol, (4) 100% dimethyl sulfoxide, (5) 100% sterol diluent, (6) distilled water, (7) 0.5% methylcellulose solution, (8) isotonic saline solution, and (9) 3% polyvinylpyrrolidone suspended in isotonic saline solution.

MATERIAL AND METHODS

Replicate groups of 10 male, Swiss albino, Caesarean-derived mice were given each solvent intraperitoneally (at 0.167 common or Briggsian log dose intervals to base 10 at lethal-dose levels and 0.5 common Briggsian log dose intervals to base 10 at effective-dose levels) and retained for observation (up to five days) until death or until normal activity returned. The room temperature was 24°–28°, with the humidity ranging from 20–30% during the day and from 60–70% during the night. The mice (in groups of 20) were housed overnight in plastic-bottomed, metal-topped cages (9×19×5 in.) prior to injection. After administration of the vehicle, the mice were transferred to smaller, plastic-bottomed, metal-topped cages (6×11×5 in.) in groups of five for observation. Food and water were available ad libitum. The weight range of the mice was 20–30 g. Time of injection, reactions displayed, and time of death were observed and recorded. The solvents with their empirical formulas are as follows (5, 21, 26, 29):

Name of solvent	Empirical formula
Ethanol	CH_3CH_2OH
Polyethylene glycol-200	$HOCH_2(OCH_2CH_2)_nOH$
Propylene glycol	$CH_3OHCHOHCH_3$
Dimethyl sulfoxide	$(CH_3)_2SO$
Sterol diluent:[1]	
Benzyl alcohol	$C_6H_5CH_2OH$
Polysorbate 80; Tween 80; Span; polyoxyethylene sorbitan	Complex mixture of polyethylene ethers of mixed partial oleic esters or sorbital

[1]To make sterol diluent add 9 mg of benzyl alcohol, 4 mg of Tween 80, 5 mg of sodium carboxymethylcellulose and 9 mg of soldium chloride to ml of water.

mono-oleate	anhydrides ($C_6H_{10}O_6R$; $C_6H_{12}O_5R$; $C_7H_9O_6R$)$_n$ R = fatty acid residue
Sodium carboxymethylcellulose	$(R_nOCH_2COONa)_xR_n$ = glucose chain. Solubility depends upon degree of substitution of carboxyl groups
Salt	NaCl
Distilled water	H_2O
Methylcellulose (4,000 cp; type HG 65, Dow Chemical)	$(R_nOCH_3)_x$(R_n = glucose chain)
Saline solution[2]	$NaCl \cdot H_2O$
Polyvinylpyrrolidone	$(-CH_2CHC_6H_4NO-)_n$

RESULTS

The present study has shown that the descending order of toxicity of the solvents is as follows: (1) 100% ethanol, (2) 100% polyethylene glycol-200, (3) 100% propylene glycol, (4) 100% dimethyl sulfoxide, (5) 100% sterol diluent, (6) distilled water, (7) 0.5% methylcellulose solution, (8) isotonic saline solution, (9) 3% polyvinylpyrrolidone (tables 1, 2, and 3).

TABLE 1. *Estimated lethal and effective dose levels of solvents tested.*

Solvent[a]	LD_0 ml/kg	ED_0 ml/kg
Polyvinylpyrrolidone	>316.0	31.6
Isotonic saline solution	>316.0	21.4
Methylcellulose (0.5%)	147.0	1.0
Distilled water	147.0	14.7
Sterol diluent	100.0	10.0
Dimethyl sulfoxide	6.8	0.32
Propylene glycol	6.8	0.1
Polyethylene glycol-200	4.6	1.0
Ethanol	0.32	0.1

[a]Administered intraperitoneally to mice.

DISCUSSION

Sterol diluent.—The authors chose sterol diluent for use in routine administration of extracts in most quantities to animals because it has good solvent properties (9) and is not very toxic (LD_0 = 100.0 ml/kg, ED_0 = 10.0 ml/kg). This mixture of benzyl alcohol, 'Tween 80, sodium carboxymethylcellulose, saline, and water is used routinely for the resuspension of extracts that will not go into solution with saline. Macht (15, 16) lists the toxicity of benzyl alcohol as about 2.0 g/kg, which is about the amount present in toxic doses of sterol diluent. Symptoms or death after the administration of benzyl alcohol occur very rapidly (10 to 20 min) (17), as they did after the administration of sterol diluent in the present study; hence, the toxicity of sterol diluent is attributed to benzyl alcohol. The rapidity of the toxic action of sterol diluent facilitates the evaluation of suspended materials, because any symptoms caused by the solvent will have occurred within 15 to 20 min. One could, therefore, administer more than 10.0 ml/kg (ED_0) to mice and still be sure that any signs observed after 30 min were caused by the extract. This is probably true for doses up to 30 ml/kg.

Methylcellulose (0.5%).—Possessing good emulsifying properties (9), methylcellulose has also been demonstrated to be highly effective when used to resuspend certain types of crystals; e.g. sulfur particles (10). Methylcellulose is not very

[2]Saline solution is 0.9% sodium chloride in water which is isotonic to human blood, esp. red blood cells.

TABLE 2. *Number of deaths out of each set of ten mice tested at various dose levels.*

Dose (interaperitoneal) ml/kg	Solvent[a]								
	A	B	C	D	E	F	G	H	I
0.1	—	—	—	—	—	—	0/10	—	0/10
0.32	—	—	—	—	—	0/10	0/10	—	0/10
1.0	—	—	0/10	—	—	0/10	0/10	0/10	1/10
1.5	—	—	—	—	—	—	—	—	10/10
2.1	—	—	—	—	—	—	—	—	8/10
3.2	—	—	0/10	—	—	0/10	—	0/10	10/10
4.6	—	—	—	—	—	—	—	0/10	—
6.8	—	—	—	—	—	0/10	0/10	4/10	10/10
10.0	—	—	—	—	0/10	4/10	5/10	10/10	—
14.7	—	—	—	0/10	—	10/10	10/10	—	—
21.4	—	0/10	—	—	—	—	—	10/10	—
31.6	0/10	0/10	—	0/10	0/10	—	—	—	—
46.5	—	—	—	—	—	—	—	—	—
68.0	0/10	—	—	—	—	—	—	—	—
100.0	—	—	—	0/10	0/10	—	—	—	—
147.0	—	—	0/10	0/10	10/10	—	—	—	—
214.0	—	—	2/10	8/10	10/10	—	—	—	—
316.0	0/10	0/10	10/10	10/10	—	—	—	—	—

[a] *A* = polyvinylpyrrolidone; *B* = isotonic saline solution; *C* = 0.5% methycellulose; *D* = distilled water; *E* = sterol diluent; *F* = dimethyl sulfoxide; *G* = propylene glycol; *H* = polyethylene glycol-200; *I* = ethanol.

TABLE 3. *Number of mice showing reactions out of each set of ten mice tested.*

Dose (intraperitoneal) ml/kg	Solvent[a]								
	A	B	C	D	E	F	G	H	I
0.1	—	—	—	—	—	—	0/10	—	0/10
0.32	—	—	—	—	—	0/10	10/10	—	3/10
1.0	—	—	0/10	—	—	10/10	10/10	0/10	10/10
1.5	—	—	—	—	—	—	—	—	10/10
2.1	—	—	—	—	—	—	—	—	10/10
3.2	—	—	10/10	—	—	10/10	—	10/10	10/10
4.6	—	—	—	—	—	—	—	10/10	—
6.8	—	—	—	—	—	10/10	10/10	10/10	10/10
10.0	—	—	—	—	0/10	10/10	10/10	10/10	—
14.7	—	—	—	0/10	—	10/10	10/10	—	—
21.4	—	0/10	—	—	—	—	—	10/10	—
31.6	0/10	10/10	—	9/10	6/10	—	—	—	—
46.5	—	—	—	—	—	—	—	—	—
68.0	8/10	—	—	—	—	—	—	—	—
100.0	—	—	—	10/10	10/10	—	—	—	—
147.0	—	—	10/10	10/10	10/10	—	—	—	—
214.0	—	—	10/10	10/10	10/10	—	—	—	—
316.0	10/10	10/10	10/10	10/10	—	—	—	—	—

[a] *A* = polyvinylpyrrolidone; *B* = isotonic saline solution; *C* = 0.5% methylcellulose; *D* = distilled water; *E* = sterol diluent; *F* = dimethyl sulfoxide; *G* = propylene glycol; *H* = polyethylene glycol-200; *I* = ethanol.

toxic (LD_0 = 147.0 ml/kg, ED_0 = 1.0 ml/kg), but it can cause decreased locomotor activity at low doses. When sterol diluent fails to give a good solution or suspenstion of crude extract, 0.5% methylcellulose is used. The present authors do not use it routinely because of its low ED_0 and because sterol diluent possess better solvent properties.

Polyvinylpyrrolidone (3%).—The least toxic of the solvents tested (LD_0 = >316.0 ml/kg, ED_0 = 31.6 ml/kg), polyvinylpyrrolidone is occasionally used in conjunction with sterol diluent when ethanol extracts are difficult to resuspend. Inherent intraperitoneal toxicity could not be determined because of the volume of fluid required. It is also doubtful that inherent intravenous toxicity could be demonstrated easily because this material is so nontoxic. Polyvinylpyrrolidone is commonly used as a human plasma extender (5).

Saline Solution.—Saline solution is not very toxic when administered intraperitoneally in the doses tested (LD_0 = >316.0 ml/kg, ED_0 = 21.4 ml/kg). It is used routinely for suspending aqueous extracts instead of polyvinylpyrrolidone because it is more easily obtained and easier to work with. Saline solution does not possess the proper solvent characteristics for resuspending materials other than water extractives. Inherent intraperitoneal toxicity could not be determined because of the volume of fluid required. Intravenous toxicity can, however, be demonstrated (27).

Distilled Water.—Although more toxic when administered intraperitoneally than saline solution, distilled water (LD_0 = 147.0 ml/kg, ED_0 = 14.7 ml/kg) can also be used for routine resuspension of aqueous extracts. Rowntree (20) has described the effects of large quantities of water when administered to mammals. The same effects were observed in mice at dose levels higher than 100 ml/kg. Distilled water is not used for resuspension of ethanol extracts because it does not have the proper solvent characteristics.

Dimethyl Sulfoxide (100%).—Dimethyl sulfoxide is toxic when administered intraperitoneally (LD_0 = 6.8 ml/kg, ED_0 = 0.3 ml/kg), but has excellent solvent characteristics and many potential uses (18). It can be used in small concentrations to aid in the dissolving of difficult extracts, but it is not suitable for use as a vehicle because it is too toxic and highly irritating. Dimethyl sulfoxide causes reversible hemolytic anemia and can cause discomfort because of heat released when it is injected (*i.e.*, from the heat of solution when mixing with water) (19). Caujolle (3) found that 50% dimethyl sulfoxide has an LD_{50} of 17.7 g/kg when administered intraperitoneally to mice.

Polyethylene Glycol-200 (100%).—Polyethylene glycol is toxic when administered intraperitoneally (LD_0 = 4.6 ml/kg, ED_0 = 1.0 ml/kg). Shideman and Procita (22) administered polyethylene glycol-400 to mice intraperitoneally and found the LD_{50} to 9.2 g/kg. Highly viscous, polyethylene glycol-200 is also too toxic for use as a vehicle when administered intravenously or intraperitoneally; however, it is five to six times less toxic when administered orally, and it could be used as a vehicle by this route (4, 8). The LD_{50} of polyethylene glycol (mol wt = 3,600) when administered intragastrically to rats and guinea pigs is 50.0 g/kg (23). When administered intravenously or intraperitoneally, polyethylene glycol causes hemolysis of red blood cells and serious kidney damage (4, 24). When administered orally or intragastrically, however, only very small quantities reach the bloodstream, and it is not as damaging to the test animal.

Propylene Glycol (100%).—Propylene glycol is similar to polyethylene glycol in that it is not very toxic when administered orally (24), but is up to six times more toxic when administered intravenously or intraperitoneally (LD_0 = 6.8 ml/kg, ED_0 = 0.1 ml/kg) (29). Davis and Jenner (6) report an LD_{50} of 11.4 g/kg and Lampe and Easterday (12) report an LD_{50} of 9.7 g/kg of propylene glycol administered intraperitoneally to mice. The high viscosity and toxicity of propylene glycol (100%) makes it undesirable for resuspending extracts that are to be injected.

Ethanol (100%).—Ethanol (100%) is the most toxic of the nine solvents tested (LD_0 = 0.32 ml/kg, ED_0 = 0.1 ml/kg). Reactions displayed by mice at nonlethal dose levels lasted up to 4 hr. Latven and Molitar (14) report that 95% ethanol was one of the most toxic and irritating solvents they tested intravenously in mice.

TABLE 4 *Literature references pertaining to vehicles.*

Name of vehicle	Per cent solution	Animal	Sex	Injection route[a]	LD_{100}	LD_{50}	Reference
Ethanol	70%	mouse	?	IV	5.0 ?	4.0 ?	14
"	"	"	?	OR	17.0 ?	14.5 ?	14
"	"	"	?	SC	18.0?	14.5 ?	14
"	95%	"	?	IV	3.0 ?	2.5 ?	14
"	"	"	?	OR	14.0 ?	12.0 ?	14
"	"	"	?	SC	12.0 ?	10.5 ?	14
"	30%	rat	?	IP	——	5.0 g/kg	1
"	"	rabbit	?	OR	——	7.5 g/kg	1
PEG[b]	50%	rat	?	IG	——	51.3 g/kg	23
PEG[c]	"	"	?	IG	——	50.0 g/kg	23
PEG[c]	"	g. pig	?	IG	——	50.0 g/kg	23
PEG-400	100%	mouse	?	IP	——	9.2 g/kg	22
PEG-1000	"	"	?	IP	——	2.0 g/kg	22
PEG-4000	"	"	?	IP	——	8.0 g/kg	22
Propylene glycol	100%	mouse	male	IP	——	11.4 g/kg	6
"	"	"	"	IP	——	9.7 g/kg	12
"	80%	g. pig	"	IG	——	18.4 g/kg	23
"	"	rat	"	IG	——	16.4 g/kg	23
"	100%	mouse	female	IP	——	9.7 g/kg	11
"	?	"	both	IG	——	22.9 ml/kg	13
"	?	rat	"	IG	——	21.0 ml/kg	13
"	?	g. pig	"	IG	——	18.9 ml/kg	13
"	100%	mouse	?	IV	8.0 ?	1.0 ?	14
"	"	"	?	OR	26.0 ?	22.0 ?	14
"	"	"	?	SC	24.0 ?	18.5 ?	14
Dimethyl sulfoxide	50%	rat	?	IP	——	12.0 g/kg	3
"	50%	mouse	?	IV	——	8.9 g/kg	3
"	"	"	?	IP	——	17.7 g/kg	3
"	100%	"	?	IV	——	5.8 g/kg	30
"	"	rat	?	IV	——	5.4 g/kg	30
"	"	mouse	?	OR	——	21.4 g/kg	30
"	"	rat	?	OR	——	28.3 g/kg	30
"	"	mouse	both	IV	——	3.8 g/kg	19
"	"	rat	"	IV	——	5.3 g/kg	19
"	"	mouse	"	SC	——	20.5 g/kg	19
"	"	rat	"	SC	——	20.5 g/kg	19
Sodium carboxymethyl-cellulose	——	——	——	——	——	——	5, 9
Methyl-cellulose	——	——	——	——	——	——	5, 9
Polyvinyl-pyrrolidone	——	——	——	——	not toxic	——	5
Water	——	——	——	——	toxic in excessive amounts	——	20
Saline	——	——	——	——	not toxic	——	27

[a] *OR* = oral; *IV* = intravenous; *SC* = subcutaneous; *IG* = intragastric; *IP* = intraperitoneal.
[b] Polyethylene glycol = *PEG*; molecular weight = 1250.
[c] Polyethylene glycol = *PEG*; molecular weight = 3600.

SUMMARY

The purpose of this study was to estimate the LD_0 and the ED_0 (respectively, the highest dosage without lethality and symptomatology) for intraperitoneal injections of nine solvents into mice.

A comparison of the toxicity (ED_0, and LD_0) of these solvents showed their descending order of toxicity to be (1) ethanol, (2) 100% polyethylene glycol-200, (3) 100% propylene glycol, (4) 100% dimethyl sulfoxide, (5) 100% sterol diluent, (6) distilled water, (7) 0.5% methylcellulose solution, (8) isotonic saline solution, and (9) 3.0% polyvinylpyrrolidone solution suspended in isotonic saline solution.

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Yoshida, M., H. Hoshi, and H. Morimoto: NUTRITIVE VALUE OF GLYCOLS FOR POULTRY FEED. Nippon Kakin Gakkaishi, Vol. 6 (2), pp. 73-81, 1969. National Institute of Animal Industry, Chiba-shi.

In view of the fact that a greater proportion of condensed animal feed consumed in Japan is imported, increased production of such feed and the development and utilization of new materials for feed preparation is imperative for the promotion of breeding industry. At this laboratory, a series of research projects on the development of new feed materials have been in progress, some resulting in the clarification of nutritive values of several materials which have never been used for animal feed, including fatty acid in physester oil /1/. At the present stage, however, various problems of both quantitative and qualitative nature hinder the search for new natural materials, and the hope for phenomenal progress in this area is extremely slim.

Under the above circumstance, another series of projects have been launched as part of a research program on new energy sources of non-agricultural or non-marine origin, in which the utilization of chemical and industrial products as feed materials is pursued. The first step of this project was to determine the nutritive value of glycols in poultry feed.

Ashida /2/ has demonstrated that 1,2-propanediol (propylene glycol; abbreviated hereafter as 1,2-PG) with a carbon number of 3 can be utilized as an energy source for white rat, and a dietary level as high as 30% be tolerated if the animal is accustomed to this compound by gradual increase in its content in the feed. In a similar experiment using chicks, Bayley et al. /3/ of Canada attempted to measure the

metabolic energy (abbreviated hereafter as ME) of this compound. It was reported that the ME value obtained in their experiment exceeded the gross energy of 1,2-PG, and the actual values have not been published. PG has been used for the treatment of ketosis in ruminant animal, and experiment from this angle has been conducted by many investigators including Hamada and Morimoto [4] whose experimental results have already been published.

Primarily for the development of non-agricultural dietary source, Miller and Dymsza [5] investigated the values of 1,3-butanediol (1,3-butylene glycol; abbreviated hereafter as 1,3-BG) with a carbon number of 4 as an energy source, using white rats. According to their report, this compound produced adverse effects when its dietary level exceeded 30%, but it could be utilized favorably at a dietary level of 20%.after it had been gradually introduced to the animals for one week. Under the latter condition, its available energy per gram was approximately 6 kcal/g. The examination of hepatic phosphohexoseisomerase, hepatic glycogen, and blood sugar values revealed that, unlike a fat, 1,3-BG was not oxidated by way of 8-hydroxy lactic acid.

In the present investigation, the nutritive values of glycols were determined by bioassay and compared, as part of the research program on artifical feed materials.

EXPERIMENTAL MATERIALS AND PROCEDURES

The present investigation can be divided into a preliminary experiment and bioassay experiments.

PRELIMINARY EXPERIMENT. The preliminary experiment was designed to determine the presence or absence of adverse effects and the upper limit of the dietary content of 1,2-PG, and the tolerance of chick toward

the compound. Using 40 newborn male White Leghorn chicks, the experiment

Table 1. Composition of basal diet

	予備試験用基礎飼料 Basal diet for prelim. trial	生物定量用基礎飼料 Basal diet for bioassay: 4週齢ヒナ用 for 4-wk-old chick	1週齢ヒナ用 for 1-wk-old chick
	%	%	%
コーンスターチ・Cornstarch	20.0	24.0	25.8
黄色トウモロコシ Yellow corn	46.1	—	—
蛋白質混合物[1] Protein mixture[1]	—	—	45.0
大豆粕 Soybean meal	29.1	32.1	—
大豆油[2] Soybean oil	—	2.0	2.0
●フスマ Wheat bran	—	38.8	—
セルロース Cellulose	—	—	22.0
無機物[3] Minerals	4.1	2.5	4.1
ビタミン[3] Vitamins	0.5	0.5	1.1
DL-メチオニン DL-methionine	0.2	0.1	—

1) 大豆粕89.6%，魚粉10.0%およびDL-メチオニン0.4%からなる
Contained 89.6% of soybean meal, 10% of fish meal and 0.4% of DL-methionine
2) BHT 0.05%を含む
Contained 0.05% of butyrated hydroxy toluene
3) 文献1参照
See Literature 1

began in March, 1966. The basal feed containing 20% of cornstarch and 18% (calculated value) of crude protein with a calculated ME of 2.86 kcal/g was used as the control diet, and cornstarch in the basal feed was replaced with 1,2-PG at 5, 10 and 20% to prepare experimental feeds. After raised on the control diet for 7 days, the chicks were randomly divided into two groups, of which one group remained on the control diet and the other group was given the experimental feed containing 5%-1,2-PG for 9 days. At 16 days of age, each group was further subdivided into two groups of 10 chicks, of which one group was given either the control deit or the 5%-1,2--PG diet, and the other group, 10% 1,2-PG diet. Nine

days later, i.e., at 25 days of age, each group was further subdivided into two groups of 5, of which one remained on the same diet and the other was given 20% 1,2-PG diet for 9 days.

During the period of the above experiment, the chicks were fed or given water upon demand, and the weight gain and feed intake were measured at 9 days intervals.

BIOASSAY. The experiments, totalling 6, are identified with arabic numerals denoting the last numeral of the year followed by two figures indicating the month in which the experiment took place. For example, Experiment 611 indicates that the experiment took place in November, 1966. The first 4 experiments used 822 4-week old male White Leghorn chicks, and the last two, 82 1-week old male White Leghorn chicks.

The first four experiments followed the procedure employed in the previous experiments [6,7]: the newborn chicks raised on the feed normally used at this laboratory for four weeks were divided into groups of 6 in such a manner that all groups showed the same average weight, and housed in separate cages for two weeks during which feed and water were given upon demand. Standard feeds were prepared by substituting specific amounts of cornstarch contained in the basal feed shown in Table 1 with soybean oil and test diets by substituting specific amounts of cornstarch with a mixture of soybean oil and test materials. The basal feed has the same composition as BD-4 in the previous experiment [6] except for the fact that chrome oxide-starch mixture of 1% in BD-4 was replaced by cornstarch since chemical analysis of excreta was not included in the experiment.

In the preparation of standard feeds, the starch in the basal feed was substituted with soybean oil at 0, 3, 6, and 9% dietary level in Experiment 606, at 0, 2.25, 4.5, 6.75, and 9% in Experiments 609 and 611.

These standard feeds were identified as SO-0, SO-2.25, etc., by amount of soybean oil.

In experiments 609 and 611 on PG and BG, experimental feeds were prepared by replacing a mixture of 5.0 parts test compound and 2.6 parts soybean oil, or a mixture of 2.0 parts test compound and 3.6 parts soybean oil with cornstarch so that, under the assumption that the test compound was completely utilized, the energy value of the experimental feeds equaled the mean value of the standard feed, i.e., the energy value of SO-4.5. In Experiment 606 testing PG polymers, 5.0 parts of test compound and 4.5 parts of soybean oil were replaced with cornstarch so that, under the assumption that the test compound was not utilized at all, the energy value of the experimental feed equaled that of SO-4.5. Such arrangement in the preparation of test feeds was designed to enhance the accuracy of bioassay.

In Experiment 706, not only the caloric value of ethanediol (ethylene glycol; abbreviated as EG) was measured by bioassay, but a new slope assay technique [8] was compared with the currently used estimation of x from y [6,7]. In this experiment, four standard feeds containing soybean oil at 0, 3, 6, and 9% were employed, each investigated with 6 groups of chicks. Two experimental feeds containing a mixture of equal parts ofEG and soybean oil at 5 and 10% were prepared, and tested with 8 and 10 groups, respectively, of chicks.

The experiments using one week old chicks followed the same bioassay technique only in a reduced scale so that the technique is adaptable to the experiments using small amounts of test compound. The chicks raised on the feed normally used at this laboratory at 3.5 g/chick/day were divided into groups of 2 (806) and 4 (809) in such a manner that

the groups all showed the same average weight, and subjected to experiments in incubators for 6 days. Standard feeds were prepared by replacing 4 and 8% of the cellulose in the basal feed for 1 week old chicks shown in Table 1 with soybean oil, and experimental feeds, by replacing 5% of cellulose with the test compound. Two groups of chicks were allocated for each of the experiments with these feeds.

TEST MATERIALS. The reagents of ethanediol, 1,2-propanediol, and 1,2-, 1,4-, and 2,3-butanediol were commercially obtained for the experiment. These compounds are oily at normal temperature. Polymers 1 and 2 of 1,2-propanediol had average molecular weights of 2,000 and 3,000, respectively, and were white powder. The dimer, trimer, and tetramer of EG and PG were obtained by forcing ethylene oxide and propylene oxide, respectively, into water, and fractionating by gas chromatography. The compositions of PG polymers are shown in Table 2. They were all colorless, transparent oily substances at normal temperature.

Table 2. Composition of polymers of propylene glycol used in Expt. 809

サンプル Sample	モノマー Monomer	ダイマー Dimer	トリマー Trimer	テトラマー Tetramer	ペンタマー Pentamer
プロピレングリコール Propylene glycol	93 %	7 %	— %	— %	— %
ジ・プロピレングリコール Di-propylene glycol	3	94	3	—	—
トリ・プロピレングリコール Tri-propylene glycol	—	8	90	2	—
テトラ・プロピレングリコール Tetra-propylene glycol	—	—	15	80	5

ANALYSIS OF DATA. The data from the bioassay were converted to dietary efficiency, i.e., the weight gain for the unit amount of feed, in order to eliminate the effects due to the difference in feed intake. As in the previous experiments [6,7], the results of Experiments 606, 609, and 611 were evaluated by the following procedure. A standard

curve was obtained from the results of the experiments in which standard feeds were used, and the curve was used to determine the feed energy corresponding to the weight gain shown by the groups fed with experimental diet, and its 95% fiducial interval. While similaranalysis was made on the overall experimental data 706, the energy of the test material in 5 types of feed used in the experiment, standard feeds containing soybean oil at 0, 3, and 5% and two experimental feeds containing an ethanediol mixture at 5 and 10% were also computed by slope assay. For this procedure, 6 groups were randomly selected from the 10 and 8 groups, respectively, fed on experimental feeds, to perform statistical analysis on the same number of cases.

The analytical operations were facilitated by the electronic computer at the Agricultural Research Computation Center, and the slope assay employed Kosaka's program [9].

The analysis of the data from Experiments 806 and 809 using one-week old chicks was facilitated by manual or electrical computer, and the estimation of 95% fiducial interval which involved complex operation was omitted. Since the primary objective of these experiments was to judge whether or not the test compounds can be utilized in chicks, measurement accuracy was sacrificed while using small quantities of sample and smaller number of subjects.

In the analysis of data, the energy of standard feeds was expressed in terms of ME in all cases, which was nitrogen-compensated by the method used by Hill and Anderson [10]. The measurement values obtained at this laboratory, 3.52 kcal/g and 9.21 kcal/g, were used as the ME of cornstarch (air-dried) and soybean oil, respectively, used as the standard materials. Thus, the value of the test compounds is evaluated in terms of ME.

MEASUREMENT OF MOISTURE CONTENT IN EXCRETA AND VOLATILE COMPONENTS OF FEED.

Themmoisture content in excreta was measured as follows. A ceramic dish of approximately 20 cm in diameter, weighed in advance, was placed under the cage to collect 24-hour excreta, which was immediately weighed. The excreta was placed in dryer at 60°C for 24 hours, then weighed. The loss in weight was assumed as due to the evaporation of moisture. Since the amount of moisture evaporating during the process of collection cannot be measured, the result is by no means real, but this method permits relative comparison among experimental groups.

For the measurement of volatile components in the feed, 20 g of feed, accurately weighed, was left standing at room temperature in a 100 ml beaker, weighed in advance, and changes in weight was observed at 24-hour intervals.

EXPERIMENTAL RESULTS

The results of the preliminary experiment are summarized in Table 3. The table shows daily weight gain per chick and feed intake for every 9 days. Since each groups was subdivided into two subgroups at 9 days intervals, the number of chicks per group was halved each time. Comparing the results of the groups fed on the feeds containing 1,2-PG at 0%,and 5% continuously, the 1,2-PG-5% group always indicated better results. The difference between the two as to dietary efficiency is actually lower since the feed intake of the 1,2-PG-5% group was always larger, but even with this taken into consideration, the outcome was more favorable. The fact that cornstarch was substituted with 1,2-PG naturally contributed to greater energy of the feed, and if the added component is assumed to have been utilized by the chicks, such result is to be expected. To say the least, the result indicates that the

experimental feed exerted no adverse effect on the chicks. When the group was divided into two subgroups, and one of the groups was given the diet containing 1,2-PG at 10%, the results were either less favorable than or the same as before. Since the larger amount of the compound had presumably increased the feed energy, this outcome should be interpreted as that the compound at this level could not be effectively utilized. When the subgroup was further halved, and 1,2-PG-20% feed was given to one of the groups, the result was notably less favorable than that of the group which remained on the same diet. The result of this group suggested no specific relationship to the dietary level of the compound in the previous diet.

The excreta of 10% or 20% 1,2-PG feeds were extremely loose and released strong, characteristic odor. From the condition of the excreta, an increase in the amount of urine was suspected, and this is assumed to be the cause of the odor.

Table 3. Average body weight gain and feed intake per day per chick in preliminary experiment

日齢 days of age	7～16	16～25	25～35
	g (%)	g (%)	g (%)
1羽1日あたり増体量: Gain/day/chick	7.0 (0)[1]	8.3 (0)	7.0 (0) 5.4 (20)
		8.5 (10)	7.7 (10) 3.3 (20)
	8.5 (5)	11.2 (5)	9.1 (5) 2.8 (20)
		9.6 (10)	8.2 (10) 5.0 (20)
1羽1日あたり飼料摂取量: Feed intake /day/chick	15 (0)	20 (0)	30 (0) 30 (20)
		23 (10)	34 (10) 30 (20)
	18 (5)	25 (5)	36 (5) 30 (20)
		24 (10)	38 (10) 29 (20)

1) 括弧内は 1,2-プロパン・ジオール配合量
Figures in parenthesis are dietary content of 1,2-propanediol

Table 4. Gain/feed and moisture in excreta in Expts. 706 and 809

実験番号 Exptl No.	飼料 Feed[1]	ヒナ羽数 No. of chicks	増体量/摂取量 Body wt. gain / Feed intake	糞排泄物水分 Moisture in excreta
		羽	g/kg	%
706	SO-0	36	272.5±15.6[2]	
	SO-3	36	311.4±10.7	76.8±0.7[3]
	SO-6	36	343.0±10.0	
	SO-9	36	364.0±10.3	
	EG-2.5	48	299.3± 9.1	81.6±1.4
	EG-5	60	324.1±18.9	85.4±1.8
809	SO-0	8	517.9	
	SO-4	8	594.8	
	SO-8	8	690.0	
	1,2-PG	8	571.8	
	di-PG	8	303.6	
	tri-PG	8	449.8	
	tetra-PG	8	342.6	

1) SO: 大豆油, BG: ブタンジオール, PG: プロパンジオール, EG: エタンジオール, 数字は配合量
SO: Soybean meal, PG: Propanediol, EG: Ethanediol. Figures indicate the content in the diet.

2) 平均値±6羽の平均値の標準偏差
mean±standard deviation of means of 6 chicks.

3) 3区の平均値±標準偏差
mean±standard deviation of 3 estimates.

These experimental results led to an assumption that 1,2-PG can be well utilized at a dietary level of 5%, but at 10%, much of it is simply excreted into urine without fully utilized, and it even exerts an adverse effect on the growth of chicks. Thus, it is unlikely that gradual introduction of this compound could increase tolerance in chicks.

Of the measured values of available energy obtained by bioassay, the data from Experiments 706 and 809 are tabulated in Table 4 as examples illustrating the analytical procedure, and the measured values of the experiments in which the test materialswere added to the feeds equally at a dietary level of 5% are given in Table 5.

A standard curve obtained from Experiment 706 using four types of standard feed is expressed by the following equation.

$$y = 179.31\,x - 217.026 \qquad (1)$$

in which x denotes the ME (kcal/g) of the standard feed and y, the weight gain (g/kg) per unit amount of the feed. In equation (1), the correlation

coefficient of x and y, r, is 0.944. The correlation coefficient, the constant term, and the regression coefficient were all significant at 1% level. The energy value of an experimental feed can be obtained by substituting the weight gain of the chick on the experimental diet in equation 1, and calculating x by reverse operation. Taking EG-5% group as an example, y is 324.1 g/kg, therefore, x is 3.0177 kcal/g. The fiducial interval is 3.0727 - 2.9630 kcal/g. The calculation of the fiducial interval was based on the number of groups on the standard diet, n, the number of groups on the experimental diet, p, and the deviation from the regression from the results of standard diet experiment, sy,x, and used equation (3) in the previous report [6]. Since the ME value of the basal feed in Table 1 (SO-0) is 2.7540 kcal/g, 0.2637 kcal, the difference from x = 3.0177 kcal, is the difference between the energy of 0.1 g cornstarch and that of a mixture of 0.05 g of EG and 0.05g of soybean oil, the substitute of the cornstarch. The ME values of air-dried cornstarch and soybean are 3.52 and 9.21 kcal/g, respectively. Thus, (0.2637 + 3.52 x 0.1 - 9.21 x 0.05)/0.05 = 3.104 kcal/g is the available energy of EG. Its 95% fiducial interval is 4.20 - 2.01 kcal/g.

Aside from this calculation, 6 groups, a total of 36 chicks, were selected randomly from the groups on experimental feeds, and the available energy of EG was obtained by 5-point slope assay technique. In this computation, soybean oil at the dietary level of 3% in the standard feed and a mixture of EG and soybean oil at equal rates at a dietary level of 5% were regarded as one unit. Thus, the feeds were considered as containing 1 and 2 units, respectively, of these components. The relationship between the amount added and the result is illustrated in Figure 1. The description of the computation method is omitted since the method is fully discuss-d in pages 142 to 145 of literature 8.

Table 5. Gross energy, available energy, availability of energy and moisture in excreta fed the sample at 5% level.

供試品 Sample	総エネルギー Gross energy (1)	利用可能エネルギー Avnilable energy (2)	95% 信頼区間 95% Fiducial interval	利用率 Availability (2)/(1)	排泄物中水分[1] Moisture in excreta[1]
	kcal/g	kcal/g	kcal/g	%	%
エタンジオール Ethanediol	4.60	2.69	3.90～1.39	58	83**
1,2-プロパンジオール 1,2-Propanediol	5.68	5.87	7.60～4.17	103	76
1,3-ブタンジオール 1,3-Butanediol	6.44	6.31	8.07～4.63	98	75
2,3-ブタンジオール 2,3-Butanediol	食べないため測定不能 not measurable due to poor appetite			0	83*
1,4-ブタンジオール 1,4-Butanediol				0	78
ジエチレングリコール Diethylene glycol	5.19	1.8		35	
トリエチレングリコール Triethylene glycol	5.51	− 0.6		0	
テトラエチレングリコール Tetraethylene glycol	5.65	− 0.4		0	
ジプロピレングリコール Dipropylene glycol	6.51	−18.1		0	
トリプロピレングリコール Tripropylene glycol	6.76	− 5.9		0	
テトラプロピレングリコール Tetrapropylene glycol	7.50	−14.8		0	
プロピレングリコール重合物 1[2] PG Polymer 1[2]	7.47	− 4.2		0	72
プロピレングリコール重合物 2[3] PG Polymer 2[3]	7.36	0.18		2	75

1) 対照区の排泄物中水分は 75%. *, ** はそれぞれ 5% と 1% 水準で対照区より高い
Moisture in excreta of control was 75%. Data with * or ** was significantly higher than that of control at 5 or 1% level, respectively.
2) 平均分子量 2,000. Average molecular weight was 2,000.
3) 平均分子量 3,000. Average molecular weight was 3,000.

表 6. 2 つの方法によるエタンジオールの利用可能エネルギー測定値の比較
Table 6. Comparison of avaiable energy of ethanediol estimated by 2 bioassay techniques.

	添加量 Dietary level	平均値 Mean	95% 信頼区間 95% Fiducial interval
	%	kacl/g	kcal/g
y から x の逆推定法 Estimation of x from y	2.5	2.87	3.25～1.03
	5.0	3.10	4.20～2.01
5 点・傾斜比法 5-point slope assay	5.0	2.69	3.90～1.39

In Experiment 809, the compounds are added to the standard feed and experimental feed in the place of cellulose in the basal feed. The available energy of cellulose is practically 0 [11]. The compounds were added at a dietary level of 5%. Thus, the following equation was

formulated as expressing the relationship between the feed energy, x kcal/g, and the weight gain per kg of feed intake, y g/kg, according to the experimental data.

$$y = 84.6 + 215.1\ x \qquad (2)$$

The correlation coefficient, r, between x and y is 0.972, significant at 1% level. At a dietary level of 5%, this equation can be converted to equation (3) which expresses the relationship between the available energy per gram of test compound, x kcal/g, and y. The energy of the test compound can be calculated directly from this equation.

$$y = 514.9 + 11.63\ x \qquad (3)$$

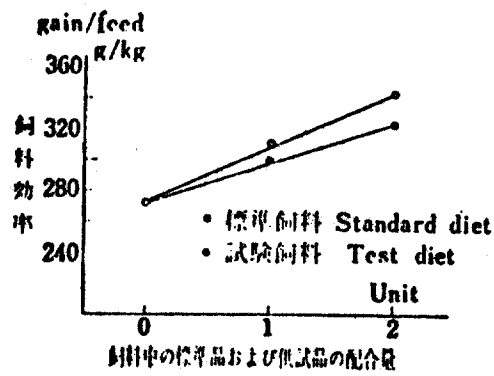

図 1. 飼料中の標準品および供試品の配合量とヒナの成績（実験 706）

Fig 1. Dietary level of standard and test materials and feed efficiency of chicks (Expt. 706)

The data from experiment 806, calculated by the same method, are not discussed here.

In the study of the moisture content in excreta, the groups given the standard feed in which the level of soybean oil was similar to that of the test compound, were chosen as the control, as shown in Table 4, and the averages of three groups from both control and experimental categories were obtained for relative comparison. The control exhibited slight variation in moisture content at each experiment, the maximum, minimum, and mean values being 76.8%, 73.7%, and 75.35%, respectively.

In Table 5, the moisture contents of experimental groups were adjusted to the values with 75% moisture content of the control as the standard so that relative comparison can be made. As evidenced in the table, the moisture increased with the addition of EG and 2,3-BG in the feed at 2.5% and 5%, causing loose droppings.

As a preliminary experiment for energy bioassay of alcohols, methyl-, ethyl-, n-propyl-, and n-butyl alcohols were added to the feed at 5%, and changes in weight of feed were measured. As a result, 80% of the alcohol was found to evaporate in 24 hours. The weight of alcohol in the control feed also showed daily variation. To determine the significance of the weight loss, changes in the weight of EG and 1,2-PG added at 5% were also examined. This experiment revealed that the weight of the feed containing EG increased by 0.8% without the weight increment of the control feed, which demonstrates that the compound possesses moisture absorbing property. Unlike EG, 1,2-PG showed no weight gain.

DISCUSSION

DIETARY VALUE OF GLYCOLS. Without nutritional constituent such as protein, vitamins, inorganic materials, etc., the dietary value of glycols can be judged only in terms of biological available energy level. The present experiment demonstrated that the gross energy of both 1,2-PG and 1,3-BG can be almost fully utilized when their dietary level was 5%. The gross energy of these compounds is 5.68 and 6.44 kcal/g, respectively. Generally speaking, the energy level is higher with larger carbon number. According to this general rule, 1,3-BG should have higher energy value than 1,2-PG. However, 1,3-BG is more viscous than 1,2-PG, which works adversely when it must be mixed with powder feed. Both materials are similar to a fat in that they provide moisture in the feed, which prevents their powdery dispersion, and increases the depth of the color of the feed. It is

assumed that these compounds also suppress the activity of fungi or bacteria, which adds to the value of the feed. It has been confirmed that neither of them cause diarrhea at a dietary level below 5%.

On the contrary, 2,3- and 1,4-BG suppressed the appetite, for the reason of which they are of no biological value. Different methods of measurement may be able to determine whether or not the energy of these compounds can be utilized, but as long as the feed containing such components are disliked by the animal, such feed would be of no practical value. It was also reported that the feed containing these compounds were avoided by white rats [2].

EG is known to be harmful to human and inhibit the growth of white rats [2], but chicks can utilize 58 to 67% of its gross energy. However, this compound causes excessive moisture in droppings when added to the feed at a level of 2.5%, which reduces the possibility of this material being directly utilized as poultry feed. These experimental findings suggest that in the preparation of animal feed using artificially produced compounds such as glycols, the nutritive value of some of the compounds vary according to the type of animal it is given to.

As compared to these glycols, EG and 1,2-PG polymers could not be utilized in chicks at all except for EG dimer, in which case 1/3 of the gross energy could be utilized. Polymers 1 and 2 of 1,2-PG having larger molecular weight were also found to be of no biological value, despite such promising properties as that they were powder as compared to all other materials tested, which were oily. This is an advantage in the preparation of feed. These polymers are linked by ether bond. It is assumed that no cleavage of ether bond takes place in the alimentary organs of chicks to liberate EG or 1,2-PG, and the compounds are discharged into excreta in the original form. The weight gain of the chicks given

tri- and tetra-EG or PG polymer 2 showed a tendency similar to that of the chicks fed on standard feed SO-O without soybean oil. These experimental results indicate that the polymers are ineffective but harmless and, as cellulose, they merely pass through the digestive organs without being utilized. This could be confirmed by the color and amount of excreta.

In Experiment 809, paralytic symptons with unstable movement of legs and lack of body-supporting ability followed as in the reactions to alcohol. Since symptoms of similar nature were also observed in 1,2-PG groups,but not in the standard-feed groups, they should be attributed to the particular samples used for these chicks. Table 5 shows minum values obtained by extrapolation using equation (3), but the reliability of these results is extremely low not only because of the extrapolation but also for the fact that the chicks were somewhat paralyzed.

ADVANTAGES OF BIOASSAY. The bioassay technique in this experiment comprised the setting up of the experimental conditions under which the weight gain was proportional only to the feed energy, and the measurement of the energy of the test materials with the energy of the standard materials as the norm. It is an optimum method for the evaluation of compounds containing no factor which would affect the growth, such as glycols. The successful formulation of this procedure was one of the motives which brought forward the project for the development of new energy sources other than agricultural and marine products. This procedure not only facilitates the measurement of the energy of a test material, but enables overall judgment of the health condition of the subject, like or dislike of the feed by the animal, and the condition of excreta. Thus, in the evaluation of dietary value of a material, this has a number of advantages over methods which determine the energy level of the material based on the measured digestion coefficient and ME only.

A question has been raised over the fact that, due to the volatilization of an undigested portion of 1,2-PG while the excreta was being dried prior to ME measurement, the ME value was higher than the gross energy [3]. In this bioassay technique, such a factor exerts no influence on the measured value. However, one of its weak points is the relatively high measurement error. The degree of error is higher when the dietary level of the test material is low. This is evident from the wide fiducial intervals shown in Table 5.

COMPARISON OF THE ESTIMATION OF X FROM Y TECHNIQUE AND THE SLOPE BIOASSAY TECHNIQUE. In experiment 706, the available energy of EG was measured by two methods. The first method comprised the formulation of equation (1) from the results of standard feed groups, and the computation of feed energy x by substituting y with the results of test feed groups in equation (1). This is called the estimation of x from y technique. In Experiment 706, a total of 204 chicks in 34 groups were used for both standard feed and experimental feed categories inclusively when the me measurement was made with a dietary level of EG set at 5%, and a total of 192 chicks in 32 groups, when the dietary level of EG was 2.5%. In the second method, called 5-point slope assay technique, two straight lines, as shown in Figure 2, were obtained, and the energy value of the test compound was calculated from their gradient. In Experiment 706, 6 groups were allocated for each point, thus, the entire experiment requiring 30 groups, a total of 180 chicks.

The measured values were all within the fiducial interval, without significant difference by method of measurement. The fiducial interval in the slope assay was broader when the EG dietary level was 5%, but the reliability of this technique should not be judged by the result of this experiment alone since this experiment used the smallest number of chicks

and the fiducial interval is also influenced by the rangeof the energy of the standard feed.

In some test compounds, the experimental result is proportional to the energy as long as the dietary level is low, but with increasing proportion of the test compound in the feed, its growth-inhibiting effects become more manifest, and the result is not necessarily proportional to the energy. In the estimation of x from y method, such characteristics of the test material cannot be revealed. Moreover, the measured value of the test material with qualities other than energy is of low accuracy. On the other hand, the reliability of the results obtained by slope assay is high, since the linearity of the relationship between the energy and the experimental results of the chicks is checked during the procedure. For this reason, the measured values obtained by slope assay were shown in Table 5.

SUMMARY

As part of a research project for the development of energy sources other than agricultural and marine products for animal feed, the dietary value of glycols was investigated.

In the preliminary experiment, 40 male White Leghorn chicks were raised on diets containing 1,2-propanediol (propylene glycol; PG) at 0, 5, 10, and 20%. The results were quite favorable at a dietary level of 5%, but less favorable at over 10%. Gradual elevation of the dietary level of the compounds failed to produce increased tolerance in chicks. Based on the above results, 822 4-week old chicks and 82 1-week old chicks were subjected to a series of experiments for the determination of biologically available energy of glycols under the condition of 5% dietary level, and the following results were obtained.

1. Chick can utilize approximately 60% of the energy provided by supposedly toxic ethanediol (ethylene glycol, E.G.), but develops loose droppings.

2. Chicks can utilize the energy of PG and 1,2-butanediol (butylene glycol, BG) almost completely.

3. The energy of 1,2-PG and 2,3-BG could not be utilized at all.

4. Approximately 35% of the gross energy of EG dimer can be utilized, but other 7 types of EG and PG polymers could not be utilized. This is presumably due to the lack of enzyme that produces the cleavage reaction of ether bond in the polymers.

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POSTSCRIPT

A paper was published by Persons et al. [12] after the completion of this report. According to their paper, the mixture of PG to poultry feed at 5% suppressed the growth of broilers and the egg-laying capacity of hen, but no adverse effect was noted at 2.5%.