MONOGRAPH

ON

LACTIC ACID

#85

Monograph on Lactic Acid #85 1/10/75

M O N O G R A P H

O N

L A C T I C A C I D

TR-72-1552-63

Submitted Under:
Contract No. FDA 72-104

January 10, 1975

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LACTIC ACID

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LACTIC ACID

Summary

Lactic acid is one of the most widely distributed acids in nature and was one of the first to be used in foods. Lactic acid is used to pack olives because it insures clarity of the brine. It is also used to acidify fruit juice in the production of wine and to improve the flavor of carbonated fruit juices. Calcium lactate has been used as a buffer, dough conditioner, and a yeast food (588).

Experiments in which L(+)-lactic acid was placed in whole stomach pouches of cats indicated that both the hydrogen ion and the lactate radical were absorbed equally; the D-isomer was thought to be absorbed similarly (558).

Kreisberg (1019) and Searle and Cavalieri (1694) determined that the virtual volume of distribution of lactate was 49.4% of the body weight and occupied a space of at least three-fourths of the body water space. Lactate diffuses readily across cell membranes by passive transport. Under certain conditions lactate distribution is either uneven or the lactate pool really consists of several smaller pools with differing rate constants (1019).

In the body, lactate is formed through anaerobic glycolysis of carbohydrate (163). The muscle and erythrocytes account for most of the lactate produced in vivo; the brain, leucocytes, renal medulla, gastrointestinal tract, and skin produce smaller quantities (1019). The lactate generated can be transplanted to other more aerobic tissues such as the liver and converted to pyruvate by lactic acid dehydrogenase. The pyruvate can then be metabolized further through the citric acid cycle; or, it can be changed to carbohydrate material as free glucose or stored as glycogen (163, 315).

Human saliva has been found to contain a significant amount of micrococcus lactilyticus which ferments lactate to propionate, acetate, carbon dioxide, and hydrogen (418).

Through infusion studies with labeled L(+)-lactic acid in man, the lactate pool size and turnover time have been estimated at 0.029 g/kg and 18.4 min, respectively. The turnover was approximately 95 mg/kg/hr with approximately 90% oxidation to carbon dioxide. The primary fate of lactate, according to Searle and Cavalieri (1694), was oxidation to carbon dioxide, not reduction to glucose. Drury and Wick (432) found a 30 min. turnover time in the rabbit. An eviscerated animal metabolized the L(+) isomer actively, but not the D(-) isomer. Since racemic lactate is almost completely utilized, the authors suggested that the D-isomer might be converted to the L-isomer or to glucose or glycogen.

Based on infusion experiments with dogs, Dies et al. (398) reported

that lactic acid was actively reabsorbed in the proximal tubule and its transport was rate-limited. Earlier, Craig (326) had found that at plasma lactate concentrations between 1 and 4 mg/ml, the rate of excretion was proportional to the glomular filtration rate. However, oral administration resulted in almost complete utilization of lactate.

Gordon (640) showed that sweat lactate was derived metabolically from serum glucose as opposed to serum lactate.

When Ballabriga (77) fed formulas acidified with L(+)- and D(-)- lactic acid, to premature infants, the D(-) form induced severe acidosis as demonstrated by weight loss, pallor, and vomiting in 4 out of 16 infants. Goldman (634) obtained similar results; he pointed out that prematures are normally on the verge of acidosis and can be made acidotic more easily than the term infant. Fazekas (515, 517, 519) reported that ingestion of lactic acid exerted an acidotic effect on rabbits leading to enlargement of the ovaries and parathyroids. In contrast, Grosz and Farmer (664) believed that infusion of lactate promoted alkalosis, based on the similarity of these symptoms to these induced by infusion of bicarbonate.

Oral administration of calcium lactate in man resulted in elevated serum calcium (448, 101, 1113) and in reduced serum phosphorus (448).

Accidental administration of 1 teaspoon of 85% lactic acid caused caustic burns of the mouth and pharynx (1887) and stricture of the esophagus (1470) in infants. Three premature infants died from the consumption of an improperly prepared formula containing an undetermined excess of lactic acid (2050).

Several short-term studies have been performed on both calcium lactate and lactic acid. When 0.25% calcium lactate was added to the diet of pullets for 15 months, no effect on egg production, egg weight, or shell thickness was observed (1573).

Wysokinska (2035) gave ten young rats increasing doses of lactic acid by gavage, beginning with 0.625 g/kg and rising to the lethal dose of 11.25 g/kg over 18 days. Autopsy revealed intensive congestion of the liver, plus a loosened gastric and duodenal mucous membrane. In subsequent experiments 1.5 g lactic acid was fed both by gavage and in the feed for 3 months. Hemoglobin content and the number of red blood cells decreased considerably.

Lactic acid concentrations of 1.1 to 10 mg/ml in the drinking water of rats decreased water consumption in both irradiated and nonirradiated rats (1448). Various salts including 2% calcium lactate dissolved in drinking water were available to young rats; no consistent selection pattern evolved (1720).

After administering per os 0.5 g of calcium lactate daily to rats, Mlynarska (1300) noted that the osmotic resistance of leukocytes increased by 19.5%. The addition of 0.115 g calcium lactate to a poor rice diet increased the growth rate of rats over a 9-week-period (1210).

Jonek (878) observed increased activity of the adrenal cortex following the consumption of 0.18 g lactic acid daily by female rabbits. Fazekas (515, 517, 519) performed a series of experiments on rabbits that were fed 0.1-0.2 g of lactic acid per kg daily for periods of up to 26 months. He found ovarian enlargement due to hypertrophy of the interstitial ovary glands and to the enlargement and maturation of follicles. In addition, the examination of the parathyroid revealed plethora, hypertrophy, and hyperplasia, indicative of hyperfunction.

Supplements of 1 g calcium lactate given daily to children on a poor rice diet increased growth over an 11-week-period (57). When 8 ml of an 87% lactic acid solution were added to 1 liter of milk, children aged 4 and 5 years occasionally produced casts in their urine (654).

Although no long-term studies were available, several special studies have been reviewed. They deal with tumor-inhibition, dental-caries, reproduction, and teratology.

Parfentjev et al. (1419) observed a slight inhibition of sarcoma 180 when mice were fed 1 ml of a 1% lactic acid solution daily for 3 weeks. Selawry and Schwartz (1699) reported a dose-related inhibition of sarcoma 180 in mice following intraperitoneal injection of D,L-lactic acid. After placing tablets containing 20-60 mg calcium lactate under the flank skin of mice with breast tumors, Dobrovolskaia-Zavadskaia (411) observed an 84% inhibition and 16% stimulation of tumor growth.

Syrian hamsters were fed a caries-producing diet plus 45.6 mg lactic acid per 100 g of diet, or 40 mg lactic acid per 100 ml of drinking water, or no supplement. While lactic acid produced some enamel decalcification, there was no significant difference in the incidence of caries between groups (649). Calcium lactate was found to be the most efficient calcium depositer as of the dentin of the lower incisors as compared to the gluconate, carbonate, or phosphate salts (1382).

D'Amour (346) determined that the consumption of 2.5 or 5.0% lactic acid by pregnant rats had no effect on the sex ratio of the offspring.

Direct application of 0.01 to 0.03 ml of a 0.01% lactic acid solution to the vitelline membrane, amniotic cavity, or subgerminal cavity of 3-day-old chick embryos led to an increased death rate as compared to controls treated with saline. Approximately 12% of the survivors from the allantoic route developed anomalies (646).

CALCIUM LACTATE

Chemical Information

I. Nomenclature

A. Common Name

Calcium lactate

B. Chemical Name

Calcium lactate

C. Trade Name

None available

D. Chemical Abstracts Registry Number

Calcium lactate 000814802

II. Empirical Formula

$C_6H_{10}CaO_6$

III. Structural Formula

$Ca(CH_3CH(OH)COO)_2 \cdot xH_2O$

IV. Molecular Weight

218.22

V. Specifications

Food Chemicals Codex

Assay	Not less than 98.0% and not more than 101.0% of $C_6H_{10}CaO_6$, after drying
Loss on drying	Pentahydrate: between 24% and 30%; Trihydrate: between 15% and 20%; Monohydrate: between 5% and 8%; Dried form: not more than 3%

V. Specifications Cont.

Limits of Impurities	
Acidity	Passes test (about 0.45%, as lactic acid
Arsenic (as As)	Not more than 3 ppm (0.0003%)
Fluoride	Not more than 15 ppm (0.0015%)
Heavy metals (as Pb)	Not more than 20 ppm (0.002%)
Lead	Not more than 10 ppm (0.001%)
Magnesium and alkali salts	Not more than 1%
Volatile fatty acids	Passes test

VI. Description

A. General Characteristics

Calcium lactate is a white to cream colored, almost odorless, crystalline power or granules, containing up to 5 molecules of water of crystallization. The pentahydrate is somewhat efflorescent.

B. Physical Properties

Calcium lactate becomes anhydrous at 120 degrees C. It is slowly soluble in cold water, quickly soluble in hot water, but almost insoluble in alcohol.

C. Stability

Store in tight containers.

VII. Analytical Methods

See D,L-lactic acid

VIII. Occurrence

Calcium lactate is prepared commercially by neutralization of lactic acid with calcium carbonate.

D,L-LACTIC ACID

Chemical Information

I. Nomenclature

A. Common Names

1. D,L-lactic acid
2. Racemic lactic acid
3. Ordinary lactic acid
4. Milchsaeure

B. Chemical Names

1. alpha-hydroxypropionic acid
2. 2-hydroxypropanoic acid

C. Trade Name

None available

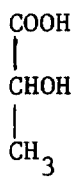
D. Chemical Abstracts Registry Number

Lactic acid	000050215
Lactic acid USP	MX8012213
Lactic acid, DL	000598823

II. Empirical Formula

$C_3H_6O_3$

III. Structural Formula



IV. Molecular Weight

90.08

V. Specifications

Food Chemicals Codex

Assay	Not less than 95.0% and not more than 105.0% of the labeled concentration of $C_3H_6O_3$
Limits of Impurities	
Arsenic (as As)	Not more than 3 ppm (0.00003%)
Chloride	Not more than 0.2%
Citric, oxalic, phosphoric, or tartaric acid	Passes test
Heavy metals (as Pb)	Not more than 10 ppm (0.001%)
Iron	Not more than 10 ppm (0.001%)
Residue on ignition	Not more than 0.1%
Sugars	Passes test
Sulfate	Not more than 0.25%

VI. Description

A. General Characteristics

Lactic acid has a crystalline form. Food grade lactic acid is available as a colorless or yellowish, nearly odorless, syrupy liquid consisting of a mixture of lactic acid and lactic acid lactate. It is usually available in solutions containing the equivalent of 50-90% lactic acid.

B. Physical Properties

Lactic acid has a melting point of 16.8 degrees C. The bp at 14-15 mm Hg is 122 degrees C and at 0.5-1 mm Hg is 82-85 degrees C. The ionization constant is 1.38×10^{-4} at 25 degrees C.

The heat of combustion at constant pressure is 3615 cal/kg. It is volatile with superheated steam. Lactic acid is soluble in water, alcohol, furfurol, and less soluble in ether; however, it is practically insoluble in chloroform, petroleum ether, and carbon disulfide. It is incompatible with oxidizing agents, iodides, nitric acid, and albumin in pharmaceuticals.

C. Stability

Store in tight containers.

VII. Analytical Methods

The official method for the determination of lactic acid in whole or skim milk, ice cream, and butter involves extraction of lactic acid with ether. Ferric chloride is added to produce a color change. The solution is then read in a spectrophotometer and compared to a standard curve.

This method can be adapted for determinations in canned vegetables, evaporated milk, and wines (2068).

VIII. Occurrence

Lactic acid occurs in sour milk as a result of lactic acid bacteria. It is also found in molasses due to partial conversion of sugars, in apples and other fruits, tomato juice, beer, wines, opium, ergot, foxglove, and several higher plants especially during germination (1262).

Lactic acid is prepared technically by "lactic acid fermentation" of carbohydrates such as glucose, sucrose, or lactose with Bacillus acidi lacti or related organisms. Such fermentation is carried out at high temperatures, and generally whey, cornstarch, potatoes, and molasses are the substrates (1262).

L-LACTIC ACID

Chemical Information

I. Nomenclature

A. Common Names

1. L-lactic acid
2. L(+)-lactic acid
3. Dextrorotatory lactic acid
4. d-lactic acid
5. Sarcolactic acid
6. Paralactic acid
7. L-milchsaeure

B. Chemical Name

L(+)-lactic acid

C. Trade Names

None available

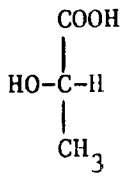
D. Chemical Abstracts Registry Number

Lactic acid	000050215
Lactic acid USP	MX8012213

II. Empirical Formula

$C_3H_6O_3$

III. Structural Formula



IV. Molecular Weight

90.08

V. Specifications

See D,L-lactic acid

VI. Description

A. General Characteristics

L-lactic acid has a crystalline form.

B. Physical Properties

L-lactic acid has a melting point of 53 degrees C, and an optical rotation of (alpha) 21-22/546.1 + 2.6 degrees (2.5 g in 100 ml water). The pK at 25 degrees was 3.79. L-lactic acid forms salts with many metals; these salts are more soluble in water than the salts of the racemic acid. Most of the salts are levorotatory.

C. Stability

Store in tight container.

VII. Analytical Methods

See D,L-lactic acid

VIII. Occurrence

L-lactic acid occurs in small quantities in the blood and muscle fluid of man and animals. The lactic acid concentration increases in muscle and blood after vigorous activity. It's also present in the liver, kidney, thymus gland, human amniotic fluid, and other organs and body fluids (1262).

D-LACTIC ACID

Chemical Information

I. Nomenclature

A. Common Names

1. D(-)-lactic acid
2. Levorotatory lactic acid
3. l-lactic acid
4. D-milchsaeure

B. Chemical Name

1. D(-)-lactic acid
2. 2-hydroxypropionic acid

C. Trade Name

None available

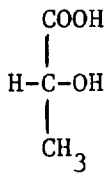
D. Chemical Abstracts Registry Number

Lactic acid	000050215
Lactic acid USP	MX8012213

II. Empirical Formula

$C_3H_6O_3$

III. Structural Formula



IV. Molecular Weight

90.08

V. Specifications

See D,L-lactic acid

VI. Description

A. General Characteristics

See D,L-lactic acid.

B. Physical Properties

D-lactic acid is soluble in water, alcohol, acetone, ether and glycerol, but practically insoluble in chloroform. It has a melting point of 52.8 degrees C and an optical rotation of (alpha) 21.5/546 - 2.6 degrees (8 g in 100 ml H_2O). D-lactic acid forms salts with many metals; most of these salts are dextrorotatory.

C. Stability

Store in tight container.

VII. Analytical Methods

See D,L-lactic acid.

VIII. Occurrence

D-lactic acid is obtained by resolution of DL-lactic acid.

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage (mg/kg)	Measurement	Ref.
Calcium Lactate	Rabbit	-	i.v.	340	MLD[x]	254
Calcium Lactate	Rabbit	-	i.v.	180	MLD[xx]	254
Calcium Lactate	Rabbit	-	i.v.	7.0	DML[xxx]	73
Calcium Lactate	Dog	-	i.v.	160	MLD[x]	254
Calcium Lactate	Dog	-	i.v.	80	MLD[xx]	254
Lactic Acid	Rat	-	Oral	3,730	LD_{50}	611
Lactic Acid	Rat	-	Oral	3,700	LD_{50}	1262

x Minimum lethal dose at 1 ml per min

xx Minimum lethal dose at 2 ml per min

xxx The distant minimum lethal dose differs from the immediate lethal dose in that the latter implies instant death.

Mice

The injection of 0.4-0.5 ml of a 9% calcium lactate solution (36-45 mg) into 3 mice produced ataxia, catalepsy, slowing of the respiration (without dyspneic symptoms), gradual immobilization and death (412).

Baldacci reported the intravenous distant minimum lethal dose to be 7 mg/kg for calcium lactate in rabbits. The distant minimum lethal dose differs from the immediate lethal dose in that the latter implies instant death. Albuminuria was observed. Autopsy revealed congestion of the thoracic and abdominal organs, distended blood vessels, and subpleural and subperitoneal hemorrhagic suffusions. The volume of urine excreted decreased (73).

Doses of 2, 4, and 6 gm of calcium lactate were administered by stomach tube to groups of 2 rabbits. The animals were from two to six weeks old and had been fasted for 48 hours. Animals were permitted to eat 6 hours after treatment and were generally sacrificed 48 hours after treatment. A gross and microscopic examination of the stomach and intestines revealed no lesions (448).

After oral administration of a lactic acid milk mixture containing 10.1 g in 30 ml to 4 rabbits, death followed in 10 min to 40 hrs. Death was attributed to acute hemorrhagic gastritis; a moderate degree of acidosis was observed (2050).

Three premature infants were administered on acid milk mixture containing an excess of lactic acid (amount unknown) and died shortly thereafter. Death was due to an acute hemorrhagic and gangrenous gastritis (2050).

An infant received one teaspoon of 87.5% lactic acid by mistake and suffered caustic burns of the mouth and pharynx (1887).

The oral administration of 1 teaspoon of 85% lactic acid to an eleven-week-old female infant caused stricture of the esophagus (1470).

Three healthy individuals received oral doses of calcium gluconate and calcium lactate on consecutive days. Doses of 20 g calcium gluconate and 10 g calcium lactate caused abdominal distress, vomiting, and diarrhea. Doses were subsequently halved. Diuresis, headache, and violent bowel movement and spasm were noted (1113).

II. Short-Term Studies

A. Calcium lactate

Chicks

Roberson fed 2 levels of ascorbic acid, 2 levels of magnesium, and 0 or 0.25% calcium lactate to pullets in a 2x2x2 factorial arrangement. The basal diet contained 3% calcium, 16% crude protein, 900 kcal of productive energy per pound, and 1015 mg of Mg per pound. Three replicates of four pullets (3 month old) were placed on each diet for 15 months. The addition of calcium lactate did not affect egg production, egg weight, Haugh score, or shell thickness. Shell roughness improved slightly (1573).

Rats

Young rats were fed a synthetic diet containing no salts for 40 days; however, various salts including 2% calcium lactate were presented in separate drinking bottles. Selection patterns of the nine animals were fairly consistant among individuals but no general pattern was determined for the entire group. The daily average intake of calcium lactate ranged from 26-122 mg (1720).

Mlynarska administered 0.5 g of calcium lactate daily by stomach tube to white rats; on the 24th day, the osmotic resistance of the leukocytes increased by 19.5% (1300).

When 0.115 g of calcium lactate was added to a rice diet similar to that of the poor in Southern India, the growth rate of the month-old

rats increased over that of rats fed only the rice diet. After nine weeks, the coats of the three lactate-fed animals were in better condition than the other animals (1210).

Man

Aykroyd and Krishnan gave per os 1 g of calcium lactate to 46 boys aged 6 to 12 years for eleven weeks. The children were maintained on a poor rice diet typical of southern India. The supplemented group had significantly greater height and weight increments (57).

B. Lactic acid

Rats

Wysokinska gave 10 six-week-old male rats increasing doses of a 50% lactic acid solution by gavage. Initially 0.25 ml (0.625 g/kg) was given; 0.25 ml was added to the dose daily until the lethal dose 4.5 ml (11.25 g/kg) was attained. Two rats died after receiving 3 ml lactic acid. Rats lost 15% of their weight within 1 week. No changes in the serum pH or carbon dioxide content was noted after the administration of large doses. Urinary pH decreased considerably. Autopsy revealed intensive congestion of the liver, plus a loosened gastric and duodenal mucous membrane (2035).

In three separate experiments, small groups of 9 to 10-week-old rats were given 3 ml of a 10% lactic acid solution (1.5 g) daily for 3 months. In two of these studies, the substance was introduced by a stomach tube, but in the third it was mixed in the feed. Control animals received either no treatment or distilled water by gavage. The hemoglobin content and the number of red blood cells decreased considerably in experimental groups; weight gain was also slower. Deaths were reported in both force-feeding studies. Swollen livers and kidneys also were found (2035).

Peters and Peters added 0.04, 0.36, 1.1, 5.0, and 10 mg/ml lactic acid to the drinking water of groups of 8 rats. After 10 days, some of the animals were exposed to 735 R total body radiation. The water consumption by both irradiated (X-rays) and non-irradiated rats was measured for 10 days before and after radiation. The water consumption decreased in all groups for the first days following radiation. In addition lactic acid concentrations of 1.1-10 mg/ml increased the inhibitory action on liquid intake in both irradiated and non-irradiated animals (1448).

Rabbits

When female rabbits consumed 0.18 g of lactic acid daily in their feed, the activity of the adrenal cortex increased. Evaluation of adrenocortical activity was based on the following findings: the presence of vacuoles in the cellular plasma; the contents of lipids in the adrenocortical layers; increased adrenal weight; and the presence of degenerative changes in various parts of the adrenal cortex. Changes were thought to result from stimulation of the hypothalamo-hypophyseal system (878).

Five female virgin rabbits aged 8 to 14 months and weighing 2.5-3.0 kg received lactic acid for 5 months. Every other day, animals were given 0.1-0.2 g/kg of lactic acid dissolved in 100-150 mls of drinking water. Three weeks of treatment were alternated with one week without treatment for the 5 month period. Fifty control animals were also examined. At the end of the study, animals were sacrificed and the ovaries were examined histologically. Ovarian weight increased significantly in the treated group; numerous enlarged, hemorrhagic follicles were visible on the ovary's surface. Ovarian enlargement was ascribed either to hypertrophy of the interstitial ovary glands and their increased lipid content, or to the enlargement and maturation of the follicles (515).

In a subsequent study, rabbits on a similar regimen were observed for 14 to 16 months. The results corresponded to those above (515).

For 5 to 16 months, 10 female rabbits received increasing doses of 0.1-0.2 g/kg of lactic acid (concentration not specified) twice daily. A three week treatment period was followed by a pause of one week. The weight of the parathyroid increased significantly more than in the control group. Histological examination revealed plethora, hypertrophy, hyperplasia, and multiplication of the light primary cells which indicated hyperfunction (517).

Six Chinchilla rabbits aged 8-10 months and weighing between 2.5 and 3.0 kg were given 0.1-0.2 g of lactic acid per kg body weight in their drinking water twice daily. Each three week treatment period was followed by two weeks without treatment for a 5 to 26 month period. Parathyroid size, increased and hyperfunction was noted (519).

When 8 ml of 87% lactic acid were added to 1 l milk, children aged 4 and 5 years occasionally produced casts in their urine (654).

III. Long-Term Studies

None available

IV. Special Studies

Tumor inhibition

Mice

Parfentjev et al. fed 1 ml of a 1% lactic acid solution daily for three weeks to 41 mice with sarcoma 180. An additional 34 mice received no treatment. Lactic acid was observed to slightly inhibit tumor growth (1419).

Selawry and Schwartz reported a dose-related inhibition of sarcoma

180 in Ha/ICR-Swiss mice following intraperitoneal injections of d-l-lactic acid. Groups of 6 mice of each sex received injections of lactic acid in saline on days 1, 2, 4, and 5 after tumor implantation. Two groups of each sex received saline only. Tumors were excised and weighed on day 6. A mean tumor inhibition of 64.5% succeeded injection of 150 mg/kg/day. Injection of 75 mg/kg/day induced a 31.5% inhibition. While all the animals at the 75 mg/kg level survived, only 58% of the 150 mg/kg group did. Comparable results were obtained with l-lactic acid, but sodium and calcium lactates were ineffective at a similar levels (1699).

Dobrovolskaia-Zavadskaia placed calcium lactate tablets containing 20-60 mg under the skin of the left groin or flanks of 24 mice. Prior to application, 23 of the mice already had breast tumors. Calcium lactate stimulated 4 tumors (16%) but inhibited 21 tumors (84%). During the study 9 new tumors were found, that is, 26.5% out of 34 breast tumors; pulmonary metastases were also found in 8 out of 23 mice. The growth of a chondrosarcoma of the perineum in one mouse was restricted. Fifteen animals died within 7 months (411).

Dental caries

Hamsters

Groups containing 8 male and 7 female 21 to 25-day-old Syrian hamsters were fed a caries-producing diet which included 40% ground yellow corn and 20% sucrose for 100 days. One group also received 0.057 ml of an 80% lactic acid solution per 100 g of diet (45.6 mg/100 g diet). A second group was given drinking water containing 0.05 ml of 80% lactic acid per 100 ml (40 mg/100 ml), while a third group received no supplement. Lactic acid produced some enamel decalcification, but there was no significant difference in the incidence or extent of caries among groups. Growth rates and general appearance were also similar (649).

Rabbits

Adult male rabbits weighing 2 kg were given per os calcium lactate, calcium gluconate, calcium chloride, precipitated calcium carbonate, and precipitated calcium phosphate in single doses of 0.46 g calcium per kg twice daily at 4-5-day intervals. Lead acetate was injected at the time of calcium administration to establish the recording time on dentin. After 4 weeks, the lower incisors dentin was decalcified. Calcium lactate was the most efficient depositer of calcium (1382).

Reproduction

Rats

D'Amour determined that the consumption of 2.5 or 5% lactic acid in a stock diet by pregnant rats had no influence on the sex ratio of the offspring (346).

Teratology

Chick

Direct applications of lactic acid solutions (0.01 to 0.03 ml of a 0.01% solution) were made to the vitelline membrane, amniotic cavity, or subgerminal cavity of 3-day chick embryos and into the allantoic cavity of 4-day chick embryos. Extensive edema followed by subcutaneous blisters and hematomas were found regardless of embryonic age or method of injection. Generally, the death rate was high (90%), but only 70% of the embryos injected by the allantoic route died. Of the 79 survivors in this group, 10 (12.7%) developed anomalies. Of the 85 surviving saline-injected controls, only one was abnormal (646).

Biochemical Aspects

I. Breakdown

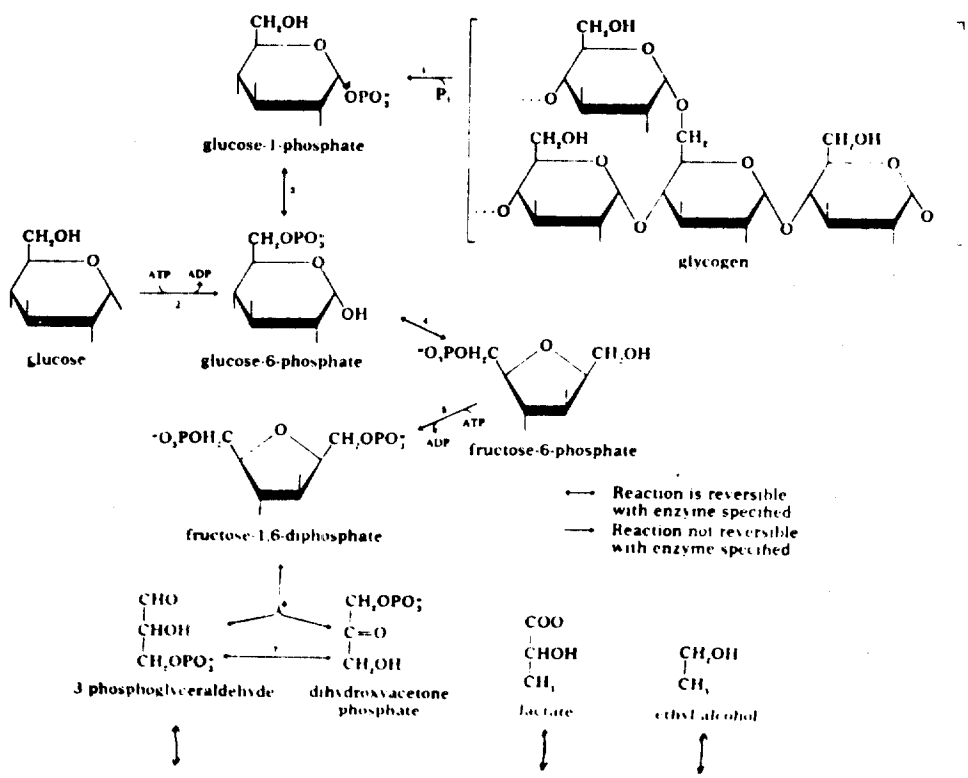
Lactic acid readily undergoes self-esterification. When a solution is heated, dehydration takes place between the alpha-hydroxyl group of one molecule and the carboxyl of another to form several polylactic acids such as lactyllactic acid, the linear trimer, and higher polymers. The products occur in all solutions containing more than 18% lactic acid. Temperature affects the relative amounts of each moiety (588).

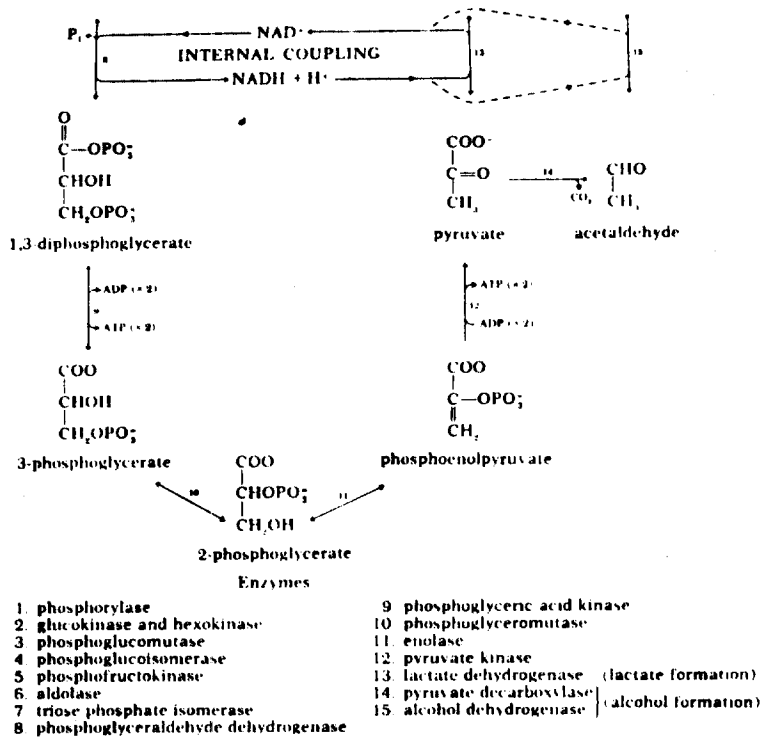
II. Absorption-Distribution

Frenning studied the absorption of lactic acid in unstimulated whole stomach pouches of cats. After instillation of 170 or 700 mM L(+)-lactic acid, the hydrogen ion and lactate concentration decreased equally. The net effluxes of hydrogen ions and lactate were also roughly equal. The absorption of D(-)-lactate was thought to function similarly. No morphological changes of the gastric mucosa were found after electron microscopic examination (558).

Lactate appears to be distributed in a space equivalent to or slightly less than the total body water. It diffuses readily across cell membranes primarily by passive transport. Under certain conditions its distribution may be uneven or the lactate pool consists of several smaller pools with differing rate constants (1019).

Lactate is formed through an anaerobic pathway of carbohydrate degradation (glycolysis) in skeletal muscle and a few select microbes. A scheme for glycolysis is portrayed below (163).

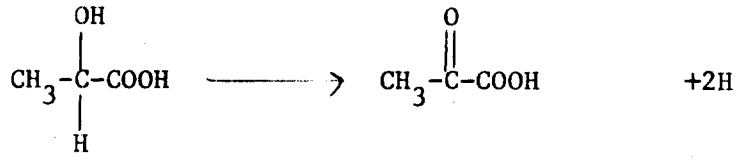




Erythrocytes and muscle account for most of the lactate produced in vivo, with smaller contributions made by brain, leukocytes, and the renal medulla. The estimated total available lactate that could be produced from these sources is 46 mg/kg-hr. However, the gastrointestinal tract and skin may also be sources. A minimum of 20-25 mg/kg-hr for a 70 kg subject should be added as an adjustment of the in vitro production rates of these tissues (1019).

III. Metabolism and Excretion

Lactic acid dehydrogenase converts lactic acid to pyruvic acid by the removal of 2 H's.



Lactic Acid Pyruvic Acid

In animals, lactate generated in the anaerobic metabolism of certain tissue such as skeletal muscle can be transported to other more aerobic tissues such as the liver. There it can be reconverted to pyruvate. The

pyruvate can then be metabolized further via the citric acid cycle or be reconverted to carbohydrate material as free glucose or stored as glycogen. In man, lactic acid dehydrogenase is widely distributed in various tissues and body fluids (163).

In 1929 Cori and Cori showed that the liver was capable of converting lactic acid to glycogen. Fasted male rats were given sodium d, l, or r-lactate by stomach tube. While feeding the dextro-isomer led to glycogen deposition in the liver, the levro-isomer formed little glycogen. However, both isomers were absorbed at similar rates. Forty to ninety-five percent of the d-lactate absorbed was retained as liver glycogen. Thirty percent of the l-lactate absorbed was excreted in the urine; none of d-lactate was excreted. The authors estimated that d-lactic acid was utilized four times faster in the cat than the l-isomer (315).

The formation of liver glycogen from lactic acid was viewed as an important link between the metabolism of the muscle and that of the liver. The cycle in brief, was described as follows: liver glycogen $\rightarrow$ blood glucose $\rightarrow$ muscle glycogen $\rightarrow$ blood lactic acid $\rightarrow$ liver glycogen (315).

Micrococcus lactilyticus was found to represent a significant portion of the bacteria indigenous to human saliva. This microorganism ferments lactate to propionate, acetate, carbon dioxide, and hydrogen (418).

Searle and Cavalieri estimated the turnover, oxidation, and reduction of lactate in man. Primed infusion studies with labeled L(+)-lactic acid indicated that the virtual volume of distribution of lactate was 49.4% of the body weight. The authors stated that it was reasonable for a freely and rapidly diffusable molecule to occupy a space greater than or equal to three quarters of the body water space. The lactate pool size and turnover time were estimated at 0.029 g/kg and 18.4 min, respectively. The turnover was approximately 95 mg/kg/hr with approximately 90% oxidation to CO_2. Searle and Cavalieri concluded that body lactate kinetics probably reflect the total flux of carbon through pyruvate, and that the primary fate of lactate was oxidation to carbon dioxide, not reduction to glucose by hepatic tissue. Such oxidation probably occurs in the peripheral tissues (1694).

Drury and Wick used C^{14}-L(+)-lactate to study the metabolism of injected lactic acid in the intact rabbit. Circulating lactic acid was used up and renewed at a rapid rate, with a turnover time of about 30 min. Most of the lactate was oxidized to carbon dioxide; however, part of the lactate could be accounted for as glucose and glycogen or by the oxidation of them. An eviscerated animal metabolized the L(+)-isomer quite actively, but not the D(-)isomer. According to the authors racemic lactate is practically completely metabolized by the intact animal. They suggested that the liver might convert the (D) isomer either to the L(+) form or to glucose or glycogen (432).

During experiments with rapid intravenous sodium-lactate loading, Dies et al. determined that tubular reabsorption of lactic acid is rate limited in the dog. Lactic acid excretion was urine flow dependent at low

filtered loads. It was concluded that lactic acid is actively reabsorbed in the proximal tubule, that its transport is rate limited, and that it is either incompletely reabsorbed at low filtered loads or partially secreted at a distal site of the nephron (398).

When sodium dl lactate was given orally to a dog, it was almost completely utilized. When the plasma lactate concentration was increased by i.v. infusion, urinary excretion was slight until the plasma concentration approached 1 mg/ml. At concentrations of 1 to 4 mg/ml, the rate of excretion was proportional to the rate of glomular filtration. The L(+) isomer, the natural form, was utilized more than the D(-) isomers in a 3 to 2 ratio (326).

Sweat lactate was derived metabolically from serum glucose as opposed to serum lactate in man. The yield of radioactive carbon in sweat lactate after an injection of labeled lactate averaged only 8% of that found after a similar injection of labeled glucose (640).

IV. Effects on Enzymes and Other Biochemical Parameters

Ballabriga et al. provided 150 premature infants with a nonacidified formula N until aged 12 days. They were then fed the following diets for the first 2 months of life: formula X (25 infants); formula X plus 0.5 g/100 ml L(+) lactic acid (21 infants); formula X plus 0.5 g/100 ml D(-) lactic acid (16 infants); formula X w/0.5 g/100 ml racemic lactic acid (29 infants); formula E with 0.5 g/100 ml lactic acid obtained by biological acidification (21 infants); formulas X and N plus 0.5 g/100 ml citric acid each (16 and 22 infants, respectively). Formula X had a higher protein content than formula N. Development was normal for groups receiving formula N, non-acidified formula X, the formulas acidified by L(+) lactic acid, and the citric acid formulas. However, the group given D(-) lactic acid formula X suffered 4 cases of metabolic acidosis as indicated by weight loss, pallor, bad overall condition, vomiting and regurgitation. The formula had to be discontinued. Three cases of mild acidosis occurred in the set fed racemic lactic acid. Acidosis was relieved by the administration of a non-acidified diet or after the intravenous injection of bicarbonate in the severe cases. The authors concluded that only L(+) lactic acid or perhaps citric acid should be used to acidify milks for feeding premature infants; racemic or D(-) lactic acid should never be used (77).

The consumption of lactic acid milk by premature infants led to acidosis and reduced weight gain. Prematures have been shown to be normally on the verge of acidosis and can be made acidotic more easily than the term infant (634).

In a series of experiments Fazekas (315, 317, 319) determined that the feeding of 0.1-0.2 g of lactic acid per kg of body weight to rabbits shifted the acid-base balance to the acid side. Although serious metabolic acidosis did not develop, enlarged ovaries and parathyroids were seen.

Parathyroid hyperfunction accompanied the increased weight.

Grosz and Farmer infused 8 ml per kg of a 500 mM sodium (DL) lactate into 10 young men; subjects suffered from paresthesia, tremor, shakiness, dizziness, and palpitation. Infused lactate was thought to be rapidly converted to bicarbonate hence induce metabolic alkalosis. However, serum pH was not measured (664).

Six infants were given 60 ml of a 10% calcium lactate solution (6g) prior to their normal morning feeding. Serum calcium, phosphorus, and total proteins were measured before and four hours after introduction. Three of the infants had mild diarrhea during the four hour period but no vomiting occurred. Serum calcium rose significantly, but serum phosphorous dropped. The serum protein concentration increased slightly (448).

Serum calcium determinations were made for a 12 hour period following oral consumption of 5 or 10 g of calcium lactate by normal fasted subjects. Serum calcium was elevated to a maximum of 14 and 28% within 5 hours for the 5 and 10 g doses, respectively (101). Similar results were obtained by Lieberman (1113).

V. Drug Interaction

No Information Available

VI. Consumer Exposure Information

Lactic acid is one of the most widely distributed acids in nature and one of the earliest used in foods. Lactic acid is used in packaging Spanish-type olives, for it insures clarify of the brine by inhibiting spoilage and further fermentation (588).

> "Lactic acid, when added to pan-dried egg whites to adjust the pH range from 4.8 to 5.1, permits rapid settling of any shell fragments, improves protein dispersion, and aids in producing a more stable dried-egg powder with superior whipping properties. A milder, more subtle taste is obtained when it is added to the vinegar in preparing certain pickles and relishes. It is also used to adjust the acidity and as a flavoring agent in the manufacture of cheese and dried food casein. Lactic acid is reported to be excellent, when used in small amounts, for acidifying fruit juice in the production of wine, and to give improved flavor to carbonated fruit juices when used in combination with other acidulants. Lactic acid is also used in certain frozen desserts to provide a mild, tart flavor without masking that of the natural fruit. It is employed in some jams and jellies, in the manufacture of beer, in mincemeat, mayonnaise, and for solubilizing pepper oleoresin (588)."

"Calcium lactate is used to preserve the firmness of apple slices during processing, to inhibit the discoloration of fruits and vegetables, as a gelling agent for demethylated pectins, and to improve the properties of dry-milk powders, condensed milk and baked food products (588)."

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 the frequency of eating, and from the USDA data on mean portion size of foods in each category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of the substance.

Table 2 reports the usage of lactic acid and calcium lactate in foods. Table 11 reports the annual poundage data. Table 13 reports the possible daily intake and total dietary, based on food consumption by total sample.

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, %
CALCIUM LACTATE NAS 0045 FEMA 3535	01	BAKED GOODS(R)	*	.00969	.01197
	03	OTHER GRAIN(R)	*	.00300	.00400
	08	PROCSD FRUT(R)	*	.00500	.00500
	10	MEAT PRODS(R)	*	.00300	.00400
	14	PROCSD VEGS(R)	*	.10400	********
	16	SOFT CANDY(R)	*	.00170	.00230
	20	GELATIN PUD(R)	*	.00200	.00200
	23	BEV TYPE I(R)	*	.19051	.19051
	33	SUGAR SUBS(R)	*	85.00000	90.00000
LACTIC ACID NAS 0103 FEMA 2611	01	BAKED GOODS(R)	27	.20413	.26408
	04	FATS OILS(R)	7	.13379	.22755
	05	MILK PRODS(R)	*	.09255	.14114
	06	CHEESE(R)	9	1.94312	3.66677
	07	FROZN DAIRY(R)	14	.00347	.03624
	10	MEAT PRODS(R)	4	.37449	.37509
	14	PROCSD VEGS(R)	*	.00239	.00258
	15	CONDM RELSH(R)	9	.71266	1.31137
	16	SOFT CANDY(R)	17	.00821	.01236
	17	CONF FROST(R)	*	.03300	.04000
	19	SWEET SAUCE(R)	*	.05000	.05000
	20	GELATIN PUD(R)	10	.07297	.10039
	21	SOUPS(R)	4	.01166	.01276
	22	SNACK FOODS(R)	*	.02785	.03686
	23	BEV TYPE I(R)	21	.00180	.00662
	24	BEV TYPE II(R)	*	.00125	.00350
	27	GRAVIES(R)	4	.17013	.17014
	28	IMIT DAIRY(R)	*	.08133	.09721
	30	HARD CANDY(R)	8	.35608	1.60562
	31	CHEWING GUM(R)	4	.01678	.02451
LACTIC ACID NAS 0103 FEMA 2611	(CONT.) 48	SEAS FLAVRS(R)	*	.00300	.01400
	49	MISC UNCLAS(R)	*	.00501	.03828

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
CALCIUM LACTATE NAS 0045 FEMA 3535	6/ 9	51,550	27,037	73,337	*	233	73,570
LACTIC ACID NAS 0103 FEMA 2611	19/ 24	582,083	773,688	953,481	57	564,151	1,517,632

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 B
CALCIUM LACTATE NAS 0045	01 BAKED GOODS(R)	*	0-5 MO.	.329460	.436050	.406980
			6-11 MO.	2.461260	5.019420	3.040380
			12-23 MO.	5.281050	8.701620	6.522650
			2-65+ YR.	13.294680	19.748220	16.422540
CALCIUM LACTATE NAS 0045	03 OTHER GRAIN(R)	*	0-5 MO.	.015000	.051000	.020000
			6-11 MO.	.291000	.858000	.388000
			12-23 MO.	.492000	1.137000	.656000
			2-65+ YR.	.834000	1.842000	1.112000
CALCIUM LACTATE NAS 0045	08 PROCSD FRUT(R)	*	0-5 MO.	.235000	.630000	.235000
			6-11 MO.	2.590000	6.450000	2.590000
			12-23 MO.	5.030000	9.985000	5.030000
			2-65+ YR.	5.915000	12.530000	5.915000
CALCIUM LACTATE NAS 0045	10 MEAT PRODS(R)	*	0-5 MO.	.033000	.097000	.044000
			6-11 MO.	.621000	1.674000	.828000
			12-23 MO.	.906000	1.557000	1.208000
			2-65+ YR.	2.352000	3.903000	3.136000
CALCIUM LACTATE NAS 0045	14 PROCSD VEGS(R)	*	0-5 MO.	1.456000	4.368000	**********
			6-11 MO.	24.960000	58.240000	**********
			12-23 MO.	40.560000	67.912000	**********
			2-65+ YR.	88.400000	148.928000	**********
CALCIUM LACTATE NAS 0045	16 SOFT CANDY(R)	*	0-5 MO.	.003400	.034000	.004600
			6-11 MO.	.037400	.115600	.050600
			12-23 MO.	.059500	.158100	.080500
			2-65+ YR.	.098600	.299200	.133400
CALCIUM LACTATE NAS 0045	20 GELATIN PUD(R)	*	0-5 MO.	.040000	.054000	.040000
			6-11 MO.	.256000	.776000	.256000
			12-23 MO.	.276000	.672000	.276000
			2-65+ YR.	.408000	1.050000	.408000
CALCIUM LACTATE NAS 0045	23 BEV TYPE I(R)	*	0-5 MO.	4.572240	6.858360	4.572240
			6-11 MO.	43.245770	148.026270	43.245770
			12-23 MO.	103.256420	309.578750	103.256420
			2-65+ YR.	198.130400	529.046270	198.130400
CALCIUM LACTATE NAS 0045	33 SUGAR SUBS(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	8.500000	17.000000	9.000000
			12-23 MO.	**********	17.000000	**********
			2-65+ YR.	68.000000	68.000000	72.000000
CALCIUM LACTATE NAS 0045	ALL CATEGORIES **********	10	0-5 MO.	6.684100	12.518410	5.322820
			6-11 MO.	82.962430	238.159290	59.398750
			12-23 MO.	155.660970	416.701470	117.030570
			2-65+ YR.	377.432620	785.346690	297.257640

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
LACTIC ACID NAS 0103	27 GRAVIES(R)	4	0-5 MO.	.170130	.510390	.170140
			6-11 MO.	2.381820	6.635070	2.381960
			12-23 MO.	6.124600	17.353260	6.125040
			2-65+ YR.	14.120790	36.237690	14.121620
LACTIC ACID NAS 0103	28 IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	1.138620	1.870590	1.362940
			12-23 MO.	.650640	2.765220	.777880
			2-65+ YR.	.731970	1.219950	.874090
LACTIC ACID NAS 0103	30 HARD CANDY(R)	8	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	.356030	1.068240	1.605620
			12-23 MO.	1.068240	3.204720	4.816860
			2-65+ YR.	2.136480	6.053360	9.633720
LACTIC ACID NAS 0103	31 CHEWING GUM(R)	4	0-5 MO.	**********	**********	**********
			6-11 MO.	.016780	.016780	.024910
			12-23 MO.	.016780	.050340	.024910
			2-65+ YR.	.033560	.067120	.049820
LACTIC ACID NAS 0103	48 SEAS FLAVRS(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	.000300	**********
			12-23 MO.	**********	.000600	**********
			2-65+ YR.	.000300	.001500	.001400
LACTIC ACID NAS 0103	ALL CATEGORIES ********** ********** **********	82	0-5 MO.	15.657090	30.460770	24.820000
			6-11 MO.	267.068400	859.157840	376.478990
			12-23 MO.	481.088150	1111.082080	713.580400
			2-65+ YR.	924.144880	1781.954660	1286.273940

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 B
LACTIC ACID NAS 0103	15 CONDM RELSH(R)	9	0-5 MO.	**********	.712660	**********
			6-11 MO.	5.701280	15.678520	10.490960
			12-23 MO.	19.954480	54.162160	36.718360
			2-65+ YR.	62.714080	151.083920	115.400560
LACTIC ACID NAS 0103	16 SOFT CANDY(R)	17	0-5 MO.	.016420	.164200	.024720
			6-11 MO.	.180620	.558280	.271920
			12-23 MO.	.287350	.763530	.432600
			2-65+ YR.	.476180	1.444960	.716980
LACTIC ACID NAS 0103	17 CONF FROST(R)	*	0-5 MO.	**********	.033000	**********
			6-11 MO.	.033000	.066000	.040000
			12-23 MO.	.066000	.231000	.080000
			2-65+ YR.	.099000	.264000	.120000
LACTIC ACID NAS 0103	19 SWEET SAUCE(R)	*	0-5 MO.	.150000	.200000	.150000
			6-11 MO.	.450000	1.550000	.450000
			12-23 MO.	1.300000	3.800000	1.300000
			2-65+ YR.	3.400000	8.950000	3.400000
LACTIC ACID NAS 0103	20 GELATIN PUD(R)	10	0-5 MO.	1.459400	1.970190	2.007800
			6-11 MO.	9.340160	28.312360	12.849920
			12-23 MO.	10.069860	24.517920	13.853820
			2-65+ YR.	14.885880	38.204250	20.479560
LACTIC ACID NAS 0103	21 SOUPS(R)	4	0-5 MO.	.023320	.174900	.025520
			6-11 MO.	2.715780	8.476820	2.973080
			12-23 MO.	4.657680	11.205260	4.440480
			2-65+ YR.	3.696220	9.852700	4.044920
LACTIC ACID NAS 0103	22 SNACK FOODS(R)	*	0-5 MO.	**********	.027850	**********
			6-11 MO.	.111400	.306350	.147440
			12-23 MO.	.306350	.862350	.405460
			2-65+ YR.	.362050	1.030450	.479180
LACTIC ACID NAS 0103	23 BEV TYPE I(R)	21	0-5 MO.	.043200	.054800	.156880
			6-11 MO.	.406600	1.398600	1.502740
			12-23 MO.	.975600	2.925000	3.588040
			2-65+ YR.	1.872000	4.998600	6.884800
LACTIC ACID NAS 0103	24 BEV TYPE II(R)	*	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	**********	.001250	**********
			12-23 MO.	**********	.002500	**********
			2-65+ YR.	.406250	1.180000	1.137500

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 B
LACTIC ACID NAS 0103	01 BAKED GOODS(R)	27	0-5 MO.	6.940420	9.185950	8.999120
			6-11 MO.	51.649020	105.739340	67.228720
			12-23 MO.	111.250850	183.308740	144.250600
			2-65+ YR.	280.056360	416.016940	369.140960
LACTIC ACID NAS 0103	04 FATS OILS(R)	7	0-5 MO.	.668950	.668950	1.137750
			6-11 MO.	3.746120	10.034250	6.371400
			12-23 MO.	8.428770	16.054800	14.335650
			2-65+ YR.	23.413250	42.277640	39.821250
LACTIC ACID NAS 0103	05 MILK PRODS(R)	*	0-5 MO.	4.997700	3.702000	7.621500
			6-11 MO.	57.751200	277.742550	68.071360
			12-23 MO.	50.439750	161.407290	76.921300
			2-65+ YR.	36.557250	111.615300	55.750300
LACTIC ACID NAS 0103	06 CHEESE(R)	9	0-5 MO.	**********	1.943120	**********
			6-11 MO.	52.484240	126.482640	99.002790
			12-23 MO.	151.563360	431.372640	286.806060
			2-65+ YR.	182.653280	458.575320	344.676380
LACTIC ACID NAS 0103	07 FROZN DAIRY(R)	14	0-5 MO.	.034700	.142270	.362400
			6-11 MO.	.329650	.916080	3.442800
			12-23 MO.	.499680	1.172860	5.218560
			2-65+ YR.	.868320	2.140990	9.277440
LACTIC ACID NAS 0103	10 MEAT PRODS(R)	4	0-5 MO.	4.115390	10.860210	4.125990
			6-11 MO.	77.519430	208.965420	77.643030
			12-23 MO.	113.095590	194.360310	113.277180
			2-65+ YR.	293.600160	487.211430	294.070560
LACTIC ACID NAS 0103	14 PROCSD VEGS(R)	*	0-5 MO.	.033450	.100380	.036120
			6-11 MO.	.573600	1.338400	.619200
			12-23 MO.	.932100	1.560670	1.006200
			2-65+ YR.	2.031500	3.422480	2.193000

LACTIC ACID

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Aykroyd-'39
Bib No. 57

Indian J. Med. Res. 27(2):409-412. 1939.

A FURTHER EXPERIMENT ON THE VALUE OF CALCIUM LACTATE FOR INDIAN CHILDREN.

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[Received for publication, November 28, 1938.]

It was observed in the Laboratories (Aykroyd and Krishnan, 1937) that the nutritive value of the poor South Indian diet for rats is increased by the addition of calcium lactate. This observation was followed up by an experiment on nursery school children aged 3 to 6 (Aykroyd and Krishnan, 1938) in which it was shown that young children given 0·5 g. of calcium lactate daily gained more rapidly in height and weight during a 5 to 6-month period than children not receiving this supplement. The present experiment, essentially similar in principle, was carried out to discover whether calcium lactate could produce the same effect on older children. Another object was to determine the relative value of calcium lactate and skimmed milk as supplements.

The investigation took place in a day school about three miles distant from the Laboratories attended by some 150 boys with ages ranging from 6 to 12, the average age being about 9. The boys were Badagas and natives of the Nilgiri Hills, consuming a diet based largely on rice and containing no milk and only small amounts of vegetables. Their state of nutrition was in general poor.

The school was divided into three groups, roughly equal as regards size and age by working down the school list, class by class, and assigning the boys in sequence to groups I, II, and III. The groups were given the following daily supplements :—

Group I :	(Control) ..	A peppermint sweet.
Group II :	.. ..	A peppermint sweet + 1 gramme of calcium lactate.
Group III :	.. ..	8 oz. (about 250 c.c.) of liquid skimmed milk, reconstituted from 30 g. of milk powder.

The peppermints were given for psychological reasons. Considerable experience has taught us that it is advisable, in carrying out school feeding experiments, to give all the children, including the control groups, some supplement. Otherwise the controls will feel neglected.

The experiment lasted 11 weeks, during which the supplements were given on 49 days. The children were weighed and measured at the beginning and end of the experimental period. Allowing for absentees, groups I, II, and III included respectively 42, 46, and 43 boys. Initial mean heights and weights in the three groups were approximately equal and analysis showed that the small differences in the initial means were not statistically significant.

The increments in weight and height in the three groups are compared in Tables I, II, and III, which include the necessary statistical constants.

TABLE I.

Groups I and II. Increments and statistical constants.

	INCREMENT OF WEIGHT, LB.		INCREMENT OF HEIGHT, INCHES.	
	GROUP I.	GROUP II.	GROUP I.	GROUP II.
	Control.	Calcium lactate.	Control.	Calcium lactate.
Number in sample	42	46	42	46
Mean of sample	−0·02	+0·80	+0·12	+0·63
Standard deviation of sample ..	0·8256	1·393	0·209	0·435
Difference between two means ..	0·82		0·21	
Standard error of difference ..	0·2417		0·0718	
Difference—				
Standard error of difference ..	3·39		2·92	
Significance	Significant.		Significant.	

TABLE II.

Groups I and III. Increments and statistical constants.

	INCREMENT OF WEIGHT, LB.		INCREMENT OF HEIGHT, INCHES.	
	GROUP I.	GROUP III.	GROUP I.	GROUP III.
	Control.	Skimmed milk.	Control.	Skimmed milk.
Number in sample	42	43	42	43
Mean of sample	−0·02	1·35	0·42	0·59
Standard deviation of sample ..	0·8256	1·415	0·209	0·279
Difference between two means ..	1·37		0·17	
Standard error of difference ..	0·25		0·0535	
Difference—				
Standard error of difference ..	5·48		3·18	
Significance	Significant.		Significant.	

TABLE III.

Groups II and III. Increments and statistical constants.

	INCREMENT OF WEIGHT, LB.		INCREMENT OF HEIGHT, INCHES.	
	GROUP II.	GROUP III.	GROUP II.	GROUP III.
	Calcium lactate.	Skimmed milk.	Calcium lactate.	Skimmed milk.
Number in sample	46	43	46	43
Mean of sample	0·80	1·35	0·63	0·59
Standard deviation of sample ..	1·593	1·415	0·435	0·279
Difference between two means ..	0·55		0·04	
Standard error of difference ..	0·298		0·077	
Difference—				
Standard error of difference ..	1·85		0·52	
Significance	Almost significant.		Not significant.	

Discussion.

The results show that weight and height increments in both the calcium l. and the skimmed milk groups were significantly greater than in the control g. While the control group did not increase in weight, the mean increases in g. II and III were 0·8 lb. and 1·35 lb. respectively. The difference between the ca lactate and skimmed milk groups as regards weight increase falls just sho statistical significance. There was no significant variation in respect of h. increments in these groups. It must be remarked, however, that the ge condition of the children receiving milk showed an improvement which wa evident in the calcium lactate groups—an improvement which we have obs again and again when poor Indian children are given extra milk. It ma concluded that, while calcium lactate is of benefit under the circumstances, i naturally a less valuable supplement than milk. Similar observations have b made on groups of rats (Aykroyd and Krishnan, 1937).

In the experiment described the period of extra feeding was somewhat sho owing to a combination of circumstances it was impossible to continue for a lo period. The experiment was, however, of value in confirming the results of earlier experiment in which a supplement of calcium lactate was found to hav beneficial effect on children in a nursery school. Half to one gramme of calc lactate could be provided to a child at a cost of about 1 anna per month.

Summary.

1. South Indian day school children given a supplement of 1 gramme calcium lactate for a period of about 11 weeks showed significantly greater hei and weight increments than children not receiving calcium.

2. In a similar group receiving skimmed milk height and weight increme were also significantly in excess of those recorded in the control group. Th skimmed milk group showed more improvement in general condition than th calcium lactate group. Weight increase in the former was somewhat larger tha in the latter but the difference was not quite significant.

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Archivo di farmacologia sperimentale 62:91-107, Sept. 1, '36

COMPARATIVE RESEARCH ON SEVERAL CALCIUM SALTS USED IN THERAPY

I. Distant minimum lethal dose of calcium chloride, calcium lactate, calcium gluconate, calcium pyruvate, administered intravenously (*)

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The introduction of calcium medication in therapy can be traced to a very distant date, and in fact, it preceded the pharmacological and physiological study of calcium.

Success has in part justified the empirical method. The materia medica of antiquity abounds in medicines based on calcium: principally used were the bones of the dead, shells, corals, all of which contain an abundant amount of calcium carbonate. These preparations, fallen out of use today, were prepared with the calcium salts that the pharmaceutical industry prepares in numerous forms, and which are tested on the most assorted diseases. Among the salts most generally adorted in therapy are calcium chloride, calcium lactate, calcium pyruvate and calcium gluconate, on which numerous clinical, but few pharmacological, experiments have been conducted with that scientific rigor and precision which are necessary in comparative research.

Therefore, I undertook a series of comparative experiments on these four calcium salts. First ofall, I believed it opportune to determine with an exact method the toxicity of each of them because, as we shall see, the less precise experiments led to ideas and opinions which did not correspond to the reality of the facts. The notes that can be collected from the literature concerning this question are very scarce and insufficient, the least lethal doses reported having been determined by means of experimental procedures which do not allow for the exact observation of the symptomatology and comparison of the respective toxicities.

The question is therefore worthy of a more precise study. Knowledge of toxicity is in fact as indispensible as that of the pharmacological and therapeutical effects, when one wants to offer a precise judgment on the value of several remedies which are similar for all the salts and have the same therapeutic indications. Advisable would be the use in practice of that medication which

(*)A preventive communication was made of this work. See Bulletin of the Italian Society of Biology, 1936, p.310.

will unite a low toxic effect with another activity.

I report succinctly the few notes which I was able to find on the argument in the vast literature available on calcium salts.

Delogu (1), in some experiments on the compared toxicity of calcium in animals, found that rabbits injected intravenously with a solution of calcium chloride would die, at a rate relative to the speed of the injection, upon the use of doses ranging from 0.26 g per kg (high speed of the injection =cc. 12.5 per kg and first minute) to 0.78 g per kg (low speed of injection =cc. 2.79 per kg and first minute). From the experiments of Delogu it is revealed that the average of the immediate minimum lethal doses is equal to 0.50 g per kg of body weight.

Rothlin (2), studying intravenous toxicity of the four calcium salts, among them chloride and gluconate, found that the average of the lethal doses in rabbits was 0.20 g per kg of the animal for chloride and 1.70 g for gluconate.

Liebermann (3), in a study on the intercedent relationships between the effect of calcium gluconate and the scillaren B on the cardiac activity and on the blood pressure, injected into the veins of dogs a solution of calcium gluconate at 10% with the speed of 4 cc per kg and first minute, and observed that, in the experimental conditions which he had preselected, the lethal dose was equal to 0.185 g ± 57 of calcium per kg of body weight.

Morton F., Mason and Harry Resnik (4) attempted to determine the toxicity of calcium chloride, calcium lactate, and calcium gluconate injected into the cisterna of dogs.

They found that doses of 0.25 mg of these salts, administered via this route, did not compromise the life of the animal; doses of 0.40 mg led, in 90% of the cases, to death in 5 or 6 seconds from respiratory paralysis. Of the salts examined, the least toxic, in respect to the immediate effects, turned out to be, according to these authors, calcium lactate.

Actually, from these notes no certain conclusion concerning toxicity can be drawn, since the experiments were conducted using methods so imprecise that they really do not allow for any reliable comparisons. The figures which I have reported all represent in fact an immediate minimum lethal dose which, perhaps, is the least suitable when one wishes to establish comparisons between the toxicity of several medicines.

According to Simon (5), in fact, when one wishes to determine the minimum lethal intravenous dose in the higher animal, it is necessary to distinguish between the immediate minimum lethal dose and the distant minimum lethal dose. By immediate minimum lethal dose is meant the smallest dose which, injected into the veins of the animals, immediately kills them. It was believed that regular curves could be obtained, from which the immediate minimum lethal dose could at once be calculated. In fact Simon (6) described particular curves for some pharmaceuticals, in which, starting from a minimal speed (very high lethal dose), increasing the speed a bit each successive time, an optimum speed could be reached, which yielded the maximum toxicity (the lowest point of

the curve). Starting from this value, with the increase of the speed, the lethal dose increased and the curve rose. That is to say, that according to the first experiment of Simon, for every medicine there existed a single speed which allowed for the certain determination of the immediate minimum lethal dose. Simon attributed the slight toxicity of the medicine with the minimal speed to the fact that the introduction of very small doses of the medicine at regular intervals gave the organism a chance to eliminate it; he interpreted the speed suitable for attaining minimal toxicity as that which allowed the medicine to become better anchored in the tissue cells and to exercise there the most complete influence: he explained the decreased toxicity which is obtained when the speed of the injection is increased with the fact that there was being introduced into the organism a quantity of the medicine greater than that which could in a unity of time reach the cells of the tissues to exercise there its toxic influence, so that a dose which actually was not able to act in its entirety appeared to be toxic. Later research of Simon (7) and other authors (8) has demonstrated, detailing a great deal in the establishing of the curves, that instead of obtaining that ideal curve described by Simon, there were obtained rather others, in which, relative to the speed of the injection, the minima are several and not only one, and one can obtain several minima with identical doses, but with various speeds. These curves assume an importance of the first order as regards the pharmacological study of the immediate effect of the medicine, and often succeed in giving us a broad view of the extremely complex mechanism through which the medicines injected into the veins cause death. If we wish to avail ourselves of them in order to compare the toxicity of various medicines, they cannot of course lead to a resolution of the problem. Therefore, these experiments, useful from a purely pharmacological point of view, are incapable of telling us the truth when we wish to arrive at the values of their comparative minimum lethal doses.

For this reason, Simon proposes, and adopted in his Institute, a method for water-soluble medicines of determining the distant toxicity via the intravenous injection in the higher animal, which consists of the intravenous injection into many animals, at a constant and of itself harmless speed, of increasing amounts of solution, always of the same concentration for any given medicine, and picking the smallest dose that kills the animal at a certain distance from the injection. This is then the distant minimum lethal dose. After administration of the medicine, the animals were kept under observation so that the symptomatology could be determined exactly. One then proceeded to the autopsy of those that died, and to the histological examination of the organs in order to determine, when possible, the cause of death. Following this method, many experiments have already been performed in the laboratory of Simon and a considerable amount of results has already been achieved.

Using this method, I determined the intravenously injected minimum lethal dose of calcium chloride, calcium lactate, calcium gluconate and calcium pyruvate.

EXPERIMENTS

Conducted on 62 healthy rabbits, all coming from the same litter. The animals were kept in the laboratory for some days before being subjected to the experiment. The solutions to be injected were prepared recently from salts whose purity was controlled. For every salt I used solutions of 0.5 N obtained by weighing the exact quantity of salt added to the solution in distilled water and in a tared balloon. For calcium gluconate, since the solubility of this substance at atmospheric temperature is 3%, the salt was mixed in cold water, then immediately distributed in a phial and stabilized with permanence in an autoclave for an hour and a half. The solutions thus obtained were injected into the marginal vein of the ear of rabbits at a speed of 0.5 cc per kg of body weight and first minute. This speed was of itself absolutely harmless. The injection finished, the animal was placed in the cage, fed, and placed under intensive observation, so that the symptomology could be watched until the animal died or had apparently recovered.

The results of the experiments are shown in tables I,II,III,IV.

CRITICAL EXAMINATION OF THE EXPERIMENTS

The examination of table I shows that the distant minimum lethal doses for the salts I studied are equal respectively to 0.0070 g per kg for calcium lactate, 0.0080 g for calcium chloride, 0.0130 g for calcium gluconate, and 0.0180 g for calcium pyruvate.

The results of direct observation, like those of the necroscopic and histological observations, are, for the animals injected with the calcium salts I studied, basically equal, since the differences consist only of the fact that some of the salts reveal a more evident influence than the others on certain functions and organs. Therefore, I will briefly summarize here the symptomatological picture presented by the animals treated with calcium lactate, calcium chloride, calcium gluconate and calcium pyruvate, clarifying the possible differences which were brought to my attention.

a) We begin by examining the effects of the non-lethal doses. These, especially if larger in comparison with the therapeutic doses, considerably modify the thermoregulation, the respiratory function, the circulatory function, the diuresis, and the diameter of the pupil of the eye.

The animals bear the injection well and show no signs of intolerance, and I never observed any phenomena of excitation; on the other hand, a depressive action, more evident as the doses increased, was always ascertainable.

During the injection and immediately after the injection, for a period varying from one and one half hours to two hours, there is always a miosis which reaches its maximum intensity one hour after the beginning of the experiment, and recedes little by little until the pupil resumes its normal diameter. I cannot

Table I

CALCIUM CHLORIDE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	Gr. eq. per Kg.	4. Esito dell'esperienza	5. Osservazioni
1	21 III 1936	1800	0,0025	a) sopravvive	A. Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	8 VII 1935	1200	0,0040	»	
3	21 III 1936	1950	0,0050	»	
4	11 V »	1220	0,0060	»	
5	19 VII 1935	2000	0,0060	»	
6	12 VII »	1840	0,0070	»	
7	16 VII »	1450	0,0075	»	
8	16 XI »	1500	0,0075	»	
9	8 I 1936	1850	0,0075	»	
10	20 XI »	1430	0,0080	b) muore dopo ore 21	
11	8 V »	1150	0,0080	» » 22	
12	25 VII 1935	1650	0,0080	» » 44	
13	24 VII »	1450	0,0085	» » 19	
14	23 VII »	1480	0,0090	» » 24	
15	29 VII »	1420	0,0090	» » 26	
16	21 X »	1600	0,0090	» » 7	
17	23 VII »	1700	0,0095	» » 32	
18	17 VII »	1500	0,0100	» » 23	
19	18 VII »	1600	0,0125	» » 6	

Distant minimum lethal dose = 0.0080 g per kg

Key:
1.= Number of experiment 2.-date 3.- weight of rabbit in g.
4.= outcome of experiment 5.= observations
a) survives
b) dies after...hours

A. No convulsive phenomena were ever noted in the experiments. In the animals that died, death occurred after cardiac arrest, followed immediately by respiratory arrest.

Table II

CALCIUM LACTATE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	Gr. eq. per Kg.	4. Esito dell'esperienza	5. Osservazioni
					A.
1	25 III 1936	1700	0,0025	a) sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	26 III »	1800	0,0050	»	
3	9 XI 1935	1250	0,0060	»	
4	14 I 1936	1180	0,0060	»	
5	10 XI 1935	1400	0,0060	»	
6	21 II 1936	1250	0,0065	»	
7	25 II »	1100	0,0065	»	
8	7 XI 1935	1330	0,0070	b) morte dopo ore 19	
9	27 II »	1470	0,0070	» » 49	
10	30 VII »	1700	0,0080	» » 2.30	
11	30 VII »	1600	0,0080	» » 2.40	
12	31 VII »	1900	0,0080	» » l'iniez.	
13	7 XI »	1430	0,0080	» » »	
14	29 VII »	1600	0,0090	» » 7	
15	29 VII »	1530	0,0095	» » 4.30	
16	20 VII »	1670	0,0100	» » 8	

Distant minimum lethal dose - 0.0070 g per kg

For key see table I.

Table III

CALCIUM GLUCONATE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	4. Gr. eq. per Kg.	Esito dell'esperienza	5. Osservazioni
					A.
1	25 III 1936	1350	0,0025	a) sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	25 III »	1560	0,0050	»	
3	5 II »	1450	0,0050	»	
4	11 XII 1935	1400	0,0080	»	
5	2 I 1936	1750	0,0100	»	
6	6 V »	1120	0,0100	»	
7	3 I »	1400	0,0110	»	
8	4 I »	1600	0,0125	»	
				b)	
9	3 II »	1420	0,0130	muore dopo ore 10	
10	29 IV »	1050	0,0130	» » 24	
11	30 IV »	1150	0,0130	» » 24	
12	21 I »	1550	0,0135	» » 5	
13	17 I »	1900	0,0135	» » l'istiez.	
14	16 I »	1640	0,0150	muore dopo ore 15	

Distant minimum lethal dose = 0.0130 g per kg

For key see table I.

CALCIUM PYRUVATE

Solution 0.5 N

1. Numero dell'esperienza	2. Data	3. Peso del coniglio in gr.	Gr. eq. per Kg.	4. Esito dell'esperienza	5. Osservazioni
1	27 III 1936	1750	0,0025	a) sopravvive	A. Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	27 III »	1700	0,0050	»	
3	21 XII 1935	1300	0,0080	»	
4	24 XII »	1620	0,0090	»	
5	27 XII »	1400	0,0100	»	
6	28 IV 1936	1150	0,0100	»	
7	8 I »	1800	0,0125	»	
8	11 I »	1570	0,0150	»	
9	14 I »	1500	0,0175	»	
10	28 I »	2000	0,0180	b) muore dopo ore 82	
11	14 IV »	1350	0,0180	» » 15	
12	23 I »	1900	0,0190	» » 48	
13	13 I »	1800	0,0200	» » 19	

Distant minimum lethal dose = 0.0180 g per kg

For key see table I.

whether the miosis is succeeded by a mydriasis, since the observations were made under varying conditions of light.

All the doses of the salts with which I experimented initially produce a drop in the temperature, which is greater in proportion with the increase of the dose; in the case of equimolecular doses, of the four salts, calcium gluconate and calcium chloride were shown to be most active from this point of view. As a rule, hypothermia is followed by hyperthermia; upon administration of doses approaching the lethal, such hyperthermia is no longer demonstrable.

The respiratory function is modified by all the salts in the sense that, as a rule, the rate of respiration decreases, sometimes notably, sometimes to a minor degree, but always evidently. Only in the case of the smallest doses -- 0.0025 g and 0.0050 g per kg -- was it impossible to demonstrate a decrease of this rate for calcium gluconate and calcium pyruvate. Bradipnea was generally followed by polypnea. When the injected dose is sufficiently high, breathing becomes particularly difficult: the animal puts its auxiliary muscles into play, and very loud rattling and hissing can be heard.

As regards the pulse, all the salts produce modifications in its rate and sometimes in its rhythm; as a rule, bradycardia develops, followed in general by a tachycardia. In this regard as well, calcium gluconate and calcium pyruvate at doses of 0.0025 and 0.0050 g were shown to have an indifferent effect on the circulatory function. I always noted a certain parallelism between the modifications of the heart and the respiration and those of the pupil: that is, they all reach their maximum intensity about one hour after the injection.

The diuresis is also considerably modified, but not in the same manner, by the various salts examined. While calcium chloride, calcium lactate and clacium gluconate produce a decrease (more evident as the doses are increased) of the volume of urine emitted in the 24 hours following the injection, calcium pyruvate, also administered in doses approaching the lethal, always allows an abundant elimination of urine. The larger doses often provoke albuminuria, most significant when calcium lactate and calcium chloride are administered, but always temporary. Glycosuria was never observed.

The animals recover quickly, so that on the day following the injection, no particular symptomatology is found upon direct examination.

b) The findings made on the animals treated with lethal doses of the four calcium salts are the following: the animals never show signs of intolerance, nor do any phenomena of motor excitement appear. The modifications of the pupil, those in the respiratory apparatus, the thermoregulatory modifications, and those of the diuresis, are qualitatively equal to those that can be observed when high doses of calcium are injected.

The drop in temperature, always notable, is particularly remarkable when calcium gluconate is injected.

The diuresis is more strongly inhibited by calcium chloride and calcium lactate; with this latter salt, complete anuria can sometimes be verified. Albuminuria, most significantly induced by calcium lactate, always accompanied the administration of

lethal doses of the salts I studied.

The general depressive action exercised by the lethal doses of the studied salts is remarkable. Freed from their cages, the animals struggle to resume their normal prone position and behave as though in a state of torpor. If they are stimulated until they are forced to move, their walk is troubled and uncertain. The musculature appears hypotonic. Placed in their cages, the animals constantly refuse the food which is offered them, and seem to be indifferent to their environment. With the advancement of the effect of the poison, the muscular hypotonia becomes more manifest: most intensely hypotonic are the flexor muscles in the limbs, so that the animals assumes a characteristic position -- the head droops inertly from its own weight, and the paws are stretched out on the floor on which the animal lies. The reflexes, though a little delayed, are present, and sensibility to pain is retained.

A progressive weakening of all the vital functions leads to the death of the animal, occurring calmly. In general, the heart stops first, while some respiratory movement may still persist.

With these lethal doses, however, we can notice a difference between the animals injected with calcium chloride, calcium lactate, and calcium pyruvate on the one hand, and those treated with calcium gluconate on the other. In the case of the former, the modifications in the thermoregulation, the pulse and the respiration, after having touched upon a miximum of intensity, generally recede, so that it seems that the animals are turning toward a recovery of their health; the rate of respiration and pulse increase again, until they reach and sometimes surpass the physiological value; the temperature, after having reached a maximum of descent, begins once again to rise, though remaining much below the normal value. In other words, it seems that the animal's powers of defenses are succeeding in prevailing over the violence of the poison. After a period varying from 38 to 72 hours, there occurs once again a weakening of all the vital functions. The breath becomes superficial, the pulse weak, and infrequent; the temperature descends progressively. The animal turns thus toward death, which arrives calmly.

However, in the rabbits that were injected with calcium gluconate, this short-lived resumption of the vital functions could not be observed. Calcium gluconate either leads rapidly and inexorably to death, or, whenever the animal succeeds in reacting against the violence of the poison, the return to normality proceeds to complete recovery of health.

<u>Autopsy and histological examination.</u> -- The autopsy, undertaken immediately after the death of the animals, revealed: strong congestion of the thoracic and abdominal organs; distended blood vessels full of liquid blood: subpleural and subperitoneal hemorraghic suffusions. Frequently, in the animals that died a certain distance from the injection, subperitoneal hematomies occupying the space located between the rectum and the bladder could be detected.

I was unable to find any differences in the effects of the four calcium salts.

c) Histological examination.-- The patches were withdrawn immediately after the death of the animal and treated with opportunely selected methods. Cuttings were executed with the congealing microtome, coloration with hematoxylin eosin, hematoxylin and Sudan III for the examination of fats. For the common preparations, the anchorage was done using the method of Zenker, the impaction in paraffin, the coloration in hematoxylin-eosin; for the connective tissues I used the coloration method of Van Gieson and the trichromic method of Mallori; for the examination of the fibrin I followed the method of Weigert; the adrenals were fixed in potassium bichromate, impacted in paraffin and colored with the method of Wiesel; the brain was fixed in alcohol, impacted in paraffin and colored with hematoxylin-eosin.

The results of the examinations are described below.

Encephalus. -- Cuttings in the bulb and cerebellum: vascular congestion; nothing of note in the parenchymal tissue.

Thymus. -- Strongly congested both in the connective vessels between the lobules and in the capillaries that penetrate the parenchyma; no hemorrhages.

Thyroid. -- Of normal aspect, also as regards vascularization.

Adrenal glands. -- Are revealed to be of normal aspect, both in the cortical and medullary sections. The various layers of the cortex are well-defined and very rich in lipoids. There is evident, as in the other organs, vascular congestion. Nothing of note in the central vein.

Heart. -- Nothing of note concerning the endocardial membrane and the epicardial serum. The aspect of the cardiac muscle is shown to be just about the same in all the levels, and shows very strong congestion of the blood vessels, which have nearly uniformly dilated walls and the membrane full of fresh, well-conserved blood. Therefore, one has the impression of a dilation of both the major and minor vessels and of the capillaries. Regressive phenomena in the cardiac muscle cell are absent.

Lung. -- Extensive vascular congestion both in the large blood vessels and in the alveolar capillaries. Nothing abnormal contained in the alveoles and in the interior of the bronchi. In the preparations prepared with the common methods of coloration, the wall of the arterial vessels appears to be slightly . This can be referred to the hypertrophy of the tunica muscularis, the colorations of Van Gieson and Mallori having demonstrated the absence of phenomena of true vasal sclerosis.

Liver. -- Imposing vasal congestion both of the large and fine capillary vessels. The epithelial elements of the organ demonstrate no findings worthy of note. The coloration with the method of Weigert for the demonstration of the fibrin yielded a negative outcome.

Kidneys. -- Vascular congestion is evident in this organ as well. With common coloration degenrative processes in the parenchymal elements can sometimes be perceived; in the case of coloration by means of Sudan III, however, no drops of fat were ever observed in the epithelial elements, for which the regressive phenomena, taken into consideration the normal colorability of the nucleus as well, can be limited to the degeneration of albumin.

Spleen. -- Congested pulp: the follicles reveal normal aspect and disposition, with slight variations in the different animals.

Intestine. -- Vascular congestion and dilation.

Preparing the preparations of the zones infiltrated with blood (see macroscopic examination) in a manner so as to involve the various organs surrounded by the same hemorraghing, it was ascertained that the vessels of the connective retro-peritoneal. area, like those of the area corresponding to the retro-bladder space are intensely dilated and full of blood; in there walls can be noted long lesions from which the blood overflows in a considerable amount, showing itself in some points to be well-conserved, in others more or less altered and in the process of coagulating. On the other hand, nothing of note was remarked in the bladder wall, in that of the rectum, and in general in the contexture of the organs surrounded by blood.

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The results of the experiments must be considered in relation with the distant minimum lethal dose and the cause of death.

From the tables presented above, we see that the scale of toxicity for the four substances is as follows:

Salt	Distant minimum lethal dose in g per kg
Calcium lactate	0.0070
" chloride	0.0080
" gluconate	0.0130
" pyruvate	0.0180

These results confirm those of Rothlin (2) only in that they also demonstrate a greater toxicity for calcium chloride as compared to calcium gluconate, but they also demonstrate that calcium gluconate is much more toxic than was shown in the experiments of that author.

Besides this, they demonstrate that calcium lactate is the most toxic of the four salts studied, and that it is followed, in order, by calcium chloride, calcium gluconate and calcium pyruvate, which last is without doubt the least dangerous. Assuming that =1 is the distant minimum lethal dose of calcium lactate, the other distant minimum lethal doses assume the values shown below:

Calcium lactate	=1
" chloride	=1.14
" gluconate	=1.85
" pyruvate	=2.57

As regards the toxicity of calcium gluconate compared to that of calcium chloride, my experiments, though generally confirming Rothlin's affirmation that calcium gluconate is less toxic than calcium chloride, also show that it is much more toxic than this author finds. In fact, from my experiments, taking =1 as the distant minimum lethal dose of calcium chloride, the corresponding dose of calcium gluconate turns out to be =1.62: Rothlin, on the other hand, finds in his research, making =1 the minimum lethal dose of calcium chloride, that that of calcium gluconate rises to 4.25. Thus, while I show that the distant minimum lethal dose of calcium gluconate is barely one and one half times that of calcium chloride, he finds that it is more than four times greater, that is, that calcium gluconate is more than four times less toxic than calcium chloride.

The discrepancy is immediately explained when one observes that in his experiments Rothlin used different speeds of injection for the two salts, while it is necessary that the speed be absolutely constant.

As concerns calcium lactate, Morton F., Mason and Harry Resnik (4) have observed that this salt, injected into the spine of dogs, is less toxic than calcium chloride or calcium gluconate. This contradiction with what I found cannot be explained easily, as the authors do not explain the considerations on which their assertions are based, limiting themselves to saying that the suspension of lactate resulted, in respect to the immediate effects, in being less toxic than the solutions of calcium chloride and calcium gluconate with an equal metal content. On the other hand, the authors also do not specify the exact quantity of the various salts which is necessary to provoke death.

If we now examine the mechanism through which the calcium salts introduced into the veins induce death, we see that the literature can illuminate very little for us on this point, since the authors are anything but concordant in this regard.

Dastre (9) observed that in the case of intravenous injections of calcium chloride, the animals die as a result of extensive intravasal congestions and therefore attributes to the salt a particular toxic effect which was negated by all the authors who later worked on the same question. They never observed the finding described by this author.

Delogu (1), injecting animals with calcium chloride intravenously, observed that death occurred with convulsive actions when the speed of the injection was increased, while a clear depressive effect leading to the death of the animals was constantly apparent.

Rothlin (2) was able to note that during the injection of calcium salts, the respiration at first accelerated and became superficial, then becoming slower and deeper. The animal is restless: violent clonicotonic cramps with opisthotonus follow, as well as cessation of breathing and esophthalmus. If the dose is not lethal, the animals recover quickly; if the dose is lethal, then they die upon the first attack of cramps or soon after. As

a rule, a quick death occurs during a convulsive attack. I believe that in this case death can be attributed above all to the excessive speed of the injection, which agrees with the observation of Delogu.

Liebermann (3), injecting calcium gluconate into the veins of dogs of whose carotid pressure he had made a manometric tracing, was able to observe that death arrived gradually with a progressive abatement of the pressure. In another work on the modifications in the blood pressure in dogs injected with equimolecular doses of calcium lactate and calcium gluconate, the same author able to observe sometimes the unforeseen death of the animal, which he interpreted as due to premortal intravasal coagulation.

Morton F., Mason and Harry Resnik (4), introducing calcium lactate, calcium chloride and calcium gluconate into the cisterna of dogs, maintained that death occurred from respiratory paralysis.

From the notes that I have reported it can be seen that the greatest uncertainty exists when it must be decided through what mechanism the calcium introduced into the veins of the animals produces death.

From the experiments which I conducted systematically on 62 animals we can exclude the possibility that calcium, injected slowly into the veins, cause convulsions: these phenomena, as Delogu showed, can be attributed to the speed of the injection.

Intravascular coagulation also can be excluded as a cause of death. The autopsy performed immediately after the death of the animal always revealed liquid blood in the vessels, and the examination of the fibrin was always negative.

The histological examination of the preparations of the organs of the animals that died following the injection yields no possible explanation of the mechanism of death. In fact, the alterations consist essentially of an enormous vascular congestion, sometimes with the rupture of small vessels of some areas.

Interesting on the other hand is the narrow analogy, the near identity of the symptoms I found upon injecting high doses of calcium at a slow speed and those observed by various authors who brought calcium directly into contact with the nervous elements in various ways.

Sabbatani (10), in his classic research on calcium, shows that this cation describes a moderating effect on the tissues. Upon the local application of a calcium salt on the cortex, the medulla, the nerves and the muscles, he always observed phenomena of depression, while, on the other hand, the application of a decalcifying reagent, tricodium citrate, always produced phenomena of excitation, which he interpreted as due to the fact that said substance forms complex ions with calcium and therefore reduces the calcium in the tissue to the state of an ion, chemically and biologically active.

Roncoroni (11) confirmed the results in his Institute: the application of salts precipitating calcium onto the cerebral cortex provoked motor action such as myoclonia and epilepsy and Ferrari (12), in his turn, observed the same depressive phenomena by injecting calcium into the carotid.

By means of the direct application of calcium to the injured-

bulum of female cats, Demole noted that the animals fell into a more or less profound sleep (13).

L. Stern and J. Chvoles (14) introducing a solution of calcium gluconate into the cerebral ventricles of dogs, found that such an injection produced in general a temporary excitation, which was followed in a few minutes by a complete loss of the muscular tonus which terminated after fifteen minutes in a profound narcosis with loss of reflexes. This state persisted for some hours, after which the animal recovered little by little. Very often however, the animal declined and ended by dying. The blood pressure would begin to drop after a few minutes and around the fifteenth to twentieth minute it was usually very low. The pulse would become slower and weaker, the breath slower and superficial. Equal doses injected into the circulus had no effect. As a consequence of these results, the authors conclude that calcium has a direct effect on the centers situated in the ventricle walls.

Bun-ichi Hasama (15), injecting 2 mmg of calcium chloride into the vicinity of the cineritious tubercle of the female cat, observed that the animals passed into a state of somnolence; the pulse and the respirationdiminished in rate; a decrease in temperature was also produced.

The results of these experiments are truly interesting because they demonstrate that the application of small amounts of calcium to encephalic centers is characterized by a symptomatology which recalls that which I observed upon the injection of high doses of calcium salts at low speed via the intravenous route.

In summary, the results of my experiments lead me to exclude the possibility that calcium salts administered intravenously at a slow speed of injection produce death through intravascular coagulation, and that the death occurs by means of convulsions. A rapid drop of the temperature, a slowing down and progressive weakening of the pulse and the respiration, an intense vascular congestion, form the symptomatological picture presented by the animals intoxicated with calcium; a symptomatological picture that can be summarized with the comprehensive term of collapse. This collapse, on the basis of the negative results of the anatomo-histological examination, can only be attributed to an intense, depressing effect of calcium on the nervous centers.

CONCLUSIONS

1 -- The distant minimum lethal intravenous dose in the rabbit is, according to Simon: 0.0070 g per kg of body weight for calcium lactate; 0.0080 g per kg for calcium chloride; 0.0130 g per kg for calcium gluconate; 0.0189 g per kg for calcium pyruvate.

2 -- These experiments, besides providing us with data which lend themselves to precise comparisons, are of value to the therapist in that they rectify opinions which were not based on sure experiments. And while up to now it has been maintained that

calcium gluconate is more than four times less toxic than calcium chloride, my research shows that the difference in toxicity is much less, little more than one and a half times. Thus it can be easily understood how the abuse of this salt in therapy, founded upon its presumed slight toxicity, was able often to produce unexpected toxic phenomena.

The disturbances observed upon the administration of calcium lactate, which is the most toxic of the four salts, can also be understood now.

3 -- The cause of death in the case of all four of the salts studied is always a depressive action of the calcium cation on the nervous centers; without doubt, this effect is opposed by the anion to a degree which the actual experiments cannot establish.

SUMMARY

The distant minimum lethal intravenous dose in the rabbit is, according to Simon: 0.0070 g per kg of body weight for calcium lactate; 0.0080 g per kg for calcium chloride; 0.0130 g per kg for calcium gluconate; 0.0180 g per kg for calcium pyruvate. The toxicity of calcium gluconate is shown by these experiments to be much greater than what was hitherto believed, and thus explains the toxic phenomena observed in the therapeutic use of this salt. The cause of death can be attributed to the depressive effect of the calcium cation on the nervous centers.

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Baldacci-'36
Bib No. 73

Arch. Farmacol. Sper. 62:91-107. 1936.

RICERCHE COMPARATIVE FRA ALCUNI SALI DI CALCIO USATI IN TERAPIA

I. - Dose minima letale lontana per via endovenosa del cloruro, lattato, gluconato, gluconato, piruvato di calcio. (*)

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L'introduzione della medicazione calcica in terapia risale a data molto lontana ed ha preceduto nel tempo lo studio farmacologico e fisiologico del calcio.

Il successo ha in parte giustificato il modo di procedere empirico. L'antica Materia medica è ricca di medicamenti a base di calcio: erano usati principalmente gli ossi delle seppie, le conchiglie, i coralli, i quali contengono in quantità abbondante il carbonato di calcio. Queste preparazioni, oggi cadute in disuso, sono state sostituite con i sali di calcio che l'industria farmaceutica prepara in forme numerose, cimentate largamente nelle più svariate malattie. Tra i sali più comunemente adoperati in terapia sono il cloruro, il lattato, il piruvato ed il gluconato di calcio, sui quali si hanno numerosissime esperienze cliniche, ma poche esperienze farmacologiche condotte con quel rigore scientifico e con quella precisione che è necessaria in ricerche comparative.

Ho perciò intrapreso appunto una serie di ricerche comparative sopra questi quattro sali di calcio e prima di tutto ho creduto opportuno determinare con un metodo esatto la tossicità di ciascuno di essi perchè, come vedremo, le esperienze poco precise hanno condotto a concetti ed opinioni non rispondenti alla realtà dei fatti. Le notizie che si possono raccogliere dalla letteratura sull'argomento sono infatti scarse ed insufficienti, le poche dosi letali che vengono riferite essendo state determi-

(*) Di questo lavoro fu fatta una comunicazione preventiva. V. Bollettino Soc. it. di Biologia. 1936. ovv. 310.

nate con procedimenti sperimentali che non ci permettono di cogliere esattamente la sintomatologia e di fare un confronto tra le rispettive tossicità.

La questione perciò merita uno studio più preciso. La conoscenza della tossicità è infatti altrettanto indispensabile quanto quella dell'attività farmacologica e terapeutica quando si voglia dare un giudizio preciso sul valore di alcuni rimedi tra loro simili ed aventi le stesse indicazioni terapeutiche. Consigliabile sarà nella pratica l'uso di quel medicamento che unirà ad un'alta attività una bassa azione tossica.

Riporto succintamente le poche notizie che ho potuto trovare in argomento sfogliando l'enorme letteratura sui sali di calcio.

Delogu (1), in alcune ricerche sulla tossicità comparata del calcio nella serie animale, trovò che i conigli iniettati endovena con soluzione di cloruro di calcio morivano, a seconda della velocità dell'iniezione usata, con dosi che da gr. 0,26 per Kg. (velocità di iniezione alta = cc. 12,5 per Kg. e per minuto primo) arrivano fino a gr. 0,78 per Kg. (velocità di iniezione bassa = cc. 2,79 per Kg. e minuto primo). Dalle esperienze di Delogu risulta che la media delle dosi minime letali immediate è uguale a gr. 0,50 per Kg. di peso corporeo.

Rothlin (2) studiando la tossicità per via endovenosa di quattro sali di calcio tra i quali il cloruro e gluconato, trovò che la media delle dosi letali nei conigli era di gr. 0,20 per Kg. di animale per il cloruro e di gr. 1,70 per il gluconato.

Liebermann (3) in uno studio sui rapporti intercedenti tra l'azione del gluconato di calcio e dello scillaren B sull'attività cardiaca e sulla pressione sanguigna, iniettando nelle vene dei cani una soluzione di gluconato di calcio al 10 % con la velocità di cc. 4 per Kg. e minuto primo osservò che, nelle condizioni sperimentali da lui prescelte, la dose letale era uguale a gr. 0,185 $\pm$ 57 di calcio per Kg. di peso corporeo.

Morton F., Mason e Harry Resnik (4) hanno tentato di determinare la tossicità del cloruro, lattato, gluconato di calcio iniettati nella cisterna dei cani.

Essi trovarono che dosi di mg. 0,25 di questi sali, somministrati per questa via, non compromettono la vita dell'animale; dosi di mg. 0,40 portano nel 90% dei casi l'animale a morte in 5 o 6 secondi per paralisi respiratoria. Tra i sali presi in esame il meno tossico rispetto agli effetti immediati risultò essere, a detta degli autori, il lattato di calcio.

Da queste notizie non si può invero trarre alcuna conclusione sicura sulla tossicità poichè gli esperimenti furono eseguiti con tecniche così poco precise che non permettono affatto confronti attendibili. Le cifre che ho riportato infatti rappresentano tutte una dose minima letale immediata la quale forse è la meno adatta quando si vogliano istituire confronti tra la tossicità di alcuni farmaci.

Secondo Simon (5), infatti, quando si vuole determinare nell'animale superiore la dose minima letale per via endovenosa, bisogna distinguere tra *dose minima letale immediata* e dose *minima letale lontana*. Per dose

minima letale immediata s'intende la dose più piccola che iniettata nelle vene degli animali li uccide subito. Si era creduto di poter ottenere delle curve regolari dalle quali si potesse senz'altro calcolare la dose minima letale immediata. Infatti SIMON (6) aveva descritto per alcuni farmaci delle curve particolari, nelle quali partendo da velocità minime (dose letale altissima), un po' per volta, aumentando la velocità, si raggiungeva una velocità *optimum* che dava la massima tossicità (punto più basso della curva). A partire da questo valore, col crescere della velocità, la dose letale cresceva e la curva saliva. Vale a dire, secondo queste prime ricerche di SIMON, per ogni farmaco esisteva una sola velocità la quale permetteva di fissare la dose minima letale immediata in modo sicuro. SIMON attribuiva la scarsa tossicità del farmaco colle velocità minime al fatto che l'introduzione di dosi molto piccole del farmaco nell'unità di tempo dava modo all'organismo di eliminarlo; interpretava la velocità atta a raggiungere la tossicità minima come quella che permetteva al farmaco di fissarsi meglio nelle cellule dei tessuti e di esercitarvi la più completa attività: spiegava la diminuita tossicità che si otteneva coll'aumento della velocità d'iniezione col fatto che s'introduceva nell'organismo una quantità di farmaco superiore a quella che poteva nella unità di tempo raggiungere le cellule dei tessuti per esercitarvi la sua azione tossica, sicchè figurava tossica una dose che realmente non aveva potuto agire tutta intera. Ulteriori ricerche di SIMON (7) stesso e di altri AA. (8) hanno dimostrato che, dettagliando molto nello stabilire dette curve, invece di ottenere quella curva ideale descritta da SIMON se ne ottengono invece altre nelle quali, a seconda della velocità d'iniezione i minimi sono parecchi e non uno solo, e si possono avere anzi parecchi minimi con dosi identiche, ma con velocità diverse. Queste curve assumono un'importanza di primo ordine per quello che riguarda lo studio farmacologico dell'azione immediata dei farmaci e riescono spesso a darci una visione larga del meccanismo estremamente complesso col quale i farmaci iniettati nelle vene dànno la morte. Se vogliamo giovarci di esse per paragonare tra loro le tossicità di vari farmaci non possono certo portarci alla risoluzione del problema. Quindi queste ricerche, utilissime dal punto di vista farmacologico puro, sono incapaci di dirci la verità quando vogliamo giungere in possesso di valori di dosi minime letali paragonabili fra loro.

Per questo SIMON per i farmaci solubili in acqua ha proposto ed adottato nel suo Istituto il metodo della determinazione della tossicità lontana per via endovenosa nell'animale superiore, il quale consiste nell'iniettare in molti animali, per via endovenosa, con velocità costante e per sè stessa innocua, volumi crescenti di soluzioni sempre della stessa concentrazione di un dato farmaco, cogliendo la più piccola dose che uccide un animale ad un certa distanza dall'iniezione. Codesta è appunto la dose minima letale lontana. Gli animali dopo la somministrazione del

farmaco vegono tenuti in osservazione in modo da poter rilevare esattamente la sintomatologia. Si procede poi all'autopsia di quelli venuti a morte ed all'esame istologico degli organi per determinare, quando è possibile, la causa della morte. Con questo metodo molte ricerche furono già eseguite nel laboratorio di SIMON ed una cospicua messe di risultati fu già raccolta.

Io con tale metodo ho determinato la dose minima letale per via endovenosa del cloruro, del lattato, del gluconato, del piruvato di calcio.

ESPERIENZE

Furono eseguite su 62 conigli sani, provenienti tutti dal medesimo allevamento. Gli animali erano tenuti qualche giorno nel laboratorio prima di essere sottoposti all'esperimento. Le soluzioni da iniettare erano preparate di recente da sali di cui fu controllata la purezza. Per ogni sale ho usato soluzioni 0,5 N ottenute pesando l'esatta quantità di sale che veniva portato in soluzione in acqua distillata ed a volume in pallone tarato. Per il gluconato di calcio, poichè la solubilità di questo prodotto a temperatura ambiente è del 3 %, il sale veniva sciolto in acqua calda, distribuito poi immediatamente in fiale e stabilizzato con la permanenza in autoclave per un'ora e mezza. Le soluzioni così ottenute erano iniettate nella vena marginale dell'orecchio del coniglio con la velocità di cc. 0,5 per Kg. di peso corporeo e minuto primo. Tale velocità è risultata essere per sè stessa assolutamente innocua. Terminata l'iniezione, l'animale veniva messo in gabbia, gli veniva somministrato il cibo e si metteva sotto attenta osservazione in modo da cogliere la sintomatologia fino a quando veniva a morte o si era apparentemente del tutto ristabilito.

I risultati delle esperienze sono raccolti nelle Tabelle I, II, III, IV.

ESAME CRITICO DELLE RICERCHE

L'esame delle Tabelle dimostra che le dosi minime letali lontane per i quattro sali da me studiati sono rispettivamente uguali a gr. eq. per Kg. 0,0070 per il lattato di calcio, gr. eq. 0,0080 per il cloruro, gr. eq. 0,0130 per il gluconato, gr. eq. 0,0180 per il piruvato di calcio.

I risultati dell'osservazione diretta, come quelli dell'esame necroscopico ed istologico sono, per gli animali iniettati con i sali di calcio da me studiati, fondamentalmente uguali poichè le differenze consistono solamente nel fatto che alcuni dei sali esplicano un'azione più evidente degli altri su determinate funzioni ed organi. Riassumo perciò qui brevemente il quadro sintomatologico presentato dagli animali trattati con lattato, cloruro, gluconato, piruvato di calcio, mettendo in luce le eventuali differenze che sono apparse alla mia attenzione

a) Incominciamo ad esaminare gli effetti delle dosi non letali. Queste, specialmente se elevate rispetto alle terapeutiche, modificano in maniera

TABELLA I

CLORURO DI CALCIO

Soluzione 0,5 N.

Numero dell'esperienza	Data	Peso del coniglio in gr.	Gr. eq. per Kg.	Esito dell'esperienza	Osservazioni
1	21 III 1936	1800	0,0025	sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	8 VII 1935	1200	0,0040	»	
3	21 III 1936	1950	0,0050	»	
4	11 V »	1220	0,0060	»	
5	19 VII 1935	2000	0,0060	»	
6	12 VII »	1840	0,0070	»	
7	16 VII »	1450	0,0075	»	
8	16 XI »	1500	0,0075	»	
9	8 I 1936	1850	0,0075	»	
10	20 XI »	1430	0,0080	muore dopo ore 21	
11	8 V »	1150	0,0080	» » 22	
12	25 VII 1935	1650	0,0080	» » 44	
13	24 VII »	1450	0,0085	» » 19	
14	23 VII »	1480	0,0090	» » 24	
15	29 VII »	1420	0,0090	» » 26	
16	21 X »	1600	0,0090	» » 7	
17	23 VII »	1700	0,0095	» » 32	
18	17 VII »	1500	0,0100	» » 23	
19	18 VII »	1600	0,0125	» » 6	

Dose minima letale lontana = gr. eq. 0,0080 per Kg.

TABELLA II

LATTATO DI CALCIO

Soluzione 0,5 N.

Numero dell'esperienza	Data	Peso del coniglio in gr.	Gr. eq. per Kg.	Esito dell'esperienza	Osservazioni
1	25 III 1936	1700	0,0025	sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	26 III »	1800	0,0050	»	
3	9 XI 1935	1250	0,0060	»	
4	14 I 1936	1180	0,0060	»	
5	10 XI 1935	1400	0,0060	»	
6	21 II 1936	1250	0,0065	»	
7	25 II »	1100	0,0065	»	
8	7 XI 1935	1330	0,0070	morte dopo ore 18	
9	27 II »	1470	0,0070	» » 49	
10	30 VII »	1700	0,0080	» » 2.30	
11	30 VII »	1600	0,0080	» » 2.40	
12	31 VII »	1900	0,0080	» » l'iniez.	
13	7 XI »	1430	0,0080	» » »	
14	29 VII »	1600	0,0090	» » 7	
15	29 VII »	1530	0,0095	» » 4.30	
16	20 VII »	1670	0,0100	» » 8	

Dose minima letale lontana = gr. eq. 0,0070 per Kg.

TABELLA III

GLUCONATO DI CALCIO

Soluzione 0,5 N.

Numero dell'esperienza	Data	Peso del coniglio in gr.	Gr. eq. per Kg.	Esito dell'esperienza	Osservazioni
1	25 III 1936	1350	0,0025	sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	25 III »	1560	0,0050	»	
3	5 II »	1450	0,0050	»	
4	11 XII 1935	1400	0,0080	»	
5	2 I 1936	1750	0,0100	»	
6	6 V »	1120	0,0100	»	
7	3 I »	1400	0,0110	»	
8	4 I »	1600	0,0125	»	
9	3 II »	1420	0,0130	muore dopo ore 10	
10	29 IV »	1050	0,0130	» » 24	
11	30 IV »	1150	0,0130	» » 24	
12	21 I »	1550	0,0135	» » 5	
13	17 I »	1900	0,0135	» » l'iniez.	
14	16 I »	1640	0,0150	muore dopo ore 15	

Dose minima letale lontana gr. eq. 0,0130 per Kg.

TABELLA IV

PIRUVATO DI CALCIO

Soluzione 0,5 N.

Numero dell'esperienza	Data	Peso del coniglio in gr.	Gr. eq. per Kg.	Esito dell'esperienza	Osservazioni
1	27 III 1936	1750	0,0025	sopravvive	Mai nelle esperienze si notarono fenomeni convulsivi. Negli animali venuti a morte questa intervenne per arresto del cuore, seguito subito dopo da arresto del respiro.
2	27 III »	1700	0,0050	»	
3	21 XII 1935	1300	0,0080	»	
4	24 XII »	1620	0,0090	»	
5	27 XII »	1400	0,0100	»	
6	28 IV 1936	1150	0,0100	»	
7	8 I »	1800	0,0125	»	
8	11 I »	1570	0,0150	»	
9	14 I »	1500	0,0175	»	
10	28 I »	2000	0,0180	muore dopo ore 82	
11	14 IV »	1350	0,0180	» » 15	
12	23 I »	1900	0,0190	» » 48	
13	13 I »	1800	0,0200	» » 19	

Dose minima letale lontana = gr. eq. 0,0180 per Kg.

sensibile la termoregolazione, la funzione respiratoria, quella circolatoria, la diuresi, il diametro pupillare.

Gli animali sopportano bene l'iniezione e non dànno segni di intolleranza e mai mi è capitato di osservare fenomeni di eccitazione; constantemente rilevabile fu invece un'azione depressiva, più evidente con le dosi più alte.

Durante l'iniezione e subito dopo l'iniezione per un tempo che varia da un'ora e mezza a due ore si ha sempre una miosi che raggiunge il massimo dell'intensità un'ora dopo l'inizio dell'esperimento e regredisce a poco a poco fino a che la pupilla riprende il diametro normale. Non posso affermare se alla miosi succeda una midriasi, l'osservazione essendo stata fatta in diverse condizioni di luce.

Tutte le dosi dei sali da me esperimentate producono inizialmente un abbassamento della temperatura, più profondo col crescere della dose; per dosi equimolecolari dei quattro sali il cloruro ed il gluconato di calcio si sono mostrati più attivi da questo punto di vista. All'ipotermia di regola segue ipertermia; con le dosi vicine alle letali tale ipertemia non è più dimostrabile.

La funzione respiratoria viene modificata da tutti i sali nel senso che viene diminuita di regola la frequenza del respiro, talvolta notevolmente, talvolta in misura minore, ma sempre in modo evidente. Solamente con le dosi più picole di gr. eq. 0,0025 e 0,0050 per Kg. non è stato possibile dimostrare per il piruvato ed il gluconato di calcio una diminuzione di tale frequenza. Alla bradipnea fa seguito in genere una polipnea. Quando la dose iniettata è sufficientemente alta il respiro si fa allora particolarmente difficile: l'animale mette in giuoco i muscoli ausiliari e si possono ascoltare ronchi e sibili fortissimi.

A carico del polso tutti i sali producono modificazioni della frequenza e talvolta del ritmo; si ha di regola una bradicardia, cui segue in genere una tachicardia. Anche da questo punto di vista il gluconato ed il piruvato di calcio alle dosi di gr. eq. 0,0025 e 0,0050 si sono dimostrati indifferenti per la funzione circolatoria. Notai sempre un certo parallelismo fra le modificazioni a carico del cuore e del respiro e quelle della pupilla: raggiungono cioè la massima intensità un'ora circa dopo l'iniezione.

La diuresi è anche sensibilmente modificata, ma non in maniera uguale dai diversi sali adoperati. Mentre il cloruro, il lattato ed il gluconato di calcio producono una diminuzione (più evidente col crescere delle dosi) del volume di orina emessa nelle 24 ore successive all'iniezione, il piruvato di calcio, anche se somministrato in dosi vicine alle letali, permette sempre un'abbondante eliminazione di orina. Le dosi elevate possono provocare sovente albuminuria, di maggior entità con lattato e cloruro, ma sempre passeggera. Non è stata mai osservata glicosuria.

Gli animali si ristabiliscono rapidamente cosicchè nel giorno successivo a quello dell'iniezione non è più rilevabile all'esame diretto alcuna particolare sintomatologia.

b) I rilievi fatti sugli animali trattati con dosi letali dei quattro sali di calcio sono i seguenti: gli animali non mostrano mai segni di intolleranza nè appaiono mai fenomeni di eccitamento motorio. Le modificazioni della pupilla, quelle a carico dell'apparato respiratorio, termoregolatore, e della diuresi, sono qualitativamente uguali a quelle che si possono osservare quando si iniettano alte dosi di calcio.

L'abbassamento della temperatura, sempre notevole, è particolarmente appariscente quando si inietta gluconato di calcio.

La diuresi è più fortemente inibita dal cloruro e dal lattato col quale ultimo sale talvolta fu riscontrata anuria completa. L'albuminuria, di maggior entità col lattato, ha sempre accompagnato la somministrazione di dosi letali dei sali da me studiati.

Appariscente è l'azione depressiva generale esercitata dalle dosi letali dei sali studiati. Liberati dall'apparecchio di contenzione, i conigli stentano a riprendere la posizione prona normale e sono come in uno stato soporoso. Se si stimolano fino a costringerli a muoversi, la deambulazione è impacciata ed incerta. La muscolatura appare ipotonica. Messi in gabbia gli animali rifiutano costantemente il cibo che loro viene offerto e sembrano indifferenti all'ambiente che li circonda. Col progredire dell'azione del tossico l'ipotonia dei muscoli si fa più manifesta: maggiormente ipotonici sono negli arti i muscoli flessori, così che l'animale assume una posizione caratteristica: la testa cade inerte per il suo peso e le zampe sono allungate e distese sul piano su cui giace l'animale. I riflessi, sebbene un po' tardi, sono presenti e la sensibilità dolorifica è conservata.

Un progressivo indebolimento di tutte le funzioni vitali porta a morte l'animale, la quale sopravviene placidamente. In genere il cuore si arresta per primo mentre ancora può sussistere qualche movimento respiratorio.

Con queste dosi letali però si nota una differenza tra gli animali iniettati con cloruro, lattato e piruvato di calcio da un lato, e quelli trattati con gluconato dall'altro. Nei primi le modificazioni a carico della termoregolazione, del polso, del respiro, dopo aver toccato un massimo d'intensità, in genere regrediscono così che pare che gli animali si avviino verso il ristabilimento della loro salute; la frequenza del respiro e del polso aumentano di nuovo fino a raggiungere e talvolta superare il valore fisiologico; la temperatura, dopo aver raggiunto un massimo di discesa, torna di nuovo a salire, pur mantenendosi parecchio al disotto del detto valore. Sembra, in altre parole, che i poteri di difesa dell'animale riescano a prevalere sulla violenza del tossico. Dopo un tempo che

oscilla da 38 a 72 ore si assiste nuovamente ad un indebolirsi di tutte le funzioni vitali. Il respiro si fa superficiale, il polso debole e raro: la temperatura discende progressivamente. L'animale si avvia così verso la morte che sopravviene placidamente.

Nei conigli invece cui è stato iniettato il gluconato di calcio non è stato possibile osservare tale effimera ripresa delle funzioni vitali. Il gluconato di calcio o conduce rapidamente ed inesorabilmente a morte, oppure, qualora l'animale riesca a reagire contro la violenza del tossico, il ritorno verso la normalità prosegue fino al ristabilimento completo.

Autopsia ed esame istologico. — L'autopsia, praticata subito dopo la morte degli animali ha dimostrato: forte congestione degli organi toracici ed addominali; vasi venosi distesi ripieni di sangue fluido; suffusioni emorragiche sottopleuriche e sottoperitoneali. Di frequente negli animali venuti a morte ad una certa distanza dall'iniezione è dato di osservare ematomi sottoperitoneali occupanti lo spazio situato tra il retto e la vescica.

Non mi fu dato di rilevare differenze fra i quattro sali di calcio.

c) *Esame istologico.* — I pezzi furono prelevati immediatamente dopo la morte dell'animale e trattati con tecniche opportunamente scelte. Furono eseguiti tagli al microtomo congelatore, colorazione con ematossilina-eosina, con ematossilina e Sudan III per la ricerca dei grassi. Per i preparati comuni il fissaggio venne fatto in ZENKER, l'inclusione in paraffina, la colorazione in ematossilina-eosina; per il connettivo si ricorse alla colorazione di VAN GIESON e a quella tricromica del MALLORY; per la ricerca della fibrina ho seguito il metodo di WEIGERT; i surreni furono fissati in bicromato potassico, inclusi in paraffina e colorati con il metodo di WIESEL; il cervello fu fissato in alcool, incluso in paraffina, colorato con ematossilina-eosina.

I risultati delle ricerche sono qui sotto descritti.

Encefalo. — Tagli nel bulbo e cervelletto: congestione vascolare; niente di notevole nel tessuto parenchimale.

Timo. — Fortemente congesto tanto nei vasi del connettivo fra i lobuli come nei capillari che si addentrano nel parenchima: mancano emorragie.

Tiroide. — Di aspetto normale anche per quanto riguarda la vascolarizzazione.

Capsule surrenali. — Si dimostrano di aspetto normale sia nella parte corticale come nella midollare. I varii strati della corticale sono bene delineati e molto ricchi di lipoidi. E' evidente, come negli altri organi, una congestione vascolare. Niente di notevole alla vena centrale.

Cuore. — Nulla di notevole per la lamina endocardica e per la sierosa epicardica. L'aspetto del muscolo cardiaco si presenta presso a poco uguale in tutti gli strati e precisamente dimostra fortissima congestione dei vasi sanguigni, che hanno pareti quasi uniformemente dilatate e il lume occupato da sangue fresco bene conservato. Si ha quindi l'impressione di una dilatazione così dei vasi maggiori come di quelli minori e dei capillari. Sono assenti fenomeni regressivi nella cellula muscolare cardiaca.

Polmone. — Cospicua congestione vascolare estesa così ai grossi vasi venosi come ai capillari alveolari. Nessun abnorme contenuto negli alveoli e nell'interno dei bronchi. Nei preparati allestiti con le comuni tecniche di colorazione la parete dei vasi arteriosi appare leggermente ispessita. Tale ispessimento è da riferirsi ad ipertrofia della tunica muscolare, le colorazioni di Van Gieson e del Mallory avendo dimostrato assenza di fenomeni di vera e propria sclerosi vasale.

Fegato. — Congestione vasale imponente tanto dei grossi come dei fini vasi capillari. Gli elementi epiteliali dell'organo non dimostrano reperti degni di nota. La colorazione col Weigert per la dimostrazione della fibrina ha dato esito negativo.

Rene. — La congestione vascolare è evidente anche in questo organo. Con la colorazione comune talvolta sembra di scorgere processi degenerativi negli elementi parenchimali; con la colorazione a mezzo del Sudan III però non si sono riscontrate mai gocce di grasso negli elementi epiteliali, per cui i fenomeni regressivi, tenuto conto anche della normale colorabilità del nucleo, debbono essere limitati alla degenerazione albuminosa.

Milza. — Polpa congesta: i follicoli hanno aspetto e disposizione normali con leggera diversità nei vari animali.

Intestino. — Congestione e dilatazione vascolare.

Allestendo i preparati nelle zone infiltrate da sangue (vedi esame macroscopico) in modo da interessare i vari organi circondati dall'emorragia stessa, si constata che i vasi del connettivo retro-peritonale come di quello corrispondente allo spazio retro-vescicale sono fortemente dilatati e ripieni di sangue; nella loro parete si notano delle lesioni di continuo da cui il sangue dilaga in cospicua quantità, presentandosi in alcuni punti bene conservato, in altri più o meno alterato ed in via di coagulazione. Niente di notevole si nota invece nella parete vescicale, in quella rettale, ed in genere nella compagine degli organi circondati dal sangue.

* * *

I risultati degli esperimenti debbono essere considerati per quello che riguarda la dose minima letale lontana e la causa della morte.

Dalle tabelle su riportate risulta che la scala di ossicità per i quattro farmaci è la seguente:

Sale	Dose minima letale lontana in gr. eq. per Kg.
lattato di calcio	0,0070
cloruro » »	0,0080
gluconato » »	0,0130
piruvato » »	0,0180

Questi risultati confermano quelli di Rothlin (2) solamente in ciò che dimostrano anch'essi una più elevata tossicità di cloruro di calcio rispetto al gluconato, ma dimostrano anche che il gluconato è assai più tossico di quello che non risultasse dalle esperienze di quest'A.

Essi inoltre dimostrano che il lattato è il più tossico fra i quattro sali studiati e che ad esso seguono, nell'ordine, il cloruro, il gluconato, il piruvato, il quale risulta senza dubbio il meno dannoso. Facendo $=1$ la dose minima letale lontana del lattato, le altre dosi minime letali infatti assumono i valori qui sotto riportati:

lattato di calcio	$=1$
cloruro » »	$=1,14$
gluconato » »	$=1,85$
piruvato » »	$=2,57$

Per quanto riguarda la tossicità del gluconato di calcio rispetto al cloruro, i miei esperimenti, pur confermando in linea generale quanto dice Rothlin, che cioè il gluconato è meno tossico del cloruro, ci mostrano che è assai più tossico di quello che non ritenesse questo A. Infatti dai miei esperimenti, ponendo $=1$ la dose minima letale lontana del cloruro, la dose corrispondente del gluconato risulta $=1,62$; Rothlin, invece, nelle sue ricerche trova, fatta $=1$ la dose minima letale di cloruro di calcio, che quella di gluconato sale a 4,25. Vale a dire che mentre io dimostro che la dose minima letale di gluconato di calcio è appena una volta e mezzo quella del cloruro, egli rileva che è oltre quattro volte maggiore, cioè che il gluconato è più di quattro volte meno tossico del cloruro.

La discrepanza s'intende subito quando si osservi che il Rothlin nelle sue esperienze usava coi due sali velocità di iniezione diverse mentre è necessario che la velocità sia assolutamente costante.

Per quello che si riferisce al lattato di calcio MORTON F., MASON e HARRY RESNIK (4) hanno osservato che questo sale, iniettato nel rachide dei cani, risulta meno tossico del cloruro e del gluconato. Tale contraddizione con quanto io rilevai difficilmente può essere interpretata poichè gli AA. non riferiscono su quale considerazioni riposino tali loro asserzioni, limitandosi a dire che la sospensione di lattato di calcio è risultata, rispetto agli effetti immediati, meno tossica delle soluzioni di cloruro e di gluconato di calcio ad uguale contenuto di metallo. D'altra parte neppure viene dai detti AA. precisata l'esatta quantità dei diversi sali che occorre per provocare la morte.

Se ora andiamo a ricercare per quale meccanismo i sali di calcio introdotti nelle vene producono la morte, vediamo che poco su questo punto ci può illuminare la letteratura, le opinioni degli AA. essendo a tale riguardo tutt'altro che concordi.

DASTRE (9) aveva osservato che per iniezioni endovenose di cloruro di calcio gli animali morivano per vaste coagulazioni intravasali ed attribuiva quindi al farmaco un'azione tossica particolare la quale fu negata da tutti gli AA. che in seguito lavorarono sull'argomento, i quali mai osservarono il reperto descritto dall'A.

DELOGU (1) iniettando endovena negli animali cloruro di calcio osservò che la morte avveniva con fatti convulsivi quando la velocità di iniezione era elevata, mentre era costantemente palese una chiara azione depressiva la quale portava a morte gli animali.

ROTHLIN (2) ebbe modo di notare che durante l'iniezione di sali di calcio il respiro dapprima si accelera e si fa superficiale, diventa poi più lento e più profondo. L'animale è irrequieto: seguono violenti crampi tonico-clonici con opistotono, arresti di respiro, esoftalmo. Se la dose non è letale gli animali si ristabiliscono rapidamente, se la dose è letale allora essi vengono a morte nel primo attacco di crampi od in qualcuno dei successivi. Di regola si ha una morte rapida durante un attacco convulsivo. Io penso che in questo caso la morte si debba attribuire sovratutto alla eccessiva velocità d'iniezione, ciò che, del resto, si accorderebbe con l'osservazione di DELOGU.

LIEBERMANN (3) iniettando nelle vene fino alla morte il gluconato di calcio in cani cui prendeva il tracciato manometrico della pressione carotidea, poteva osservare che la morte sopravveniva gradualmente con un progressivo abbassarsi della pressione. Lo stesso A. in un'altro lavoro sulle modificazioni della pressione sanguigna in cani cui erano state iniettate dosi equimolecolari di lattato e gluconato di calcio, ebbe talvolta ad osservare la morte improvvisa dell'animale, ch'egli interpretò come dovuta a coagulazione intravasale premortale.

MORTON F. MASON e HARRY RESNIK (4) introducendo lattato, cloruro

e gluconato di calcio nella cisterna dei cani sostennero che la morte avveniva per paralisi respiratoria.

Dalle notizie che ho riportato si può rilevare che la più grande incertezza esiste quando si debba decidere per quale meccanismo il calcio introdotto nelle vene degli animali produca la morte.

Dalle mie esperienze condotte sistematicamente su 62 animali si può senz'altro escludere che il calcio iniettato lentamente nelle vene dia luogo a fatti convulsivi; questi fenomeni, come ha dimostrato Delogu, sono da attribuirsi all'alta velocità d'iniezione.

Neppure la coagulazione intravascolare può essere chiamata in causa come determinante la morte. L'autopsia praticata subito dopo la morte dell'animale ha sempre dimostrato sangue fluido nei vasi e la ricerca della fibrina fu sempre negativa.

L'esame istologico dei preparati degli organi degli animali venuti a morte in seguito ad iniezione è muta per un'eventuale spiegazione del meccanismo della morte. Le alterazioni infatti consistono essenzialmente in una congestione vascolare enorme, talvolta con rottura di piccoli vasi di qualche distretto.

Interessante invece appare la stretta analogia, la quasi identità dei sintomi rilevati da me iniettando con velocità lenta alte dosi di calcio e quelli osservati da vari AA. i quali hanno portato in varie maniere il calcio direttamente a contatto degli elementi nervosi.

Sabbatani (10) nelle sue classiche ricerche sul calcio dimostra che questo catione spiega un'azione moderatrice sui tessuti. Con l'applicazione locale sulla corteccia, sul midollo, sui nervi, sui muscoli, di un sale di calcio egli osservava sempre fenomeni di depressione mentre, all'opposto, l'applicazione di un reattivo decalcificante, il citrato trisodico, produceva sempre fenomeni di eccitazione, che interpretava come dovuti al fatto che il detto farmaco forma col calcio joni complessi e sottrae quindi ai tessuti il calcio allo stato di jone, chimicamente e biologicamente attivo.

Roncoroni nel suo Istituto (11) confermava i risultati: l'applicazione di sali precipitanti il calcio sulla corteccia cerebrale provocava azione motoria come la mioclonia e l'epilessia e Ferrari, alla sua volta (12) osservava gli stessi fenomeni depressivi iniettando il calcio nella carotide.

In seguito Demole mediante l'applicazione diretta di calcio sull'infundibolo dei gatti notò che questi cadevano in un sonno più o meno profondo (13).

L. Stern e J. Chvoles (14), introducendo nei ventricoli cerebrali di cani una soluzione di gluconato di calcio, rilevavano che tale iniezione produceva in generale una passeggera eccitazione, cui seguiva dopo pochi minuti una perdita completa del tono muscolare ed uno stato di torpore che terminava dopo quindici minuti in una narcosi profonda

con perdita dei riflessi. Questo stato si prolungava qualche ora, dopo di che l'animale si rimetteva poco a poco. Assai spesso però l'animale deperiva e finiva per morire. La pressione sanguigna cominciava ad abbassarsi dopo qualche minuto ed al quindicesimo-ventesimo minuto era in genere molto bassa. Il polso diveniva più lento e più debole, il respiro più lento e superficiale. Dosi uguali iniettate nel circolo restavano senza effetto. In seguito a questi risultati gli AA. concludono per un effetto diretto del calcio sui centri situati nelle pareti ventricolari.

Bunichi Hasama (15) iniettando in prossimità del tuber cinereo dei gatti mmgr. 2 di cloruro di calcio osservò che gli animali cadevano in uno stato di sonnolenza; il polso ed il respiro diminuivano di frequenza; si produceva una diminuzione della temperatura.

I risultati di queste ricerche sono veramente interessanti perchè dimostrano che l'applicazione di piccole quantità di calcio sui centri encefalici, è caratterizzata da una sintomatologia la quale richiama quella da me osservata iniettando dosi elevate di sali di calcio con piccole velocità per via endovenosa.

Riassumendo, i risultati delle mie esperienze mi portano ad escludere che i sali di calcio somministrati per via endovenosa con lenta velocità di iniezione, producano la morte per coagulazione intravascolare e che la morte avvenga in mezzo a fatti convulsivi. Una rapida caduta della temperatura, un rallentarsi ed indebolirsi progressivo del polso e del respiro, una congestione vascolare intensa è il quadro sintomatologico presentato dagli animali intossicati con calcio; quadro sintomatologico che si può riassumere col termine comprensivo di collasso. Tale collasso, in seguito ai risultati negativi dell'esame anatomo-istologico, non può attribuirsi che ad un'azione elettiva, deprimente del calcio sopra i centri nervosi.

CONCLUSIONI

1° — La dose minima letale lontana per via endovenosa secondo Simon è, nel coniglio: per il lattato di calcio di gr. eq. 0,0070 per Kg. di peso corporeo; per il cloruro di gr. eq. 0,0080 per Kg.; per il gluconato di gr. eq. 0,00130 per Kg.; per il piruvato di gr. eq. 0,00180 per Kg.

2° — Queste esperienze, oltre a fornirci dati che si prestano a confronti precisi, sono preziose per il terapista in quanto rettificano opinioni non basate su esperimenti sicuri. E mentre finora si riteneva che il gluconato di calcio fosse oltre quattro volte meno tossico del cloruro, le mie ricerche dimostrano che la differenza di tossicità è molto minore, poco più di una volta e mezzo. Si comprende bene perciò come l'abuso di questo sale in terapia, fondato sulla sua presunta scarsa tossicità, abbia potuto produrre sovente fenomeni tossici inaspettati.

E si comprendono anche i disturbi osservati con la somministrazione di lattato di calcio, che è il più tossico fra i quattro sali.

3° — La causa della morte per tutti e quattro i sali studiati è sempre un'azione depressiva del catione calcio sui centri nervosi alla quale si oppone senza alcun dubbio l'anione in misura che le attuali esperienze non possono stabilire.

RIASSUNTO

La dose minima letale lontana per via endovenosa secondo SIMON, *è nel coniglio: per il lattato di calcio = gr. eq. 0,0070 per Kg. corporeo, per il cloruro di calcio = gr. eq. 0,0080 per Kg., per il gluconato gr. 0,0130 per Kg., per il piruvato gr. eq. 0,0180 per Kg. La tossicità del gluconato di calcio risulta da queste esperienze assai più elevata di quanto finora si credesse e spiega i fenomeni tossici osservati nell'uso terapeutico di questo sale. La causa della morte si deve attribuire all'azione depressiva del catione sui centri nervosi.*

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Helv. Paediat. Acta 25(1):25-34. 1970.

Ballabriga-'70
Bib No. 77

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Metabolic response of prematures to milk formulas with different lactic acid isomers or citric acid

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Received on July 30, 1969

When, some years ago, Goldman et al. [1] described metabolic acidosis in prematures receiving a milk acidified by lactic acid, it was hypothetically supposed that the acidosis could be due to the fact that the D(−) isomer is not metabolised.

Various clinical and biochemical studies carried out on commercial milks acidified by lactic acid (Ofteringer [2], Hungerland [3], Vesterdal [4], Ballabriga [5, 6]) showed that such milks did not cause clinically discernible acidosis and that the acid-base balance values remained within normal limits. Cevini [7] encountered metabolic acidosis using racemic lactic acid.

The aim of this study is to examine the metabolic and clinical responses of prematures fed various milk formulas with varying protein contents and acidified with racemic acid, L(+) isomer, D(−) isomer or a large amount of citric acid.

Material and methods

The study was made on 150 premature infants, all of whom were considered to be "normal" from the clinical point of view, without any signs of distress. The biochemical investigations were carried out from the 12th day of life when calory intake had reached a level of 120 cal. kg bodyweight day, it being considered that beforehand even the normal premature infant may show considerable alterations in acid-base balance, such alterations being caused by his adaptation to his new surroundings.

A micro-Astrup apparatus, model Amei, was used to study the acid-base balance and the values were calculated with the new pH-log pCO_2 nomogram revised by Siggaard-Andersen [8] in 1962.

All the blood samples were taken from the heel, using heparinized capillary tubes. A double determination was made in every case and the blood samples were taken at the same time of day in order to avoid any possible variations. The double determinations were considered to be valid when the actual pH values or the pH values after saturation of the samples, did not differ by more than 0.005.

In those cases in which the blood sample was taken whilst the infant was crying loudly, the double determination was not accepted. The analysis was carried out at once, after the sample had been shaken. A total of 600 double determinations were made, the actual pH, pCO_2, standard bicarbonate and base excess values being calculated.

Table 1

Composition of the formulas used, in g/100 ml

Formula	N	X	X+D−	X+L+	X+ racemic	E	X+ citric	N+ citric
Fats	3.4	1.4	1.4	1.4	1.4	1.3	1.4	3.4
Proteins	1.62	3.19	3.19	3.19	3.19	2.9	3.19	1.62
Lactose	7.34	4.43	4.43	4.43	4.43	3.8	4.43	7.34
Starch	0	0	0	0	0	1.6	0	0
Dex. maltose	0	6.0	6.0	6.0	6.0	4.8	6.0	0
Mineral salts	0.2	0.64	0.64	0.64	0.64	0.6	0.64	0.2
Lactic acid	0	0	0.5D−	0.5L+	0.5R	0.5L+	0	0
Citric acid	0	0	0	0	0	0	0.5	0.5
Calories	68.25	68.8	68.8	68.8	68.8	66.0	68.8	68.25
pH	6.65	6.71	4.98	5.1	4.9	4.6	4.8	4.1

The 150 premature infants who served as the object of this study were fed 8 different types of foods and the differences in the composition of these products are given in Table 1.

All the subjects regularly received an equivalent of 120 cal. kg bodyweight day from the age of 12 days, irrespective of the product administered. The food was administered by stomach tube or as a bottle feed, a total of 8 feeds per day were given. Vitamin C daily intake was 60 mg kg.

All the prematures received the non-acidified formula X until aged 12 days, then the following diets: formula X: 25 cases; formula X with L(+) lactic acid: 21 cases; formula X with D(−) lactic acid: 16 cases; formula X with racemic lactic acid: 29 cases; formula E with L(+) lactic acid obtained by biological acidification: 21 cases; formula X with citric acid: 16 cases; formula N with citric acid: 22 cases.

Each infant received the diet for 12 days during which time four determinations of the acid-base balance were taken: the first whilst the infant was still receiving formula N without lactic acid; on the same day he was transferred to one of the experimental acidified formulas and the acid-base balance was taken on the 4th, 8th and 12 days of this diet.

The D(−) lactic acid used was the one produced by Kyowa Hakko Kogyo (Japan) at 52% of which 70% was the D(−) form. A 30% D(−) lactic acid free acid from the Sigma Chemical Co., St. Louis, was also used.

80% racemic lactic acid was obtained from Koge Chemical Works, Copenhagen and L(+) lactic acid from Boehringer Sohn, Ingelheim. The Chemische Fabrik Schweizerhall, Basel, supplied the citric acid dead free.

The amounts of calories, proteins, liquid and acid administered per kg bodyweight day with these diets were as follows: see Tab. 2.

Urinary elimination of organic acids was studied only in the boys of each group by twodimensional chromatography. The urine samples for 24 h were collected and put in a refrigerator until analysis. Finally, 75 prematures were chosen and four determinations were made on each of them, the first at the beginning of the experiment whilst they were still receiving the acid free N Formula (sample no. 1) then on the 8th and 12th day of the diet containing the different lactic and citric acids (samples nos. 2 and 3). The acidified milk diet was stopped after 12 days and the Formula N diet regiven for a period of 10 days after which the final determination of eliminated organic acids was made (sample no 4).

Table 2

	Calories	Calories % from proteins	Liquid cm³	Proteins g	T.A* mEq/kg
Formula N	120	9.7	175	2.84	1.03
Formula X	120	18.9	174	5.5	1.35
Formula X with D(—)	120	18.9	174	5.5	9.37
Formula X with L(+)	120	18.9	174	5.5	8.09
Formula X with racemic	120	18.9	174	5.5	9.15
Formula E	120	17.0	175	5.0	9.03
Formula X with citric	120	18.9	174	5.5	10.61
Formula N with citric	120	9.7	175	2.84	10.62

*TA = Titrable acid expressed in mEq/kg/day obtained by titrating the milk to end point pH 7.4 using the radiometer automatic titrator TTT 1.

The NORDMAN and NORDMAN [9, 10] method of twodimensional chromatography was used with a solvent of alcohol/ammonia/water 400:25:75 and eucalyptol/propanol/formic acid/water 200:200:80:18. Organic acids were identified by comparison with internal standards and studying the run, fluorescence and colour in front of various dye solutions. Acridine and bromocresol green were generally used as dyes and either p-aminobenzoic aldehyde, iodised starch, silver nitrate, p-nitroaniline or sulphanilic acid as develop reagents.

When the chromatogram showed abnormal pattern, ketonic acids in the same urine samples was measured using the CAVALLINI and FRONTALI [11–13] method to detect the possible presence of p-hydroxyphenyl-pyruvic acid. One-dimensional chromatography with the following two solvents was used:

no 1: amylic alcohol/absolute alcohol/water saturated (40:40:

no 2: butanol/ethanol/water 400:25:75, to detect ketoglutaric acid run in the same direction.

We studied the phenolic acid elimination by the ARMSTRONG [14] method adapted to one-dimensional chromatography using benzene/propionic acid/water 200:140:10 as solvent. Results were interpreted by internal standards and specific developers to confirm the existence of p-hydroxyphenyl lactic and p-hydroxyphenyl-acetic acids.

When a rise in phenolic acid excretion was observed the urinary tyrosine elimination was analysed by high tension electrophoresis: 7,000 volts for 45 min, pH 1.9 (Holzel Technik Apparatus, Munich) [15], ninhydrine and Pauly reagents for tyrosine were used as staining methods. Statistical treatment of data have been made with a Sharp I.C. Compet 32 electronic desk calculator.

Results

The study of the acid-base balance in each group gave the following results:

a) Comparison of the pH, standard bicarbonate, pCO_2 and base excess values in 25 prematures fed formula N, then the non acidified formula X for 12 days.

$\bar{x}$ pH Formula N = 7.352 $s = \pm 0.04$ $s_{\bar{x}} = \pm 0.008$

$\bar{x}$ pH Formula X = 7.339 $s = \pm 0.037$ $s_{\bar{x}} = \pm 0.007$

p between 0.3 and 0.2

$\bar{x}$ St. B. Formula N = 22.12 $s = \pm 2.29$ $s_{\bar{x}} = \pm 0.45$
$\bar{x}$ St. B. Forpula X = 21.25 $s = \pm 1.94$ $s_{\bar{x}} = \pm 0.38$
p between 0.2 and 0.1

$\bar{x}$ pCO_2 Formula N = 42.4 $s = \pm 3.52$ $s_{\bar{x}} = \pm 0.7$
$\bar{x}$ pCO_2 Formula X = 41.9 $s = \pm 3.84$ $s_{\bar{x}} = \pm 0.76$
p between 0.3 and 0.2

$\bar{x}$ B. E. Formula N = −2.25 $s = \pm 2.9$ $s_{\bar{x}} = \pm 0.53$
$\bar{x}$ B. E. Formula X = −3.54 $s = \pm 3.69$ $s_{\bar{x}} = \pm 0.53$
p between 0.2 and 0.1

b) Comparison of the pH, standard bicarbonate, pCO_2 and base excess values in 21 prematures who received non acidified formula N followed by a period of 12 days with formula X with L(+) lactic acid.

$\bar{x}$ pH Formula N = 7.351 $s = \pm 0.04$ $s_{\bar{x}} = \pm 0.008$
$\bar{x}$ pH Formula X = 7.343 $s = \pm 0.028$ $s_{\bar{x}} = \pm 0.006$
with L(+) p between 0.6 and 0.5

$\bar{x}$ St. B. Formula N = 22.9 $s = \pm 2.5$ $s_{\bar{x}} = \pm 0.54$
$\bar{x}$ St. B. Formula X = 22.1 $s = \pm 1.37$ $s_{\bar{x}} = \pm 0.3$
with L(+) p between 0.3 and 0.2

$\bar{x}$ pCO_2 Formula N = 43.76 $s = \pm 4.9$ $s_{\bar{x}} = \pm 1.07$
$\bar{x}$ pCO_2 Formula X = 43.43 $s = \pm 4.1$ $s_{\bar{x}} = \pm 0.91$
with L(+)
p between 0.9 and 0.8

$\bar{x}$ B. E. Formula N = −1.23 $s = \pm 2.82$ $s_{\bar{x}} = \pm 0.61$
$\bar{x}$ B. E. Formula X = −2.36 $s = \pm 1.7$ $s_{\bar{x}} = \pm 0.37$
with L(+) p between 0.2 and 0.1

c) Comparison of the pH, standard bicarbonate, pCO_2 and base excess values in 16 prematures who received non acidified formula N followed by a period of 12 days with formula X with D(−) lactic acid.

$\bar{x}$ pH Formula N = 7.365 $s = \pm 0.039$ $s_{\bar{x}} = \pm 0.09$
$\bar{x}$ pH Formula X = 7.322 $s = \pm 0.07$ $s_{\bar{x}} = \pm 0.017$
with D(−) $p < 0.05$

$\bar{x}$ St. B. Formula N = 22.68 $s = \pm 2.12$ $s_{\bar{x}} = \pm 0.53$
$\bar{x}$ St. B. Formula X = 20.37 $s = \pm 3.28$ $s_{\bar{x}} = \pm 0.8$
with D(−) $p < 0.05$

$\bar{x}$ pCO_2 Formula N = 42.34 $s = \pm 4.37$ $s_{\bar{x}} = \pm 1.09$
$\bar{x}$ pCO_2 Formula X = 40.12 $s = \pm 5.05$ $s_{\bar{x}} = \pm 1.2$
with D(−) p between 0.3 and 0.2

$\bar{x}$ B. E. Formula N = −1.16 $s = \pm 2.8$ $s_{\bar{x}} = \pm 0.7$
$\bar{x}$ B. E. Formula X = −5.13 $s = \pm 5.08$ $s_{\bar{x}} = \pm 1.2$
with D(−) $p < 0.05$

d) Comparison of the pH, standard bicarbonate, pCO_2 and base excess values in 29 prematures who received non acidified formula N followed by a period of 12 days with formula X with racemic lactic acid.

$\bar{x}$ pH Formula N = 7.36 $s = \pm 0.04$ $s_{\bar{x}} = \pm 0.007$
$\bar{x}$ pH Formula X = 7.331 $s = \pm 0.04$ $s_{\bar{x}} = \pm 0.008$
with racemic $p < 0.02$

$\bar{x}$ St. B. Formula N	= 22.95	$s = \pm 2.14$	$s_{\bar{x}} = \pm 0.39$
$\bar{x}$ St. B. Formula X	= 21.8	$s = \pm 2.21$	$s_{\bar{x}} = \pm 0.41$
with racemic		$p < 0.05$	
$\bar{x}$ pCO_2 Formula N	= 43.0	$s = \pm 4.2$	$s_{\bar{x}} = \pm 0.77$
$\bar{x}$ pCO_2 Formula X	= 44.4	$s = \pm 4.56$	$s_{\bar{x}} = \pm 0.84$
with racemic		p between 0.3 and 0.2	
$\bar{x}$ B. E. Formula N	= -0.72	$s = \pm 2.16$	$s_{\bar{x}} = \pm 0.4$
$\bar{x}$ B. E. Formula X	= -2.99	$s = \pm 2.89$	$s_{\bar{x}} = \pm 0.53$
with racemic		$p < 0.01$	

e) Comparison of the pH, standard bicarbonate, pCO_2 and base excess values in 21 cases who received non acidified formula N followed by a period of 12 days with formula E containing L (+) lactic acid obtained by biological acidification

$\bar{x}$ pH Formula N	= 7.331	$s = \pm 0.04$	$s_{\bar{x}} = \pm 0.009$
$\bar{x}$ pH Formula E	= 7.329	$s = \pm 0.031$	$s_{\bar{x}} = \pm 0.006$
with L (+)		p between 0.9 and 0.8	
$\bar{x}$ St. B. Formula N	= 20.48	$s = \pm 1.75$	$s_{\bar{x}} = \pm 0.38$
$\bar{x}$ St. B. Formula E	= 21.05	$s = \pm 1.74$	$s_{\bar{x}} = \pm 0.38$
with L (+)		p between 0.3 and 0.2	
$\bar{x}$ pCO_2 Formula N	= 40.33	$s = \pm 1.42$	$s_{\bar{x}} = \pm 0.3$
$\bar{x}$ pCO_2 Formula E	= 41.9	$s = \pm 1.44$	$s_{\bar{x}} = \pm 0.3$
with L (+)		p between 0.2 and 0.1	
$\bar{x}$ B. E. Formula N	= -4.59	$s = \pm 2.43$	$s_{\bar{x}} = \pm 0.53$
$\bar{x}$ B. E. Formula E	= -3.85	$s = \pm 2.3$	$s_{\bar{x}} = \pm 0.5$
with L (+)		p between 0.4 and 0.3	

f) Comparison of the pH, standard bicarbonate, pCO_2 and base excess values in 16 prematures who received non acidified formula N followed by a period of 12 days with formula X with citric acid.

$\bar{x}$ pH Formula N	= 7.331	$s = \pm 0.036$	$s_{\bar{x}} = \pm 0.009$
$\bar{x}$ pH Formula X	= 7.281	$s = \pm 0.079$	$s_{\bar{x}} = \pm 0.19$
with C.Ac.		$p < 0.05$	
$\bar{x}$ St. B. Formula N	= 21.81	$s = \pm 2.0$	$s_{\bar{x}} = \pm 0.5$
$\bar{x}$ St. B. Formula X	= 18.86	$s = \pm 3.8$	$s_{\bar{x}} = \pm 0.96$
with C.Ac.		p between 0.02 and 0.01	
$\bar{x}$ pCO_2 Formula N	= 44.93	$s = \pm 5.01$	$s_{\bar{x}} = \pm 1.25$
$\bar{x}$ pCO_2 Formula X	= 40.94	$s = \pm 5.22$	$s_{\bar{x}} = \pm 1.3$
with C.Ac.		$p < 0.05$	
$\bar{x}$ B. E. Formula N	= -2.81	$s = \pm 2.73$	$s_{\bar{x}} = \pm 0.68$
$\bar{x}$ B. E. Formula X	= -6.94	$s = \pm 5.12$	$s_{\bar{x}} = \pm 1.2$
with C.Ac.		$p < 0.01$	

g) Comparison of the pH, standard bicarbonate, pCO_2 and base excess values in 22 prematures who received non acidified formula N followed by a period of 12 days with formula X with citric acid.

$\bar{x}$ pH Formula N	= 7.345	$s = \pm 0.03$	$s_{\bar{x}} = \pm 0.006$
$\bar{x}$ pH Formula N	= 7.344	$s = \pm 0.037$	$s_{\bar{x}} = \pm 0.008$
with C.Ac.		p between 0.9 and 0.8	

$\bar{x}$ St. B. Formula N = 22.21	s = ± 2.02	$s_{\bar{x}}$ = ± 0.44
$\bar{x}$ St. B. Formula N = 21.77	s = ± 2.16	$s_{\bar{x}}$ = ± 0.47
with C.Ac.	p between 0.6 and 0.5	
$\bar{x}$ pCO_2 Formula N = 43.47	s = ± 3.87	$s_{\bar{x}}$ = ± 0.84
$\bar{x}$ pCO_2 Formula N = 41.87	s = ± 2.57	$s_{\bar{x}}$ = ± 0.56
with C.Ac.	p between 0.2 and 0.1	
$\bar{x}$ B. E. Formula N = -2.11	s = ± 2.66	$s_{\bar{x}}$ = ± 0.58
$\bar{x}$ B. E. Formula N = -2.83	s = ± 2.68	$s_{\bar{x}}$ = ± 0.58
with C.Ac.	p between 0.4 and 0.3	

We compared the urine chromatograms of the organic, ketonic and phenolic acids to detect the presence of intense tyrosyluria (increase of tyrosine, p-hydroxyphenylpyrovic, p-hydroxyphenyllactic, p-hydroxyphenylacetic acids in the urine) or an increase in the excretion of organic acids, especially lactic acid. The results of this comparison are set out in Table 3. Only the substantial and intense alterations of the chromatograms were taken into consideration.

Development was normal from the clinical point of view in the groups receiving formula N and non acidified formula X, the formulas acidified by L(+) lactic acid (X with L(+) and E), or the formulas with large amount of citric acid.

Amongst the group of prematures receiving the D(—) lactic acid formula X, there were four cases with severe clinical signs of metabolic acidosis i.e. loss of weight, pallor, bad overall condition, vomiting and regurgitation which necessitated the discontinuation of the diet and the correction of the acidosis with intravenous bicarbonate infusion.

In the group receiving the formula acidified by racemic lactic acid there were three cases with similar clinical signs but milder which disappeared when the formula was discontinued. The infants receiving formula X with a high citric acid content showed no signs of clinical anomaly inspite of much lower acid-base balance values than those obtained during the non acidified formula N diet.

Table 3

Formula	Protein intake g/kg/d	Acid mEq/kg/d	Cases	Number of chromatograms	Very intensive tyrosyluria	Organic aciduria	Lactic acid excretion increased
N	2.84	1.03	73	146	0	0	0
E	5.0	9.03	5	10	0	0	0
X	5.5	1.35	10	20	1	0	0
X + lactic acid L(+)	5.5	8.09	5	10	0	0	0
X + racemic	5.5	9.15	10	20	4	2	1
X + cit. ac.	5.5	10.61	11	22	4	0	0
X + lactic acid D(—)	5.5	9.37	7	14	2	1	2
N + citric	2.84	10.62	11	22	0	0	0
N + racemic	2.84	8.83	5	10	0	1	3
N + lactic acid D(—)	2.84	9.05	9	18	2	2	2

Discussion

There was no significant difference between the acid-base values of the prematures fed a non acidified relative protein diet (5.5 g/kg/d) and those fed a low protein diet (2.84 g/kg/d).

When the high protein diet was supplemented with L(+) lactic acid or biologically acidified, no significant differences occurred in the pH, pCO_2, standard bicarbonate or base excess values compared with the low protein non acidified formula.

"Biochemical" metabolic acidosis with acid-base balance values significantly lower than those observed with the low or high protein non acidified diess was noted in those groups fed the acidified, high protein, racemic acid or D(−) isomer diet. In some cases the metabolic acidosis was revealed by clinical signs, such as extreme pallor, depression, bad overall condition, loss of weight, regurgitations and vomiting necessitating the discontinuation of the diet.

Biochemical and clinical metabolic acidosis disappeared when a non acidified, low protein diet was administrated, or after intravenous injection of bicarbonate in the very severe cases.

The high protein diet with a large amount of citric acid (10.62 mEq/kg/d) induced biochemical metabolic acidosis without clinical signs, whereas the same amount of citric acid with a low protein diet did not. The manufactured formulas generally contain much less citric acid (approximately 4.3 mEq/kg/d) and do not induce metabolic acidosis.

On the whole a large number of prematures fed high protein diets seem to border on biochemical metabolic acidosis without clinical signs and the administration of a semi-metabolised acid such as racemic lactic acid, or the D(−) isomer, is sufficient to produce complete biochemical metabolic acidosis with or without clinical signs. The same result can occur with doses of citric acid higher than those generally used in manufactured milks. The significant differences between groups receiving the high protein diets with racemic lactic acid or D(−) are attributable to the differences in the degree of metabolisation of the administered isomer.

BEIN's [16] experiments on animals using various C14 labelled isomers showed that a significant amount of D(−) lactate was oxidised with a broad maximum peak at about the end of the first hour after injection. SCHIMASSEK's [17] experimental studies showed that in the isolated rat liver the rate of D(−) lactate oxidation is nearly the same as the conversion of L(+) lactate. Pyruvate, formed by the oxidation of D(−) lactate from mitochondria D(−) oxidase, is neither immediatley oxidised nor converted to L(+) lactate. Experiments on animals by DRURY and WICK [18], who also used C14 labelled isomers, showed that eviscerated tissues metabolise L(+) quite actively and are able to oxidase a limited quantity of the D(−) isomer.

The authors established that racemic lactate is almost completely metabolised by the intact animal and it is probable that the liver converts D(—) isomer either into the L(+) form, glucose or glycogen.

In infants, during the first weeks of life, it is extremely probable that acidosis occurs on administration of racemic or D(—) lactic acid because the isomer is given in excessive amounts and cannot be completely metabolised, due to the fact that the immature liver cannot convert the D(—) isomer into either the L(+) form, glucose or glycogen. This explains why some prematures are perfectly able to tolerate formulas with a fairly D(—) isomer content, whereas others have severe metabolic acidosis. On the other hand, if the infants are fed a high protein diet which may produce a condition approaching biochemical acidosis, the administration of a non-metabolised isomer facilitates the appearance of clinically recognisable acidosis in the majority of the cases. For this reason metabolic acidosis is much less frequently observed in prematures fed a low protein diet, even one acidified by racemic or D(—) lactic acid.

The clinical symptoms of cases with severe metabolic acidosis after administration of D(—) isomer are the same as DUNLOP and HARRIS [19] described in ruminants, i.e. abdominal disorders, dehydration, depression, fall of pH, bicarbonate and progressive increase in the concentration of blood D(—) lactate.

The study of the urinary organic acid elimination revealed three kinds of alterations: a) intensive tyrosyluria; b) increase in organic acid excretion; c) strong elimination of lactic acid.

During the neonatal period the occurrence of tyrosyluria with varying intensity can be attributed to the following factors: a) protein intake; b) vitamin C intake; c) p-hydroxyphenylpyruvate oxidase activity in the liver.

Amongst the prematures receiving the low protein formula N, the high protein non-acidified formula X or the formula acidified by L(+) lactic acid, there was only one case of intense tyrosyluria (receiving the non-acidified formula X) as opposed to a much higher incidence in the groups receiving the high protein formula with racemic acid D(—) isomer or a large amount of citric acid (Tab. 3). There are, therefore, border line cases of tyrosyluria during the neonatal period in prematures receiving a high protein formula, which frequently develop into intense tyrosyluria when additional incompletely metabolised acids are administered. The latter may have a toxic effect and temporarily inhibit p-hydroxyphenylpyruvate-oxidase activity in the liver. Strong tyrosyluria also occurs, but much less frequently, with low protein diets (2.84 g/kg/d), containing racemic lactic acid or D(—) isomer. During the period of intense tyrosyluria there is a drop in the excretion of acids of the Krebs cycle, but a notable increase occurs when a low protein non acidified diet is administered.

In the groups receiving racemic lactic acid or the D(—) isomer there was a general increase in the urinary excretion of organic acids in some cases, especially in the spot corresponding to lactic acid elimination, representing an excess of non metabolised D(—) isomer. This was not observed in the prematures fed the citric acid formula. The alterations in organic acid elimination observed in the infants receiving the D(—) isomer, with intense metabolic acidosis and dehydration, are difficult to interpret given that there was possible impairment of kidney function. As in the tyrosyluria cases AVERY et al. [20] studied during the neonatal period, we found no correlation between the existence of metabolic acidosis and the intensity or frequency of tyrosyluria, nor between the latter and the presence of depression and lethargy. No clinical signs indicating metabolic acidosis accompany acute tyrosyluria. The cases of intense lactaciduria, fed the D(—) isomer diet had varying degrees of metabolic acidosis.

Summary

Prematures fed milks acidified with racemic lactic acid or D(—) lactic acid during the first two months of life develop "biochemical" metabolic acidosis, frequently accompanied by the following clinical signs: bad overall condition, loss of weight, dehydration, regurgitation and vomiting. The pH, standard bicarbonate and base excess values are significantly lower during the period of the racemic and D(—) isomer diets than with milk acidified by L(+) lactic acid or a non acidified milk with the same protein content.

A diet containing a large amount of citric acid (10.62 mEq/kg/d) could induce biochemical metabolic acidosis without clinical signs.

Tyrosyluria occurred at times in the infants receiving the high protein diets and was much more frequent and intense with the diet containing racemic lactic acid or the D(—) isomer. This intense tyrosyluria does not depend on the presence of metabolic acidosis and is without clinical signs. In some of these cases we observed an increase in the urinary elimination of organic acids, especially lactic acid. The mechanism responsible for these alterations is discussed.

The conclusion to be drawn from this work is that when acidified milks are used in premature feeding, the formulas must contain exclusively L(+) lactic acid or possibly citric acid provided the dose is below 4.5 mEq/kg/d. Racemic lactic acid and D(—) lactic acid should not be used in infant feeding.

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retracted, the floor of the ulcers revealed a clean granulating surface. In twenty four days after the onset of treatment the patient was discharged as cured.

SUMMARY

Cases of phagedenic ulcerations of the genitals that previously had not responded to local therapeutic measures were rapidly healed by the intravenous injections of antimony and potassium tartrate. This treatment should not be employed for simple chancroids, but reserved for those types of genital lesions which deep and rapid ulceration has rendered unsuitable for routine local measures.

ABSTRACT OF DISCUSSION

DR. ALDO CASTELLANI, New Orleans: Granuloma inguinale is a so-called tropical disease, but as a matter of fact it is more commonly encountered in the subtropics than in the true tropics. It was first found in India in 1889 by McLeod, and later in the West Indies by Conyers and Daniels, in 1896. As regards the etiology, I have no doubt that the so-called Donovan bodies are the real causative agents of the disease. In fact, I think that the presence of Donovan bodies in scrapings clinches the diagnosis. I agree with the authors that antimony and potassium tartrate is a specific for the disease, but in my experience it must be given in large doses and for long periods of time. I do not think that local applications do much good. They certainly do not cure the condition, unless antimony and potassium tartrate is given intravenously. It has been applied externally in ointments and in solutions, but, as a rule, both the ointment and the solution produce a severe inflammation, and I gave up their use long ago. What I have found useful in certain cases is the local application of phosphorated oil. Phosphorus in my experience has a specific action in dermal leishmaniosis, and by giving 4 or 5 drops of phosphorus oil (1 per cent) hypodermically every other day for three or four weeks, the results are very good. In granuloma inguinale phosphorus has no distinct specific action, but its local application is at times useful.

DR. HOWARD FOX, New York: I should like to say a few words about the use of antimony and potassium tartrate in the treatment of granuloma inguinale. It is generally agreed that its action in this disease is specific, though, as Dr. Castellani has said, it generally requires a large amount to produce healing. In many cases, an apparent cure is followed by relapse. It has been suggested that the drug be continued for several months after healing to increase the chances of a permanent cure. Antimony and potassium tartrate is generally well borne in the usual dosage; fatalities have, however, been reported by Johns and Gage of New Orleans from dosage larger than the average. In the two cases reported, the symptoms were those of antimony and potassium tartrate poisoning—generalized hemorrhage of the skin, yellow atrophy of the liver, and failure of postmortem clotting. It is therefore evident that the drug is not entirely devoid of danger. From the recent work of Randall, it would seem that better results can be obtained by the newer synthetic compounds of antimony than by antimony and potassium tartrate. The preparations that he used were antimony sodium thioglycollate and antimony thiosemicarbamide. In the nine cases in which they were used, there was a prompt and complete healing. Some of these cases had proved refractory to treatment with antimony and potassium tartrate.

DR. SAMUEL M. PECK, New York: It is true, as Dr. Castellani said, that the patients must be treated over long periods of time and given large doses. One of these patients was treated every other day for seven months. The reason the patient received no further treatment was that all available veins were thrombosed. We could give no more intravenously, and the other methods were too painful. As to the newer drugs that Dr. Fox mentioned, we are using them now and hope that we can report our results in the near future.

THE EFFECT OF CALCIUM LACTATE INGESTION ON ~~SERUM~~ CALCIUM*

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Kahn and Roe's[1] report on calcium absorption from the intestinal tract in human subjects appeared just as we were beginning to make hourly serum calcium observations after the ingestion of known amounts of calcium lactate. As our studies did not confirm those made by Kahn and Roe, we have continued our work. Since then we have completed the study of seventeen normal persons, nine after the ingestion of 5 Gm. and eight after the oral administration of 10 Gm. of calcium lactate. Cases of endocrine disturbances have also been studied, but they will be reported in a later communication.

Kahn and Roe speak of the number of milligrams of calcium per hundred cubic centimeters of blood—not stating whether they mean whole blood, serum or plasma. They used a method for determination that they had previously reported.[2] All calcium determinations in our series of observations were made according to the Clark modification[3] of the Kramer-Tisdall method. We have used this method daily for about a year and find that we can check our results as well as known calcium solutions within the percentage of error allowed by the titration method. The blood was collected in centrifuge tubes, the serum allowed to separate, and 2 cc. of this serum was then used for the analysis.

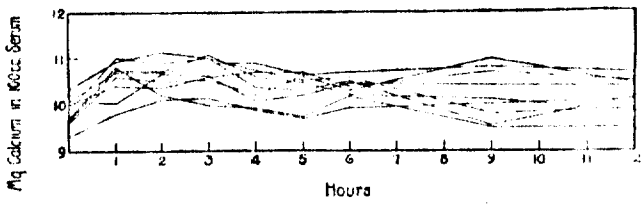

Chart 1.—Hourly calcium determinations in nine normal subjects after the ingestion of 5 Gm. of calcium lactate; the maximal elevation is 14 per cent, the lowest 5 per cent.

REPORT OF TEST IN NORMAL PERSONS

Nine normal subjects, who had fasted for at least twelve hours, were given 5 Gm. of calcium lactate followed by approximately 250 cc. of water, a control serum calcium having been obtained prior to the ingestion of the calcium lactate. Calcium determinations were then made every hour for the first six hours and at the eighth and twelfth hours after the ingestion. At the end of the sixth hour, the subjects were allowed to take a light lunch containing from 25 to 35 mg. of calcium. The ingestion of this test amount of calcium lactate did have a definite effect on the serum calcium levels as is shown in chart 1. The maximal elevation obtained in this group was 14 per cent, the average maximal rise being 8 per cent, and the lowest elevation 5 per cent.

* From the Medical Laboratories of the Massachusetts General Hospital.

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The maximal rises occurred as follows: two at the end of the first hour, two at the second, four at the third, and one at the fourth. The average curve of the nine observations is shown in chart 2.

This procedure was repeated, under similar conditions, on eight normal persons, 10 Gm. of calcium lactate instead of 5 being used. The eight subjects showed a much more striking rise of the serum calcium than did those receiving 5 Gm. Here the maximal elevation was 28.5 per cent as compared to 14 per cent,

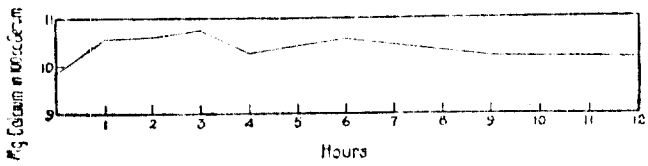

Chart 2.—Average curve obtained from the nine persons after taking 5 Gm. of calcium lactate; the average elevation in the serum calcium is 8 per cent.

and the lowest maximal was 5 per cent, the same as in the preceding series. These curves are shown in chart 3. The average maximal elevation obtained was 14 per cent in contrast to 8 per cent in the other group. The average curve for these eight persons is shown in chart 4. The maximal rise occurred in two subjects at the end of the first hour, in two at the second, in three at the third, and in one at the fifth.

COMMENT

Various workers have attempted to raise the calcium content of the blood by oral administration of calcium salts. Neither Clark,[4] working with rabbits, Kramer and Howland,[5] with rats, nor Denis and Minot,[6] studying human subjects, were able to demonstrate any rise in the serum calcium after feeding calcium lactate.

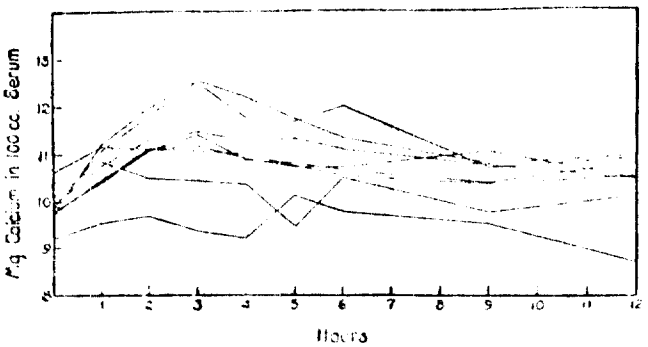

Chart 3.—The types of curve observed in eight normal subjects after the oral administration of [illegible] calcium lactate; the [illegible] elevation obtained is 28.5 per cent, the lowest 5 per cent.

Salvesen, Hastings and McIntosh[7] report a 20 per cent rise in serum calcium in a dog after the oral administration of 7.5 Gm. of calcium chloride daily. Jansen[8] found calcium bicarbonate to be the most efficient calcium salt in raising the serum calcium of man, and calcium lactate the least serviceable. Hjort,[9] working with dogs, obtained his highest elevation of serum calcium with calcium lactate (17 per cent and 48 per cent), the next highest with calcium chloride (32 per cent), and about the same rise with both calcium glycerophosphate (8 and 9 per cent) and carbonate (7 per cent). Using calcium bicarbonate, he could not raise the serum calcium level. These dogs received 0.2727 Gm. of the calcium salt per kilogram of body weight.

In the present series of observations on human subjects, the highest rise in serum calcium obtained was 28.5 per cent in a single case after 10 Gm. of calcium lactate had been given. The average elevation for eight normal persons was 14 per cent. The maximal rise occurred anywhere from the first to the fifth hour. Seven of this group remained above their preingestion level at the end of the twelve hour period.

The maximal elevation obtained in nine persons after taking 5 Gm. of calcium lactate was 14 per cent, the average being 8 per cent. Only three of the nine subjects were back to their normal serum calcium level at the end of twelve hours. The rise of serum calcium obtained in our studies is certainly very small when compared to maximal rises of 43, 104, 105 and 238 per cent as reported by Kahn and Roe. Why there is such

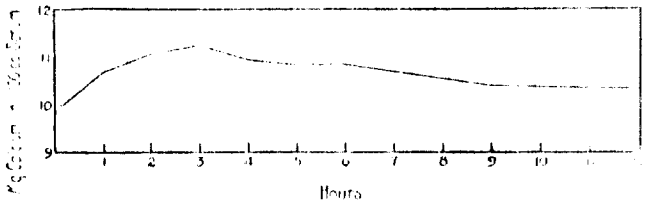

Chart 4.—Average curve for eight subjects after the ingestion of 10 Gm. of calcium lactate; the average maximal elevation is 14 per cent.

a marked discrepancy between our studies and those of Kahn and Roe is hard to say, for they state they have checked the Kramer-Tisdall method with their own chemical methods.

These observations in normal persons show that the fasting serum calcium level can be raised by the oral administration of calcium lactate, and that greater absorption is obtained after the ingestion of 10 Gm. than after the giving of 5 Gm. In the majority of subjects studied, this increase in serum calcium had not reached the preingestion level at the end of twelve hours. From our work it is impossible to say how much calcium is actually retained by the body, because no observations of calcium excretion were made.

SUMMARY

1. Calcium lactate administered in 5 Gm. doses to nine normal subjects gave a maximal elevation of 14 per cent of the serum calcium. This maximal rise comes between the first and fourth hours after ingestion, but some elevation is usually maintained over a period of more than twelve hours.

2. Calcium lactate given in 10 Gm. doses to eight normal subjects produced a similar, but more pronounced, increase in serum calcium, the maximal rise being 28 per cent, and occurring between the first and fifth hour. Again, some elevation was maintained above the fasting level for a period of twelve hours.

3. Our studies are in disagreement with those recently reported by Kahn and Roe, who have [illegible]

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increases in the blood calcium as high as 108 per cent after the ingestion of 5 Gm. of calcium lactate. This is more than seven times greater than we obtained in our maximal elevation after the same amount.

4. Similar observations on calcium absorption are being carried out on various types of endocrine disturbances to see if there is any deviation from the normal, and these will be reported at a later date.

INFANTILE TETANY

REPORT OF TWENTY-ONE CASES *

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The cases included in this series represent the total number of cases of infantile tetany treated in the Children's Hospital of Philadelphia during 1924 and the first nine months of 1925. These cases were studied at the instigation and under the direction of Dr. J. P. C. Griffith, to whom we are indebted for the privilege of reporting them. The cases have been studied from the aspects of etiology, symptomatology, blood studies and treatment.

ETIOLOGIC FACTORS

Eighteen of the infants were colored; three were white, of whom one was Italian, and two of Anglo-Saxon parentage.

Twelve infants, or 57.1 per cent, were males; nine, or 42.9 per cent, were females. That is, there were four males to every three females.

The age at which symptoms began was noted. From table 1, it will be seen that no cases began after the

TABLE 1.—*Age at Which Symptoms of Infantile Tetany Began*

2 months	5 cases	8 months	3 cases
3 months	1 case	9 months	1 case
4 months	1 case	10 months	3 cases
5 months	2 cases	12 months	1 case
6 months	2 cases	14 months	1 case
7 months	1 case		

fourteenth month, and that eleven cases, or 52.4 per cent, began before the seventh month. Five cases, or 23.8 per cent, began before the age of 3 months.

Klose[1] says that most authors place the age of onset too high and states definitely that the disease never occurs under the age of 2 months. Table 2 shows the

TABLE 2.—*Frequency of Infantile Tetany in Winter, Spring and Summer Months*

December	[illegible]	April	5 cases
January	[illegible]	May	2 cases
February	[illegible]	June	3 cases
March	[illegible]	July	1 case

frequency of infantile tetany in the winter, spring and summer months.

Rickets was present in slight degree as indicated by costal beading, in two cases; in moderate degree, as indicated by costal beading plus epiphyseal enlargement, in eleven cases; in severe degree, as indicated by the foregoing signs plus marked chest deformity, in six cases. In two cases, no clinical diagnosis of rickets could be made.

The length of time of breast feeding is given in table 3. The proportion of the presymptomatic period

TABLE 3.—*Length of Time Infants Were Breast Fed*

No breast feeding	4 cases	Breast fed 6 months	1 case
Breast fed 1 month	2 cases	Breast fed 8 months	1 case
Breast fed 2 months	5 cases	Breast fed 12 months	1 case
Breast fed 4 months	3 cases	Breast fed 13 months	1 case
Breast fed 5 months	1 case	Not stated	1 case

during which breast feeding was continued is given in table 4. Nearly one half (43 per cent) of the infants were breast fed when symptoms first occurred. No artificial feeding had been given to eight of the infants. Two had been fed on condensed milk dilutions. In three cases, the infants had been fed on both whole

TABLE 4.—*Proportion of Presymptomatic Period During Which Breast Feeding Was Continued*

In 9 cases	100 per cent	In 1 case	25 per cent
In 1 case	80 per cent	In 1 case	20 per cent
In 1 case	72 per cent	In 1 case	14 per cent
In 1 case	62 per cent	In 4 cases	No breast feeding
In 1 case	56 per cent	In 1 case	Not stated

milk and condensed milk dilutions. In one case, the type of feeding was not stated.

Fifteen infants had had no cod liver oil; four infants had been given cod liver oil in an irregular and inadequate manner; in two infants, this was not determined.

SYMPTOMATOLOGY

Carpopedal spasm was present in four of the infants, and was not seen in sixteen. In one of the infants' records, carpopedal spasm is not mentioned and probably was not present.

The facial reflex, or Chvostek's sign, was present in fifteen of the infants, and was absent in six.

The trousseau sign was present in six infants, absent in thirteen, and doubtful in two.

Laryngospasm was present in nine cases and absent in eleven. In one case, there is no mention as to whether or not laryngospasm was present.

Generalized convulsions were present in all twenty-one cases of the series.

In order to find out whether Erb's phenomenon was present, the threshold to electrical stimulation was

TABLE 5.—*Levels at Which Cathodal Closing Contractions Were Obtained*

[illegible]	[illegible]	4 [illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	3 [illegible]		

tested in thirteen of the twenty-one cases, according to the method described by Holmes.[2] The cathodal closing contraction was obtained at the levels shown in table [illegible]. Of the thirteen cases so studied, 84.6 per cent gave contractions below 5 milliamperes and 63.8 per cent gave contractions below 4 milliamperes.

BLOOD STUDIES

The large majority of cases showed a blood calcium of less than 9 mg. per hundred cubic centimeters, which

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Rev. Asoc. Med. Argentina 55(453/454):26-29. 1941.

Carratala-'41
Bib No. 254

COMPARATIVE TOXICITY OF THE PRINCIPAL SALTS OF CALCIUM

"El calcio en toxicología. Toxicidad comparada de sus principales sales"

Dr. Rogelio Carratala

I

As a constant element of the organism, the calcium ion is of great interest due to its metabolism, its quantitative variations in the tissues in relation to the different morbid states, and the repercussions of this variation in the organic content on the innermost physiological functions. It is well known that defective calcium metabolism is the cause of various incapacities.

Pharmacologically, the action of the calcium ion as a stimulant of the circulatory system was tested, measuring the increase in the number and the amplitude of cardiac movement, the increase in blood pressure. Also studied were its relation to the vagus, the parasympathetic system and the sympathetic terminations; its regulation of the conductivity and excitability of the neuro-muscular system; its property of decreasing the alkaline

reserve, acting in an acidic sense; its intervention in the processes of blood coagulation; its effect on the digestive system; its property of retarding inflammatory processes, etc. On the other hand, besides the ionic antagonism it causes in certain circumstances, its antitoxic function, more evident every day, should be considered a consequence of its pharmacological properties. The therapeutic use of calcium for intoxications should be based on the knowledge of the alteration that will result in its complicated metabolism. Since there are many toxic factors and circumstances capable of affecting calcemia, amounts of calcium in the blood, blood tests will serve effectively in that situation for the differential diagnostic. Besides the disturbance of the metabolism of calcium in the course of certain intoxications, there are various modalities in its metabolism, which are favorable or not to its therapeutic use in poisonings: a) it may act by fixing toxins, as occurs with some metals, and impeding their noxious effects in the circulatory torrent. This, in certain cases (lead), is considered a danger because calcium facilitates the accumulation of the toxic in the organism, b) it is effective for intoxications, such as those caused by carbon tetrachloride, due in part to a decrease in hepato-cellular lesions and a reduction in the alteration of the functional activity of Kupffer's cells, c) in some cases it aids in the elimination of some toxins; d) in various circumstances it increases tolerance to certain poisons, e) on occasion brings about normality in the presence of increase in nervous irritability; f) the decrease in the toxicity of certain substances determined by an excess of calcium is explained, at times by the reduction in cellular permeability; g) calcium tends to protect the heart from the effects of some toxins. However, the tone of the

miocardia increases: the calcium salt, when injected into the circulation, makes it possible for the heart to beat more vigorously; vasoconstriction has been verified, blood pressure goes up; h) of equal importance are the relationships of calcium with respect to modification of respiration caused by certain substances.

It is these diverse considerations that have established the therapeutic use of calcium in toxicology. The functional changes caused by alcohol, cocaine, magnesium, various metals, carbon tetrachloride, barbiturates, aspirin, digitalis, etc. are neutralized to a greater or lesser degree by calcium.

We have attempted to establish the therapeutic value for intoxications caused by cocaine (1), (2); alcohol (3); and barbiturates (4). In general calcium chloride, acting as an antitoxin, considerably attenuates motor excitation phenomena, cardiac and circulatory disturbances, psychological and sensitive disorders, and respiratory disturbances caused by acute cocaine intoxication. The effectiveness of intravenous injections of 10 % solutions of calcium chloride in the treatment of the effects produced by alcohol has been shown clinically and experimentally. We explain the neutralization of calcium by the barbiturates experimentally by the decrease in sympathetic and vagal activity resulting from barbituric anaesthesia.

Calcium therapy has been shown to be effective in cases of metallic intoxication. With its use, not only has a better tolerance to arsenical preparations been observed (Salvarsán, Neosalvarsán, etc.); but a lessening of the symptoms resulting from intoxications due to antimony, tartar emetic, and lead in saturnism has been established. The favorable action of calcium on the concomitant effects of gold salt therapeutics have been confirmed repeatedly. Accidents, stomatitis, etc. during the course of antisyphi-

litic treatments with mercury and bismuth compounds, except those due to lesions of the urinary apparatus, improve noticeably with use of calcium. Injections of calcium salts (chloride or gluconate) are indicated for thallium intoxications. Serious osseous and dental manifestations caused by flourine point out the importance of correct calcium metabolism. For cases of acute phosphoric intoxication, the use of calcium solutions aids blood coagulation and helps to prevent dangerous hemorrhages caused by the toxin as a result of the process of degeneration of the fat on the walls of the vessels, lack of blood coagulation, hepatic lesions, etc.

In cases of intoxication due to oxalic acid, the use of calcium is very helpful, even for stomach lavage. The lavage should be carried out using calcium chloride solutions of 10 parts per 1000, aiding the transformation of the toxic into calcium oxalate which is insoluble and less toxic. The intravenous injection of 10 cubic centimeters of 10 % calcium gluconate, which may be administered several times per day, is recommended. It, like 10 % calcium chloride administered intravenously and in the same dose, acts as an antitoxin.

In cases of intoxications due to certain gases, the favorable effect of calcium is due to a certain inhibition of the permeability of the epithelium of the pulmonary alveoli. The exanthems due to barbituric acid tend to be neutralized by calcium. It has been found to have favorable effects for quinine overdose. Calcium gluconate seems to act as a protector in aspirin intoxication. An increase in tolerance to carbon tetrachloride is observed with its use. In all cases carbon tetrachloride intoxication requires the administration of calcium, the defficiency of which is an important factor favoring the intoxication. In serious cases 10 % calcium gluconate solution should be injected intravenously at the rate of about one gram per day. In

less serious cases oral administration of repeated doses of calcium chloride is acceptable. In addition, the action of the calcium in this type of intoxication reduces the toxin's noxious effects on the hepatic tissue.

Digitalis and, in general, the glucosides with cardiovascular action and calcium are comparable in their action on the vessels and blood pressure.

In the therapeutic selection of the calcium salts, several factors should be considered: 1.) their calcium content. For instance, calcium gluconate contains 13 % CaO or 9.3 % calcium which is less than that contained in calcium chloride; 2.) their different rate of assimilation by the organism; 3.) their different toxicities.

Calcium has to be administered in the form in which it appears in vegetables if it is to be assimilated. Calcium must be absorbed in the ion state, in which form it is incorporated in the cells. The organism's use of calcium depends on the conditions under which it can be released. For that reason, neither organic nor inorganic calcium compounds can be assimilated by the organism if the dissociation does not occur. Calcium glycerophosphate, for example, which is easily dissociated by the digestive juices, is assimilable by the cells and is fixed in the tissues.

II

Experimental

DETERMINATION OF THE TOXICITY OF THE SALTS OF CALCIUM

Methodology. - The experiments were performed on not very young dogs and rabbits of similar size. The rabbits weighed more than one and a half kilos, and the dogs were of medium size. The calcium salts used were lactate, chloride and gluconate. The solutions were prepared just prior to

use and were prepared with distilled water and salts of known purity. The concentration was in all cases 10 %. In all cases the salts were administered intravenously to the immobilized animals; in the marginal vein of the rabbits' ears and in veins of the rear legs of the dogs. Two rates of injection were adopted: fast and slow. The slow rate was set at one cubic centimeter of solution per minute. The fast rate was set at two cubic centimeters per minute. The mortal and the minimum mortal doses were determined for each salt.

Results. - The effects produced by the mortal doses of these calcium salts were translated into respiratory, circulatory, temperature, diuresis, and pupil diameter changes. The symtomatology was severe with these doses. In some cases death, by syncope, is explosive. In general, changes in the frequency and rhythm occur. Extrasystoles, alternating cardiac arrests, and finally complete heart failure. These circulatory manifestations are always sharper and more intense with the lactate and chloride than with the gluconate.

From the point of view of respiration, the accentuated quickness of the stoppage of respiratory movements, similar to those of the heart movements, is observed. In other circumstances the high doses, in modifying the respiration, show typical Cheyne-Stokes. It was observed that calcium gluconate caused reduction of respiratory frequency with the smallest intensity.

The antipyretic effect was noted with all the calcium salts used and is more pronounced as the quantity of calcium injected increases. The drop in temperature, as observed in the rectum, was almost eight degrees from the physiological value. It was verified that, in general, under these conditions hypothermia is followed by hyperthermia.

Myosis, which is observed immediately upon injection of high doses, tends to persist for two to three hours, then slowly receding in the non-

mortal cases. Myosis seemed to us to be more pronounced in the rabbits than in the dogs.

Active intestinal movements were also observed, and there seemed to be a decrease in the diuresis. The animals receiving the calcium doses at the higher rate tended to experience convulsions, jerks, shivers. The high doses, however, caused depression. The minimum mortal doses, which are shown in the Tables, were an average of the various observations. They result in death by cardiac arrest alternating with periods of bradycardia and extrasystole. Myosis and depression are, at that point, very pronounced.

The results given in Table I show that, of the three 10 % calcium salt solutions injected intravenously to dogs and rabbits at the rate of one cubic centimeter per minute, the lactate is the most toxic. Its minimum mortal doses are 0.16 and 0.34 grams per kilo of weight for the dog and rabbit, respectively. Calcium chloride is the second most toxic with minimum mortal doses of 0.18 and 0.40 grams per kilo of weight for the dog and rabbit, respectively. Calcium gluconate is the least toxic with minimum mortal doses of 0.29 and 0.62 grams for the dog and rabbit, respectively.

Taking the minimum mortal doses of the lactate as unity, the doses of the other salts become: lactate, 1; chloride, 1.17; and gluconate, 1.82. These ratios show that the minimum mortal dose of calcium gluconate is almost twice that of the lactate and one and a half that of the chloride.

Toxicity Ratios

(Minimum Mortal Doses)

Lactate	1
Chloride	1.17
Gluconate	1.82

TABLE I

Determination of mortal and minimum mortal doses of 10 % calcium salt solutions per kilogram of weight and at a rate of 1 cubic centimeter per minute

Animal	Mortal Doses	Minimum Mortal Doses
	Calcium Lactate	
Rabbit	0.28 to 0.39 grams	0.34 grams
Dog	0.11 to 0.24 grams	0.16 grams
	Calcium Chloride	
Rabbit	0.35 to 0.50 grams	0.40 grams
Dog	0.14 to 0.30 grams	0.18 grams
	Calcium Gluconate	
Rabbit	0.51 to 0.66 grams	0.62 grams
Dog	0.18 to 0.34 grams	0.29 grams

TABLE II

Determination of mortal and minimum mortal doses of 10 % calcium salt solutions per kilogram of weight and at a rate of 2 cubic centimeters per minute

Animal	Mortal Doses	Minimum Mortal Doses
	Calcium Lactate	
Rabbit	0.12 to 0.24 grams	0.18 grams
Dog	0.06 to 0.13 grams	0.08 grams
	Calcium Chloride	
Rabbit	0.20 to 0.35 grams	0.26 grams
Dog	0.08 to 0.16 grams	0.10 grams
	Calcium Gluconate	
Rabbit	0.24 to 0.46 grams	0.38 grams
Dog	0.11 to 0.21 grams	0.16 grams

Upon increasing the speed of the intravenous injection (2 cubic centimeters of the solutions per minute) the toxicity of the calcium is observed to increase noticeably.(Table II). The minimum mortal doses of calcium lactate are now 0.08 and 0.18 grams per kilogram of weight for the dog and rabbit, respectively. For calcium chloride they are now 0.10 and 0.26 grams per kilogram of weight for the dog and rabbit, respectively. For calcium gluconate they are 0.16 and 0.38 grams per kilogram of weight for the dog and rabbit, respectively. These values not only demonstrate the greater toxicity of calcium with increased rate of injection; but also show that among the salts tested, the lactate still has the greatest toxicity. Calcium chloride is still second and the gluconate is the least toxic. Naturally, this must be a consequence of the varying amounts of calcium in each of these salts. The gluconate is the one with the smallest amount.

The ratios of the minimum mortal doses are now: 1, for the lactate; 1.44, for the chloride; and 2.11 for the gluconate. Consequently, the minimum mortal dose of calcium gluconate is slightly more than twice that of the lactate and almost one and a half times that of the chloride.

Toxicity Ratios

(Minimum Mortal Doses)

Lactate	1
Chloride	1.44
Gluconate	2.11

Conclusions

I) Toxicologically, knowledge of the action of calcium is important due to its metabolism, its pharmacological effects on the different organic

systems, and its favorable or unfavorable therapeutic uses for intoxications. With respect to this, the importance of its application in numerous intoxications caused by metals, metalloids, oxalic acid, alcohol, cocaine, aspirin, certain gases, barbiturates, carbon tetrachloride, etc. grows day by day.

II) The different calcium content, assimilation and toxicity are factors in the therapeutic selection of the calcium salts.

III) Determination of the mortal and minimum mortal doses of the 10 % solutions of calcium lactate, chloride and gluconate injected intravenously to dogs and rabbits at a rate of 1 cc. per minute revealed that the lactate is the most toxic of the three, followed by the chloride and lastly the gluconate. Taking the minimum mortal dose of the lactate as unity, the doses of the other salts become: lactate, 1; chloride, 1.17; and gluconate, 1.82. These ratios show that the minimum mortal dose of the gluconate is almost twice that of the lactate and one and a half times that of the chloride.

IV) Increase in the rate of the intravenous injection of calcium results in greater intensity of the pharmacological and toxic symptoms. The administration of 2 cc. of the solutions per minute yields the following ratios: lactate, 1; chloride, 1.44; and gluconate, 2.11. Consequently, the toxicity of calcium gluconate is still the least of the three. Its minimum mortal dose is slightly more than twice that of the lactate and almost one and a half times that of the chloride. These differences are due to different amounts of calcium in each of the salts.

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Rev. Asoc. Med. Argentina 55(453/454):26-29. 1941.

Carratala-'41
Bib No. 254

EL CALCIO EN TOXICOLOGIA TOXICIDAD COMPARADA DE SUS PRINCIPALES SALES (*)

POR EL

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I

Como elemento constante del organismo, el ión calcio interesa sobremanera por su metabolismo, sus variaciones cuantitativas en los tejidos en relación a los diferentes estados mórbidos, por las repercusiones de esta variación en el contenido orgánico sobre las más íntimas funciones fisiológicas. Es bien sabido que el metabolismo defectuoso del calcio es la causa generadora de diversas incapacidades.

Farmacológicamente, se examina la acción del ión calcio, como estimulante del aparato circulatorio con aumento en el número y en la amplitud de los movimientos cardíacos, con aumento de la presión sanguínea; por sus relaciones con el vago, el sistema orto-simpático o parasimpático y las terminaciones simpáticas; por la regulación de la conductibilidad y de la excitabilidad del aparato neuro-muscular; por la propiedad de disminuir la reserva alcalina, actuando en sentido acidósico; por su intervención en los procesos de coagulación sanguínea; por su acción sobre el aparato digestivo; por su propiedad de retardar los procesos inflamatorios, etc. Por otra parte, además del antagonismo iónico que determina en ciertas circunstancias, ha de considerarse como consecuencia de sus propiedades farmacológicas, la fundamental función antitóxica, cada día más evidente. El empleo terapéutico del calcio en las intoxicaciones debe basarse, sobre todo, en el conocimiento de la alteración que intervenga en su complicado metabolismo. Desde que son muchos los factores y las circunstancias tóxicas capaces de afectar la calcemia, las cifras sanguíneas en calcio, el examen de la sangre servirá eficazmente, en esa situación, para el diagnóstico diferencial. Además de los trastornos del metabolismo del calcio en el curso de ciertas intoxicaciones, en la acción del mismo, ha de reconocerse modalidades diversas, favorables o no a su empleo terapéutico en los envenenamientos: a) puede actuar fijando xicos, como ocurre con algunos metales, e impidie su acción nociva en el torrente circulatorio. Esto ciertos casos (plomo), se considera un peligro tente ya que el calcio facilita la acumulación del xico en el organismo; b) ejerce acción favorable intoxicaciones, como la determinada por el tetra ruro de carbono, debido en parte, por lo menos, disminución de las lesiones hépato-celulares y a disminución de la alteración de la actividad fun nal de las células de Kupfer; c) actúa, en otros sos, favoreciendo la eliminación de algunos tóxi d) en diversas circunstancias, aumentaría la tole cia a determinados venenos; e) en ocasiones, pro la normalidad en presencia del acrecentamiento la irritabilidad nerviosa; f) la disminución de la xicidad de ciertas substancias determinada por exceso de calcio se explica, a veces, por la disminu de la permeabilidad celular; g) el calcio suele teger al corazón de la acción de algunos tóxicos. A entonces, aumenta el tono del miocardio: la sal calcio, inyectada en la circulación, hace que el c zón pueda latir más vigorosamente; se compru vasoconstricción, la presión sanguínea se eleva; h) sultan igualmente importantes las relaciones del cio con respecto a las modificaciones impresas a respiración por determinadas substancias.

Son estas diversas consideraciones, las que han tablecido el empleo terapéutico del calcio en tox logía. Las modificaciones funcionales a que lle el alcohol, la cocaína, el magnesio, diversos met el tetracloruro de carbono, los barbitúricos, la asp na, la digital, etc., en sus acciones tóxicas, son neu lizadas, en mayor o menor proporción, por el cal

Hemos contribuído a establecer ese valor terap co en las intoxicaciones determinadas por la co na (1), (2); por el alcoholismo (3); y por los ba túricos (4). En general, el cloruro de calcio ate considerablemente, actuando como antitóxico y c antichoc, los fenómenos de excitación motora, los t tornos cardíacos y circulatorios, los desórdenes sitivos y psíquicos y los trastornos respiratorios, p ducidos por la *cocaína* en la intoxicación aguda. perimental y clínicamente, se ha demostrado la cacia de las inyecciones endovenosas de cloruro calcio, en solución al 10 %, en el tratamiento de manifestaciones producidas por el *alcohol*. Respe a la neutralización del calcio por los *barbitúricos* explicamos, experimentalmente, por la disminu de la actividad vagal y simpática a que lleva la a tesia barbitúrica.

En las *intoxicaciones metálicas*, la calcioterapia ha mostrado eficaz. Con su empleo, no sólo se ha ñalado la mejor tolerancia de *preparaciones ars cales* (Salvarsán, Neosalvarsán, etc.), sino que se establecido la atenuación de los síntomas en las i xicaciones por el *antimonio*, por el *tártaro emét* por el *plomo*, en el saturnismo. Se ha confirmado, petidas veces, la acción favorable del calcio sobre efectos concomitantes de la terapéutica por las *s de oro*. Los accidentes, estomatitis, etc., en el cu de tratamientos antisifilíticos con *preparados mer riales y bismúticos*, salvo los que se deben a lesio del aparato urinario, mejoran sensiblemente con calcio. Las inyecciones de sales de calcio (cloru lactato o gluconato) están indicadas en el curso de

(*) Comunicado a la Sociedad de Medicina Legal y Toxicología, en la sesión del 8 de Mayo de 1940.

intoxicaciones por el *talio*. Las serias manifestaciones óseas y dentarias, ocasionadas por el *fluor*, establecen la necesidad del metabolismo cálcico correcto. En la intoxicación *fosfórica* aguda, el empleo de soluciones de calcio favorecerá la coagulación de la sangre y contribuirá a evitar las peligrosas hemorragias que determina el tóxico a consecuencia del proceso de degeneración grasa de las paredes de los vasos, de la incoagulabilidad de la sangre, con lesiones hepáticas, etc.

En la intoxicación producida por el *ácido oxálico* es de acentuada conveniencia el empleo del calcio, aun para el lavado de estómago. Para ello, es de aconsejar tal lavado con soluciones de cloruro de calcio al 10 por 1000, facilitando de tal manera la transformación del tóxico en oxalato de calcio insoluble y menos tóxico. Se aconseja la inyección de 10 centímetros cúbicos de gluconato de calcio al 10 %, por vía intravenosa, que se podrá repetir varias veces al día y que, como el cloruro de calcio al 10 %, administrado por igual vía y dosis, actúa como antitóxico.

En intoxicaciones ocasionadas por algunos *gases*, la acción favorable del calcio se establecería por cierta inhibición de la permeabilidad del epitelio de los alveolos pulmonares. Los exantemas del *ácido barbitúrico* suelen ser neutralizados por el calcio. Se encuentra favorable acción del mismo en la hiperdosificación de la *quinina*. El gluconato de calcio, se viene mostrando como protector en la intoxicación determinada por la *aspirina*. Con su empleo se reconoce mejoría de la tolerancia del *tetracloruro de carbono*. En todos los casos, esta última intoxicación requiere la administración de calcio, el que neutralizará la deficiencia del mismo, factor importante que favorece la intoxicación. Se inyectará, por vía venosa, en los casos graves, gluconato de calcio en solución al 10 %, en la proporción de un gramo diario más o menos. En las formas leves, se podrá acudir a la vía bucal administrando el calcio (cloruro) en dosis repetidas. Por otra parte, la acción del calcio en esta intoxicación disminuye los efectos nocivos del tóxico sobre el tejido hepático.

La *digital* y, en general, los *glucósidos* de acción cardiovascular y el calcio, se equiparan en su acción sobre los vasos y sobre la presión sanguínea.

Ahora bien; en la elección terapéutica de las sales de calcio se han de considerar diversos factores que la harán variar: 1º) por su contenido en calcio. Así, el gluconato de calcio contiene 13 por 100 de CaO ó 9.3 por 100 de calcio, cantidad inferior a la contenida en el cloruro de calcio; 2º) por su diferente asimilación por el organismo; 3º) por su diversa toxicidad.

El calcio, para que pueda ser asimilado, debe ser administrado bajo la forma en que se presenta en los vegetales. El calcio debe ser absorbido al estado de ión, forma en que se incorpora a las células. La utilización del calcio por el organismo depende de las condiciones en las que puede ser liberado. Por ello, una combinación cualquiera de calcio, orgánico o no, no podrá ser absorbida por el organismo, si la disociación no se establece. El glicerofosfato de calcio, por ejemplo, fácilmente disociable por los jugos digestivos, es asimilable por las células, fijado en los tejidos del organismo.

II

Experimental

DETERMINACION DE LA TOXICIDAD DE SALES DE CALCIO

Método. — Las determinaciones se han realizado en conejos y perros, no muy jóvenes; de talla aproximadamente similar: los conejos de más de un kilo y medio; los perros de talla mediana. Las sales de calcio empleadas han sido el lactato, el cloruro y el gluconato. Las soluciones, en agua destilada, preparadas en el momento de usarlas, con sales cuya pureza fué previamente establecida. La concentración en todos los casos lo fué de 10 %. La inyección, en animales inmovilizados, se realizó, en todas las circunstancias, por vía venosa: en la vena marginal de la oreja del conejo y en el perro en una vena de la pata posterior. Se adoptaron dos tipos de velocidad de la inyección: lenta y alta. La velocidad lenta se ha realizado en proporción de 1 centímetro cúbico de la solución por minuto; la alta, doblando la dosis, de dos centímetros de la solución, por cada minuto. Se han determinado, para cada sal, las dosis mortales y las dosis mínimas mortales.

Resultados. — Los efectos producidos por las dosis mortales de estas sales de calcio se traducen en alteraciones respiratorias, circulatorias, de la temperatura, de la diuresis y del diámetro de la pupila. La sintomatología, con estas dosis, es severa. En algunos casos, la muerte, por síncope, es fulminante. En general, se aprecian modificaciones en la frecuencia y a veces en el ritmo. Se comprueban extrasístoles; paros cardíacos alternados y, finalmente, completo paro cardíaco. Estas manifestaciones circulatorias son siempre más nítidas e intensas con el lactato y cloruro de calcio que con el gluconato.

Desde el punto de vista respiratorio, se observa en ciertos casos, al igual que lo comprobado con los movimientos del corazón, la acentuada rapidez del paro de los movimientos respiratorios. Las altas dosis, en otras circunstancias, al modificar la respiración, permiten comprobar típico Cheyne-Stokes. Se observa que el gluconato de calcio es el que con menos intensidad determina disminución de la frecuencia respiratoria.

La acción antipirética se revela con todas las sales de calcio empleadas y es tanto más acentuada a medida que se eleva la cantidad de calcio inyectada. El descenso de temperatura, observada en el recto, alcanza en ciertos casos de intoxicación mortal con dosis elevadas, hasta ocho grados del valor fisiológico. Se comprueba que, en general, en estas condiciones la hipotermia es seguida de hipertermia.

La miosis, comprobada desde que se practica la inyección de dosis altas, se suele mantener hasta dos o tres horas; luego va cediendo paulatinamente en los casos no mortales. Esta miosis nos ha parecido más pronunciada en el conejo que en el perro.

Se comprueba también movimientos intestinales activos y parece demostrarse una disminución de la diuresis. Los animales que reciben las dosis de calcio a mayor velocidad suelen demostrar rápidamente fenómenos convulsivos, sacudidas, temblores, sobresaltos. Las dosis altas, mortales, llevan, en cambio a la acción depresiva. Las dosis mínimas mortales, ex

presadas en los cuadros, resultado término medio de diversas observaciones, llevan a la muerte por paro del corazón, alternando con períodos de definida bradicardia y con extrasístole. La miosis y la depresión es, entonces, muy pronunciada.

Los resultados mencionados en el cuadro I revelan que, de las tres sales de calcio, en solución al 10 %, empleadas en perros y conejos, por vía venosa y a velocidad de 1 centímetro cúbico de las mismas por minuto, el lactato se muestra más tóxico siendo la dosis mínima mortal de 0.gr.16 y 0.gr.34, por kilo de peso, para el perro y el conejo, respectivamente. Le sigue en toxicidad el cloruro de calcio que presenta como dosis mínima mortal la de 0.gr.18 y 0.gr.40, por kilo de peso, en el perro y en el conejo respectivamente. El gluconato de calcio demuestra ser el menos tóxico: las dosis mínima mortales son de 0.gr.29 y 0.gr.62, para el perro y el conejo, respectivamente.

CUADRO I

Determinación de dosis mortales y mínimas mortales de sales de calcio en solución al 10 %, por kilo de peso, y a velocidad de 1 centímetro cúbico por minuto

Animal	Dosis mortales	Dosis mínimas mortales
	Lactato de calcio	
Conejo ...	0.g.28 a 0.g.39	0.g.34
Perro	0.g.11 a 0.g.24	0.g.16
	Cloruro de calcio	
Conejo ...	0.g.35 a 0.g.50	0.g.40
Perro	0.g.15 a 0.g.30	0.g.18
	Gluconato de calcio	
Conejo ...	0.g.51 a 0.g.66	0.g.62
Perro	0.g.18 a 0.g.34	0.g.29

Relación de toxicidad

(Dosis mínimas mortales)

Lactato	1
Cloruro	1.17
Gluconato	1.82

Considerando como la unidad la dosis mínima mortal del lactato, las dosis de las otras sales se transforman en: lactato, 1; cloruro, 1.17 y gluconato, 1.82. Estas proporciones, revelan que la dosis mínima mortal del gluconato de calcio es casi dos veces aquella del lactato y una vez y media aquella del cloruro.

CUADRO II

Determinación de dosis mortales y mínimas mortales de sales de calcio en solución al 10 %, por kilo de peso, y a velocidad de 2 centímetros cúbicos por minuto

Animal	Dosis mortales	Dosis mínimas mortales
	Lactato de calcio	
Conejo ...	0.g.12 a 0.g.24	0.g.18
Perro	0.g.06 a 0.g.13	0.g.08
	Cloruro de calcio	
Conejo ...	0.g.20 a 0.g.32	0.g.26
Perro	0.g.08 a 0.g.16	0.g.10
	Gluconato de calcio	
Conejo ...	0.g.24 a 0.g.46	0.g.38
Perro	0.g.11 a 0.g.21	0.g.16

Relación de toxicidad

(Dosis mínimas mortales)

Lactato	1
Cloruro	1.44
Gluconato	2.11

Al acentuar la velocidad de la inyección endovenosa (2 centímetros cúbicos de las soluciones, por minuto), se observa (cuadro II) que la toxicidad del calcio se aumenta en forma pronunciada. Ahora, para el lactato de calcio, la dosis mínima mortal es de 0.g.08 y 0.g.18, por kilo de peso, para el perro y el conejo, respectivamente. Para el cloruro de calcio, de 0.g.10 y 0.g.26, por kilo de peso, para el perro y el conejo, respectivamente. Para el gluconato de calcio, de 0.g.16 y 0.g.38, por kilo de peso, para el perro y el conejo, respectivamente. Estos totales, si bien demuestran la mayor toxicidad del calcio en proporción a la velocidad, revelan también que, entre las sales examinadas, el lactato sigue siendo el de mayor toxicidad; le sigue el cloruro y, por último, el de menor toxicidad resulta ser el gluconato. Esto, como es natural, tiene que ser la consecuencia de la diferente proporción en calcio de cada una de las sales; el gluconato es el que contiene la menor cantidad.

La proporción aquí, de las dosis mínimas mortales, es: 1, para el lactato; 1.44, para el cloruro; y 2.11, para el gluconato. En consecuencia, la dosis mínima mortal del gluconato de calcio es algo más de dos veces aquella del lactato y casi una vez y media aquella del cloruro.

Conclusiones

I) Toxicológicamente, el conocimiento de la acción del calcio, interesa por su metabolismo, sus acciones farmacológicas sobre los distintos sistemas orgánicos, por las distintas modalidades, favorables o no, para su empleo terapéutico en las intoxicaciones. A este respecto, se acentúa, día a día, la importancia de su aplicación en numerosas intoxicaciones determinadas por metales, metaloides, el ácido oxálico, el alcohol, la cocaína, la aspirina, ciertos gases, los barbitúricos, el tetracloruro de carbono, etc.

II) En la elección terapéutica de las sales de calcio interviene el diferente contenido en calcio de las mismas; su diversa asimilación y su distinta toxicidad.

III) La determinación de las dosis mortales y mínimas mortales del lactato, cloruro y gluconato de calcio, en solución al 10 %, empleadas en perros y conejos, por vía venosa y a velocidad de 1 cc. de las mismas por minuto, revela que el lactato es el más tóxico de los tres, siguiéndole el cloruro y, por último, el gluconato de calcio. Considerando como la unidad la dosis mínima mortal del lactato, las dosis de las otras sales se transforman en: lactato, 1; cloruro, 1.17 y gluconato, 1.82. Estas proporciones revelan que la dosis mínima mortal del gluconato es casi dos veces aquella del lactato y una vez y media aquella del cloruro.

IV) El factor de la velocidad de las inyecciones intravenosas de calcio determina mayor intensidad de los síntomas farmacológicos y tóxicos. En efecto, la administración de dos centímetros cúbicos de las soluciones, por minuto, lleva a la siguiente proporción: lactato, 1; cloruro, 1.44; y gluconato, 2.11. En consecuencia, la toxicidad del gluconato de calcio,

siempre menor que la de las otras sales, se revela en que la dosis mínima mortal de esta sal es de algo más de dos veces aquella del lactato y casi una vez y media aquella del cloruro. Estas diferencias, encuentran su explicación en la diferente proporción en calcio de cada una de las sales.

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J. Biol. Chem. 81(2):389-403. 1929.

Cori-'29
Bib No. 315

GLYCOGEN FORMATION IN THE LIVER FROM *d*- AND *l*-LACTIC ACID.

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(Received for publication, December 14, 1928.)

The question as to whether or not the liver is able to form glycogen from lactic acid is of importance for the relation between the carbohydrate metabolism of the muscles and that of the liver. It is known that the process glycogen ⇄ lactic acid occurs in the muscles, while the process glycogen ⇄ glucose occurs in the liver. Glucose derived from liver glycogen is convertible into muscle glycogen; it is, however, not definitely known whether lactic acid derived from muscle glycogen is convertible into liver glycogen. If this should prove to be the case, the glucose molecule would be capable of a complete cycle in the body; it could in turn be liver glycogen, blood sugar, muscle glycogen, blood lactic acid, and again liver glycogen. If, on the other hand, the liver is unable to form glycogen (or glucose) from lactic acid, this cycle would be interrupted as soon as the glucose molecule was deposited as muscle glycogen.

Mandel and Lusk (1) found that *d*-lactic acid, when administered to phlorhizinized dogs, leads to the excretion of extra glucose in the urine. This might be interpreted in the sense that, since lactic acid is convertible into glucose in the liver of the diabetic animal, it should also be able to form liver glycogen in the normal animal. However, the literature is contradictory on this point. Röhmann (2) states that ammonium and sodium lactate, when fed to rabbits fasted previously for 3 to 4 days, leads to glycogen deposition in the liver. Parnas and Baer (3) concluded that glycogen synthesis occurred during perfusion of the isolated turtle liver with sodium lactate. Barrenscheen (4), on the other hand, reported negative results in perfusion experiments on isolated livers of rabbits and dogs. Izume and Lewis (5) injected sodium lactate subcutaneously into two fasting rabbits and observed glycogen deposition in the liver. Takane (6) in Meyerhof's laboratory, obtained carbohydrate synthesis from sodium lactate which was added to sections of liver tissue suspended either in Ringer's solution

or blood serum. It appears from a remark on p. 417 of Takane's paper that glucose rather than glycogen was formed from the added sodium lactate, which this author ascribes to the special conditions of his *in vitro* experiments. Abramson, Eggleton, and Eggleton (7), working on dogs anesthetized with ether and amytal, were unable to demonstrate glycogen synthesis in the liver following intravenous injection of sodium *r*-lactate, though the injection of glucose, under the same conditions, led to glycogen formation in the liver.

The writers became interested in the question of glycogen formation from lactic acid when it was found that epinephrine when injected into 24 hour fasting rats, causes a simultaneous disappearance of muscle glycogen and an increase in liver glycogen in approximately equivalent amounts (8). It was suggested that part of the disappearing muscle glycogen was converted into liver glycogen with lactic acid as an intermediary stage, since there was no other obvious source for the newly formed liver glycogen and since carbohydrate oxidation was too low to account for the muscle glycogen which disappeared. In view of these results the experiments reported in the present paper were undertaken.

Meyerhof and Lohmann (9) found a marked difference in the utilization of *d*- and *l*-lactic acid in isolated mammalian tissues. Whereas *d*-lactic acid, when added to sections of liver, kidney, and brain tissue, increased the respiration, *l*-lactic acid had a doubtful effect. Liver tissue was able to synthesize carbohydrate from *d*-lactic acid but hardly from *l*-lactic acid.

The rate at which sodium *d*-lactate could be infused intravenously into rats without causing an appreciable rise in the lactic acid content of blood and urine was 95± 5 mg. per 100 gm. of body weight per hour (10). When the experiments were repeated with sodium *r*-lactate, a considerable amount of lactic acid was excreted in the urine (10). This suggested that there was also a difference in the utilization of the *d*- and *l*-lactic acid in the intact animal. It was therefore decided to use both forms of lactic acid in the investigation of glycogen formation in the liver and to include observations about the rate of absorption from the intestine and lactic acid content of blood and urine.

Methods.

The experiments were made on male rats after a fasting period of 24 hours. The weight of the animals at the time of feeding

varied between 100 and 160 gm. Sodium *d*-, *r*-, or *l*-lactate was fed by stomach tube in the manner described in a previous paper (11). The amount of fluid introduced was 2.2 cc. In some experiments 2.5 to 3.5 cc. of a 6 per cent solution of free lactic acid were fed without ill effects. When sodium lactate was injected subcutaneously, three equal doses were given with an interval of 1 hour between each injection. When it became desirable to feed a constant amount of lactic acid per unit of body weight, the following procedure was adopted. The concentration of the lactic acid in the stock solution was determined by titration with phenolphthalein as indicator and by the Friedemann, Cotonio, and Shaffer method (12) with very satisfactory agreement. The desired amount of lactic acid solution was delivered from a burette into a small beaker. After addition of a small drop of methyl red, the solution was neutralized with 20 per cent NaOH. The fluid in the beaker was drawn up with a syringe and injected through the catheter into the stomach. Beaker, syringe, and catheter were then washed out with a small quantity of water. All animals were killed 3 hours after the administration of lactic acid.

Blood for duplicate lactic acid determinations was collected after decapitation. The muscular movements during the collection of blood make the lactic acid values slightly too high, because, when blood is collected in the same manner from animals under amytal anesthesia, lower values for blood lactic acid are found. The average for six determinations on normal rats under amytal anesthesia was 26.8 mg. per cent (10), while in the present series of determinations in Table I the average was 41.5 mg. per cent. Urine was collected by placing the small wire screen cages, in which the animals were kept, on plates. Urine remaining in the bladder after the death of the animals was added to that voided spontaneously. The urine was made up to a volume of approximately 20 cc. in a 25 cc. volumetric flask, and 2.5 cc. each of 10 per cent $CuSO_4$ and 5 per cent $Ca(OH)_2$ were added in order to remove interfering substances. After 30 minutes standing the solution was filtered and analyzed. Extraction of the lactic acid from the urine with ether was found unnecessary, since the direct method gave only slightly higher values than after ether extraction.

After collection of blood, the liver was removed as quickly as

possible, frozen with CO_2 delivered from a tank, weighed in the frozen state, cut into small pieces, and introduced into boiling 60 per cent KOH. Glycogen was determined according to Pflueger's method. After hydrolysis of the glycogen with 2.2 per cent HCl, neutralization, and filtration, sugar was determined by means of the Bertrand method. If less than 10 mg. of sugar was present in 20 cc. of the solution to be analyzed, a known amount of glucose was added before the determination was carried out.

For the determination of the amount of lactic acid absorbed from the intestine, a method was used which had been worked out previously for the determination of sugar absorption (11). The difference between the amount of lactic acid fed and the amount of lactic acid recovered from the whole intestinal tract represents the amount of lactic acid absorbed. It is necessary to apply a slight correction because the intestine of a rat weighing 100 gm. contains on an average 10.9 mg. of lactic acid (Table I). After the death of the animal, the esophagus was tied and the whole intestinal tract was removed and placed in a beaker. Stomach and intestines were cut open and extracted with repeated portions of hot water on a water bath at 100°. The washings were poured into a 250 cc. volumetric flask, and before being made up to the mark, 12 cc. of colloidal iron and a few drops of a saturated solution of sodium sulfate were added. An aliquot part of the filtrate was treated with copper sulfate and lime in order to remove interfering substances. The final filtrate used for the lactic acid analysis was water-clear. Control experiments showed that lactic acid is not included in the colloidal iron precipitate.

All lactic acid determinations were carried out by the Friedemann, Cotonio, and Shaffer method (12). When this method was first used, and occasionally later, analyses of zinc lactate solutions containing known amounts of lactic acid were made. The recovery of lactic acid was of the same magnitude as reported by the above authors. Recently, the modification of Kendall and Friedemann (13), in which the permanganate used for the oxidation is substituted by colloidal manganese, was used with equal success.

Preparation of d- and l-Lactic Acid.[1]

Pederson, Peterson, and Fred (14) found that certain bacteria produce only the *d* form of lactic acid from glucose. Dr. Peter-

[1] The older designation of the sarcolactic acid as *d*-lactic acid is used in this paper.

son kindly consented to isolate a sufficient quantity of *d*-lactic acid from the bacteria cultures for our purpose. His method of preparation was as follows: The fermented media, glucose-yeast water, were evaporated to a small volume, acidified, and the lactic acid extracted with ether. The acid was converted into the zinc salt by boiling with zinc carbonate. A sample for analysis gave 13.1 per cent water of crystallization. The zinc salt was dissolved in water, acidified, and extracted with ether. After the ether was removed, the solution was decolorized with norit and concentrated to about 38 per cent of lactic acid. A sample was converted into the zinc salt and an analysis gave 12.6 per cent water of crystallization. A sample of zinc lactate prepared by us 18 months later from the same *d*-lactic acid solution gave the following analytical results:

$Zn(C_3H_5O_3)_2 + 2\ H_2O$.

Found.	H_2O,	13.06	per cent;	ZnO,	29.09	per cent.
Calculated.	"	12.89	" "	"	29.11	" "

(The racemic zinc lactate, which crystallizes with 3 molecules of water, yields 18.17 per cent H_2O and 26.73 per cent ZnO.) The rotation of a 2.5 per cent solution of the water-free salt in a 2.2 dm. tube was $\alpha = -0.45°$. $[\alpha]_D^{25} = -8.2°$. The magnitude of rotation of the *d*- and *l*-zinc lactate depends very much on the concentration. Jungfleisch and Godchot (15) reported for the same concentration (2.5 per cent of the water-free salt) $-8.0°$. Meyerhof and Lohmann (9) found for the *l*-zinc lactate $+8.1°$ and Neuberg (16) $+8.2°$.

The *l*-lactic acid was prepared according to Irvine's method (17), consisting of a resolution of the racemic acid with morphine. A concentrated solution of C. P. lactic acid was diluted to 20 per cent and was heated for 6 hours under a reflux condenser in order to destroy the anhydride. The hot solution was neutralized with morphine, filtered, and allowed to crystallize. A second crop of morphine *l*-lactate was obtained upon concentration of the mother liquor.[2] The salt was recrystallized from 50 per cent alcohol, dissolved in water, and decomposed with ammonia. After filtration and acidification, the free lactic acid was extracted with

[2] Up to this point, the preparation was carried out by Dr. Pucher of the Department of Biochemistry of the University of Buffalo.

ether in a continuous extraction apparatus. After the ether was removed, the solution was concentrated to 20 per cent of lactic acid. A sample was converted into the zinc salt by boiling with $ZnCO_3$ and was analyzed with the following results.

Air-dry (20 hours at 37°).....	0.3146 gm.	$Zn(C_3H_5O_3)_2 + 2H_2O$.
Heated for 4 hours at 120°....	0.2737 "	
Difference.....	0.0409 "	= 13.00 per cent H_2O.
Air-dry before ashing.....	0.2649 "	
After ashing.....	0.0778 "	= 29.37 per cent ZnO.
$[\alpha]^{20}_{D}$.....	+ 8.0°	(c = 2.5 per cent of water-free salt, l = 2.2 dm., α = + 0.44°).

TABLE I.

Glycogen Content of Liver of 24 Hour Fasting Rats.

Average body weight 116 ± 10 gm.

Per 100 gm. body weight.			Liver glycogen.	Blood lactic acid.
Weight of liver.	Glycogen in liver.	Lactic acid in intestine.		
gm.	*mg.*	*mg.*	*per cent*	*mg. per cent*
3.73	4.2	12.2	0.11	47.0
3.21	2.1	8.3	0.06	46.5
3.35	2.8	12.0	0.08	34.8
3.40	1.8	10.7	0.05	43.4
3.46	4.0	11.2	0.11	42.5
3.43	2.9	11.0	0.08	35.1
3.25*	1.9		0.06	
3.18*	7.7		0.24	
3.38	3.4	10.9	0.10	41.5

* Fed 2.5 cc. of saline; killed 3 hours later.

Results.

The rats were subjected to a fasting period of 24 hours before lactic acid was fed in order to reduce the liver glycogen to a low level. The glycogen content of the liver of 24 hour fasting rats was determined on a series of eight control rats (Table I). The average was 0.1 per cent liver glycogen, or, since the liver weight was 3.38 per cent of the body weight, 3.4 ±1.4 mg. of glycogen for the liver of a 100 gm. rat. In previous determinations on 24 hour

fasting rats the average for sixteen experiments was 0.2 per cent or 7 ±2 mg. of liver glycogen per 100 gm. of rat (8). The values found by Macleod and collaborators (18) on rats fasted previously for 24 hours are in substantial agreement. In forty-eight experiments the average glycogen content was 0.16 per cent. The liver weight was not recorded, but assuming it to be on an average the same as in our experiments, this would correspond to 5.4 mg. of liver glycogen per 100 gm. of rat. It need hardly be pointed out that the remarkable constancy of the liver glycogen of 24 hour fasting rats makes such animals well suited for a study of glycogen formation. This rules out many uncertainties which are met with when glycogen formation in the liver of larger species is investigated. The possibility of determining absorption in the rat is a further advantage.

A comparison of the data in Tables I and II shows that the liver is able to form glycogen from lactic acid. There was only a small difference in the amount of glycogen formed during absorption of *d*- and *r*-lactate (on an average 53 mg. against 41 mg.). It would, however, be wrong to conclude from this that *l*-lactic acid is able to form liver glycogen as rapidly as *d*-lactic acid. This is not the case, as will be shown later. In order to explain the small difference between *d*- and *r*-lactate, the absorption from the intestine must be taken into consideration. The rats receiving *d*-lactate absorbed on an average 89.7 mg., while the rats receiving inactive lactate absorbed 115.1 mg., one-half of which (57.5 mg.) is *d*-lactic acid. The difference between the absorption of 89.7 and 57.5 mg. is not great enough to affect appreciably the rate of glycogen formation in the liver. The same is true for the experiments in which free *d*-lactic acid was fed, where the average absorption amounted to only 62.7 mg., while the amount of glycogen formed in the liver was 43 mg.

The percentage of absorbed lactic acid which is retained as glycogen in the liver is surprisingly high (Table II). It amounted on an average to 61.8 per cent when sodium *d*-lactate was fed and to 72.2 per cent when free *d*-lactic acid was given. In two cases, more than 95 per cent of the absorbed lactic acid was retained in the liver as glycogen. The percentage retention after feeding racemic lactate is only 34.2 per cent, because one-half of the lactic acid absorbed, namely the *l*-lactic acid, forms practically no

liver glycogen. Of glucose, fructose, and dihydroxyacetone 18, 38, and 21 per cent respectively of the amounts absorbed are retained as liver glycogen (19). On a percentage basis *d*-lactic acid is therefore a better glycogen former in the liver than any of these three sugars. It should be mentioned however that sodium lac-

TABLE II.

Glycogen Content of Liver 3 Hours after Lactic Acid Feeding.

Per 100 gm. body weight.				Liver glycogen.	Liver glycogen in per cent of amount absorbed.	Blood sugar.	Remarks.
Amount fed.	Amount absorbed.	Weight of liver.	Glycogen in liver.				
mg.	*mg.*	*gm.*	*mg.*	*per cent*		*mg. per cent*	
89	69.6	3.31	66.7	2.01	95.8	115	Sodium *d*-lactate. Average body weight, 145 ± 7 gm.
116	86.7	3.20	36.8	1.15	42.4	100	
125	90.1	3.28	57.8	1.76	64.1	113	
138	112.4	3.76	50.4	1.34	44.8	108	
117	89.7	3.39	52.9	1.56	61.8	109	
148	84.0	3.05	25.0	0.82	28.8	105	Sodium *r*-lactate. Average body weight, 150 ± 9 gm.
172	91.3	3.06	24.8	0.81	27.2	96	
251	135.2	3.74	54.6	1.46	40.4	116	
267	150.0	3.65	58.8	1.61	39.2	108	
209	115.1	3.37	40.8	1.18	34.2	106	
98		3.28	32.1	0.98			*d*-Lactic acid. Average body weight, 147 ± 9 gm.
102	43.1	3.65	34.3	0.94	79.5		
112	46.5	2.76	24.0	0.87	51.6		
120	61.0	3.68	59.2	1.61	97.0		
150	84.2	4.10	50.8	1.24	60.3		
210*	79.0	3.55	57.2	1.61	72.3		
132	62.7	3.50	42.9	1.21	72.2		

* One-half neutralized with NaOH.

tate is absorbed approximately 7 times more slowly than glucose and $3\frac{1}{2}$ times more slowly than fructose.

The blood sugar level is hardly changed during lactic acid absorption and the same is true following subcutaneous injection of lactate. This confirms the work of Janssen and Jost (20), Riegel (21), and Abramson, Eggleton, and Eggleton (7), who in-

jected sodium lactate intravenously. Izume and Lewis (5) state that sodium lactate in doses less than 2.0 gm. per kilo did not induce any appreciable hyperglycemia in fasting rabbits, while larger doses produced an increase in blood sugar.

The mechanism of absorption of lactic acid needs further investigation. In marked contrast to glucose and other sugars (11), the rate of absorption of sodium lactate and of free lactic acid depends on the amount fed. This is shown in Table II, in which the experiments are arranged according to the amount fed. Another striking difference exists between the absorption of sugars and lactic acid. Whereas isomeric sugars are absorbed at widely different rates from the intestine, for instance, mannose is absorbed 5 times more slowly than glucose (11), *d*- and *l*-lactate are absorbed at nearly the same rate (Table III). There is still one point which should be mentioned in connection with the experiments in Table II. For an equal amount fed, free lactic acid is absorbed more slowly than sodium lactate, but this is probably due to the acid reaction in the former case rather than to an intrinsic difference.

In order to afford a better comparison between optically active and inactive lactate, a standard amount of lactic acid was fed in all further experiments, namely 170 mg. per 100 gm. of body weight (Table III). This led to the absorption of nearly the same amounts of lactic acid in the three cases, the average being 111 mg. for *d*-lactate, 124 mg. for *l*-lactate, and 108 mg. for *r*-lactate. For an equal amount absorbed, *r*-lactic acid forms definitely less liver glycogen than *d*-lactic acid (26.1 mg. against 43.8 mg.). This is due to the fact that glycogen formation from *l*-lactic acid is almost entirely absent. It will be noted in Table III that the livers of the rats receiving *l*-lactate contained 10 $\pm$ 2.8 mg. of glycogen, while the livers of the control rats in Table I contained 3.4 $\pm$1.4 mg. This is perhaps not an entirely negative result, though the difference is very slight indeed. The *l*-lactic acid used in these experiments, on the basis of the analyses made, is regarded as sufficiently pure to exclude an appreciable admixture of *d*-lactic acid, which, if it were present, would account for the small amount of liver glycogen formed. It is possible therefore that *l*-lactic acid is able to form liver glycogen at a very slow rate.

Another striking difference between *d*- and *l*-lactic acid is found when the figures for blood and urine lactic acid are compared (Table III). Whereas during 3 hours of absorption of *d*-lactate only 0.5 mg. of lactic acid is excreted, 36.5 mg. appear in the urine

TABLE III.

Comparison of d-, l-, and r-Lactic Acid.

170 mg. of lactic acid per 100 gm. of body weight were fed in each case. The rats were killed 3 hours after the feeding.

Per 100 gm. body weight.				Liver glycogen.	Liver glycogen in per cent of amount absorbed.	Blood lactic acid.	Remarks.
Weight of liver.	Amount absorbed.	Amount excreted.	Glycogen in liver.				
gm.	*mg.*	*mg.*	*mg.*	*per cent*		*mg. per cent*	
3.78	108	0.4	52.0	1.37	48.1	43.6	Sodium *d*-lactate. Average body weight, 110 ± 9 gm.
3.59	103	0.4	56.5	1.57	54.8	53.8	
3.60	118	0.6	32.6	0.91	27.6	63.7	
3.58	116	0.7	34.2	0.95	29.4	54.6	
3.64	111	0.5	43.8	1.20	39.6	53.9	
3.48	104	27.9	8.6	0.24		113.8	Sodium *l*-lactate. Average body weight, 103 ± 3 gm.
3.14	132	28.6	12.7	0.40		78.9	
3.13	126	49.0	5.5	0.18		81.0	
3.21	135	41.4	13.2	0.41		87.1	
3.77	113	41.9	12.5	0.33		65.5	
3.32	134	30.5	7.5	0.23		93.1	
3.34	124	36.5	10.0	0.30		86.6	
3.28	107	1.2	22.5	0.68	21.0	53.2	Sodium *r*-lactate. Average body weight, 107 ± 5 gm.
3.26	96	1.6	35.1	1.08	36.5	68.2	
3.10	105	0.9	30.8	1.00	29.3	75.0	
3.35	123	2.8	16.0	0.47	13.0	83.2	
3.25	108	1.6	26.1	0.80	24.9	70.0	

when *l*-lactate is fed. This corresponds to an excretion of 29.4 per cent of the amount absorbed. The increase in blood lactic acid after the *l*-lactate feeding corresponds to a retention of 18 per cent of the amount absorbed, if it is assumed that the blood lactic acid is in equilibrium with 50 per cent of the body weight.

This leaves roughly 50 per cent of the amount absorbed or 62 mg. of *l*-lactic acid which, presumably, were utilized in the body in the course of 3 hours. Since *d*-lactate may be injected intravenously at a rate of 95 mg. per 100 gm. of rat per hour without causing an appreciable increase in blood lactic acid, or excretion in the urine (10), *l*-lactic acid is utilized approximately 4 times more slowly in the rat than *d*-lactic acid. A comparison of the experiments with *d*- and *r*-lactate in Table III, when based on excretion in the urine, does not reveal that there exists such a marked difference in the utilization of *d*- and *l*-lactic acid. This is due to the fact that after *r*-lactate feeding only half as much *l*-lactic acid is absorbed as after *l*-lactate feeding. In the latter case the rate of absorption of *l*-lactic acid was found to be 50 per cent higher than the rate of utilization. In the former case the rate of absorption does not exceed the rate of utilization and consequently the excretion in the urine is very small.

It remains to be determined how far a kidney factor might be involved in the different utilization of *d*- and *l*-lactic acid. Hewlett, Barnett, and Lewis (22) found in men that the threshold for lactic acid excretion is between 30 and 40 mg. per cent of blood lactic acid. This coincides fairly well with the values observed on rats under amytal anesthesia, when *d*-lactate was infused intravenously (10). As stated under "Methods," the lactic acid values in the present paper are approximately 15 mg. per cent too high, because muscular movements during the collection of blood were not abolished by an anesthetic, but this does not affect the following considerations. In Table III the average blood lactic acid after *d*-lactate feeding was 12.4 mg. higher than that of the control rats in Table I. Since after *r*-lactate feeding only half as much *d*-lactic acid is absorbed, it might be assumed that the increase in blood lactic acid was almost entirely due to *l*-lactic acid. This would give a concentration of 28.5 mg. per cent of *l*-lactic acid in the blood, at which level no appreciable quantity of lactic acid is excreted in the urine. After *l*-lactate feeding the average blood lactic acid was 45.1 mg. per cent higher than that of the control rats, and at this concentration in the blood lactic acid was excreted in the urine. The threshold for the excretion

of *l*-lactic acid is therefore not much different from that of *d*-lactic acid.

Experiments in which sodium *d*-lactate was injected subcutaneously are summarized in Table IV. A smaller amount of liver glycogen was formed than after *d*-lactate feeding. This may be

TABLE IV.

Glycogen Content of Liver after Subcutaneous Injection of Sodium d-Lactate.

160 mg. of lactic acid per 100 gm. of body weight, divided into three doses, were given.

Per 100 gm. body weight.			Liver glycogen.	Blood lactic acid.	Blood sugar.
Weight of liver.	Amount excreted.	Glycogen in liver.			
gm.	*mg.*	*mg.*	*per cent*	*mg. per cent*	*mg. per cent*
3.51	2.8	26.6	0.76	56.1	101
3.34	2.9	16.4	0.49	70.2	102
3.21	1.3	14.0	0.44	69.5	111
3.24	1.6	29.5	0.91	48.8	108
3.22	0.8	28.0	0.87	43.1	
3.25	2.9	28.4	0.88	46.2	
3.29	2.0	23.8	0.72	55.6	105

due in part to the fact that absorption from the subcutaneous tissue was not completed when the animals were killed.

DISCUSSION.

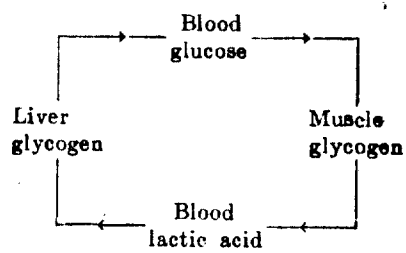
The main result of the present investigation is that *d*-lactic acid can be deposited as liver glycogen and that it is utilized several times faster in the rat than *l*-lactic acid. Also, the levo isomer is hardly able to form liver glycogen. This is another example of the discrimination of the body cells between two optical isomers. As stated in the introduction, Meyerhof and Lohmann (9) obtained the same results on isolated tissues of the rat. It is possible that narcosis abolishes the faculty of the liver to synthesize glycogen from lactic acid, because Abramson, Eggleton, and Eggleton (7) obtained negative results with dogs under ether and amytal anesthesia. Another contributory factor was probably the strong

alkalosis which they produced in their animals by intravenous administration of sodium lactate. The fact that glucose was still able to form liver glycogen under these abnormal conditions does, of course, not prove that lactic acid is unable to do so under more physiological conditions. The same authors state that there is no marked difference in the utilization of *d*- and *l*-lactic acid in the dog. However, they performed most of their experiments with *r*-lactic acid and did not compare *d*-and *l*-lactic acid directly. In the present experiments in which samples of *d*- and *l*-lactic acid of known purity were used, the difference in utilization was very marked.

The demonstration of glycogen synthesis in the liver from lactic acid links together some recently established experimental results. Himwich, Koskoff, and Nahum (23) found on decerebrate dogs, by an analysis of the arterial and venous lactic acid content of various organs, that the main site of lactic acid formation was the muscle, while the organ chiefly concerned with the removal of lactic acid from the blood was the liver. It seemed very probable that the liver formed glycogen from the lactic acid escaping from the muscles. Olmsted and Coulthard (24) actually observed a prolonged increase in liver glycogen in decerebrate cats. They explained this by a new formation of glycogen from an unknown carbohydrate existing in the body, but what actually took place was a conversion of muscle glycogen via lactic acid into liver glycogen. Epinephrine injections, which cause a disappearance of muscle glycogen in normal rats, also lead to glycogen formation in the liver from lactic acid (8). Geiger and Schmidt (25) showed recently that extra sugar in phlorhizinized dogs following epinephrine injections can be accounted for by the muscle glycogen which disappears. They failed, however, to realize that it was lactic acid and not glucose which was carried away by the blood stream to be converted into glucose in the liver.

Formation of liver glycogen from lactic acid is thus seen to establish an important connection between the metabolism of the muscle and that of the liver. Muscle glycogen becomes available as blood sugar through the intervention of the liver, and blood sugar in turn is converted into muscle glycogen. There exists

therefore a complete cycle of the glucose molecule in the body, which is illustrated in the following diagram.



Epinephrine was found to accelerate this cycle in the direction of muscle glycogen to liver glycogen and to inhibit it in the direction of blood glucose to muscle glycogen; the result is an accumulation of sugar in the blood. Insulin, on the other hand, was found to accelerate the cycle in the direction of blood glucose to muscle glycogen, which leads to hypoglycemia and secondarily to a depletion of the glycogen stores of the liver. It will be investigated to what extent this cycle plays a rôle in the preservation of liver glycogen and hence of a normal blood sugar level during fasting. There is also a possibility that other hormones besides epinephrine and insulin influence this cycle.

SUMMARY.

1. Sodium *d*-lactate, when fed by mouth or injected subcutaneously, leads to glycogen deposition in the liver. Sodium *l*-lactate, though it is absorbed at the same rate from the intestine as the dextro isomer, hardly forms any liver glycogen. Of *d*-lactate 40 to 95 per cent of the amount absorbed in 3 hours is retained as liver glycogen.

2. Of *l*-lactate 30 per cent of the amount absorbed is excreted in the urine, while no excretion occurs during *d*-lactate absorption. It is estimated that *l*-lactic acid is utilized 4 times more slowly in the rat than *d*-lactic acid.

3. The rôle of the cycle, liver glycogen → blood glucose → muscle glycogen → blood lactic acid → liver glycogen, as an important phase of carbohydrate metabolism, is emphasized.

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Craig-'46
Bib No. 326

Am. Journ. Physiol. 146:146-159. 1946.

RENAL TUBULAR REABSORPTION, METABOLIC UTILIZATION AND ISOMERIC FRACTIONATION OF LACTIC ACID IN THE DOG

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Received for publication December 27, 1945

Hardly any of the lactic acid formed in severe muscular exercise is lost in the urine even though the concentration in the blood may be considerably increased (1). Little is known about the mechanism whereby lactic acid is retained within the body. It warrants study for its importance in metabolism and for an understanding of kidney function.

Lactic acid exists in two stereoisomeric forms of which only the l(+) isomer (2) is formed in the body (3). Because of this specificity, a study of the excretion of lactic acid should include a comparison of the isomers. The use of sodium dl lactate in the alkali therapy of acidosis in various conditions (4) including burns (5) brings added interest to the study of the excretion of the d(−) isomer as well as the natural one.

The experimental plan was to infuse intravenously dl mixtures in which the proportion of l(+) to d (−) varied, to measure the rates of glomerular filtration and urinary excretion, and to determine the isomeric composition of the lactic acid in the infusion, blood and urine.

METHODS. Four well-trained normal female dogs weighing from 10 to 16 kgm. were the subjects. Of these G (previously designated Gt), H and M had been used in a study of anesthesia (6). During the experiments the dogs were supine on a padded dog board; a minimum of restraint was applied. About 50 cc. of water per kgm. was given by mouth and an infusion was established (7), usually at about 2 cc. per minute, into a vein of the ear or foreleg. The latter was more suitable for rapid, concentrated, or acid infusions. Blood samples were taken during alternate urine collection periods from a retention needle placed in a femoral artery after subcutaneous injection of 1 cc. of 2 per cent novocain. Coagulation and glycolysis were retarded by the use of 2 drops of saturated potassium oxalate per 10 cc. of blood (8), and the blood was placed in the centrifuge immediately. After 10 minutes of centrifugation the plasma was pipetted off as a further precaution against glycolysis by the cells (9) and to avoid possible exchange between cells and plasma (10). Urine was collected from the bladder at measured intervals of about 10 minutes by means of an indwelling catheter. The bladder was washed out with 10 cc. of water at each collection. Urine flows varied from 3 to 10 cc. per minute.

The rate of glomerular filtration was given by the clearance of creatinine (11). Creatinine plasma levels of 0.1 to 0.3 mgm. per cc. were obtained with a priming infusion of 1 gram in 20 cc. of 0.85 per cent NaCl and a sustaining infusion of 5 to 10 mgm. per minute with the sodium lactate.

Sodium lactate was prepared by neutralizing with NaOH a sample of 85 per

cent lactic acid (reagent grade). Hydrolysis of lactides was accomplished by refluxing a dilute solution of the acid or by boiling during the addition of NaOH and testing with phenol red until reacidification (12) was complete.

Suitable concentrations of lactate in the blood were obtained with a priming infusion of 20 per cent and a sustaining infusion of about 10 per cent lactate. Since about 60 per cent of the infused lactate was metabolized under these conditions, it was necessary to infuse rapidly in order to maintain rates of glomerular filtration adequate to saturate the reabsorptive mechanism. The range of loads that could be presented to the tubules for reabsorption was limited by the fact that when the infusion rate was of the order of 300 mgm. per minute or greater vomiting occurred. This limitation did not appear to be related necessarily to the disturbance of acid base balance, for vomiting was not avoided by the use of incompletely neutralized lactic acid. The only fatality in this series of experiments occurred after one dog went into convulsions during an infusion of 620 mgm. per minute at pH 4.5 (expt. H 7–17, table 4). The plasma concentration here was 4.7 mgm. per cc. and the tubular reabsorption of lactate was only 28 mgm. per minute, but the pH of the blood was 7.34, and of the urine, 7.40. More acid infusions, as illustrated by experiment G 2–15 in table 1, produced hemolysis, but neither the blood pH nor the tubular reabsorptive mechanism was disturbed. Neutralized or slightly alkaline infusions as illustrated by experiment H 3–1 in table 1, raised the blood pH slightly; the pH of the urine did not rise above 8. The progressive increase in urine pH was duplicated in another experiment with a slightly alkaline infusion in which the excretion of lactate did not rise, as it did in H 3–1. The delayed excretion of base in the anesthetized dog following sodium lactate (Abramson and Eggleton, 13) suggests that the progressive rise in urine pH is related to an increased excretion of $NaHCO_3$.

Analyses were made in duplicate upon diluted filtrates of plasma and urine obtained following precipitation of proteins with $CdSO_4$. Creatinine was determined according to the alkaline picrate method (14). Glucose was determined by a modification of the Folin method without yeasting (6). Lactic acid was determined by the method of Barker and Summerson (15). Nitrate free sulfuric acid was used (16). In a series of 27 dilutions of four standard solutions, the average deviation of the gravimetric values from those obtained from the standard curve was 5 per cent. The standard solutions were prepared from zinc lactate from which the water of crystallization had been driven off by heating in an oven at 130° overnight. The values reported are for the lactate radical of molecular weight 89 and so do not include the cation.

The isomeric composition of lactate samples was calculated from the optical rotation of a solution of sodium lactate in water (method 1) or a solution of lactic acid benzimidazole in ethanol after weighing the silver salt (method 2) (Moore, Dimler and Link, 17). In method 1 the calculation was based on a molecular rotation of 14° reported by Bancroft and Davis (12). Agreement between the two methods was obtained within 2 per cent. Optical rotations, measured at temperatures between 25° and 30° in 2 dm. tubes of 2 or 14 cc. capacity, were observed in a polarimeter equipped with a sodium vapor lamp. Seven readings

were made for each solution and a water or ethanol blank; the null point was approached from alternate directions. The range of variation of the readings was not more than 0.03°.

TABLE 1

Excretion of lactate during acid and slightly alkaline infusions

TIME*	URINE VOLUME	CREATININE CLEARANCE	PLASMA LACTATE CONCENTRATION	PLASMA pH	LACTATE EXCRETION	URINE pH	LACTIC ACID REABSORPTION
			Experiment G 2-15†				
min.	*cc./min.*	*cc./min.*	*mgm./cc.*		*mgm./min.*		*mgm./min.*
0				7.34		6.08	
26				7.37			
38			3.12				
49	2.17	43.7			72	6.36	63
51			2.89				
54				7.40			
59	2.60	52.5			85	6.35	66
			Experiment H 3-1‡				
32				7.35			
37			0.067				
46	3.80	57.7			0.96	6.88	4
55			0.077				
59	3.32	52.1			0.82	6.81	4
93			1.58				
96	3.78	64.2			50	7.71	50
99				7.43			
108	4.78	64.6			61	7.82	47
113			1.86				
118	5.53	63.5			66	7.91	50
120				7.43			

* When urine volume is given, time represents time of urine collection.

† Experiment G 2-15, 10.3 kgm., sitting height 91 cm., surface area 0.61 sq. m. Sustaining infusion containing 97 mgm. per cc. ½th neutralized lactic acid at pH 3.49 and 7 mgm. per cc. creatinine at 1.76 cc. per min., begun at t = 0. At t = 11, priming infusion of 1 gram creatinine in 20 cc. lactate and at t = 19, priming infusion of 40 cc. of 20 per cent sodium lactate at pH 7.50. Urine cherry red after t = 48.

‡ Experiment H 3-1, 11.4 kgm., sitting height 91 cm., surface area 0.63 sq. m. Sustaining infusion containing 6 mgm. creatinine per cc. in 0.85 per cent NaCl at 1.90 cc. per min., begun at t = 0. At t = −17, 500 cc. water by mouth. Priming infusion, 1 gram creatinine in 20 cc. 0.85 per cent NaCl at t = 8. Second sustaining infusion containing 105 mgm. lactate and 6 mgm. creatinine per cc. at pH 7.80 at 1.75 cc. per min. begun at t = 60. Second priming infusion of 20 cc. of 20 per cent sodium lactate at pH 7.08 in at t = 65.

The preparation of urine and blood for polarimetry was as follows: Acid urines were made alkaline to phenol red indicator with NaOH. Dilute urines were concentrated to about 25 cc. by boiling. The alkaline urines were read in method 1 after removal of cloudy and colored material by treatment with finely divided carbon (Norit A). Although one sample of bladder urine collected

before the beginning of an experiment had a rotation of $-0.03°$, control samples of urine produced at a rate of more than 3 cc. per minute during an infusion of creatinine and 0.85 per cent NaCl showed no appreciable rotation, so that blank corrections for urine were regarded as unnecessary.

Plasma proteins were precipitated by adding 100 cc. of plasma to a solution of 500 cc. of $CdSO_4$ (20.8 grams $3CdSO_4 \cdot 8H_2O$ + 100 cc. 1 N H_2SO_4 in 1 liter) and 300 cc. of water and neutralizing with 100 cc. of 1.1 N NaOH. After half an hour the precipitate was removed by centrifugation. The slightly alkaline filtrate was concentrated to 25 cc. by boiling, and made acid with H_2SO_4 to a pH below 2 (thymol blue as indicator). An ether extraction was made by shaking the concentrated filtrate with twice the volume of freshly opened anesthesia ether for 5 minutes and repeating the procedure five times with additional portions of ether. The ether was then removed by evaporation by vacuum (water pump). In method 2, both urine and blood samples were extracted with ether to make the lactic acid available in minimum volume. The extract (method 1) was neutralized to phenol red with NaOH, decolorized with Norit A and diluted to 5 cc. in a volumetric flask. The solution was read in the polarimeter and then analyzed for lactic acid.

The loss in lactic acid from boiling and treatment with carbon was no greater than the error in the analyses. As much as 7 per cent of lactic acid, however, may be removed by finely divided carbon under some conditions (18), so that polarimeter samples were analyzed for lactic acid after decoloration. The ether extraction was about 75 per cent complete.

Recoveries were performed by adding 10 cc. of 10 per cent sodium lactate containing 70 per cent l(+) lactate to 90 cc. of control plasma containing 20 mgm. of endogenous lactate. The fraction of l(+) lactate in the extract obtained by the above procedure agreed with the fraction of l(+) lactate in the original mixture within 2 per cent. This demonstrated the absence of any significant error from fractionation of the isomers during the preparation of samples for polarimetry.

Results. Representative data for the concentration of lactic acid in blood and urine of the dog under resting conditions appear in table 1. When sodium lactate was given by stomach tube in amounts comparable to those used in the treatment of burns (5), the concentration of lactate in the plasma increased in an hour to a maximum about three times the resting value. Doubling the dose did not bring about any considerable increase in the maximum. Utilization of the ingested lactic acid was nearly complete as far as this could be determined from the urinary excretion (table 2). When the concentration of lactate in the blood of the resting dog was increased by intravenous infusion, little lactate was lost in the urine until the concentration in the plasma approached 1 mgm. per cc. At greater plasma concentrations, the rate of excretion was proportional to the rate of glomerular filtration of lactate. At high plasma concentrations, the ratio of the concentration in the urine to the concentration in the plasma averaged 4.7 in 26 experiments of 2 or more collection periods; the range of variation was from 2.4 to 10.9.

The possible binding of lactate to plasma proteins was tested by the collodion bag method (19).[1] Sodium lactate was not bound appreciably by the plasma proteins; thus the concentration in the plasma is an adequate measure of the concentration in the glomerular filtrate. The rate of glomerular filtration of lactate, therefore, is the product of the plasma concentration of lactate and the clearance of creatinine.

TABLE 2

Fate of sodium lactate given by mouth*

TIME† AFTER LACTATE	PLASMA LACTATE CONCENTRATION P_L	URINE VOLUME V	URINE pH	LACTATE EXCRETION U_LV	LACTATE CLEARANCE U_LV/P_L	SUMMATION OF LACTATE EXCRETION
Experiment H 4–19‡						
min.	*mgm./cc.*	*cc./min.*		*mgm./min.*	*cc./min.*	*grams*
0	0.17					
17	0.24					
19		4.03		0.89	3.5	0.028
34	0.48					
49		5.97		6.46	12.6	0.222
64	0.56					
82		3.92		7.74	14.3	0.408
94	0.53					
Experiment G 5–3§						
0	0.15		5.72			
12		0.21	5.95	0.26	1.1	0.006
20	0.32					
41		0.17	5.91	0.75	1.7	0.028
48	0.50					
70		0.23	5.77	1.12	2.4	0.062
80	0.43					
101		0.23	5.62	1.08	3.1	0.095
112	0.30					

* Containing 34 per cent l(+) and 66 per cent d(−) lactate.

† When urine volume is given, time is midpoint of urine collection period.

‡ Experiment H 4–19, weight 13.8 kgm., sitting height 91 cm., surface area 0.68 sq. m. given 550 cc. M/6 lactate or 8.25 grams.

§ Experiment G 5–3, weight 13.6 kgm., sitting height 88 cm., surface area 0.66 sq. m., given 250 cc. M/6 lactate or 3.75 grams.

Representative values for glomerular filtration, urinary excretion, and tubular reabsorption at different concentrations of lactate in the plasma are given by

[1] Bags containing 10 cc. of oxalated dog plasma were floated in test tubes containing 20 cc. of phosphate buffer. The plasma contained 2 mgm. per cc. of sodium lactate (about 34 per cent l(+) lactate, and 0.2 mgm. per cc. of creatinine. The initial concentrations in the buffer were 20 per cent higher in two tubes and 40 per cent lower in two other tubes than the concentrations in the plasma. After 24 hours of equilibration at room temperature, the ratios of concentration outside to concentration inside the bags averaged 1.08 for sodium lactate and 1.06 for creatinine.

experiment H 6–25 (fig. 1). In this experiment, when a high plasma concentration of lactate had been reached, the lactate-creatinine infusion was replaced by a mannitol-creatinine infusion to maintain the urine flow, and clearances were measured at decreasing plasma lactate concentrations.

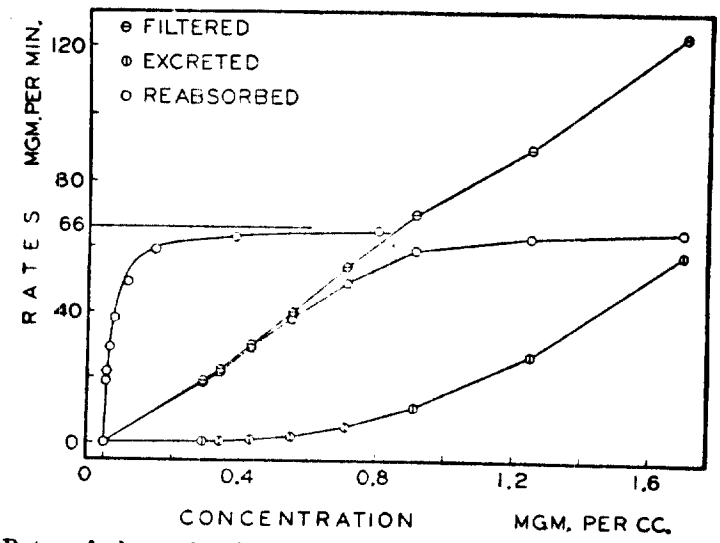

Fig. 1. Rates of glomerular filtration, urinary excretion, and tubular reabsorption of lactic acid at various concentrations in the plasma, from experiment H 6–25. The lactic acid in the infusion was 86 per cent l(+).

The smoothed curve at the left represents the rate of reabsorption (T) as a function of the concentration in the renal tubular fluid distal to the region of reabsorption, (A) in the seventh equation of Shannon (20):

$$KT = A(Tm - T) \tag{1}$$

in which $K = 0.018$ mgm. per cc. and $Tm = 66$ mgm. per min. The asymptote inserted in the figure at $T = 66$ was obtained by adding 1 to the highest observed value of T. The open circles represent experimental data. The values of T are those plotted at the right against plasma concentration (P_L). The values of A were calculated according to Shannon (20):

$$A = P_L - (T/C_{Cr}) \tag{2}$$

in which C_{Cr} is the observed clearance of creatinine in cc. per min. The variation in C_{Cr} in this experiment is evident from the irregularities in the line joining the horizontal barred circles.

The values of K in figure 1 and figure 2 should be multiplied by 100 for comparison with those of Shannon and Fisher (21) for glucose since their unit of volume was 100 cc. K for lactic acid then is found to be about 10 times as great as K for glucose in the dog.

It is interesting to note that the hyperbolic curve of equation (1) is not peculiar to renal excretion, but satisfies much of the data for other biological saturation phenomena such as cellular respiration (22, 23) and photosynthesis (24).

In order to provide a wide range of filtration rates, various speeds of infusion of lactate were used up to the maximum that the dogs could tolerate. Rates of filtration and reabsorption in individual clearance periods for dogs G and H collected over a period of six months are set forth in figure 2. The rate of re-

absorption became independent of the rate of glomerular filtration of lactate at a ratio of filtration to reabsorption of about 1.6. The maximum rate of tubular reabsorption (Tm) was reproducible within the limits of variation shown in figure 2. Only 2 out of the 64 data in the range of filtration rates between 100 and 240 fell outside ± 30 per cent of the mean Tm. The variation in the creatinine clearance was of the same order of magnitude, but there was no correlation between Tm and the creatinine clearance.

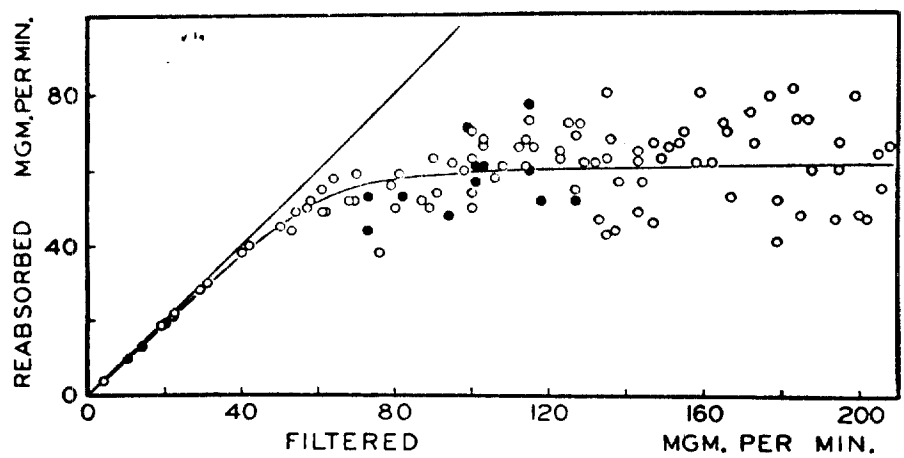

Fig. 2. Rates of tubular reabsorption and glomerular filtration of dl lactic acid, dogs G and H. Each datum represents an individual urine collection period. Solid circles represent periods in which plasma glucose concentration was increased by infusion sufficiently to saturate the reabsorption mechanism for glucose. The diagonal line represents complete reabsorption of the filtered lactic acid.

The smoothed curve represents the rate of reabsorption (T) as a function of the rate of glomerular filtration of sodium lactate (P_LC_{Cr}) in the eighth equation of Shannon (20):

$$KC_{Cr}T = (P_LC_{Cr} - T)(Tm - T) \tag{3}$$

when $K = 0.028$ mgm. per cc., $Tm = 62$ mgm. per min. and $C_{Cr} = 61$ cc. per min. In the series of 24 experiments represented in this figure, the mean C_{Cr} was 61 for both dogs. For values of P_LC_{Cr} between 100 and 234, the mean values of T were 63 for dog G and 61 for dog H. In order to calculate the values of T in the smoothed curve, equation (3) was solved for T by means of the binomial theorem:

$$2T = P_LC_{Cr} + KC_{Cr} + Tm - \sqrt{(P_LC_{Cr} + KC_{Cr} + Tm)^2 - 4P_LC_{Cr}Tm} \tag{4}$$

In contrast to figure 1, most of the lactic acid infused in this series contains a preponderance of the d(−) isomer.

Simultaneous reabsorption of glucose and lactic acid. Because of the relationship between glucose and lactic acid in metabolism, it was thought these substances might interfere with each other in tubular reabsorption. Glucose and sodium lactate were infused separately and together to provide plasma concentrations adequate for tubular saturation. In 12 collection periods for dogs G and H without infused lactate and 12 periods with high plasma lactate (more than 1

mgm. per cc.) in all of which the ratio of filtration to reabsorption of glucose was above 1.36, the means for glucose Tm and standard errors of the means were 168 ± 2.7 and 152 ± 2.6, respectively. The difference was significant but amounted to only 10 per cent. Data for lactic acid reabsorption in the presence of glucose filtration rates adequate for saturation of the glucose reabsorption mechanism appear in figure 2 as solid circles. Neither glucose nor lactic acid interfered to any conspicuous extent with the reabsorption of the other.

Fractionation of dl lactic acid. By purchasing samples of lactic acid at random, mixtures containing from 35 to 71 per cent of l(+) lactic acid were obtained. In 9 experiments various dl mixtures of sodium lactate were infused and blood and urine samples collected for determination of the isomeric composition of the lactate. About 200 cc. of blood was drawn in several portions during the urine collection period so that the values obtained were essentially simultaneous. The composition of the urine lactate shifted in favor of the d(−) form by one or two per cent in the course of 3 urine collection periods, but this was within the analytical error. In the series of experiments in table 3 for which complete data were available, the mean l(+) concentration in the lactate of the infusions, bloods and urines was 52, 43, and 38 per cent respectively. From these data the share of each isomer in glomerular filtration, urinary excretion, tubular reabsorption and metabolic utilization was calculated. The rates of glomerular filtration and tubular reabsorption are shown in figure 3; the rates of intravenous infusion and metabolic utilization[2] in figure 4. From the data in table 4 there was no indication that the Tm of the dl mixture was influenced by its isomeric composition, although the l(+) concentration in the reabsorbed lactate varied from 31 to 90 per cent.

For the purpose of comparing the efficiency of handling the two isomers, the reabsorption fraction defined as the rate of reabsorption divided by the rate of filtration, and the utilization fraction defined as the rate of utilization divided by the rate of infusion, were established for each isomer and the dl mixtures. The average values of these fractions are represented in figures 3 and 4 by diagonal lines. The utilization fractions were independent of the rate of infusion in the range available; at low rates of infusion, complete utilization would be expected (table 2). The reabsorption fractions vary widely since the fraction reabsorbed is a dependent function of the filtration rate when Tm has been exceeded. The relative efficiency in handling the two isomers is given by the ratio of the d(−) fraction to the l(+) fraction, calculated for each experiment in table 4. The d(−)/l(+) ratios for 9 experiments had mean values of 0.68 for reabsorption and 0.65 for utilization.

Discussion. The experiments represented in figure 2 demonstrated that the

[2] When the plasma lactate concentration remains constant the rate of utilization is given by the difference in the rates of infusion and urinary excretion if none is excreted by other routes. The degree to which these conditions are met is as follows. In 8 experiments the plasma concentration increased by 0.73 mgm. per 100 mgm. per minute on the average; in the other 5 experiments there was an average decrease of 0.54 mgm. per 100 mgm. per minute. In anesthetized dogs given from 10 to 30 grams of sodium lactate, Abramson and Eggleton (25) found in the intestine not more than 120 mgm. or a maximum of 1.2 per cent.

mechanism for the renal tubular reabsorption of lactic acid was limited in capacity, was not influenced by the simultaneous saturation of the glucose reabsorption mechanism, and was stable in the sense that Tm was reproducible. The fact

TABLE 3

Experiment, dog, date	Isomeric composition of lactic acid from: Infusion				Blood				Urine			
	Method†	Observed rotation	Concentration	Per cent l(+)	Method†	Observed rotation	Concentration	Per cent l(+)	Method†	Observed rotation	Concentration	Per cent l(+)
			mgm./cc.				mgm./cc.				mgm./cc.	
D 9–11	2	0.80	70.1	35	1	0.23	14.4	25	1	1.24	62.6	19
M 9– 4	1	0.97	97.4	34	1	0.21	17.4	31	1	1.61	98.8	24
H 7–17	2	0.71	61.8	35	2	0.40	18.6	31	2	0.73	44.2	29
D 7– 9	1	−0.12	122.3	52	1	0.09	16.3	33	1	0.63	59.3	33
M 6–13	1	−0.03	97.3	50	1	0.10	14.5	39	1	1.42	180.0	37
H 5–16	1	−0.04	79.5	51	1	0.05	9.4	41	1	0.79	85.0	35
G 9–18	2	−1.19	70.5	72	1	−0.06	12.4	58	1	0.14	31.9	43
M 8– 7	2	−1.15	66.1	72	2	−0.16	18.6	61	2	−0.56	61.5	62
D 8–28	2	−1.11	71.1	70	2	−0.15	12.6	65	2	−0.18	38.6	56
av.				52				43				38
G 2–15	1	0.69	92.8	38					1	0.34	19.3	22
H 3– 1	1	0.81	105.0	38					1	0.19	10.7	22
G 4– 5	1	0.68	93.0	38					1	0.22	10.7	17
G 3– 8	1	0.80	123.0	40					1	0.22	10.3	16
*H 6–25	1	−4.41	195.0	86								
*G 10–23	1	−0.47	18.6	90	1	−0.16	6.2	91	1	−0.15	5.1	96

* In these experiments the sodium lactate was prepared from samples of commercial zinc d lactate which contained 98 and 95 per cent l(+) lactate respectively according to analysis by method 2. The zinc was removed by precipitation with H_2S, but after this step the percentage of d(−) lactate increased as shown in the table above.

† In method 1, the percentage of l(+) lactic acid in the mixture is equal to

$$50 - \frac{1589 \times \text{observed rotation}}{\text{concentration of dl lactate in water}}$$

In method 2, the percentage of l(+) lactic acid in the mixture is equal to

$$50 - \frac{1275 \times \text{observed rotation}}{\text{concentration of dl lactic acid benzimidazole in ethanol}}.$$

that Tm did not vary significantly with the isomeric composition of the dl mixtures although the ratio of d(−) to l(+) ranged from 3.0 to 0.5, suggests that both isomers are handled by the same mechanism. This view is supported by evidence of competition between the isomers for reabsorption. When the rates

of filtration and reabsorption were presented for each isomer separately in figure 3, two features were observed. At high filtration rates the reabsorption rates of the isomers did not differ significantly and failed to approach the Tm for the dl

TABLE 4

Relative activity of lactic acid isomers in metabolic utilization and renal tubular reabsorption when supplied in dl mixtures

EXPERIMENT, DOG, DATE	ISOMERIC COMPOSITION OF INFUSED LACTIC ACID	CONCENTRATION OF LACTIC ACID IN PLASMA	RATE OF CHANGE OF PLASMA LACTIC ACID CONCENTRATION	RATES FOR dl MIXTURES					PER CENT d(−) REABSORBED / PER CENT l(+) REABSORBED	PER CENT d(−) UTILIZED / PER CENT l(+) UTILIZED
				Infusion	Glomerular filtration	Urinary excretion	Renal tubular reabsorption	Metabolic utilization		
	per cent l(+)	*mgm./cc.*	*1000 × mgm./mgm./min.*	*mgm./min.*	*mgm./min.*	*mgm./min.*	*mgm./min.*	*mgm./min.*		
D 9-11	35	1.43	2	180	137	72	65	108	0.75	0.65
M 9- 4	34	1.87	7	169	105	73	32	96	0.52	0.74
H 7-17	35	4.74	5	620	244	216	28	404	0.53	0.87
D 7- 9	52	3.19	−6	323	195	128	67	195	0.53	0.60
M 6-13	50	3.85	−4	325	201	154	47	171	0.75	0.62
H 5-16	51	3.33	−5	206	208	134	74	72	0.64	0.25
G 9-18	72	1.17	−6	167	90	38	52	129	0.61	0.61
M 8- 7	72	1.91	25	425	138	112	26	313	1.11	0.83
D 8-28	70	1.43	6	132	92	46	46	86	0.65	0.68
av.									0.68	0.65
G 2-15	38	2.98	−6	170	143	78	65	92		0.57
H 3- 1	38	1.68	10	184	108	59	49	125		0.74
G 4- 5	38	2.26	3	176	149	90	59	86		0.40
G 3- 8	40	2.25	0	232	156	84	72	148		0.60
H 6-25	86	1.70	−24	0	123	58	65			
G 10-23	90	0.78	2	135	64	6	58	129	1.12	1.03

mixture. At low filtration rates of one isomer and high filtration rates of the other, considerable amounts of each isomer failed to be reabsorbed. That is, there is no indication that a relationship between filtration and reabsorption of the type exhibited by the dl mixture or for glucose exists for either isomer considered independently of the other.

It will be noticed that in contrast to the glucose Tm of the dog (21), lactic acid Tm was approached gradually as the rate of glomerular filtration was increased. Hence a sharp renal threshold for lactic acid is not to be expected.

Two explanations of the rounding or splay of the filtration-reabsorption curve

have been advanced. Shannon (20) has interpreted it in terms of the kinetics of the reaction between the substance being reabsorbed and the reabsorbing mechanism, K in his equation being proportional to the equilibrium constant of the reaction. That the results for lactic acid are susceptible of this interpretation is evident from figures 1 and 2. On the other hand, the explanation of Smith (26) appears to be equally valid. In analyzing kidney function in hypertensive patients, Smith has assumed that in any individual nephron there is no splay (or K is very small), and has ascribed the splay in the curve for the kidneys as a whole to variations from one group of nephrons to another in the ratio of filtration

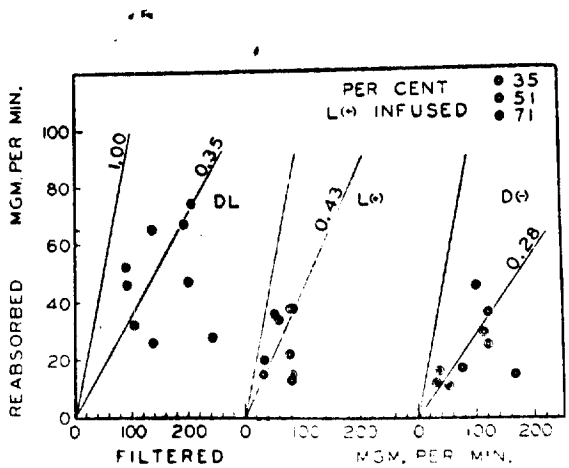

Fig. 3

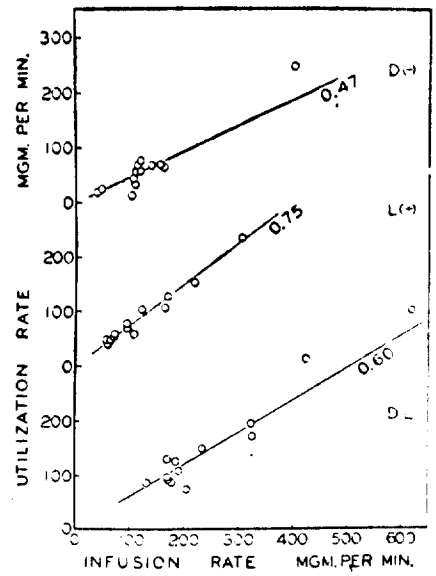

Fig. 4

Fig. 3. Rates of glomerular filtration and renal tubular reabsorption for dl mixtures and the l(+) and d(−) components of the mixtures calculated from the data in tables 3 and 4. The diagonal lines of slope equal to one represent complete reabsorption. The mean fraction reabsorbed is indicated by lines of smaller slope.

Fig. 4. Rates of intravenous infusion and metabolic utilization for dl mixtures and the l(+) and d(−) components of the mixtures calculated from the data in tables 3 and 4. The mean fraction utilized is indicated by the diagonal lines.

to reabsorption, so that groups of nephrons become saturated at different plasma levels. When the values for filtration and reabsorption from the smooth curve of figure 1 were analyzed in this way, the frequency distribution curve of glomerular activity resembled very closely the one published by Smith for a series of glucose kidney titrations in man, in respect to the height of the maximum, the position of the maximum along the r/R axis, and the skew to the left. The values from the curve in figure 2 yielded a somewhat flatter but essentially similar frequency distribution curve. In the absence of arguments to the contrary it may be assumed that both kinetic and distributional factors are operating simultaneously; a problem of apportioning the splay between them remains.

According to the kinetic theory, the difference in efficiency of reabsorption of the isomers may be interpreted as an expression of a greater affinity of the reabsorption mechanism for the l(+) lactic acid than for the d(−). Pitts (27) has pointed out that any circumstance favoring the *combination* of the reabsorptive mechanism with its substrate over the *splitting off* of the substrate should reduce the value of K. From this one might expect a smaller value of K with l(+) lactic acid than with d(−). An indication in this direction is seen in the fact that in one experiment with nearly pure l(+) lactic acid (fig. 1) K = 0.018, whereas in the series made with lactic acid in which the d(−) predominated (fig. 2) K = 0.028. These values of K differ by a factor of 0.64 which is of the right order of magnitude. Since the difference in the shape of the curves defined by these constants is so small, however, a great many experiments might be required to establish its significance statistically.

Although the fate of lactic acid in metabolism was not considered in these experiments, the existence of a non-oxidative route of disposal is suggested by the following. If it is assumed that a dog 0.6 sq. m. in surface area consumes 72 cc. of oxygen per minute under basal conditions (28) and 100 cc. during the infusion (29), then there is enough oxygen available for the combustion of only 133 mgm. of lactic acid per minute, whereas rates of utilization up to 400 mgm. per minute were observed.

As was found in table 2, small amounts of dl lactic acid were almost completely utilized. The same is true of rabbit (30), rat (31), and man (4). Under the same circumstances, however, when the isomers were supplied in pure form, in the rabbit (30) and the rat (31), the l(+) lactic acid was almost completely retained, while from 30 to 50 per cent of the d(−) was rejected in the urine. In the anesthetized dog (25), on the other hand, when relatively larger amounts of both were involved as much of the d(−) lactic acid was retained as of the dl mixture. In experiments with the rabbit (32) in which pure isomers were infused separately, the utilization fractions were constant over a wide range of utilization rates as was seen in figure 4 in the dog. The ratio of utilization fraction for d(−) and l(+) lactic acid was 0.42 as compared with 0.65 for the dog.

The striking feature of the experiments dealing with isometric fractionation was the similarity between reabsorption and utilization in the degree of preference for the l(+) or natural isomer as idicated by d(−)/l(+) ratios of 0.68 for the reabsorption fractions and 0.65 for the utilization fractions. The metabolic ratio may be merely the resultant of widely different ratios characteristic of different metabolic processes such as oxidation or synthesis, or characteristic of different tissues such as liver or muscle. The only subdivision of utilization available was made for the rat by Cori and Cori (31). They found that the degree of preference for the l(+) isomer was the same in glycogen synthesis as in total utilization. The agreement in the degree of preference between utilization and renal reabsorption in the dog and between utilization and glycogen synthesis in the rat permits the tentative suggestion that isomeric fractionation of lactic acid throughout the body may have a common explanation. The nature of the fractionating mechanism is obscure.

SUMMARY

Sodium dl lactate given to the normal dog by mouth was almost completely retained. When the plasma lactate concentration was increased by intravenous infusion, urinary excretion was quite small until the plasma concentration approached 1 mgm. per cc. At greater plasma concentrations up to 4 mgm. per cc., the rate of excretion was proportional to the rate of glomerular filtration of lactate.

The mechanism for renal tubular reabsorption of lactic acid exhibited a reproducible maximum capacity. The excretion of lactate at rates of filtration insufficient for complete saturation of the reabsorptive mechanism was discussed in relation to existing theories.

Simultaneous saturation of the reabsorptive mechanisms for both glucose and lactic acid had no conspicuous effect on the reabsorption of either substance.

In 9 experiments dl mixtures containing from 35 to 71 per cent of the l(+) isomer were infused. The isomeric composition of lactate from samples of blood and urine collected over the same period was determined; the mean l(+) fraction in the infusion, blood, and urine lactate was 52, 43, and 38 per cent respectively.

The l(+) and d(−) components of the dl mixtures were compared according to the fraction of the filtered lactic acid that was reabsorbed and the fraction of infused lactic acid that was utilized. The ratio of the d(−) fraction to the l(+) fraction was substantially the same for both reabsorption and utilization; the means were 0.68 and 0.65, respectively. This suggests a widespread preference in the dog for the "natural" over the d(−) isomer in the ratio of 3 to 2.

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D'Amour-'34
Bib No. 346

SPECIAL ARTICLES

THE NATURE OF LYSOZYME ACTION[1]

THE lytic action on certain bacteria of a supposed enzyme, lysozyme, present in tears, egg white and various body tissues and fluids, has been described and studied by Fleming and others.[2] We have tried to determine whether the action of lysozyme is physical or enzymatic. A polypeptide in the form of an amorphous white powder, obtained by the purification of egg white, completely dissolved a suspension of air sarcinae (barium sulfate standard No. 8) in a concentration of 0.12 gamma per cc. An aqueous solution of the purified lysozyme did not change the surface tension of water and had no proteolytic, lipolytic or amylolytic action. It did not activate the action of papain or of the endoproteases of the bacteria. It did not act on lecithin or on the alcohol-ether soluble fraction of the sensitive sarcinae; it gave no evidence of a phosphatase action. It did split off a reducing sugar from ovomucoid and from a polysaccharide obtained by hydrolysis of the test organisms. The corresponding mucoid of the bacteria has not yet been isolated. The defatted bacteria are extremely insoluble, apparently consisting chiefly of a mucoid yielding a large carbohydrate fraction. Cartilage and chitin were not attacked.

Apparently lysozyme is an enzyme or an enzyme mixture which splits a reducing sugar from certain mucoids and from the polysaccharides derived from them. Its occurrence in tears, nasal, bronchial and gastro-intestinal mucus, egg white and semen[3, 4] can thus be understood, the bacteriolytic action being incidental. The same enzyme was obtained from a polypeptide fraction of the sensitive bacteria. The possible relation of this factor to bacteriophage action is being investigated. It is possible that this ferment may furnish an important tool for the study of mucins. It is to be expected that a series of such mucinases[5, 6] will be found in various tissues and organisms. A commercial pepsin preparation was found to split gastric mucin independently of peptic activity. It is possible that the specific bacterial polysaccharides are derived from capsular mucoids and that the enzyme described by Dubos and Avery[7] which decomposes the capsule of Pneumococcus III and hydrolyses its specific polysaccharide belongs to this group of ferments.

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[1] From the Biochemical and Bacteriological Laboratories of the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York City.

[2] A. Fleming, *Proc. Roy. Soc. Med.*, 71: 26, 1932. Review.

[3] Kurzrok and Miller have shown that semen dissolves the mucous plug of the cervical canal.

[4] R. Kurzrok and G. Miller, *Am. Jour. Obstet. and Gynec.*, 56: 15, 1928.

[5] The term "mucinase" has already been applied to a ferment which coagulates mucin. The existence of such a ferment is not, however, well established.

[6] Oppenheimer-Kuhn, "Die Fermente und ihre Wirkungen," Vol. 2, Leipzig, 1925.

[7] R. Dubos and O. T. Avery, *Jour. Exp. Med.*, 54: 51, 73, 1931.

EFFECTS OF FEEDING SODIUM BICARBONATE OR LACTIC ACID UPON THE SEX RATIO IN RATS

SEVERAL articles have appeared recently in the newspapers to the effect that the ingestion of sodium bicarbonate during pregnancy influences the sex of the offspring both in the human and in the dog, males being produced exclusively. While there appears to be no physiological basis for such a belief, the necessity of increasing our rat colony gave an opportunity to test the question experimentally.

Animals to be bred were placed upon our stock diet, which consists of a mixture of ground grains, dried milk, mineral salts and cod liver oil, with which was incorporated either sodium bicarbonate or lactic acid. Following breeding, the animals were continued upon the same diet until parturition. The food seemed perfectly palatable, 15 to 20 grams being consumed per day.

The results are given in the following table:

Material and concentration	No. of litters	Males	Females	Total
2½ per cent. sodium bicarbonate	15	61	67	128
5 " " " "	20	85	101	186
2½ " " lactic acid	10	38	42	80
5 " " " "	28	107	128	235
Totals	73	291	388	629

Sex ratio, sodium bicarbonate animals, $\frac{\text{Females}}{\text{Males}} = \frac{168}{146} = 1.15$

Sex ratio, lactic acid animals, $\frac{\text{Females}}{\text{Males}} = \frac{170}{145} = 1.17$

In a group of 14 control litters the sex ratio of females to males was 1.03. Donaldson[1] quotes a table from King which includes data covering some 815 litters, showing a variation in female to male sex ratio of from 1.06 to .66.

[1] "The Rat," page 25, table 6.

In view of this variation, and of the close agreement between the sex ratios of the two groups in our study, we conclude that, for the rat at least, the sex ratio is not affected by the feeding of base or acid.

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THE EFFECTS OF THALLIUM SULFATE UPON SOILS

QUESTION has lately been raised concerning the ultimate effects, upon the soil, of the thallium compounds used for rodent control. S. C. Brooks[1] has warned land owners of the possibility that the soil might be sterilized by this practise. Obviously, such a problem merits investigation. The writer has developed critical methods during studies on weed control and is preparing a detailed report on tests with thallous sulfate. Meanwhile, he presents the following summary of pertinent results.

Using four California soils, the writer studied the initial toxicity, the decrease of toxicity with time and cropping, the saturation capacity of the soils and the effects of thallium-coated grain upon adjacent plants. Oat seedlings used as indicator plants were harvested 30 days after planting in the greenhouse tests.

In equimolecular concentrations, thallous sulfate proved many times as toxic as arsenic trioxide and sodium chlorate. Whereas toxicity of the latter soil sterilants decreased with successive cropping, that of thallous sulfate remained unchanged through three such treatments.

Thallium toxicity was tested through a range of 25 ppm to 2,000 ppm in the soil solution. It proved greatest in soils of low fertility but could not be correlated with soil type or water-holding capacity. The chemical was strongly fixed in all soils, the saturation capacity of Yolo clay being about 10,000 ppm on a dry weight basis. Leaching with 200 cm of distilled water had practically no effect on the location or toxicity of thallous sulfate in the soil.

Thallous sulfate is an extremely effective soil sterilant, it is strongly fixed and it resists leaching. Although these facts explain Brooks' observations and would seem to justify his warning, their aspect is changed by quantitative studies. Thallium-treated "potted" (hulled) barley had practically no effect upon germination or growth of oats planted in the same soil and spaced within ½ centimeter of the barley grains. When the spacing was decreased to ¼ centimeter, growth was reduced. Except where the dosage was excessive, oat seedlings were unaffected by the application of treated barley to the soil, followed by irrigation.

Thallium-treated grain also affected vegetation in a pasture area very little. The heaviest application, equivalent to 27 pounds of thallous sulfate per acre, reduced growth less than 50 per cent.

In concentrations of 100 ppm or more (on a dry weight basis) thallous sulfate should, apparently, be fully toxic in most soils. At this rate, about 30 pounds would be required to sterilize an acre inch.

Calculations on the area sterilized by the grains comprising a squirrel bait (approximately 20 grams of poisoned grain) show that over 100,000 baits would be required to cover an acre. This is equivalent to 5,000 pounds of poisoned grain bearing 50 pounds of thallous sulfate and might sterilize the top 1½ inches of soil. Under natural conditions, however, the chemical would be fixed in a much shallower layer. The baits, if taken, would be distributed through the top four feet of soil and would have little sterilizing effect.

The disparity between these figures and the amounts used in field practise is striking. As shown by a brief survey of ground-squirrel control in California, the average initial dosage of poisoned grain bearing one per cent. Tl_2SO_4 is about 1/3 pound per acre; later applications are lighter. In one county the dosage has decreased to 1/35 of a pound in five years. The success of this material should permit similar reductions in other regions, so that the amount of chemical becomes totally insignificant as far as soil sterilization is concerned.

The writer observed no loss of fertility in range lands successfully treated for five successive years. The only denuded areas found were the open burrows, fresh mounds and beaten trails of squirrels in untreated fields. Regardless of other objections to thallium compounds in rodent control, the possibility of losing agriculturally valuable land through sterilization seems remote.

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Dies-'69
Bib No. 398

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AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 216, No. 1, January 1969. *Printed in U.S.A.*

Renal excretion of lactic acid in the dog

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DÍES, FEDERICO, GUADALUPE RAMOS, ESTHER AVELAR, AND MIGUEL LENNHOFF. *Renal excretion of lactic acid in the dog.* Am. J. Physiol. 216(1): 106–111. 1969.—The renal excretion of lactic acid was studied under different conditions in mongrel dogs. Previous reports that tubular reabsorption of lactic acid is rate limited were confirmed during experiments with rapid intravenous Na-lactate loading. Lactic acid-to-creatinine clearance ratios did not decrease below 2–3% even when the filtered load of the acid was decreased to about 30% of normal. Lactic acid excretion was urine flow dependent at low filtered loads. Osmotic diuresis increased fractional excretion of lactic acid. Extracellular fluid expansion with isotonic saline increased lactic acid excretion more than was expected from the levels of urine flow attained. Stop-flow studies showed that lactic acid was reabsorbed against a concentration gradient in "proximal samples." It was concluded that lactic acid is actively reabsorbed in the proximal tubule, that its transport is rate limited, and that it is either incompletely reabsorbed at low filtered loads or partially secreted at a distal site of the nephron.

renal tubular transport; proximal tubule; stop flow; mannitol diuresis; furosemide; saline diuresis

FEW PUBLICATIONS have dealt with the mechanism of urinary excretion of lactic acid (6). In particular, there is a lack of information regarding the site of tubular reabsorption of lactic acid along the nephron. Such information is essential for the proper interpretation of experiments showing a corticomedullary concentration gradient of lactic acid in dog kidney tissue (7).

Craig (6) had shown previously that lactic acid reabsorption in the dog is rate limited. Initial studies of lactic acid net uptake by the dog kidney performed in our laboratory showed that lactic acid was not completely reabsorbed at normal blood levels, even when the filtered load was considerably reduced by decreasing GFR. This observation suggested that lactic acid reabsorption might be gradient limited as well.

The present investigation was undertaken to further characterize the renal excretion of lactic acid. Results indicated that lactic acid reabsorption occurs in the proximal tubule against a concentration gradient, that it is rate limited, and that some lactic acid is excreted even at low filtered loads.

METHODS

All experiments were performed on female mongrel dogs weighing from 12 to 15 kg, anesthetized with intravenous sodium pentobarbital (30 mg/kg body wt). After appropriate priming doses, a sustaining infusion containing creatinine (1.7 mg/ml) in either 0.85 or 0.45% NaCl solution was given at a constant rate of 0.3 ml/kg per min. Urine was collected from one kidney through a ureteral catheter for two to three consecutive 10-min periods. In the middle of each period of urine collection, blood was allowed to flow spontaneously from a catheter placed in the femoral artery, into heparinized tubes and into tubes containing 10% perchloric acid.

In 16 dogs, DL-Na-lactate was infused instead of NaCl at rates from 20 to 250 μmoles/kg per min. A priming dose of 1 μmole/kg lactate was injected at the beginning of each lactate infusion.

A saline load was administered to six dogs during the infusion of the creatinine solution mentioned above, and following two to three control clearance periods. The saline solution (120 mM NaCl, 20 mM $NaHCO_3$, mM KCl) was given intravenously at a constant rate of 2 ml/kg per min for the first 20 min and of 1 ml/kg per min for another 60 min prior to sampling.

Furosemide (kindly supplied by Dr. Wilfried De Jong, Química Hoechst de México) was administered to another five dogs, following control periods. After an iv priming dose of 10 mg/kg, the diuretic drug was infused continuously at a rate of 0.15 mg/kg per min. Two to three consecutive urine and blood samples were taken when maximal diuresis had been reached. A 100 mM NaCl-3 mM KCl solution was infused simultaneously in adequate amounts to maintain fluid balance.

Glomerular filtration rate was decreased stepwise through the elevation of the ureteral catheter to 30, 45, and 60 cm above the level of the table, in each of six animals.

Stop-flow experiments were carried out on four dogs

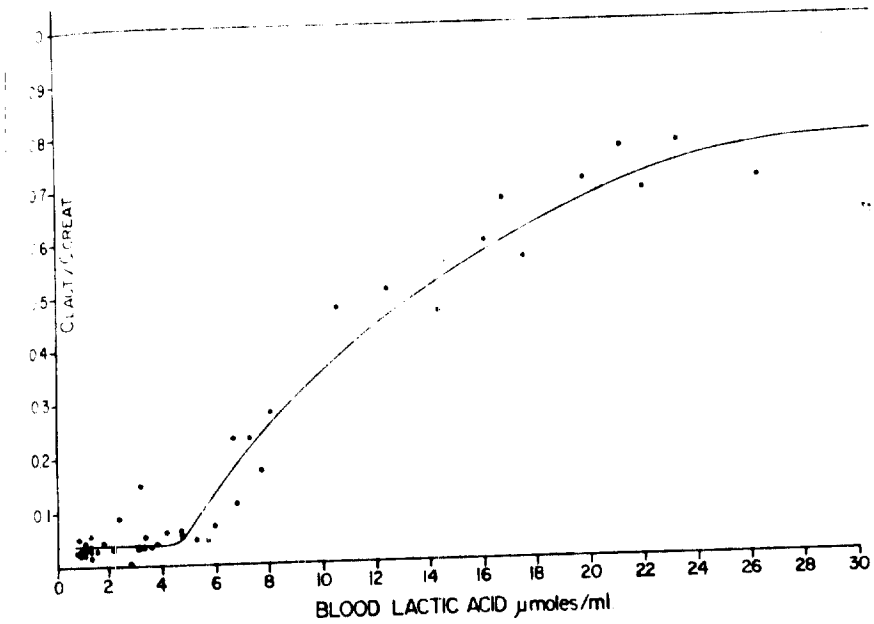

FIG. 1. Lactic acid-to-creatinine clearance ratio as a function of blood lactic acid concentration. No threshold concentration of lactic acid was found. Smooth curve was drawn freehand.

using the technique described by Malvin et al. (14). In these studies creatinine and PAH were incorporated into a 20% mannitol solution, along with enough DL-Na-lactate to deliver from 100 to 200 μmoles/kg per min. This solution was injected at a constant rate of 0.6 ml/kg per min after the administration of a suitable priming solution. Control clearance periods were obtained before and after the occlusion of the ureter, which was 6 min in duration.

All solutions containing sodium lactate were titrated to pH 5 with HCl, so that the metabolism of the lactate ion would not produce alkalosis. Blood pH ranged from 7.32 to 7.45 in most experiments. Cyanosis, tachycardia, and muscular rigidity often occurred when lactate was infused at the highest rates and despite artificial ventilation with a Harvard respiration pump. Some animals (not included in this report) died during this condition.

Whenever a variable was introduced in the experimental protocols, a period of at least 45 min was allowed for equilibration. All values shown in figures and tables are the average of two to three consecutive clearance periods.

Creatinine and PAH were analyzed in urine and protein-free plasma filtrates with the methods of Bonsnes and Taussky (2) and of Bratton and Marshall (3), respectively. Sodium was measured with a flame photometer. Lactic acid was estimated in blood and urine with the enzymatic method of Lundholm et al. (13). Rabbit muscle lactic dehydrogenase (Sigma Chemicals Co., type I) was used for the assay. The recovery of lactate added to blood was 101.0% ± 1.73 SD and of that added to urine 98.7% ± 1.62 SD. The coefficient of variation of the analysis was 0.14%. At least 0.1 μmole/ml of lactate can be detected accurately with this method. Blood for lactic acid analysis was collected directly into a centrifuge tube containing 10% cold perchloric acid in order to stop the metabolic production of lactic acid by the erythrocytes in vitro. The analysis was performed on the neutralized protein-free filtrate. The ratio of whole blood to plasma lactic acid concentration, as determined in a separate series of experiments with lactic acid concentrations ranging from approximately 1 to 20 μmoles/ml, ranged from 0.847 to 0.905 and averaged 0.862. Since whole blood concentrations of lactic acid were used throughout to calculate filtered loads, there is a constant error of approximately 15% in these values.

RESULTS

Net tubular reabsorption of lactic acid. As shown in Fig. 1, at blood concentrations of lactic acid ranging from normal endogenous values (0.92 ± 0.15 SD μmole/ml) to about 5 μmoles/ml, the urinary excretion of this compound averaged about 3% of the filtered load. As the filtered load was raised, the rate of excretion increased sharply and the clearance of lactic acid approached that of creatinine asymptotically. Net secretion of lactic acid was never observed even at blood concentrations some 30 times higher than basal values.

Net tubular reabsorption of lactic acid showed the kinetics of a rate-limited transport mechanism, as shown in Fig. 2 (closed circles). Lactic acid Tm averaged 28 ± 3.6 SD μmoles/min per kg body wt and was attained at filtered loads of approximately 224 ± 29 SD μmoles/min per kidney.

Effect of urine flow on lactic acid excretion. The excretion of lactic acid was correlated to urine flow. Figure 3 shows a statistically significant direct correlation between

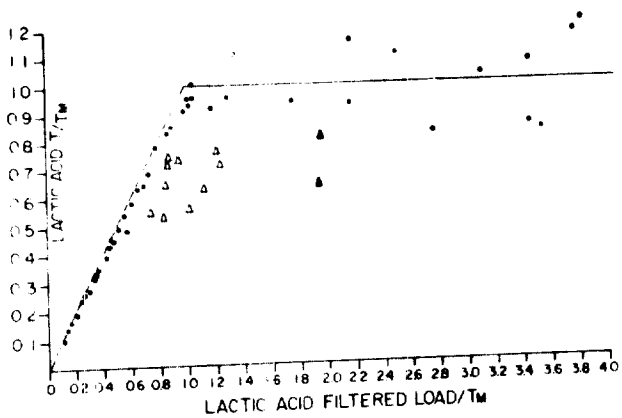

FIG. 2. Relationship between lactic acid reabsorption and filtered load. Triangles indicate results of experiments performed under simultaneous lactate and 20% mannitol loading. Dots correspond to experiments in dogs given a lactic acid load but no mannitol.

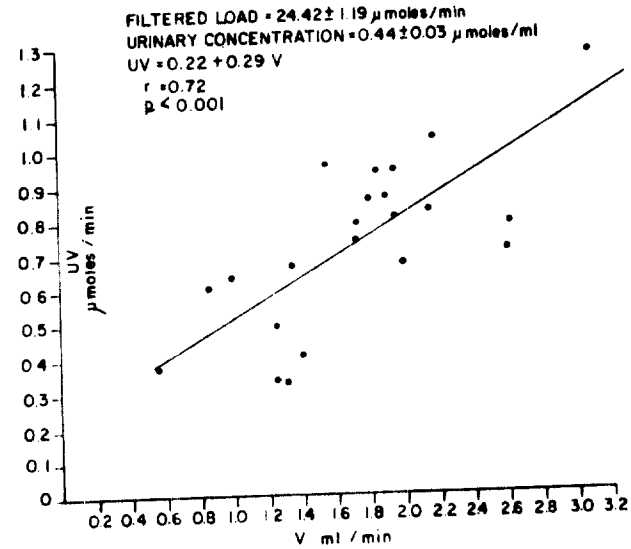

FIG. 3. Relationship between urinary excretion of lactic acid and urine flow at low filtered loads. Regression line was calculated by the least-squares method (11). Vertical standard deviation of the regression line at the point $x, \bar{y}$ is ± 0.17.

lactic acid excretion and urine flow in animals with a low filtered load. In these experiments differences in urine flow represent spontaneous variations in the diuretic response of the animals to the infusion of the control solution.

Table 1 summarizes the results obtained during saline- and drug-induced diuresis. It can be observed that the excretion of lactic acid increases markedly during forced diuresis. However, according to the regression equation of Fig. 3, the expected excretion of lactic acid during saline diuresis (1.22 μmoles/min) was lower than actual excretory rates (1.90 μmoles/min); this difference was statistically significant ($t = 2.59$, $P < 0.05$). In the furosemide diuresis experiments,

TABLE 1. *Effect of saline- and drug-induced diuresis on lactic acid excretion*

	Control* (N = 11)	Saline† (N = 6)	Furosemide‡ (N = 5)
A_{lact}, μmoles/ml	0.83±0.11	0.75±0.10	0.95±0.[illegible]
V, ml/min	0.65±0.15	3.45±0.51	5.74±0.[illegible]
C_{creat}, ml/min	24±1.2	29±3.4	21±2
U_{lact}, μmoles/ml	0.60±0.09	0.55±0.14	0.35±0[illegible]
UV_{lact}, μmoles/min	0.39±0.07	1.90±0.26	2.01±0[illegible]
F_{lact}, μmoles/min	19.9±1.3	21.8±2.6	20.0±2[illegible]
C_{lact}, ml/min	0.47±0.09	2.53±0.43	2.12±0[illegible]
C_{lact}/C_{creat}	0.020±0.[illegible]1	0.087±0.015	0.101±0[illegible]

All values are means ± SEM. * Prime: creatinine [illegible]0 mg/ml, PAH 4 mg/ml; 2 ml/kg. Sustaining infusion: creati[illegible]e 1.73 mg/ml, PAH 0.6 mg/ml, NaCl 8.5 mg/ml; 0.3 ml/kg [illegible]er min for 60 min prior to sampling. † Same sustaining in-fusion as in control studies plus NaCl 120 mM, $NaHCO_3$ 20 mM, KCl 3 mM; 2 ml/kg per min for the first 20 min and 1 ml/kg per min for another 60 min prior to sampling. ‡ Furosemide was given in a continuous infusion (0.15 mg/kg per min) and a priming dose of 10 mg/kg. Samples were collected when maximal diuresis was attained. Urinary losses were immediately replaced by constantly infusing adequate volumes of a 100 mM NaCl-3 mM KCl solution.

expected lactic acid excretion (1.88 μmoles/min), agreed closely with actual values (2.01 μmoles/min).

Effect of reducing filtered load on lactic acid excretion. The filtered load of lactic acid was decreased below basal levels by reducing GFR in six animals. Table 2 shows the results of two representative experiments. It can be observed that the concentration of lactic acid in the urine remained above a limiting value of about 0.4 μmole/ml, and that the excretion of the acid did not fall below 3% of the filtered load, despite the fact that the load of lactic acid was reduced to about one-third of normal values. In contrast, the urinary concentration of sodium decreased as GFR fell, so that C_{Na} was depressed disproportionately to C_{creat} (Table 2). In all these experiments blood concentrations of lactic acid and of sodium remained approximately constant.

Effect of osmotic diuresis on lactic acid excretion. Under conditions of simultaneous loading with lactic acid (100–200 μmoles/kg per min) and 20% mannitol (0.6 ml/kg per min), lactic acid reabsorption was significantly depressed below "nondiuretic" control values. These results are plotted in Fig. 2 (open triangles). In these experiments the lactic acid concentration of blood ranged from 5.09 to 10.80 μmoles/ml and that of the urine from 4.32 to 15.28 μmoles/ml. Urine-to-blood concentration ratios varied between 0.5 and 2.3. Lactic acid-to-creatinine clearance ratios varied from 0.15 to 0.69. Urine flow ranged from 5.3 to 16.6 ml/min per kidney.

Localization of the site of lactic acid reabsorption. In four stop-flow experiments (Table 3, Fig. 4) it was observed that a marked concentration gradient for lactic acid developed in those samples which are interpreted to correspond to the fluid that was in contact with the proximal tubular epithelium during the period of flow detention. In all experiments the minimal urine lactic

TABLE 2. *Effect of reducing filtered load on excretion of lactic acid*

Exp. No.	V_{lact}, μmoles/ml	C_{creat}, ml/min	V_{i}, ml/min	U_{lact}, μmoles/ml	UV_{lact}, μmoles/min	F_{lact}, μmoles/min	C_{lact}/C_{creat}	U_{Na}, μEq/ml
XXII		Prime: creatinine 20 mg/ml, PAH 4 mg/ml, mannitol 100 mg/ml; 2 ml/kg						
18 kg body wt		Infusion: creatinine 1.73 mg/ml, PAH 0.6 mg/ml, mannitol 30 mg/ml, NaCl 8.5 mg/ml, 0.3 ml/kg per min for 60 min prior to sampling						
	0.77	37	1.73	0.45	0.78	28	0.03	89
		Ureter elevation to 30 cm above table level for 45 min prior to sampling						
	0.82	28	1.05	0.54	0.57	23	0.03	43
		Ureter elevation to 45 cm above table level for 45 min prior to sampling						
	0.75	19	0.80	0.54	0.43	14	0.03	36
		Ureter elevation to 60 cm above table level for 45 min prior to sampling						
	0.75	11	0.55	0.51	0.28	8	0.03	19
XXI		Prime and infusion as in exp XXII						
25 kg body wt	0.66	31	2.15	0.69	1.48	20	0.07	176
		Ureter elevation to 30 cm above table level for 45 min prior to sampling						
	0.68	23	1.50	0.66	0.99	16	0.06	99
		Ureter elevation to 45 cm above table level for 45 min prior to sampling						
	0.72	16	1.10	0.69	0.76	12	0.07	26
		Ureter elevation to 60 cm above table level for 45 min prior to sampling						
	0.74	10	0.70	0.72	0.50	7	0.07	19

Each value is the average of two consecutive 10-min clearance periods.

acid concentration coincided exactly with the maximal PAH concentration which was used as a marker for the proximal tubule. The reabsorption of sodium against a concentration gradient in the same experiments occurred in more "distal" samples.

DISCUSSION

The main point of interest of the present investigation lies in the demonstration that lactic acid reabsorption along the nephron occurs in the proximal tubule. Although it is acknowledged that the stop-flow technique does not lend itself to the precise study of phenomena taking place in the proximal convolution, this is mostly true of transport mechanisms that do not result in the development of concentration gradients. The perfect coincidence of the maximal lactic acid and PAH concentration gradients in the same stop-flow samples, can be taken as sufficient evidence to conclude that lactic acid reabsorption occurs in the proximal tubule. This was an expected result since most organic compounds which are reabsorbed by the renal tubules are transported by the proximal tubular epithelium. It is also the case for other organic acids such as citric acid (18), alpha-ketoglutaric acid (5), malic acid (17), uric acid (12), and various amino acids (4, 15).

The infusion of hypertonic sodium lactate solutions during our stop-flow experiments undoubtedly expanded the extracellular volume. This is probably the explanation for the moderately high values of the minimal concentration that were observed (Table 3). Extracellular expansion depresses both proximal (8) and distal sodium reabsorption (9). The latter effect apparently decreases the capacity of the distal tubule to lower the urinary concentration of sodium and can be detected in stop-flow studies (1).

In the present experiments it was possible to confirm Craig's observation that the net reabsorption of lactic acid is rate limited (6), since the reabsorptive mechanism exhibited saturation kinetics (Fig. 2). This observation along with the large concentration gradient developed for lactic acid in the proximal tubule, would indicate that lactic acid is actively transported across the tubular epithelium. This conclusion is particularly warranted if indeed there is no transtubular electrical potential difference in the proximal tubule (16).

The inability of the kidney to reabsorb lactic acid completely, even at very low filtered loads (Table 2), suggests that lactic acid transport may be gradient, as well as rate limited. (There might be some doubt that the small quantities of lactic acid being excreted at endogenous blood lactate levels is in fact lactate. This is unwarranted since the assay method employed was quite specific. Apart from L-(+)-lactic acid, only α-hydroxybutyrate and β-chlorolactate react to a slight extent with heart muscle lactic dehydrogenase, and these two compounds do not occur naturally.)

The depression of lactic acid reabsorption during mannitol diuresis is compatible with the concept of gradient limitation. Indeed, if lactic acid reabsorption in the proximal tubule is gradient limited, it would be expected that the enhancement of proximal tubular volume flow and the dilution of the solutes in the proximal tubular fluid, as effected by mannitol, would decrease the reabsorptive rate of lactic acid. However, this is not the only possible explanation for the enhanced excretion of lactic acid during osmotic diuresis.

One distinct feature of lactic acid excretion was its direct correlation to the rate of urine flow. This was observed regardless of the means of producing osmotic diuresis. This phenomenon might be explained if gradient limited reabsorption of lactic acid occurred in the distal tubule. However, this was not substantiated by the stop-flow experiments. Another possible explanation is that lactic acid might diffuse from the medullary interstitium where it accumulates, into the tubular lumen at a distal portion of the nephron as suggested by Dell and Winters (7). Such a phenomenon would ex-

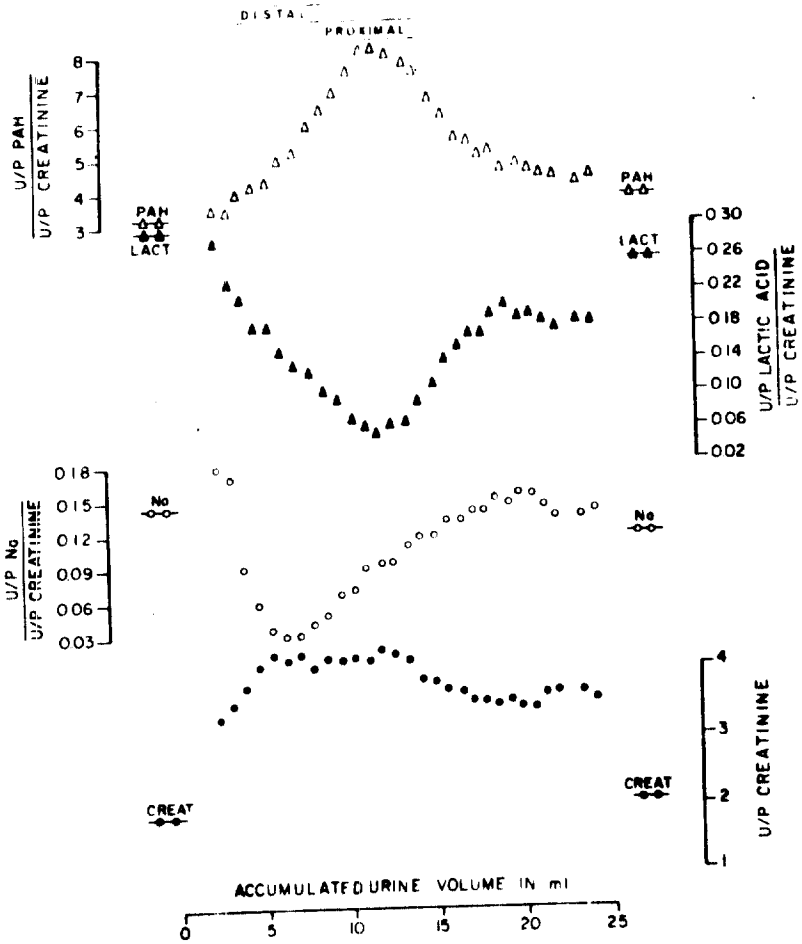

FIG. 4. *Experiment E* showing the st[illegible] flow patterns for PAH, lactic acid, sodium [illegible] and creatinine. Prestop-flow values are sh[illegible] on the left and poststop-flow values on th[illegible] right. Sustaining solution (0.85 mg/ml cre[illegible]nine, 0.3 mg/ml PAH, 200 mg/ml mannitol [illegible] M sodium lactate), infused at a constant [illegible]te of 0.6 ml/kg per min. Urine flow prior to occlusion = 8.4 ml/min; postocclusion = 6.9 ml/min. Mean blood lactic acid concentration 9.3 μmoles/ml. Net reabsorption of lactic acid prior to occlusion = 89.7 μmoles/min, postocclusion = 96.4 μmoles/min. Ureteral occlusion time = 6 min. Collection time = 220 sec. Dog weight = 12 kg.

TABLE 3. *Stop-flow experiments of lactic acid reabsorption*

Substance	Determinations	Experiment A	C	D	E
Lactic acid	Min conc., μmoles/ml	1.68 (9.27)	1.78 (12.37)	1.49 (13.12)	2.28 (10.05)
	U/P	0.252	0.208	0.143	0.246
	$(U/P_{lac})/(U/P_{creat})$	0.038	0.037	0.034	0.057
p-Aminohippuric acid	Max conc., mg/ml	1.68 (9.27)	0.166 (12.37)	0.680 (13.12)	0.730 (10.05)
	U/P	41	11	42	36
	$(U/P_{PAH})/(U/P_{creat})$	6.62	1.92	10.10	8.46
Sodium	Min conc., μEq/ml	5.2 (3.92)	11.0 (4.03)	11.1 (5.40)	15.4 (1.67)
	U/P	0.033	0.088	0.110	0.123
	$(U/P_{Na})/(U/P_{creat})$	0.053	0.015	0.024	0.030

Values in parentheses refer to the accumulated volume (in ml) corresponding to the sample where the minimal or maximal concentrations of each compound were found.

plain the apparently fixed lactate concentration in the final urine at low filtered loads (Tables 1 and 2). The amount of lactate diffusing in such a manner would be a function of the concentration gradient between the medullary interstitium and the tubular fluid delivered from the proximal convolution into the distal tubule and of the permeability of the epithelium to lactate. In our stop-flow experiments net secretion of lactate was not observed. However, these experiments were done under conditions of lactic acid loading. The lactate concentration in the fluid trapped in the distal segment may have been too high to permit diffusion of the acid into the tubular lumen.

During saline loading, lactic acid excretion increased more than would be predicted by the augmentation of urine flow. This result suggests that extracellular fluid expansion depresses the reabsorption of lactic acid, as it has been shown for sodium (8) and for bicarbonate (10).

In summary, it is concluded that lactic acid is actively reabsorbed in the proximal tubule of the dog kidney that this transport mechanism is rate limited, and that

lactic acid is either incompletely reabsorbed at low filtered loads, or partially secreted at a distal site of the nephron.

This work was supported by a research grant from Norwich Pharmacal Co.

Received for publication 5 February 1968.

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The role of certain products of the intermediate metabolisms in the cancerous process.

By N. Dobrovolskaia-Zavadskaia.

We had occasion to observe in a sacroma patient an accumulation of pyruvic acid which progressed with the advance of the disease: This observation, which was confirmed repeatedly in various cancer patients, gave us the idea of verifying the influence of two intermediate metabolites: pyruvic acid and lactic acid, on the development of spontaneous cancers in the breast of mice.

The interest in such a verification is justified, in the first place due the particulars of the energy metabolism of the cancerous cell, which were put in evidence by Warburg and his coworkers (1924), by Dickens and Simer (1930), by Dickens and Weil-Talherbe (1943) etc. These investigations established that the cancer cells show a metabolism which is different from that of the normal corresponding tissues in that they combine a high aerobic and anaerobic glycolysis with a respiratory quotient above one: they consume consequently less free oxygen, but get their vital energy in the fermentation of carbohydreates with formation of lactic acid. Warburg called this phenomenon cellular asphyxia and considered it a cause of the cancerization.

But further studies have not confirmed this latter conclusion: the tendency to resort to glyclosis rather than to respiration appeared not only in the cancer cell, but equally in other cells when they are being altered by the degenerative processes or suffer in the pursuit of their functioning. In addition, it was found that certain normal tissues have a glycolysis comparable to that of grafted tumors. It is first of all the retina, then the medular part of the kidney and the serum of certain species; in the jejunal mucous membrane of the rat, the glycolysis proved to be as active in a tumor with vigorous proliferation, and the respiratory quotient was 0.85.

Nevertheless the fermentative action on the sugar remains in the cancerous

tissue 70 to 80 times higher than that of the liver and 100 times higher than in the blood. Dreyfus (1940) found in his studies on the humane dometrium that its cancerization yields the lowest respiratory quotient and that the glycolysis, particularly the anaerobic type, is much higher than in all the other conditions studied. Dickens and Weil-Malherbe observed in the hepatomas caused in the rat by p-dimethylaminoazobenzene, a high anaerobic glycolysis, a more moderate aerobic glycolysis, and a respiratory quotient of less than 1.

The precancerous states, determined in the liver by p-dimethylamino-azobenzene, show only a slight increase of the aerobic glycolysis, and in some cases only, a slight anaerobic glycolysis as well. The differentiated spontaneous hepatomas of the agouti mice show, on the other hand, the same respiration, glycolysis and respiratory quotient as the adjacent normal hepatic tissue. In correlation, the highly specialized functions - formation of urea and ammonia and (l(+)alamine, formation of acetoacetic acid and caprilic acid, oxidation of pyruvic acid - remain almost intact in these benign hepatomas, while they are more or less completely suppressed in the malign tumors produced by p-dimethylaminoazobenzene; only the synthesis of urea is maintained, in general, within very reduced but measurable limits. When all the other functions listed above have completely stopped in the malign neoformed tissue. Orr and Stickland on their part confirmed in tumors of the same type the fact that the typical glycogenolysis of the adult hepatic tissue is replaced in the differentiated neoplastic tissues by the fermentative degradation of the glucose. Consequently, though the glycolytic capacity was not at the origin of the neoplastic process, it is nevertheless a very characteristic metabolic particularity.

The degradation of the glucides is the principal source of the energy necessary for the functioning of the organism. Under normal conditions, as well as under pathological conditions, the glycogen (the fuel of life of Macleod) - liberates its energy not by a direct combustion, like coal in

a steam engine, which would yield temperatures incompatible with life, but by oxidation of hydrogen; this is a stingy supply of energy for the necessities: "the cell needs small change" according to the expression by Szent-Geoergyi. When this degradation comprises a consumption of molecular oxygen and the elimination of water and CO_2, it is called respiration; particularly effective from a calorigenic point of view, it constitutes, however, only one of the forms of a more general biological process, comprising the fermentations, that is, the vital oxidations which are produced without the aid of atmospheric oxygen.

In the more developed concept of cellular respiration (Polonvosky, 1944) the discharge of carbon dioxide is not indissolubly connected with the oxidation of the carbon atom; this is a secondary phenomenon, relating the decarboxylation (under the influence of carboxylase) of certain alpha-ketonic acids (for example, pyruvic acid). As far as the oxidation itself is concerned, it is considered principally as a dehydroxygenation of various substrates, catalyzed by a certain number of dehydrases, which transport the hydrogen levels from stage to stage to combine them finally with oxygen, activated by other diastatic systems of the type of the oxidases. Consequantly, respiration and glycolysis affect two complimentary aspects of the same process.

The role of lactic acid, CH_3 CHOHCOOH, and of pyruvic acid, CH_2 CO COOH, in the energetic degradation of the matter is important. We have known for a long time that lactic acid is formed with each muscle contraction, in the course of the secretory work of the glands, of the functioning of the nervous system, that sugar disappears from the blood and is transformed into lactic acid, etc. During the first half of this century we became aware of the importance of certain types of insaturation, of the role of the double bond of the carbonyl group, and so our attention was centered on other

intermediate metabolites and their mutual relations. As far back as 1912, Mayer observed the appearance of lactic acid in the urine of animals which had been given pyruvic acid per os. In 1913 Neuberg found a conversion of methyl glyoxal to lactic acid in the tissues, and Lavene and Meyer in the leucocytes. At the same time Dakin and Dudley demonstrated the existence of a glyoxalase enzume which converted the alpha ketonic aldehydes into corresponding hydroxy acids. Finally Case and Cook (1931) established the presence of methyl glyoxal and pyruvic acid among the products of the muscular metabolism.

The different problems of the intermediate metabolism aroused more and more the interest of scientific researches (First International Conbress of Biochemistry in Cambridge 1949). The reciprocal relations between the two metabolites studied by us are not quite clear. We take from Case (1932) the diagram on the following page which seems to us to summarize best the present state of the concepts on this subject. The left part of this diagram concerns the reduction of methyl glyoxal to lactic acid by means of methyl glyoxidase with gluthathion as a co-ferment- a simple addition of the ions of water H and OH (hydrogenation) is followed here by the dismutation of methyl glyoxal hydrated to lactic acid. The right part of the diagram leads by oxidation of methyl glyoxal to pyruvic acid; the decarboxylation of the latter to acetaldehyde and the reduction of acetaldehyde to alcohol (the last stages of the alcoholic fermentation) did not take place in the animal organism.

The importance of pyruvic acid in the intermediate metabolism is due to the fact that it is the end stage of the degradation not only of the glucides, but also of certain protides and lipids as well, and the starting point of the synthetic reconstitution of the specific products of the organism. The cancer's cell is according to its origin connected with the normal

cells. Though it does not serve any useful purpose in the organism, it draws off from there its nutritional materials and releases there the residues of its own metabolism. We know little about these residues; but it is strange that Mendle, Bauch and Strelitz have found (1931) that the neoplastic cells produce pyruvic acid in large quantities.

Personal observations.- In order to demonstrate the effective potentialities of the two metabolites under study with regard to cancerous cells, we used the method of overloading the organism with these same metabolites. The control was established individually for each tumor before the start of the treatment by calculating the growth index $a = \frac{dn - do}{t}$, where do and dn are the mean diameters of the tumor at the start and at the end of an observation period, and t indicates the number of days of this period; the index a is thus expressed in mm/day. The further calculation of this index in the course of the treatment permitted us to detect the slightest effects of penicillin, vitamins and many other agents studied by us.

These experiments can be divided into two principal groups, depending on the metabolite used:

Group 1.- Sodium pyruvate, in the form of a freshly prepared 10% solution was injected under the skin of 86 mice, first every second day and then every day; the doses were: 0.5 and 0.7 cc of this solution. Even before the start of the treatment, 84 mice had a total of 103 mammary adenocarcinomas; three of these carcinomas were not influenced by the pyruvate and continued to grow with the same growth index as in the control period: 35 tumors reacted by increasing their index a, a stimulating effect was found in 34%, and 65 tumors (63%) were inhibited. In the course of the treatment, 27 new locations made their appearance, that is 20.8% related to 130, the definite number of tumors. Out of 84 mice with adenocarcinoma of the breast, 39 (46%) developed pulmonary metastases.

Of the two remaining mice one had pavement epithelioma which was stimu-

lated by the pyruvate, and the other - a generalized lymphadenoma where certain subcutaneous nodules started to dry and to form crusts. The survival rate of all these animals from the start of the treatment is as follows: 40 mice (47%) were dead in the course of the first month; 28 (33%) in the course of the second month; 12 = 14% in the course of the third month; 5 (6%) in the course of the fourth month and 1 in the course of the sixth month.

Group II: - Lactic acid was used in the form of its two salts:

a) Sodium lactate in 2%, 4.7% or 5.4% solutions was contained in ampoules and heated until it was clear at the time of the injection. Daily doses of 0.5 or 0.7 cc for the strongest solutions and 1 cc for the 2% solution, were administered to 23 mice; 21 of these mice were carriers of 27 adenocarcinomas of the breat which reacted to the treatment as follows: none of these tumors remained indifferent to Na-lactate, that is, none survived without changing its growth index; 10 tumors (37%) reacted in the sense of stimulation and 17 (63%) in the sense of inhibition of their proliferation. More or less after the start of the treatment appeared new locations, which amounted to 26.6% relative to 34, the total number of breast cancers in the mice treated with sodium lactate. In 21 mice carrying these cancers, adenocarcinoma metastases were found in the lung six times = 29%.

We also had in this series of experiments 2 cases of lymphadenoma limited to the ganglions. These mice did not react by any clear local change, but their survival lasted up to four months and the generalization of their morbid process did not take place. In general, the death rate was distributed in this group over the months following the start of the treatment as follows: 1st month: 8 cases = 35%; 2nd month: 5 cases = 22%; 3rd month: 4 cases = 17%; and 4th month: 6 cases = 26%.

b) Calcium lactate was introduced under the skin of the left groin or of the flanks in the form of tablets. A small incision which was made for this

introduction which was closed by means of clamps. A single dose varying from 20 to 60 mg was applied to 24 mice.

Before this application 23 mice already had breast tumors which reacted as follows to the implantation of Ca-lactate:4 (16%) in the sense of stimulation and 21 (84%) in the sense of inhibition, only one with a week's delay. In the course of the further survival of these mice, 9 new locations were found, this is 26.5% out of a total of 34 breast cancers. Pulmonary metastases were found in 8 out of 23 mice, that is 35%.

The last mouse of this series had a chondro-sarcoma of the perineum which also reacted in the sense of a growth restriction, which increases the definite inhibition percentage to 85%. This mouse died 27 days after the application of the lactate. The other mice died after the intervention as follows: 1st month: 8 cases = 35%; 2nd month: 2 cases = 9%; 4th month: 2 cases = 9%; 5th month: 2 cases = 9%; 7th month: 1 case = 4%.

The implantation of calcium lactate under the skin was not followed by any local complication: the operative wounds healed normally, and the product was then resorbed progressively.

Discussion of the results obtained in the light of the microscopic study of the tumors.

In order to be judiciously interpreted the numerical data reported above must be compared with the changes produced in the tumors by the metabolites under study.

In group I, the mammary adenocarcinomas increased the rate of their growth in 34% of the cases under the influence of the pyruvate, under the microscope we find changes of a circulatory order, which are frequent in these cases and which can be contributed to this increase. In the figure on the opposite side (fig. 1a and b) we distinguish manifestations of a particular edema, which consists in the appearance of empty spaces all around the neoplastic cordons, as if there was here an accumulation of trans-

parent liquid between the cancerous tubes of the stroma. In certain figures, these cords continue to be full, but in certain others they are already on the way of dislocation. An adenocarcinoma with pavement metaplasia of the Borrel-Harland type presents an edema similar to the corneous globes (fig. 1c). The pulmonary metastases are generally in direct contact with the tissue of the organ; in this test series they are frequently detached by empty spaces (fig. 1b).

This circulatory anomaly is very characteristic of the effect of sodium pyruvate: it is found in the brain in the form of a perivascular edema, in the liver - in the form of a pericapillary edema, in the intestine - around the glandular tubes, in the heart, between the fasculi of the myocardium etc. We do not mean to say that a real stimulating effect of the pyruvate does not exist; it exists, we see its manifestation in the mitoses on the sections and in the growth of the tumoral mass; One case, that of 56752 LII, is an extremely demonstrative form this point of view. We had this mouse under preliminary observation for 189 days for three small nodules (1 x 2 mm) which did not develop. Soon after the start of the injections one of these tumors began to grow; it increaed regularly with a growth index of 0.27, and in 54 days of treatment it attained dimensions of 12 x 20mm. In the histological section (fig. 1e), we seen, in addition to a considerable peri- and intracordonal edema, numerous mitoses despite their not always regular nature, they ensured a considerable neoplastic proliferation. On the other hand, the other modules in the same mouse began to regress from the start of the treatment and no trace of them remained in the autopsy. This different reaction of different localizations in the same animal is in agreement with our findings in collaboration with I. Nekhorochef (1943) on 43 issues of our breeding. In studying the behavior of the index a in these different issues, it was found that the rate of growth of a

tumor depends less on a hereditary factor (that is on the terrain) than on the constitution of the tumor itself, that is, on the particular development potential.

This particular development potential played certainly a role in the 3 tumors where the reaction to the pyruvate did not manifest itself. These three tumors belonged to three mice, two of which also had other tumors; two of these tumors reacted in the sense of stimulation and one in the sense of restriction.

We now pass to the inhibition reaction, which was observed in 63% of the tumors treated with the pyruvate. This inhibition was always real, for lack of another mechanism that could determine the slowdown of the growth. Under the microscope the phenomon, which predominates in the histological sections, is the frequency of angiomateus and serous cavities, of various size. At the origin of these cavities we find the centers of hyperemia which are scattered from the start of the treatment. This arrangement in centers is a probable effect of the corresponding vasomotor nerves exited by the pyruvate. The antibiotic effect of these centers is reminiscent of that obtained at the level of hyperemia caused by penicillin, namely: the disappearance of the mitoses, the arrest of the proliferation neoplastic and the installation of the state of hypobiosis in the proximity of the dilated vessels in which the blood keeps circulating. We see in fig. 1d the progressive disappearance of the basophilic substance of the nucli, which become acidophilic; the changed cells continue to maintain their individuality, contrary to what happens in coagulant necrosis.

When the hyperemic vessels continue to dilate, they can be transformed into cavities and the progressive nature of this process explains the variety of size of the anginomatous cavities in the tumors treated with the pyruvate. In the course of this process certain vessels burst in the interior of the

neoplastic tubes; despite the dislocation by the extravescuar blood, the cancerous cells keep their chromatin better than in the vicinity of a vessel distended by the circulating blood. Could it be the oxygen of the circulating blood which exercises this antibiotic action on the cancerous cell? The extravasated blood is deprived of it since it has lost its connection with the pulmonary circulation.

The fact that the hyperemia and the formation of angiomatous cavities have not contributed to the growth of the tumors shows that the proliferation itself of the neoplastic cells had been involved. In fact, the sections of the tumors with a reduced growth index do not show in the majority of cases any conclusive mitosis; on the other hand, the nuclei in the course of a pyknosis appeared sometimes in small, more or less tufty layers. In rare cases we find on the bottom of the clearly differentiated nuclei isolated cells with dense masses of chromatin inside, which are reminiscent of metaphasias, but with blended chromoscomes. Anaphases are extremely rare; small dense balls side by side seem sometimes to indicate the end of an anaphase (fig. 1f).

It is logical to conclude from the preceding considerations that there is a clear correlation between the effect of the pyruvate on the growth indices and on the mitoses. This correlation manifests itself both in tumors which are inhibited in their growth and in tumors which are stimulated.

On the other hand, the action of the pyruvate entrains only to a very insignificant extent the manifestations of the means of defense against the cancerous process: tubulation, mesenchymatization, collagenization, as well as the active intervention on the part of the stroma.

The action of lactic acid was studied first in the form of its sodium salt. With regard to the preceding group of experiments, there was therefore only a change as far as the acid radical is concerned. The portion of the tumors stimulated by sodium lactate is a little higher, 37% compared to

34% in the group of tumors treated with the pyruvate. The microscopic study of the section showed far fewer edemas, almost now detachment of the neoplastic cords, but on the other hand much more hyperemia centers, which are more massive and more extended (fig. 2a), more voluminous angiomatous cavities and intratissular, more destructive hemorrhages than in the pyruvate group. These circulatory changes could have been at least partly responsible for the accelerated growth of the tumors.

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a) female 56343 XXXIX, Na-pyruvate 76 days, pericordonal edema.

b) female 56495 XXXIX, pyruvate 39 days, edematized pulmonary metastases

c) female 57522 XLVI, pyruvate 49 days, adenocarc inoma with pavement metal-plasia, edea at the level of the corneousglobes

d) female 57901 XXXIX, pyruvate 120 days, hyperemia center, hypobiotic state

e) female 56752 LII, pyruvate 54 days, stimulation, numerous mitroses

f) female 56642 XLI, pyruvate 42 days, inhibition, abortive mitoses.

Fig. 2.

a) female 57314 XLVIII, Na-lactate, 90 days + 8 days without treatment: massive hyperemia, deep hypobiosis of the tissue;

b) female 56736 XLVI, Ca-lactate 50 days, sclerosis, tubulation

c) female, 58181 IV B, Ca-lactate, 50 days, "lacteal zone", hypobiotic.

In correlation with the extent of the local circulatory manifestations we find the effect produced at these levels on the neoplastic tissue itself. Fig. 2a, which is characteristic of this effect, belongs to female 57314 XLIII whose tumor was apparently stimulated. This mammary adenocarcinoma showed in the section extended zones of massive hyperemia with an extremely

intensive antibiotic effect: no cell preserved, no circulating vessel, the general preserved topography discloses only the phenomenon produced. On the periphery of the tumor we find the tissue more vegetating with a rarefied acinous structure, a fibrous capsule all around the tumor. No mitosis. Tissue more compact and less congested than the two small tumors of recent origin; an effect of the shorter treatment or of the acquired increased tolerance? This mouse died after 107 days of treatment; its tumors measured at this time 14 x 18 mm (2 x 4 at the beginning), 6 x 8 and 3 x 4. Consequently these tumors grew without a stimulating effect on the nuclei having been established.

In another tumor of the same lot (female 58295 IV B) we found immediately after the start of the treatment a clear hyperemia at the periphery of the tumor and at certain points of its vegetating part numerous mitoses with a pyknotic tendency. Hyperemia, angiomatization and a hypobiotic state were also found in the pulmonary metastases. No tumor remained indifferent to the action of sodium lactate. The remaining tumors (63%) all reacted by restriction of their growth. The phenomena of hyperemia, angiomatization, intratissular hemorrhages and hypobiosis are as frequent as in the stimulated tumors. In one case with rapid growth before the treatment (a = 0.47 for 17 days) we found in the section (17 days of treatment) numerous mitoses, but all with blended chromosomes (a = 0.58 during the last 8 days).

Contrary to the observations in the pyruvate group, the sclerotic effect of sodium lactate on the stroma manifested itself particularly in the cases with prolonged survival (fig. 2b). Drying and drop of a tumor were followed by recidivation on the spot within a month. In some cases tubulation and mesenchymatization were observed.

The administration of calcium lactate was effected in the form of a single

massive dose, introduced under the skin; the reaction was again produced in both directions with marked predominance of the inhibition (85%). The changes found under the microscope are similar in the majority of cases to those described for sodium lactate. Nevertheless it was possible to detect in some cases a particular effect limited to a defined zone or presenting several similar centers concentrated in a part of the tumor. The morphological modalities of these "zones" are in correlation with the characteristic changes of the lactate effect mentioned above,namely:
1) accumulation of large angiomatous cavities grouped side by side; 2) state of cytolysis, hypobiosis or hemorrhagic nephrosis predominant in one region of the tumor; 3) the manifestations of sclerosis which are in most cases added to the preceding changes and which are generally accompanied by the phenomena of cytolysis in the vicinity (fig. 2c).

The analogy with the "penicillinate zone" obtained with little purified penicillin (1944) and the "gravidic zone" following gestation and lactation, was called by us the "lacteal zone", described above. The phenomenon of "zone" indicates the instantaneous effect of an agent, operating for a limited time, on the tumor which exists at that moment. Once this action is terminated, the tumor resumes its usual behavior and furnishes thus a morphological individual control (that is, on the same tumor) of what has been done. The "zone" effect appeared also as absolute proof that the agent used had an affinity for the neoplastic process.

In our calculations we have disregarded the "new locations", that is, those which appeared after the start of the treatment. The reason is that the locations of a mammary adenocarcinoma seems to be determined by its hereditary disposition rather than by other agents. In studying (with Mme. Adamova 1939) the correlation between the endogenous agents and the exogenous agents (radon and 1-2-5-6-dibenzanthracene) we have seen frequently that the mammary adenocarcinomas did not appear at the levels of the agent,

but elsewhere, as if there were predestined places for them. The hereditary transmission of a specific location (that of the nape in a mother and its two daughters, that of the right armpit in two sisters (fig. 3c) etc), also argues in favor of the existence of a constitutional localizing factor. This idea finds its confirmation in the present test series; with the various agents used, we obtained very close new location percentages: 21% for pyruvate, 21% for sodium-lactate and 26% for Ca-lactate.

The behavior of the pulmonary metastases, on the other hand, is more in correlation with the evolutional potentials of the tumors, and one can say to a certain degree with the product administered: the highest value (46%) was obtained for the pyruvate, then 35% for Ca-lactate, and 29% for Na-lactate.

For the survival of the animals, as well as for the metastases the pyruvate proves most unfavorable; 80% of the mice treated died in the course of the first two months; there were only 6% which lived to the fourth month, and one mouse died in the sixth month. The mean portion belongs again to Ca-lactate: 70% dead in the course of the first two months, but the mice which survived this period and consequently withstood the resorption of the implanted Ca-lactate, disappeared progressively, the last mouse being dead in the 7th month. With Na-lactate, 57% died in the course of the first two months and 26% lived up to the 4th month.

From the foregoing considerations we arrive at the conclusion that among the multiple factors which play a role in the cancerous process, the metabolites studied can exert at certain times a decisive influence on its development. Don't they also play a part in its pathology?

A test series made with the pyruvate on animals of a non-cancerous strain showed no case of cancerization; being a normal intermediate element of the economy of the organism, the pyruvate proved incapable of producing a cancer in the absence of a hereditary disposition, contrary to dibenzanthracene and randon, which produced locally the sarcomas and pavement epitheliomas

(no adenocarcinoma) in the same strain. This does not prove, however, in the constellation of circumstances which lead to cancerization that the intermediate metabolite does not contribute in any way to it.

With the discovery of the role of the "milk factor" in the pathology of the mammary adenocarcinoma in the mouse we have finally arrived at the idea of the virus. Graft, Moore, Stanley, Randall and Haagensen recently isolated this milk "factor" from our strain R 111 and from mice of the strain C 57, which were lactated with nurses R 111. The electronic microphotography used in this study showed particles of varied sizes, more or less rounded and isolated, or united in conglomerates; these conglomerates are more numerous and more voluminous for the milk of nurslings (fig. 3a). This variety of form, and particularly the dimensions are not in agreement with the idea of an exogenous virus, that is, a living virus which is itself capable of producing cancer. For the living creatures, not only the form, but the definite dimensions are likewise specific; the variations is assumed within the narrow limits of a Gauss curve.

The idea of an endogenous virus would perhaps be more acceptable, but it is still less specific. In our research on the fractioning of the extracts of cancerous tissues and of the organs rich in cells, we are struck by the difficulty of getting rid of the salts. In order to identify them, we used a method of micro-crystallization by evaporating a drop of the production in clear solution on a blade. Under the microscope we saw then the crystals we were looking for, but also sometimes figures of extraordinary complexity (and even beauty), composed of rounded particles, very much reminiscent of the lacteous agent. (fig. 3b). Dr. Deicha examined for us these particles under the polarizing microscope and they were found to be spherolites, that is, formations having a crystalline nature. We must ask ourselves if the product which we isolated from the milk of the strain R

111 does not perhaps also represent an intermediate stage of protein degradation, more specifically of that of nucleo-proteins. This does not prove that this product has in addition a special character: it is formed in an organism predisposed to cancer, that into a particular physiological mechanism which is characteristic of this state of predisposition; and it is the final stage. If we want to make it act in a strain less predisposed (strain C 57 with 1.9% cancer) we have to introduce at the beginning of its life (up to 12 days) that is, before the mechanism corresponding to this strain is established. Its action in a strain highly predisposed to cancer was completely suppressed (White and Andervont 1943) by a diet free of cystine (no tumor) and reduced from 97.3% to 25% by a diet free of lysine (White and White, 1944). All this shows that the milk factor did not act alone, it is only one of the mechanism which play a role in the cancerization and which are transmitted by heredity, sometimes only by cystoplasmic heredity.

The same holds true for the hormonal factor; its particular mechanism in cancerous strains was put in evidence by an additional application of estrogen hormones (acassagne, 1938), but this application had no effect in non-cancerous strains; the cooperation of the milk factor seems to be indispensable.

Fig. 3.

a) "lacteous virus", electronic American microphotography

b) product of fractioning of a protein extract, microcrystallization in the form of spherolites

c) simultaneous appearance of mammary cancer in the right armpit of two sisters: female 56402 and 56403 of the strain XXXIX.

Nevertheless neither the hormonal factor nor the milk factor can explain the extraordinary efficacy of the localizing factor mentioned above, which determines probably at the same time the histologic form. The manifestations of this factor were not changed to any degree by our interventions for the mammary cancer. But for the sarcomas and the pavement epitheliomas its role has been assumed by the exogenous factor: the reaction on the carcinogen was local. And moreover the sisters with the same locations (fig. 3c), developed their tumors simultaneously, which proves still another factor namely the time factor. The collaboration of all these factors is necessary if a cancerous form similar to that of the mother is to be produced in the descendants. This explains the rarity of such a complete reproduction, particularly with regard to the time factor. In a family descending from a cancerous mother, the daughters develop the tumors frequently with various delays. It can thus be assumed that, contrary to the localizing factor, the time factor is less vigorous in its manifestations and that it can be influenced by certain other factors. Among these factors, the interior conditions of the economy of the organism, the particulars of its metabolism must play a role. We observe actually a recrudescence of cancer cases, and we ask ourselves what is the cause? Is it not in fact an activation of the time factor by abnormal conditions of the war period, by too one-sided nutrition, by continued intestinal fermentation etc.?

After a period of latency in the cancerous process, the tumors begin to develop more rapidly than under normal conditions, and this manifests itself in the impression of a general increase of the cancers. The possibility of advancing or delaying the start of the cancerization has been repeatedly observed in the course of various experiments on strains predisposed to cancer.

This immense war-time experience on the human population works, it seems to us, not only in new fields of study, but it also shows at the same time perspectives not attended to by preventive medicine. In fact, if we found

a means of suppressing the intestinal fermentation by a proper diet, of activating the oxido-reduction reactions in the tissues, of improving the respiration in general, etc. and thus of delaying the onset of the cancerization beyond say 80-90 years, the cancer problem could be considered at least partly as solved.

Consequently the present experiments have shown the close relationship that exists between certain products of the intermediate metabolism and the development of mammary cancers in mice.

The neoplastic reaction, in response to an overload of the mechanism by sodium pyruvate, sodium lactate or calcium lactate, manifested itself either by the effect of stimulation (increase of the growth index numerous mitoses, or by the effect of inhibition (reduction of the growth rate and even regression of certain tumors, mitoses with confluence chromosomes).

The circulatory changes, precordonal edema, hyperemia centers for the pyruvate, more pronounced angiomatization and manifestations of sclerosis for the lactates were likewise recorded. The phenomena of hypobiosis, particularly for the lactates, were frequently observed.

In the series with calcium lactate, the regional effect under the influence of a single, more or less strong dose was observed; we called it "lacteous zone" in analogy to the "pencillinate zone" and the "gravidic zone". This "zone" presents decisive proof in favor of the intervention of the lactate in the neoplastic development.

Though these manifestations can be considered as means of defense against the neoplastic process (drying of the tumors) they have not determined any definite cure under the conditions of our experiments.

The multiplicity of factors, already established for the pathogeny of mammary cancer, finds once more its confirmation in the manifestation of the localizing factor (histological structural factor at the same time): the

percentage of new locations was not substantially influenced by the various metabolites. Among the other specific mechanisms which determine the functioning of these factors,the intermediate metabolism can nevertheless have some significance.

It is very likely that, what can be isolated as the "lacteous virus" falls itself into the category of degradation products of the proteins, or more specifically of the nucleo-proteins. This product would be a final stage of a particular mechanism playing its part in the neoplastic pathogeny.

The role of metallic ions requires an additional study.

The behavior of the various factors playing a role in the cancerization is varied. Some pertaining to the terrain present a character of extraordinary rigidity and can not be influenced by exogenous factors; this is the localizing factor for mammary adenocarcinoma. On the other hand, for sarcomas and pavement epitheliomas caused by carcinogenic agents, the location is determined by the carcinogen used, which holds thus the place of a hereditary localizing factor. The possibility of such a substitution is accounted for by the local reaction of the conjunctive tissue and of the pavement epithelioma to the cancerogenic effect.

Among the other factors belonging likewise to the terrain, but less rigid, it is the time factor which is of the greatest interest; the possibility of influencing it manifests itself in the delay of the appearance of tumors in a predisposed strain by a diet with a reduced caloric value; by the sudden appearance of breast cancer in a woman who has received a blow in the chest; it is probably also this factor which plays a role in the increase of cancers in the post-war period etc. The possibility of delaying the time factor works in a wide field of study and shows new perspectives in the field of preventive medicine for cancers.

Translated by Carl Demrick Associates, Inc./IE/db

Bull. Assoc. Franc. Etude Cancer 37:57-75. 1950.

Dobrovolskaia-
Zavadshaia-5
Bib No. 411

Le rôle de certains produits du métabolisme intermédiaire dans le processus cancéreux

PAR

N. DOBROVOLSKAÏA-ZAVADSKAÏA

Nous avons eu l'occasion d'observer, chez un malade sarcomateux, une accumulation de l'acide pyruvique qui progressait avec l'aggravation de la maladie. Cette observation qui a été confirmée depuis à plusieurs reprises, sur des malades cancéreux variés, nous a donné l'idée de vérifier l'influence de deux principaux métabolites intermédiaires : l'acide pyruvique et l'acide lactique, sur l'évolution des cancers spontanés de la mamelle chez les souris.

L'intérêt d'une telle vérification est justifié, en premier lieu, par les particularités du métabolisme énergétique de la cellule cancéreuse qui ont été mises en évidence par Warburg et ses collaborateurs (1924), par Dickens et Simer (1930), par Dickens et Weil-Malherbe (1943), etc. Ces investigations ont établi que les cellules cancéreuses présentent un métabolisme distinct de celui des tissus normaux correspondants en ce qu'elles combinent une glycolyse aérobique et glycolyse anaérobique élevées avec le quotient respiratoire au-dessous de l'unité : elles consomment, en conséquence, moins d'oxygène libre, mais puisent leur énergie vitale dans la fermentation des hydrates de carbone avec formation de l'acide lactique. Warburg a donné le nom de l'asphyxie cellulaire à ce phénomène et le considérait comme cause de la cancérisation.

Les recherches ultérieures n'ont pourtant pas confirmé cette dernière conclusion : la tendance à recourir à la glycolyse plutôt qu'à la respiration s'est montrée propre non seulement à la cellule cancéreuse, mais également à d'autres cellules quand elles viennent d'être altérées par les processus dégénératifs ou souffrent dans la poursuite de leur fonctionnement. En outre, il a été trouvé que certains tissus normaux possèdent la glycolyse comparable à celle des tumeurs greffées : c'est en premier lieu la rétine, puis la partie médullaire du rein et le sérum de certaines espèces ; dans la muqueuse jéjunale du rat, la glycolyse s'est montrée aussi active que dans une tumeur à prolifération vigoureuse, et le quotient respiratoire était de 0,85.

Néanmoins, l'action fermentative sur le sucre reste, dans le tissu cancéreux, soixante-dix à quatre-vingts fois plus élevée que celle du foie et cent vingt-quatre fois plus élevée que celle du sang. Dreyfus (1940) a trouvé, dans ses études sur l'endométrium humain, que sa cancérisation donne le quotient respiratoire le plus bas et la glycolyse, surtout anaérobie, beaucoup plus élevée que dans tous les autres états étudiés. Dickens et Weil-Malherbe ont observé, pour les hépatomes provoqués chez le rat par le p-dimethylaminoazobenzène, une glycolyse anaérobie élevée, une glycolyse aérobie plus modérée et le quotient de respiration au-dessous de l'unité.

Les états précancéreux, déterminés dans le foie par le p-diméthylaminoazobenzène, ne montrent qu'une petite augmentation de glycolyse aérobie et, dans quelques cas seulement, une légère glycolyse anaérobie également. Les hépatomes spontanés, bien différenciés, des souris agouti avaient, par contre, la respiration, la glycolyse et le quotient respiratoire comme dans le tissu hépatique adjacent normal. En corrélation, les fonctions hautement spécialisées — formation de l'urée de l'ammoniaque et de 1 (+)-alanine, formation de l'acide acétoacétique de l'acide caprilique, oxydation de l'acide urique, synthèse des hydrates de carbone fermentables de l'acide pyruvique — restent presque intactes dans ces hépatomes bénins, tandis qu'elles se trouvent plus ou moins complètement supprimées dans les tumeurs malignes produites par le p-diméthylaminoazobenzène : seule la synthèse de l'urée se maintient, en général, dans les limites très réduites, mais mesurables quand toutes les autres fonctions, énumérées plus haut, se sont complètement éteintes dans le tissu néoformé malin. Orr et Stickland de leur côté confirment, sur les tumeurs du même genre, le fait que la glycogénolyse typique du tissu hépatique adulte est remplacée, dans les tissus néoplasiques dédifférenciés, par la dégradation fermentative du glucose. Par conséquent, si même la capacité glycolytique n'était pas à l'origine du processus néoplasique, elle en est, tout de même, une particularité métabolique très caractéristique.

La dégradation des glucides présente la source principale de l'énergie nécessaire pour le fonctionnement de l'organisme. Dans les conditions normales, aussi bien que pathologiques, le glycogène — ce combustible de la vie (the fuel of life de Macleod) — libère son énergie non pas par une combustion directe comme le charbon dans une machine à vapeur, ce qui donnerait des températures incompatibles avec la vie, mais par oxydation de l'hydrogène ; c'est un débit parcimonieux de l'énergie au fur et à mesure des nécessités : « la cellule a besoin de petite monnaie » suivant l'expression de Szent-Gyorgyi. Quand cette dégradation comporte une consommation d'oxygène moléculaire et une élimination de l'eau et de CO_2, on la désigne du nom de respiration ; spécialement efficace au point de vue calorigène, elle ne constitue, pourtant, qu'une des formes d'un processus biologique plus général, comprenant les

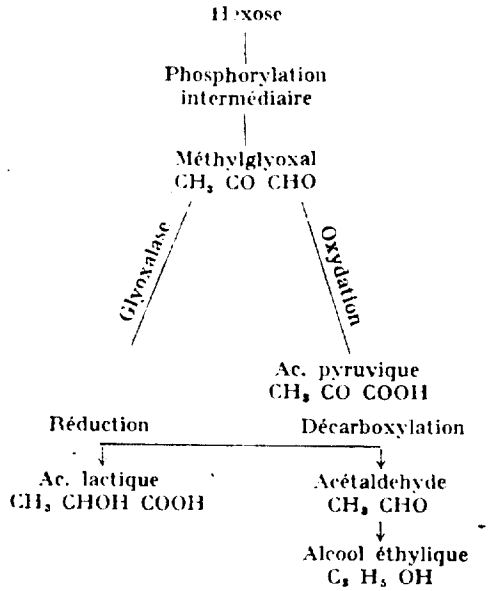
fermentations, c'est-à-dire, les oxydations vitales se produisant sans le concours de l'oxygène de l'air.

Dans la conception plus évoluée de la respiration cellulaire (Polonovsky, 1944), le départ de gaz carbonique n'est plus indissolublement lié à l'oxydation de l'atome de carbone ; c'est un phénomène secondaire, tenant à la décarboxylation (sous l'influence de carboxylase) de certains acides α-cétoniques (par exemple, acide pyruvique). Quant à l'oxydation elle-même, on la considère principalement comme une déshydrogénation des divers substrats, catalysée par un certain nombre de déshydrases, qui transportent de palier en palier, les molécules d'hydrogène, pour finalement les unir à l'oxygène, activé par d'autres systèmes diastasiques, du type d'oxydases. Par conséquent, la respiration et la glycolyse affectent deux aspects complémentaires d'un processus unique.

Le rôle de l'acide lactique CH_3 CHOH COOH et de l'acide pyruvique CH_3 CO COOH, dans la dégradation énergétique de la matière, est capital. Depuis longtemps, l'on sait que l'acide lactique se forme à chaque contraction musculaire, au cours du travail sécrétoire des glandes, du fonctionnement du système nerveux, que le sucre disparaît du sang en se transformant en acide lactique, etc. Au cours de la première moitié de ce siècle, on a commencé à se rendre compte de l'importance de certains types d'insaturation, du rôle de la double liaison du groupe carbonile, et c'est ainsi que l'attention a été attirée vers d'autres métabolites intermédiaires et leurs relations mutuelles. Déjà en 1912, Mayer a observé l'apparition de l'acide lactique dans l'urine des animaux, auxquels on a donné de l'acide pyruvique par la bouche. En 1913, Neuberg a constaté une conversion du méthylglyoxal en acide lactique dans les tissus et Lavène et Meyer, dans les leucocytes. En même temps, Dakin et Dudley ont démontré l'existence d'un enzyme glyoxalase, qui convertit les aldéhydes α-cétoniques en hydroxy-acides correspondants. Enfin, Case et Cook (1931) ont établi la présence du méthylglyoxal et de l'acide pyruvique parmi les produits du métabolisme musculaire.

Les différents problèmes du métabolisme intermédiaire suscitent de plus en plus l'intérêt des chercheurs scientifiques (Ier Congrès International de Biochimie à Cambridge, 1949). Les relations réciproques entre les deux métabolites que nous étudions ne sont pas encore bien élucidées. Nous empruntons à Case (1932) le schéma ci-contre qui nous semble résumer le mieux l'état actuel des conceptions à ce sujet. La branche gauche de ce schéma concerne la réduction du méthylglyoxal en acide lactique, à l'aide de méthylglyoxalase avec glutathion comme co-ferment — une simple addition des ions d'eau H et OH (hydrogénation) est suivie ici par la dismutation du méthylglyoxal hydraté en acide lactique. La branche droite du schéma mène, par oxydation du méthyglyoxal, à l'acide pyruvique ; la décarboxy-

lation de ce dernier en acétaldéhyde et la réduction de l'acétaldéhyde en alcool (derniers stades de la fermentation alcoolique) n'ont pas lieu dans l'organisme animal.



L'importance de l'acide pyruvique dans le métabolisme intermédiaire tient à ce qu'il est le terme d'aboutissement de la dégradation non seulement des glucides, mais de certains protides et lipides également, et le point de départ de la reconstitution synthétique des produits spécifiques de l'organisme. La cellule cancéreuse, d'après son origine, est apparentée aux cellules normales. Quoiqu'elle ne remplisse aucune tâche utile dans l'organisme, elle y puise ses matériaux nutritifs et y dégage les résidus de son propre métabolisme. On sait peu de choses au sujet de ces résidus : mais, fait curieux, Mendel, Bauch et Strelitz (1931) ont trouvé que les cellules néoplasiques produisent de l'acide pyruvique en grande quantité.

Observations personnelles. — Pour mettre en relief les potentialités effectives, vis-à-vis des cellules cancéreuses, des deux métabolites en étude, nous nous sommes servis de la méthode de surcharge de l'organisme par ces mêmes métabolites. Le contrôle a été établi, individuellement pour chaque tumeur, avant le début du traitement, par le calcul de l'indice d'accroissement $a = \frac{dn - do}{t}$, do et dn étant les diamètres moyens de la tumeur au début et à la fin d'une période d'observation et t, nombre de jours de cette période ; l'indice a est

ainsi exprimé en mm/jour. Le calcul ultérieur de cet indice, au cours du traitement, nous a permis de détecter les moindres effets de la pénicilline, des vitamines et de beaucoup d'autres agents étudiés.

Ces expériences peuvent être réparties en deux groupes principaux, suivant le métabolite utilisé :

Groupe I. *Le pyruvate* de sodium, sous forme de solution à 10 p. 100 fraîchement préparée, a été injecté sous la peau de 86 souris, d'abord tous les deux jours et ensuite tous les jours ; les doses étaient : 0,5 et et 0,7 cm³ de cette solution. Déjà avant le début du traitement, 84 de ces souris présentaient au total 103 adénocarcinomes mammaires : 3 de ces tumeurs n'ont pas été influencées par le pyruvate et continuaient à croître avec le même indice de croissance qu'à la période de contrôle : 35 tumeurs ont réagi par augmentation de leur indice α, effet stimulant en 34 p. 100, et 65 tumeurs (63 %) ont été inhibées. Au cours du traitement, 27 nouvelles localisations ont fait leur apparition, c'est-à-dire, 20,8 p. 100 par rapport à 130, nombre définitif de tumeurs. Sur 84 souris porteuses d'adénocarcinome de la mamelle, 39 (46 %) ont développé des métastases pulmonaires.

Sur 2 souris qui restent, l'une avait un épithélioma pavimenteux qui a été stimulé par le pyruvate, et l'autre — un lymphadénome généralisé dont certains nodules sous-cutanés ont commencé à sécher et se transformer en croûtes. La survie de tous ces animaux depuis le début du traitement se présente comme suit : 40 souris (47 %) sont mortes au cours du premier mois ; 28 (33 %) au cours du deuxième mois ; 12 = 14 p. 100 — au cours du troisième mois ; 5 (6 %) au cours du quatrième mois et 1 au cours du sixième mois.

Groupe II. *L'acide lactique* a été utilisé sous forme de ses deux sels :

a) *Lactate de sodium* en solutions colloïdales à 2, à 4,7 ou à 5,4 p. 100, gardées en ampoules et chauffées jusqu'à l'éclaircissement, au moment de l'injection. Les doses quotidiennes de 0,5 ou de 0,7 cm³ pour les solutions plus fortes, et de 1 cm³ pour la solution à 2 p. 100, ont été administrées à 23 souris ; 21 de ces souris étaient porteuses de 27 adénocarcinomes de la mamelle qui ont réagi sur le traitement de la façon suivante : aucune de ces tumeurs n'est restée indifférente au lactate de Na, c'est-à-dire, aucune n'a gardé sans changement son indice de croissance : 10 tumeurs (37 %) ont réagi dans le sens de la stimulation et 17 (63 %) dans le sens de l'inhibition de leur prolifération. Plus ou moins après le début du traitement, sont apparues 7 nouvelles localisations, ce qui fait 20,6 p. 100 par rapport à 34, nombre total de cancers mammaires chez les souris traitées par le lactate de sodium. Chez 21 souris porteuses de ces cancers, les métastases d'adénocarcinome ont été trouvées dans le poumon six fois = 29 p. 100.

Nous avons encore dans cette série d'expériences, 2 cas de lymphadénome limité aux ganglions. Ces souris n'ont réagi par aucune modification locale nette, mais leur survie durait jusqu'au quatrième mois,

et la généralisation de leur processus morbide n'a pas eu lieu. En général les cas de morts se répartissent dans ce groupe sur les mois qui suivent le début du traitement de la façon suivante : mois I 8 cas = 35 p. 100 ; mois II = 5 cas = 22 p. 100 ; mois III — 4 cas = 17 p. 100, et mois IV — 6 cas = 26 p. 100.

b) *Lactate de calcium* a été introduit, sous la peau de l'aine gauche ou des flancs, sous forme de comprimés ; une petite incision qui a servi pour cette introduction fut fermée à l'aide d'agrafes. La dose unique variant de 20 à 60 milligrammes, a été appliquée ainsi à 24 souris.

Avant cette application, 23 souris présentaient déjà 25 tumeurs mammaires qui ont tautes réagi à l'implantation du lactate de Ca : 4 (16 %) dans le sens de la stimulation et 21 (84 %) dans le sens de l'inhibition, dont l'une seulement avec une semaine de retard. Au cours de la survie ultérieure de ces souris, 9 nouvelles localisations ont été constatées, ce qui fait 26,5 p. 100 sur le total de 34 tumeurs mammaires. Les métastases pulmonaires ont été trouvées dans 8 cas sur 23 souris, ce qui fait 35 p. 100.

La dernière souris de cette série a eu un chondro-sarcome du périnée qui a également réagi dans le sens de restriction de croissance ce qui fait remonter le pourcentage définitif d'inhibition à 85 p. 100. Cette souris est morte vingt-sept jours après l'application du lactate. Les autres souris mouraient dans les proportions suivantes au cours des mois qui se sont succédés après l'intervention : mois I — 8 cas = 35 p. 100 ; mois II — 8 cas = 35 p. 100 ; mois III — 2 cas = 9 p. 100 ; mois IV — 2 cas = 9 p. 100 ; mois V — 2 cas = 9 p. 100 ; mois VII — 1 cas = 4 p. 100.

L'implantation du lactate de calcium sous la peau n'a été suivie d'aucune complication locale : les plaies opératoires guérissaient normalement et le produit se résorbait progressivement par la suite.

Discussion des résultats obtenus à la lumière de l'étude microscopique des tumeurs traitées.

Les données numériques que nous relatons plus haut nécessitent, pour être judicieusement interprétées, d'être rapprochées des modifications produites dans les tumeurs par les métabolites en étude.

Dans le groupe I, les adénocarcinomes mammaires ont augmenté, sous l'influence du pyruvate, la rapidité de leur croissance dans 34 p. 100 des cas. Or, on découvre au microscope les modifications d'ordre circulatoire, fréquentes dans ces cas, qui pouvaient contribuer à cette augmentation. Sur les figures ci-contre (fig. 1 *a* et *b*), on distingue les manifestations d'un œdème particulier qui consiste dans l'apparition d'espaces vides tout autour des cordons néoplasiques, comme s'il y avait accumulation d'un liquide transparent entre les boyaux cancéreux et le stroma. Sur certaines figures, ces cordons continuent à être pleins, mais sur certaines autres ils se trouvent déjà en voie de dislocation. Un adénocarcinome à métaplasie pavimenteuse,

type Borrel-Haaland, présente un œdème semblable au niveau des globes cornés (fig. 1 *c*). Les métastases pulmonaires sont en général en contact immédiat avec le tissu de l'organe ; dans cette série d'expériences, elles sont souvent détachées par des espaces vides (fig. 1 *b*).

Cette anomalie circulatoire est très caractéristique de l'effet du pyruvate de sodium : on la retrouve dans le cerveau sous forme d'œdème périvasculaire, dans le foie — sous forme d'œdème péricapillaire, dans l'intestin — autour des tubes glandulaires, dans le cœur, entre les faisceaux du myocarde, etc. Par cela nous ne voulons pas dire que l'effet stimulateur réel du pyruvate n'existe pas ; il existe, on voit sa manifestation dans les mitoses sur les coupes et dans l'accroissement de la masse tumorale. Un cas, celui de la ♀ 56752 LII est, à ce point de vue extrêmement démonstratif. Nous avions cette souris en observation préliminaire pendant cent quatre-vingt-neuf jours pour 3 petits nodules (1 × 2 mm) qui n'évoluaient pas. Aussitôt après le début des injections, l'une de ces tumeurs a commencé à grossir ; elle grossissait régulièrement, avec l'indice d'accroissement de 0,27, et en cinquante-quatre jours de traitement atteignit les dimensions de 12 mm × 20. A la coupe histologique (fig. 1 *e*) on voit, à côté d'un œdème péri- et intracordonal considérabe, de nombreuses mitoses ; malgré leur caractère pas toujours régulier, elles sont arrivées à assurer une prolifération néoplasique considérable. Par contre, les deux autres nodules situés sur la même souris commencèrent à régresser dès le début du traitement et à l'autopsie, il n'en restait aucune trace. Cette variété de réaction des différentes localisations sur un même animal, vient en accord avec ce que nous avons établi, en collaboration avec I. Nekhorochef (1943) sur 43 lignées de notre élevage. En étudiant le comportement de l'indice *a* dans ces différentes lignées, il a été trouvé que la rapidité de croissance d'une tumeur dépend moins d'un facteur héréditaire (c'est-à-dire propre au terrain), que de la constitution de la tumeur elle-même, c'est-à-dire de sa potentialité évolutive particulière.

Cette potentialité évolutive particulière a joué sûrement dans les 3 tumeurs où la réaction au pyruvate ne s'est pas manifestée. Ces 3 tumeurs appartenaient à 3 souris dont 2 avaient encore d'autres tumeurs : 2 de ces tumeurs ont réagi dans le sens de la stimulation et l'une dans le sens de la restriction.

Nous passons maintenant à la réaction d'inhibition qui a été observée dans 63 p. 100 des tumeurs traitées par le pyruvate. Cette inhibition était toujours réelle, faute d'autre mécanisme qui pourrait déterminer le ralentissement de croissance. Au microscope, le phénomène qui prédomine sur les coupes histologiques est la fréquence de cavités, angiomateuses et séreuses, de taille variée. A l'origine de ces cavités semblent se trouver les foyers d'hypérémie que l'on trouve disséminés dès le début du traitement. Cette disposition en foyers est un effet probable des nerfs vaso-moteurs correspondants

altérés par le pyruvate. L'effet antibiotique de ces foyers rappelle celui qui a été obtenu au niveau de l'hypérémie provoquée par la pénicilline, à savoir : la disparition des mitoses, l'arrêt de la prolifération néoplasique et l'installation de l'état d'hypobiose au voisinage des vaisseaux dilatés dans lesquels le sang reste circulant. On voit, sur la figure 1 *d*, la disparition progressive de la substance basophile des noyaux qui deviennent acidophiles ; les cellules ainsi modifiées continuent à garder leur individualité, contrairement à ce qui se passe avec la nécrose coagulante.

Quand les vaisseaux hypérémiés continuent à se dilater, ils peuvent se transformer en cavités, et le caractère progressif de ce processus explique la variété de taille des cavités angiomateuses dans les tumeurs traitées au pyruvate. Au cours de ce processus, certains vaisseaux éclatent à l'intérieur des boyaux néoplasiques ; malgré la dislocation par le sang extravasé, les cellules cancéreuses gardent mieux leur chromatine qu'au voisinage d'un vaisseau distendu par le sang circulant. Ne serait-ce pas l'oxygène du sang circulant qui exerce cette action antibiotique sur la cellule cancéreuse ? Le sang extravasé en serait privé, ayant perdu sa liaison avec la circulation pulmonaire.

Le fait que l'hypérémie et la formation des cavités angiomateuses n'aient pas contribué à l'accroissement des tumeurs montre que la prolifération même des cellules néoplasiques avait été compromise. En effet, les coupes des tumeurs à l'indice de croissance diminué ne présentent, dans la majorité des cas, aucune mitose probante ; par contre, les noyaux en voie de pycnose apparaissent quelquefois en petites nappes plus ou moins touffues. Dans les cas plus rares, on trouve, sur le fond des noyaux différenciés clairs, des cellules isolées avec des masses denses de chromatine à l'intérieur, rappelant des métaphases, mais à chromosomes conflués. Les anaphases sont extrêmement rares : deux petites boules denses l'une à côté de l'autre semblent être parfois l'aboutissement d'une anaphase (fig. 1 *f*).

Il est logique de conclure, de ce qui précède, qu'il existe une corrélation nette entre l'effet du pyruvate sur les indices de croissance, et sur les mitoses. Cette corrélation s'est manifestée aussi bien pour les tumeurs inhibées dans leur évolution que pour les tumeurs stimulées.

Par contre l'action du pyruvate n'entraîne pas, dans une proportion

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FIG. 1.

a) 56343 XXXIX, pyruvate de Na 76 jours, œdème péricordonal.
b) 56495 XXXIX, pyruvate 39 jours, métastases pulmonaires œdématiées.
c) 57522 XLVI, pyruvate 49 jours ad.-carc. à métaplasie pavimenteuse, œdème au niveau des globes cornés ;
d) 57901 XXXIX, pyruvate 120 jours : foyer d'hypérémie, état hypobiotique ;
e) 56772 LII, pyruvate 54 jours, stimulation, mitoses nombreuses ;
f) 56642 XLI, pyruvate 42 jours, inhibition, mitoses abortives.

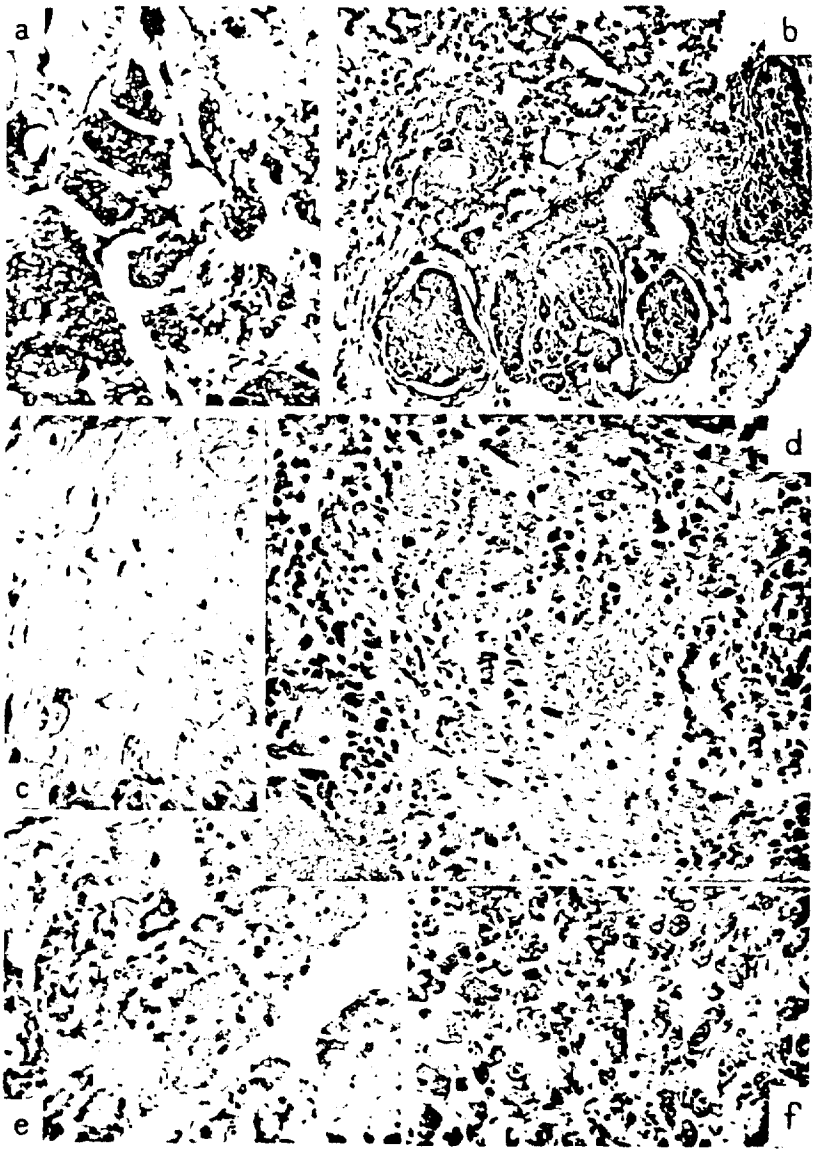

FIG. 1.

quelque peu significative les manifestations des moyens de défense contre le processus cancéreux : la tubulation, la mésenchymatisation, la collagénisation aussi bien que l'intervention active du côté du stroma.

L'action de l'*acide lactique* a été étudiée tout d'abord sous forme de son sel de *sodium*. Par rapport au groupe précédent d'expériences, il n'y avait, par conséquent, de changement qu'à l'égard du radical acide. La proportion de tumeurs stimulées par le lactate de sodium est un peu plus élevée, 37 p. 100 vis-à-vis de 34 p. 100 dans le groupe de tumeurs traitées par pyruvate. L'étude microscopique des coupes a révélé beaucoup moins d'œdème, presque pas de décollement des cordons néoplasiques, mais, en revanche, beaucoup plus de foyers d'hypérémie, plus massive et plus étendue (fig. 2 *a*), des cavités angiomateuses plus volumineuses et des hémorragies intratissulaires plus destructives, que dans le groupe du pyruvate. Ces modifications d'ordre circulatoire auraient pu être, du moins en partie, responsables de l'accélération d'accroissement des tumeurs.

En corrélation avec l'importance des manifestations circulatoires locales, se trouve l'effet produit, à ces niveaux, sur le tissu néoplasique lui-même. La figure 2 *a* caractéristique de cet effet, appartient à la ♀ 57314 XLIII dont la tumeur fut apparemment stimulée. Cet adénocarcinome mammaire présentait, à la coupe, des zones étendues d'hypérémie massive à l'effet antibiotique extrêmement intense : aucune cellule conservée, aucun vaisseau circulant, la topographie générale conservée trahit seule le phénomène qui s'est produit. A la périphérie de la tumeur se trouvait le tissu plus végétant à structure acineuse raréfiée, capsule fibreuse tout autour de la tumeur. Pas de mitose. Tissu plus compact et beaucoup moins hypérémié dans les 2 petites tumeurs d'origine plus récente : effet du traitement moins prolongé ou bien de la tolérance augmentée, acquise ? Cette souris est morte après cent sept jours de traitement ; ses tumeurs mesuraient à ce moment 14 mm $\times$ 18 (2 $\times$ 4 au début), 6 $\times$ 8 et 3 $\times$ 4. Par conséquent, ces tumeurs grossissaient sans qu'un effet stimulant sur les noyaux put être établi.

Dans une autre tumeur du même lot (♀ 58295 IV B), on a trouvé, immédiatement après le début du traitement, une hypérémie nette à la périphérie de la tumeur et, à certains points de sa partie végétante, de nombreuses mitoses à tendance pycnotique. L'hypérémie, l'angiomatisation et l'état hypobiotique ont été également trouvés dans les métastases pulmonaires. Aucune tumeur n'est demeurée indifférente à l'action du lactate de sodium. Les tumeurs qui restent (63 %) ont toutes réagi par restriction de leur croissance. Les phénomènes d'hypérémie, d'angiomatisation, d'hémorragies intratissulaires et d'hypobiose sont aussi fréquents que dans les tumeurs stimulées. Dans un cas à croissance rapide avant le traitement (α = 0,17 pendant 17 jours) on trouve sur la coupe (17 jours de traitement) des mitoses nombreuses,

FIG. 2.

a) ♀ 57311 XLVIII, lact. Na 90 j. — 8 j. sans traitement : hypérémie massive, hypoplasie profonde du tissu ;
b) ♀ 56736 XLVI, lact. Ca 50 jours : sclérose, tubulation ;
c) ♀ 58181 IV 15, lact. Ca 50 jours : « zone lactée » hypoplastique.

mais toutes à chromosomes confluants (a = — 0,58 pendant les 8 derniers jours).

Contrairement à ce qui a été observé dans le groupe au pyruvate, l'effet sclérosant du lactate de sodium sur le stroma a été manifeste, surtout dans les cas à survie plus prolongée (fig. 2 *b*). Dessèchement et chute d'une tumeur ont été suivis de récidive sur place en l'espace d'un mois. Dans quelques cas, des phénomènes de tubulation et de mésenchymatisation ont été enregistrés.

L'administration du *lactate de calcium* a eu lieu sous forme d'une dose massive unique, introduite sous la peau, la réaction s'est de nouveau produite dans les deux sens avec prédominance plus marquée d'inhibition (85 %). Les modifications découvertes au microscope, sont semblables, dans la majorité des cas, à celles qui viennent d'être décrites pour le lactate de sodium. Néanmoins, il a été possible de déceler, dans certaines tumeurs, un effet particulier, limité à une zone déterminée, ou bien présentant plusieurs foyers semblables, concentrés dans une partie de la tumeur. Les modalités morphologiques de ces « zones » sont en corrélation avec les modifications caractéristiques de l'effet du lactate, mentionnées plus haut, à savoir : 1° accumulation de grosses cavités angiomateuses groupées les unes à côté des autres ; 2° l'état de cytolyse, d'hypobiose ou de nécrose hémorragique prédominant dans une région de la tumeur ; et 3° les manifestations de sclérose qui s'ajoutent le plus souvent aux altérations précédentes, sont en général accompagnées des phénomènes de cytolyse au voisinage (fig. 2 *c*).

Par analogie avec la « zone pénicillée » obtenue avec la pénicilline peu purifiée (1944) et la « zone gravidique » observée à la suite de la gestation et de l'allaitement, nous avons appelé la « zone lactée » celle qui vient d'être décrite. Le phénomène de « zone » traduit l'effet momentané d'un agent, opérant pendant un temps limité, sur la tumeur qui existe à ce moment. Une fois cette action terminée, la tumeur reprend son allure habituelle et fournit ainsi un contrôle morphologique individuel (c'est-à-dire, sur la même tumeur) de ce qui a été fait. L'effet de « zone » apparaît ainsi comme une preuve absolue que l'agent utilisé avait une affinité pour le processus néoplasique.

Nous avons mis à part, dans nos calculs, les « nouvelles localisations » c'est-à-dire celles qui sont apparues après le début du traitement. La raison en est que la localisation d'un adénocarcinome mammaire semble être beaucoup plus déterminée par sa disposition héréditaire que par d'autres agents. En étudiant (avec Mme Adamova, 1939) la corrélation entre les agents endogènes et les agents exogènes (radon et 1-2-5-6-dibenzanthracène) nous avons vu souvent les adénocarcinomes mammaires apparaître non pas au niveau de l'agent appliqué, mais ailleurs, comme s'il y avait pour elles des places prédestinées. La transmission

héréditaire d'une localisation déterminée (celle de la nuque chez une mère et ses deux filles, celle de l'aisselle droite chez deux sœurs, (fig. 3 c), etc.), plaide également en faveur de l'existence d'un facteur localisateur constitutionnel. Cette idée retrouve sa confirmation dans la série actuelle d'expériences ; avec les agents divers utilisés, on a obtenu des taux de nouvelles localisations très rapprochés : 21 p. 100 pour le pyruvate, 21 p. 100 pour le lactate de sodium et 26 p. 100 pour le lactate de Ca.

Le comportement des métastases pulmonaires, par contre, se trouve plutôt en corrélation avec des potentialités évolutives des tumeurs et, peut-être à un certain degré, avec le produit administré : le chiffre le plus élevé (46 %) a été obtenu pour le pyruvate, puis 35 p. 100 pour le lactate de Ca et 29 p. 100 pour le lactate de Na.

Pour la survie des animaux, aussi bien que pour les métastases, le pyruvate se montre le plus défavorable : 80 p. 100 de souris traitées meurent au cours des deux premiers mois ; ce n'est que 6 p. 100 qui ont vécu jusqu'au quatrième mois et une souris est morte au sixième mois. La place moyenne appartient de nouveau au lactate de Ca : 70 p. 100 de morts au cours des deux premiers mois, mais les souris qui ont traversé cette période et, par conséquent ont supporté la résorption du lactate de Ca implanté, disparaissent progressivement, la dernière souris étant morte au 7e mois. Avec le lactate de Na, 57 p. 100 sont mortes au cours des deux premiers mois et 26 p. 100 ont atteint le quatrième mois.

De tout ce qui précède, nous arrivons à la constatation que, parmi les facteurs multiples qui jouent dans le processus cancéreux, les métabolites étudiés peuvent exercer, à certains moments, une influence décisive sur son évolution. N'ont-ils pas leur part également dans sa pathogénie ?

Une série d'expériences, faites avec le pyruvate sur les animaux d'une lignée non cancéreuse, n'a donné aucun cas de cancérisation : étant un élément intermédiaire normal de l'économie de l'organisme, le pyruvate ne s'est pas montré capable de provoquer un cancer en l'absence de la prédisposition héréditaire, contrairement au dibenzanthracène et au radon qui ont fait surgir localement des sarcomes et des épithéliomas pavimenteux (pas d'adénocarcinome) dans cette même lignée. Cela ne prouve pourtant pas que, dans la constellation de circonstances qui mène à la cancérisation, le métabolisme intermédiaire n'ait aucune part contributive.

Avec la découverte du rôle de « facteur lait » dans la pathogénie de l'adénocarcinome mammaire chez la souris, on a été finalement amené à l'idée de virus. Graff, Moore, Stanley, Randall et Haagensen ont récemment isolé ce « facteur » du lait de notre lignée R III et des souris de la lignée C 57 qui ont été allaitées par des nourrices R III. La microphotographie électronique, jointe à ce travail, représente des

particules de tailles assez variées, plus ou moins arrondies et isolées, ou bien, réunies en conglomérats ; ces conglomérats sont plus nombreux et plus volumineux pour le lait de nourrissonnes (fig. 3 *a*). Cette variété de forme et surtout de dimensions ne s'accorde pas bien avec l'idée de virus exogène, c'est-à-dire un être vivant, capable à lui seul de provoquer le cancer. Pour les êtres vivants, non seulement la forme, mais les dimensions définitives sont également spécifiques ; la variation est admise dans les limites étroites d'une courbe de Gauss.

L'idée d'un virus endogène serait, peut-être, plus acceptable, mais elle est encore moins précisée. Dans nos recherches sur le fractionnement des extraits du tissu cancéreux et des organes, riches en cellules, nous nous sommes heurtés à la difficulté de nous débarrasser des sels. Pour les repérer, nous nous sommes servis d'une méthode de microcristallisation, en laissant une goutte de produit en solution limpide s'évaporer sur une lame. Au microscope, on a vu alors les cristaux qu'on cherchait, mais, en outre, parfois des figures d'une complexité (et même d'une beauté) extraordinaire, composées de particules arrondies, rappelant beaucoup celles de l'agent lacté (fig. 3 *b*). Le Dr Deicha nous a examiné ces particules au microscope polarisant, et elles se sont montrées être des sphérolites, c'est-à-dire, des formations ayant une nature cristalline. Il y a lieu de se demander si le produit que l'on a isolé du lait de la lignée R III ne présente pas également un stade intermédiaire de dégradation protidique et, plus spécialement, de celle de nucléo-protéines. Cela n'empêche pas que ce produit ait, en outre, un caractère spécial : il se forme dans un organisme prédisposé au cancer, grâce à un mécanisme physiologique particulier propre à cet état de prédisposition, et il en est le terme d'aboutissement. Si l'on veut le faire agir dans une lignée moins prédisposée (la lignée C 57 avec 1,9 % de cancers) il faut l'introduire au début de la vie (7 à 12 jours) c'est-à-dire avant que le mécanisme correspondant propre à cette lignée, soit établi. Son action dans une lignée très prédisposée au cancer mammaire a été complètement supprimée (White et Andervont, 1943) par une diète carencée en cystine (aucune tumeur), et réduite de 97,4 p. 100 à 25 p. 100 par celle carencée en lysine (White et White, 1944). Tout ceci montre que le facteur lait n'agit pas seul, il n'est qu'un des mécanismes qui jouent dans la cancérisation et qui sont transmis par l'hérédité, ne fût-ce parfois que par l'hérédité cytoplasmique.

De même pour le facteur hormonal ; son mécanisme particulier

FIG. 3.

a) Virus lacté ; microphotographie électronique américaine ;
b) [illegible] de fractionnement d'un extrait protidique, microcristallisation sous [illegible] sphérolites ;
[illegible] du cancer mammaire dans l'aisselle droite chez deux [illegible] 6402 et [illegible] 6403 de la lignée XXXIX.

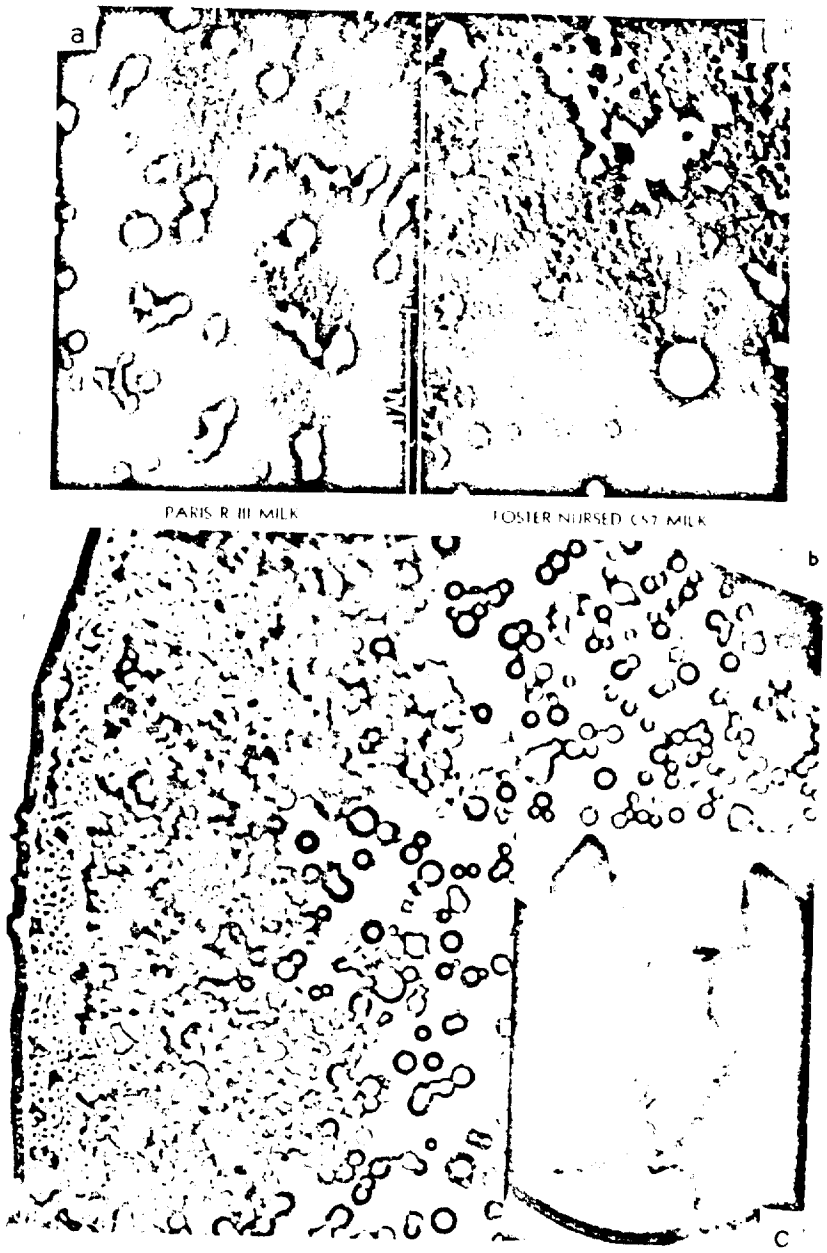

FIG. 3.

dans les lignées cancéreuses a été mis en évidence par un apport supplémentaire des hormones œstrogènes (Lacassagne, 1938), mais cet apport est resté sans effet dans les lignées non cancéreuses ; le concours du facteur lait semble être indispensable.

Néanmoins, ni le facteur hormonal, ni le facteur lait ne peuvent nullement rendre compte de l'efficacité extraordinaire du facteur localisateur, dont il était question plus haut, et qui détermine probablement, en même temps la forme histologique. Les manifestations de ce facteur n'ont été changées par notre intervention en aucun degré pour le cancer mammaire. Mais pour les sarcomes et les épithéliomas pavimenteux, son rôle a été assumé par le facteur exogène : la réaction sur le cancérigène a été locale. Et de plus, les sœurs avec les mêmes localisations (fig. 3 *c*) ont développé leurs tumeurs simultanément, ce qui met en évidence encore un facteur, facteur temps. La collaboration de tous ces facteurs est nécessaire afin qu'une forme cancéreuse tout à fait semblable à celle de la mère soit reproduite chez les descendants. Cela explique la rareté d'une telle reproduction intégrale, surtout à l'égard du facteur temps : dans une famille descendant d'une mère cancéreuse, les filles développent très souvent les tumeurs avec des délais variés. D'où l'on peut supposer que, contrairement au facteur localisateur, le facteur temps est moins rigoureux dans ses manifestations et qu'il peut être influencé par certains autres facteurs. Parmi ces facteurs, les conditions intérieures de l'économie de l'organisme, les particularités de son métabolisme ne peuvent ne pas jouer un rôle. On observe actuellement une recrudescence de cas de cancer, et on se demande quelle en est la cause ? Ne serait-ce pas, en effet, une activation du facteur temps par des conditions anormales de la période de guerre, par une alimentation trop unilatérale, par la fermentation intestinale continuelle, etc. ?

Après une période de latence propre au processus cancéreux, les tumeurs commencent à se développer plus précocement que dans les conditions normales, et cela se traduit par l'impression d'une augmentation de cancers en général. La possibilité d'avancer, ou bien de retarder le début de la cancérisation a été à maintes reprises observée au cours des expériences variées sur les lignées prédisposées au cancer.

Cette immense expérience de guerre sur la population humaine ouvre, nous semble-t-il, non seulement des champs d'étude nouveaux, mais elle montre en même temps des perspectives inattendues pour la médecine préventive. En effet, si l'on trouvait le moyen de supprimer par un régime approprié la fermentation intestinale, d'activer les réactions d'oxydo-réduction dans les tissus, d'améliorer la respiration en général, etc., et de retarder ainsi le début de la cancérisation au delà de nonante ans, le problème du cancer pourrait être considéré, au moins en partie, comme résolu.

En conséquence, les expériences actuelles ont mis en relief l'affinité

étroite qui existe entre certains produits du métabolisme intermédiaire et l'évolution des cancers mammaires chez les souris.

La réaction néoplasique, en réponse à la surcharge de l'organisme par le pyruvate de soude, lactate de soude ou lactate de calcium, s'est manifestée soit par l'effet de stimulation (augmentation de l'indice d'accroissement, mitoses nombreuses), soit par l'effet d'inhibition (diminution de la rapidité de croissance et même régression de certaines tumeurs, mitoses à chronosomes confluents).

Cette dualité d'effet n'est pas une particularité des métabolites, elle est commune à tous les agents chimiques et physiques qui se sont montrés capables d'intervenir activement dans le processus cancéreux.

Les modifications d'ordre circulatoire, œdème péri-cordonal, foyers d'hypérémie pour le pyruvate, angiomatisation plus prononcée et manifestations de sclérose pour les lactates ont été également enregistrées. Les phénomènes d'hypobiose, surtout pour les lactates, ont été souvent observés.

Dans la série à lactate de calcium, l'effet régional sous l'influence d'une dose unique plus ou moins forte, a été observé ; nous l'avons nommé par analogie avec la « zone pénicillée » et la « zone gravidique », la « zone lactée » ; cette « zone » présente une preuve décisive en faveur de l'intervention du lactate dans l'évolution néoplasique.

Quoique ces manifestations puissent être considérées comme des moyens de défense contre le processus néoplasique (dessèchement des tumeurs), elles n'ont déterminé, dans les conditions de nos expériences, aucune guérison définitive.

La multiplicité des facteurs, déjà établie pour la pathogénie du cancer mammaire, trouve encore une fois sa confirmation dans la manifestation du facteur localisateur (facteur de structure histologique en même temps) : les taux de nouvelles localisations n'ont pas été sensiblement influencés par les divers métabolites. Parmi les autres mécanismes spécifiés qui déterminent le fonctionnement de ces facteurs, le métabolisme intermédiaire peut avoir néanmoins une signification.

Il est très probable que ce qu'on a isolé comme « virus lacté » rentre, lui aussi, dans la catégorie des produits de dégradation des protides ou, plus spécialement, des nucléoprotéides ; ce produit serait un terme d'aboutissement d'un mécanisme particulier, ayant sa part dans la pathogénie néoplasique.

Le rôle des ions métalliques nécessite une étude supplémentaire.

Le comportement des différents facteurs jouant dans la cancérisation, est varié. Les uns, appartenant au terrain, présentent un caractère de rigidité extraordinaire et ne se laissent pas influencer par des facteurs exogènes ; tel est le facteur localisateur pour l'adénocarcinome mammaire. D'autre part, pour les sarcomes et les épithéliomas pavimenteux provoqués à l'aide des agents cancérigènes, la localisation est déterminée par le cancérigène utilisé, qui tient ainsi place du

facteur localisateur héréditaire ; la possibilité d'une telle substitution s'explique par la réaction locale du tissu conjonctif et de l'épithéliome pavimenteux à l'attaque cancérigène.

Parmi les autres facteurs, appartenant également au terrain, mais moins rigides, c'est le facteur temps qui présente le plus d'intérêt : la possibilité de l'influencer se manifeste dans le retardement de l'apparition des tumeurs, dans une lignée prédisposée, par la diète à valeur calorique diminuée ; par le déclenchement subit d'un cancer du sein chez une femme ayant reçu un coup dans la poitrine ; c'est lui aussi probablement qui joue dans l'augmentation des cancers dans la période d'après guerre, etc. La possibilité de retarder le facteur temps ouvre un large champ d'étude et montre des perspectives nouvelles dans le domaine de la médecine préventive des cancers.

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Calcium lactate

Compt. Rend. 224:1658-1660. 1947.
French publication Academie des Sciences, Seance du 9 Juin, 1947, pp.1658-60

Biology - The effect of Na and Ca lactates on the mouse, and comparison thereof with that of pyruvate Note (1) by Mme. Nadine Dobrovolskaya-Zavadskaia and M. Vladimir Momsikoff

Lactic acid (CH_3CH OH COOH) has long been considered as one of the prime factors responsible for manifestations of beri-beri and avian polyneuritis; a rise in the quantity thereof in the brain has been found in the course of B1 avitaminosis, (2) (3).

More recently, there has been further concentration on the chemical study of pyruvic acid (CH_3CO COOH) (4) (5). We reported (6) a syndrome reproducing, by sodium pyruvate alone and in the absence of avitaminosis, experimental beriberi on young mice.

In order to elucidate the role of lactic acid as well, we ran a series of analogous experiments (see figures).

1) Male, grey-wild, 13.8 g Injection at 4:40 pm of 1 cc of 4.7% Na lactate; at 5:00 pm, staggers, respiration slowed down, rubs muzzle with paws, stretches itself (itching); 5:55 pm, scratches ears, licks itself; 5:25 pm urine, 7 drops at a time, 5:35 pm, begins to recover. Fresh injection of 1 cc, same solution; 5:50 pm, cataleptic state (photos 1 and 2 without retention); escapes afterwards, runs. No convulsive movement; 6:10 pm, cataleptic state continues; 6:30 pm, no longer ill. Next day, weight: 13.6 g, good condition.

2) Female 61,813 XXXV, weight 9.3 g; 11:30 am, injection of 0.5 cc of 9% Ca lactate; 12:15 pm, wavering walk, rear paws spread; 2:10 pm, slow breathing, no spasmodic manifestation; 3:30 pm, catalepsy, photo without retention (fig. 3); 5:00 pm, paralysis of rear paws; 5:40 pm, dies without convulsions (6 hours 10 minutes after injection).

3) Male 61,184 XXV beige, 9.3 g; 11:25 am, injection of 0.4 cc same solution; 12:10 pm, paralysis of rear paws, wavering walk, slow breathing; 2:05 pm, respiration not as slow as before (smaller dose); 3:10 pm, catalepsy (fig.4); 5:00 pm no convulsive manifestation, paralysis of rear paws; 6:00 pm, still breathing; 6:30, dead.

Fig.1 and 2 - Cataleptic state: animal resting on stomach and back "a bougie" in unaccustomed position with paws drawn out as it was placed. Complete recovery afterward.

Fig. 3 - Cataleptic state, animal remains in abnormal position with right front paw drawn out. Dead 2 hours afterward without convulsion.

Fig. 4 - Immobilization 3 hours and 45 minutes after injection. Dead 2 hours afterward without convulsion, but with rear paws paralyzed.

Fig. 5 - Paralysis of paws 2 hours 30minutes after injection. Dead without convulsion.

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4) 61,115, silver grey; 6.7 g; 12:00 noon, injection of .4 cc of 9% Ca lactate; 12:20 pm, rear paws spread out, walks slowly; 2:00 pm, very stricken but does not remain on back; 2:10 pm, left rear paw drawn out, paralyzed; right rear paw remains folded and in movement; 2:30 pm, left rear paw and right front paw are paralyzed, drawn out (fig.5); 2:35 pm, dead without convulsion 2 hours and 35 minutes after injection.

To summarize, the effect of lactic acid manifests itself by ataxia, catalepsy, slowing of respiration (without dyspneic symptom) and gradual immobilization of the animal.

Contrary to what was observed with the pyruvic animal, we noted the complete absence of spasmodic and convulsive phenomena (no hyperextension of the neck). The animal represented in fig. 1 and 2 still manifested a few symptoms of anxiety and irritation (itching, unassustomed diuresis);

in spite of a pronounced cataleptic state it recovered and lived for several more months. All the other animals died with serious paralytic manifestations but no convulsive attack. Pyruvic acid, therefore, remains the most probable immediate cause of the spasmodic and convulsive phenomena. The synergism of lactic acid in the paralytic phenomena and perhaps in certain internal manifestations is quite probable.

This difference in action is explained (7) (4) by the fact that pyruvic acid is not a derivative of lactic acid. According to the expression of E.M. Case (7), it is not an intermediate precurser or lactic acid, nor a product of direct oxidation of the latter; the immediate precurser of pyruvic acid should be methyl glyoxal ($CH_3CO\ COH$) from which pyruvic acid is derived by oxidation.

(Translated by Carl Demrick Associates, Inc/GCT/t)

Compt. Rend. 224:1658-1660. 1947.

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Zavadskaia-'47
Bib No. 412

BIOLOGIE. — *L'effet de lactates de* Na *et de* Ca *sur la Souris et son rapprochement de celui du pyruvate.* Note (1) de Mme **Nadine Dobrovolskaïa-Zavadskaïa** et M. **Vladimir Momsikoff.**

L'acide lactique ($CH_3CHOHCOOH$) a été longtemps considéré comme l'un des principaux responsables des manifestations du béribéri et du polyneurite aviaire; une augmentation de sa quantité dans le cerveau a été trouvée (2) (3) au cours de l'avitaminose B_1.

Plus récemment, on s'est concentré davantage sur l'étude chimique de l'acide pyruvique ($CH_3COCOOH$) (4) (5). Nous avons relaté (6) un syndrome reproduisant, par le pyruvate de sodium seul et en l'absence de l'avitaminose, le béribéri expérimental sur les souriceaux.

Pour élucider également le rôle de l'acide lactique, nous avons réalisé une série d'expériences analogues (voir figures).

1° ♂ gris-sauvage de 13^g^,8. Injection à 16^h^40^m^ de 1^cm^3^ de solution de lactate de Na à 4,7 %; 17^h^, chancelle, respiration ralentie, se frotte le museau avec les pattes, se gratte (démangeaison); 17^h^55^m^, se gratte les oreilles, se lèche; 17^h^25^m^, urine 7 gouttes à la fois; 17^h^35^m^, commence à se rétablir. Nouvelle injection de 1^cm^3^, même solution; 17^h^50^m^, état cataleptique (photos 1 et 2, sans rétention); se sauve après, court. Aucun mouvement convulsif; 18^h^10^m^, l'état cataleptique continue; 18^h^30^m^, ne va pas plus mal. Le lendemain, poids : 13^g^,6, bon état.

2° ♂ 61183 XXXV, poids 9^g^,3; 11^h^30^m^, injection de 0^cm^3^,5 de lactate de Ca à 9 %; 12^h^15^m^, marche en oscillant, pattes postérieures écartées; 14^h^10^m^, respire lentement, pas de mani-

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(1) Séance du 9 juin 1947.

(2) H. W. Kinnersley et R. A. Peters, *Biochem. Journ.*, 23, 1929, pp. 1126 et 1930; 24, pp. 711 à 712.

(3) R. Lecoq, *C. R. Soc. Biol.*, 119, 1935, p. 276.

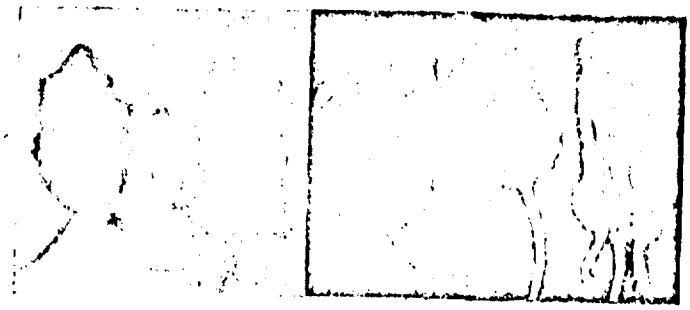
(4) V. P. Meikeljon, B. Passmore et R. A. Peters, *Biochem. Journ.*, 26, 1932, pp. 1872-1879.

(5) R. A. Peters et R. H. S. Thompson, *Biochem. Journ.*, 28, 1934, pp. 916 à 925.

(6) N. Dobrovolskaïa-Zavadskaïa et collab., *Comptes rendus*, 222, 1946, pp. 248, 611 et 935; 223, 1946, p. 1034.

festation spasmodique; 15h30m, catalepsie, photo sans rétention (*fig.* 3); 17h, paralysie des pattes postérieures; 17h40m, meurt sans convulsion (6 heures 10 minutes après l'injection).

3° ♂ 61184 XXXV beige, de 9g,3; 11h25m, injection de 0cm3,4, même solution; 12h10m, paralysie des pattes postérieures, marche en oscillant, respire lentement; 14h5m, respiration moins ralentie que chez le précédent (plus petite dose); 15h10m, catalepsie (*fig.* 4); 17h, aucune manifestation convulsive, paralysie des pattes postérieures; 18h, respire encore; 18h10m, meurt.



1. 2. 3. 4. 5.

Fig. 1 et 2. — État cataleptique : l'animal reste sur le ventre et sur le dos (a bougé) en position insolite avec les pattes étirées comme on l'avait mis. Rétablissement complet par la suite.

Fig. 3. — État cataleptique, l'animal garde la position anormale avec la patte antérieure droite étirée. Mort 2 heures après, sans convulsion.

Fig. 4. — Immobilisation 3 heures 45 minutes après l'injection. Mort 2 heures après sans convulsion, mais avec les pattes postérieures paralysées.

Fig. 5. — Paralysie des pattes, 2 heures 30 minutes après l'injection. Mort sans convulsion.

4° 61115 XXXV gr. arg. de 6g,7; 12h, injection de 0cm3,4 de lactate de Ca à 9 %; 12h20m, pattes postérieures écartées, marche lentement; 14h, très frappé, mais ne reste pas sur le dos; 14h10m, patte postérieure gauche étirée, paralysée; la patte postérieure droite reste pliée et fait des mouvements; 14h30m, la patte postérieure gauche et la patte antérieure droite sont paralysées, étirées (*fig.* 5); 14h35m, mort sans convulsion 2 heures 35 minutes après l'injection.

En résumé, l'effet de l'acide lactique s'est manifesté par l'ataxie, la catalepsie, le ralentissement de la respiration (sans symptôme dyspnéique) et l'immobilisation progressive de l'animal.

Contrairement à ce qui a été observé avec l'acide pyruvique, on a constaté l'absence complète de phénomènes spasmodiques et convulsifs (aucune hyperextension du cou). L'animal représenté sur les figures 1 et 2 manifesta encore quelques symptômes d'inquiétude et d'irritation (démangeaison, diurèse insolite); malgré un état cataleptique prononcé, il s'est rétabli et a vécu encore plusieurs mois. Tous les autres animaux sont morts avec des manifestations paralytiques graves, mais sans aucune crise convulsive. L'acide pyruvique reste, par conséquent, la cause immédiate la plus probable des phénomènes spasmodiques et convulsifs. Le cinergisme de l'acide lactique dans les phéno-

mènes paralytiques et, peut-être, dans quelques manifestations internes, est tout à fait probable.

Cette différence d'action s'explique ([7]) ([4]) par le fait que l'acide pyruvique n'est pas un dérivé de l'acide lactique. Suivant l'expression de E. M. Case ([7]), il n'est pas un précurseur intermédiaire de l'acide lactique, ni un produit d'oxydation directe de ce dernier; le précurseur immédiat de l'acide pyruvique doit être le méthylglyoxal (CH_3COCOH) duquel l'acide pyruvique est dérivé par oxydation.

J. Dent. Res. 29:304. 1950.

Douglas-'50
Bib No. 418

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ON THE OCCURRENCE OF THE LACTATE FERMENTING ANAEROBE, MICROCOCCUS LACTILYTICUS, IN HUMAN SALIVA

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THE microorganism now known as *Micrococcus lactilyticus** (Foubert and Douglas, 1948a), was first observed and partially described by Lewkowicz (1901) who isolated one strain from the mouth of an infant. The subsequent work of Hall and Howitt (1925) on the anaerobic flora of the mouth added much to our knowledge of this microorganism, and indicated that it was present in large numbers in saliva. However, the methods used by these workers were not suitable for accurately determining the numbers of these bacteria in saliva. This problem was greatly simplified when it was found that *M. lactilyticus* carried out an active fermentation of lactate (Foubert and Douglas, 1948a), a property which is rare among microorganisms, and which made possible the design of more specific cultural methods for determining its incidence in human saliva.

As most treatises (Wilson and Miles, 1946; Appleton, 1944) which deal with the normal flora of the oral cavity either fail entirely to mention the presence of *M. lactilyticus* or at most give it but scanty attention, it seemed desirable to publish our findings on the occurrence of this bacterium in saliva. Since our results show that *M. lactilyticus* is quantitatively one of the most important of the bacteria present in saliva, this fact is bound to be of significance to those interested in the microbial ecology of the oral cavity.

METHODS

Two or three ml. of saliva were collected aseptically from students and colleagues in the laboratory. Serial dilutions were prepared in sterile tap water and plated on Mediums 1 and 2. Colony counts were made after the plates had been incubated anaerobically (95 per cent H_2, 5 per cent Co_2) at 37° C. for 48 hours. Medium 1 consisted of 0.5 per cent Difco yeast extract, 1.0 per cent sodium lactate, and 1.5 per cent agar. Medium 2 contained 2.0 per cent Difco peptone, 0.5 per cent Difco yeast extract, 1.0 per cent glucose, and 1.5 per cent agar. Both media were adjusted to pH 7.0.

M. lactilyticus develops readily on Medium 1, the colonies, which are compact and lens shaped, attaining a diameter of approximately 1.0 mm. in 48 hours. Streptococci also develops anaerobically on this medium but since the

This work was supported in part by a grant from the School of Dentistry of the University of Washington.

Received for publication, August 1, 1949.

*Other names applied to this organism in the past have been *Micrococcus anaerobius alkalescens anerobius* (Lewkowicz, 1901), *M. anaerobius* (Hall and Howitt, 1925), and *Veillonella anaerobius* (Prevot, 1933).

colonies rarely exceed 0.1 mm. in diameter they may be easily distinguished from *M. lactilyticus*. Colonies of *M. lactilyticus* may also be identified by the gram reaction, which is negative at this stage, and by the formation of abundant gas within 24 hours in subcultures made by stabbing freshly exhausted deeps of Medium 1.

TABLE I

THE OCCURRENCE OF M. LACTILYTICUS IN SALIVA

SUBJECT	MILLION PER ML.	
	M. LACTILYTICUS*	STREPTOCOCCI AND FACULTATIVELY ANAEROBIC MICROCOCCI†
A	7	7.5
B	60	55
C	37	63
D	10	57
E	13	65
F	90	200
G	17	60
H	55	125
I	7	10
J	24	60
K	340	385
L	24	60
M	53	90
N	6	30
O	3	7
P	60	140
Q	90	135

*Determined from the large colony count on Medium 1.
†Determined from the total colony count on Medium 2.

Medium 2, when inoculated with high dilutions of saliva and incubated anaerobically, yields practically nothing but streptococci and facultatively anaerobic micrococci. *M. lactilyticus* occurs only rarely on plates of this medium.

TABLE II

CHANGES IN pH FOLLOWING THE GROWTH OF A CULTURE OF M. LACTILYTICUS IN 1.0 PER CENT YEAST EXTRACT, 0.5 PER CENT SODIUM LACTATE BROTH AT DIFFERENT INITIAL pH VALUES

INITIAL pH	pH AFTER 24 HOURS' INCUBATION
4.7	no growth
4.8	5.32
5.0	6.0
5.35	6.46
6.20	6.80
7.0	6.92

RESULTS

The results obtained by applying the plating methods described above to saliva collected from seventeen individuals are summarized in Table I. It can be seen that *M. lactilyticus* was present in all of the saliva samples, in numbers varying from three million to 340 million per milliliter. In some cases 50 per cent of the total flora capable of developing under these conditions consisted of this microorganism. These facts leave little doubt that *M. lactilyticus* constitutes a significant portion of the bacterial flora indigenous to human saliva.

Very little is known concerning the ecological relationships of the various groups of microorganisms normally resident in the mouth, but it seems probable that the occurrence of *M. lactilyticus* in this habitat is dependent upon the presence of other bacteria that convert sugars to lactic acid. This appears to be true because *M. lactilyticus* is capable of fermenting only lactate, or the related compounds, pyruvate and malate. Of these substances only lactate would be expected to be present in significant amounts in saliva, and its origin there is undoubtedly due to bacterial fermentation of sugars (Neuwirth and Klosterman, 1940). Since it has been established that *M. lactilyticus* ferments lactate to propionate, acetate, carbon dioxide, and hydrogen (Foubert and Douglas, 1948), it is reasonable to conclude that *M. lactilyticus* carries out a similar biochemical conversion in the mouth. Probably the most significant effect of this fermentation is a resultant increase in pH which is quite marked when the process takes place in an initially acid environment. This is clearly shown in Table II and is due to the fact that the salt of a stronger acid (lactic) is converted to salts of weaker acids (propionic and acetic).

Whether the biochemical activity of *M. lactilyticus* in the mouth is a significant factor in deterring the production of dental caries is not known, but this question deserves the attention of those interested in this particular problem.

The author wishes to express his appreciation to Mr. Richard Layton for his very capable assistance.

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Drury-'65
Bib No. 432

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Ann. N. Y. Acad. Sci. 119(3):1061-1069. 1965.

CHEMISTRY AND METABOLISM OF L(+) AND D(−) LACTIC ACIDS*

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We have been studying the metabolism of lactic acid in rabbits, both in the intact and in the eviscerated animal. In the intact animal we studied the turnover rate and routes of disposal of the body lactate. We determined the rate by injecting a tracer dose of L(+) C^{14} uniformly labeled isomer having high radioactive potency. This was prepared by the method of Brin.§ Then, after allowing time for mixing in the body, we determined the rate of decay of the specific activity of the plasma lactate. TABLE 1 shows the rate of decay of the specific activity of the plasma lactate in two intact animals after a single injection of tracer amount of the labeled material. The first plasma sample was taken after allowing 20 minutes for mixing. The change in specific activity results from the addition to the body pool of endogenous nonlabeled lactate which is constantly being produced. At the same time, the tissues take lactate from the pool at a rate approximately equal to the rate of addition. We know this because the plasma lactate concentrations remain essentially constant. To such a situation we may apply the equation $L_t = L_0 e^{-st}$, where L_0 is the beginning S.A., L_t is the specific activity at time t, and s is the constant of disappearance. In both animals the specific activity drops in 30 minutes to about 37 per cent of the original, which would indicate a turnover rate of body lactate in 30 minutes.

Next, we investigated the routes of disposal of the circulating lactate in intact animals. The oxidation rate may be estimated by measuring the radioactivity in the respired CO_2. The "one-shot" type of experiment cannot be used for this because, as a result of body pooling of CO_2, one cannot tell whether a given molecule of $C^{14}O_2$ eliminated at the end of the run was produced when the lactate S.A. was high (at the beginning) or at the end when the S.A. was low. One must keep the S.A. of the body lactate constant.

Knowing the rate of turnover of body lactate, we were able to plan the experimental conditions whereby a constant S.A. of the body lactate could be maintained. This could be effected by giving a priming dose of labeled lactate and then giving a constant injection of the labeled lactate at such a rate that

*This work was supported in part by a research grant of the National Institutes of Health, No. AM-00052.

†Deceased May 15, 1965.

‡This work was supported in part by a research grant of the National Institutes of Health, No. 5685.

§BRIN, W. 1953. Biochemical Preparations. 3: 61. Wiley. New York, N. Y.

TABLE 1
S.A. OF LABELED PLASMA LACTATE AFTER ONE SHOT I.V. INJECTION. FIRST SAMPLE WAS TAKEN 20 MINUTES AFTER INJECTION

Rabbit 1		Rabbit 2	
Time (min.)	S. A. of plasma lactate	Time (min.)	S. A. of plasma lactate
0	250	0	230
30	95	30	80
		60	38

TABLE 2
S.A. OF THE BLOOD LACTATE IN INTACT RABBITS INJECTED WITH L(+) LABELED LACTATE

Rabbit no.	Time after initial injection (min.)	Blood lactate (mg. %)	S. A. blood lactate
1	41	25	340
	101	22	308
2	30	8	1110
	62	10	1075

At the start a priming dose of lactate was given and then the lactate was given by constant I.V. injection so that the amount of lactate injected in 30 minutes equalled that of the priming dose.

the amount injected in 30 minutes would equal the priming dose. Two experiments were carried out in this way. The results are given in TABLE 2. The activity per milligram of the plasma lactate remained essentially constant; thus we could determine the lactate oxidation rate from the activity in the respired CO_2 in a run of four hours or more. The plasma lactate S.A. was determined by the method of Drury and Wick.*

The detailed values for the elimination of labeled CO_2 by these two animals are given in TABLE 3. The rapid approach to a plateau in the S.A. of the respired CO_2 supports the direct evidence of constancy of the S.A. of the circulating lactate from the start of the test. This indicates that we were replacing activity at the rate the body was disposing of it. This maintained level

*DRURY, D. R. & A. N. WICK. 1956. Am. J. Physiol. 184: 304.

TABLE 3
SPECIFIC ACTIVITY IN SUCCESSIVE PERIODS OF RESPIRATORY CO_2 OF RABBITS PRESENTED IN TABLE 2

Rabbit 1		Rabbit 2	
Time (min.)	CO_2 S. A.	Time (min.)	CO_2 S. A.
0-15	77	0-17	195
15-36	146	17-30	242
36-46	163	30-60	239
46-72	176	60-97	276
72-101	198		

of plasma lactate activity can then be taken as indicating that the steady state of plasma lactate S.A. was essentially that which was produced by the priming dose. The rapid rise in respired CO_2 S.A., reaching a plateau in about 30 minutes, indicates the lactic acid must be disposed of very quickly.

Not all the activity disappearance can be accounted for by direct oxidation to CO_2. There are other routes of disposal which have been proposed in the past: conversion of lactate to glucose, and to liver and muscle glycogen. The investigators used classical metabolic methods in obtaining the results upon which they based their conclusions. An important point in evaluating the results of this work is that when the investigators administered lactate, it was almost always in racemic form given by stomach tube. This work was done before C^{14} was available. We felt that this matter should be reinvestigated now that C^{14}-labeled lactate is available. We determined how much of the injected activity was disposed of by these routes. From the two animals in which body lactate was maintained by constant infusion, a sample of muscle and the entire liver were taken at the end of the run. These were dissolved in hot alkali for preparation of glycogen by the Pflüger procedure. The amounts of radioactivity of the glycogens are given in TABLE 4. The total muscle glycogen is calculated on the assumption that muscle constitutes 50 per cent of the body

TABLE 4
DISPOSAL OF CARBON RADIOACTIVITY OF INJECTED LACTATE (PER CENT)

Animal no.	Injected lactate	CO_2	Glycogen and glucose
1	281,000	65	5
2	372,000	65	4

weight. It is apparent that only a small fraction of the administered lactate is disposed of as stored glycogen. TABLE 5 shows a breakdown of the disposal of the injected labeled lactate. It is apparent that a large fraction of the lactate is quickly oxidized to $C^{14}O_2$.

TABLE 5
DISPOSAL OF LACTATE IN 1.75 HOURS

	Counts
Respired CO_2	243,000
Body CO_2	37,000
Body lactate	41,000
Glycogen	3,000
Glucose and derivatives	25,000
Total accounted for	349,000
Injected	370,000

We had to determine whether injected lactate was metabolized by being first converted to glucose by the liver before metabolization. TABLE 6 indicates that little of the lactate which is oxidized to CO_2 would have been metabolized by first being converted to glucose and then this glucose being oxidized. We checked this by giving the same amount of C^{14} in lactate and glucose in two separate experiments and observing the relative rates of elimination of $C^{14}O_2$ after glucose and after lactate. Normal intact rabbits were given a single injection of one of the compounds and the respired CO_2 was collected and its C^{14} content was determined. The results are shown in TABLE 7. It is quite apparent that these two substances are burned by the body in quite different ways. The specific activities of the circulating material in both would be at a maximum immediately after injection, and these would

TABLE 6
PER CENT OF ADMINISTERED ISOTOPE EXHALED AS CO_2

Lactate given		Glucose given	
30 min.	60 min.	30 min.	60 min.
22	40	2	8
22	48	2	5
21	41	2	8

TABLE 7
COMPARATIVE RATES OF OXIDATION OF L(+) LACTATE AND GLUCOSE AFTER I.V. INJECTION OF THE LABELED COMPOUND IN TWO SEPARATE EXPERIMENTS; THE SAME AMOUNT OF C^{14} WAS GIVEN IN BOTH RUNS

Period (min.)	Per cent of injected counts in CO_2 respired	
	After lactate	After glucose
0 - 15	8.5	0.5
15 - 30	12.0	1.1
30 - 45	14.0	2.0
45 - 60	10.5	3.0
60 - 75	8.4	3.4

decrease with dilution by material elaborated by the body. This could explain the rapid rise in activity due to the burning of lactate and the decrease afterward. The $C^{14}O_2$, after glucose injection, does not show maximal elimination until after an hour, which suggests that this substance is relatively sluggishly burned. If lactate had to be converted to glucose in order to be oxidized, the labeled CO_2 from it would have come off even more slowly than that from the labeled glucose, since injected lactate could not be immediately converted to glucose.

We have also studied the metabolism of lactate in the eviscerated nephrectomized rabbit. In this preparation, the important tissues that contribute to metabolism are heart, brain, endocrine glands, and muscles of respiration. The inactive muscles have a relatively low metabolic rate. This preparation has a metabolic rate of some 50 per cent of the intact rabbit. As a result of the extensive surgery, the concentration of the circulating lactate is five to ten times that of the intact animal under basal conditions. Because of this, one would not expect the turnover rate of the body lactate to be as high as in the intact animal. We determined the decay rate of labeled lactate in the same way as we did in the intact animal, and the results indicate a turnover time of body lactate of four hours, in contrast to that of the intact animal which is 30 minutes. As the usual body lactate concentration in these preparations is 100 to 200 mg. per cent or even more, this turnover time still represents an active metabolism of this compound (see TABLE 8).

TABLE 9 gives the results of an experiment planned to measure the oxidation rate of the extra hepatic tissues (i.e., that of the eviscerated nephrectomized animal). The animal was given a priming dose of potent radioactive lactate in order to bring the activity of the body lactate up to that of the lactate solution that we were planning to give by constant infusion. Despite the injection of labeled lactic acid at the rate of 333 mg./hr. for four hours, the specific activity of the body lactic acid (terminal) was less than the specific activity of the injected material. This suggests an appreciable addition of

TABLE 8
RESULTS AFTER SINGLE INJECTION OF L(+)-LABELED LACTATE IN EVISCERATED RABBIT

Time after injection (min.)	Plasma lactate conc. (mg. %)	Plasma radioactivity cts./ml.
30	226	237
150	214	144
270	200	108

TABLE 9
DATA SHOWING THE OXIDATION RATE OF THE NATURAL FORM L(+)- OF LACTIC ACID WHEN GIVEN BY CONSTANT INJECTION

Hour	S. A. plasma lactate	Expired CO_2 total counts	Lactic acid oxidized mg./kg./hr.
1st	166	19,400	230
2nd	175	18,600	200
3rd	188	12,200	122
4th	213	10,600	94

unlabeled lactate from sources in the body. The respiratory figures indicate quite a high rate of oxidation of lactate, and we calculate it as at least 50 per cent of total metabolism. It may be noted that the amount of lactate oxidized steadily drops as the run progresses. The reason for this is apparent from examination of the record. The specific activity of the respired CO_2 remains constant or rises somewhat as the experiment progresses. When these figures are corrected for the change in specific activity of the circulating lactate, we get a value indicating that lactate continues to comprise a large proportion of total body metabolism throughout. The drop with time in amount of lactic acid oxidized is due to the progressive drop in metabolic rate which we observed in all animals in these experiments.

Results with D(−) lactic acid. We studied the metabolism of D(−) lactate in the eviscerated animal. We felt that little could be learned about how the extrahepatic tissues deal with this isomer by using the intact animal, because of the probability of the liver changing the D(−) form to the L(+) form or to glucose or glycogen. The metabolism of D(−) lactic acid was followed by both the one injection and the constant infusion methods. The single shot experiments give us information about turnover rate.

TABLE 10 gives the comparative respiration results in two experiments after single injections of the L(+) and the D(−) forms. The animals were

TABLE 10
RESPIRED CO_2 ACTIVITY AFTER SINGLE I.V. INJECTION OF LABELED L(+) AND D(−) LACTATE. THE SAME AMOUNT OF C^{14} WAS GIVEN IN THE TWO SEPARATE EXPERIMENTS

After L(+) injection		After D(−) injection
Time (hr.)	Relative activity	Relative activity
1	110	25
2	145	50
3	100	53
4	78	58
5	58	68
	40	62

given the same amount of C^{14}. With the L(+) form, the specific activity of the respired CO_2 quickly rises to a peak and then steadily falls, due to dilution with lactic acid produced by the body. With D(−) form the specific activity of respired CO_2 tends to level off after the initial rise. This indicates that the unnatural form is diluted little, if any, by body metabolism. As a result, one can calculate its rate of oxidation without having to estimate the S. A. of the plasma lactate as with the natural isomer. The S. A. of the compound in the body will simply be that of the injected material. TABLE 11 gives the respiratory data on an animal is given the D(−) form by constant injection. From this we see that the eviscerated animal can oxidize a definite, though limited, amount of the D(−) isomer. The steady rise in plasma lactate supports this conclusion and contrasts with the drop in plasma concentration in the case of the L(+) isomer, despite the injection at a higher rate (TABLE 9). A part of the rise in plasma lactate concentration must have been due to accumulation of the D(−) isomer. This is borne out by the rise in plasma radioactivity which paralleled the rise in the plasma lactate concentration. With this rise in

TABLE 11
DATA SHOWING OXIDATION RATE OF D(−)-LACTATE WITH CONTINUOUS INFUSION (EVISCERATE)

Hr.	Plasma lactate (mg. %)	Plasma R. A. (cts./ml.)	Lactate oxidized (kg./hr.)	CO_2 from lactate (%)
1	136	182	11	1
2	180	344	20	3
3	242	344	19	4
4	318	466	20	5

concentration, there is no increase in oxidation of the unnatural form, suggesting that the rate of oxidation of this isomer is not affected by its concentration in this range.

Summary

We have studied the metabolism of lactic acid in the intact rabbit, making use of C^{14}-labeled L(+) lactate. Our results indicate that circulating lactate is being used up and renewed at a rapid rate. The turnover time is about 30 minutes. A large part of the lactate is disposed of by oxidation to carbon dioxide. Only a small part of it can be accounted for as glucose and glycogen or by the oxidation of them. The tissues of the eviscerated animal metabolize L(+) quite actively. They can oxidize a limited amount of the D(−) isomer. Since it has been well established that racemic lactate is practically completely metabolized by the intact animal, it seems likely that the liver converts the D(−) isomer either to the L(+) form or to glucose or to glycogen. It could possibly effect all three conversions.

Discussion of the Paper

N. R. ALPERT *(University of Illinois, Chicago, Ill.)*: I noticed that you had accounted for a substantial portion of the radioactivity of your lactate. In contrast, in some of the earlier experiments that were done by the Harvard group (I believe it was Conant and Hastings and co-workers), they were unable to account for all of their lactate oxidation.

DRURY: This is isotope work.

ALPERT: Theirs was too. They were using a C^{14} isotope, I believe, at that time.

DRURY: Theirs was tissue slice work.

ALPERT: No, I believe this was injected into the intact animal in their early experiments.

DRURY: I don't know of very much animal work that they have done. They specialize in tissue slices.

ALPERT: They also did tissue slice work afterward.

Do you think that any of the lactate that you are unable to account for might show up as a group of oligosaccharides?

DRURY: You are talking about the intact animal?

ALPERT: Yes, the intact animal.

DRURY: Of course; I showed you the balance sheet. We could account for practically all. However, there was a factor in there—glucose and derivatives.

ALPERT: Yes.

DRURY: But, even so, that was less than five per cent. But, certainly, they could have been in the form that you speak of.

ALPERT: So you were able to account for most of your lactate.

DRURY: We accounted for 85 to 90 per cent. I don't dispute your question: it is very likely that some of it goes into that form.

ALPERT: Of course, they were working in rats and this may make a difference, I don't know.

R. H. DUNLOP *(New York State Veterinary College, Cornell University, Ithaca, N. Y.)*: What was the concentration of lactate in plasma in the intact animal when you had the balance sheet.

DRURY: Between 10 and 20 mg. per cent.

DUNLOP: Is there any difference in the use of L-lactate for oxidation versus synthesis that is concentration-dependent. That is, in the higher concentrations that would be attained after exercise, would you get more synthesis than in these lower concentrations in the resting state.

DRURY: We have not studied that directly. It could be expected though, because if the metabolism is largely muscle metabolism, skeletal muscle metabolism, the delivery of lactate by the circulation to these active muscles is going to be of greater concentration. If the blood lactate is only 5 mg. per cent, the circulation just can't deliver enough lactate, even though the diffusion of lactate in capillaries of muscle is very fast. There is a circulatory problem in the relationship between concentration and utilization. Acetate, we know, depends on the concentration of circulating acetate. But if you don't have any acetate in the blood, or it is half a milligram per cent, there is not much acetate burned by the active muscles. I think the same thing applies to lactate.

O. N. MILLER *(Tulane University, New Orleans, La.)*: I think a point to be considered is that the low level of lactate in the blood, presumably, is related to the lactate levels in the tissues. It has been shown, I believe, that perhaps the limiting step in gluconeogenesis is the carboxy-kinase activity. And to overcome this, there must be certain levels in the perfusate to get a net synthesis of glucose. So the point is that if these experiments are run at a very low level of lactate in the blood, it would not be surprising to me that you get a very small incorporation in the glucose, because you have not overcome this first rate-limiting reaction.

DRURY: You are talking about an animal, the liver? I agree with your point. The liver is certainly the organ that converts lactate to glucose or other things, too. I doubt, though, whether it would apply to the eviscerated animal.

Durlacher-'46
Bib No. 448

Yale J. Biol. Med. 18:135-143. 1946.

THE EFFECTS OF CALCIUM CHLORIDE AND OF CALCIUM LACTATE ADMINISTERED BY GAVAGE

STANLEY H. DURLACHER, WILLIAM HARRISON JR., AND DANIEL C. DARROW

Post-mortem examination of two infants who had received calcium chloride by gavage while under treatment on the private service of the New Haven Hospital revealed extensive damage to the mucosa of the stomach and upper intestinal tract. These findings stimulated investigation to determine whether similar lesions could be produced experimentally. Although it is frequently stated in the literature that oral administration of calcium chloride is irritating and may cause vomiting,[6, 8] no description of the lesions produced has been encountered. Numerous clinical experiences similar to those cited in the third case described here confirm the impression that the doses of calcium chloride usually administered in tetany neonatorum are excessive and perhaps that calcium chloride is always a dangerous drug during the early months of life.

Case I

White female. Age 36 days. Autopsy by Dr. J. J. McGillicuddy. No. 5391.

Clinical history:

This 2900-gram infant was born spontaneously after a normal pregnancy. The first two days of life were not remarkable. On the morning of the third day, tremors (especially of the right arm and leg) were noted. The Chvostek, Trousseau, and peroneal signs were absent, and the physical examination was otherwise normal. Four grams of calcium chloride in 25 cc. of water (16 per cent solution) were administered by gavage. Blood-stained vomitus appeared two hours later, followed shortly thereafter by blood in the stool. Shock occurred in 4½ hours, and peritonitis was suspected. An x-ray revealed free fluid but no air in the peritoneal cavity. On the following day, exploratory laparotomy confirmed the presence of considerable clear

* From the Departments of Pathology and of Pediatrics, Yale University School of Medicine. A portion of the material here presented is derived from the thesis of W. H., Jr., submitted to the faculty of the Yale University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine.

fluid in the abdominal cavity, but no changes of the abdominal viscera were noted. Examination of the fluid revealed no cells or organisms by smear and culture. The postoperative course was characterized by disruption of the wound, peritonitis, and the development of a fecal fistula. Terminally the child vomited repeatedly and died on the thirty-sixth day of life. The clinical diagnosis was "Intestinal obstruction, cause unknown."

Autopsy findings:

The body was emaciated and skin turgor was diminished. An organizing suppurative peritonitis with metastatic abscesses in the lungs, subcutaneous tissues, and viscera was found. The serosa of the stomach was dull and covered by fibrinous adhesions. The mucosa was replaced by opaque, yellow, calcareous plaques, which were brittle and firmly attached to the submucosa. Plaques of similar consistency were seen in the small intestine, particularly in the duodenum and upper jejunum. Microscopic examination revealed ulceration and necrosis of the mucosa and submucosa of stomach and small intestines. The stomach wall was composed of plaques of hyaline scar tissue, here and there infiltrated by small round cells. Many of these scars contained granular material which stained black in the von Kossa preparation indicating the presence of calcium. Organizing and calcified thrombi occluded the arterial vessels of the submucosa (Fig. 1), and there were similar lesions in the small intestine. Focal necrosis and calcification were present in the media of many large arteries (Fig. 2).

Case II

White male. Age 7 days. Autopsy by Dr. S. H. Durlacher. No. 5452.

Clinical history:

The baby, born by low forceps, weighed 3060 grams. The first two days of the infant's life were uneventful. On the evening of the second day shaking of the arms and legs as well as tremors of the eyelids were noted. The physical examination revealed hyperactive reflexes and positive plantar and Chvostek responses bilaterally. The serum calcium was 6.8 mg. %. A gavage containing 3 grams of calcium chloride in 18 cc. of water (16.6 per cent solution) was administered, and one gram of calcium chloride was added to each four-hour feeding thereafter. On the following day the infant vomited the feedings and respiratory difficulty was observed. This was attributed to aspiration. Signs of collapse ensued, and despite transfusions and other supportive measures, death occurred on the seventh day of life. The clinical diagnosis was "Aspiration pneumonia; Icterus neonatorum; Tetany neonatorum."

Autopsy findings:

The clinical diagnosis of aspiration pneumonia was confirmed at autopsy. The other striking findings were confined to the stomach, which was slightly

dilated and contained approximately 200 cc. of curdled milk. The serosa was smooth and glistening. The mucosa along the lesser curvature was pale and intact, but that of the greater curvature, especially in the fundic portion, was bright red and ulcerated. The bases of these ulcers were filled by white opaque, firm, friable material which had the consistency of an egg-shell. Microscopically, sections of the damaged portion of the stomach showed necrosis and disappearance of the mucosa with edema, polymorphonuclear cellular infiltration, and necrosis of the blood vessel walls in the submucosa and muscularis (Figs. 3 and 4).

Case III

White female. Newborn. N.H.H. No. B19725.

A full-term infant was born spontaneously September 5, 1941, in good condition. During the pregnancy the mother drank only one pint of milk a day, and developed extensive dental caries. The baby, breast fed for two days, was then placed on formula. When seven days old, a clonic convulsion which lasted six minutes occurred. A similar convulsion followed soon thereafter. Physical examination was essentially negative; Chvostek and Trousseau signs could not be elicited. The serum calcium was 6.2 mg.%. On the eighth day, through a misunderstanding of the ruling that medication should not be added to infants' milk mixtures, the infant was given two grams of calcium chloride in water before the 10 A.M. and 2 P.M. feedings. Both feedings were promptly vomited, and the vomitus was blood streaked. She took and retained one-half of the 6 P.M. feeding, which contained 2 grams of calcium chloride. Saline clysis, transfusion, and hykinone were given because of the hematemesis. The baby did well on the regular formula without added calcium, and saline clyses for the next two days. No convulsions occurred. On the eleventh day of life, 0.5 cc. of 50 per cent calcium chloride were added to each feeding, and continued for two days without incident. Discharge diagnosis was hypocalcemic tetany. The infant has gained weight since discharge and has shown no subsequent gastro-intestinal symptomatology.

The above histories suggest that calcium chloride produces gastro-intestinal lesions. In the first case, the long interval before death may make interpretation somewhat difficult but the other two cases leave little room for doubt. It should be emphasized that these three infants received some of the calcium chloride in aqueous solution at a time when the stomach was empty. Doses of the drug administered in milk mixtures were better tolerated. The following experimental work shows the effect of similar and smaller doses of calcium chloride in rabbits.

Experimental

Methods: Forty-two rabbits, from two days to six weeks of age, were used. The animals were fasted for 48 hours before gavage and permitted to eat six hours after gavage. A soft rubber catheter (French No. 8) was employed and the animals were sacrificed 48 hours after treatment, except as noted in table 1. Anhydrous calcium chloride was administered in 5, 7.5, 10, 15, and 20 per cent aqueous solutions; calcium lactate in 5 and 10 per cent aqueous solutions; ammonium chloride in 5, 10, and 20 per cent aqueous solutions; and three rabbits were treated with combined solutions of ammonium chloride and calcium lactate as shown in table 1. Two rabbits were intubated and the catheter manipulated roughly to determine whether this procedure alone would cause trauma. Gross and microscopic study of the stomach and intestines was performed in all instances.

Results: The results of these experiments are outlined in table 1. Rough intubation caused no lesions. All animals, independent of weight and age, receiving 20 per cent solution of calcium chloride had severe gastric damage, which consisted of mucosal necrosis and ulceration (Fig. 5), submucosal edema, cellular infiltration (Fig. 6), and angionecrosis. Necrosis and exudation occasionally extended through all coats and fibrinous exudate was present on the serosal surface. Perforation of the stomach of one of the larger rabbits occurred. The lesions were most marked along the greater curvature of the stomach, especially in the region of the fundus. No lesions were produced in the small or large intestine. With doses of 15 per cent calcium chloride, lesions were not found in the older rabbits but severe ulcers, similar to those described above, occurred in the unweaned animals receiving over 0.75 gm. per kg. Similar results were obtained with the 10 and 5 per cent solutions.

Calcium lactate in doses of 6 gm. per kg. produced no changes, even in the most immature animals. Concentrations higher than 10 per cent could not be given because of the limited solubility of the drug.

Animals receiving ammonium chloride in 20 per cent aqueous solution showed no morphological changes in the viscera. Those receiving doses greater than 1.5 gm. per kg. had convulsions and died within one hour after the administration. Those animals receiving both calcium lactate and ammonium chloride were likewise free of anatomical lesions.

The above experiments show that calcium chloride is quite irritating to the stomach of rabbits. When the weaker concentrations

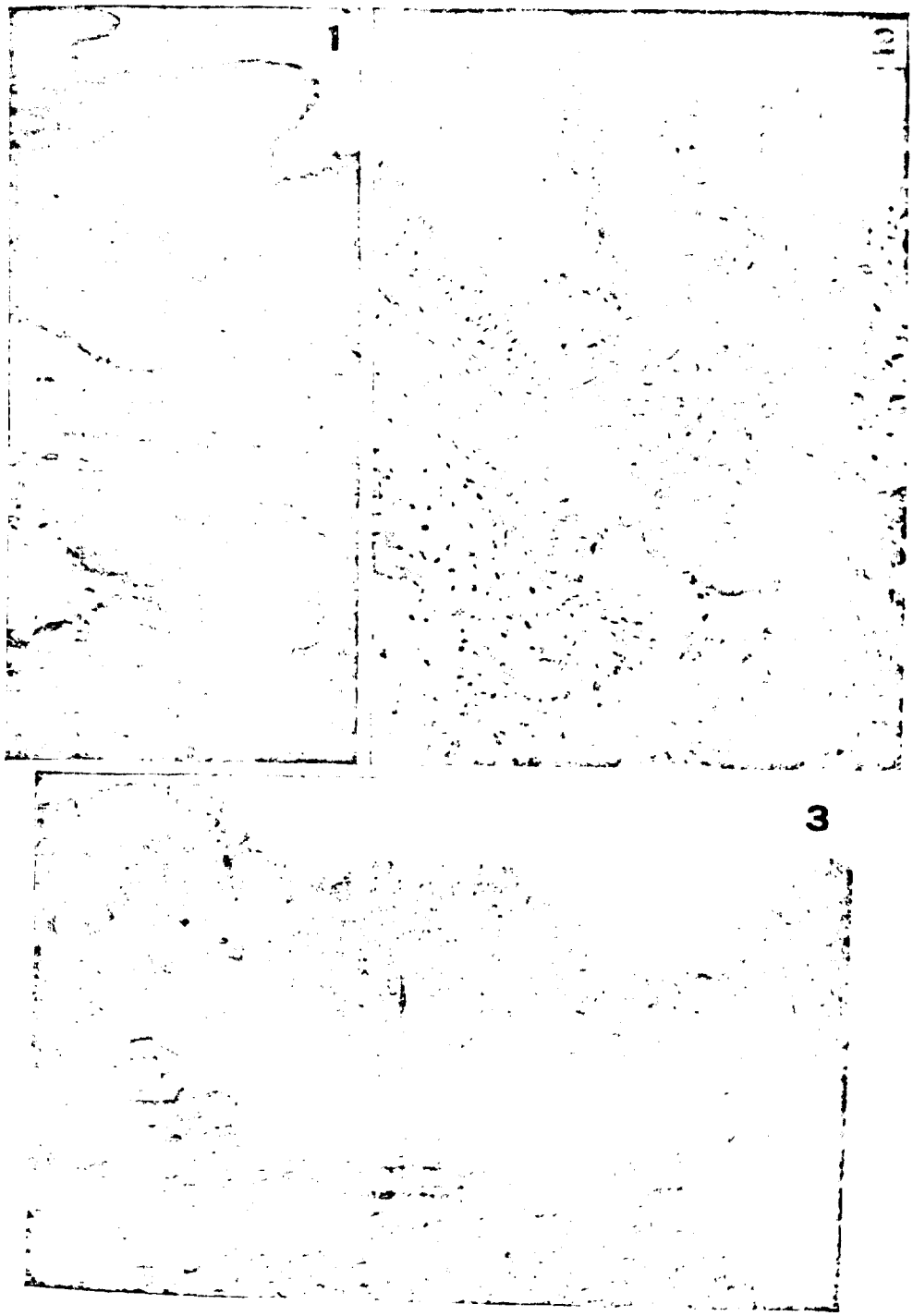

FIG. 1. Case I. Low-power view of stomach wall, showing ulceration and necrosis of mucosa with arterial necrosis and calcification. ×35

FIG. 2. Case I. Arterial [illegible] and [illegible]. ×175

FIG. 3. Case II. Low-power view of stomach wall, showing necrosis of mucosa, cellular infiltration, and vascular thrombosis in submucosa and muscularis. ×45

4
6

TABLE 1

EFFECTS OF CERTAIN SALTS ADMINISTERED TO RABBITS BY GAVAGE

Rabbit	*Weight (gm.)*	*Salt*	*Dose (gm./kg.)*	*Concentration (%)*	*Manner of death*	*Edema*	*Mucosal necrosis*	*Submucosal necrosis*
1	60	—			S	—	—	—
2	80	—			S	—	—	—
3	215	$CaCl_2$	0.75	20	S	+++	++	—
4	180	$CaCl_2$	1.50	20	D	+++	+++	++
5	275	$CaCl_2$	1.50	20	S	+++	++	—
6	900	$CaCl_2$	1.50	20	S***	+++	+++	+++
7	1000	$CaCl_2$	1.50	20	D	+++	+++	+++
8	80	$CaCl_2$	0.68	15	S	—	—	—
9	80	$CaCl_2$	1.25	15	S	+++	++	—
10	60	$CaCl_2$	1.25	15	D	+++	+++	—
11	60	$CaCl_2$	1.25	15	S	+++	++	++
12	80	$CaCl_2$	2.50	15	S	+++	+++	+++
13	160	$CaCl_2$	1.50	15	S	+++	+++	+++
14	375	$CaCl_2$	1.50	15	S*	+	—	—
15	780	$CaCl_2$	0.75	15	S	+	—	—
16	810	$CaCl_2$	1.50	15	S	+	—	—
17	920	$CaCl_2$	1.50	15	S	+	—	—
18	150	$CaCl_2$	1.50	10	D	+++	+++	—
19	330	$CaCl_2$	1.50	10	S	++	—	—
20	775	$CaCl_2$	0.75	10	S	—	—	—
21	810	$CaCl_2$	1.50	10	S	+	—	—
22	1000	$CaCl_2$	1.50	10	S	—	—	—
23	60	$CaCl_2$	1.25	7.5	S	—	—	—
24	760	$CaCl_2$	0.75	5	S	—	—	—
25	110	$CaCl_2$	1.50	5	D	++	++	+
26	810	$CaCl_2$	1.50	5	S	—	—	—
27	1100	$CaCl_2$	1.50	5	S	—	—	—
28	120	Ca lact.	4.0	10	S	—	—	—
29	275	Ca lact.	6.0	10	S	—	—	—
30	340	Ca lact.	6.0	10	S*	—	—	—
31	650	Ca lact.	2.0	10	S	—	—	—
32	700	Ca lact.	2.0	10	S	—	—	—
33	645	Ca lact.	4.0	5	S	—	—	—
34	200	NH_4Cl	0.5	20	S	—	—	—
35	342	NH_4Cl	1.5	20	D**	—	—	—
36	315	NH_4Cl	0.5	10	S	—	—	—
37	320	NH_4Cl	1.0	10	S	—	—	—
38	320	NH_4Cl	0.5	5	S	—	—	—
39	344	NH_4Cl	1.0	5	S	—	—	—
40	280	NH_4Cl	2.0	10	D**	—	—	—
		Ca lact.	2.0	10				
41	210	NH_4Cl	4.0	10	D**	—	—	—
		Ca lact.	4.0	10				
42	240	NH_4Cl	1.0	5	S	—	—	—
		Ca lact.	2.0	10				

S — Sacrificed after 48 hours.
D — Died before 48 hours.
* — Sacrificed after [illegible] hours.
** — Died of [illegible] after 1 hour.
*** — Perforation of stomach wall found at autopsy

TABLE 2

CHANGES IN SERUM CALCIUM, PHOSPHORUS, AND TOTAL PROTEIN FOLLOWING THE ADMINISTRATION OF CALCIUM LACTATE TO NEWBORN INFANTS

Infant	*Weight* (gm.)	*Ca.** (mg.%)	*Ca.*† (mg.%)	*Difference* (mg.%)	*Phos.** (mg.%)	*Phos.*† (mg.%)	*Difference* (mg.%)	*Total protein** (gm.%)	*Total protein*† (gm.%)	*Difference* (gm.%)	*Rise in Ca accounted for by rise in protein*
1	3045	10.1	11.6	1.5	6.2	5.2	−1.0	5.6	5.8	0.2	0.15
2	3175	11.7	12.4	0.7	6.8	5.7	−1.1	5.7	5.7	0.0	0.0
3	3105	9.4	12.4	3.0	7.6			5.9	6.8	0.7	0.5
4	3725	9.6	11.4	1.8	8.6	7.9	−0.7	5.7	6.4	0.7	0.5
5	3450	9.8	12.5	2.7	7.5	5.0	−2.5	5.5	5.7	0.2	0.15
6	5960	10.0	13.2	3.2	5.3	5.0	−0.3	5.7	6.3	0.6	0.45

* Values before 10 A. M. feeding.

† Values before 2 P. M. feeding; 4 hours after receiving 60 cc. of 10 per cent calcium lactate by gavage.

are administered the lesions are produced only in very young rabbits. Amounts of calcium lactate of equivalent calcium content produced no lesions, but the solubility of the calcium lactate is so low that concentrations equivalent only to 5 per cent $CaCl_2$ could be tested.

The effect of calcium lactate on the serum calcium in infants

The following work was undertaken in order to determine whether calcium lactate is effective in raising the concentration of calcium in the serum.

Methods: Six infants were studied; five were newborn, the sixth was four months old. Ten cubic centimeters of venous blood were withdrawn from the normal neonatal infants during the hour preceding their regular 10 A.M. feeding. Immediately following this they were gavaged with 60 cc. of a 10 per cent calcium lactate solution. The regular morning feeding was allowed. Preceding the 2 P.M. feeding, the second blood sample was taken. Blood samples were taken at similar intervals from the four-month-old baby. Blood serum was analyzed for calcium by the Clark-Collip modification of the Kramer-Tisdal method.[4] Serum phosphorus was determined by the Fiske-Subbarow colorimetric method, using a photoelectric colorimeter.[5] Total proteins were determined by the method of Barbour and Hamilton.[1]

Results: Three of the babies had mild diarrhea during the four hour period following gavage. No vomiting occurred. Significant rise in the serum calcium (see table 2) occurred in all six cases. Initial calcium levels ranged from 9.4 to 11.7 mg.%. Levels taken four hours after gavage ranged from 11.4 to 13.2 mg.%. The largest increase occurred in the four month's old baby. The levels of phosphorus were found to drop as the calcium level rose. This drop varied from 0.3 to 2.5 mg.%, or from 6 to 33 per cent of the original level. The serum proteins showed very small increases in concentration, varying from 0 to 0.7 gm.%, or from 0 to 12 per cent of the original value. The last column of table 2 represents the rise in serum calcium which might be due to the concomitant rise in proteins, calculated according to the equation $Ca = 0.75\ P + 6.8$. This equation uses the mean value of those found by five authors, as reported by McLean and Hastings.[11] It can be seen in Chart I that the average rise in serum calcium is not accompanied by a proportional rise in serum protein. The rise in protein could account for only a very small fraction of the calcium rise, the remaining rise being due solely to unbound calcium. The graph charts this rela-

tionship on the scale indicated by the above formula: i.e., 0.75 gm. of protein equals 1 mg. of calcium, protein equals 0 when calcium equals 6.8.

Discussion

It appears that present pediatric practice employs excessive and dangerous doses of calcium chloride for the newborn infant. For the symptomatic treatment of tetany, many pediatric text-books[2,7,9,12] recommend doses of calcium chloride ranging from 2 to 4 gm., followed by repeated smaller doses administered in 10 per cent solution or even higher concentrations. This dangerous practice probably arose from the fact that these doses were originally used for older infants suffering from rachitic tetany. Under these circumstances the material was relatively harmless. Improved knowledge and practice of infant nutrition has reduced the incidence of rachitic tetany, and at present tetany of the newborn is the commonest type treated in New Haven. A dose of calcium chloride not particularly excessive for an older child apparently is dangerous when administered to a newborn infant. The production of ulcers of the stomach in newborn rabbits with a 5 per cent solution of calcium chloride indicates the danger of this salt to the mucosa of the stomach of unweaned animals. Calcium chloride added to milk undoubtedly is less irritating than are aqueous solutions. Nevertheless, our experience indicates that calcium chloride, in any vehicle, is probably dangerous to newborn babies.

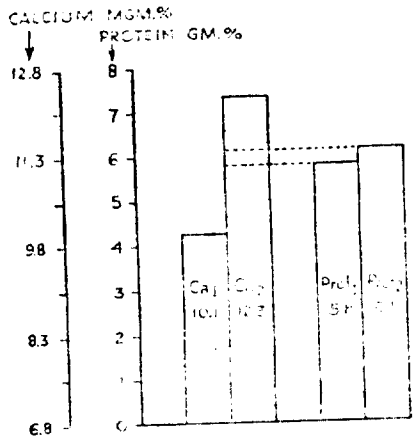

Chart 1

The chart shows the average concentrations of calcium and protein in serum before and four hours after administration of calcium lactate. The scale of the protein is equivalent to the calcium scale to serum protein and permits direct comparison of the rise in calcium due to rise in serum protein concentration. The chart illustrates that administration of calcium lactate produced a rise in both and calcium.

It is noteworthy that other calcium salts are ineffective when given by mouth in the treatment of tetany of infants. This lack of therapeutic result is not explained by the lower calcium content, which is roughly one-third and one-fourth, respectively, for the

lactate and gluconate. The absence of a rise in serum calcium in cases of tetany is hard to reconcile with the easily demonstrated rise in serum calcium following oral administration of calcium lactate in normal newborn babies, adults,[2,10] and experimental animals.[8] In five recent trials, calcium gluconate (5 to 10 cc. of a 10 per cent solution) given intravenously has immediately relieved convulsions, but in two cases signs of tetany and low serum calcium have persisted for several days. The method of treatment of tetany of the newborn needs re-examination in order to find a method as effective as oral calcium chloride without its potential dangers.

Summary and conclusions

Necrosis and ulceration of the stomach, and in one instance of the small intestine, occurred in two infants who died after receiving calcium chloride by gavage. Similar lesions were reproduced in rabbits with comparable and with smaller doses of calcium chloride. Equivalent and larger doses of calcium lactate produced no anatomical changes in rabbits. The latter salt administered to six newborn infants was effective in raising the serum calcium.

Calcium chloride, as commonly recommended, is damaging to the stomach and intestines. Its administration by gavage to newborn infants is contraindicated.

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Fazekas-'49
Bib No. 515

Orvosi Hetilap 90:777-781. 1949.
Published by The Forensic Medicine Institute of the Szeged University of Sciences. Director of the Institute: Dr. Julius I. FARKAS, honorary lecturer.

Experimental data on simple compounds used for influencing ovarian functions.

By Dr. Julius I. FAZEKAS.

During the last two decades, the following substances were mainly used, more or less successfully, for replacing or restoring the function of the ovary when its functioning was reduced: the folliculus hormone and the corpus luteus hormone or the gonadotropic hormone of the front lobe of the hypophysis (prolan A, prolan B). Some observed good results after the administration of stilbene derivatives. A few authors found results favorable in this field after X-ray treatment of the overy, others after X-ray treatment of the hypophysis; some found that the results were favorable with a carbohydrate-free ketogenic (meat) diet (LORALEK), etc. Since it is not always possible to restore the ovary function with these methods on the one hand, and since hormone treatment in particular is rather costly on the other hand, it appears desirable to search for a treatment method which would eliminate the disadvantages of the therapeutic methods applied to date.

In my earlier works I disclosed my investigations on the basis of which I showed that when adequate dosages of certain chemical compounds are supplied, the suprarenal glands of various animals become enlarged and the cortical substance function of the same intensifies, as compared to normal functioning. In the course of these tests I observed facts which indicate that it is also possible to act on the ovary function without using any hormones, by applying adequate dosages of simple compounds.

My first findings in this respect were made in 96 tame rabbits, treated with NH_4OH. During these tests, female tame (domestic) rabbits, weighing 2500 to 3500 gr, kept separately , which were sexually mature (aged 8 to 14 months) were given every second day 50 to 60 ccm ½% NH_4OH per os; they were killed after 1 to 3 months. We then found suprarenal gland cortical substance hyperthrophy as well as folliculus maturation in the ovary, hemorrhaged folliculi and the formation of yellow corpuscles, together with a certain degree of enlargement. Our second observations concerned geese; for the purpose of increasing the function of the suprarenal glands, we supplied every second day 50 to 70 ccm ½% NH_4OH through the stomach tube in increasing doses during 4 weeks to 10 geese originating from the same hatching. We gave the same feed, without treatment, to 10 other geese from the same hatching for control purposes. The geese were killed after 4 weeks; numerous nut-size and larger eggs (ovules) were found in the ovaries of the geese treated with NH_4OH, which in each goose formed a total mass having the size of a man's fist. As compared to the above, the eggs (ovules) present in the ovaries of the geese which had received the same feed but without treatment were less than green-pea-size.

We performed additional tests on the basis of these preliminary findings, during which sexually mature (aged 8 to 14 months) female, virgo intacta, separately kept tame rabbits of the same breed (chinchilla) were used; they received the same food. Tame rabbits are especially adequate for tests intended for the artifical stimulation of the ovary function. We know from investigations performed by VAN BENEDEN and HEAPE, as well as from the tests performed by others that in tame rabbits ovulation, or the rupture of the folliculus occurs only after copulation.

It is, however, known that folliculus rupture occurs in tame rabbits after "sterile mating" as well. Activated by the corpus luteum which then forms, characteristic changes occur in the genitalia (enlargement of the womb, decidua formation, enlargement of the teats). This condition is usually defined as "false pregnancy" or "apparent pregnancy". The false pregnancy condition lasts for 13 to 14 days according to ANCEL and BOUEN, and for 16 to 19 days according ti HAMMOND; it lasts for 16 days according to ANAUS; then it regresses as a result of corpus luteum degeneration. To avoid a "false pregnancy", the tame rebbits must be kept separated for at least 1 month before testing of the ovarian function or the condition of the genitalia respectively, is started.

In the course of the tests reported below, we isolated the control rabbits already at the age of 5 months, i.e. 1 month before they reached sexual maturity (6 months), and we kept them separately for 3 to 8 months before using tham for the test. As a matter of course, the separation continued during the test period as well. The purpose of this procedure was to have ovaries of various age available for examination which , while sexually mature, had not yet undergone --for natural reasons--any follicle maturation and rupture, nor corpus luteum formation; we thus could evaluate the effect of the treatment with maximal precision.

For the first test series we used female tame rabbits, with an initial weight of 2500 to 3500 g and aged 8 to 14 months; we assigned them to groups including 5 to 7 animals each (kept separately); we used different compounds for the treatment of each group. Each animal received, in gradually increasing doses, 0,1 to 0,2 g per kg of body weight of the active agent, dissolved in 100 to 150 ccm of drinking water, applied every second day. The duration of the treatment was 5 months, carried out as follows: a period of one week without treatment was inserted in all cases after a treatment period of three weeks. The following compounds were used for the treatment: ammoniumchloride, ammoniumsulfate, ammoniumcarbonate, sodium-ammonium phosphate, ammoniumacetate, ammoniumlactate, calciumchloride, acidum hydrochloricum (25%), acidum lacticum cc, acidum aceticum, sodium dihydrophosphate, ammonium hydrophosphate.

With the applied treatment, the acid base equilibrium of the animals periodically shifts to a lesser extent towards acidity; but no serious acidosis, as currently interpreted, did occur. The animals ate with good appetite and gained weight throughout the (test) period. For control purposes we kept 50 female, virgo intacta tame rabbits of the same age, breed and intial weight likewise separated; they received the same quality and quantity feed.

After 5 months we killed the treated and the control rabbits by means of air embolims; we found that the ovaries of the treated animals, as compared to those of the controls, were more or less enlarged in all cases; on their surface , visible to the unaided eye, numerous enlarged and mature hemorrhaged follicles or corpora lutea were in evidence.

Enlargement of the ovaries of tame rabbits as a consequence of treatment with various compounds.

1 Sorszám	2 A kezelés módja	3 Az esetek száma	4 A 2 ovarium súlya cg			8 A 2 ovarium súlygyarapodása középértékben		9 S. d.
			5 maximum	6 minimum	7 középérték	cg	%	
1.	Kontroll	50	15	52	38.66	—	—	—
I.	Ammonium hydroxyd	96	42	150	63.24	24.58	63.57	6.92
2.	Ammonium chlorid	50	53	108	70.82	32.16	88.36	5.82
3.	Ammoniumsulfát	5	68	128	95.50	56.84	147.00	7.63
4.	Ammonium carbonát(!)	7	60	92	80.70	52.04	134.60	6.34
5.	Na.-ammon. phosph.	7	67	111	95.66	57.00	150.00	9.20
6.	Ammonium acetat	6	54	121	87.30	48.64	125.81	8.32
7.	Ammonium lactat	5	68	146	104.66	66.00	173.30	9.24
8.	Calcium chlorid	5	60	146	102.20	63.54	164.35	8.06
9.	Ac. hydrochloricum	5	73	115	93.60	54.94	142.10	7.38
10.	Ac. lacticum	5	64	118	96.40	57.74	150.00	7.65
11.	Ac. aceticum	6	57	102	87.62	48.96	125.80	7.14
12.	Natrium dihydrophosphat	5	92	162	126.33	87.67	229.35	11.32
13.	Ammon. hydrophosphat	5	81	132	112.00	73.34	190.47	8.27

Sodium

1) Serial No.
2) Treatment method
3) Number of cases
4) Weight of the 2 ovaries, cg
5) maximum 6) minimum 7) average value
8) Weight increase of the 2 ovaries in average values - cg - %
9) Significance

The data of the table show convincingly that under the effect of treatment with the applied compounds, a considerable increase of the ovary weight occurs, reaching an average value of 63.57 - 229.35%. The fact that the weight increase is significant (S.D.) is proved by the performed statistical(probability) calculations. The enclosed photograph (No.I) is a good illustration of the volumetrical increase, of the hemorrhaged follicles and of the corpora lutea. On said illustration the last (VIth) line shows the ovaries of control rabbits of the same age and with identical weight, without treatment; in the Ist to Vth line the enlarged ovaries of treated rabbits are demonstrated, with a different compound used for each line.

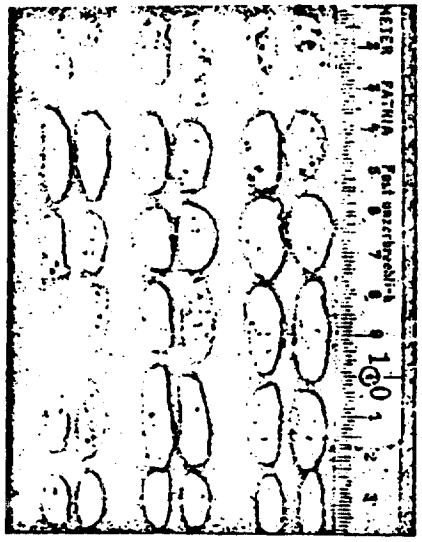

Illustration I. enlargement, folliculus maturation and hemorrhage, corpus luteum formation in the ovaries of virgo intacta tame rabbits treated for 5 months with various compounds.

Line I.	Calcium chloride	treatment	93 cg	127 cg	146 cg
" II.	ammonium acetate	"	83 "	100 "	121 "
" III.	" lactate	"	68 "	100 "	146 "
" IV.	" sulfate	"	85 "	88 "	128 "
" V.	sodium-ammonium-phosphate	"	80 "	90 "	111 "
" VI.	without treatment (control)	"	37 "	40 "	40 "

Histological examination. The sections of the ovaries from the untreated control rabbits show large numbers of primary follicles, crowded closely to each other; between these, maturing but not yet mature, comparatively small follicles are visible (10 to 14 in each section); the interstitial ovary glands (medullary substance) are thus restricted to a comparatively very small area. No completely mature follicles, ready to rupture, are detectable. (Illustration 2.).

On the other hand, in virgo intacta rabbits treated for 5 months and kept separately, the major part of the enlarged ovary consists of interstitial ovary glands (medullary substance); the cells of these glands are conspicuously larger than normal and their number is likewise considerably increased; in their protoplasm, the lipoids multiplied, forming smaller and larger drops, and the nucleus is frequently displaced laterally. In some cases these cells are so rich in lipoids within each spot-like area that they appear very similar to the lutein cells. It is likewise remarkable that the ovaries of some animals show numerous mature follicles, hyperemia, and hemorrhaged follicles (Illustration 3), while the ovaries of other animals show numerous corpora lutea (Illustration 4). Mature and hemorrhaged follicles are found not only on the surface, but also in the deeper layers. The cortical substance of some ovaries narrowed, while others show no narrowing. We did not find folliculus degeneration or ovary cell degeneration in the ovaries of the treated rabbits. This seems to indicate that the applied

treatment does not damage the follicles, or the ovary respectively. The primary follicles and the maturing follicles on the ovaries of the treated rabbits are conspicuously less dense, at a greater distance from each other, as compared to the control ovaries. This can be explained by the fact that on the one hand, the follicles seem to be pushed apart as a result of the enlarged volume and surface of the ovary, thus taking up a larger area; on the other hand, the absolute number of the follicles will of necessity decrease as well, as a consequence of intensified follicle maturation which occurs in the course of the treatment.

Illustration 2. Section of the ovary from an untreated (control) tame rabbit, weight 3000 g, virgo intacta, age 13 months. The total weight of the 2 ovaries is 25 cg.

Illustration 3 . Hemorrhaged and mature follicles in the ovary of a 13 months old, virgo intacta rabbit, after treatment with calcium chloride during 5 months. The total weight of the two ovaries is 146 cg. Initial body weight: 3100 g. Body weight at the termination of the treatment: 4200 g. Hemotoxyline-eosin dye.

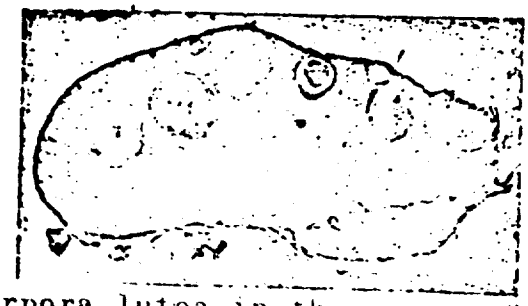

Illustration 4. Corpora lutea in the ovary of a tame rabbit, virgo intacta, age 13 months, after treatment with ammonium lactieum during 5 months. The total weight of the 2 ovaries: 146 cg. Initial body weight: 3100 g. Body weight at the termination of the treatment: 4700 g. Van Gieson dye.

The above statements lead to the conclusion that the observed ovary enlargement can on the one hand be ascribed to the hyperthrophy of the interstitial overy glands and to the increase of their lipoid content; on the other hand, it can be ascribed to the enlargement, maturation and hemorrhage of the follicles or the corpora lutea formation respectively.

In the course of another test series under the aforementioned test conditions we likewise treated 5 to 7 rabbits (aged 8 to 10 months) with the same compounds (80 to 100 doses); we observed them during 14 to 16 months; they were then killed by means of air embolims and their ovaries were subsequently examined. Substantially similar symptoms were found in the ovaries of these animals as those found after a treatment period of 5 months; I therefore omit a detailed report. It is merely noted that we did not find any degenerative symptoms in the ovaries of these animals.

According to data in literature, an effect causing the maturation of the follicles in the ovary and the formation of corpora lutea was primarily found to be caused by the gonadotropic hormones produced by the front lobe of the hypophysis.

Numerous tests undoubtedly proved that the function of the ovaries is hormonally directed by the front lobe of the hypophysis.

Some authors contend, on the basis of various tests, that the basophylic cells of the hypophyisis produce the hormone which maturates the follicle, while the acidophylic cells produce the hormone which forms the corpora lutea. HERLANT observed a follicle-maturing effect on the occasion of a basophyilic cell transplant which (cells) had multiplied during the SELYE alarm-reaction. After implanting the zone of oxen- and hog-hypophysis which contains basophylic cells into female, sexually mature rats, GIROUD and MARTINET observed a considerable ovary enlargement, follicle maturation and formation of corpora lutea. Following the transplant of an acidophilic cell group, they found that a minor follicle enlargement only developed, while no corpora lutea formed. According to their tests, they attribute the follicle-maturing effect to the basophylic cells of the front lobe of the hypophysis; they believe that the basophylic cells are likewise involved in the formation of corpora lutea.

The formation of corpus luteum was observed by HOHLWEG in young rats and by KLAFTEN, PATAKY and others in ovaries of sexually mature, isolated tame rabbits following the injection of a larger dose of folliculus hormone. They explain this as follows: large quantities of follicle hormore inhibit the hormone (prolan A) production of the front hypophysis lobe, while promoting the production of the corpus luteum-forming hormone (prolan B).

After injecting the urine of a pregnant woman (which was boiled and therefore did not contain any prolan), BAUMANN observed corpus luteum formation in animal ovaries; he attributes this to the effect of the large volume of folliculine in the urine of the pregnant woman, which acts through the hypophysis. HOHLWEG and others share this view. According to the investigations of ZONDEK, CLAUBERG and others the corpus luteum formation is always preceded by a certain degree of follicle maturation, triggered by prolan. AXEL-WESTMANN likewise pointed out that no corpus luteum formation takes place without follicle maturation.

After injecting the urine of men and women kept on a ketogenic diet into infantile white mice, JULESZ observed in the animals follicle maturation and edematous swelling; but no follicle hemorrhage or corpus luteum formation took place. JULESZ explains this phenomenon by stating that the ketosis stimulates the front lobe of the hypophysis to intensified prolan production.

We wish to state additionally that LUIGI caused significant follicle maturation in 'infantile' tame rabbits with electrical stimulation of the hypophysis; FREEGOOD and PINCUS achieved the same in guinea pigs with electrical stimulation of the neck sympaticus.

The results of our own reported tests indicate that it is possible to obtain follicle maturation, follicle hemorrhage and corpus luteum formation in the ovaries of tame rabbits without hormone application, with suitable dosages of simple compounds .

The question is raised: which mechanism of the compounds used for our tests is instrumental in triggering the symptoms observed in the ovary? Since the detected changes fully coincide with those resulting during the pregnancy reaction of ZONDEK-ASCHHEIM and FRIEDMANN respectively, and with the symptoms respectively which usually occur after the administration of the gonadotropic hormones of the hypophysis, it is highly probable that the compounds used by us cause the changes observed in the ovary likewise through the front lobe of the hypohysis. However, since the applied compounds include organic and inorganic compounds as well as ammoniacal and non-ammoniacal compounds, it is obvious that their specific chemical composition cannot be held responsible for the effect on the hypophysis, or for the effect on the ovary respectively, through the hypophysis. It is a shared characteristic of these compounds that they shift the acid base equilibrium of the organism towards acidity. This has been confirmed by our earlier investigations and by the examinations performed by others.

We are therefore of opinion that the minor periodical shift of the acid base equilibrium towards acidity ,resulting from the effect of the applied compounds, most probably stimulates the corresponding cells of the front hypophysis lobe, which then react with intensified gonadotropic hormone production; the latter cause follicle maturation, the formation of coropra lutea and ovary enlargement. This coincides with the findings of SELYE and HERLANT during "alarm reaction", when after the intravenous injection of hydrochloric acid an increased number of basophilic cells of the front hypophysis lobe was observed, together with follicle maturation in the ovary.

We mentioned earlier that follicle rupture occurs in tame rabbits only 10 to 18 hours after mating. This is due to the fact that in rabbits (and in cats) the front hypophysis lobe produces follicle-maturing hormone only under the effect of the nervous excitation caused by the mating. HARRIS (1941) injected cuprum-acetate into the third cerebral chamber of tame rabbits; this triggered ovulation through the excitation of the hypothalamicus centers. It is necessary in this context to take the fact into consideration as well that the compounds administered by us, by shifting the acid base equilibrium towards acidity, excite the hypothalamicus center of the hypophysis, which causes the front hypophysis lobe to produce gonadotropic hormones; the latter then cause the symptoms observed in the ovary.

It can be assumed on the basis of our rabbit tests that the ovaries of other animals and eventually those of women the function of which is reduced can likewise be induced to regular or intensified functioning with the suitable therapeutic administration of the used compounds or of other compounds having an acidotic effect. However, further tests are needed to reach a final decision in this respect.

Our findings concerning geese indicate that the ovulation process of poultry can be accelerated with the applied treatment, eventually making increased egg production possible. The confirmation of the above would be of noteworthy economic importance.

I wish to state, for the sake of completeness, that we were able to verify, besides changes of the ovary, hyperthrophy of the suprarenal glands, a gradual enlargement of the teats (proliferatic), followed first by plasma secretion and later by milk secretion which lasted for months, enlargement of the womb, enlargement of the hypophysis,etc. I will report in detail on these symptoms in another paper.

—

Fazekas-'49
Bib No. 515

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Orvosi Hetilap 90:777- 781. 1949.

A szegedi Tudományegyetem Törvényszéki Orvostani Intézetének közleménye.
Intézetvezető: Fazekas I. Gyula dr. egyet. magántanár.

Kísérleti adatok a petefészekműködés befolyásolására egyszerű vegyületekkel*

Irta: FAZEKAS I. GYULA dr.

A csökkent működésű petefészek működésének pótlására vagy helyreállítására az utóbbi két évtizedben főként a folliculus hormont és a corpus luteum hormont vagy a hypophysis elülső lebenyének gonadotrop hormonjait (prolan A, prolan B) alkalmazzák, több-kevesebb sikerrel. Némelyek a stilben származékok adagolásától észleltek jó eredményt. Egyes szerzők a petefészkek, mások a hypophysis Röntgen besugárzásától, némelyek pedig szénhydratmentes étrendben (húsok) dietától (Leralek) stb. láttak kedvező eredményt ezen a téren. Mivel ezen eljárások alkalmazásával egyrészt nem mindig lehet a petefészek működését helyreállítani, másrészt pedig különösen a hormon kezelés meglehetősen költséges, kívánatos olyan kezelési mód után kutatni, amely az eddigi eljárások hátrányait kiküszöbölné.

Korábbi munkáimban ismertettem azokat a vizsgálataimat, amelyekben kimutattam, hogy bizonyos vegyületek megfelelő adagolására a különböző állatok mellékvesei megnagyobbednak és azok kéregállománya fokozottabban működik a rendesnél. Ezen kísérleteim során olyan megfigyeléseket tehettem, amelyek arra utaltak, hogy minden hormonadás nélkül, egyszerű vegyületek megfelelő adagolása által is lehetséges a petefészkek működését befolyásolni.

Első ilyenirányú megfigyeléseimet NH_4OH-

* Az Orvos-Egészségügyi Szakszervezet szegedi Tud. Csoportjának 1949. év február 2-iki ülésén tartott előadás.

val kezelt 96 házinyúlon tettem. E kísérleteinkben 2500—3500 gr súlyú, elkülönítve tartott, nemileg érett (8—14 hónapos) nőstény házinyulaknak másodnaponkint per os 50—60 ccm ½%-os NH_4OH-t adva, 1—3 hónap mulva levágtuk, amikor mellékvesekéreg-túltengés mellett, a petefészkeken folliculusérést, vérzéses folliculusokat és sárgatest-képződést észleltünk, bizonyos fokú megnagyobbodás kíséretében. Második megfigyeléseink libákon történtek, amikor 10 egyköltésből származó libának mellékveseműködés-fokozás céljából másodnaponkint 50—70 ccm ½%-os NH_4—OH-t adagoltunk gyomorsondán keresztül, fokozatosan emelkedő adagokban 4 héten át, más 10, ugyanazon költésből származó testvérlibát pedig kezelés nélkül kontrollként ugyanúgy táplálunk. A libákat 4 hét mulva levágva, az NH_4OH-val kezelt libák petefészkében számos diónyi és ennél nagyobb tojássárgáját (pete) találtunk, amelyek egy-egy libában együttesen jókora férficöllnyi tömeget képeztek. Ezzel szemben a kezelés nélkül ugyanúgy táplált libák petefészkében alig zöldborsó nagyságú peték (tojássárgája) voltak.

Ezen előzetes megfigyelések alapján újabb kísérleteket végeztünk, amelyekben nemileg érett (8—14 hónapos), nőstény, virgo intacta, elkülönítve tartott azonos fajú (csincsilla) házinyulakat alkalmaztunk és azonos táplálékon tartottunk. Házinyulak kiválóan alkalmasak olyan kísérletekre, amelyekben a petefészkek működését mesterséges úton kívánjuk befolyásolni. *Van Beneden* és *Heape*, valamint mások vizsgálataiból ugyanis tudjuk, hogy házinyulakon az ovulatio, illetve folliculus repedés csak a párzás után jön létre.

Ismeretes azonban, hogy házinyulakon »steril hágás« után is bekövetkezik a folliculusrepedés, aminek nyomán corpora luteak képződnek. Az ilyenkor keletkező corpus luteumok hatására a genitaliákon jellemző elváltozások (méhnagyobbodás, decidua képződés, emlő-megnagyobbodás) jelentkeznek. Ezt az állapotot »álterhességnek« vagy »látszatterhességnek« szokás nevezni. Az álterhességi állapot *Ancel* és *Bouin* szerint 13—14 napig, *Hammond* szerint 16—19 napig, *Knaus* szerint pedig 16 napig tart, amikor a sárgatestek degeneratiója miatt visszafejlődik. Ezért az »álterhesség« elkerülése végett a petefészek mu[illegible]sére, illetve a genitaliák állapotára vo[illegible] kísérletek előtt a házinyulakat legalább 1 hónap[illegible] elkülönítve kell tartani.

Mi az alábbi kísérletek során úgy a kezelés[illegible]szánt, mint a kontroll-nyulakat már 5 hónap[illegible] korukban, tehát az ivarérés (6 hónap) előtt 1 h[illegible] nappal elkülönítettük és a kísérletbefogás[illegible] is már 3—8 hónapig elkülönítve tartottuk. Az [illegible] különítés természetesen a kísérlet ideje alatt [illegible] tartott. Ezzel az eljárással azt kívántuk el[illegible] hogy különböző korú olyan petefészkeket vi[illegible] hassunk, amelyek ivarérettek ugyan, de [illegible] természetes okokból tüszőérés és repedés, valam[illegible] corpus luteumképződés még ne legyen, és így a kezelés hatását a legexactabb módon tudjuk le[illegible]

Az első kísérletsorozatban 2500—3500 gr kezdeti súlyú 8—14 hónapos nőstény házinyul[illegible]kat 5—7 állatból álló (de külön tartott) c[illegible]tokba osztottuk s minden csoport állatait m[illegible] más vegyülettel kezeltük. Minden állat fo[illegible]san emelkedő adagokban másodnaponkint test súlykilogrammonkint 0.1—0.2 gr hatóanyag[illegible] kapott 100—150 ccm ivóvízben oldva. A kezelé[illegible] 5 hónapon át tartott, úgy, hogy három heti kezelés után mindig egy heti kezelésmentes időszakot tartottunk közbe. Kezelésre a következő vegyülete[illegible]ket alkalmaztuk: Ammoniumchlorid, ammoniumsulfát, ammoniumcarbonat, natrium-ammoniumphosphat, ammoniumacetat, ammoniumlactat, calciumchlorid, acidum hydrochloricum (25%-os), acidum lacticum cc., acidum aceticum, natrium dihydrophosphat, ammonium hydrophosphat. Az alkalmazott kezeléssel az állatok savbasis egyensúlya időszakosan enyhébb mértékben a sav irányba tolódik el, a köznapi értelembe vett (súlyosabb) acidosis azonban nem jön létre. Az állatok mindvégig jó étvággyal táplálkoztak és ittak. Kontrollként 50 hasonló korú, fajú és kezdeti súlyú virgo intacta nőstény házinyulat ugyanolyan minőségű és mennyiségű táplálékon ugyanúgy elkülönítve tartottunk.

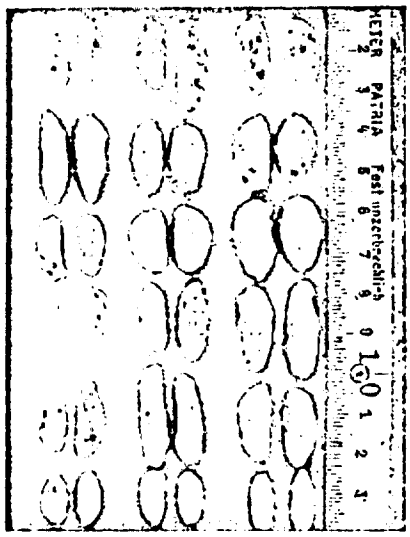
A kezelt és kontroll-nyulakat 5 hónap mulva légemboliával megöltük és azt találtuk, hogy a

Házinyulak petefészkének megnagyobbodása különböző vegyületekkel történt kezelés következtében

Sorszám	A kezelés módja	Az esetek száma	A 2 ovarium súlya cg			A 2 ovarium súlygyarapodása középértékben		S. d
			minimum	maximum	középérték	cg	%	
1.	Kontroll	50	15	52	38.60	—	—	
1.	Ammonium hydroxyd	96	42	150	63.24	24.58	63.57	[illegible]
2.	Ammonium chlorid	50	53	108	70.82	32.16	88.36	[illegible]
3.	Ammoniumsulfát	5	68	128	95.50	56.84	147.00	[illegible]
4.	Ammonium carbonát(?)	7	60	92	80.70	52.04	134.60	[illegible]
5.	Nat.-ammon. phosph.	7	67	111	95.66	57.00	150.00	[illegible]
6.	Ammonium acetat	6	54	121	87.30	48.64	125.81	[illegible]
7.	Ammonium lactat	5	68	146	104.06	66.00	173.39	[illegible]
8.	Calcium chlorid	5	60	146	102.20	63.54	164.45	[illegible]
9.	Ac. hydrochloricum	5	73	115	93.60	54.94	142.10	[illegible]
10.	Ac. lacticum	5	64	118	96.40	57.74	150.00	[illegible]
11.	Ac. aceticum	6	57	102	87.62	48.96	125.80	[illegible]
12.	Natrium dihydrophosphat	5	92	162	126.33	87.67	220.35	[illegible]
13.	Ammon. hydrophosphat	5	81	132	112.00	73.34	190.47	8.17

...lt állatok petefészkei a kontrollokéhoz viszo-...va minden esetben kisebb-nagyobb mérték-...s megnagyobbodtak, súlyosabbak voltak, felü-...kon pedig már szabadszemmel számos meg-...yobbodott érett és bevérzett folliculus, vagy ...pus luteum volt látható.

A táblázat adatai meggyőzően mutatják, ...y az alkalmazott vegyületekkel végzett kezelés ...ására a petefészkek jelentékeny, középérték-...a 63.57—229.35%-os súlynövekedése következ-...t be. A súlynövekedés significans voltát (S. D.) ...lvégzett statisztikai (valószínűségi) számítások ...zolják. A térfogatos növekedést, valamint az ...ett folliculusokat, bevérzett folliculusokat és a ...rpus luteumokat jól szemlélteti a mellékelt (1.



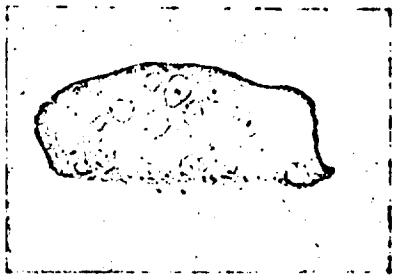
1. *kép.* Megnagyobbodás, folliculus érés és bevérzés, ...rpus luteum képződés különböző vegyületekkel 5 hó-...napig kezelt virgo intacta házinyulak petefészkein.

I. sorban	Calciumchlorid kezelés	93 cg	127 cg	146 cg
II. »	Ammonium acetat »	83 »	100 »	121 »
III. »	» lactat »	68 »	100 »	146 »
IV. »	» sulfát »	85 »	88 »	128 »
V. »	natrium-ammonium-phosphat kezelés	80 »	90 »	111 »
VI. »	kezelés nélkül (kontr.)	37 »	40 »	40 »

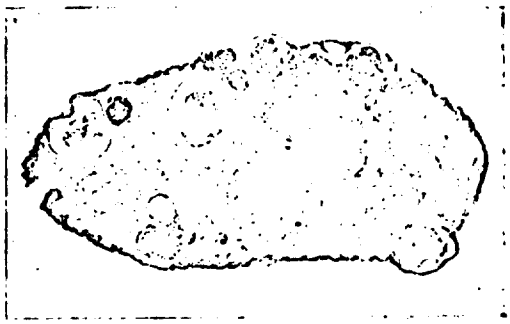
...) fénykép, amelyen a legalsó (VI.-ik) sorban az ...nos korú és súlyú kezeletlen kontroll-nyulak ...tefészkei, az I—V-ik sorban pedig soronkint ...más-más vegyülettel kezelt nyulak megnagyob-...dott petefészkei láthatók.

Szövettani vizsgálat. A nem kezelt, *kontroll* házi-...nyulak petefészkének metszetein nagyszámú, sűrűn egy-...más mellett elhelyezkedő primaer folliculus közt szá-...mos (egy egy metszeten 10—14) érőfélben levő, de még ...nem érett, aránylag nem nagy folliculus látható, úgy, ...gy az interstitialis petefészekmirigyek (velőállomány) ...aránylag csak igen kis területre szorítkoznak. Teljesen ...ett, repedésre kész folliculusok nem észlelhetők. ...2. kép.)

Ezzel szemben az 5 hónapon át *kezelt*, elkülönítve ...tott virgo intacta házinyulak megnagyobbodott pete-...fészkének nagyobb részét az interstitialis petefészek-...mirigyek (velőállomány) alkotják, amelyeknek sejtjei szembetűnően nagyobbak a rendesnél és számuk is lényegesen megszaporodott, protoplasmájukban a lipoidok megszaporodtak, kisebb nagyobb cseppeket képeznek, a sejtmag pedig gyakran oldalra tolódott. Némely esetben egy-egy foltszerű területen ezek a sejtek annyira lipoid-dúsak, hogy erősen hasonlítanak a lutein sejtekhez. Feltűnő emellett, hogy az állatok egy részének petefészkében számos érett folliculus, vérbőség és *bevérzett folliculus* (3. kép), más állatok petefészkeiben pedig számos *corpus luteum* (4. kép) látható. Érett és bevérzett tüszők, valamint corpora luteak nemcsak a felszínen, hanem a mélyebb rétegekben is vannak. Egyes petefészkekben a kéregállomány megkeskenyedett, másokban nem. A kezelt nyulak petefészkeiben folliculus degeneratiót vagy petesejt degeneratiót nem észleltünk. Ez



2. *kép.* 3000 gr súlyú virgo intacta 13 hónapos nem kezelt (kontroll) házinyúl petefészkének metszete. A két petefészek együttes súlya 25 cg.



3. *kép. Bevérzett és érett folliculusok* 13 hónapos virgo intacta házinyúl petefészkében *5 hónapig tartó calciumchlorid kezelés után.* A két petefészek együttes súlya 146 cg. Kezdeti testsúly: 3100 gr. Testsúly a kezelés végén: 4200 gr. Haematoxylin-eosin festés.

4. *kép. Corpus luteumok* 13 hónapos virgo intacta házinyúl petefészkében, *5 hónapig tartó ammonium lacticum kezelés után.* A két petefészek együttes súlya: 146 cg. Kezdeti testsúly: 3100 gr. Testsúly a kezelés végén: 4700 gr. Van Gieson festés.

amellett látszik szólani, hogy az alkalmazott kezelés nem károsítja a folliculusokat, illetve a petesejteket. A kezelt nyulak petefészkein szembetűnő, hogy a primaer folliculusok és az érőfélben levő folliculusok a kontroll petefészekhez viszonyítva ritkábban, egymástól távolabb helyezkednek el. Ez azzal magyarázható, hogy egyrészt a petefészek tömegének és felületének megnagyobbodása miatt a folliculusok egymástól mintegy szétterülnek és nagyobb területen helyezkednek el, másrészt pedig a kezelés során bekövetkező fokozott folliculus érés miatt a folliculusok absolut száma is természetszerűleg csökken.

Az elmondottakból megállapítható, hogy az észlelt petefészekmegnagyobbodás egyrészt az interstitialis petefészekmirigyek túltengésére és azok lipoid tartalmának szaporodására, másrészt pedig a folliculusok megnagyobbodására, érésére, bevérzésére, illetve a corpora luteák képződésére vezethető vissza.

Másik kísérletsorozatban az előbbiekkel azonos kísérleti körülmények mellett ugyanesak 5—7 (8—10 hónapos) nyulat ugyanazon vegyületekkel (80—100 adag) kezeltünk és 14—16 hónapig figyeltünk meg, majd légembóliával történt leölésük után megvizsgáltuk petefészkeiket. Ezen állatok petefészkein lényegileg hasonló jelenségeket láttunk, mint az 5 hónapig tartó kísérleti idő után, miért is ezek részletes ismertetését mellőzöm. Csupán annyit jegyzek meg, hogy degeneratiós jelenségeket ezen állatok petefészkein sem észleltünk.

Az irodalmi adatok szerint a petefészken folliculus érlelő és sárgatestképző hatást elsőszerban a hypophysis mellső lebenye által termelt gonadotrop hormonok hatására észleltek.

Nagyszámú vizsgálatok kétségtelenül bebizonyították, hogy a petefészkek működését a hypophysis mellső lebenye hormonalis úton irányítja.

A szerzők egy része különböző kísérletek alapján azt tartja, hogy a hypophysis basophil sejtjei termelik a folliculus érlelő hormont, az acidophil sejtek pedig a corpus luteum képző hormont. *Herlant* a *Selye*-féle alarm-reactióban megszaporodott basophil sejtek átültetése alkalmával folliculus érlelő hatást észlelt. *Giroud* és *Martinet* ökörés sertés-hypophysis basophil sejteket tartalmazó zónájának nőstény ivarérett patkányokba történt implantálására a petefészek jelentékeny megnagyobbodását, folliculus-érést és corpus luteum-képződést figyelt meg. Az *acidophil* sejtcsoport átültetésére csak kisfokú petefészek megnagyobbodást és csekély folliculus-megnagyobbodást látták kifejlődni, corpus luteumok azonban nem keletkeztek. Kísérletek alapján a hypophysis mellső lebeny basophil sejtjeinek tulajdonítanak folliculus-érlelő hatást, a corpus luteum-képződésben pedig ugyanesak szerepet tulajdonítanak a basophil sejteknek is.

Hohlweg fiatal patkányok, *Kloften*, *Pataky* és mások ivarérett, elkülönített házinyulak petefészkén nagyobb adag *folliculus hormon* befecskendezésre corpus luteum-képződést figyeltek meg. Ezt úgy magyarázzák, hogy a tüszőhormon nagy mennyiségben gátolja a hypophysis mellső lebenyének tüszőérlelő hormon (prolan A) termelését, ezzel szemben elősegíti a sárgatestképző hormon (prolan B) képződést. *Baumann* terhes nő felforralt, tehát prolant nem tartalmazó vizeletének befecskendezésére állatok petefészkében sárgatestképződést észlelt, amit a terhes nő vizeletének nagymennyiségű folliculinnak a hypophysis keresztül kifejtett hatására vezet vissza. [illegible] *Hohlweg* és mások. *Zondek*, *Claubergh* és mások vizsgálatai szerint a sárgatestképződést mindig [illegible] nyos fokú tüszőérés előzi meg, amit a prolan váltt ki. *Axel—Westmann* vizsgálatai is [illegible] tak arra, hogy tüszőérés nélkül nincs [illegible] képződés.

Julesz ketogen diétán tartott nők [illegible] vizeletének infantilis fehér egerekbe [illegible] az állatokban folliculus-érést és vizenyős duzzadást észlelt, folliculus bevérzés és [illegible] luteum képződés azonban nem jött létre. [illegible] e jelenséget ugy magyarázza, hogy a ketogen [illegible] hypophysis mellső lebenyét fokozott prolan termelésre ingerli.

Megemlítjük még, hogy *Luigi* infantilis nyulakon a hypophysis, *Friedgood* és *Pozner* tengeri malacokon a nyaki sympaticus [illegible] ingerlése által idézett elő jelentős tüszőérés.

Saját ismertetett vizsgálataink [illegible] azt mutatják, hogy hormonadás nélkül, [illegible] vegyületek megfelelő adagolásával is [illegible] házinyulak petefészkén folliculus-érést, [illegible] bevérzést és corpus luteum-képződést [illegible]

Felvetődik a kérdés, hogy a kísérletben alkalmazott vegyületek milyen mechanismusán váltják ki a petefészken észlelt jelenségeket. Mivel a talált elváltozások mindenben megegyeznek, a *Zondek-Aschheim*, illetve a *Friedman* terhességi reactióban keletkezőkkel, illetve [illegible] kal a jelenségekkel, amelyek a hypophysis gonadotrop hormonjainak adagolására szokatlan [illegible] minden valószínűség amellett szól, hogy [illegible] lunk alkalmazott vegyületek is a hypophysis [illegible] lebenyén keresztül idézik elő a petefészken [illegible] elváltozásokat. Mivel azonban az alkalmazott vegyületek közt szerves és szervetlen, továbbá [illegible] moniák és nem ammoniak vegyületek szerepelnek, nyilvánvaló, hogy nem specialis vegyületek [illegible] lük tehető felelőssé a hypophysisre, illetve [illegible] át a petefészekre gyakorolt hatásért. E vegyületek közös tulajdonsága azonban az, hogy [illegible] adagokban a szervezet savbasis egyensúlyát [illegible] irányba tolják el. Ezt saját korábbi és mai vizsgálatai bizonyítják. Ezért nézetünk szerint a nagyobb valószínűséggel a savbasis-egyensúly az adagolt vegyületek hatására bekövetkezett sékelt fokú időszakos savanyú irányú [illegible] izgatólag hat a hypophysis mellső lebenyének megfelelő sejtjeire, amelyek azután fokozott gonadotrop-hormontermelésel válaszolnak. Az utóbbiak idézik elő a folliculus-érést, [illegible] képződést és petefészekmegnagyobbodást [illegible] hangban áll ez *Selye* és *Herlant* adatai szerint tett észleletével, amikor intravenás [illegible] kendezés után a hypophysis mellső lebenyének sejtjeinek megszaporodása mellett [illegible] túltengést és a petefészken folliculus-érést [illegible] tek meg.

Említettük már, hogy házinyulakban [illegible] culus-repedés csak a párzás után 10—18 [illegible] következik be. Ennek oka az, hogy nyulak [illegible]

-ocskákban) a hypophysis mellső lebeny tüszőérő hormont csak a párzás okozta idegizgalom hatására termel. *Harris* (1941) házinyulak harmadik agykamrájába cuprum-acetat oldatot fecskendezett be, ami a hypothalamicus központok ingerlése révén ovulatiót váltott ki. Ennek alapján azt is lehet gondolni, hogy az általunk adagolt vegyületek a savbasis-egyensúly savi irányba tolása által izgatják a hypophysis hypothalamicus központját és ez váltja ki a hypophysis mellső lebenyének gonadotrop hormontermelését, ez utóbbi pedig a petefészken észlelt jelenségeket.

Nyúlkísérleteink alapján feltehető, hogy az alkalmazott vagy más acidoticus hatású vegyületek megfelelő gyógyszeres adagolása által egyéb állatok és esetleg nők csökkent működésű petefészke is rendes vagy fokozottabb működésre serkenthető. Ennek eldöntésére azonban még további vizsgálatok szükségesek.

Libákon tett megfigyeléseink arra mutatnak, hogy az alkalmazott kezeléssel szárnyasok petérési folyamatát gyorsítani lehet, ami esetleg a tojástermelés fokozását tehetné lehetővé. Ennek igazolása figyelemreméltó gazdasági jelentőséggel bírna.

A teljesség kedvéért megemlítem, hogy kezelt nyulainkon a petefészek elváltozásai mellett a mellékvesék túltengését, az emlők fokozatos megnagyobbodását (proliferatio), majd előbb savó, később pedig hónapokon át tartó tejelválasztást (secretio), méhmegnagyobbodást, a hypophysis megnagyobbodását stb. állapíthattuk meg. E jelenségekről részletesebben más munkánkban számolunk be.

Összefoglalás: 1. Virgo intacta, elkülönítve tartott nemileg érett házinyulaknak másodnaponkint 3—5—16 hónapon át 13 olyan szerves és szervetlen ammoniak és más vegyületeket adagoltunk, amelyek közös tulajdonsága az, hogy a szervezet savbasis-egyensúlyát a savi irányba tolják.

2. E kezelés hatására a házinyulak petefészkén 63—229%-os súlynagyobbodás, térfogatnövekedés, folliculus-érés, folliculus-bevérzés és corpus luteum-képződés következett be.

3. A petefészek térfogatának növekedése részben az interstitialis petefészekmirigyek (velőállomány) hyperplasiájára, részben pedig a folliculusok megnagyobbodására és a corpus luteumok képződésére vezethető vissza.

4. Az interstitialis petefészekmirigyek sejtjeinek megnagyobbodása azok lipoid tartalmának jelentékeny megszaporodására vezethető vissza. Emellett e sejtek száma is megnövekedett.

5. Libákon tett megfigyeléseink szerint az alkalmazott kezeléssel az állatok petefészkén feltűnő petefészgyorsulás és fokozódás volt észlelhető, ami arra utal, hogy ily módon esetleg szárnyasok tojástermelését fokozni lehetne, ami gazdasági szempontból bírna jelentőséggel. Erre időzve azonban még további vizsgálatok szükségesek.

6. Vizsgálataink eredménye és az irodalmi adatok alapján arra lehet következtetni, hogy az észlelt petefészekelváltozások minden valószínűség szerint a hypophysis mellső lebenyének a kezelés hatására bekövetkező fokozott gonadotrop-hormon termelésének következményei.

7. Nézetünk szerint az alkalmazott vegyületek nem specifikus vegyi tulajdonságaik révén hozzák létre a hypophysis mellső lebenyének fokozott gonadotrop-hormon termelését, hanem azáltal, hogy a szervezet savbasis-egyensúlyát időszakonkint és mérsékelt fokban a savanyú irányba tolják el, ami azután vagy közvetlenül hat a hypophysisre, vagy pedig a hypophysis hypothalamusban levő idegközpontjának izgatása révén váltja ki a fokozott gonadotrop-hormon termelést.

8. Kísérleteink eredményei alapján feltehető, hogy az alkalmazott, vagy más acidotikus hatású vegyületek megfelelő adagolása által más állatok és esetleg nők csökkent működésű petefészkei is fokozottabb működésre serkenthetők és ezáltal azok tevékenysége rendessé tehető. Ennek eldöntésére még további vizsgálatok szükségesek.

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Fazekas-'54
Bib No. 517

Virchow's Archive, Vol. 324, p. 531 to 542 (1954)

From the Institute of Forensic Medicine of the Szeged University (Hungary).
(Director: Prof. Dr. I.Gy. FAZEKAS).

Enlargement of the parathyroid caused by simple acidoctic compounds.

By
I.Gy. FAZEKAS

With 3 text illustrations.

(Received on July 1953).

Reports on the enlargement of parathyroid in man were published first by ERDHEIM (1903) and subsequently by CLAUDE and BAUDOUIN, REINHARD and CREUZFELD, BALLET and LAVASTINE, JOSEPHSON, CUSHING and DAVIDOFF, HOFF and others in conjunction with basophil adenomas of the hypophysis front lobe (HVL), as well as by LLOYD in connection with the primary cell adenomas of the hypophysis. In 1904 RECKLINGHAUSEN prepared a pathography of osteosis fibrosa generalisata which develops under the influence of the increased adrenal gland function due to adenomatous or hyperplastic causes. The correlation between the modification or hyperfunction respectively, of the bones and adrenal glands was verified first by MANDL (1926) and later by others on the basis of the fact that patients recovered after the surgical removal of their adenomatous or hyperplastic parathyroid. The so-called secondary hyperplasia of the parathyroid can occur as a compensating reaction in case of an increased para-hormone need of the organism, for example in case of bone metastases of a carcinoma, in case of myeloma multiplex, rachitis, CUSHING's disease, acute kidney diseases (CAMERON, VERZAR), in case of gravidity (ARVAY), partial or total kidney extirpation, as well as in case of a phosphorus content increase of the diet (COURNOT).

An enlargement of the parathyroid was observed by ANSELMINO, HOFFMANN and HEROLD after the application during several days of HVL-extract in rats, later in other animals; HERTZ and KRANES observed the same in rabbits, HAM and HAIS in dogs. They assumed, on the basis of these findings, that HVL forms a hormone which stimulates the parathyroid This coincides fully with the findings of SMITH in rats, KOSTER, HOUSSAY and BIASOTTI and those of HOUSSAY and SAMMARTINO as well as VERNETTI who found in dogs after the extirpation of the hypophysis an atrophy and a degenerative atrophy of the parathyroid. LIVON and PEIRON, as well as ASCHNER (in dogs) and subsequnetly COLLIP (in rats), on the other hand, found no parathyroid atrophy after the removal of the hypophysis. Using rabbits, CATTANEO did not obtain any enlargement of the parathyroid with the hypophysis extract. In 1949 TORNBLOM found a high-grade enlargement of the parathyroid in rabbits after the administration of large quantities of sodium and ammonium phosphate, simultaneously with a calcium-deficient diet during 12 weeks.

Investigations carried out to date clearly indicate that the increased or reduced function of the parathyroid causes various pathological conditions through the radical changes brought about in the calcium- and phosphorus metabolism of the organism. It would therefore be desirable to be able to influence the parathyroid function with a method which would eliminate diseases resulting from decreased function by re-establishing said function.

It was found during earlier investigations (FAZEKAS) that the continued administration of compounds with acidotic effect can cause the following in rabbits, geese,. . . (illegible word), swine and in goats: hypertrophy and increased function of the adrenal gland core, enlargement of the ovaries and uterus, follicle. maturity (illeg.), and follicle hemorrhage, formation of yellow bodies ~~corpuscles~~, cyclic changes of the mucous membrane of the uterus tubes, obesity, enlargement of the breasts and milk secretion , as well as enlargement of the hypophysis and multiplication of the basophil cells of the HVL. The following investigations are intended to show a possibility for chemical action on the parathyroid.

The compounds listed on the Table were used for the treatment of the animals. The parathyroid of 362 treated rabbits (including animals from previous tests as well) were examined. Size, weight and histological . . (illeg.) were compared with the corresponding data of the control animals. 50 similar rabbits of the same age and weight were used for control. -- The survey table shows that the parathyroid of the untreated control animals 6 to 14 had an average weight of 9,2 mg; the parathyroid of the treated rabbits, on the other hand, showed a considerable weight increase.

1. Ammonium-hyroxide was used for treating 96 female and 64 male animals during 4 - 5 - 16 months. Each animal received twice daily 50 to 80 cm^3 1/2% of NH_4OH through the stomach probe in gradually increasing doses. 3 weeks of therapy were always followed by a period of one week without therapy. The body weight of the animals increased gradually during treatment; at the time when they were killed, it amounted to 3100 to 4400 g. - The adrenal glands weighed 17 to 39 mg, 26 mg on the average, which is 16.8 mg, i.e. 182.6% more as compared to the weight of the adrenal glands of the controls. According to the statistical calculations the significant difference amounted to 10.84; therefore the weight increase of the parathyroid is specific.

2. Ammonium-chloride was used for treating 50 female and 30 male rabbits during 3 - 5 - and 16 months. Every second day the animals received 0,1 to 0,2 g/kg of NH_4Cl dissolved in 100 cm^3 of drinking water, in gradually increasing doses. A 3-week treatment was always followed by an interruption of 1 week . During the treatment period the weight of the animals increased gradually; at the time when they were killed, the weight with air embolism amounted to 3500 to 4500 g. The weight of the parathyroid was 16,5 - 57,5 mg, i.e. 25,3 mg more than for the controls; this corresponds to a weight increase of 274,6%. The significance was found for 9,03.

Table 1. Weight data of the parathyroid for the treated rabbits and for the controls.

Tabelle 1. *Gewichtsangaben der Nebenschilddrüsen bei behandelten Kaninchen und Kontrollen.*

		1 Zahl der Tiere	Gewicht der Nebenschilddrüsen in mg 3 minimal	4 maximal	5 Mittelwert	Gewichtszunahme der Nebenschilddrüsen im Mittelwert %	mg	S. D.
	Kontrollen	50	6	14	9,2	–	–	–
1	Ammoniumhydroxyd	160	17,0	39,0	26,0	16,8	182,6	10,84
2	Ammoniumchlorid	80	16,5	57,5	34,5	25,3	274,6	9,03
3	Ammoniumsulfat	10	15,0	33,0	27,5	18,3	198,9	7,04
4	Ammoniumcarbonat	14	15,0	40,0	25,0	15,8	171,9	6,86
5	Natriumammoniumphosphat	14	19,0	51,0	32,5	23,3	254,3	6,50
6	Ammoniumacetat	12	18,0	49,0	34,5	25,3	274,6	9,73
7	Ammoniumlactat	10	17,5	52,0	29,5	20,3	219,5	6,15
8	Calciumchlorid	10	21,0	54,0	32,5	23,3	251,0	7,76
9	Acidum hydrochloricum	10	16,0	48,0	28,5	19,3	210,8	6,66
10	Acidum lacticum	10	22,5	43,0	29,5	20,3	219,5	5,27
11	Acidum aceticum	12	20,0	36,0	28,6	19,3	209,7	12,12
12	Natriumdihydrophosphat	10	24,0	50,0	36,5	27,3	294,5	10,72
13	Ammoniumhydrophosphat	10	16,5	43,0	30,5	21,3	234,8	7,10

1) Number of animals
2) Weight of the parathyroid in mg
3) minimal 4) maximal 5) average
6) weight increase of the adrenal glands, average value

3. Ammonium-sulfate was administered to 10 female rabbits during 5 - 16 months with the same method and in identical doses. When killed, their body weight was 4100 to 4800 g. The weight of the parathyroid was 15 to 33 mg, and 27,5 on the average, i.e. 18,3% more than the weight for the controls, which means a plus of 98,9%. Differentia significans: 7,04.

4. Ammonium-carbonate was used for treating 14 female rabbits during 5 to . . . (illeg.) months. Same treatment method. Body weight at the time when killed: 4000 to 4700 g. Weight of the parathyroid: 15 to 40 mg, and 25 mg on the average, i.e. 15,8 mg more than for the controls. Accordingly, the weight increase was 171,9%, with a significance of 6,86.

5. Sodium-ammonium-phosphate was likewise applied to 14 female rabbits during 5 to 14 months, with the same method. Body weight at the time when killed: 3800 to 4700 g. Weight of the parathyroid: 19 to 51 mg and 32,5 mg on the average, i.e. 23,3 mg more than for the untreated control animals. This is a weight increase with a significant difference of 6,5.

6. Ammonium-acetate was used for treating 12 female rabbits during 5 to 16 months. Identical treatment method. Body weight at the time when killed: 3500 to 4800 g. Weight of the parathyroid: 18 to 49 mg, average value: 34,5 mg, i.e. 25,3 more than for normal animals, which means a weight plus of 274,6%; significance: 9,73.

7. Ammonium lactate was used for the treatment of 10 female rabbits during 5 - 16 months. Same treatment method. Body weight at the time when killed: 3350 to 1700 g. Weight of the parathyroid : 17,5 to 52 mg and 29,5 on the average, i.e. 20,3 mg more than for the controls, which means a weight increase of 219,5%. Significance: 6,15.

8. Calcium-chloride was used for treating 10 female rabbits during 5 - 16 months with the same method. Body weight when killed: 3700 to 5050 g, weight of the parathyroid 21 to 54 mg, 32,5 mg on the average, i.e. 23,3 mg more (251%) than for the controls Differentia significans: 7,76.

9. Acidum hydrochloricum was used for the treatment of 10 female rabbits during 5 to 16 months. The animals received twice daily 0,5 to . . . (illeg.) 25% HCL in 100 to 150 cm^3 of drinking water, in gradually increasing doses. A 3-week treatment period was also followed here by a pause of one week without treatment. Body weight when killed: 3500 to 4500 g. Weight of the parathyroid 16 to 48 mg, and 28,5 mg on the average, i.e. 19,3 mg more than for normal animals. This means a weight plus of 210%, with a significance of . . . (illegible).

10. Acidum lacticum was applied to 10 female rabbits during 5 to 16 months. The animals received increasing doses of 0,2 -0,5 cm^3 active substance twice daily in 100 to 150 cm^3 of drinking water. A 3-week treatment period was followed by a pause of one week. When killed, the body weight was 3700 to 4800 g.--Weight of the parathyroid 22,5 to 43 mg, and 29,5 mg on the average, i.e. 20,3 mg (219,5%) more than for the controls. Significant difference: 5.27.

11. Acidum aceticum was used for the treatment of 12 female rabbits during 5 to 16 months. The animals received every second day increasing doses of . . . (illeg.) 0,5 cm^3/kg concentrated acetic acid in 100 to 150 cm^3 of drinking water. Body weight at the time when killed: 3600 to 4600 g. Weight of the parathyroid 20 to 36 mg, average value 28,6 mg, i.e.19,3 mg more than for the normal rabbits, which corresponds to an increase of 209,7%. Differentia significans: 12,12.

12. Sodium hydrophosphate was used for the treatment of 10 female rabbits during 5 to 16 months. These animals received, however, 0,3 to 0,7 g/kg of the active substance twice daily in increasing doses, in 100 to 150 cm^3 of drinking water. Body weight when killed: 4000 to 4500 g. Weight of the parathyroid 24 to 50 mg, average value: 36,5 mg, i.e. 27,3 mg more than for the controls. This means a 294,5% weight increase, with a significance of 10,72.

13. Ammonium-hydrophosphate was administered to 10 female rabbits for 5 to 16 months. Each animal received 0,3 to 0,7 g/kg of the compound twice daily in increasing doses, in 100 to 150 cm^3 of drinking water. Body weight at the time when killed: 3900 to 4800 g. Weight of the parathyroid 16,5 to 43 mg, average value: 30,5 mg, i.e. 21,3 mg (234,8%) more than for the controls. Significant difference: 7,1.

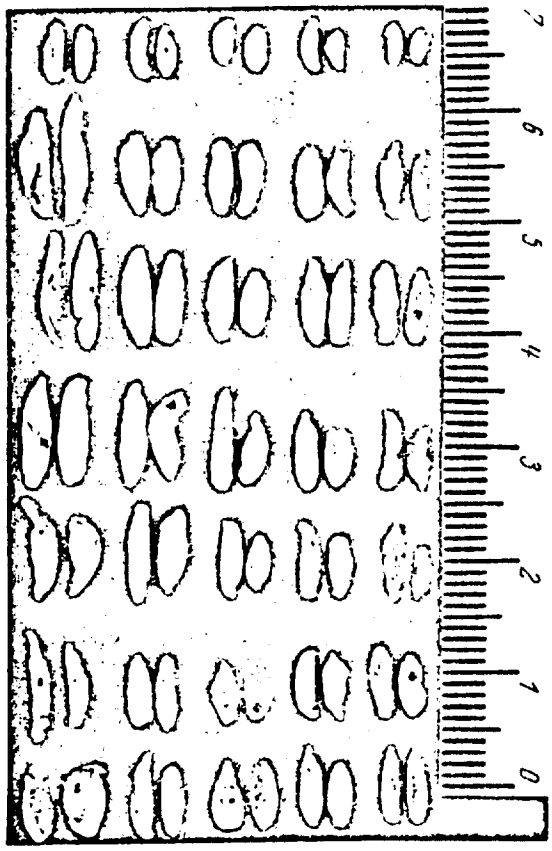

Illustration 1. Enlargement of the parathyroid in rabbits treated with various compounds during 5 months.
Line I: ammonium-chloride treatment: 16,5 - 18 - 21,5 - 57,5 mg.--Line II: ammonium-sulfate treatment: 15 - 17 - 20 - 27,5 mg.--Line III: ammonium carbonate treatment: 15 -18 - 20 - 27,5 to 40 mg.-- Line IV: Na-ammoniumphosphate treatment: 19 - 20,5 -37,5 - 51 mg. Line V: ammonium acetate treatment: 18 - 22.5 - 24 - 39 mg. Line VI: ammonium lactate treatment: 17,5 - 20 - 21 - 30 - 52 mg. Line VII: controls without treatment: 6 - 7 - 8 to 9,5 - 10 mg.

It can be stated in view of the cited data that under the effect of the applied compounds the weight of the parathyroid increases considerably, i.e (its increase) is maximal with. . .(illeg.)sodiumhydrophosphate (294,5%), ammonium-chloride and ammonium-acetate (274,6%) respectively; it is minimal in animals treated with ammonium-carbonate (171,9%0 and ammonium-hydroxide (182,6%). On the basis of the statistical computation results the weight increase of the adrenal glands can unanimously be qualified as specific. --The weight increase coincided with a conspicuous enlargement of the adrenal gland volume and dimensions respectively.

<u>Illustration 2.</u> Histological aspect of the parathyroid in untreated control rabbits. Dark primary cells of the gland substance predominate. The . . .(illeg.) primary cell number is comparatively small; their size is normal. Hematoxyline-eosin pigment. 1000-fold enlargement.

It was found in the course of the histological examination that the parathyroid vessels of the untreated controls were generally moderately plethoric and moderately wide. The main mass of the glands consisted of the so-called dark primary cells; oxyphilic cells could be found at gland edge sectors (III.2). -- In the treated rabbits, on the other hand, the larger vessels, but also the capillaries were wide and filled with blood already after a 3-month treatment period; the main mass of the glands consisted of enlarged and also inumerically increased so-called light primary cells. The plasma of these cell types was enlarged, light and it contained vacuoles. Their nucleus was likewise large and light when colored . Nuclear division could be observed in several of these cells. Only a comparatively small number of dark primary cells was detected in the parathyroid of the treated animals.

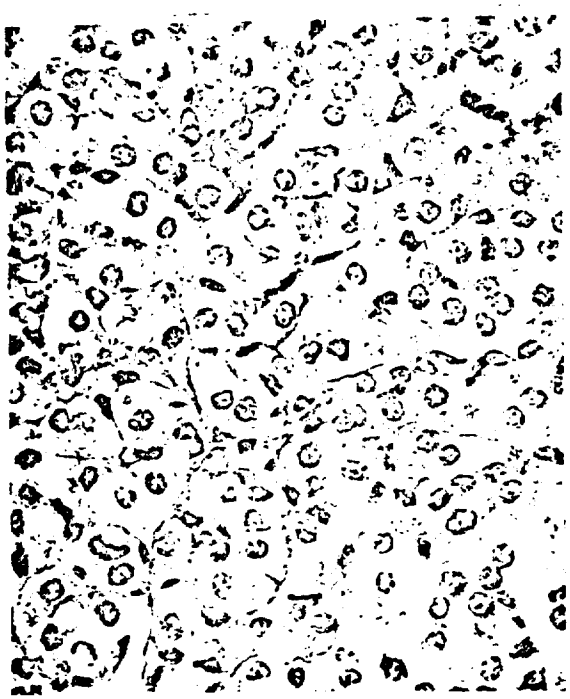

Illustration 3. Histological aspect of the enlarged parathyroid of a rabbit treated during 3 months with NH_4Cl. The gland substance is principally formed by light primary cells with enlarged plasma. Hemotoxyline-eosin coloring. 1000-fold enlargement.

A similar histological aspect was found also by ANSELMINO, HOFFMANN and HEROLD in rats, by HERTZ and KRANES in rabbits, by HOUSSAY in dogs and rabbits after the application of a watery HVL-extract. The authors are of opinion that this symptom is a consequence of the stimulating effect of HVL on the function of the adrenal glands. According to HOUSSAY's findings atrophy of the cell protoplasm, decrease of the cells number and indistinct cell limits were observable already 5 days after hypophysectomy. The regular epithelial structure of the glands disappeared simultaneously, and a trabecular, bundle-like cell system developed. Said atrophy of the parathyroid was especially conspicuous in dogs which had undergone simultaneous surgical removal of the hypophysis and of the pancreas. VERNETTI observed a considerable increase of the connective tissues in atrophied parathyroid of dogs after hypophysectomy.

In the course of my own tests , plethora, hypertrophy and hyperplasia, a considerable multiplication of the light primary cells and symptoms of nuclear division were evident in the adrenal glands of treated rabbits. On the strength of all this, the conclusion could be reached that an intensified function of the parathyroid occurs, caused by the treatment which was used. However, we will be able to state a definite oponion on the above only after subjecting the enlarged adrenal glands to function tests.

The question now arises: what constitutes the function mechanism of the observed enlargement of the parathyroid caused by treatment? Since the 13 applied compounds include organic and inorganic, ammoniacal and non-ammoniacal compounds as well as acids and lye solutions, it is unconceivable that the described adrenal gland enlargement could result from the specific effect of said compounds. Efforts must therefore be made to detect a common cause related to the action mechanism, which always occurs in the organism under the effect of said compounds.

It is generally known that the acids and acid . . . (illegible word) shift the acid-base equilibrium of the organism towards acidity (acidosis); it is less well-known, however, that the lye solutions can cause acidosis as well. FAZEKAS demonstrated that NH_4OH and NaOH--despite their lye characteristics--cause acidosis in the organism. His statements were also confirmed by VENULET, GOEBEL and FISLOWITZ, ALVALI and GEIGER , as well as by HAZARD and VAILLE.The shared characteristic of the used compounds therefore consists in their ability to shift the acid-base equilibrium of the organism in the acidotic direction. We believe that the shift of the blood chemistry towards acidity has an irritating effect, either directly on the HVL, or by way of the hypothalamus center of the hypophysis, affecting the corresponding cells of the HVL, thereby stimulating them into intensified functioning.

Then the intensified hypophysis function causes the enlargement and intensified function respectively, of the parathyroid Our findings to the effect that the hypophysis of such treated animals is likewise enlarged and that the basophil cell number of the HVL increases, speaks in favor of the above. Our previous statements according to which the effect of these compounds in rabbits causes enlargement and intensified functioning of the suprarenal gland core, follicle maturing and (follicle) hemorrhage in the ovaries as well as the formation of yellow corpuscles,also speak in favor of an increased HVL-function.

The following data in literature refer to an intensified function of the hypophysis under acidoctic effect: JULESZ found follicle maturation in infantile mice ovaries after injecting male and female urine and with ketogenic diet. SELYE and HERLANT observed similar symptoms, besides hylerplasia of the suprarenal gland core after injecting hydrochloric acid intravenously. HARRIS injected a cuprunacetate solution into the 3. cerebral chamber of rabbits, which triggered ovulation by irritating the hypothalamus center.A chronic acidosis is determinable mostly for those diseases of man during which, according to data in literature, an enlargement of the adrenal glands was found; for example: in cases of chronic kidney ailments,pregnancy, partial or total kidney extirpation,etc.

After the administration of sodium- and ammonium-phosphate to rabbits TORNBLUM observed enlargement of the adrenal glands, simultaneous decrease of the blood calcium and a considerable increase of phosphorus in the blood. He repeated the treatment, using animals whose hypophysis had been surgically removed; he subsequently found a decrease of the blood calcium- and phosphorus content, but no parathyroid enlargement took place. TORNBLUM concluded on the basis of the above that the enlargement of the adrenal gland is caused by hyperphosphatemia. He used other test animals to investigate which hormone effect is correlated to the modification of the phosphoremia. He removed the parathyroid and the thyroid glands of one animal group, and removed the thyroid glands, parathyroid and hypophyses of another group. The hypocalemia and hyperphospatemia of these animals was less extensive. However, when the animals without thyroid- and adrenal glands received hypophysis extract, the hylocalcemia did not change, but the hyperphospatemia was considerably higher. In his opinion, this indicates that the hypophysis intensifies the phosphoremia, without requiring the intervention of the adrenal glands. TORNBLUM demonstrates the effect of the hypophysis schematically as follows: hypophysis - hyperphosphatemia - hyperfunction of the adrenal gland. In his view, the adrenal gland is not directly irritated by the hypophysis; instead, this occurs through the humoral factor, by increasing the blood phosphorus level.

The result of our own tests seems to contradict TORNBLOM's opinion, since we applied--besides 3 types of phosphates--10 other phosphorus-free compounds and observed an enlargement of the parathyroid caused by them. It was stated in the course of previous investigations (FAZEKAS) that the inorganic blood phosphorus content of rabbits is considerably increased by a single administration of a larger dose of NH_4OH and NaOH, while the serum-calcium and serum-chlorine decreases, hyperglycemia occurs, the alkali reserve of the blood serum declines (!) and the concentration of the hydrogenion increases, i.e. acidosis is generated. Therefore hyperphosphatemia can likewise occur without phosphorus supply. According to our experiences, an increase of the inorganic blood phosphorus content in case of acidotic conditions due to various causes (intoxications, diseases) is almost always detectable. This fact seems to confirm TORNBLOM's interpretation. According to BALO's extensive and thorough investigations the inorganic phosphorus content of the blood serum increases considerably in case of a subsequent blood acidification in vitro as well, without being influenced by the hypophysis here. Accordingly, the phosphorus content of the blood serum can increase without hypophysis action (the organically bound P of the red corpuscles is transformed into an inorganic bond and enters the blood serum). All this indicates that the increase of the blood phosphorus content can be regarded as a partial symptom of the acidosis, and that its presence alone offers no incontestable evidence (to confirm the assumption) that the enlargement of the parathyroid and their intensified function respectively, are caused by hyperphosphatemia.

In our opinion--on the basis of our own and other test results--the enlargement and the increased function respectively, of the parathyroid occurs as follows: the shift of the blood chemistry towards acidity due to various causes has an irritating effect on HVL. either directly, or by way of the hypothalamus center of the hypophysis;,it then stimulates this organ, intensifying its function, which subsequently causes the enlargement of the adrenal glands through the formation of parathyrcotrophormone.

Present investigations offer new data to show that the inner-secretory gland system can be chemically influenced and its functtion changed respectively, without hormone application, through the administration of simple compounds. -- Decisive statements regarding the adrenal gland, however, can be made on the basis of new results only. Pertinent investigations are in course and it is hoped that the hypofunction of the parathyroid as well as pathologic conditions caused by the same can likewise be remedied.

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SUMMARY

1. 362 rabbits were given twice daily for 3-5-16 months organic and inorganic ammonium-and other compounds (0.1 - 0.2 gl kg). These compounds had the same quality in that they shifted the acid-base equilibrium of the organisms in the acid direction. The weight, size and histological picture of the parathyroids of the treated rabbits were compared with the corresponding information about 50 untreated control animals of the same age, species and weight.
2. The minimum weight of the parathyroid in the controls amounted to 6 mg, the maximum 14 mg, and the acerage value 9.2 mg.
3. In contrast, the minimum weight of the parathyroids of the acid-treated rabbits amounted to 15-25 mg, the maximum 33-57.5 mg, and the average value 25-36.5 mg. The average weight showed, in proportion to the control animals, an increase of 15.8-27 mg, that is 171.9-294.5%, which clearly signified statistically a significant difference of 5.27-12.12.
4. Histologically, the substance of the parathyroids of the control rabbits consisted mainly of dark primary cells; light primary cells existed in a comparatively small number; oxyphilic cells were scattered in the edge sectors. In the enlarged parathyroids of the treated animals a wealth of blood was ascertained, the dark primary cells decreased in number considerably; the oxphilic cells were missing or likewise decreased in number; the light primary cells were considerably increased and enlarged, in several places, with vacuole formation, such that the primary mass of the gland consisted of the light primary cells.

5. By reason of the enlargement and of the histological pictures of the parathyroids, it was concluded that their function increased. However, we will be able to state a definite opinion on the above only after subjecting the enlarged parathyroids to function tests.

6. Our earlier assertions - that enlargement of the hypophysis, increase of the basophilic cells of the HVL, enlargement of the adrenal cortex and intensification of its functions, enlargement of the ovaries, follicle maturity and bleeding, corpus lutem formation, enlargement of the uterus and milk secretion can be produced in animals treated such - indicated that these changes as well as the enlargement of the parathyroids are connected with the increased function of the HVL.

7. The increased function of the HVL, in our opinion, did not result from the specific effect of the applied compounds, but rather resulted from the moderates, periodic, acidic influence caused by them.

8. Our investigations therefore offer a new proof that the function of the inner-secretory gland-system can be transposed or increased through administration of simple compounds with "acid effects". By reason of our results it is hoped that the function of the hypo-functioning parathyroids (and other inner-secretory glands) can be restored or made normal and at the same time the pathological conditions caused by this hypofunction can be remedied.

Fazekas-'54
Bib No. 517

Virchows Archiv, Bd. 324, S. 531—542 (1954).

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Aus dem Institut für gerichtliche Medizin der Universität Szeged (Ungarn)
(Direktor: Prof. Dr. I. Gy. Fazekas).

Vergrößerung der Nebenschilddrüsen durch einfache acidotische Verbindungen.

Von

I. Gy. Fazekas.

Mit 3 Textabbildungen.

(Eingegangen am 7. Juli 1953.)

Über Vergrößerung menschlicher Nebenschilddrüsen wurde zuerst von Erdheim (1903), später von Claude und Baudouin, Reinhard und Creuzfeld, Ballet und Lavastine, Josephson, Erdheim, Cushing und Davidoff, Hoff u. a. in Verbindung mit basophilen Adenomen des Hypophysenvorderlappens (HVL) sowie von Lloyd in Verbindung mit Hauptzellenadenomen der Hypophyse berichtet. Recklinghausen beschrieb 1904 das Krankheitsbild der Osteitis fibrosa generalisata, die sich unter dem Einfluß der adenomisch oder hyperplastisch bedingten gesteigerten Nebenschilddrüsenfunktion ausbildet. Der Zusammenhang zwischen dieser Knochen- und Nebenschilddrüsenveränderung bzw. -hyperfunktion wurde zuerst von Mandl (1926) und später von anderen dadurch bewiesen, daß die Kranken nach operativer Entfernung ihrer adenomischen oder hyperplastischen Nebenschilddrüsen gesund wurden. Die sog. *sekundäre Hyperplasie* der Nebenschilddrüsen kann als Kompensationsreaktion bei gesteigertem Parathormonbedürfnis des Organismus vorkommen, so z. B. bei Knochenmetastasen eines Carcinoms, bei einem Myeloma multiplex, einer Rachitis, Osteomalacie, Cushingschen Krankheit, akuten Nierenerkrankung (Cameron, Verzár), bei Gravidität (Árvay), bei partieller oder totaler Nierenexstirpation sowie bei einem Calcium- und Phosphorgehaltanwuchs der Speiseordnung (Cournot).

Anselmino, Hoffmann und Herold beobachteten nach mehrtägiger Verabreichung von HVL-Extrakt an Ratten, später auch an anderen Tieren. Hertz und Veranes an Kaninchen, Ham und Hais an Hunden Vergrößerungen der Nebenschilddrüse. Auf Grund dieser Beobachtungen nahmen sie an, daß der HVL ein die Nebenschilddrüsenfunktion stimulierendes Hormon bildet. Damit stehen in vollem Einklang die Beobachtungen, nach denen Smith an Ratten, Köster, Houssay und Biasotti, sowie Houssay und Sammartino, aber auch Vernetti an Hunden nach Exstirpation der Hypophyse eine Atrophie und degenerative Atrophie der Nebenschilddrüsen vorfinden konnten. Livon und Peyron sowie Aschner sahen hingegen an Hunden, später Collip an Ratten keine Schilddrüsenatrophie nach Entfernung der Hypophyse. Thompson und Cushing konnten an Hunden, Cattaneo an Kaninchen durch Hypophysenextrakt keine Vergrößerung der

Nebenschilddrüsen erzielen. 1949 beobachtete TÖRNBLOM an Kaninchen nach Verabreichung von großer Menge Natrium- und Ammoniumphosphat bei einer 12wöchigen calciumarmen Speisefolge eine hochgradige Vergrößerung der Nebenschilddrüse.

Die bisherigen Untersuchungen stellten klar, daß die gesteigerte oder verminderte Funktion der Nebenschilddrüsen durch die im Calcium- und Phosphorstoffwechsel des Organismus verursachten tiefgreifenden Veränderungen verschiedene pathologische Zustände hervorruft. Es wäre also wünschenswert, die Funktion der Nebenschilddrüse auf chemischem Wege so beeinflussen zu können, daß die durch ihre verminderte Funktion bedingten Erkrankungen durch die Wiederherstellung der Funktion zu beheben wären.

Im Laufe früherer Untersuchungen (1938—1950) wurde schon festgestellt (FAZEKAS), daß durch dauerhafte Verabreichung zahlreicher acidotisch wirkender Verbindungen in Kaninchen, Gänsen, Schweinen und Ziegen Hypertrophie und gesteigerte Funktion der Nebennierenrinde, Vergrößerung der Ovarien und des Uterus, Follikelreifung und -blutung, Gelbkörperbildung, cyclische Veränderungen an der Schleimhaut der Uterushörner, gesteigertes Fettwerden, Vergrößerung der Brüste und Milchsekretion sowie Vergrößerung der Hypophyse und Vermehrung der basophilen Zellen des HVL erzielt werden konnten. — Auf die Möglichkeit der chemischen Beeinflussung der Nebenschilddrüsen sollen folgende Untersuchungen hinweisen.

Zur Behandlung der Tiere wurden die in der Tabelle angeführten Verbindungen verwendet. Die Nebenschilddrüsen von 362 behandelten Kaninchen (darunter befanden sich auch Tiere aus vorhergehenden Versuchen) wurden untersucht, Größe, Gewicht und histologischer Befund mit den entsprechenden Angaben der Kontrolltiere verglichen. Als Kontrollen dienten 50 gleichaltrige und -artige Kaninchen mit gleichem Gewicht. — Aus der Übersichtstabelle geht es hervor, daß die Nebenschilddrüsen der unbehandelten Kontrolltiere 6—14 mg, im Mittelwert 9,2 mg wogen, die der behandelten Kaninchen hingegen an Gewicht erheblich zunahmen.

1. Mit *Ammoniumhydroxid* wurden 96 weibliche und 64 männliche Tiere 4—5—16 Monate lang behandelt. Jedes Tier bekam 2täglich 50 bis 80 cm³ $^1/_2$%iges NH_4OH durch Magensonde in allmählich zunehmenden Dosen. Nach einer 3wöchigen Behandlung folgte immer eine 1wöchige behandlungsfreie Periode. Das Körpergewicht der Tiere nahm im Laufe der Behandlung allmählich zu und betrug bei der Tötung mit Luftembolie 3100—4400 g. — Die Nebenschilddrüsen wogen 17—39 mg, im Mittelwert 26 mg, was im Verhältnis zum Gewicht der Nebenschilddrüsen der Kontrollen um 16,8 mg, d. h. um 182,6% mehr war. Auf

Tabelle 1. *Gewichtsangaben der Nebenschilddrüsen bei behandelten Kaninchen und Kontrollen.*

		Zahl der Tiere	Gewicht der Nebenschilddrüsen in mg			Gewichtszunahme der Nebenschilddrüsen im Mittelwert		S. D.
			minimal	maximal	Mittelwert	%	mg	
	Kontrollen	50	6	14	9,2	—		
1	Ammoniumhydroxyd	160	17,0	39,0	26,0	16,8	182,6	10,84
2	Ammoniumchlorid	80	16,5	57,5	34,5	25,3	274,6	9,03
3	Ammoniumsulfat	10	15,0	33,0	27,5	18,3	198,9	7,04
4	Ammoniumcarbonat	14	15,0	40,0	25,0	15,8	171,9	6,86
5	Natriumammoniumphosphat	14	19,0	51,0	32,5	23,3	254,3	6,50
6	Ammoniumacetat	12	18,0	49,0	34,5	25,3	274,6	9,73
7	Ammoniumlactat	10	17,5	52,0	29,5	20,3	219,5	6,15
8	Calciumchlorid	10	21,0	54,0	32,5	23,3	251,0	7,76
9	Acidum hydrochloricum	10	16,0	48,0	28,5	19,3	210,8	6,66
10	Acidum lacticum	10	22,5	43,0	29,5	20,3	219,5	5,27
11	Acidum aceticum	12	20,0	36,0	28,6	19,3	209,7	12,12
12	Natriumdihydrophosphat	10	24,0	50,0	36,5	27,3	294,5	10,72
13	Ammoniumhydrophosphat	10	16,5	43,0	30,5	21,3	234,8	7,10

Grund der statistischen Rechnungen betrug die signifikante Differenz 10,84, die Gewichtszunahme der Nebenschilddrüsen ist also spezifisch.

2. Mit *Ammoniumchlorid* wurden 50 weibliche und 30 männliche Kaninchen 3—5—16 Monate behandelt. Zweitäglich bekamen die Tiere 0,1—0,2 g/kg NH_4Cl in 100—150 cm³ Trinkwasser gelöst, in allmählich zunehmenden Dosen. Nach 3wöchiger Behandlung wurde immer eine Behandlungspause von 1 Woche eingeschaltet. Das Gewicht der Tiere nahm während der Behandlungsdauer allmählich zu und betrug bei der Tötung mit Luftembolie 3500—4500 g. Das Gewicht der Nebenschilddrüsen betrug 16,5—57,5 mg, im Mittelwert 34,5 mg, das ist um 25,3 mg mehr als bei den Kontrollen, das einer 274,6%igen Gewichtszunahme entspricht. Die Signifikanz wurde für 9,03 gefunden.

3. *Ammoniumsulfat* wurde 10 weiblichen Kaninchen 5—16 Monate auf gleiche Art und in gleichen Dosen verabreicht. Körpergewicht bei Tötung 4100—4800 g. Gewicht der Nebenschilddrüsen 15—33 mg, im Mittelwert 27,5 mg, um 18,3 mg mehr als bei den Kontrollen, was 198,9%iges Plus bedeutet. Differentia significans: 7,04.

4. Mit *Ammoniumcarbonat* wurden 14 weibliche Kaninchen 5 bis 16 Monate behandelt. Gleiche Behandlungsmethode. — Körpergewicht bei Tötung: 4000—4700 g. Gewicht der Nebenschilddrüsen 15—40 mg,

im Mittelwert 25 mg, um 15,8 mg mehr, als bei den Kontrollen. Die Gewichtszunahme betrug also 171,9% mit einer Signifikanz von 6,86.

5. *Natriumammoniumphosphat* wurde gleichfalls 14 weiblichen Kaninchen 5—14 Monate mit gleicher Methode gegeben. Körpergewicht bei Tötung: 3800—4700 g. Gewicht der Nebenschilddrüsen 19—51 mg, im Mittelwert 32,5 mg, d. h. um 23,3 mg mehr als bei den unbehandelten Kontrolltieren. Das ist eine 254,3%ige Gewichtszunahme mit einer signifikanten Differenz von 6,5.

6. *Ammoniumacetat* wurde 12 weiblichen Kaninchen 5—16 Monate lang verabreicht. Behandlungsmethode wie vorher. Körpergewicht bei Tötung: 3500—4800 g. Die Nebenschilddrüsen wogen 18—49 mg, im Mittelwert 34,5 mg, um 25,3 mg mehr als bei den normalen Tieren: ein Gewichtsplus von 274,6%. Differentia significans 9,73.

7. Mit *Ammoniumlactat* wurden 10 weibliche Kaninchen während 5—16 Monate behandelt. Gleiche Behandlungsmethode. Körpergewicht bei Tötung: 3350—4700 g. Gewicht der Nebenschilddrüsen 17,5 bis 52 mg, im Mittelwert 29,5 mg, das ist um 20,3 mg mehr als bei den Kontrollen, das eine 219,5%ige Gewichtszunahme bedeutet. Signifikanz: 6,15.

8. Mit *Calciumchlorid* wurden 10 weibliche Kaninchen 5—16 Monate gleicherweise behandelt. Körpergewicht bei Tötung: 3700—5050 g. Gewicht der Nebenschilddrüsen 21—54 mg, im Mittelwert 32,5 mg, d. h. um 23,3 mg (251%) mehr als bei den Kontrollen. Differentia significans: 7,76.

9. Mit *Acidum hydrochloricum* wurden 10 weibliche Kaninchen 5 bis 16 Monate lang behandelt. Diese Tiere bekamen 2täglich 0,5—1,0 cm³ 25%iges HCl in 100—150 cm³ Trinkwasser, in allmählich zunehmenden Dosen. — Auch hier folgte nach einer Behandlung von 3 Wochen eine 1wöchige behandlungsfreie Pause. Körpergewicht bei Tötung der Tiere: 3500—4500 g. Gewicht der Nebenschilddrüsen 16—48 mg, im Mittelwert 28,5 mg, um 19,3 mg mehr als bei den normalen Tieren. Das bedeutet ein Gewichtsplus von 210,8%, mit einer Signifikanz von 6,66.

10. *Acidum lacticum* wurde 10 weiblichen Kaninchen 5—16 Monate hindurch gegeben. Den Tieren wurde 2täglich in zunehmenden Dosen 0,2—0,5 cm³/kg Wirkungsstoff in 100—150 cm³ Trinkwasser verabreicht. Nach 3 Wochen Behandlung folgte 1 Woche Pause. Bei Tötung betrug das Körpergewicht 3700—4800 g. — Gewicht der Nebenschilddrüsen 22,5—43 mg, im Mittelwert 29,5 mg, also um 20,3 mg, d. h. um 219,5% mehr als bei den Kontrollen. Signifikante Differenz 5,27.

11. Mit *Acidum aceticum* wurden 12 weibliche Kaninchen 5—16 Monate behandelt. Die Tiere bekamen 2tägig in zunehmenden Dosen 0,2 bis 0,5 cm³/kg konzentrierte Essigsäure in 100—150 cm³ Trinkwasser. Körpergewicht bei Tötung 3600—4600 g. Gewicht der Nebenschild-

drüsen 20—36 mg, im Mittelwert 28,6 mg, d. h. um 19,3 mg mehr als bei den normalen Kontrolltieren, was einer Zunahme von 209,7% entspricht. Differentia significans 12,12.

12. Mit *Natriumdihydrophosphat* wurden 10 weibliche Kaninchen 5—16 Monate lang behandelt. Diese Tiere bekamen aber 0,3 bis 0,7 g/kg Wirkungsstoff 2täglich in zunehmenden Dosen in 100 bis 150 cm³ Trinkwasser. Körpergewicht bei Tötung: 4000—4500 g. Gewicht der Nebenschilddrüsen betrug 24—50 mg, im Mittelwert 36,5 mg, also um 27,3 mg mehr als bei den Kontrollen. Das bedeutet eine 294,5%ige Gewichtszunahme mit einer Signifikanz von 10,72.

13. *Ammoniumhydrophosphat* wurden 10 weiblichen Kaninchen 5 bis 16 Monate hindurch verabreicht. Ein Tier bekam 0,3 bis 0,7 g/kg Verbindung 2täglich in zunehmenden Dosen in 100 bis 150 cm³ Trinkwasser. Körpergewicht bei Tötung: 3900—4800 g. Gewicht der Nebenschilddrüsen 16,5—43 mg, im Mittelwert 30,5 mg, d. h. um 21,3 mg mehr (234,8%) als bei den Kontrollen. Signifikante Differenz: 7,1.

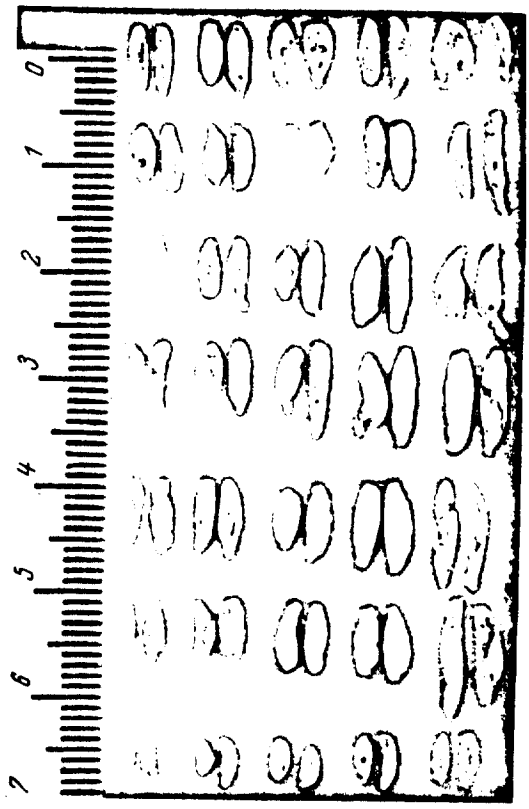

Abb. 1. Vergrößerung der Nebenschilddrüsen bei 5 Monate lang mit verschiedenen Verbindungen behandelten Kaninchen. — I. Reihe: Ammoniumchloridbehandlung: 16,5—18—24,5—26—57,5 mg. — II. Reihe: Ammoniumsulfatbehandlung: 15—16—17—20—27,5 mg. — III. Reihe: Ammoniumcarbonatbehandlung: 15—18—20—27,5 bis 40 mg. — IV. Reihe: Na-Ammoniumphosphatbehandlung: 19—20,5—25—37,5—51 mg. — V. Reihe: Ammoniumacetatbehandlung: 18—22,5—24—39—43 mg. — VI. Reihe: Ammoniumlactatbehandlung: 17,5—20—24—30—52 mg. VII. Reihe: Kontrollen ohne Behandlung: 6—7—8 bis 9,5—10 mg.

Mit Hinsicht auf die angeführten Angaben kann behauptet werden, daß die Nebenschilddrüsen unter Wirkung der verwendeten Verbindungen

an Gewicht erheblich zunehmen, und zwar maximal bei den mit Natriumdihydrophosphat (294,5%), Ammoniumchlorid bzw. Ammoniumacetat (274,6%), minimal bei den mit Ammoniumcarbonat (171,9%) und Ammoniumhydroxid (182,6%) behandelten Tieren. Auf Grund der statistischen Rechnungsergebnisse kann die Gewichtszunahme der Nebenschilddrüsen einstimmig für spezifisch geh...

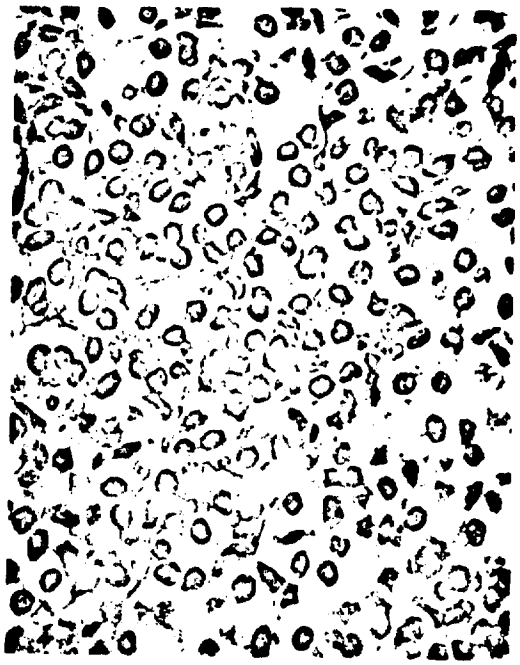

Abb. 2. Histologisches Bild der Nebenschilddrüsen bei unbehandelten Kontrollkaninchen. In überwiegender Mehrheit bilden dunkle Hauptzellen die Drüsensubstanz. Die hellen Hauptzellen sind in verhältnismäßig kleiner Zahl und von normaler Größe. — Hämatoxylin-Eosinfärbung. 1000fache Vergrößerung.

werden. — Die Gewichtszunahme ging mit einer auffallenden Vergrößerung der Nebenschilddrüsen an Volumen bzw. Dimensionen einher (Abb. 1).

Im Laufe der *histologischen Untersuchung* wurde beobachtet, daß die Gefäße der Nebenschilddrüsen bei den *unbehandelten* Kontrollen im allgemeinen mittelmäßig blutreich und mittelmäßig weit waren. Die Hauptmasse der Drüsen bildeten die sog. *dunklen Hauptzellen*, an den Drüsenrandteilen konnten zerstreut *oxyphile Zellen* vorgefunden werden (Abb. 2). — In den Nebenschilddrüsen der *behandelten* Kaninchen hingegen waren die größeren Gefäße, aber auch die Capillaren, schon nach 3monatiger Behandlung weit und blutvoll, die Hauptmasse der

Drüsen bestand aus vergrößerten und auch an Zahl vermehrten sog. *hellen Hauptzellen*. Das Plasma dieser Zelltypen war vergrößert, hell, oft befanden sich darin Vacuolen. Ihr Kern war gleichfalls groß und färbte sich hell. In mehreren solchen Zellen waren Kernteilungen zu sehen. Dunkle Hauptzellen wurden in den Nebenschilddrüsen der behandelten Tiere nur in verhältnismäßig geringer Zahl vorgefunden.

Abb. 3. Histologisches Bild der vergrößerten Nebenschilddrüsen eines 3 Monate lang mit NH_4Cl behandelten Kaninchens. Die Drüsensubstanz ist besonders von hellen Hauptzellen mit vergrößertem Plasma gebildet. — Hämatoxylin-Eosinfärbung. 1000fache Vergrößerung.

Die oxyphilen Zellen fehlten fast vollkommen in den 3—5monatigen Fällen, in den Fällen von 16 Monaten waren sie aber schon meistens aufzufinden. Vermehrung des Bindegewebes konnte nicht einmal nach 16monatiger Behandlung in den Nebenschilddrüsen der Tiere beobachtet werden (Abb. 3).

Gleiches histologisches Bild fanden auch ANSELMINO, HOFFMANN und HEROLD bei Ratten, HERTZ und KRANES bei Kaninchen, HOUSSAY bei Hunden und Kaninchen nach Verabreichung wäßrigen HVL-Extraktes. Verfasser betrachten diese Erscheinungen als Folge der auf die Funktion der Nebenschilddrüsen ausgeübten stimulierenden HVL-Wirkung. Nach den Beobachtungen von HOUSSAY konnten schon 5 Tage

nach der Hypophysektomie Atrophie des Zellprotoplasmas, Verminderung der Zellen und Verschwommenheit der Zellgrenze festgestellt wurden. Gleichzeitig verschwand die gleichmäßige epitheliale Struktur der Drüsen, und es bildete sich eine trabeculare, bündelartige Anordnung der Zellen aus. Solche Atrophie der Nebenschilddrüsen war besonders bei Hunden auffallend, an denen Hypophysektomie und Pankreasexstirpation gleichzeitig durchgeführt worden sind. VERNETTI beobachtete in den atrophischen Nebenschilddrüsen hypophysektomisierter Hunde auch erhebliche Vermehrung des Bindegewebes.

Im Laufe eigener Untersuchungen zeigten sich in den Nebenschilddrüsen der behandelten Kaninchen Blutreichtum, Hypertrophie und Hyperplasie, erhebliche Vermehrung der hellen Hauptzellen und Kernteilungserscheinungen. Auf Grund all dessen könnte auf die gesteigerte Funktion der infolge der verwendeten Behandlung vergrößerten Nebenschilddrüsen geschlossen werden. Darüber werden wir aber eine endgültige Meinung nur auf Grund der an den vergrößerten Nebenschilddrüsen durchgeführten Funktionsuntersuchungen äußern können.

Es ergibt sich nun die Frage, worin besteht der Funktionsmechanismus der beobachteten, behandlungsbedingten Vergrößerung der Nebenschilddrüsen. Da sich unter den 13 verabreichten Verbindungen organische und anorganische, ammoniakalische und nichtammoniakalische Verbindungen sowie Säuren und Laugen befinden, kann nicht daran gedacht werden, daß die beschriebene Vergrößerung der Nebenschilddrüsen durch die spezifische Einwirkung dieser Verbindungen bedingt werden könne. Es muß also, in betreff des Wirkungsmechanismus nach einer gemeinsamen Ursache gesucht werden, die im Organismus unter dem Einfluß dieser Verbindungen immer auftritt.

Es ist im allgemeinen bekannt, daß die Säuren und sauren Salze das Säurebasengleichgewicht des Organismus in die saure Richtung verschieben (Acidose), weniger bekannt ist es aber, daß auch die Laugen eine Acidose verursachen können. FAZEKAS wies nach, daß NH_4OH und NaOH — trotz ihrem Laugencharakter — im Organismus eine Acidose hervorrufen. Seine Behauptungen fanden auch von VENULET, GOEBEL und TISLOWITZ, ALVALL und GEIGER sowie von HAZARD und VAILLE Bestätigung. Die gemeinsame Eigenschaft der verwendeten Verbindungen besteht also darin, daß sie das Säurebasengleichgewicht des Organismus in die acidotische Richtung verschieben. Unserer Ansicht nach wirkt die Verschiebung des Blutchemismus in die Säurerichtung reizend, entweder unmittelbar auf den HVL oder auf dem Weg über das Hypothalamuszentrum der Hypophyse auf die entsprechenden Zellen des HVL und stimuliert sie dadurch zur gesteigerten Funktion. Die gesteigerte Hypophysenfunktion ruft dann die Vergrößerung bzw. die gesteigerte Funktion der Nebenschilddrüse hervor

Dafür spricht unser Befund, daß sich auch die Hypophyse bei solch behandelten Tieren vergrößert und die basophilen Zellen des HVL an Zahl zunehmen. Gleichfalls für die gesteigerte HVL-Funktion sprechen unsere früheren Behauptungen, daß durch die Einwirkung dieser Verbindungen bei Kaninchen Vergrößerung und gesteigerte Funktion der Nebennierenrinde, in den Eierstöcken Follikelreifung und -blutung sowie Gelbkörperbildung hervorgerufen werden.

Auf die Funktionssteigerung der Hypophyse unter acidotischem Einfluß weisen auch folgende Literaturangaben hin: JULESZ fand an infantilen Mäuseovarien Follikelreifung nach Einspritzung von Urin von Männern und Frauen an ketogener Diät. SELYE und HERLANT beobachteten, außer Hyperplasie der Nebennierenrinde, gleiche Erscheinungen nach intravenöser Einspritzung von Salzsäure. HARRIS spritzte, in die 3. Hirnkammer von Kaninchen, eine Cuprumacetatlösung, was durch die Reizung der Hypothalamuszentren eine Ovulation auslöste. Eine chronische Acidose ist meistens bei jenen menschlichen Erkrankungen nachzuweisen, wo laut den Literaturangaben eine Vergrößerung der Nebenschilddrüsen beobachtet wurde: z. B. bei chronischen Nierenerkrankungen, Schwangerschaft, partieller oder totaler Exstirpation der Nieren usw.

TÖRNBLOM beobachtete bei Kaninchen nach Verabreichung von Natrium- und Ammoniumphosphat Vergrößerung der Nebenschilddrüsen, gleichzeitige Abnahme des Blutcalciums und hochgradige Zunahme des Blutphosphors. Die Behandlung wurde von ihm an hypophysektomisierten Tieren wiederholt, danach eine Herabsetzung des Blutcalcium- und -Phosphorgehaltes festgestellt, die Nebenschilddrüsen vergrößerten sich aber nicht. TÖRNBLOM schloß daraus, daß die Vergrößerung der Nebenschilddrüse durch die Hyperphosphatämie verursacht wurde. In anderen Versuchsserien forschte er nach, von was für Hormoneinwirkungen die Veränderung der Phosphorämie abhänge. Er führte an einer Kaninchengruppe Schilddrüsen- und Nebenschilddrüsenentfernung, an der anderen Schilddrüsen-, Nebenschilddrüsen- und Hypophysenexstirpation durch. Bei diesen Tieren war die Hypocalcämie und Hyperphosphatämie kleiner. Wenn aber den schilddrüsen- und nebenschilddrüsenberaubten Tieren Hypophysenextrakt verabreicht wurde, veränderte sich die Hypocalcämie nicht, die Hyperphosphatämie war aber wesentlich höher. Das weist — seiner Meinung nach — darauf hin, daß die Hypophyse die Phosphorämie verstärkt, ohne die Vermittlung der Nebenschilddrüsen in Anspruch genommen zu haben. TÖRNBLOM veranschaulicht den Einfluß der Hypophyse auf die Nebenschilddrüse mit folgendem Schema: Hypophyse—Hyperphosphatämie—Hyperfunktion der Nebenschilddrüse. Nach seiner Auffassung geschieht die Reizung der Nebenschilddrüse nicht unmittelbar durch die Hypophyse, sondern auf dem Weg über einen humoralen Faktor durch die Steigerung des Blutphosphorniveaus.

Das Ergebnis unserer eigenen Versuche scheint dieser Ansicht von TÖRNBLOM zu widersprechen, da wir außer 3 Phosphatarten noch 10 andere phosphorfreie Verbindungen verabreicht haben und danach eine Vergrößerung der Nebenschilddrüse beobachten konnten. In vorigen Untersuchungen wurde behauptet (FAZEKAS), daß der anorganische Blutphosphorgehalt bei Kaninchen durch einmalige Verabreichung einer größeren Dosis von NH_4OH und NaOH erheblich anwächst, das Serumcalcium und -chlor abnimmt, eine Hyperglykämie auftritt, die Alkalireserve des Blutserums absinkt (!) und die Konzentration des Hydro-

genious zunimmt, d. h. eine Acidose hervorgerufen wird. Eine Hyperphosphatämie kann also auch ohne Phosphorzufuhr zustande kommen. Laut unseren Erfahrungen ist der Zuwachs des anorganischen Blut-Phosphor-Gehaltes bei den infolge verschiedenster Ursachen (Vergiftungen, Erkrankungen) auftretenden acidotischen Zuständen fast immer nachzuweisen. Diese Tatsache scheint TORNEBLOMS Auffassung zu unterstützen. Laut den ausgedehnten und gründlichen Untersuchungen v. BALÓ nimmt der anorganische Phosphorgehalt des Blutserums auch im Falle des nachherigen Sauerwerden des Blutes in vitro erheblich zu, ohne über durch die Hypophyse beeinflußt zu sein. Demnach kann als der Phosphorgehalt des Blutserums auch ohne Hypophyseneinwirkung ansteigen (das organisch gebundene P der roten Blutkörperchen geht in eine anorganische Bindung über und gelangt in das Blutserum). A. das weist darauf hin, daß die Steigerung des Blutphosphorgehaltes Teilerscheinung der Acidose angesehen werden kann und ihr Dasein an sich keinen Beweis außer allem Zweifel dafür bietet, als ob die Vergrößerung der Nebenschilddrüsen bzw. ihre Funktionssteigerung durch die Hyperphosphatämie bedingt wäre.

Auf Grund eigener und anderer Versuchsergebnisse kommt die Vergrößerung bzw. gesteigerte Funktion der Nebenschilddrüse — unserer Ansicht nach — so zustande, daß die durch verschiedene Ursache bedingte Verschiebung des Blutchemismus in die Säurerichtung entweder unmittelbar oder auf dem Weg über das Hypothalamuszentrum der Hypophyse auf den HVL reizend wirkt, dieses Organ zur gesteigerten Funktion stimuliert, was dann durch die gesteigerte Bildung von Parathyreotrophormon die Vergrößerung der Nebenschilddrüsen bzw. ihre gesteigerte Funktion verursacht.

Vorliegende Untersuchungen dienen mit neuen Angaben darüber, daß das innersekretorische Drüsensystem ohne Hormondarreichung bloß durch Zufuhr von einfachen Verbindungen auf chemischem Wege beeinflußt bzw. seine Funktion umgestellt werden kann. — Angesichts der Nebenschilddrüse kann aber Endgültiges nur auf Grund neuer Ergebnisse ausgesprochen werden. Die diesbezüglichen Untersuchungen sind im Gange und hoffentlich können Hypofunktion von Nebenschilddrüsen und auch dadurch bedingte pathologische Zustände behoben werden.

Zusammenfassung.

1. 362 Kaninchen wurden 2täglich 3—5—16 Monate hindurch organische und anorganische Ammoniak- und andere Verbindungen (0,1 bis 0,2 g/kg) verabreicht. (Die gemeinsame Eigenschaft dieser Verbindungen besteht darin, daß sie das Säurebasengleichgewicht des Organismus in die Säurerichtung verschieben.) Das Gewicht, die Größe und das his-

logische Bild der Nebenschilddrüsen solch behandelter Kaninchen wurde mit den entsprechenden Angaben von 50 unbehandelten gleichaltrigen Kontrollkaninchen von gleicher Art und gleichem Gewicht verglichen.

2. Das minimale Gewicht der Nebenschilddrüsen bei Kontrollen betrug 6 mg, das maximale 14 mg, im Mittelwert wurden 9,2 mg vorgefunden.

3. Im Gegensatz betrug das minimale Gewicht der Nebenschilddrüsen der acidotisch behandelten Kaninchen 15—25 mg, das maximale 33—57,5 mg, im Mittelwert also 25—36,5 mg. Diese Gewichtszunahme weist, im Verhältnis zu den Kontrolltieren, im Mittelwert 15,8—27 mg, d. h. 171,9—294,5%, auf, was laut statistischer Berechnungen eine signifikante Differenz von 5,27—12,12 bedeutet.

4. Histologisch bestand die Substanz der Nebenschilddrüsen bei den *Kontroll*kaninchen besonders aus *dunklen Hauptzellen*, helle Hauptzellen waren in verhältnismäßig kleiner Zahl vorhanden, an den Randzellen lagerten auch oxyphile Zellen zerstreut. In den vergrößerten Nebenschilddrüsen der *behandelten Kaninchen* hingegen war Blutreichtum festzustellen, die dunklen Hauptzellen nahmen an Zahl erheblich ab, die oxyphilen Zellen fehlten sogar oder nahmen gleichfalls an Zahl ab, die *hellen Hauptzellen* vermehrten und vergrößerten sich aber erheblich, an mehreren Stellen mit Vacuolenbildung, so daß die Hauptmasse der Drüsen von den hellen Zellen gebildet wurde.

5. Auf Grund der Vergrößerung und des histologischen Bildes der Nebenschilddrüsen kann auf ihre gesteigerte Funktion geschlossen werden. Darüber werden wir uns aber nur nach der funktionalen Untersuchung der vergrößerten Nebenschilddrüsen aussprechen können.

6. Unsere früheren Behauptungen, daß bei so behandelten Tieren Hypophysenvergrößerung, Vermehrung der basophilen Zellen des HVL, Vergrößerung und gesteigerte Funktion der Nebennierenrinde, Vergrößerung der Eierstöcke, Follikelreifung und -blutung, Corpus luteum-Bildung, Uterusvergrößerung und Milchsekretion hervorgerufen werden können, weisen darauf hin, daß diese Veränderungen, aber auch die Vergrößerung der Nebenschilddrüsen durch die gesteigerte Funktion des HVL bedingt werden.

7. Die gesteigerte Funktion des HVL ist, unserer Ansicht nach, nicht auf die spezifische Wirkung der verwendeten Verbindungen zurückzuführen, sondern vielmehr auf die von ihnen ausgelöste, periodische acidotische Einwirkung von mäßigem Grade.

8. Unsere Untersuchungen bieten einen neuen Beweis dafür, daß die Funktion des innersekretorischen Drüsensystems durch entsprechende Verabreichung einfacher, acidotisch wirkender Verbindungen umgestellt bzw. gesteigert werden kann. Auf Grund unserer Ergebnisse steht es zu hoffen, daß gegebenenfalls die Funktion der vermindert

funktionierenden Nebenschilddrüsen (und andrer innersekretorischen Drüsen) wiederhergestellt bzw. normal gemacht und zu gleicher Zeit die durch diese unzulängliche Funktion bedingten pathologischen Zustände behoben werden können.

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Fazekas-'54
Bib No. 519

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Endokrinologie 32:45-57. 1954.

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THE EFFECT OF THE PARATHYROID GLAND FUNCTION (Serum Ca and P) BY ACIDOTIC COMPOUNDS

by I. Gy. Fazekas

Mac Callum and Voegtlin detected a reduction in the blood-Ca content in dogs after removal of the parathyroid glands. Greenwald as well as Hastings and Murray, Reed, Zachy and Payte, on the other hand, observed that the P content of the blood in such animals increased. Collip et al. found that after injection of parathyroid extract (pharahormone) there was an increase in the Ca content of the blood serum both in normal dogs and cats and in parathyroidectomized dogs and cats. It is known that in humans suffering from Recklinghausen disease (osteitis fibrosa generalisata) caused by an enlargement and increased function of the parathyroid glands the Ca content of the blood serum increases considerably, the inorganic P content decreases considerably, however. Similar changes in the serum Ca and P content were observed also in acute hyperparathyroidism (Olivier, Smith , Hanes). In various cases of hypoparathyroidism there is an increase in the serum p. also in humans along with a decrease in the Ca content of the blood serum. The parathyroid glands therefore play a first-class role in the regulation of the Ca and P metabolism in the organism.

Fazekas determined that the parathyroid glands of rabbits enlarged by 171 to 294% through lengthy application of acidotic-acting compounds. The histological finding indicates the increased function of the parathyroid glands which have been thus enlarged . This cannot be decided, however, solely based on the histological picture. In order to investigate the function of the parathyroid glands thus enlarged new animal experiments were implemented. Since according to the medical data published there is a close relationship between the Ca and inorganic P content of the blood and the parathyroid function, it was to be assumed that information could be obtained,by systematic investigation of the blood Ca and P content in animals which had been treated with compounds having an acidotic effect and causing a parathyroid hypertrophy, concerning the function of the parathyroid glands.

In our tests 72 similar-type (Chinchilla) and similar-aged (8 to 10 month old) female and male rabbits with an initial weight between 2500 to 3000 grams were treated for from 5 to 26 months with compounds producing an acidotic effect. Fifty animals, however, which did not receive such treatment, were used as control animals. The animals were fed with the same amount of oats and dry clover. The following compounds were administered: ammonium chloride, ammonium sulphate, ammonium hydrophosphate, ammonium acetate, ammonium lactate, sodium hydrophosphate, acidum hydrochloricum (hydrochloric acid), acidum lacticum (lactic acid), acidum aceticum (acetic acid),and calcium chloride. Six animals were treated with each compound. Every animal was given twice a day for a period of three weeks in weekly increasing doses 0.1-0.2 g per kilogram of body weight of the corresponding compound dissolved in 50 to 80 ccm of drinking water. After that there follows a two week period during which no treatment was given. Thus the treatment period and treatment pause were repeated for 5 to 26 months. Six rabbits were treated in the same way with the following compound mixture: 30 g ammonkum chloride, 5 g ammonium acetate, 5 g ammoniumhydrophosphate, 10 g calcium chloride, 5 g hydrochloric acid, 5 g lactic acid, with 1000 g of drinking water.(aquae fontis) . . In 1 ccm of this solution there is 0.06 g of effective substance mixture. These animals were treated for 16 . Six other rabbits were treated with the same compound mixture and in the same way but for 26 months.

In the laboratory animals the Ca content of the blood serum and the inorganic P content of the total blood were determined before, during and after the treatment for a period of two weeks. The control rabbits were also subjected to the same tests. The Ca (calcium) content was determined in 1 ccm of blood serum by means of the oxydimetric method of Clark and Collip. The inorganic P (phosphorus) content of the blood, however, was determined in 0.2 ccm full blood with the colorimetric method of Kuttner and Cohen by means of a Stankosch electrophotometer. The mean value of 2 determination processes conducted in parallel always gave the result. For purposes of brevity the individual data will not be reported, but in the attached table only the mean values of the individual animal groups will be given. Since in the 11th animal group during the first 12 months the same characteristic serum Ca and P changes were found as in the 1st to 10th animal groups, these data will not be discussed individually, but only the results of the 11th, 12th and 13th treatment periods (13-16 months). In the case of the 12th animal group which was treated with the compound mixture the blood serum Ca and inorganic P content of the blood over the course of the 18-month treatment showed changes of the same nature as in the preceding groups. In the case of the 12th animal group a pause of 3 months was given after 18 months of treatment. During this time the serum Ca and inorganic P returned to the normal initial values. Then the treatment of the animals was continued in the same way for a further 5 months (4 treatment periods).

The detailed evaluation of the various Ca and P changes will be omitted here. As an example we would like to cite only the evaluation of the treatment with ammonium sulphate as well as the 26-month treatment with the compound mix.

Ammonium Sulphate Treatment

1. Treatment period. Serum-Ca. Prior to the treatment 14.92 mg%. After the first week of treatment 9.39 mg %, which signifies in comparison to the normal initial value a drop of 5.53 mg % (37.06%). After two weeks of treatment 17.35 mg % , that is 2.45 mg % more (16.4%), after three weeks of treatment 18.45 mg %, i.e. 3.53 mg % (23.6) more. After a one-week treatment pause 19.88 mg % , after a two week pause 16.42 mg % , which in comparison with the normal initial value signifies an increase of 4.69 mg % (31.9%) and 1.52 mg % (10.0%). Blood-P: Prior to the treatment 3.31 mg %. After one week of treatment 4.13 mg %, which means an increase of 0.82 mg % (26.1%). After two weeks of treatment 2.19 mg % , after 3 weeks' treatment 1.99 mg %, that is to say 1.12 mg % (33.8%) and 1.32 mg % (39.9%) less than the normal initial value. After a one-week treatment pause 3.62 mg % , after a two-week pause 4.97 mg % , i.e. 1.63 mg % (81.9%) and 2.98 mg (149.7 %) greater than after 3-week treatment and 0.31 mg % (9.3%) and 1.66 mg % (50.1%) greater than the normal initial value.

II. Treatment period. Serum-Ca: after 1-week treatment 16.24 mg % , after 2-week's treatment 21.44 mg %, after 3 weeks 23.11 mg %, i.e. 2.32 mg % (15.55%), 6.52 mg % (43.6%) and 8.19 mg % (54.9%) more than the normal initial value and 5.02 mg % (30.5%) and 6.69 mg % (40.7) greater than the value after 2-week pause of the preceding treatment period. After one week treatment pause 20.77 mg %, after two-week pause 16.26 mg %, i.e. 5.85 mg % (39.2%) and 1.34 mg % (8.9%) greater than the normal initial value. This signifies in comparison with the initial treatment value an increase of 2.34 mg % (10.1%) and 6.85 mg % (29.9%). Blood-P: after one week treatment 2.35 mg % , after two week treatment 1.44 mg %, after three week treatment 1.62 mg %, which amounts to 0.96 mg % (29.0%), 1.87 mg % (56.5%) and 1.69 mg % (51.0%) less than the normal initial value. After a one-week pause 3.14 mg %, after two-week pause 3.2 mg % , i.e. 0.17 mg % (5,1 %) and 0.11 mg % (3.3%) less than the normal initial value.

Third Treatment Period. Serum-Ca: after one-week treatment 16.9 mg % , after two weeks 20.51 mg %, after three weeks treatment 22.66 mg %. These values are 1.98 mg % (13.3%), 5.69 mg % (37.6%) and 7.77 mg % (51.8%) greater than the normal initial value. After one-week treatment pause 20.1 mg %, after two-week pause 17.23 mg %, which is 5.18 mg % (35.3%) and 2.21 mg % (14.8%) greater than the normal initial value, but on the other hand 2.56 mg % (11.2%) and 5.43 mg % (23.9%) less than the value after three weeks of treatment of this period. Blood-P: after one-week treatment 2.59 mg %, after

two-week treatment 2.22 mg % , after three-week treatment 1.8 mg %. These values are 0.62 mg % (15.7%), 1.09 mg % (32.9%) and 1.51 mg % (45.6%) lower than the normal initial value. After one-week treatment pause 2.66 mg %, after 2-week pause 3.46 mg %, i.e. 0.65 mg % (16.6%) less and 0.15 mg % (4.5) more than the normal initial value. The P value therefore returned somewhat to the normal value.

Fourth Treatment Period. Serum-Ca: After one-week treatment 18.31 mg % , after two-week treatment 20.42 mg %, after 3-week treatment 22.14 mg %, i.e. 3.39 mg % (22.7%), 5.50 mg % (36.8%) and 7.22 mg % (48.2 %) higher than the normal initial value and 1.08 mg % (6.2%), 3.19 mg % (18.5%) and 4.91 mg % (28.5%) greater than the value after the two-week treatment pause of the preceding treatment period. After one-week treatment pause 18.60 mg % , i.e. 3.68 mg % (24.6%) greater than the normal initial value, but 3.54 mg % (15.9%) less than after the three week treatment of this period. Blood-P: After 1-week treatment 2.23 mg %, after two weeks of treatment 2.32 mg %, after three weeks of treatment 1.75 mg %, i.e. 0.98 mg % (29.6%), 0.99 mg % (29.9%) and 1.56 mg % (47.1%) less than the normal initial value. After one-week treatment pause 2.63 mg % , i.e. 0.82 mg % (45.2%) more than the value after three-week treatment of this period, but 0.68 mg % (20.5%) less than the normal initial value.

Twelfth Treatment Group (compound mixture)

Eighteenth (XVIII) treatment period. Serum-Ca: Prior to the treatment 15.81 mg %, after one week treatment 11.92 mg %, which signifies in comparison to the normal initial value a decrease of 3.89 mg (24.6%) . This decrease was not as great, however, as at the start of the first treatment (37.2%). After two-week treatment 17.81 mg %, after three-week treatment 22.86 mg %, i.e. 1.0 mg % (6.3%) and 7.05 mg % (44.59%) more than the normal initial value. After one week treatment pause 20.27 mg %, after two-week pause 16.35 mg %, i.e. 2.59 mg % (11.3%) and 6.51 mg % (29.3%) less than after three-week treatment of this period, but 4.46 mg % (28.2%) and 0.54 mg % (3.4%) more than the normal initial value. Blood P: before the treatment 3.72 mg %. After one week of treatment 4.58 mg %, i.e. 0.86 mg % (23.1%) more than the normal initial value. After the second week of treatment 2.77 mg %, after the third week of treatment 1.88 mg % , i.e. 0.95 mg % (25.5) and 1.84 mg % (49.4%) less than the,normal initial value. After one week treatment pause 3.07 mg %, i.e. 0.85 mg % (22.8%) and 0.65 mg % (17.4%) less than the normal initial value, but 0.99 mg % (52.6%) and 1.19 mg % (63.1%) more than after the third week of treatment of this period.

Nineteenth (XIX) Treatment Period. Serum-Ca: After one week treatment 15.66 mg %, i.e. almost the same as the normal initial value. This is evidence of the fact that the organism was already able to ward off the Ca-reducing effect of the treatment. After two week treatment 19.84 mg %, after 3 week treatment 22.63 mg %, which in comparison to the normal initial value signifies a Ca increase of 4.03 mg % (23.4%) and 6.82 mg % (43.1%). After one week treatment pause 20.60 mg % and after 2 week pause 16.41 mg %, which in comparison with the value after the 3 weeks of treatment of this period signifies a drop of 2.03 mg % (8.4%) and 6.22 mg % (27.4%), but which amounts to 4.79 mg % (30.3%) and 60 mg % (3.7%) more than the normal initial value. Blood-P: After one-week treatment 3.23 mg %, after 3-week treatment 2.30 mg %, i.e. 0.49 mg % (13.1%) , 1.2[illegible] mg % (34.4%) and 1.-2 mg % (38.1%) less than the normal initial value. After a one week pause 3.05 mg % and after a two week treatment pause 3.39 mg %, i.e. 0.67 mg % (18.0%) and 0.33 mg % (8.8%) less than the normal initial value, but o.75 mg % (32.6ç) and 1.09 mg % (47.3%) more than after the third week of treatment of this period.

Twentieth (XX) Treatment Period. Serum-Ca: After one week treatment 16.93 mg %, after two week treatment 19.88 mg %, after 3 weeks treatment 21.99 mg %, i.e. 1.12 mg % (7.0%), 4.07 mg % (26.3%) and 6.18 mg % (39.0%) greater than the normal initial value. After one week treatment pause 20.06 mg %, after 2 week pause 17.21 mg %, i.e. 1.93 mg % (8.7%) and 4.78 mg % (21.4%) less than after the three week treatment of this period, but 4.25 mg % (26.8%) and 1.40 mg % (9.4%) more than the normal initial value. Blood P: after one week treatment 2.61 mg %, after two week treatment 2.28 mg %, after 3 week treatment 2.10 mg % , signifying 1.11 mg % (29.8%0, 1.54 mg % (41.3%) and 1.62 mg % (43.5%) less than the normal initial value. After one week treatment pause 2.53 mg %, after two week pause 2.75 mg %, i.e. 1.19 mg % (31.9%) and 0.97 mg % (26.0%) less than the normal initial value, but 0.43 mg % (20.4%) and 0.65 mg % (30.9%) more than after the three weeks of treatment of this treatment period.

TWENTY FIRST (XXI) Treatment Period. Serum-Ca: after one week treatment 17.22 mg %, the same value as after two week treatment pause of the previous period, which also means that the organism was able to compensate for the Ca-reducing action of the 1 week treatment. After 2 weeks of treatment 19.38 mg %, after three weeks of treatment 22.05 mg %, which is 3.57 mg % (28.9%) add 6.24 mg % (39.4%) more than the normal initial value. After one week treatment pause 18.94 mg %, i.e. 3.11 mg % (19.7%) less than after the third week treatment of this period, but 3.13 mg % (19.8%) more than the normal initial value. Blood-P: After one week treatment 2.72 mg %, after two week treatment 2.28 mg %, after 3 week treatment 1.78 mg %, i.e. 1.0 mg % (26.5%), 1.44 mg % (38.6%) and 1.94 mg % (52.1%) less than the normal initial value. After one week treatment pause 2.67 mg %, i.e. 1.07 mg % (28.7) less than the normal initial value, but 0.87 mg % (49.4%) more than after three weeks of treatment of this period.

The investigations with the other compounds also showed the same changes in Ca and P. In the case of the control rabbits, however, it was not possible to determine any similar and equivalent changes in the serum Ca and in the inorganic P.

The results show therefore that under the effect of the acidotic compounds which were used , after the first week of treatment of the first period, the serum Ca dropped considerably below the initial value (24% to 37%), but the blood-P increased at the same time 20 to 42% above the normal initial value. This is in agreement with our earlier finding that under the effect of a dose of NH_4OH or NaOH the serum Ca decreases and the blood-P increases, however. In the 2nd week of treatment there is an increase in the Serum-Ca (10-35%) and it reaches its maximum after 3 weeks of treatment (Ca:20-51%; P: 24-50%. After the 1st and 2nd weeks of treatment pause the serum-Ca value gradually decreases in comparison with the value of the 3 week treatment, but usually remains higher even after 2 weeks (7-21%) than the normal initial value, the blood P increases again, however, over the normal (by 50-67%). During the subsequent treatment periods the serum-Ca value never drops below the normal value after the first week of treatment, but rather it remains 12-22% higher during that period of time, the blood P, however, drops considerably (20-30%) below the normal value. In the 2nd and third weeks of treatment thus the increase in the serum-Ca (50-65) and the decrease in the blood P (30-56%) is still more evident than during the same time of the first treatment period. During the course of the two week treatment pause the serum- Ca gradually decreases, but the blood-P increases in comparison with the value of the 3 week treatment. The serum-Ca, however, is then still greater and the inorganic P still lower than the normal initial value, that is to say that the serum-Ca goes higher and the blood P, however, goes lower than before the start of the treatment.

In the case of the rabbits which had been administered the compound mixture for a period 16 months long and in the case of those rabbits which were treated anew after an 18-month treatment and after the subsequent 3-month treatment pause it was possible to observe Ca and P changes of the same nature.

After the laboratory animals were sacrificed we found a 160.8-277.7 % parathyroid gland enlargement (mean values)! In the parathyroid glances , in agreement with our earlier tests, we were able to observe histologically blood filling, increase and enlargement of the bright cells, nucleus division phenomena, i.e. the signs of a hyperfunction.

Other researchers also detected a parathyroid gland enlargement during long-lasting administration of ammonium chloride, glucose and hydroxylamine (acidosis). They did not study the change in the blood Ca and in the blood P, however, and thus they were also not able to prove anything with regard to the functional increase in the expanded (enlarged) parathyroid glands. It is known that in certain cases of chronic kidney insufficiency there occurs a secondary hyperplasia and an increase in the function of the parathyroid glands.

which can then cause characteristic bone changes. The bone system of our laboratory animals, after supplementing with newer tests, was subjected to a histological, x-ray and chemical examination. The results of this examination will be given in another article.

The enlargement and the histological picture of the parathyroid glands as well as the increase in the serum Ca and the drop in the inorganic blood P content point to the fact that the function of the parathyroid glands which expanded under the effect of the acidotic treatment increased.

In the evaluation of our experimental results, however, it is necessary to take into consideration that the blood Ca and P content is affected not only by the parathyroid glands but also by the hypophysis, (pararenals) and ovaries.

Koster and Geesink, as well as Vernetti and Gegerly observed in dogs, and Riddle in pigeons, and Hogben, Charles and Slome as well as Schapiro and Zwarenstein in frogs, the decrease in the blood Ca after hypophysectomy. Vernetti and Gegerly also determined a parathyroid atrophy and they attributed this to a lack of the hypophyseal function. Perla and Sandberg, as well as Collip, detected in hypophysectomized rats the negative trend of the Ca equilibrium, i.e. in those animals a Ca loss occurred. Putnam, Benedikt and Teel, and also Schapiro, observed in cats, Hoffmann and Anselmino observed in dogs, Pirolli in dogs, Pugsley and Anderson as well as Friedgood in guinea pigs and rats, Riddle in pigeons, that the blood-Ca with feeding of HVL extract the blood picture increased and at the same time there was an enlargement in the parathyroid glands. Anselmino and Hoffmann, as well as Berowitsch, found in dogs that the rise in the blood Ca with feeding of HVL extract did not occur when a removal of the parathyroid gland was performed in advance . This indicates that the hypophysis extract causes the blood Ca to increase by increasing the function of the parathyroid glands. Other researchers indicated that the HVL extract also causes a Ca increase in the blood of animals which have been subjected to removal of the hypophysis (hypophysectomy) (Schapiro and Zwarenstein, Charles, as well as Hogben, Charles and Slome).

Bernabeo and Parra observed in one-sided (partial) extirpation of the pararenals a decrease in the blood Ca. Others, in contrast, found that after both-sided (bilateral) removal of the pararenals there was an increase in the Ca content of the blood (Hastings and Compere, Lucas, as well as Rogoff and Stewart, Suginato; Swingle and Pfiffner, Zwarenstein, Amantia). This agrees with the fact that Geene, as well as Sears, and furthermore Thaddea and Auersbach in certain groups of Addison's disease also found an increase in the blood serum Ca content. Leicher, Fiandaca , as well as Mirvish and Bosmann observed after administration of pararenal cortex hormone preparations a decrease in the blood serum Ca content.

Lorincz, Santha and Szabo found in healthy men and women as well as in women in the 2nd and 3rd months of pregnancy a small degree of decrease in the serum Ca under the effect of the administration over a period of ten days of desoxycorticosterone acetate. Thaddea was able to prevent the increase in the blood serum Ca in adrenalectomized animals and in Addison disease by administering pararenal cortex hormone. Fiandaca and Capizzi were able to reduce the serum Ca content in Addison diseases by means of the"pararenal"cortex extract(translator: probably suprarenal cortex).

Other scientists observed after hypophysectomy a decrease in the inorganic P content in the blood (Anderson and Oestter, Jones and Shinowara, Li, Geschwind and Evans). Li, Geschwind and Evans found that the injection of the growth hormone of the hypophysis in hypophysectomized rats prevented the decrease in the inorganic serum P and even increased the P level above that of the control animals. Forsham and associates observed in normal humans after a large dose of ACTH (adrenocorticotropic hormone) an increase in the inorganic serum P content . Tornblom found a decrease in inorganic P in hypophysectomized rabbits, but after ACTH was administered an increase took place. Tornblom fed HVL extract to hypophysectomized and thyreoparathyroidectomized rabbits and he observed, along with an increase in the inorganic serum P a decrease in the serum Ca. Similar Ca and P changes occurred when a pararenal cortex extract was fed to such rabbits. Tornblom attributed this effect of the HVL extract partially to the growth hormone and also partially to the ACTH which comes into play on the course through the suprarenal glands .

As to the role of the ovaries, the observations indicate that the blood Ca increases considerably in birds during the laying time (Riddle and Reichart, Pfeifer and Gardner, Zondek and Marks) and this is taken to be a typical estrogen action. An increase in the blood C content could also be determined in mice, rats and dogs (Riddle and Reichart, Anselmino and Hoffmann). This increase does not occur, however, in the case of parathyroidectomized animals. This indicates that the estrogen exerts its action on its way through the parathyroid glands. In agreement with this, Dustin and Bullough observed in the parathyroid glands of estrogen-treated mice an increase in the mitotic cell division phenomena. In our opinion the follicle hormone also exerts its effect on the parathyroid glands on its way through the hypothalamus-hypophysis system.

In accordance with the data given above (in the medical literature) the Ca reduction and the P rise in the 1st week of the acidotic treatment could be explained by the increased function of the HVL. At this time the ACTH forms to a greater extent and through the increase in the function of the suprarenal cortex this causes the rise in P and the drop in Ca. This agrees well with the earlier finding of Fazekas, namely that under the effect of acidotic treatment not only do the parathyroid glands become larger but also the ovaries, the suprarenal glands and the hypophysis and they cause an increased

function. It appears that in the 1st week of treatment the parathyreotropic hormone is still not increased to the amount which would correspond to the increased demands and therefore a transition hypo function of the parathyroid glands occurs at that time (Ca decrease, P increase). In the 2nd and 3rd weeks of the acidotic treatment, on the other hand, and even later for two weeks after the treatment has been discontinued, there is an increase in Ca but a decrease in P, which indicates that the increased parathyreotropic hormone formation of the hypophysis causes at this time an increase in the function of the parathyroid glands and later even an enlargement, as was the case for Anselmino et al. after administering parathyreotrophic hormone. Some researchers hold that the existence of the parathyreotrophic hormone is not a proven fact. The explanation of this problem, however, would go beyond the scope of our work and therefore we would not like to become concerned with it at this time. Our results lead us to hope that perhaps the reduced function of the parathyroid glands can be made normal and that the pathological conditions which are caused by the reduced function can be eliminated.

SUMMARY

1. The author treated 72 male and female rabbits (in groups of 6 animals each) twice a day with acidotis-producing compounds for a period of time varying from 5 to 26 months in length. A period of three weeks of treatment was always followed by a pause in the treatment lasting 2 weeks. Afterwards, however, the treatment was continued in the same way. The Ca of the blood serum and the inorganic P content of the total blood were determined weekly.

2. After the first week of treatment in the 1st treatment period the serum Ca decreased add the inorganic P of the blood increased, however. In the 2nd and 3rd weeks of the treatment, on the other hand, there occurred a gradual increase in the serum Ca and a reduction in the inorganic P. During the treatment pause the serum Ca decreased gradually in comparison with the values of the 3-week treatment and even after the two-week pause its value was higher than the normal initial value. The inorganic P content of the blood increased once again during the first treatment pause above the normal initial value.

3. During the course of the next treatment period the serum Ca increased already during the first week of treatment and still more during the second and third weeks. The inorganic P content, however, decreased. The Ca increase and the P decrease were also noticed during the treatment pauses, although to a smaller extent than during the time of the treatment. The serum Ca turned out to be greater and the inorganic P content of the blood smaller, however, than before the treatment.

4. In agreement with our earlier experiments, there was a 160 to 277% enlargement of the parathyroid glands of the animals. Histologically there was an enrichment in blood, an increase and enlargement of the light main cells as well as nucleus division phenomena, i.e. the signs of a hyperfunction.

5. Since the Ca and P metabolism of the organism is primarily controlled by the functioning of the parathyroid glands, the experiments carried out by the author indicate that the function of the parathyroid glands at the time of the Ca decrease and P increase in the first week of treatmentdecreases temporarily. During the course of the further treatment at the time of the Ca increase and P decrease, however, it (the function) increases. The function of the parathyroid glands can therefore be affected in two ways by means of the acidotic treatment: 1. in the 1st week of treatment it can be reduced and from the second week on it can be increased. It is to be assumed that the function disturbances of the parathyroid glands could perhaps be favorably influenced through acidotic treatment.

figure on pp. 54 and 55

1= no.
2= chemicals which were used
3= no. of animals
4= mg % of Ca and P
5= pre-treatment
6= after
7= after
8= 1 2 3 weeks of treatment
9= 1 and 2 week treatment pause
10= after 3 weeks of treatment
11= after
12= after
13 = after
14= after
15= after
16= 1 2 3 weeks of treatment
17= 1 2 weeks of treatment pause
18= 1 2 3 weeks of treatment
19= 1 2 weeks of treatment pause
20= 1 2 3 weeks of treatment
21= 1 2 weeks of treatment pause
22= controls
23= 1 ammonium chloride
24= 2 ammonium sulphate
25= 3 sodium dihydr. phosphate
26= 4 hydrochloric acid
27= 5 lactic acid
28=6 acetic acid
29= 7 ammonium hydrophosphate
30= 8calcium chloride
31= 9 ammonium acetate
32= 10 ammonium lactate
33 = 11 Mixture of no. 1,4,5,7 8 and 9
34= Mixture of No. 1,4,5,7, 8 and 9

Fazekas-'54
Bib No. 519

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Endokrinologie 32:45-57. 1954.

Aus dem Insittut für gerichtliche Medizin der Universität Szeged, Ungarn
(Direktor: Prof. Dr. I. Gy. Fazekas)

Beeinflussung der Nebenschilddrüsenfunktion (Serum-Ca u. -P) durch azidotische Verbindungen

Von I. Gy. Fazekas

Mac Callum und Voegtlin wiesen bei Hunden nach Entfernung der Nebenschilddrüsen die Verminderung des Blut-Ca-Gehaltes nach. Greenwald sowie Hastings und Murray, Reed, Zachy und Payte beobachteten hingegen, daß der P-Gehalt des Blutes bei solchen Tieren zunimmt. Collip u. a. stellten fest, daß sich nach Einspritzung von Nebenschilddrüsenextrakten (Parathormon) der Ca-Gehalt des Blutserums so bei normalen wie bei parathyreoidektomisierten Hunden und Katzen vermehrt, der anorganische P-Gehalt sich aber vermindert. Es ist bekannt, daß bei Menschen, die an einer, durch Vergrößerung und gesteigerte Funktion der Nebenschilddrüsen bedingten Recklinghausen-Krankheit (Osteitis fibrosa generalisata) leiden, der Ca-Gehalt des Blutserums erheblich ansteigt, der anorganische P-Gehalt aber wesentlich abfällt. Gleiche Veränderungen des Serum-Ca- und P-Gehaltes wurden auch bei akutem Hyperparathyreoidis-

mus beobachtet (Olivier, Smith, Hanes). In verschiedenen Fällen des Hypoparathyreoidismus vermehrt sich das Serum-P auch bei Menschen neben Abnahme des Ca-Gehaltes des Blutserums. Die Nebenschilddrüsen spielen also in der Regulierung des Ca- und P-Stoffwechsels im Organismus eine Rolle ersten Ranges.

Fazekas stellte fest, daß sich die Nebenschilddrüsen von Kaninchen durch dauerhafte Verabreichung von azidotisch wirkenden Verbindungen um 171—294 % vergrößern. Der histologische Befund weist auf die gesteigerte Funktion der so vergrößerten Nebenschilddrüsen hin, das kann aber allein auf Grund des histologischen Bildes nicht entschieden werden. Um die Funktion der so vergrößerten Nebenschilddrüsen zu untersuchen, wurden neue Tierversuche angestellt. Da nach den Schrifttumsangaben zwischen dem Ca- und anorganischen P-Gehalt des Blutes sowie der Nebenschilddrüsenfunktion ein enger Zusammenhang besteht, war es anzunehmen, daß die systematische Untersuchung des Blut-Ca- und -P-Gehaltes bei Tieren, die mit azidotisch wirkenden, eine Nebenschilddrüsenhypertrophie verursachenden Verbindungen behandelt wurden, über die Funktion der Nebenschilddrüsen Auskunft geben werde.

In unseren Versuchen wurden 72 gleichartige (Chinchilla) und gleichaltrige (8—10 Monate alt) weibliche und männliche Kaninchen mit 2500 bis 3000 g Anfangsgewicht 5—26 Monate lang mit azidotisch wirkenden Verbindungen behandelt, 50 Tiere aber ohne Behandlung dienten zu gleicher Zeit als Kontrollen. Die Tiere wurden mit gleicher Menge von Hafer und trockenem Klee genährt. — Es wurden folgende Verbindungen verabreicht: Ammonium-chlorid, -sulfat, -hydrophosphat, -azetat, -laktat, Natriumdihydrophosphat, Acidum-hydrochloricum, -lacticum, -aceticum, Kalziumchlorid. — Mit jeder Verbindung wurden je 6 Tiere behandelt. Jedes Tier bekam 2 mal täglich 3 Wochen hindurch, in wöchentlich zunehmenden Dosen ,0,1—0,2 g/1 kg Körpergewicht von der entsprechenden Verbindung, in 50—80 ccm Trinkwasser gelöst. Danach folgte eine 2wöchige behandlungsfreie Periode. So wiederholten sich Behandlung und Behandlungspause während 5—26 Monate. 6 Kaninchen wurden auf gleiche Art und Weise mit folgendem Verbindungsgemisch behandelt: 30 g Ammoniumchlorid, 5 g Ammoniumazetat, 5 g Ammoniumhydrophosphat, 10 g Kalziumchlorid, 5 g Acidum hydrochloricum, 5 g Acidum lacticum ad 1000 g aquae fontis. In 1 ccm dieser Lösung befindet sich 0,06 g Wirkstoffgemisch. Diese Tiere standen 16 Monate hindurch unter Behandlung. 6 andere Kaninchen wurden mit gleichem Verbindungsgemisch auf gleiche Weise, aber 26 Monate lang behandelt.

Bei den Versuchstieren wurden Ca-Gehalt des Blutserums und anorganischer P-Gehalt des Gesamtblutes vor, während und nach der Behandlung 2 Wochen lang wöchentlich bestimmt. Auch die Kontrollkaninchen wurden denselben Untersuchungen unterworfen. Der Ca-Gehalt wurde in 1 ccm Blutserum mit der oxydimetrischen Methode von Clark und Collip, der anorganische P-Gehalt des Blutes aber in 0,2 ccm Voll-

blut mit der kolorimetrischen Methode von Kuttner und Cohen mittels eines Stankoschen Elektrophotometers bestimmt. Der Mittelwert von je 2 parallel ausgeführten Bestimmungsgängen ergab immer das Resultat. Der Kürze wegen werden die Einzelangaben nicht mitgeteilt, sondern in beiliegender Tabelle lediglich die Mittelwerte der einzelnen Tiergruppen angeführt. Da bei der 11. Tiergruppe während der ersten 12 Monate die gleichen charakteristischen Serum-Ca- und -P-Veränderungen vorgefunden wurden als bei den 1.—10. Tiergruppen, werden diese Daten vereinzelt nicht besprochen, sondern nur die Ergebnisse der XI., XII. und XIII. Behandlungsperiode (13—16 Monate) angegeben. Bei der mit Verbindungsgemisch behandelten 12. Tiergruppe zeigte der Blutserum-Ca- und anorganische P-Gehalt des Blutes im Laufe der 18monatigen Behandlung Veränderungen gleichen Charakters wie bei den vorigen Gruppen. Bei der 12. Tiergruppe wurde nach 18monatiger Behandlung eine Pause von 3 Monaten eingeschaltet. Während dieser Zeit kehrte das Serum-Ca und anorganische P auf den normalen Ausgangswert zurück. Nachdem wurde die Behandlung der Tiere während 5 Monate (4 Behandlungsperioden) auf gleiche Art fortgesetzt.

Die ausführliche Auswertung der verschiedenen Ca- und P-Veränderungen soll hier wegbleiben, als Beispiel möchten wir nur die Auswertung der Behandlung mit Ammoniumsulfat sowie der 26monatigen Behandlung mit dem Verbindungsgemisch mitteilen.

Ammoniumsulfat-Behandlung

I. Behandlungsperiode. — Serum-Ca: Vor der Behandlung 14,92 mg-%. Nach 1wöchiger Behandlung 9,39 mg-%, was im Verhältnis zum normalen Anfangswert eine Senkung von 5,53 mg-% (37,06 %) bedeutet. Nach 2wöchiger Behandlung: 17,35 mg-%, d. h. um 2,45 mg-% (16,4 %), nach 3wöchiger Behandlung: 18,45 mg-%, d. i. um 3,53 mg-% (23,6 %) mehr. Nach 1wöchiger Behandlungspause 19,88 mg-%, nach 2wöchiger Pause 16,42 mg-%, was im Verhältnis zum normalen Anfangswert um 4,69 mg-% (31,9 %), bzw. 1,52 mg-% (10,0 %) mehr ist. — Blut-P: Vor der Behandlung 3,31 mg-%. Nach 1wöchiger Behandlung 4,13 mg-%, was einen Anwuchs von 0,82 mg-% (26,1 %) bedeutet. Nach 2wöchiger Behandlung 2,19 mg-%, nach 3wöchiger Behandlung 1,99 mg-%, d. h. um 1,12 mg-% (33,8 %) bzw. 1,32 mg-% (39,9 %) weniger als der normale Anfangswert. Nach 1wöchiger Behandlungspause 3,62 mg-%, nach 2wöchiger Pause 4,97 mg-%, d. i. um 1,63 mg-% (81,9 %) bzw. 2,98 mg-% (149,7 %) höher als nach 3wöchiger Behandlung und um 0,31 mg-% (9,3 %) bzw. 1,66 mg-% (50,1 %) höher als der normale Anfangswert.

II. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 16,24 mg-%, nach 2wöchiger Behandlung 21,44 mg-%, nach 3 Wochen 23,11 mg-%, d. i. um 2,32 mg-% (15,55 %), um 6,52 mg-% (43,6 %) bzw. um 8,19 mg-% (54,9 %) mehr als der normale Anfangswert und um 5,02 mg-% (30,5 %) bzw. um 6,69 mg-% (40,7 %) höher als der

Wert nach 2wöchiger Pause der vorigen Behandlungsperiode. — Nach 1wöchiger Behandlungspause 20,77 mg-%, nach 2wöchiger Pause 16,26 mg-%, d. i. um 5,85 mg-% (39,2 %) bzw. 1,34 mg-% (8,9 %) höher als der normale Anfangswert. Das bedeutet im Verhältnis zum anfänglichen Behandlungswert einen Abstieg von 2,34 mg-% (10,1 %) bzw. 6,85 mg-% (29,9 %). — Blut-P: Nach 1wöchiger Behandlung 2,35 mg-%, nach 2wöchiger Behandlung 1,44 mg-%, nach 3wöchiger Behandlung 1,62 mg-%, was um 0,96 mg-% (29,0 %), 1,87 mg-% (56,5 %) bzw. 1,69 mg-% (51,0 %) niedriger als der normale Anfangswert. Nach einer Woche Pause 2,14 mg-%, nach 2wöchiger Behandlungspause 3,2 mg-%, d. h. um 0,17 mg-% (5,1 %) bzw. 0,11 mg-% (3,3 %) weniger als der normale Anfangswert.

III. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 16,9 mg-%, nach 2 Wochen 20,51 mg-%, nach 3wöchiger Behandlung 22,66 mg-%. Diese Werte sind um 1,98 mg-% (13,3 %), 5,69 mg-% (37,6 %) bzw. 7,77 mg-% (51,8 %) höher als der normale Anfangswert. Nach 1wöchiger Behandlungspause 20,1 mg-%, nach 2wöchiger Pause 17,23 mg-%, was um 5,18 mg-% (35,3 %) bzw. 2,21 mg-% (14,8 %) höher ist als der normale Anfangswert, um 2,56 mg-% (11,2 %) bzw. 5,43 mg-% (23,9 %) hingegen niedriger als der Wert nach 3wöchiger Behandlung dieser Periode. — Blut-P: Nach 1wöchiger Behandlung 2,59 mg-%, nach 2wöchiger Behandlung 2,22 mg-%, nach 3 Wochen Behandlung 1,8 mg-%. Diese Werte sind um 0,62 mg-% (15,7 %), 1,09 mg-% (32,9 %) bzw. 1,51 mg-% (45,6 %) niedriger als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 2,66 mg-%, nach 2wöchiger Pause 3,46 mg-%, d. i. um 0,65 mg-% (16,6 %) weniger bzw. um 0,15 mg-% (4,5 %) mehr als der normale Anfangswert. Der P-Wert kehrte also etwa aufs Normale zurück.

IV. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 18,31 mg-%, nach 2wöchiger Behandlung 20,42 mg-%, nach 3wöchiger Behandlung 22,14 mg-%, d. i. um 3,39 mg-% (22,7 %) 5,50 mg-% (36,8 %) bzw. 7,22 mg-% (48,2 %) höher als der normale Anfangswert und um 1,08 mg-% (6,2 %), 3,19 mg-% (18,5 %) bzw. 4,91 mg-% (28,5 %) höher als der Wert nach der 2wöchigen Behandlungspause der vorigen Behandlungsperiode. — Nach 1wöchiger Behandlungspause 18,60 mg-%, d. i. um 3,68 mg-% (24,6 %) höher als der normale Anfangswert, aber um 3,54 mg-% (15,9 %) niedriger als nach der 3wöchigen Behandlung dieser Periode. — Blut-P: Nach 1wöchiger Behandlung 2,33 mg-%, nach 2wöchiger Behandlung 2,32 mg-%, nach 3wöchiger Behandlung 1,75 mg-%, was um 0,98 mg-% (29,6 %), 0,99 mg-% (29,9 %) bzw. 1,56 mg-% (47,1 %) weniger als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 2,63 mg-%, d. i. um 0,82 mg-% (45,2 %) mehr als der Wert nach 3wöchiger Behandlung dieser Periode, aber um 0,68 mg-% (20,5 %) weniger als der normale Anfangswert.

12. Behandlungsgruppe (Verbindungsgemisch)

XVIII. Behandlungsperiode. Serum-Ca: Vor der Behandlung 15,81 mg-%, nach 1wöchiger Behandlung 11.92 mg-%, was im Verhältnis zum normalen Anfangswert einen Abstieg von 3.89 mg-% (24.6 %) bedeutet. Diese Abnahme war aber nicht so hochgradig als bei Beginn der ersten Behandlung (37.2 %). Nach 2wöchiger Behandlung 17.81 mg-%, nach 3wöchiger Behandlung 22.86 mg-%, d. i. um 1.0 mg-% (6,3 %) bzw. 7,05 mg-% (44,59 %) mehr als der normale Anfangswert. Nach 1wöchiger Behandlungspause 20.27 mg-%, nach 2wöchiger Pause 16.35 mg-%, d. i. um 2.59 mg-% (11.3 %) bzw. 6.51 mg-% (29.3 %) weniger als nach 3wöchiger Behandlung dieser Periode, aber um 4,46 mg-% (28,2 %) bzw. 0,54 mg-% (3.4 %) mehr als der normale Anfangswert. — Blut-P: Vor der Behandlung 3.72 mg-%. Nach 1wöchiger Behandlung 4.58 mg-%, d. i. um 0.86 mg-% (23.1 %) höher als der normale Anfangswert. Nach 2wöchiger Behandlung 2.77 mg-%, nach 3wöchiger Behandlung 1.88 mg-%, d. i. um 0,95 mg-% (25.5 %) bzw. 1.84 mg-% (49.4 %) weniger als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 2.87 mg-%, nach 2wöchiger Behandlungspause 3.07 mg-%, d. i. um 0,85 mg-% (22.8 %) bzw. 0,65 mg-% (17,4 %) weniger als der normale Anfangswert, aber um 0,99 mg-% (52,6 %) bzw. 1,19 mg-% (63.1 %) mehr als nach der 3wöchigen Behandlung dieser Periode.

XIX. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 15,66 mg-%, d. i. fast gleich mit dem normalen Anfangswert. Ein Beweis dafür, daß der Organismus die Ca-vermindernde Wirkung der Behandlung schon abzuwehren vermag. Nach 2wöchiger Behandlung 19,84 mg-%, nach 3wöchiger Behandlung 22.63 mg-%, das im Verhältnis zum normalen Anfangswert eine Ca-Zunahme von 4,03 mg-% (23,4 %) bzw. 6,82 mg-% (43,1 %) bedeutet. — Nach 1wöchiger Behandlungspause 20,60 mg-%, nach 2wöchiger Pause 16,41 mg-%, was im Verhältnis zum Wert nach der 3wöchigen Behandlung dieser Periode einer Senkung von 2,03 mg-% (8,4 %) bzw. 6,22 mg-% (27,4 %) entspricht, aber um 4,79 mg-% (30,3 %) bzw. 60 mg-% (3,7 %) mehr beträgt als der normale Anfangswert. — Blut-P: Nach 1wöchiger Behandlung 3,23 mg-%, nach 2wöchiger Behandlung 2,44 mg-%, nach 3wöchiger Behandlung 2,30 mg-%, d. i. um 0,49 mg-% (13,1 %), 1,28 mg-% (34,4 %) bzw. 1.42 mg-% (38.1 %) weniger als der normale Anfangswert. — Nach einer Woche Pause 3,05 mg-%, nach 2wöchiger Behandlungspause 3,39 mg-%, d. i. um 0,67 mg-% (18.0 %) bzw. 0,33 mg-% (8,8 %) weniger als der normale Anfangswert, aber um 0,75 mg-% (32,6 %) bzw. 1,09 mg-% (47,3 %) mehr als nach der 3wöchigen Behandlung dieser Periode.

XX. Behandlungsperiode. — Serum-Ca: Nach 1wöchiger Behandlung 16,93 mg-%, nach 2wöchiger Behandlung 19,88 mg-%, nach 3wöchiger Behandlung 21,99 mg-%, d. h. um 1,12 mg-% (7,0 %), 4,07 mg-% (26,3 %) bzw. 6,18 mg-% (39,0 %) höher als der normale An-

fangswert. — Nach 1wöchiger Behandlungspause 20,06 mg-%, nach 2wöchiger Pause 17,21 mg-%, d. i. um 1,93 mg-% (8,7 %) bzw. 4,78 mg-% (21,1 %) weniger als nach der 3wöchigen Behandlung dieser Periode, aber um 1,25 mg-% (26,8 %) bzw. 1,40 mg-% (9,4 %) mehr als der normale Anfangswert. Blut-P: Nach 1wöchiger Behandlung 2,61 mg-%, nach 2wöchiger Behandlung 2,28 mg-%, nach 3wöchiger Behandlung 2,10 mg-%, d. i. um 1,11 mg-% (29,8 %), 1,51 mg-% (41,3 %) bzw. 1,62 mg-% (43,5 %) weniger als der normale Anfangswert. Nach 1wöchiger Behandlungspause 2,53 mg-%, nach 2wöchiger Pause 2,75 mg-%, d. i. um 1,19 mg-% (31,9 %) bzw. 0,97 mg-% (26,0 %) weniger als der normale Anfangswert, aber um 0,43 mg-% (20,4 %) bzw. 0,65 mg-% (30,9 %) mehr als nach der 1wöchigen Behandlung dieser Behandlungsperiode.

X. 1. Behandlungsperiode. Serum-Ca: Nach 1wöchiger Behandlung 17,22 mg-%, gleicher Wert als nach 2wöchiger Behandlungspause der vorigen Periode, was auch bedeutet, daß der Organismus die Ca-vermindernde Wirkung der 1wöchigen Behandlung zu kompensieren vermag. Nach 2wöchiger Behandlung 19,38 mg-%, nach 3wöchiger Behandlung 22,05 mg-%, was um 3,57 mg-% (28,9 %) bzw. 6,24 mg-% (39,4 %) höher ist als der normale Anfangswert. — Nach 1wöchiger Behandlungspause 18,94 mg-%, d. i. um 3,11 mg-% (19,7 %) weniger als nach der 3wöchigen Behandlung dieser Periode, aber um 3,13 mg-% (19,8 %) mehr als der normale Anfangswert. — Blut-P: Nach 1wöchiger Behandlung 2,72 mg-%, nach 2wöchiger Behandlung 2,28 mg-%, nach 3wöchiger Behandlung 1,78 mg-%, d. i. um 1,0 mg-% (26,5 %), 1,44 mg-% (38,6 %) bzw. 1,94 mg-% (52,1 %) weniger als der normale Anfangswert. Nach 1wöchiger Behandlungspause 2,65 mg-%, d. i. um 1,07 mg-% (28,7 %) weniger als der normale Anfangswert, aber um 0,87 mg-%, (49,4 %) höher als nach 3wöchiger Behandlung dieser Periode.

Auch die Untersuchungen mit den übrigen Verbindungen zeigten gleiche Ca- und P-Veränderungen. Bei den Kontrollkaninchen konnten aber keine ähnlichen und gleichmäßigen Veränderungen des Serum-Ca und anorganischen P festgestellt werden.

Die Ergebnisse zeigen also, daß unter dem Einfluß der verwendeten azidotischen Verbindungen, nach der erstwöchigen Behandlung der ersten Periode, das Serum-Ca wesentlich (24—37 %) unter den Anfangswert sinkt, das Blut-P aber zu gleicher Zeit 20—42 % über den normalen Wert ansteigt. Das steht im Einklang mit unserer früheren Feststellung, daß unter dem Einfluß einer Dosis von NH_4OH bzw. NaOH das Serum-Ca abnimmt, das Blut-P aber zunimmt. In der 2. Behandlungswoche tritt eine Steigerung des Serum-Ca (10—35 %) und ein Absinken des Blut-P (11—36 %) auf, was nach 3wöchiger Behandlung sein Maximum erreicht (Ca: 20—51 %; P: 24—50 %). Nach der 1- und 2wöchigen Behandlungspause nimmt das Serum-Ca im Verhältnis zum Wert der 3wöchigen Behandlung allmählich ab, aber bleibt meistens auch noch nach 2 Wochen höher (7—21 %) als der normale Anfangswert, das Blut-P erhebt sich hin-

gegen wiederholt über das normale (50—67 %). Während der folgenden Behandlungsperioden sinkt das Serum-Ca nach der erstwöchigen Behandlung niemals unter den normalen Wert, sondern bleibt auch zu dieser Zeit um 12—22 % höher, das Blut-P aber fällt wesentlich (20—30 %) unter den Normalwert. In den 2. und 3. Behandlungswochen ist so die Zunahme des Serum-Ca (50—65 %) wie die Abnahme des Blut-P (30—56 %) noch deutlicher als zur selben Zeit der ersten Behandlungsperiode. Im Laufe der 2wöchigen Behandlungspausen nimmt das Serum-Ca allmählich ab, das Blut-P aber zu, im Verhältnis zum Wert der 3wöchigen Behandlung. Das Serum-Ca ist aber auch noch dann höher, das anorganische P aber niedriger als der normale Anfangswert, d. h. das Serum-Ca stellt sich auf höher, das Blut-P aber auf niedriger ein, als es vor dem Behandlungsbeginn stand.

Bei den mit dem Verbindungsgemisch 16 Monate lang behandelten Kaninchen und bei denen, die nach einer 18monatigen Behandlung und darauffolgenden 3monatigen Behandlungspause aufs neue behandelt wurden (insgesamt 26 Monate), konnten Ca- und P-Veränderungen gleichen Charakters beobachtet werden.

Nach Tötung der Versuchstiere wurde eine 160,8—277,7 %ige Nebenschilddrüsenvergrößerung (Mittelwerte!) vorgefunden. In den Nebenschilddrüsen konnten histologisch, im Einklang mit unseren früheren Untersuchungen, Blutfülle, Vermehrung und Vergrößerung der hellen Zellen, Kernteilungserscheinungen, also die Anzeichen einer Hyperfunktion, beobachtet werden.

Andere Verfasser verzeichneten gleichfalls eine Nebenschilddrüsenvergrößerung bei dauerhafter Verabreichung von Ammonium chlorid, Glukose und Hydroxylamin (Azidose). Die Veränderung des Blut-Ca und -P untersuchten sie aber nicht und so konnten sie auch die Funktionssteigerung der vergrößerten Nebenschilddrüsen nicht beweisen. Es ist bekannt, daß in gewissen Fällen der chronischen Niereninsuffizienz eine sog. sekundäre Hyperplasie und gesteigerte Funktion der Nebenschilddrüsen entsteht, was dann charakteristische Knochenveränderungen verursachen kann. Das Knochensystem unserer Versuchstiere wurde, nach Ergänzung mit neueren Versuchen, einer histologischen, röntgenologischen und chemischen Untersuchung unterzogen. Über das Ergebnis wird in einer anderen Arbeit berichtet.

Die Vergrößerung und das histologische Bild der Nebenschilddrüsen sowie der Anstieg des Serum-Ca und das Sinken des anorganischen Blut-P-Gehaltes weisen darauf hin, daß sich die Funktion der unter dem Einfluß der azidotischen Behandlung vergrößerten Nebenschilddrüsen steigerte.

Bei der Auswertung unserer Versuchsergebnisse muß aber in Betracht gezogen werden, daß der Blut-Ca- und -P-Gehalt nicht nur durch die Nebenschilddrüsen, sondern auch durch die Hypophyse, Nebennieren und Ovarien beeinflußt werden.

Köster und Geesink sowie Vernetti, Gegerly beobachteten an Hunden, Riddle an Tauben, Hogben, Charles und Slome sowie Schapiro und Zwarenstein an Fröschen die Abnahme des Blut-Ca nach Hypophysektomie. Vernetti, Gegerly stellten dabei noch eine Nebenschilddrüsenatrophie fest und führten diese auf den Mangel der Hypophysenfunktion zurück. Perla und Sandberg sowie Collip wies bei hypophysektomisierten Ratten das Negativwerden des Ca-Gleichgewichtes nach, d. i. bei solchen Tieren tritt ein Ca-Verlust auf. Putnam, Benedikt und Teel, ferner Schapiro beobachteten an Katzen, Hoffmann und Anselmino an Hunden, Pirolli an Kaninchen, Pugsley und Anderson sowie Friedgood an Meerschweinchen und Ratten, Riddle an Tauben, daß sich das Blut-Ca bei Verabreichung von HVL-Extrakt erhöht und auch die Nebenschilddrüsen sich zu gleicher Zeit vergrößern. Anselmino und Hoffmann sowie Berowitsch fanden bei Hunden, daß der Anstieg des Blut-Ca bei Verabreichung von HVL-Extrakt nicht eintrifft, wenn vorher an den Tieren eine Nebenschilddrüsenentfernung durchgeführt worden ist. Das weist darauf hin, daß das Hypophysenextrakt den Anstieg des Blut-Ca durch die Steigerung der Nebenschilddrüsenfunktion hervorruft. Andere Forscher wiesen nach, daß das HVL-Extrakt auch bei hypophysektomierten Tieren eine Ca-Steigerung des Blutes hervorruft (Schapiro u. Zwarenstein, Charles sowie Hogben, Charles u. Slome).

Bernabeo und Parra beobachteten nach einseitiger Nebennierenexstirpation die Abnahme des Blut-Ca, andere stellten hingegen fest, daß nach beiderseitiger Entfernung der Nebennieren der Ca-Gehalt des Blutserums ansteigt (Hastings und Compere, Lucas sowie Rogoff u. Stewart, Suginato; Swingle u. Pfiffner, Zwarenstein, Amantia). Damit steht in Einklang, daß Geene sowie Sears, ferner Thaddea und Auersbach bei gewissen Gruppen der Addison-Kranken gleichfalls eine Steigerung des Blutserum-Ca-Gehaltes vorfanden. Leicher, Fiandaca sowie Mirvish und Bosmann beobachteten nach Darreichung von Nebennierenrindenhormonpräparaten das Sinken des Blutserum-Ca-Gehaltes. Lörincz, Sántha und Szabó fanden bei gesunden Männern und Frauen sowie bei Frauen im 2.—3. Schwangerschaftsmonat eine kleingradige Abnahme des Serum-Ca unter dem Einfluß des 10 Tage lang verabreichten Desoxycorticosteronacetat. Thaddea gelang bei adrenalektomisierten Tieren und bei Addison-Kranken den Anstieg des Blutserum-Ca durch Verabreichung von Nebennierenrindenhormon zu verhindern. Fiandaca und Capizzi konnten gleichfalls den Serum-Ca-Gehalt bei Addison-Kranken durch Nebennierenrindenextrakt vermindern.

Andere Forscher beobachteten nach einer Hypophysektomie die Abnahme des anorganischen P-Gehaltes im Blut (Anderson u. Oestter, Jones u. Shinowara, Li, Geschwind u. Evans). Li, Geschwind u. Evans wiesen nach, daß die Einspritzung des Wachstumshormons der Hypophyse bei hypophysektomierten Ratten das Absinken des anorga-

nischen Serum-P verhindert, sogar das P-Niveau über das der Kontrollen ansteigt. Forsham u. Mitarbeiter beobachteten bei normalen Menschen nach großer ACTH-Dosis den Anstieg des anorganischen Serum-P-Gehaltes. Törnblom stellte bei hypophysektomisierten Kaninchen die Abnahme des anorganischen P des Serums fest, das aber nach ACTH-Darreichung anstieg. Törnblom verabreichte hypophysektomisierten und thyreoparathyreoidektomisierten Kaninchen HVL-Extrakt und beobachtete neben Anstieg des anorganischen Serum-P die Abnahme des Serum-Ca. Gleiche Ca- und P-Veränderungen zeigten sich, wenn solchen Kaninchen ein Nebennierenrindenextrakt gegeben wurde. Törnblom führt diese Wirkung des HVL-Extraktes teils auf das Wachstumshormon, teils aber auf das ACTH zurück, das auf dem Wege über die Nebennieren zur Geltung kommt.

Auf die Rolle der Ovarien weisen die Beobachtungen hin, nach denen sich das Blut-Ca bei Vögeln während der Legeperiode stark vermehrt (Riddle u. Reichart, Pfeifer u. Gardner, Zondek u. Marks), was als typische Östrogenwirkung aufgefaßt wird. Nach Verabreichung von Östrogen konnte auch bei Mäusen, Ratten und Hunden eine Zunahme des Blut-Ca-Gehaltes festgestellt werden (Riddle u. Reichart, Anselmino u. Hoffmann), dieser Anstieg bleibt aber bei parathyreoidektomisierten Tieren weg. Diese Tatsache weist darauf hin, daß das Östrogen seine Wirkung auf dem Wege über die Nebenschilddrüsen ausübt. In Einklang damit beobachteten Dustin und Bullough in den Nebenschilddrüsen von östrogenbehandelten Mäusen die Vermehrung der mitotischen Zellteilungserscheinungen. Unserer Ansicht nach übt auch das Follikelhormon seinen Einfluß auf die Nebenschilddrüsen auf dem Wege über das Hypothalamus-Hypophysensystem aus.

Im Spiegel der obigen Schrifttumsangaben könnte das Ca-Sinken und der P-Anstieg in der 1. Woche der azidotischen Behandlung mit der gesteigerten Funktion des HVL erklärt werden. Zu dieser Zeit bildet sich das ACTH nämlich in größerem Maße und das ruft durch die Funktionssteigerung der Nebennierenrinde den P-Anstieg und die Ca-Abnahme hervor. Das ist mit der früheren Behauptung (Fazekas) gut zu vereinbaren, daß nämlich unter dem Einfluß azidotischer Behandlung nicht nur die Nebenschilddrüsen, sondern auch die Ovarien, Nebennierenrinde und Hypophyse größer werden und eine gesteigerte Funktion entfalten. Es hat den Anschein, daß das Parathyreotrophormon in der 1. Behandlungswoche von der Hypophyse noch nicht in den gesteigerten Ansprüchen entsprechender Menge gebildet wird, weshalb eine Übergangshypofunktion der Nebenschilddrüsen zu dieser Zeit entsteht (Ca-Abnahme, P-Anstieg). In der 2. und 3. Woche der azidotischen Behandlung hingegen, sogar auch später während 2 Wochen nach Abbrechung der Behandlung, nimmt das Ca zu, das P aber ab, was darauf hinweist, daß die gesteigerte Parathyreotrophormonbildung der Hypophyse zu dieser Zeit eine Funktionssteigerung der Nebenschilddrüsen, später auch ihre Vergrößerung hervorruft, wie es bei

Anselmino u. Mitarbeiter nach Verabreichung von Parathyreotrophormon der Fall war. (Einige Verfasser halten die Existenz des Parathyreotrophormons für nicht bewiesen; die Erörterung dieser Frage würde aber den Rahmen unserer Arbeit übertreten, weshalb wir uns damit nicht zu beschäftigen wünschen.) Unsere Ergebnisse lassen hoffen, daß gegebenenfalls die verminderte Funktion der Nebenschilddrüsen normal gemacht und die durch verminderte Funktion bedingten pathologischen Umstände aufgehoben werden können.

Zusammenfassung

1. Verfasser behandelte 72 männliche und weibliche Kaninchen (in Gruppen mit je 6 Tieren) 2täglich mit azidotisch wirkenden Verbindungen 5—26 Monate lang. Nach 3wöchiger Behandlung wurde immer eine 2wöchige behandlungsfreie Periode eingeschaltet, nachher aber die Behandlung auf gleiche Weise fortgesetzt. Das Ca des Blutserums und der anorganische P-Gehalt des Gesamtblutes wurden wöchentlich festgestellt.

Nr.	Verwendete Chemikalien	Zahl der Tiere	mg-% Ca und P	Vor Behandlung	I nach 1 wöchiger Behandlung	I nach 2 wöchiger Behandlung	I nach 3 wöchiger Behandlung	I nach 1 wöchiger Behandlungspause	I nach 2 wöchiger Behandlungspause
	Kontrollen	50	Ca	15,60	15,70	15,55	15,62	15,70	15,80
			P	3,65	3,65	3,50	3,70	3,60	3,72
1	Ammonium chlorid	6	Ca	14,82	9,81	16,39	17,88	19,93	17,29
			P	3,07	3,78	2,73	2,33	4,28	5,14
2	Ammonium sulfat	6	Ca	14,92	9,39	17,37	18,45	19,88	16,42
			P	3,31	4,13	2,19	1,99	3,62	4,97
3	Natr. dihydr. phosphat	6	Ca	14,19	10,27	18,78	19,57	20,41	15,55
			P	3,55	4,27	1,92	1,85	3,56	4,64
4	Acid. hydrochlor.	6	Ca	16,52	12,42	22,22	24,96	21,27	18,92
			P	3,80	5,27	2,81	2,08	2,78	3,21
5	Acid. lacticum	6	Ca	15,40	10,90	20,10	23,10	21,17	18,70
			P	3,82	5,49	2,55	1,92	2,68	3,09
6	Acid. aceticum	6	Ca	15,48	10,61	16,78	17,56	21,27	18,53
			P	3,09	4,55	2,60	2,22	4,16	4,91
7	Ammon. hydrophosphat	6	Ca	15,08	9,58	16,84	17,97	18,73	16,22
			P	3,53	3,95	1,94	1,88	3,12	5,36
8	Calcium chlorid	6	Ca	14,19	8,85	15,57	17,40	20,31	17,42
			P	3,19	3,98	2,92	2,46	4,32	5,28
9	Ammon. acetat.	6	Ca	15,38	9,96	17,05	18,14	20,09	16,51
			P	3,09	3,98	2,85	2,54	4,85	5,25
10	Ammon. lactat	6	Ca	14,95	9,79	18,52	19,50	19,82	15,70
			P	3,49	4,65	2,01	1,96	3,97	4,77
							XI		
11	Gemisch von Nr. 1, 4, 5, 7, 8 und 9	6	Ca	16,19	18,60	22,36	25,18	21,99	19,45
			P	3,86	5,05	3,38	2,00	3,11	3,71
							XVIII		
12	Gemisch von Nr. 1, 4, 5, 7, 8 und 9	6	Ca	15,81	11,92	17,81	22,86	20,27	16,35
			P	3,72	4,78	2,77	1,89	2,57	3,07

2. Nach 1wöchiger Behandlung der 1. Behandlungsperiode nahm das Serum-Ca ab, das anorganische P des Blutes aber zu. In der 2. und 3. Woche der Behandlung hingegen vermehrte sich allmählich das Serum-Ca und verminderte sich das anorganische P. Während der Behandlungspause sank das Serum-Ca allmählich im Verhältnis zu den Werten der 3wöchigen Behandlung, auch nach der 2wöchigen Pause war sein Wert höher als der normale Anfangswert. Der anorganische P-Gehalt des Blutes erhob sich während der ersten Behandlungspause wieder über den normalen Anfangswert.

3. Im Laufe der weiteren Behandlungsperiode vermehrte sich das Serum-Ca auch schon während der erstwöchigen Behandlung, noch mehr während der 2. und 3. Woche, der anorganische P-Gehalt nahm aber ab. Die Ca-Vermehrung und die P-Herabsetzung war auch während der Behandlungspausen festzustellen, obwohl in kleinerem Maße als zur Zeit der Behandlung. Das Serum-Ca stellte sich also höher, der anorganische P-Gehalt des Blutes aber niedriger ein als vor der Behandlung.

Behandlungsperiode:																			
II					III					IV									
nach			nach		nach			nach		nach			nach					
1	2	3	1	2	1	2	3	1	2	1	2	3	1	2					
wöchiger Behandlung			wöchiger Behandlungspause		wöchiger Behandlung			wöchiger Behandlungspause		wöchiger Behandlung			wöchiger Behandlungspause						
15,60	15,82	15,51	15,42	15,70	15,45	15,80	15,50	15,81	16,00	15,70	15,62	15,90	15,45	15,90					
3,80	3,73	3,80	3,70	3,65	3,50	3,55	3,50	3,60	3,62	3,55	3,63	3,70	3,73	3,69					
16,70	21,41	24,02	21,38	16,17	16,78	21,12	22,74	20,26	17,44	17,68	20,44	22,31	19,37	18,50					
2,43	1,41	1,89	3,01	3,22	2,84	2,34	2,18	2,79	3,44	2,50	2,28	1,77	2,82	3,20					
16,24	21,44	23,11	20,77	16,26	16,90	20,51	22,66	20,10	17,23	18,31	20,42	22,14	18,60	17,90					
2,35	1,44	1,02	3,14	3,20	2,59	2,22	1,80	2,66	3,46	2,33	2,32	,75	2,68	2,81					
15,01	22,78	23,41	21,19	17,15	17,65	21,34	22,27	20,31	17,37	18,39	20,67	22,16	18,67	18,08					
2,54	1,57	1,84	2,81	3,07	2,53	2,16	1,96	2,78	3,81	2,20	2,25	1,69	2,72	2,90					
19,02	21,75	24,02	21,60	20,02	19,17	22,02	23,97	21,30	20,30	19,30	22,37	24,70	21,70	20,10					
3,43	2,69	2,05	2,44	2,91	2,50	2,26	1,94	2,50	3,19	2,67	2,38	2,01	2,41	2,85					
17,67	20,65	23,00	20,27	19,10	18,40	21,40	23,07	20,92	19,40	18,50	21,20	23,70	21,20	19,80					
3,36	2,68	2,45	2,55	2,81	2,64	2,22	2,00	2,44	3,14	2,73	2,45	2,12	2,48	2,82					
16,59	22,20	23,36	20,17	15,14	16,45	21,34	22,57	20,62	17,34	17,87	19,86	22,76	19,37	19,20					
2,24	1,27	1,50	2,76	3,20	2,75	2,61	2,42	2,87	3,73	2,47	2,41	1,73	2,90	3,12					
15,39	22,55	22,97	21,08	15,42	16,43	21,34	22,32	20,65	17,62	17,92	19,68	21,86	18,62	18,20					
2,27	1,50	1,87	3,31	3,22	2,79	2,35	2,15	2,59	3,49	2,16	2,70	1,73	2,44	2,90					
16,80	21,43	25,24	22,60	17,18	17,60	22,75	23,80	21,35	18,35	18,10	21,30	22,85	21,25	20,00					
2,60	1,58	1,25	3,30	3,10	2,80	2,85	2,00	2,75	3,40	2,45	2,10	1,82	2,90	2,85					
16,99	21,89	23,16	21,37	16,34	16,68	22,01	22,91	19,70	16,74	17,37	19,80	22,21	19,10	18,60					
2,59	1,18	1,37	3,10	3,27	2,74	2,25	2,25	2,68	3,57	2,53	2,20	1,61	2,77	2,90					
14,67	21,69	22,40	21,19	17,21	17,71	19,99	22,68	19,83	17,19	17,92	21,60	22,31	17,96	17,60					
2,75	1,36	1,38	2,72	2,84	2,58	2,25	2,01	2,68	4,42	2,32	2,17	1,73	2,69	2,85					
XII					XIII														
20,31	21,71	23,95	21,24	19,44	19,81	21,08	24,92	21,73	19,73										
3,49	2,80	2,30	3,11	3,74	3,01	2,62	2,10	2,62	2,87										
XIX					XX					XXI									
15,65	19,84	22,63	20,60	16,41	16,23	19,88	21,99	20,06	17,21	17,22	19,38	22,05	18,94	18,50					
3,23	2,44	2,30	3,05	3,39	2,61	2,26	2,10	2,59	2,75	2,72	2,28	1,78	2,65	2,70					

4. An den Nebenschilddrüsen der Tiere zeigten sich — im Einklang mit unseren früheren Untersuchungen — eine 160—277 %ige Vergrößerung, histologisch ein Blutreichtum, Vermehrung und Vergrößerung der hellen Hauptzellen sowie Kernteilungserscheinungen, d. i. Anzeichen einer Hyperfunktion.

5. Da der Ca- und P-Stoffwechsel des Organismus hauptsächlich durch die Funktion der Nebenschilddrüsen reguliert wird, weisen die Versuche des Verfassers darauf hin, daß sich die Funktion der Nebenschilddrüsen zur Zeit der Ca-Abnahme und P-Vermehrung in der 1. Behandlungswoche übergänglich vermindert, im Laufe der weiteren Behandlung zur Zeit der Ca-Vermehrung und P-Abnahme aber steigert. Die Funktion der Nebenschilddrüsen kann also durch azidotische Behandlung in zwei Richtungen beeinflußt werden: in der 1. Behandlungswoche kann sie vermindert, von der 2. Woche ab gesteigert werden. Es ist anzunehmen, daß die Funktionsstörungen der Nebenschilddrüsen durch azidotische Behandlung vielleicht günstig beeinflußt werden könnten.

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Acta Physiol. Scand. 85(3):362-373. 1972.

Frenning-72
Bib No. 558

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Acta physiol. scand. 1972. 85. 362—373

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Gastric Absorption of L(+) and D(−) Lactic Acid and their Effects on the Transmucosal Ion Transport in Innervated, Non-secreting Cat Stomachs

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Received 2 December 1971

Abstract

FRENNING, B. *Gastric absorption of L(+) and D(−) lactic acid and their effects on the transmucosal ion transport in innervated non-secreting cat stomachs.* Acta physiol. scand. 1972. *85.* 362—373.

On instillation of 170 mM lactic acid (L or D) into unstimulated whole stomach pouches in cats the hydrogen ion concentration decreased more slowly than on instillation of 170 mM hydrochloric acid. When 700 mM lactic acid (L or D) was instilled the reverse was found. On instillation of 170 mM HCl subsequent to an instillation of 170 mM lactic acid (L or D) the changes in concentration of electrolytes as well as the net fluxes of electrolytes were of the same order of magnitude as during a preceding control instillation of HCl. On instillation of 170 mM HCl subsequent to an instillation of 700 mM lactic acid (L or D) the decrease in hydrogen ion concentration and the increase in sodium ion concentration were significantly larger than in the control instillation of HCl. The net influx of sodium ions was significantly increased but not the net efflux of hydrogen ions. After exposure of the gastric mucosa to 700 mM lactic acid (L or D) it produced a mucoid fluid that contained mainly sodium and chloride ions. This explained the results obtained when HCl was instilled subsequent to an instillation of 700 mM lactic acid. No morphological changes were found on examination in the scanning electron microscope of gastric mucosae exposed to 6 ml of 700 mM L lactic acid.

On instillation of relatively small amounts (5—10 ml) of 170 mM hydrochloric acid into tied-off unstimulated cat stomachs the hydrogen and chloride ion concentrations in the instillate decrease and the sodium and potassium ion concentrations increase, usually without any appreciable net movement of fluid. This was shown by Teorell (1933, 1939) and has been repeatedly confirmed. Expts. on cats with innervated whole stomach pouches have shown that exposure of the mucosa to acetic or acetylsalicylic acid increases its permeability to ions (Flemström, Frenning and Öbrink 1964, Flemström and Frenning 1968, Frenning 1971). A different view is held by Davenport (1964, 1967 and 1970), who from the result of instillations of relatively large amounts (30 ml) of acid test solutions into denervated (Heidenhain) gastric pouches in dogs claims that the normal mucosa offers barriers against trans-

mucosal transport of electrolytes. Subsequent to repeated instillations of relatively large amounts of acetic, propionic, butyric, acetylsalicylic or salicylic acid he obtained large net losses of hydrogen ions and large net gains of sodium ions on instillation of the acid test solution, an effect attributed to "breaking of the barriers". Differences in experimental techniques and in nomenclature probably account for the different interpretations of the effect of these acids.

A permeability increasing effect of acetic or propionic acid has been shown also on the isolated frog gastric mucosa and evidence supporting transient intracellular accumulation of acid, presumably in an ionized form, after exposure of the secretory side of the mucosa to acetic, propionic or lactic acid (pH = 4.00) has been presented (Flemström 1971). Frenning and Öbrink (1971) examined in the scanning electron microscope cat gastric mucosae exposed to acetic or acetylsalicylic acid and found that the surface epithelial cells, in contrast to what was found in normal stomachs, were swollen and that the intercellular junctions (see Farquhar and Palade 1963) appeared to be partially severed. Against this background it was considered of interest to determine whether L(+) or D(−) lactic acid (α-hydroxypropionic acid) also influenced the gastric mucosal permeability for ions and whether exposure to lactic acid changed the morphology of the gastric mucosa as observed in the scanning electron microscope.

Methods

Experimental animals

The expts. were performed on cats (mean wt 3.0 kg, range 2.2—4.4 kg, n = 25) which had been starved for at least 18 h but given free access to water. Anesthesia was induced with Fluothane® (Halothane®) or chloroform and maintained with chloralose (70 mg/kg b.wt.) and urethane (0.2—0.6 g), both given i.v. The stomach was isolated by ligatures at the cardia and the pylorus, care being taken not to disturb the gastric blood and nerve supplies. A glass cannula was inserted in the pyloric end of the stomach and the abdomen was closed. The stomach was then rinsed with physiological saline. Before starting the expts. there was a resting period of 2—3 h during which the secretory condition of the stomach was checked. The body temperature was 38.1 ± 0.2° C at the start of the expts. and 38.2 ± 0.2° C at the end (mean ± S.E., n = 22). The mean blood pressure was initially 143 ± 6 mm Hg and at the end of the expts. 128 ± 5 (n = 21).

Analysis

Acidity determination. 0.05 ml samples were diluted in 5 ml distilled water and titrated with 10 mM NaOH (indicator bromthymol blue).

Chloride was determined electrometrically on the same samples as were used for acidity determination (Auto Burette Unit, type ABU12, pH Meter Type PMH 26c, Radiometer, Copenhagen, Denmark). 5 mM $AgNO_3$ was used for titration.

Sodium and potassium were determined flame photometrically after apropriate dilution with distilled water (flame photometer Eppendorf, Netheler and Hinz, GMBH, Hamburg).

L(+) lactate was determined enzymatically according to Scholtz, Schmitz, Bücher and Lampen (1959). The reagents were obtained from Boehringer & Soehne, GMBH, Ingelheim, Germany. *D(−) lactate* was not determined.

The coefficient of variation for sodium determination was ± 2 % at 100 mM. For the acidity, chloride and lactate determinations the coefficients of variation were smaller. (The coefficients were determined from analysis of 10 samples from the same solutions.)

Chemicals

The D(−) lactic acid used contained 3 % L(+) lactic acid. The L(+) lactic acid used was of highest available purity (98—99 %). Both were obtained form Sigma Chemical Co, St Louis, Mo, USA.

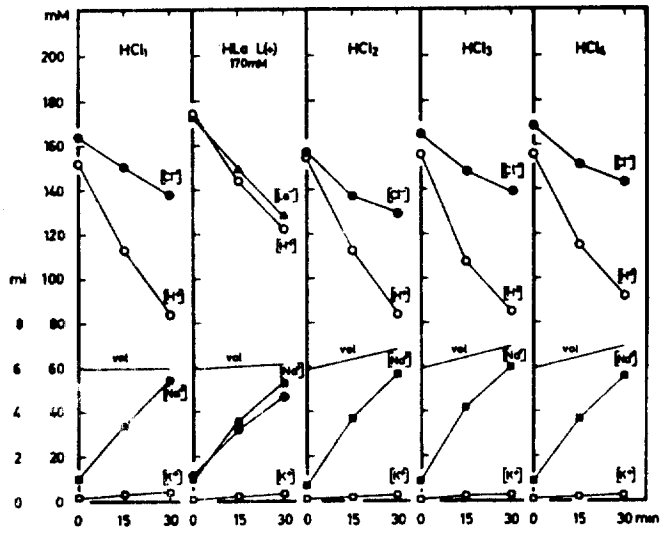


Fig. 1. The result of an expt. in which 6 ml of 170 mM HCl was instilled into a tied-off nonsecreting cat stomach once before and repeatedly after an instillation of 6 ml of 170 mM L(+) lactic acid.

Experimental procedures

The electrolyte output in the last 30 min of the resting period (the basal electrolyte output) was determined. The expts. were then begun with a 30 min instillation of 6 ml of 170 mM HCl. This period is referred to as HCl_1 or control period. 6 ml of 170 or of 700 mM L(+) or D(−) lactic acid was then instilled for an equal length of time. This was followed by 2 or 3 consecutive 30 min instillations of 170 mM HCl, referred to as HCl_2, HCl_3 and HCl_4. (As a 4th HCl instillation was not performed in all of the expts. and the results of those that were performed did not give any further information, they are not reported in detail.) Initial samples were taken within 1 min. At 15 min samples were taken without complete draining of the stomach. At 30 min the stomach was drained as completely as possible, the volume measured to the nearest 0.1 ml in a fine-graded measuring cylinder and samples taken. On each occasion a total of 0.1 ml was used for determination of hydrogen, chloride, sodium and potassium. A further 0.05 ml was used for L(+) lactate determination. Before HCl was instilled subsequent to instillation of 700 mM lactic acid the stomach was rinsed with HCl; otherwise no rinsing was performed between the different instillation periods. The changes in concentration of hydrogen, sodium, potassium, chloride and lactate given comprise the differences in concentration between the final and the initial samples. The reported changes in volume of the instilled solutions are corrected for the volume of the samples taken. The net fluxes of ions reported comprise the differences between recovered and instilled amounts.

Scanning electron microscopy

Specimens from 3 stomachs fixed immediately after a 30 min instillation of 700 mM L(+) lactic acid and from untreated stomachs were examined in a Jeol scanning electron microscope (JSM-U3), operated at 15 kV. For description of the preparatory procedure used, see Frenning and Öbrink (1971).

Results

Absorption of lactic acid

On instillation of 170 or 700 mM L(+) lactic acid the decreases in hydrogen ion and lactate concentration were essentially equal (Fig. 1 and 3, Table I). The net effluxes of hydrogen ions and lactate were also roughly equal (see Table II). Note the different ways in which the changes in concentration and the net fluxes of ions were determined. No determinations of D(−) lactate were performed, but as the decrease in hydrogen ion concentration and the increases in sodium, potassium and

TABLE I. Changes in concentration of electrolytes and in volume on 30 min instillations of 6 ml of 170 mM hydrochloric acid or of lactic acid into unstimulated whole gastric pouches in cats. HCl_1 is the control period. HCl_2 and HCl_3 were performed 0–30 and 30–60 min, respectively, after removal of the lactic acid. The values given are mean ± S.E.

Experimental period	n	ΔH^+ (mM)	ΔCl^- (mM)	ΔNa^+ (mM)	ΔK^+ (mM)	ΔLactate (mM)	ΔV (ml)
HCl_1		−65 ± 4	−20 ± 3	+41 ± 2	+3 ± 1		+0.3 ± 0.4
170 mM L(+) lactic acid	5	−52 ± 1	+48 ± 4	+43 ± 1	+4 ± 0	− 56 ± 5	+0.5 ± 0.3
HCl_2		−75 ± 3	−29 ± 1	+50 ± 2	+4 ± 1		+0.5 ± 0.5
HCl_3		−71 ± 1	−25 ± 3	+49 ± 2	+4 ± 1		+0.8 ± 0.6
HCl_1		−67 ± 6	−21 ± 3	+46 ± 4	+3 ± 0		+0.9 ± 0.2
700 mM L(+) lactic acid	6	−292 ± 8	+51 ± 3	+61 ± 2	+5 ± 0	−287 ± 8	+2.1 ± 0.4
HCl_2		−100 ± 4	−30 ± 1	+70 ± 3	+4 ± 0		+3.0 ± 0.4
HCl_3		− 89 ± 6	−25 ± 2	+64 ± 2	+3 ± 0		+2.7 ± 0.4
HCl_1		−68 ± 6	−19 ± 4	+47 ± 3	+3 ± 0		+0.4 ± 0.3
170 mM D(−) lactic acid	3	−54 ± 3	+49 ± 1	+46 ± 1	+3 ± 0		+0.6 ± 0.4
HCl_2		−73 ± 6	−29 ± 5	+48 ± 3	+3 ± 0		+1.0 ± 0.1
HCl_3		−71 ± 4	−21 ± 1	+51 ± 2	+3 ± 0		+1.2 ± 0.2
HCl_1		−60 ± 5	−18 ± 2	+41 ± 2	+3 ± 0		+0.3 ± 0.2
700 mM D(−) lactic acid	3	−292 ± 15	+49 ± 3	+64 ± 4	+5 ± 1		+1.4 ± 0.1
HCl_2		− 97 ± 12	−28 ± 1	+70 ± 3	+3 ± 0		+2.2 ± 0.6
HCl_3		− 81 ± 1	−22 ± 1	+61 ± 4	+3 ± 0		+3.0 ± 0.3

TABLE II. The mean net fluxes of electrolytes ± S.E. in the same expts. as presented in Table I. The mean basal electolyte output in 30 min preceding the start of the expts. is also given.

Experimental period	n	H^+ net (μEq/30 min)	Cl^- net (μEq/30 min)	Na^+ net (μEq/30 min)	K^+ net (μEq/30 min)	Lactate net (μEq/30 min)	ΔV (ml)
Basal output		+11 ± 8	+83 ± 4	+65 ± 5	+7 ± 1		+0.4 ± 0.1
HCl_1		−436 ± 36	−77 ± 66	+348 ± 33	+33 ± 4		+0.3 ± 0.4
170 mM L(+) lactic acid	5	−310 ± 34	+387 ± 43	+326 ± 22	+28 ± 3	−333 ± 17	+0.5 ± 0.3
HCl_2		−446 ± 40	−205 ± 48	+368 ± 36	+31 ± 5		+0.5 ± 0.5
HCl_3		−420 ± 42	−46 ± 87	+394 ± 53	+32 ± 6		+0.8 ± 0.6
Basal output		+11 ± 4	+76 ± 15	+62 ± 10	+4 ± 1		+0.5 ± 0.1
HCl_1		−374 ± 49	+16 ± 47	+408 ± 37	+28 ± 3		+0.9 ± 0.2
700 mM L(+) lactic acid	6	−1691 ± 206	+524 ± 52	+495 ± 39	+42 ± 5	−1789 ± 251	+2.1 ± 0.4
HCl_2		−359 ± 40	+195 ± 72	+704 ± 41	+42 ± 4		+3.0 ± 0.4
HCl_3		−412 ± 28	+233 ± 63	+697 ± 55	+34 ± 2		+2.7 ± 0.4
Basal output		+6 ± 2	−45 ± 16	+35 ± 13	+2 ± 1		+0.3 ± 0.1
HCl_1		−456 ± 48	−39 ± 47	+361 ± 21	+24 ± 1		+0.4 ± 0.3
170 mM D(−) lactic acid	3	−336 ± 33	+401 ± 49	+351 ± 34	+22 ± 1		+0.6 ± 0.4
HCl_2		−399 ± 1	−74 ± 17	+381 ± 22	+22 ± 1		+1.0 ± 0.1
HCl_3		−395 ± 21	+6 ± 23	+402 ± 15	+23 ± 1		+1.2 ± 0.2
Basal output		+19 ± 7	+111 ± 27	+86 ± 20	+8 ± 2		+0.7 ± 0.2
HCl_1		−406 ± 41	−67 ± 40	+331 ± 10	+26 ± 3		+0.3 ± 0.2
700 mM D(−) lactic acid	3	−1757 ± 238	+431 ± 33	+485 ± 33	+39 ± 5		+1.4 ± 0.1
HCl_2		−367 ± 34	+39 ± 74	+658 ± 63	+38 ± 6		+2.2 ± 0.6
HCl_3		−382 ± 19	−250 ± 28	+699 ± 81	+39 ± 4		+3.0 ± 0.3

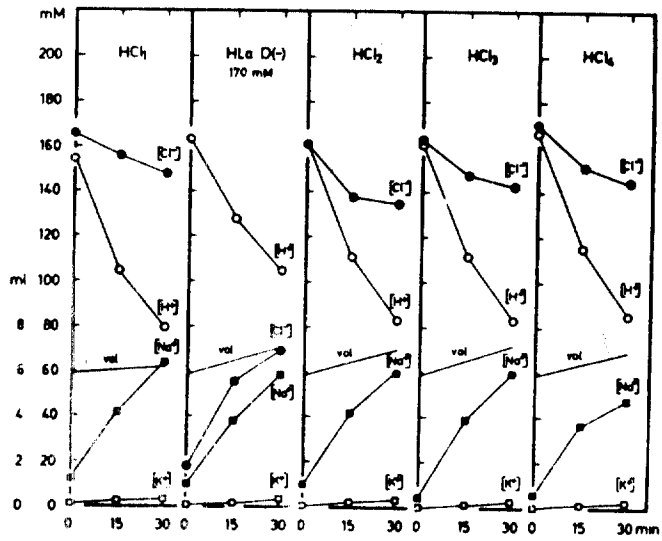


Fig. 2. An expt. similar to that shown in Fig. 1, the only difference being that the D(−) isomer of lactic acid was used in the intervening instillation.

chloride ion concentration were of the same order of magnitude on instillation of 170 or 700 mM D(−) lactic acid as on instillation of 170 or 700 mM L(+) lactic acid, respectively, the absorption of D(−) lactate probably also equalled the absorption of L(+) lactate. The decrease in hydrogen ion concentration was smaller on instillation of 170 mM lactic acid than on instillation of 170 mM HCl (Fig. 1 and 2, Table I). When 700 mM lactic acid was instilled, *i.e.* when the lumen to blood concentration difference was considerably increased, the reverse was found. Neither during instillation of 170 mM L(+) nor of 170 mM D(−) lactic acid did any decrease in volume occur, though the solutions were hypoosmotic. 30 min instillations of 700 mM L(+) or D(−) lactic acid resulted in mean volume increases which exceeded those in the corresponding control periods by 1.2 and 1.1 ml, respectively.

170 mM lactic acid and gastric transmucosal ion transport

On instillation of 170 mM HCl subsequent to an instillation of 170 mM L(+) lactic acid the decreases in hydrogen and chloride ion concentration and the combined increase in sodium and potassium ion concentration were of the same magnitude as in the control period. The same was found in the following HCl instillations (see Fig. 1 and Table I). The net effluxes (from the gastric lumen) of hydrogen and chloride ions and the net influxes (into the gastric lumen) of sodium and potassium ions on instillation of HCl were also unchanged after exposure of the mucosa to 170 mM L(+) lactic acid (see Table II). There was essentially no difference when the stomach was exposed to the same concentration of the D(−) isomer (see Fig. 2 and Table I and II). Thus an instillation of 170 mM lactic acid (L or D) did not in any respect influence the net gastric transmucosal ion transport on subsequent instillation of hydrochloric acid.

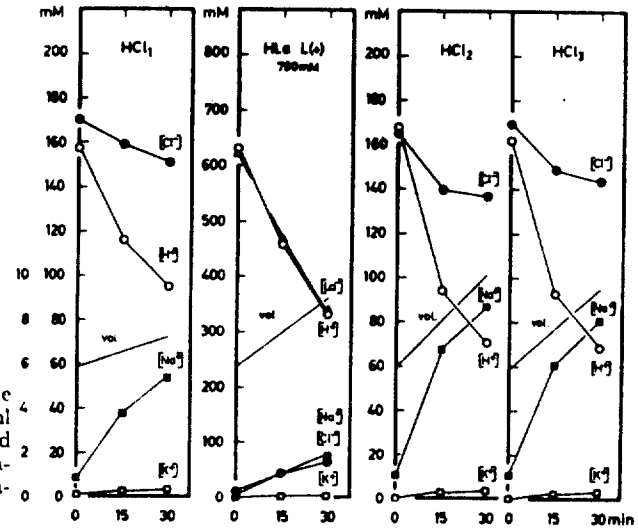


Fig. 3. An expt. showing the effect of an instillation of 6 ml of 700 mM L(+) lactic acid on the changes in concentration of electrolytes on instillation of 170 mM HCl.

700 mM lactic acid and gastric transmucosal ion transport

The results are presented in Fig. 3—5 and Table I, II and III. On instillation of 170 mM HCl subsequent to an instillation of 700 mM L(+) lactic acid (Fig. 3, Table I) the decreases in hydrogen and chloride ion concentration and the increase in sodium ion concentration were significantly larger than in the control instillation of HCl ($p < 0.01$, 0.05 and 0.02, respectively). The increase in volume was significantly larger than in the control period ($p < 0.01$). During HCl_3 the decrease in hydrogen ion concentration and the increase in sodium ion concentration and in

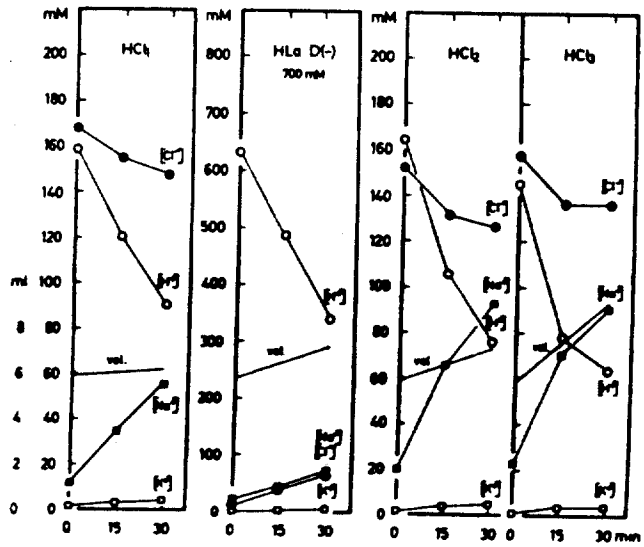


Fig. 4. An expt. similar to that shown in Fig. 3, the only difference being that the D(−) isomer of lactic acid was used in the intervening instillation.

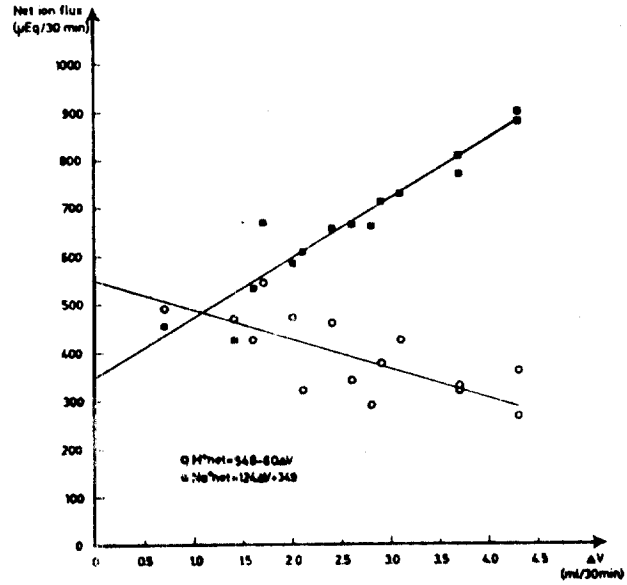


Fig. 5. The net fluxes of hydrogen and sodium ions from all instillations of 6 ml of 170 mM HCl performed subsequent to an instillation of 6 ml of 700 mM L(+) lactic acid, plotted against the change in volume in the corresponding instillation period. The regression lines were calculated by the Bartlett method.

volume still were significantly larger than the control values ($p < 0.05$, 0.01 and 0.01, respectively). The net efflux of hydrogen ions, however, was not increased in HCl_2 (Table II) but large net influxes of sodium, potassium and chloride ions occurred ($p < 0.01$, 0.05 and 0.05, respectively). In HCl_3 the net influxes of chloride and sodium ions still were significantly larger than before exposure of the mucosa to 700 mM L(+) lactic acid ($p < 0.02$ and 0.01, respectively). In 3 of the expts. a 4th HCl instillation was performed. The mean net efflux of hydrogen ions was 418 μEq/30 min, the mean net influx of sodium ions 629 μEq/30 min and the mean increase in volume 1.6 ml/30 min, a result similar to that in the preceding period.

After exposure of the gastric mucosa to 700 mM of the D(−) isomer of lactic acid the result was essentially the same (Fig. 4, Table I and II). In HCl_2 the net influx of sodium ions was significantly larger than in the control period ($p < 0.05$) and in HCl_3 the net influxes of sodium, potassium and chloride ions were all significantly increased ($p < 0.05$, 0.01 and 0.01). In the latter period the increase in volume was also significantly larger than in the control period ($p < 0.01$). The net effluxes of hydrogen ions were of the same magnitude in all periods.

After completion of the control periods the recovered fluid contained only negligible amounts of visible mucus. On instillation of 700 mM lactic acid (L or D), however, and especially on subsequent instillations of HCl, the amount of visible mucus in the recovered solutions was markedly increased in most of the expts.

The net fluxes of sodium and hydrogen ions obtained on instillation of HCl subsequent to an instillation of 700 mM L(+) lactic acid were plotted against the corresponding ΔV and the regression lines calculated by the Bartlett (1949) method

TABLE III. Electrolyte content in the fluid produced in unstimulated whole gastric pouches in cats in 30 min periods before and subsequent to a 30 min instillation of 6 ml of 700 mM L(+) lactic acid. The values given are mean ± S.E. n = 3.

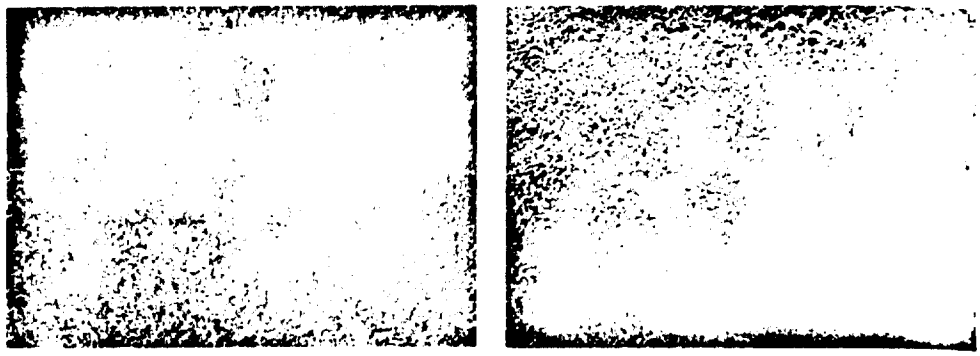
Collection period	H^+ (μEq/30 min)	Na^+ (μEq/30 min)	K^+ (μEq/30 min)	Cl^- (μEq/30 min)	Lactate (μEq/30 min)	secretion rate (ml/30 min)
Control	20 ± 10	93 ± 2	5 ± 1	115 ± 11		0.7 ± 0.1
post lactic acid						
0–30	127 ± 17	321 ± 87	37 ± 20	327 ± 51	111 ± 6	2.4 ± 0.6
30–60	26 ± 9	226 ± 51	9 ± 1	214 ± 33	11	1.6 ± 0.3
60–90	36 ± 59	280 ± 78	15 ± 6	353 ± 151		1.9 ± 0.5

(see Fig. 5). The net influx of sodium ions increased and the net efflux of hydrogen ions decreased on increase in volume. The net effluxes of hydrogen ions in the control period, in HCl_2 and in HCl_3 were corrected for the influence of changes in volume (Corrected H_{net} = Observed $H_{net} + k\ \Delta V$, k being the regression coefficient for the period in question calculated by the method of Bartlett (1949)). The thus obtained mean net effluxes ± S.E. at zero net change in volume for these periods were 482 ± 43, 511 ± 33 and 608 ± 12 μEq/30 min, respectively. There was a statistically significant difference between the thus corrected net effluxes of hydrogen ions in HCl_3 and in the control period ($p < 0.05$). When the mean regression coefficient for the net efflux of hydrogen ions for all instillations of HCl subsequent to an instillation of 700 mM L lactic acid (Fig. 5) was used to calculate the net efflux of hydrogen ions at $\Delta V = 0$ the corrected net effluxes of hydrogen ions in HCl_2 was calculated to be 541 ± 33 and that in HCl_3 to be 577 ± 14 μEq/30 min. On comparison between these values and that in the control period (corrected as described above) no statistically significant difference was obtained ($0.1 < p < 0.5$ and $0.05 < p < 0.1$, respectively). It must be considered that in both cases the corrections to zero net change in volume also involves an uncertainity due to extrapolation over a relatively large distance (Fig. 5), and that the relation between H_{net} and ΔV is not necessarily linear, though linearity appears to be a good approximation when the changes in volume are relatively large.

In 3 cats the basal electrolyte output in 30 min was determined and a 30 min instillation of 700 mM L(+) lactic acid was then given. The stomach was then drained and the fluid produced collected at 30 min intervals. The fluid produced was mucoid and as can be seen in Table III it contained mainly sodium and chloride ions. (2 expts. with D(−) lactic acid gave essentially the same result). As no rinsing with HCl was performed after the lactic acid instillation there was a large amount of lactic acid in the first collection period.

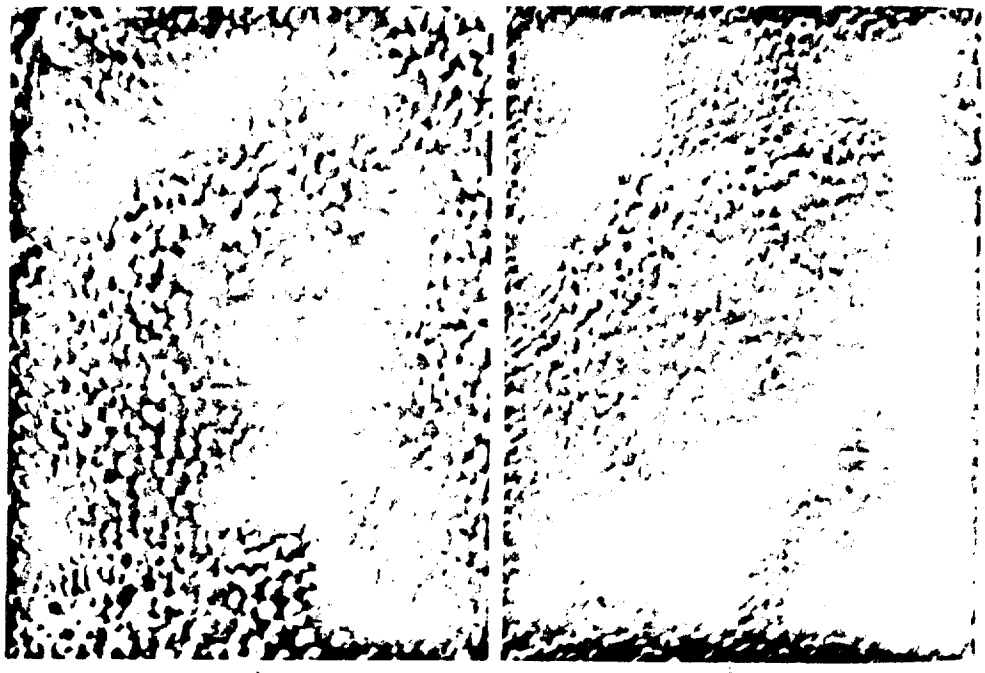
L(+) lactic acid and gastric mucosal surface morphology

Specimens from 3 stomachs exposed to 6 ml of 700 mM L(+) lactic acid for 30 min and from untreated controls were examined in the scanning electron microscope.



A B

Fig. 6 A and B. The appearance of gastric mucosal surface epithelial cells in the scanning electron microscope. A is from an untreated control, B from a mucosa fixed immediately after exposure to 700 mM L(+) lactic acid. The cell surfaces are polygonal. No separations in the intercellular junctions can be seen. (Magnification ×2,250).



A B

Fig. 7 A and B. Surface epithelial cells at a higher magnification. Numerous knob-like microvilli are seen on the cell surfaces which are slightly convex upwards. The cells appear closely attached to adjacent cells. A control, B post lactic acid. (Magnification ×9,100).

In contrast to the findings in stomachs exposed to acetic or acetylsalicylic acid (Frenning and Öbrink 1971) no changes of the surface morphology of the gastric mucosa were found (Fig. 6 A and B, 7 A and B).

Discussion

According to our present theories and knowledge the prerequisite for a weak acid to change the permeability properties of the gastric mucosa is that it accumulates intracellularly to such a degree as to cause cellular swelling (due to an increased intracellular osmolality and/or to intracellular acidosis or to a specific action of its anion) and partial separations in the junctions between the mucosal cells and possibly also an increase in the intercellular spaces in the mucosa (Flemström and Frenning 1968, Flemström 1971, Frenning 1971, Frenning and Öbrink 1971, Flemström and Marsden 1972, *cf.* Martin 1963). The results of Hingson and Ito (1971), who studied the fine structure of the surface epithelium in mouse stomachs exposed to some carboxylic acids, also confirm the occurrence of cellular swelling subsequent to exposure to these acids. They did not report any changes in the intercellular junctions until the process of cell degradation was far advanced. Their results do not, however, exclude the possibility that incomplete separations in these junctions occurred as a consequence of cellular swelling, as appeared to be the case in cat gastric mucosae exposed to acetic or acetylsalicylic acid on examination in the scanning electron microscope (Frenning and Öbrink 1971). Flemström (1971) observed an increase in the acid output from histamine stimulated frog gastric mucosa *in vitro* when it had previously been exposed to 10 mM L(+) lactic acid (pH = 4.00) added to the mucosal side solution. No such effect was obtained subsequent to treatment with D(−) lactic acid or lactate (L or D, pH = 7.12). His results strongly suggest, qualitatively, that L(+) lactic acid transiently accumulated in the mucosal cells and there is no reason to believe that the distribution of the D(−) isomer was different. The gastric mucosal permeability for ions still remained unchanged after a 30 min instillation of 6 ml of 170 mM L(+) or D(−) lactic acid. This would seem to indicate that the intracellular concentrations of lactate and hydrogen ions never reached such a magnitude as to cause cellular swelling. The disappearance rate of 170 mM lactic acid from the stomach was lower than that of 170 mM HCl (see Fig. 1 and 2), whereas 170 mM acetic acid disappears faster than HCl (Teorell 1939, Flemström, Frenning and Öbrink 1964, Flemström and Frenning 1968). If lactic acid is less lipid soluble than acetic acid this may explain the results. The partition ratio for L(+) lactic acid between isopropylether and 1 M HCl is considerably smaller than that for acetic acid (0.05 and 0.2, respectively, Camien, Fowler and Dunn 1959), which in fact indicates that lactic acid has a lower solubility than acetic acid in organic solvents. For 3 barbituric acid derivatives with similar pK_a values Schanker *et al.* (1957) showed that the higher the partition coefficient chloroform—HCl and heptane—HCl, the higher was the disappearance rate from the stomach. This may be due to different affinities of the mucosal lipids for the acids although it is possible that the affinity of the mucosa for weakly polar acids is not due to the lipids since the highly hydrophilic polysacharide dextran gels have high affinities for such solutes (Marsden 1972).

After exposure of the stomach to 700 mM L or D lactic acid it produced a mucoid fluid containing mainly sodium and chloride ions. This caused a larger increase in sodium ion concentration and a larger decrease in hydrogen ion concentration on

subsequent instillations of hydrochloric acid in comparison with the control hydrochloric acid instillation. It thus acted as a diluting secretion in the sense of the recent formulation of the two-component hypothesis (Makhlouf, McManus and Card 1966). The net efflux of hydrogen ions was, however, not increased on instillation of HCl subsequent to an instillation of 700 mM lactic acid (Table II) and, further, it decreased on increase in volume (Fig. 5). These findings appear to exclude any neutralization of importance.

Possible reasons for the reduction of the net efflux of hydrogen ions on increase in volume are that the fluid produced reduced the lumen to blood concentration difference and possibly also that the flow of fluid reduced the rate of diffusion of hydrochloric acid and into the gastric pits and tubules (*cf.* Rehm, Schlesinger and Dennis 1953). If the volumes of the recovered instillates were somewhat overestimated due to admixture of mucus the values for the net effluxes of hydrogen ions would have been falsely low and those for the net influxes of sodium ions falsely high. Some contributory effects of this kind cannot be excluded. The composition of the fluid produced after exposure of the gastric mucosa to 700 mM lactic acid (Table III) and the normal surface morphology of lactic acid treated gastric mucosa appear to exclude the possibility that acid secretion was stimulated and the composition of the secretion altered due to an increased diffusional transport over the mucosa (*cf.* Frenning 1971).

On comparison of the present expts. with 170 mM lactic acid and similar expts. with 170 mM acetic acid (Flemström and Frenning 1968) it would appear that although both acids enter the cells (Flemström 1971) the amount of acetic acid that enters is sufficient to change the permeability of the mucosa for ions but the amount of lactic acid is not (for discussion of possible reasons to this vide supra). As there was no difference between the effects of the L and D isomers of lactic acid it is considered less probable that the difference in effect of acetic and lactic acids was due to a more rapid metabolism of lactate than of acetate.

Not even on instillation of HCl subsequent to an instillation of 700 mM L lactic acid (*i.e.* in HCl_2) was the gastric mucosal permeability to ions increased. In the following instillation period (HCl_3) the mucosal ion permeability was possibly increased—when correction was made to zero net flow of fluid the net efflux of hydrogen ions was possibly higher than that in the control period. The explanation for this, apart from the possibility that the value of H_{net} at $\Delta V = 0$ was determined falsely high due to the uncertainty of this correction, might be that though a relatively large amount of lactic acid probably entered the cells on instillation of 700 mM lactic acid no cellular swelling occurred because the luminal sides of the cells were exposed to a hyperosmotic solution. On subsequent instillation of 170 mM HCl there might have been a moderate cellular swelling that increased the mucosal ion permeability in the next period.

The present results further imply that an increased net influx of sodium ions into the gastric lumen in combination with an increase in volume (or a less than normal decerase in volume) taking place after any kind of instillation does not necessarily mean that the gastric mucosal permeability to ions is increased.

My thanks are due to Mrs Kerstin Eklund and Mrs Lena Westlund for invaluable technical assistance. Financial support was given by the Medical Faculty, University of Uppsala and the Swedish Medical Research Council project nr 14X-151.

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Gerarde-'64
Bib No. 611

Intern. Congr. Occupational Health, 14th, Madrid 1963(2):723-727.
Pub. 1964.

ASPIRATION HAZARDS OF PETROLEUM PRODUCTS

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We became interested in the aspiration hazards of petroleum products when it came to our attention in reviewing poisoning reports among children that kerosine and other petroleum solvents and furniture polish containing mineral seal oil stood second only to aspirin as a cause of accidental poisoning in children. Our interest was to know more about the cause of these poisonings, and determine what could be done to prevent or treat them. Analysis of the reported cases disclosed that serious lung damage usually developed in these children, but if they survived the first week or so, complete recovery without sequelae was the rule. Generally these children drank kerosine carelessly left by their parents in accessible places in water glasses or 'coke' bottles. Furniture polish was gulped from an open furniture polish container left on a low table while mother was occupied elsewhere – often times only for a few brief moments on the telephone. By far the majority of the cases occurred in children under 5, often 2 or under.

A review of the literature indicated that there were two schools of thought on this problem. The first believed that the kerosine or furniture polish entered the lung directly by aspiration at the time of swallowing, or by subsequent vomiting. The other believed they were absorbed into the blood stream in a sufficiently high concentration to cause injury to the pulmonary capillaries from the high blood concentration.

Our approach to this problem, developed by one of us (HWG), was a direct experimental one. First, kerosine was administered to experimental animals by stomach tube in high doses (10 cc/kg). Figure 1 shows two rats which received 40 doses of 5 cc each of kerosine by stomach tube. Except for some excoriations around the anus, these animals are quite healthy. Examination of their lungs showed perfectly normal lungs, as in the center lungs of Figure 2. Contrast this, however, with the lungs on either side removed from rats which died following the instillation of 0.1 cc of kerosine directly into the trachea. Here, hepatization of the lungs has occurred, with acute cor pulmonale having developed. Figure 3 contrasts the microscopic picture of normal lung tissue with the bloody exudation into the lungs, following direct instillation of small amounts (0.1-0.2 cc) of kerosine directly into the trachea.

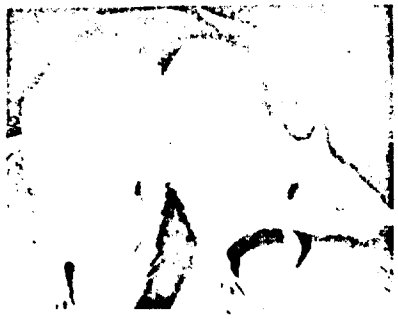

Fig. 1. Two rats which received 40 doses of 5 ml each of kerosine by stomach tube.

Fig. 2. Gross appearance of rat heart and lungs after aspiration of 0.2 ml of kerosine (right and left; center normal).

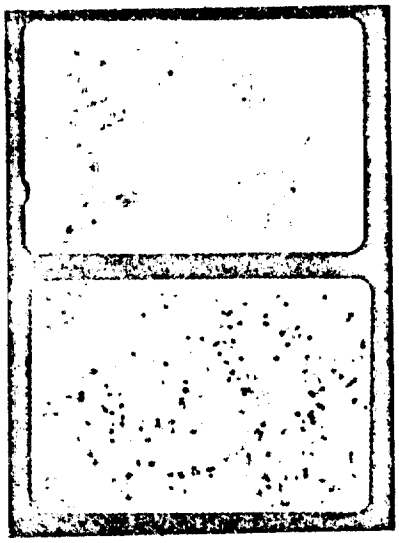

Fig. 5. Microscopic appearance of rat lungs after aspiration of 0.2 ml kerosine (upper one normal). Magnification ×430.

Figure 4 shows a rabbit dosed with so much kerosine by stomach tube that his fur actually became wet with kerosine excreted from the anus. Yet this rabbit was otherwise healthy. A fraction of this dose placed directly in the trachea resulted in death of the rabbit with extensive lung damage. Similar experiments have been performed on chickens, with comparable results. Figure 5 shows extensive damage in the medial dependent portions of chicken lungs from kerosine placed directly in the trachea.

We believe the lessons learned from these simple, direct experiments are so self evident that they need only emphasis. Petroleum products which enter the stomach and remain there are essentially nontoxic. Thus, the *oral* LD_{50} of kerosine (Figure 6) is 28,350 milligrams/kg. On the other hand, if the kerosine is aspirated into the lungs, either due to gagging at the time of ingestion, or by subsequent vomiting, the LD_{50} (Figure 7) is between 300 and 600 milligrams/kg. It thus seems self-evident to us that kerosine in the stomach should be left there, because the hazard of leaving it there is nil, but the hazard of aspiration during attempts to remove it from the stomach is so great as to seriously endanger the patient. If aspiration into the lungs has already occurred, the only thing to be accomplished by inducing vomiting or passing a stomach tube is the possibility of aspirating more kerosine into the lungs with further lung damage. Since this has been established experimentally in all species of animals tested, there is no reason to doubt that the same principles apply in the human. Supportive therapy, with oxygen and antibiotics, should be given if aspiration has already occurred.

Extending this work further, Dr. Gerarde has developed a standardized test for the aspiration hazard of a petroleum hydrocarbon. A rat weighing between 200 and 300 grams is anesthetized with ether. Its jaws are held open with forceps (Figure 8) and the tongue pulled forward. 0.2 ml of the test material is placed at the back of the pharynx and the nares held shut during inspiration. This causes aspiration of the material into the lung. Mortality at 24 hours is measured and found (Figure 9) to be a function of viscosity. If the viscosity exceeds 90 or 100 SSU at 100°F, the material produces no deaths at 24 hours. On the other hand, if the viscosity is less than this, aspiration with subsequent deaths does occur. If the lungs are removed and weighed 24 hours after dosing in this fashion, it is found that lung weights are increased for those materials which produce deaths (Figure 10). If the lung weight (normal for the rat 1-1.5 gms) does not exceed 3 grams, deaths do not occur. Using this technique, mixtures of kerosine and lubricating oil were found to present no hazard if the viscosity exceeded 58 SSU at 100 F. Thus no mortality (Figure 11) or increased lung weight (Figure 12) occurred when the viscosity exceeded 58. We believe that this clearly demonstrates, that the aspiration hazard of a petroleum hydrocarbon is related to its viscosity, and can be simply tested by this technique.

Finally, a modification of this technique was developed to test the aspiration hazard of petroleum hydrocarbon aerosols. Figure 13 shows kerosine being sprayed directly into an anesthetized rat's mouth. As shown in Figure 14, the actual spraying is done with the nozzle close to or actually in the animal's mouth. As much as 1 cc of kerosine (5 times the amount which causes death and lung damage when instilled as a liquid directly in the trachea) was without mortality or increase in lung weight when aspirated as an aerosol (Figure 15). We thus conclude that petroleum hydrocarbon aerosols are without hazard from aspiration.

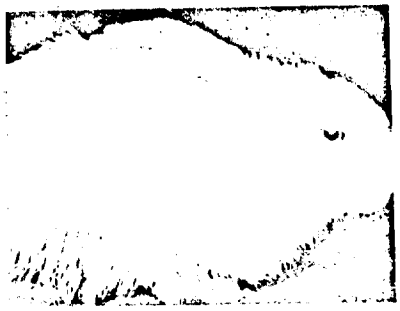

Fig. 4. Rabbit dosed with large doses of kerosine. Fur wet with kerosine excreted through anus but rabbit otherwise healthy.

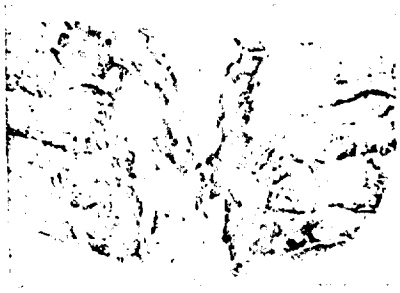

Fig. 5. Chicken lungs after aspiration of kerosine. Note hemorrhagic areas in medial and dependent regions.

ORAL TOXICITY OF COMMON LIQUID CHEMICALS

CHEMICAL	LD-50 (mg/kg)	TOXICITY CLASS
ACETIC ACID (RAT)	3,310	SLIGHTLY TOXIC
LACTIC ACID (RAT)	3,730	SLIGHTLY TOXIC
ISOPROPYL ALCOHOL (RUBBING ALCOHOL/RAT)	5,840	PRAC. NON-TOXIC
ACETONE (RAT)	8,750	PRAC. NON-TOXIC
ETHYL ALCOHOL (RAT)	13,660	PRAC. NON-TOXIC
GLYCERINE (RABBIT)	27,000	REL. HARMLESS
KEROSENE (RABBIT)	28,350	REL. HARMLESS

Fig. 6. Oral toxicity of some common materials, including kerosine.

MALE ALBINO RATS
INCREASING DOSES OF KERUSINE (ASPIRATION)

Dose (ml)	Mortality (Hours After Dosing)						
	1	2	6	24	48	72	16 DAYS
0.05	0/10	0/10	0/10	0/10	0/10	0/10	*1/10
0.10	0/10	1/10	3/10	4/10	4/10	4/10	4/10
0.15	0/10	2/10	6/10	7/10	8/10	9/10	9/10
0.20	0/10	3/10	7/10	9/10	9/10	9/10	9/10
0.25	4/10	7/10	10/10	10/10	—	—	—

*DIED ON 10th DAY

Fig. 7. Mortality of rats (numerator) over number of rats treated (denominator) with increasing doses of kerosine.

Fig. 8. Method of administering liquids for testing aspiration hazard in the rat.

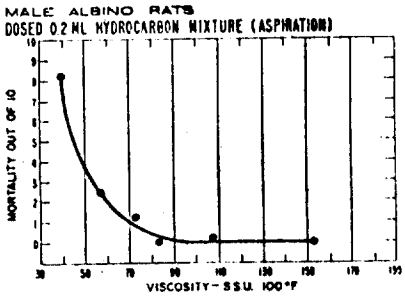

Fig. 9. Relationship of mortality of rats after aspiration to viscosity of the hydrocarbon aspirated.

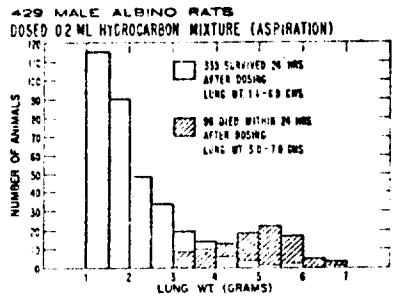

Fig. 10. Relationship of lung weight in rats 24 hours after aspiration to survival. Deaths occur only if the lung weights exceed 3 gm.

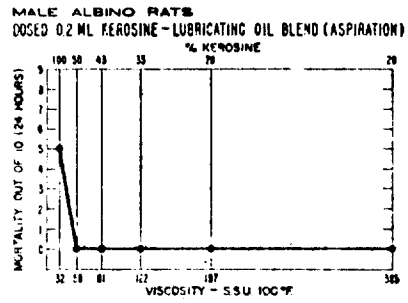

Fig. 11. Effect of increasing the viscosity of kerosine by adding lubricating oil on the survival of rats which aspirated the mixture. Note that 50% of kerosine with a viscosity of 58 SSU at 100°F caused no deaths.

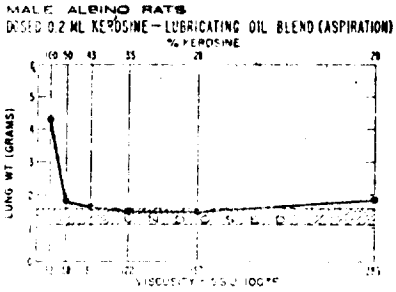

Fig. 12. Relationship of lung weight to viscosity of aspirated kerosine-lubricating oil mixture. Note that lung weights are not significantly increased if viscosity exceeds 58 SSU at 100°F.

Fig. 13. Method of administering aerosol.

Fig. 14. Same as Figure 13, but in practice the aerosol orifice is placed close to rat's mouth.

MALE ALBINO RATS
ASPIRATED 1.0 ML OF KEROSINE AEROSOL
(Sacrificed 24 Hours After Dosing)

Mortality	Clinical Observations After Dosing	Lung Weights (Grams)	Avg	Gross Pathology
0/10	NO EVIDENCE OF SYSTEMIC INTOXICATION OR PULMONARY DISTRESS LOCAL IRRITATION AROUND EYES & NOSE CLEARED RAPIDLY	1.18, 1.27, 1.22, 1.06, 1.43, 1.28, 1.47, 1.16, 1.43, 1.30	1.28	8 LUNGS NORMAL ON INSPECTION

LUNG WEIGHTS UNDOSED: RANGE 1.1-1.5 GRAMS AVG 1.3

Fig. 15. No mortality or increased lung weight in rats following aspiration of an aerosol of 1.0 ml of kerosine. Contrast this with Figures 2 and 7.

Conclusions

Light petroleum hydrocarbons, such as kerosine and certain solvents, or mineral seal oil, have a very low order of toxicity if ingested and kept in the stomach. However, if aspirated directly into the lungs they cause extensive lung damage and death. Therefore, the induction of vomiting or pumping out the stomach is contraindicated following petroleum hydrocarbon ingestion unless extreme precautions, such as the insertion of an intratracheal tube, are taken to avoid aspiration. Heavier petroleum hydrocarbons are without aspiration hazard (if the viscosity exceeds 60–100 SSU at 100°F). A simple test for determining the aspiration hazard using mortality and lung weights at 24 hours is described. Tests of petroleum hydrocarbon aerosols show them to be without aspiration hazard. Kerosine or other petroleum solvents should not be left in open, unmarked containers, such as water glasses or 'coke' bottles where they can be accidentally ingested and aspirated by small children. Furniture polish likewise should not be left opened when small children are around.

Resumen, Abstract, Résumé, Vol. IV, No. 196

Amer. J. Dis. Children 100(5):705-707. 1960.

Goldman-'60
Bib No. 634

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26. Lactic Acid Acidosis. HERBERT I. GOLDMAN, M.D.*; SAMUEL KARELITZ, M.D.; ELI SEIFTER, PH.D.*; HEDDA E. ACS, M.D.,* AND NORMAN B. SCHELL, M.D.* Department of Pediatrics, Long Island Jewish Hospital, New Hyde Park, N.Y.

A previous investigation revealed that premature infants fed a lactic acid milk (Pelargon) gained less weight than prematures fed isocaloric isovolumetric amounts of nonacidified milk mixtures.

In the present study, 36 infants of average birth weight 1,479 gm. and average age 22 days were divided into two groups of 18 infants. Both groups contained infants of similar birth weight and age and both had been fed a half-skimmed milk mixture (Alacta). For a period of seven days, one group was changed to isocaloric amounts of Pelargon, while the control group remained on the half-skimmed mixture. The average blood pH of the acid milk group fell from 7.39 to 7.25 during the week, and the average plasma CO_2 fell from 19.6 mEq/L. to 14.8 mEq/L. Corresponding averages for the control group are pH 7.38 and 7.40, and CO_2 19.1 and 20.4.

Studies were also performed comparing premature infants fed half-skimmed mixtures with infants fed the same mixture acidified with lactic acid. The results are similar to the above except for a less striking drop in pH. Infants were also made acidotic with human milk acidified with lactic acid. These studies indicate that the acidosis is the result of the added lactic acid.

The "Spontaneous Acidosis in Premature Infants" reported by McBryde and Branning occurred in infants most of whom were fed lactic acid milk. The protein milk with which Darrow et al. produced acidosis in prematures was also a lactic acid milk. This probably was a contributory factor hitherto not included in their explanation of the mechanism of the acidosis.

The mechanism of production of acidosis by lactic acid was investigated. Studies were done on infants receiving nonacidified milk and repeated when the infants were changed to lactic acid milk. The average rise in blood lactate was 0.5 mEq/L.; the urinary lactate excretion rose 0.3 to 2.5 mEq/day; the urinary sodium and ammonia excretion rose, and the magnitude of the combined increase approximates the urinary lactate increase.

Several prematures were given sodium lactate by intravenous injection. Blood and urine lactate levels were determined and compared to those reported by Hartmann and Senn in a similar study with children 5 to 14 years of age. At two hours, elevated blood lactate levels were found in the premature, whereas the children had normal values at this time.

The acidosis produced in prematures therefore appears dependent on two factors:

1. Increased vulnerability of the premature to acidosis. Prematures fed a cows' milk formula have been shown to be normally on the verge of acidosis and to be made acidotic more easily than the term infant. The relatively small amounts of fixed base required for the excretion of the lactate in the urine and the slight rise in blood lactate are sufficient to render the premature acidotic.

2. A relative deficiency in the metabolism of racemic lactate by the premature.

Discussion

DR. DANIEL C. DARROW (Kansas City): Well, I think we all are aware that there are a lot of booby traps in studies of acid-base equilibrium and I think it is perfectly clear that we did not adequately consider the fact that the racemic lactic acid which we add to milk is the dextro form, which is not readily metabolized, and is undoubtedly played a role in the development of acidosis in the infants we studied.

Those studies probably should be followed up by some studies indicating whether the premature infant is more handicapped in the oxidation of the dextro form of lactic acid than is the older group of infants.

* By invitation.

I think everybody has wondered about the use of the protein milk in diarrhea which Finkelstein developed a number of years ago. Results have been disappointing in young infants. Finkelstein, however, never recommended protein milk for infants under 3 months of age.

The buttermilk often used in Europe is a lactic acid milk. Because of the high osmotic load and lactic acid content, it is probably not a good food for patients with diarrhea.

This study, I think, also brings up the question of whether we are correct in continuing to use sodium lactate when we want to give bicarbonate. We could use sodium acetate, which we know will be readily and rapidly oxidized.

DR. ROBERT USHER (Montreal): I have found this a fascinating paper, and the relationships between the findings here in the second and third weeks of life and those which we have found in the first week of life are very interesting.

Premature infants who have respiratory distress syndrome have a low pH and a drop in bicarbonate during the first three days of life. Urines collected from 40 infants during the first three days of life showed a markedly elevated sodium output. The increment of sodium output when compared to that of healthy premature infants was approximately the same as you produced by feeding your infants lactic acid.

I would wonder whether the catabolic process going on in babies with respiratory distress syndrome is releasing organic acids which during the first three days perhaps cannot be buffered even as well as they can after one week of age. If these are then excreted with base there might then be a relatively normal plasma level of lactic and other organic acids but with a marked depletion of plasma bicarbonate and of buffer base reserve.

DR. WILLIAM M. WALLACE (Cleveland): I would like to ask Dr. Goldman whether he has considered and has any information about the fecal excretions of cations during the feeding of these two kinds of milk.

DR. CHARLES U. LOWE (Buffalo): I would like to add a little bit to what Dr. Darrow said in terms of pitfalls. Some of these we have experienced ourselves.

The first one has to do with methodology. I know the author said he used standard methods, but there are at least two for lactate, one of which is open to considerable error. The Barker-Summerson method is not specific for lactate. If the author did use the lactic acid dehydrogenase method, I think we have to be prepared to accept his values for lactate as indicating lactate. On the other hand, if the Barker-Summerson method was used, it is at least possible that he is measuring a constellation of organic acids, not only lactate.

The second problem—again, I'm sure, one he has considered—has to do with the time of measurement of lactate in relationship to feeding. There is a normal flux of blood lactate in blood in relation to feeding. It rises postprandially and then falls in normal infants.

It is conceivable there was a difference in the handling of these babies, insofar as the time after feeding at which lactate measurements were made. If this did not occur—that is, if the measurements were made at the same time after feeding—there is yet another possibility for error. This has to do with gastric emptying. It is conceivable that the acidified formula affected the rate of the emptying of the intestine, and the rise of lactate in relation to feeding bears a predictable relationship to the rise of blood sugar. It seems to me this must be controlled.

A third problem—and it seems to me this is the type of control that should have been used—has to do with the administration of an acid other than lactate. If the effect of lactate was due to its acidity, then it seems to me the results could have been produced with some other acid.

DR. GILBERT B. FORBES (Rochester, N.Y.): I would like to ask Dr. Goldman to comment on the dose of lactic acid used in these studies. As I recall, this was almost twice as much as the usual amount recommended for infant formulas.

DR. GOLDMAN (Closing Discussion): I want to thank Dr. Darrow for pointing out what I neglected to mention—that the lactic acid used in all commercial lactic acid milks is a racemic mixture of the *d* and *l* isomers, and that it is probably the *d* isomer which is causing most of the trouble.

Dr. Wallace, we did no stool analyses. However, the studies of Darrow and Hoffman include stool analyses. The amount of sodium present in infants receiving lactic acid milk was negligible, so we concluded that this was not an important factor.

Dr. Lowe, the method used for lactate determination was the method of Barker and Summerson. Blood specimens were drawn at the same time on the matched pairs of infants in an attempt to control the variables relating to feeding. We could not, of course, control the activity of the infants. This study of blood lactate levels in the two groups did not involve a very large number of infants and, in our opinion, is more important for what it failed to demonstrate than what it did demonstrate. This study did not show a very large increase in blood lactate levels which would be sufficient to account for the acidosis.

Dr. Forbes, the amount of lactic acid used in the formula, 1 ml/100 ml. formula, is comparable to the amount recommended by Marriott, 1 teaspoon/pint. This is approximately twice the concentration present in the proprietary lactic acid milk that we used but is as strong as several of the other commercially available lactic acid milks.

Gordon-'71
Bib No. 640

JOURNAL OF APPLIED PHYSIOLOGY
Vol. 31, No. 5, November 1971. *Printed in U.S.A.*

Sweat lactate in man is derived from blood glucose

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GORDON, ROBERT S., JR., RONALD H. THOMPSON, JOSEPH MUENZER, AND DEL THRASHER. *Sweat lactate in man is derived from blood glucose.* J. Appl. Physiol. 31(5): 713–716. 1971.—That sweat lactate is derived metabolically from blood glucose has been shown by the use of tracer metabolites in normal human subjects. The yield of radioactive carbon in sweat lactate after an injection of labeled lactate averages only 8% of that found after a similar injection of labeled glucose.

gland function; temperature regulation

TWO DECADES AGO, Weiner and Van Heyningen (6), in a study of the concentrations of lactate found in normal human sweat, inferred that this substance was a product of glandular metabolism, rather than representing blood lactate which had been concentrated and secreted by the glands. Their best evidence for this hypothesis was the demonstration that the lactate concentration of sweat was not at all affected by exercise, whereas the corresponding concentration of lactate in the blood increased by as much as 10-fold. They also drew attention to the fact that the production of the lactate in human sweat, with either blood glucose or glandular glycogen as the metabolic precursor, would liberate enough free energy to satisfy classical thermodynamic requirements for the energy of formation of sweat from isotonic body fluid. More recently, Wolfe et al. (7) studied the formation of lactate in microdissected human sweat glands incubated in vitro. Under these experimental circumstances, it could be shown that the lactate was actually being produced by the glands themselves, that lactate output was increased either by addition of glucose to the incubation medium, or by addition of pharmacologic agents which are known to stimulate sweating in vivo, and that the rate of lactate production in vitro agreed well with that observed in vivo during normal sweating. Thus it would appear that lactate production is related to the process of secretion of sweat, and that glucose from the medium is utilized as a precursor.

We undertook the present investigation in order to extend the above observations, and to demonstrate by the use of radioisotopes in the intact healthy human subject that the metabolic precursor of sweat lactate in vivo is, as expected, blood glucose.

EXPERIMENTAL METHODS

The subjects for these studies were normal college students, both male and female, who were admitted to and housed in the Clinical Center of the National Institutes of Health under the normal volunteer patient program. Each had had a thorough medical checkup to rule out unsuspected illness, and each was free of intercurrent disease at the time of the study. None was on any unusual diet, or was receiving any medication on the day of study. After receiving an explanation of the nature and purpose of the experiment and indicating his consent, the volunteer was asked to report to the laboratory at about 9:00 AM, after showering thoroughly and rinsing with distilled water. Wearing a nylon racing-type swimsuit, the subject entered the National Institute of Arthritis and Metabolic Diseases metabolic and environmental chamber, where the conditions had been adjusted to 120° F and 10–12% relative humidity. He reclined for the duration of the study on a nylon mesh hammock (L. L. Bean Co., Freeport, Me.) which was suspended on a Brookline metabolic balance modified for the continuous monitoring and recording of weight losses in the range expected under these circumstances. Fifteen to twenty minutes after entering the chamber, each volunteer had achieved a constant rate of weight loss, in the range of 450–800 g/hr (varying primarily with the size of the subject). At this point, the test dose of radioactive substrate was injected intravenously. After the skin around the injection site had been meticulously cleaned to dispose of any radioactive contamination of the surface, the subject was allowed to sweat, at rest, for the duration of the experiment. In the desertlike atmosphere of the chamber, sweat evaporated almost immediately. After the time specified below had elapsed, the volunteer climbed into a portable fiberglass tub (Bioscience Industries, Asbury Park, N. J., model DT-1), fitted with a recirculating pump and a shower head, and was washed down thoroughly with two 5-L aliquots of distilled water. The hammock was put in and washed concurrently. The pooled washes were then weighed to determine their total volume. The total volume of sweat secreted was assumed to be equal to the volunteer's weight loss, the small amount of water loss through the respiratory tract being neglected. After an aliquot of the pooled washings had been reduced in volume 10- to 30-fold with a rotating vacuum evaporator (Rinco Rotavapor), we had available for analysis a large sample of reconstituted sweat at 1–2 times its original concentration, the concentration factor being known from the figures given above.

Glucose-U-^{14}C and sodium *l*-lactate-1-^{14}C were purchased from the New England Nuclear Co., Boston, Mass., and were prepared for parenteral use by the Radiopharma-

ceutical Service, National Institutes of Health, Clinical Center. Processing included chromatographic demonstration of purity, preparation and calibration of an isotonic solution containing 1.0 μc/ml, and sterilization and safety testing of the resulting product.

All measurements of radioactivity were carried out in a Packard Tri-Carb model 3320 liquid scintillation spectrometer. Aqueous samples were mixed with Bray's solution for counting; the efficiency for ^{14}C at the discriminator settings we employed averaged 60%. When necessary, we corrected results for quenching on the basis of the count rate observed with the automatic external standard.

The yield of radioactivity in sweat after the injection of 5 μc of glucose-^{14}C was compared with that after an equal amount of lactate-^{14}C in studies on nine subjects, four males and five females. In the first two subjects (*HB* and *LD*) the duration of the period of sweating was 90 min, whereas for all other subjects it was 180 min. The longer period was adopted after we learned that secretion of radioactivity in sweat continued well into the 3rd and 4th hr after injection of glucose, and that our volunteers tolerated 3 hr of heat exposure without discomfort. Results of these studies are given in Table 1.

The efficiency of our method of collecting sweat solutes by washdown and the accuracy of the concentration and reconstitution procedures were tested in experiments in which a dose of 0.5 μc of lactate-^{14}C solution was spread about on the skin of volunteers who had not received systemic ^{14}C-labeled materials recently. The volunteer then sweated in the chamber for 90 or 180 min as in the experiments described above. Table 2 summarizes the results of these studies. The recovery of radioactivity in the majority of the volunteers was approximately two-thirds of the dose applied, but in a few cases it exceeded 80%. Since earlier recovery experiments based on the chemical determination of unlabeled phosphate (an ion present in only minute amounts in normal sweat (2)) had indicated that our recoveries were usually nearly 95%, we tested the recovery of lactate-^{14}C after a sweating period of only 5 min, and observed the nearly quantitative yield that we had expected. Because of the apparent nonlinearity of the time course of loss of superficially applied lactate-^{14}C, we also carried out a number of experiments in which the subject was exposed to the chamber for 90 min, the labeled lactate was applied, and he then sweated for another 90 min (180 in all). Under these circumstances, the recovery of radioactivity was somewhat (and significantly) better than it had been when the isotope was applied at the initiation of the period of sweating. We did not feel it necessary to pursue further the causes of these losses of radioactivity, but feel that degradation of labeled lactate by skin bacteria must have been a major factor. Losses of up to 30% of sweat radioactivity in the foregoing experiments would not be of any significance in relation to the conclusions to be drawn.

TABLE 1. *Percent of injected ^{14}C recovered in sweat*

Subj	*A*: Given as Lactate-^{14}C	*B*: Given as Glucose-^{14}C	*A/B*, %
HB	0.054	1.35	4.0
LD	0.070	1.91	3.7
DR	0.11	2.09	5.3
TF	0.075	1.85	4.0
JS	0.41	2.20	18.5
KL	0.29	1.72	16.9
KP	0.14	2.00	7.1
SF	0.19	2.83	6.7
CP	0.18	2.46	7.1
Mean values ±SE	0.17 ±0.04	2.05 ±0.14	8.15 ±1.87

TABLE 2. *Percent recovery in both of lactate-^{14}C applied to skin*

5 Min		90 Min		180 Min		Second 90 Min	
Subj	%	Subj	%	Subj	%	Subj	%
SB	93.8	*AD*	71.1	*SB*	81.6	*SB*	94.7
AC	97.6	*LE*	68.4	*AC*	73.3	*DG*	70.0
DF	95.4	*JH*	72.3	*DF*	73.3	*CH*	74.3
JJ	94.4	*JJ*	71.5	*SK*	82.3	*MM*	94.6
SK	99.2	*WR*	60.1	*RN*	65.6	*RN*	74.2
RN	97.1	*JS*	70.6	*RR*	76.2	*KS*	88.1
RR	95.2	*MW*	61.6	*RW*	68.1	*SS*	86.8
WR	92.9						
RW	96.1						
Mean ±SE	95.7 ±0.66		67.9 ±1.9		74.3 ±2.4		83.2 ±3.9

In the experiments just described, only the total yield of radioactivity in sweat was noted. To demonstrate that the radioactive compound recovered in sweat was indeed lactate, studies were done in five other subjects, each of whom received only a single injection of 10 μc of glucose-^{14}C. These subjects sweated for 90 min and were then washed with only one aliquot of approximately 2.5 L of distilled water, so as to yield a maximal concentration of the radioactive metabolite, although the collection was not quantitative. After vacuum evaporation to about 5 times initial sweat concentration, the sweat solutes were fractionated as follows. The first fraction (neutral or alkaline ether-soluble materials) was extracted overnight by continuously refluxing diethyl ether, the concentrated sweat having been alkalinized to a pH of approximately 12 by the addition of 0.02 M sodium carbonate. The second fraction (ether-soluble acids) was obtained by continuing the ether extraction a second night, after acidifying the aqueous phase to a pH between 1 and 2 by the addition of 0.05 M sulfuric acid. The water-soluble residue constituted the third fraction. Table 3 shows the distribution of radioactivity into these fractions in the five subjects used for this phase of the investigation, as well as the behavior of our labeled glucose and lactate preparations when mixed with isotonic sodium chloride containing 10 mEq/L of carrier lactate and fractionated similarly.

We made an attempt to characterize the organic acid fraction from the above studies by paper chromatography, but even after evaporation of the ether phase and concentration of the residue in a small volume of water, we did not find a sufficiently high level of radioactivity to permit the demonstration of clear peaks. We therefore utilized material derived from two other volunteers to determine the partition coefficients of the radioactive metabolite in two systems: equal volumes of diethyl ether and 0.05 M

sulfuric acid, and equal volumes of chloroform and 0.05 M sulfuric acid. Ether and chloroform were chosen because of their convenient volatility, and because data exist in the older chemical literature, summarized by Seidell (3), on the distribution of many organic acids in these systems. The extraordinary water solubility of lactic acid and its virtual insolubility in chloroform are unique among commonly encountered products of metabolism. Table 4 summarizes the results of our experiments with the radioactive metabolites obtained from two normal volunteers, and our findings when authentic labeled lactic acid was added to the same systems as an internal standard. In addition, the published data on the distribution of lactic and pyruvic acids are shown for comparison. Other organic acid metabolites cited by Seidell all have a higher relative solubility in ether and chloroform. The observed values are in good agreement with those predicted on the assumption that essentially all of the radioactivity in the organic acid fraction of sweat is lactic acid.

The chemical nature of the radioactive water-soluble metabolite identified in Table 3 as fraction three remains unknown. Our attempts to identify it further have been limited by the very low level of radioactivity remaining in the solution after extraction of the acidic components. We did demonstrate (using material derived from *subjects AD* and *JJ*) that the labeled material equilibrates easily across a cellophane membrane on standing overnight in the refrigerator. To test the hypothesis that this labeled material might be urea, radioactive as a result of the incorporation of $^{14}CO_2$ derived from the oxidation of glucose, we treated the fraction derived from four of the five subjects with urease. Using conditions under which authentic urea-^{14}C was totally hydrolyzed to $^{14}CO_2$ and discharged to the atmosphere, we found no loss of radioactivity from the solution of the unknown metabolite.

TABLE 3. *Chemical fractionation of ^{14}C sweat metabolites*

Subj	Percent of Sweat ^{14}C Recovered in:		
	Fraction 1 (ether soluble, high pH)	Fraction 2 (ether soluble, low pH)	Fraction 3 (water soluble)
JJ	0	93	8
WR	0	95	3
AD	0	94	9
MW	0	91	9
JH	0	94	3
Glucose*	0.2	1	94
Glucose*	0	0.3	106
Lactate*	0	91	0.4
Lactate*	0	99	1.0

* Fractionations done with samples of authentic radioactive material.

TABLE 4. *Organic solvent partition of ^{14}C sweat metabolites*

Material	Partition Coefficient (Organic/Aqueous)	
	Ether	Chloroform
Metabolite from *subj RR*	0.081	< .03
Metabolite from *subj MM*	0.079	< .01
l-Lactic acid-1-^{14}C	0.081	< .01
Lactic acid*	0.084	0.016
Pyruvic acid*	0.16	0.06

* Results of classic chemical studies reported by Seidell (3).

CONCLUSIONS AND DISCUSSION

It is immediately apparent, from inspection of the results in Table 1, that labeled glucose is more efficient as a precursor for sweat lactate than is labeled lactate itself. This indicates at once that most, if not all, of the lactate found in sweat is a product of glandular metabolism, as Weiner and Van Heyningen supposed, and that little if any is derived from circulating lactate.

Because of the well-known biologic interconversion of glucose and lactate (the Cori cycle), both blood metabolites must have become labeled when either one was injected in isotopic form. There have been a number of studies of the kinetics of this process in intact man; one of the most recent and most relevant to the evaluation of our results is that of Waterhouse and Keilson (4). Using normal volunteers who differed from our subjects in being older, on the average, and in having had no breakfast on the day of study, these authors found that 7–55% of the blood pool of lactate (and pyruvate) eventually returns to circulation as glucose and that, during the first 2.5 hr after the injection of labeled pyruvate, one might expect to find from 1.5 to 10% of the dose of radioisotope in circulation at any one moment as glucose. Our subjects having been permitted to eat, we should expect that the replacement rate of blood glucose would be higher than in fasting subjects (5) and also that the appearance of radioactive carbon derived from lactate in the blood glucose pool would be delayed, due to its deposition into and mixing with the larger quantity of glycogen stored in the liver. Nevertheless, it seems likely that the conversion of lactate to glucose through the Cori cycle, with subsequent utilization of this glucose for the production of sweat lactate, accounts for most if not all of the observed excretion of labeled sweat lactate after the injection of ^{14}C-labeled lactate as a precursor. Unfortunately, it is not possible to analyze these processes more quantitatively in the absence of data on blood radioactivity in our subjects, and without closely timed analyses of sweat suitable for kinetic studies. However, we have yet to develop methods permitting the simultaneous collection of frequent closely timed blood and sweat samples, without contamination of one by the other and without imposing excessive discomfort on the subject.

In the light of our finding that the lactate found in human sweat is indeed a product of glandular metabolism, it is interesting to reconsider the implications of the thermodynamic calculations of Weiner and Van Heyningen (6). Not only might the glands liberate an amount of energy sufficient to account for the osmotic work of sweat secretion by glycolysis of glucose or glycogen, it now appears that they must do so. Yet with the cutaneous vasodilation and high blood flow that always occur in sweating subjects, the microenvironment of the sweat glands must be very well oxygenated. This is, of course, evidenced by the bright

pink color of the skin of any healthy person who is overheated and sweating. We believe, therefore, that the high rate of glycolysis observed in human sweat gland tissue both in vitro and in vivo implies that in some way, as yet undetermined, lactate production is linked to the secretory process. In an earlier publication (1), we suggested one possible mechanism by which this might occur. The present results neither confirm nor detract from that hypothesis, but are consistent with the possibility that lactate production in sweat gland tissue represents something other than a means of producing ATP under circumstances where oxidative metabolism is inadequate for functional needs

Received for publication 17 May 1971.

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Grabowski-'61
Bib No. 646

Science 134:1357-1360. 1961.

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Reports

Lactic Acid Accumulation as a Cause of Hypoxia-Induced Malformations in the Chick Embryo

Abstract. Most hypoxia-induced malformations are caused by a syndrome involving tremendous edema followed by formation of clear blisters and hematomas. These, in turn, mechanically interfere with development. Studies of blood *p*H and lactic acid indicate that lactate accumulation initiates this syndrome. The effects of lactic acid injections parallel those of subjection to hypoxia, confirming this conclusion.

Studies of the teratogenic and lethal effects of hypoxia on chick embryos in this laboratory (*1*) have centered around (i) a comprehensive quantitative survey of the effects of graded doses of hypoxia, and (ii) a morphogenetic and biochemical analysis of these effects. Certain conclusions are now fitting together into a total picture, which is reported here.

The earlier studies (*2*, *3*) showed that the effects of oxygen deficiency could be studied quantitatively. Furthermore, within established limits, the effects were proportional to the treatment. It was also shown that, in embryos of a given age group, different levels of hypoxia (produced by a flowing mixture of air and N_2) could induce qualitatively different kinds of anomalies (*3*, *4*). These differences were due to different modes of action of hypoxia. For instance, in 3-day embryos, exposure to 1 percent O_2 for 3 hours produces a variety of severe head anomalies caused by extensive cell death in this region. Mild hypoxia (6 to 12 hours at 10 to 12 percent O_2) induces little else than rumplessness of unknown cause. But malformations, especially of eyes, beak, and extremities, are induced most readily by moderate hypoxia (6 to 12 hours at 4 to 8 percent O_2). These malformations are caused not by any direct action of oxygen deficiency but by an indirect sequence of events (*5*). The primary effect of the hypoxia is to produce a tremendous edema (embryo volume increases up to tenfold) which persists for several hours. Numerous subcutaneous blisters appear over the head and trunk. Most of them disappear, but some persist either as clear blisters or as hematomas after neighboring blood vessels rupture into them. Additional hematomas may appear, especially in the eye cup and in head mesenchyme. Persistent blisters and hematomas are evidently responsible for the maldevelopment of adjacent structures, since (i) incidence of malformations of a given region agreed with incidence of hematomas found 24 hours after treatment, and (ii) abnormal development of these structures does not begin until 2 or 3 days after treatment.

Further study was concentrated on this circulatory syndrome, since (i) it was responsible for most of the observed anomalies and (ii) it seemed amenable to experimental analysis. It was postulated that the primary effect —edema—may be due to the accumulation of metabolites from prolonged anaerobiosis, such as lactic acid, and resulting disturbances in *p*H and salt balance. Consequently, the *p*H of venous blood samples from over 50 embryos, 3 and 4 days of age, were checked before and after treatment. A Beckman *p*H meter with capillary glass electrode was used. Normal *p*H was 8.0 to 8.2. This level was maintained in most embryos checked immediately

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* 125, 16 (1957)].

after mild and moderate subjection to hypoxia except for an occasional individual in which *p*H dropped to 7.6 or even to 6.8. This drop was always associated with a very feeble heartbeat and other signs of imminent death. If the circulatory disturbance was caused by the accumulation of acidic metabolites, it was apparent that the buffering capacity of the embryonic blood stream could, up to a certain point, keep the *p*H constant.

Direct assay of the lactic acid content of blood of 3- and 4-day embryos was then attempted, with 0.01-ml samples and a method sensitive to ± 0.4 μg of lactate (*6*). The lactic acid level in the blood of 10 controls ranged from 50 to 150 μg/ml. The lactate levels of embryos subjected to hypoxia ranged from 400 to 1300 μg/ml, and this in embryos swollen to 10 times normal volume. The average for embryos subjected to moderate hypoxia was around 800 μg/ml. This is equivalent to a concentration of 0.08 percent—certainly enough to affect the salt balance of an embryo containing 0.85 percent of total salts. Clearly, lactic acid accumulates in quantity in the blood stream of embryos subjected to hypoxia.

Was this increase in blood lactate a significant effect of hypoxia? Direct applications of lactic acid solutions (0.01 to 0.03 ml of 0.01 percent lactic acid in saline) were made to the vitelline membrane, amniotic cavity, or subgerminal cavity of 3-day embryos and into the allantoic cavity of 4-day embryos. Regardless of method of injection or age of embryo, all the early effects of lactic acid injection precisely paralleled those of subjection to moderate hypoxia—namely, extensive edema followed by the appearance of subcutaneous blisters and hematomas. The death rate was generally high, around 90 percent, but only 70 percent of embryos injected with lactic acid by way of the allantoic route died. Of the 79 survivors in this group, 10 (12.7 percent) developed anomalies. Only one of the 85 surviving saline-injected controls was abnormal. Furthermore, the malformations obtained with lactic acid injections were the same ones induced by hypoxia at this age—namely, defective eyelids, exocephaly, and short upper beaks. Many details remain to be studied, but the conclusion is clear. The simple accumulation of lactic acid—the physiological consequence of prolonged anaerobiosis—is responsible for most of the abnormal development which follows exposure to oxygen-deficient atmospheres.

The significance of these data may extend beyond that of interest in chick embryos subjected to hypoxia. Vascular anomalies have been described in mouse fetuses subjected to anoxia (*7*). A comparable syndrome of edema, subcutaneous blisters, hematomas, and maldevelopment of adjacent tissues has been described in mammalian and avian embryos in deficiencies of pantothenic and linoleic acids, after administration of the redox dye trypan blue, as well as in Little and Bagg's famous mutant strain of mice (*8*). Is it not possible that some of these agents (and others too) and hypoxia have a common mode of action—that is, an interference with oxidative metabolism and the accumulation of metabolites?

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References and Notes

1. The studies reported here were supported by a grant from the Easter Seal Research Foundation. The investigation was initiated at the University of Pittsburgh and continued at the University of Miami. I acknowledge the technical assistance of J. Beck, R. Moyers, and M. Milan in various phases of this program.
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26 July 1961

J. Dental Res. 28:282-287. 1949.

Granados-'49
Bib No. 649

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OBSERVATIONS ON EXPERIMENTAL DENTAL CARIES

III. The Effect of Dietary Lactic Acid

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THE decalcification of enamel by acids of bacterial origin, particularly lactic acid,[1] is still thought by many investigators to be the original lesion in dental caries. McClure[2] has observed a pronounced destructive effect of dilute solutions of hydrochloric and lactic acids on the enamel and dentin of rat molars. Gortner, Restarski, Bieri, and McCay[3] have also shown that drinking solutions of lactic and other acids produces marked decalcification of the rat and hamster molars.

On the other hand, Gortner, McCay, Restarski, and Schlack[4] have observed that oxalic acid solutions, unlike solutions of other acids, do not produce etching of the rat enamel. On the contrary, oxalic acid or its sodium salt diminishes or prevents the enamel decalcification that might be expected by the simultaneous ingestion of phosphoric acid and citric acid solutions. This protective role of oxalic acid against enamel decalcification was found to be due to the formation of calculuslike deposits, presumably of calcium oxalate, on the molars. Taking into consideration these and some other observations, Schlack, Howell, Taylor, Berzinskas, and Aborn[5,6] have investigated the effect of oxalates, administered in the food and in the drinking water, on caries activity in the albino and cotton rats. These workers found no protective action of the oxalates against the incidence or extent of carious lesions.

We are reporting here the results of an experiment on the effect of dietary lactic acid, incorporated in the food, and in the drinking water, on caries activity in the hamster.

EXPERIMENTAL

Forty-five Syrian hamsters, between 21 and 25 days of age, from litters of a colony maintained on a diet of Purina Laboratory Chow* and raw milk, were distributed into three groups of fifteen animals each (eight males and seven females). The animals were set in screen bottom cages without bedding, and reared for 100 days on the following rations:

Group 1 (Diet 1): ground yellow corn† 40 per cent, sucrose 20 per cent, cornstarch 5 per cent, potato starch 5 per cent, powdered whole milk 19 per cent, ether-extracted yeast 5 per cent, alfalfa meal 5 per cent, and sodium chloride 1 per cent.

Group 2 (Diet 2): to each 100 gm. of Diet 1 were added, and thoroughly mixed, 0.057 c.c. of lactic acid (80 per cent). Groups 1 and 2 were given distilled water to drink.

Received for publication, August 6, 1948.

*From Ralston Purina Co., St. Louis, Mo., U.S.A.

†The particle size of the ground yellow corn was distributed as follows: 100 per cent through a 12.5-mesh screen; 63 per cent through a 25-mesh screen, and 31 per cent through a 50-mesh screen.

Group 3 (Diet 3): this group was fed Diet 1, but here each 100 c.c. of the distilled water given to drink contained 0.050 c.c. of lactic acid (80 per cent). The diets as well as the drinking fluids were all available ad libitum, and the animals were weighed every week. These experiments were carried out between the months of November and February.

The amounts of lactic acid given in the diet to Group 2 and that supplied in the water to Group 3 were calculated in such a way that the animals of these two groups would ingest daily the same amount of lactic acid. This calculation was made after observation of the average daily amount of food and water consumed by the hamster. The pH of the diets mixed with distilled water was determined with a glass-electrode potentiometer. The diets and the fluids had the following pH: Diet 1, 5.55; Diet 2, 5.12; distilled water, 6.8; distilled water + lactic acid, 3.1. Throughout the whole experimental period the lactic acid was added to the food (Diet 2) and to the water (Diet 3) every day.

On completion of the experimental period the animals were sacrificed and autopsy was performed on them. After fixation in 10 per cent formalin, the jaws were prepared for examination in the usual way. The degree of enamel decalcification was evaluated in accord with the method devised by Restarski, Gortner, and McCay,[7] and the carious lesions were recorded and scored using the chart described by Keyes.[8] Ground sections were prepared from several molars of the different groups.

RESULTS

The animals from the three groups exhibited the same healthy appearance. Likewise, the growth rate of the three groups was essentially the same, as it can be seen in Fig. 1. Møllgaard, Lorenzen, Hansen, and Christensen[9, 10] have reported that lactic acid, either incorporated in the diet or formed in the intestines in increased amounts by ingestion of skim milk acidified with lactobacillus, produces in the pig a marked increase in the absorption of calcium and phosphorus, and considerably promotes the growth and health of this animal. However, under the conditions of our experiment we found that, in the hamster dietary, lactic acid does not improve the growth or health of the animal. This difference may be due to the fact that all or most of the lactic acid supplied to the hamsters was constantly neutralized or combined in such a way that it could not reach the intestines in the free state, and there exert any apparent beneficial action on the growth. Otherwise, some physiological and biochemical differences with respect to lactic acid that might exist between the two animal orders could account for this discrepancy.

The autopsy revealed no gross changes other than those found in the oral cavity. Various degrees of alveolar resorption were found in several animals, but there was not significant difference in the incidence or extent of resorption among the three groups.

In Group 1 the common yellow-brown stain of the enamel cuticle, unrelated to caries activity, was found on all surfaces of the molars. In this group there was no evidence of enamel decalcification in any of the molar surfaces (grade 0).

In Group 2 the stain was present mainly on the lingual surfaces of both upper and lower molars. Some buccal and many occlusal surfaces appeared stainless and slightly chalky; this was especially true in the occlusal fossae. This indicates that the lactic acid incorporated in the food acted, at least slightly, on the enamel of those surfaces which are most favorable for food retention. In accord with Restarski, Gortner, and McCay's method[7] this group was also grade 0. In Group 3 the stain was almost completely absent. The enamel of most surfaces appeared stainless, somewhat chalky, and sometimes slightly etched. The cusps of the molars appeared somewhat shorter, thus giving a shallower appearance to the fossae. In this group, furthermore, lines of fracture and brittleness of

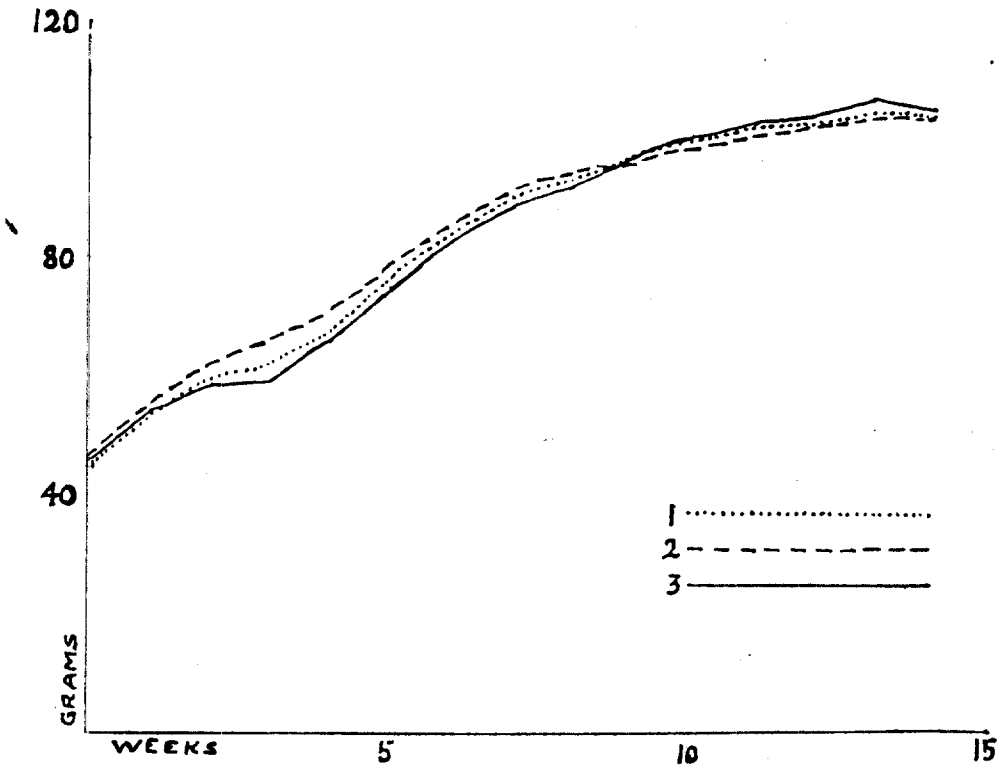

Fig. 1.—Average growth curves of the three groups.

the enamel were frequently observed. It is apparent that all these changes, found in both jaws with about the same intensity, were due to the lactic acid contained in the water given to Group 3. The enamel decalcification of the lingual surfaces in this group was grade 2.

As it can be seen from Table I, there was no significant difference at all in the incidence or extent of carious lesions among the three groups. Comparing the caries activity in the two jaws (Table II), Group 1 exhibited slightly higher incidence and extent of lesions in the maxilla. In Group 2 the caries activity also was significantly higher in the maxilla. In Group 3 the incidence of lesions

was only slightly higher in the maxilla, but the extent of the lesions was significantly higher than in the mandible. Comparing the incidence of occlusal, buccal, and lingual carious lesions in the various groups, Table III shows that the lingual surfaces were the least affected in all the three groups, and that there was a significant higher incidence of lesions in the occlusal than in the buccal surfaces, this being especially so in Group 2.

TABLE I
CARIES INCIDENCE IN THE THREE GROUPS

	GROUP 1			GROUP 2			GROUP 3		
	♂♂	♀♀	♂♂ + ♀♀	♂♂	♀♀	♂♂ + ♀♀	♂♂	♀♀	♂♂ + ♀♀
Number of experimental animals	8	7	15	8	7	15	7*	7	14
Number of animals affected	8	7	15	8	7	15	7	7	14
Number of carious molars	62	62	124	62	51	113	57	60	117
Average number of carious molars	7.7	8.8	8.2	7.7	7.3	7.5	8.1	8.6	8.3
Number of carious lesions	90	90	180	98	73	171	90	89	179
Average number of carious lesions	11.2	12.8	12	12.3	10.4	11.4	12.9	12.7	12.8
Average scores of carious lesions	7.0	6.8	6.9	8.7	5.9	7.3	7.9	7.9	7.9

*One of the eight original males of this group died at the beginning of the experiment.

Table I also shows that although in Group 2 there was a higher incidence and extent of caries in the males than in the females, this was not the case in Groups 1 and 3. Thus, in these experiments, no sex difference was consistently found in relation to caries activity. Through the microscopic study of ground sections, signs of acid action (transverse striations of the prisms, cloudy enamel) were found, combined or not with caries, at the very bottom of many occlusal fossae in Groups 2 and 3.

TABLE II
CARIES INCIDENCE IN THE TWO JAWS

	GROUP 1 15 ANIMALS		GROUP 2 15 ANIMALS		GROUP 3 14 ANIMALS	
	MAXIL.	MANDIB.	MAXIL.	MANDIB.	MAXIL.	MANDIB.
Number of carious molars	67	57	70	43	66	51
Average number of carious molars	4.5	3.8	4.7	2.9	4.7	3.6
Number of carious lesions	93	87	106	65	96	83
Average number of carious lesions	6.2	5.8	7	4.3	6.9	5.9
Average scores of carious lesions	7.3	6.8	10.4	4.5	8.5	4.8

TABLE III
PERCENTAGE OF OCCLUSAL, BUCCAL, AND LINGUAL CARIOUS LESIONS IN THE THREE GROUPS

LESIONS	GROUP 1	GROUP 2	GROUP 3
Occlusal	76.2	88.3	76.0
Buccal	22.6	9.4	22.9
Lingual	1.2	2.3	1.1

DISCUSSION

The incidence of caries in the various surfaces in the three groups may be compared and correlated with the signs of enamel decalcification present in certain surfaces in Groups 2 and 3. Groups 1 (water) and 3 (lactic acid in the water) exhibited essentially the same incidence of caries in the various surfaces. Thus the lactic acid solution given to Gorup 3 was able to produce an enamel decalcification which did not alter the susceptibility of the decalcified surfaces to dental caries. On the other hand, in Group 2 (lactic acid in the food), the occlusal surfaces, being less affected by acid than in Group 3 but more than in Group 1, exhibited higher caries incidence than in Groups 1 and 3, at expenses of much lower incidence in the buccal surfaces (Table III). This difference, the origin of which is not clear, is, however, within nearly the same total incidence and extent of caries present in all the three groups.

Thus, under the conditions of our experiment, dietary lactic acid produced enamel decalcification but failed to alter, in any direction, the incidence or extent of carious lesions. On the other hand, if the incidence of caries were to be at least partially increased by the presence of lactic acid in the bottom of the occlusal fossae, one could say that Group 2 exhibited a higher incidence of occlusal lesions than the other groups; for in Group 2 the lactic acid mixed in the food was forced rapidly during the process of mastication into the occlusal fossae, without the saliva having had time to neutralize it. Perhaps this might explain the higher incidence of occlusal cavities in Group 2, but not the decrease of buccal lesions in this group (Table III) to a level far below the percentage of buccal lesions in Groups 1 and 3.

The fact that we found gross and microscopic evidence of acid action in many occlusal fossae, including the bottom of them, and combined or not with caries, in Groups 2 and 3, leaves no doubt that the dietary lactic acid in many instances reached the bottom of the fossae. In spite of this, we have seen that there was no significant difference in the incidence or extent of lesions among the three groups. This fact shows that, in these experiments, the dietary lactic acid, which could reach even the bottom of the fossae, did not play any important role in the development or progress of dental caries.

SUMMARY

The effect of dietary lactic acid on dental caries was studied in three groups of newly weaned hamsters, which were reared for 100 days on the experimental diets. Group 1 (control) was fed the cariogenic ration, and distilled water. Group 2 received the cariogenic diet to which had been added 0.057 c.c. of lactic acid (80 per cent) per 100 gm. of food, and distilled water. Group 3 was fed the cariogenic diet, and distilled water to which had been added 0.050 c.c. of lactic acid (80 per cent) per 100 c.c. of water.

No significant differences were found in the incidence or extent of carious lesions among the three groups. The animals from all the groups exhibited essentially the same health appearance and the same growth rate.

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EFFECT ON THE URINE OF ADDITION OF ACIDS AND ALKALIS TO THE DIET OF INFANTS*

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Because of the marked buffer action of cow's milk and its consequent inhibitory action on hydrochloric acid, various methods have been used to promote the efficacy of the gastric juice in normal infants, as well as in those with digestive disturbances. Such aids are milk acidified with tenth-normal hydrochloric acid, lactic acid, lemon juice and bacterial cultures. Good results have been obtained with these additions, namely, a steady gain in weight and an improvement in digestive disturbances. Little attention, however, has been paid to the changes resulting in the urine when acid has been added to the milk.

Von Bernuth and Duken [1] observed a series of spasmophilic and non-spasmophilic infants who were given 260 cc. and 400 cc. tenth-normal hydrochloric acid to a liter of milk. In fourteen of twenty-three infants, or 61 per cent, casts developed in the urine; most of these were finely granular, although there was an occasional hyaline cast; sometimes there was a trace of albumin. These urinary changes disappeared rapidly when the acid was omitted from the diet.

Feuchtwanger and Lederer [2] showed that the most important condition for the appearance of casts in the urine is an acid reaction of the urine. When a definite acidity is reached, casts appear. Freudenberg [3] observed that after additions of ammonium chloride a strongly acid urine with casts resulted; these pathologic factors disappeared when this substance was omitted.

The present investigation embraces an extensive study of the urinary changes in infants and in older children who received one of the following acids or alkalis in the milk ingested: tenth-normal and thirtieth-normal hydrochloric acid, lactic acid, lemon juice, sodium bicarbonate or sodium hydroxide in varying quantities and concentrations. A small number of children was selected in order that the effect of these various substances could be observed on the same child for a long period.

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* Received for publication, Aug. 13, 1927.

* From the Home for Hebrew Infants (service of Dr. Alfred F. Hess).

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2. Feuchtwanger, A., and Lederer, M.: Zur Frage der Entstehung von Harnzylindern bei Säuglingen, Jahrb. f. Kinderh. **112**:7, 1926.

3. Freudenberg, E.: Einfluss der Ionen auf die Diurese beim Saügling, Ztschr. f. Kinderh. **39**:608, 1925.

Since 1924, when Hess[4] reported the good results obtained with "lemon-juice-milk," all the infants in one ward at this institution (about twenty-five) have received lemon juice in a dilution of 3 per cent mixed with their milk formula. Eight normal infants were selected for study from this ward in order to ascertain whether the acidity of lemon juice leads to any abnormalities in the urine. Examinations of many specimens of urine showed that the reaction was acid and that abnormal chemical or microscopic changes had not occurred. The hydrogen ion concentration of the urine of three infants for a period of six days showed a range from 5.5 to 7.[5] It is evident, therefore, that the citric acid contained in the 3 per cent dilution of lemon juice did not irritate the kidneys in any way.

The investigation was then continued with three normal infants, aged 7½, 8 and 11 months, whose urine had been normal on several occasions; one of these had been receiving "lemon-juice-milk." Two hundred and forty cubic centimeters of thirtieth-normal hydrochloric acid was added to the milk formula. For the first few days, changes were not noted in the urine. After from two to six days, microscopic examinations showed red blood cells and many finely granular and occasionally hyaline casts in the urine of all three infants; coarsely granular casts were found in two instances and a faint trace of albumin in one. When the acid was omitted from the diet, the urine became normal from three to six days later. As was to be expected, the greater the number of casts, the more frequently granular casts appeared in the urine. In fact, as many as ten casts were not observed in any instance when only hyaline casts were present. In other words, when + + + or + + + + are designated in table 1, granular as well as hyaline casts are indicated. It should be added, however, that not infrequently granular casts appeared when the total number was less than ten, that is to say, when the change has been designated as + +. It was surprising that with the development of casts in the urine, showers of them in some instances, albumin was so rarely observed; even when red blood cells were present, they were rarely noted. Apparently casts, usually the hyaline variety, are the first indication of irritation of the kidney.

To the milk given an infant, aged 3 months, who had tetany, 240 cc. of tenth-normal hydrochloric acid was added. Twenty-four hours later, the urine showed a faint trace of albumin and a few finely granular

4. Hess, A. F., and Matzner, M. J.: The Value of Milk Acidified with Lemon Juice, J. A. M. A. **82**:1604 (May 17) 1924.

5. The p_H of the urine was determined by means of the colorimetric method. For the upper range of hydrogen ions (from 6.0 to 8.5) phenolsulphonphthalein was used. For the concentrations below 6.6, special standards were prepared, for p_H 4 (brom phenol blue), for p_H 5 (brom cresol green) and for p_H 6, (brom phenol red).

TABLE 1.—*Appearance of Casts Following the Addition of Varying Strengths of Hydrochloric Acid to Milk* *

Name.....	S. F.	B. K.	H. W.	M. M.	E. R.	N. F.	A. W.	C. W.	M. F.
Age.....	11 mo.	5 mo.	7½ mo.	3½ mo.	4½ yr.	4 yr.	5 yr.	4 yr.	4 yr.
3/3/27.....	240 cc. N/30 HCl added	240 cc. N/30 HCl added	240 cc. N/30 HCl added	.....	.....	.....	.....	.....	.....
3/7/27.....	++	0	0	.....	.....	.....	.....	.....	.....
3/8/27.....	+++	++	0	.....	.....	.....	.....	.....	.....
3/9/27.....	++	+	0	.....	.....	.....	.....	.....	.....
3/10/27.....	0	0	++	.....	.....	.....	.....	.....	.....
3/11/27.....	0	0	++	.....	.....	.....	.....	.....	.....
3/15/27.....	N/30 HCl omitted	N/30 HCl omitted	N/30 HCl omitted	240 cc. N/10 HCl 8 Gm. calcium lactate added	.....	.....	.....	.....	.....
3/16/27.....	.....	.....	.....	+	.....	.....	.....	.....	.....
3/17/27.....	.....	.....	.....	++++	.....	.....	.....	.....	.....
3/18/27.....	0	0	0	++++	.....	.....	.....	.....	.....
3/19/27.....	0	0	+	.....	.....	.....	.....	.....	.....
3/22/27.....	0	+	0	.....	.....	.....	.....	.....	.....
3/28/27.....	45 cc. N/10 HCl added	45 cc. N/10 HCl added	45 cc. N/10 HCl added	45 cc. N/10 HCl added	.....	.....	.....	.....	.....
3/29/27.....	++	0	++	0	.....	.....	.....	.....	.....
3/30/27.....	0	0	+	.....	.....	.....	.....	.....	.....
4/1/27.....	++	++	+	.....	.....	.....	.....	.....	.....
4/2/27.....	0	0	0	++++	.....	.....	.....	.....	.....
4/3/27.....	+	0	0	++	.....	.....	.....	.....	.....
4/4/27.....	++	++	+	++++	.....	.....	.....	.....	.....
4/5/27.....	++	0	0	++++	.....	.....	.....	.....	.....
4/5/27.....	N/10 HCl omitted	N/10 HCl omitted	N/10 HCl omitted	N/10 HCl omitted	.....	.....	.....	.....	.....
4/6/27.....	0	.....	0	++++	.....	.....	.....	.....	.....
4/7/27.....	0	+	0	.....	.....	.....	.....	.....	.....
4/8/27.....	25 cc. N/10 HCl added	25 cc. N/10 HCl added	25 cc. N/10 HCl added	.....	100 cc. N/10 HCl added	100 cc. N/10 HCl added	.....	.....	.....
4/9/27.....	0	.....	0	+++	0	0	.....	.....	.....
4/11/27.....	0	0	0	0	++	0	.....	.....	.....
4/12/27.....	0	+	0	+++	0	0	.....	.....	.....
4/13/27.....	0	+	0	0	0	0	.....	.....	.....
4/14/27.....	0	+	0	0	0	.....	.....	.....	.....
4/15/27.....	N/10 HCl omitted	.....	N/10 HCl omitted	.....	.....	.....	.....	.....	.....
4/16/27.....	0	0	0	++++	240 cc. N/10 HCl added	.....	.....	.....	.....
4/17/27.....	0	0	0	+++	0	.....	.....	.....	.....
4/18/27.....	0	0	0	++++	++	.....	.....	.....	.....
4/19/27.....	0	0	0	++++	0	.....	.....	.....	.....
4/20/27.....	0	0	0	++++	+	.....	.....	.....	.....
4/21/27.....	45 cc. N/10 HCl added	45 cc. N/10 HCl added	45 cc. N/10 HCl added	Calcium lactate omitted	.....	.....	100 cc. N/10 HCl added	.....	.....
4/23/27.....	++++	+	0	0	0	.....	++	.....	.....
4/24/27.....	++	+	0	+	0	.....	+	100 cc. N/10 HCl added	.....
4/25/27.....	0	++	0	0	++	.....	++++	++	.....
4/26/27.....	0	++	0	+	0	.....	++	0	.....
4/27/27.....	0	++	+	0	+	.....	.....	0	.....
4/28/27.....	0	0	0	0	+	.....	+	0	.....
4/29/27.....	0	0	+	0	+	.....	.....	0	.....
4/30/27.....	0	0	0	0	++	.....	.....	++	.....
5/1/27.....	N/10 HCl omitted	N/10 HCl omitted	N/10 HCl omitted	.....	.....	.....	N/10 HCl omitted	.....	240 cc. N/10 HCl added
5/2/27.....	0	0	0	.....	+	.....	++	0	++
5/3/27.....	.....	.....	.....	.....	0	.....	.....	+	0
5/6/27.....	.....	.....	.....	.....	0	.....	+	0	++++
5/7/27.....	.....	.....	.....	.....	0	.....	.....	0	+++
5/8/27.....	.....	.....	.....	.....	++	.....	.....	0	++
5/9/27.....	.....	.....	.....	.....	.....	.....	.....	0	++
5/10/27.....	.....	.....	.....	.....	.....	.....	.....	0	+
5/11/27.....	.....	.....	.....	.....	.....	.....	.....	0	+++
5/12/27.....	.....	.....	.....	.....	.....	.....	.....	0	++
5/13/27.....	.....	.....	.....	.....	.....	.....	.....	0	++++
5/14/27.....	.....	.....	.....	.....	.....	.....	.....	0	++

* In the table, + = 1 to 2 casts; ++ = 3 to 10 casts; +++ = 11 to 20 casts; ++++ = more than 20 casts per 1 square centimeter.

casts; subsequently numerous casts of various kinds appeared, as shown in table 1. This infant was also given 8 Gm. of calcium lactate daily. The urine of two older children, 4 and 4½ years of age, who likewise received 240 cc. of tenth-normal hydrochloric acid with their milk, showed hyaline casts from twenty-four to forty-eight hours later.

The effect of a smaller quantity (100 cc.) of tenth-normal hydrochloric acid was next observed. This amount was added to the milk drunk by four older children, aged 4, 4, 4½ and 5 years. One of these did not show any changes in the urine after five days, at which time he left the institution. A few casts were noted at varying intervals in the urine of the three remaining children.

Four infants were next given a still smaller quantity (45 cc.) of tenth-normal hydrochloric acid with their milk. Casts in small numbers appeared in the urine of all these infants within from one to four days. In the case of the infant receiving calcium lactate in addition, casts appeared in larger numbers. When the acid was discontinued, the urine of all the infants cleared up rapidly with the exception of that of the last one; the calcium factor was responsible for the severe urinary changes in this infant, as will be shown subsequently.

As my coworkers and I were interested in determining the minimal amount of tenth-normal hydrochloric acid that could be added to milk without producing urinary changes, the quantity added was reduced to 25 cc. One of the three infants receiving this amount of acid showed merely from one to two casts on three occasions, and the others did not show any changes over periods of from seven to twelve days, respectively. The acid was then omitted from the diet, the urine was examined for five days, and all specimens became normal.

It was thought that possibly this development of casts on the addition of acid and their disappearance when the acid was discontinued might be merely a chance occurrence. In order to assure ourselves of the causal realtionship of the addition of acid, one more test was made. Accordingly, the three infants were given 45 cc. tenth-normal hydrochloric acid daily. After two days in the case of two of the infants and six days in the third, the casts reappeared approximately to the same extent as during the period when this amount of acid had been given previously.

These results indicate that the changes in the urine associated with additions of tenth-normal hydrochloric acid to milk occur even when small quantities of acid are added.

We have noticed also that urine which shows casts on the addition of acid may become normal and remain so indefinitely even though the feeding of acid milk is continued; this leads us to infer that the kidney accustoms itself to the irritant. This was illustrated by E. R., who passed casts intermittently for two weeks when he received 240 cc. of

tenth-normal hydrochloric acid, but whose urine was normal for the seven following days. A similar occurrence was noted in C. W., who received 100 cc. of tenth-normal hydrochloric acid; the urine showed casts intermittently for six days and then became normal for a period of two weeks.

It was evidently of importance to note the hydrogen ion concentration of the urine under these conditions. It was found that the urine of infants receiving 45 cc. of tenth-normal hydrochloric acid and that of older children who received 100 cc. ranged uniformly between a p_H of 4 and 5 with an occasional specimen with a p_H of 6. In the case of E. R., who received 240 cc. of tenth-normal hydrochloric acid, the acidity was only slightly greater than when less acid was given. Apparently the urine cannot readily be acidified beyond a p_H of 4.

TABLE 2.—*Hydrogen Ion Concentration of Urine Following the Addition of Varying Amounts of Tenth-Normal Hydrochloric Acid to Milk*

Name	S. F.	B. K.	H. W.	A. W.	C. W.	E. R.	M. F.
4/16/27	...	...	...	...	...	240 cc. N/10 HCl added	...
4/21/27	45 cc. N/10 HCl added	45 cc. N/10 HCl added	45 cc. N/10 HCl added	100 cc. N/10 HCl added	...	...	...
4/22/27	4-5	4-5	6	4	5	4	...
4/23/27	5-6	4-5	5	4	4-5	4	...
4/24/27	4-6	5	5	4-5	100 cc. N/10 HCl added	4	...
4/25/27	5	5	5	5	5	4-5	...
4/26/27	4-5	5	5-6	5	4-5	4-5	...
4/27/27	5	5	5	4-5	4	5	...
4/28/27	5	4-5	5	5	4	5	...
4/29/27	5-6	5	5-6	5	4	5	...
5/ 1/27	...	...	...	...	...	...	240 cc. N/10 HCl added
5/ 9/27	...	5	...	...	6	...	4-5
5/10/27	...	5	...	...	6	...	4
5/11/27	...	6	...	...	5	...	4
5/12/27	...	5-6	...	...	4	...	4
5/13/27	...	5-6	...	...	4	...	5
5/14/27	...	5-6	...	...	4-5	...	4-5

THE EFFECT OF THE ADDITION OF CALCIUM LACTATE

That calcium may also act as an irritant of the kidneys was reported by Fuechtwanger and Lederer.[2] They observed granular and hyaline casts in the urine a few hours after giving calcium chloride; it disappeared when this medication was omitted. In the present study, the following instance shows the effect of the addition of calcium lactate: Infant M. M., aged 3½ months, suffering from typical tetany with severe convulsions, was given, in addition to 240 cc. of tenth-normal hydrochloric acid, 8 Gm. of calcium lactate daily. After twenty-four hours, his urine showed casts and red blood cells, which continued to be present for the following two days. A somewhat similar result was observed when only 45 cc. of this acid was added. When the acid was omitted, the urine still contained casts on eight different occasions, over a period

of thirteen days. When, however, the calcium lactate was discontinued, the urine became normal within fifteen hours and remained normal thereafter.

THE EFFECT OF THE ADDITION OF LACTIC ACID

A recent paper by Hottinger[6] gave interesting information concerning the excretion of some organic acid in infants. He found that even when large amounts of citric acid were fed, 95 per cent was consumed within the body, whereas when lactic acid was given in the form of buttermilk, a decidedly larger quantity of organic acid was excreted in the urine. The effect of lemon juice has been already discussed.

The effect of lactic acid was studied in two of the older children (4 and 5 years of age). To a quart of milk, two teaspoonfuls of lactic acid (U. S. P. 87 per cent) and 1 ounce of Karo syrup were added, according to the method of Marriott. In one instance, a few casts developed in the urine after three days; casts were not seen for the next six days, but they reappeared on the tenth, fourteenth and sixteenth day; from the seventeenth to the twenty-first day the urine was normal. In the case of the second child, a few casts were noted on the fourth and fifth day, but the urine remained normal thereafter.

The manifestations described in the urine resulting from the addition of acid or calcium to the milk raise the important question as to whether the casts result from an injury to the kidney or from a temporary irritation. It is generally believed that granular casts denote degenerative changes in the tubules of the kidney. In infants, however, conditions are not the same, for the kidney is far more sensitive to minor insults than in the case of the adult, as is illustrated by the well known pathologic changes which accompany such clinical conditions as acute intestinal intoxication, trauma in the new-born and "orthostatic albuminuria." The prompt disappearance of these changes when the source of the irritation has been removed is convincing evidence that the damage to the kidney has been slight and temporary. It would seem that this holds true for the fleeting pathologic conditions which we have occasioned. Seegal[7] recently published an experimental study of the effect on the kidney of feeding of tenth-normal hydrochloric acid. Although the rabbits passed casts and albumin throughout the experimental period, the only demonstrable pathologic change at necropsy was acute degeneration of the convoluted tubules. In those instances in which administration of acid had been stopped, it was found that the kidney had promptly returned to a normal condition. Similar results were obtained in experiments on dogs.

6. Hottinger, A.: Studien über Säuren- Basenhaushalt im kindlichen Organismus, Monatschr. f. Kinderh. **30**:497, 1925.

7. Seegal, B. C.: Chronic Acidosis in Animals: Relation to Kidney Pathologic Change, Arch. Int. Med. **39**:550 (April) 1927.

THE EFFECT OF SODIUM BICARBONATE

It seemed that it would also be interesting to note the effect of additions of an alkali to milk. With this in view, two infants were given 240 cc. of tenth normal sodium bicarbonate with their milk; the taste of the milk was not appreciably altered, and the infants took their formulas well. Examinations carried out for six days failed to show any abnormal changes in the urine. These infants were next given 240 cc. of fifth-normal sodium bicarbonate with their milk; they likewise did not evince any urinary changes. These two infants, as well as the older children, were then given the same amount of a double normal sodium bicarbonate solution. Even this concentration of alkali did not prove irritating; examinations of urine were carried out daily for the following nine days. The reaction of the urine to litmus was alkaline, and the hydrogen ion

TABLE 3.—*Hydrogen Ion Concentration of Urine Following the Addition of Two Hundred and Forty Cubic Centimeters of Double Normal Sodium Bicarbonate to Milk*

Name	M. F.	C. W.	H. W.	S. F.
5/15/27	240 cc. double normal sodium bicarbonate	240 cc. double normal sodium bicarbonate	240 cc. double normal sodium bicarbonate	240 cc. double normal sodium bicarbonate
5/16/27	6.6	7.3	7.8	7.7
5/17/27	6.6	7.4	7.2	7.8
5/18/27	6.6	7.1	7.6	7.6
5/19/27	6.6	7.4	7.6	7.6
5/20/27	7.8	7.6	7.7	7.6
5/21/27	7.1	7.7	6.7*	6.7*
5/23/27	7.7	7.9	7.3	7.8
5/24/27	7.6	7.0	7.5	7.6
5/25/27	7.7	7.9	7.2	8.0

* Sodium bicarbonate omitted.

concentration ranged from 7.1 to 8 when the double normal solution was used. One exception should be noted in this regard, that of one of the older children, who continued to pass acid urine with a p_H of 6.6 in spite of the fact that these large amounts of alkali were being added to the milk. In fact, the experience with sodium bicarbonate, an experience extending over a period of twenty-one days, has made it evident that large amounts of this alkali must be employed when one desires to bring about prompt and definite alkalinization of the urine. In this connection, attention may be called to the paper of Nassau[8] who gave some older children sodium bicarbonate in varying amounts from 7 to 10 Gm. in order to note the effect on "orthostatic albuminuria." Apart from its laxative influence on patients with this condition, it is worthy of note that this amount of alkali did not lead to signs of irritation of the kidney.

8. Nassau, E.: Ueber die Bedeutung der Reaktion des Harnes für das Auftreten der Statischen Albuminurie im Kindesalter. Ztschr. f. Kinderh. **33**:158, 1922.

THE EFFECT OF SODIUM HYDROXIDE

The effect of the addition of a stronger alkali, sodium hydroxide, was next studied. Two infants and two older children were each given 100 cc. of tenth-normal sodium hydroxide with their milk; their urine was examined for the next six days. It was found to be normal on each occasion. The reaction was acid to litmus, and the hydrogen ion concentration ranged from 5 to 7.2 with a preponderance on the acid side, much to our surprise. The amount of sodium hydroxide was now

TABLE 4.—*Hydrogen Ion Concentrations of Urine Following the Addition of Tenth-Normal Sodium Hydroxide in Varying Amounts to Milk*

Name	M. F.	C. W.	H. W.	S. F.
5/26/27	100 cc. tenth normal sodium hydroxide	100 cc. tenth normal sodium hydroxide	100 cc. tenth normal sodium hydroxide	100 cc. tenth normal sodium hydroxide
5/27/27	6.5	7.0	5.0	6.4
5/28/27	6.4	7.0	6.4	6.4
5/29/27	6.0	5.0	6.0	6.8
5/31/27	6.2	6.0	5.5	6.6
6/ 1/27	6.4	4.5	7.2	6.6
6/ 3/27	6.4	6.4	6.6	6.6
6/ 4/27	6.6	5.0	6.6	6.6
6/ 5/27	240 cc. tenth normal sodium hydroxide	240 cc. tenth normal sodium hydroxide	240 cc. tenth normal sodium hydroxide	240 cc. tenth normal sodium hydroxide
6/ 5/27	7.4	7.2	7.0	7.1
6/ 6/27	6.6	7.0	6.6	7.0
6/ 7/27	6.6	7.0	6.6	6.8
6/ 8/27	6.6	6.6	...	7.4
6/ 9/27	7.6	7.1	...	7.0
6/10/27	7.2	7.6	...	6.6
6/11/27	6.4	6.6	...	6.6
	240 cc. tenth normal sodium hydroxide; fat content of diet reduced	240 cc. tenth normal sodium hydroxide; fat content of diet reduced	...	240 cc. tenth normal sodium hydroxide; fat content of diet reduced
6/12/27	7.4	8.4	...	7.7
6/13/27	7.1	7.5	...	7.0
6/14/27	8.5	6.8	...	7.0
6/15/27	6.8	7.3	...	6.9
6/16/27	7.3	7.6	...	7.3
6/17/27	6.8	6.6	...	7.5
6/18/27	6.6	7.0	...	7.5

increased to 240 cc. Daily microscopic examinations for a period of fourteen days showed normal specimens of urine in each instance. The reaction was acid to litmus on many occasions and slightly alkaline less often, the hydrogen ion concentration ranging from 5 to 7.6 with a preponderance on the acid side. The stools of these children were alkaline and soapy.

It was apparent that the sodium hydroxide was being lost to the body, and that it was being excreted by way of the alimentary tract as evidenced by the alkaline and soapy stools. Our next step, therefore, was to prevent this elimination by the reduction of the fat intake of the food, which was accomplished by substituting skimmed milk and omitting

the butter from the diet. After this change, the reaction of the urine promptly became more alkaline, and the hydrogen ion concentrations rose to a range from 6.6 to 8.5 with a preponderance on the alkaline side.

CONCLUSIONS

1. Hydrochloric acid in small amounts, 45 cc. of a tenth-normal solution, when added to a liter of milk is followed by the appearance of casts and sometimes of red blood cells in the urine of infants. In older children, about 100 cc. of this dilute acid is required to bring about similar irritative manifestations.

2. When the tenth-normal hydrochloric acid is omitted from the diet, the pathologic urinary changes disappear in about forty-eight hours.

3. When 8 Gm. of calcium lactate was added to the milk, a similar result occurred.

4. Lactic acid (U. S. P. 87 per cent), when added in quantities of 8 cc. to a liter of milk, only occasionally produces casts in the urine.

5. Lemon juice in a concentration of 3 per cent does not lead to any evidences of irritation.

6. Sodium bicarbonate when given to infants and older children in large doses, 240 cc. of a double normal solution, the equivalent of 10.1 Gm. or 152 grains daily, does not have the same irritating effects on the urinary tract that acid does.

7. Sodium hydroxide, 240 cc. of a tenth-normal solution in quantities of 100 cc., does not produce the urinary changes noted when tenth-normal hydrochloric acid is added. The result is a slightly alkaline or slightly acid urine, which may account for the lack of irritative effects.

8. The addition of sodium hydroxide leads to the formation of the typical soapy stool.

9. When the fat content of the diet was reduced by skimming the milk, the addition of sodium hydroxide rendered the urine markedly alkaline.

Br. J. Psychiatry 120(557):415-418. 1972.

Grosz-'72
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J. Psychiat. (1972), **120**, 415-18

Pitts' and McClure's Lactate-Anxiety Study Revisited*

By HANUS J. GROSZ and BARBARA B. FARMER

Introduction

Pitts and McClure (Pitts and McClure, 1967; Pitts, 1969) have recently suggested that all symptoms of anxiety and anxiety neurosis itself are caused by a raised blood and body fluids lactate level. As the biochemical mechanism underlying anxiety neurosis they proposed that the excess lactate complexes with, and reduces, the concentration of ionized calcium at the surface of excitable membranes to an extent that causes a disordered nerve activity to manifest itself in the form of anxiety symptoms. In support of this speculation, according to which anxiety neurosis is a biological disease essentially brought on by a lactate-induced hypocalcaemia, Pitts and McClure (1967) quote investigations that have shown that on standard exercise anxiety neurotics tend to produce higher blood lactate levels than do normal controls, and their own lactate infusion study. As pointed out elsewhere (Grosz and Farmer, 1969), neither the former nor the latter, however, can be considered satisfactory evidence.

The purpose of this paper is to report our attempt to replicate Pitts and McClure's infusion study in a group of normal subjects but with a crucial modification in the control experiment. Pitts and McClure had infused intravenously into a group of anxiety neurotics and into a group of healthy normal controls, in random order, solutions of sodium (DL) lactate, sodium (DL) lactate with calcium chloride, and glucose saline. They reported that the infusion of sodium (DL) lactate produced more, and more severe, symptoms of anxiety in the experimental subjects than it did in the control group. In both groups, the symptoms of anxiety were attenuated by the addition of calcium chloride to the lactate solution. They concluded that the symptoms of anxiety were due to a raised blood lactate level.

Ignored in this experimental design is the fact that an infusion of sodium (DL) lactate initiates a major shift in the acid-base balance of body fluids: indeed, as we have demonstrated (Grosz and Farmer, 1969) the infusion by Pitts and McClure of 500 millimoles sodium (DL) lactate, 10 ml./kg. of body weight, given over a period of 20 minutes, leads quite rapidly to a pronounced metabolic alkalosis—the result of the well known conversion, mole for mole, of sodium lactate to sodium bicarbonate. Even before the end of the infusion it was not unusual for us to find a blood pH of 7·6 and a blood bicarbonate level 50 per cent above the pre-infusion level.

Such changes in the acid-base balance of the blood raise the question whether the symptoms produced by the sodium (DL) lactate infusion are not in some measure due to the induced metabolic alkalosis. Anxiety neurotics are notoriously prone to suffer from anxiety symptoms associated with an involuntarily self-induced state of hyperventilatory alkalosis, and forced voluntary hyperventilation also often succeeds in triggering symptoms of anxiety. It is possible, therefore, that an experimentally induced alkalosis produced by the infusion of any alkalizing agent may have a similar effect. Moreover, since an alkalosis leads to a definite reduction in the free ionized calcium concentration it would be understandable on this ground alone that the addition of calcium chloride effectively lessened the severity of the hypocalcaemic symptoms.

In the present study we compared in a double blind experiment the symptomatic effects of infusing sodium (DL) lactate with the symptomatic effects produced by the infusion of another

* Based on a paper presented at the annual meeting of The American Psychiatric Association in San Francisco, in May 1970.

alkalinizing agent, namely sodium bicarbonate. We decided to infuse only normal subjects because Pitts and McClure (1967) reported that the infusion of sodium (DL) lactate induced anxiety symptoms also in their normal controls and it therefore seemed unnecessary to expose patients with anxiety neurosis to the distressing experience of this experiment.

Materials and Methods

Our subjects were ten young healthy men unfamiliar with the purpose of our study who for pay volunteered to undergo three infusion experiments. The three infusions which were administered double-blind in random order and on different days were: 500 millimolar sodium (DL) lactate, 500 millimolar sodium bicarbonate, and 555 millimolar glucose in 150 millimolar sodium chloride. All solutions were infused as a dose of 8 ml. per kg. of body weight given over a period of thirty minutes.

Before and immediately after the infusions the subjects were asked to record the presence or absence of twenty symptoms explained in lay terms. With a couple of minor exceptions the list of symptoms was identical with the inventory of anxiety symptoms that Pitts and McClure had administered to their subjects. In addition, all subjects were interviewed to record their personal experiences and any unusual responses evoked by the infusions.

Findings

Fig. 1 shows separately for each of our ten subjects the distribution of symptoms produced by the lactate and the bicarbonate infusions. The former produced a total of 51 symptoms, the latter a total of 42 symptoms. The difference between the number of responses to the two treatments is not statistically significant. In contrast, the glucose saline infusions produced only three symptoms in two subjects: shakiness on two occasions and nervousness on one occasion.

Of the 51 symptoms produced by the lactate infusion, 25 symptoms, or 50 per cent, were also produced in the same subjects by the bicarbonate infusions. Similarly, 60 per cent of the

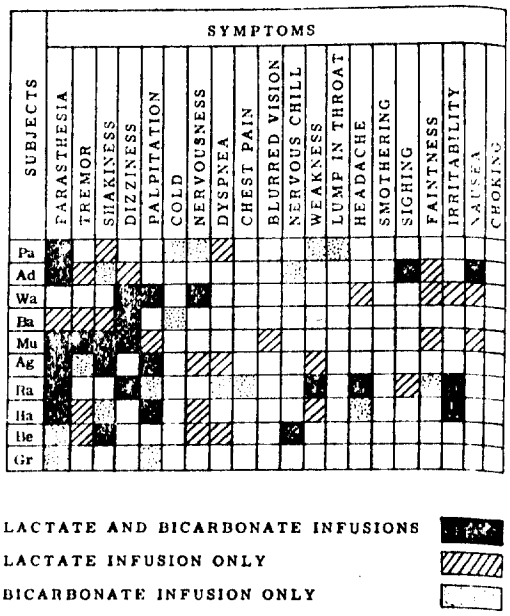

Fig. 1.—The symptomatic effect of sodium (DL) lactate and sodium bicarbonate infusion on ten subjects.

bicarbonate induced symptoms were also evoked by the lactate infusions.

The identity of effects was most pronounced in the five most commonly reported symptoms, namely paraesthesia, tremor, shakiness, dizziness and palpitation. These symptoms were also most commonly recorded in Pitts and McClure's group of anxiety neurotics. In our study, over 60 per cent of these symptoms associated with the lactate infusions were also evoked by the bicarbonate infusions. Paraesthesia, perhaps the commonest of all alkalotic hypocalcaemic symptoms, was reported by all except one of the ten subjects: six subjects complained of it following the infusion of both alkalinizing agents.

If we treat these findings as a 2×2 contingency table (Table I) and test for the significance of the correlation, that is, we ask what is the probability that out of the 50 opportunities (10 subjects $\times$ 5 symptoms) 17 double responses and 17 double non-responses would occur by chance, we find this to be less than ·02. In other words, there is a significant tendency among the ten subjects to respond either to both or to neither of the two infused alkalinizing agents.

TABLE I

Symptomatic responses of ten subjects on five items to infusions of sodium lactate and sodium bicarbonate

		Response to bicarbonate		Total	Proportion of opportunities on which a response to lactate occurred
		Yes	No		
Response to lactate	Yes	17	9	26	26/50 = ·52
	No	7	17	24	
	Total	24	26	50	
Proportion of opportunities on which a response to bicarbonate occurred		24/50 = ·48			

DISCUSSION

Our present findings, as well as our previously reported observations (Grosz and Farmer, 1969), leave little doubt that Pitts and McClure's (1967) experiments lacked the necessary controls to substantiate their claim that it is the elevated blood lactate concentration, and particularly its direct effect on ionized calcium, that is responsible for the symptoms produced by the massive and rapid infusions of sodium (DL) lactate. Thus our present study demonstrates that a significant proportion of the common symptoms associated with anxiety triggered by a sodium (DL) lactate infusion can be just as well produced by the infusion of another alkalinizing agent such as sodium bicarbonate. For example, both solutions were equally effective in provoking paraesthesia. This alkalotic hypocalcaemic symptom was strongly present because in a state of alkalosis there is a reduction in the physiologically active ionized calcium level. Besides, we found that the infusions lowered the total blood calcium level by over 10 per cent. This would naturally increase the likelihood of hypocalcaemic symptoms, especially with a superimposed alkalosis. Yet Pitts singles out this one symptom to exemplify the specific effect of the lactate ion. 'It is noteworthy', he writes (Pitts, 1969), 'that with the lactate infusion all subjects in both groups experienced paraesthesia.' More than that; Pitts (1969) was led to erect a whole theory of anxiety neurosis on the occurrence of these largely alkalotic hypocalcaemic symptoms which he erroneously assigned to the calcium-binding action of the lactate ion.

Pitts' and McClure's (1967) reasoning was based essentially on two assumptions: one, that there is associated with these symptoms of anxiety a blood lactate level of 12 to 15 millimoles per litre, and two, that these blood lactate levels can lower to a clinically significant degree the concentration of ionized calcium.

Both assumptions are false (Grosz and Farmer, 1969). In the first place, owing to its rapid conversion to bicarbonate, the infused lactate does not lead to any really marked blood lactate elevations. Admittedly, Pitts and McClure (1967) reported that the venous lactate levels were raised by 12 to 15 millimoles per litre. But this turns out to have been *at the end of the infusion* and not at the onset of the symptoms, which came on within a minute or two after the infusion was started. At that crucial phase of the infusion the subjects received no more than about 10 per cent of the administered sodium lactate. Had Pitts and McClure measured the venous lactate levels then, when the symptoms appeared, they would have found, as we did (Grosz and Farmer, 1969), that the blood lactate levels were barely 3 millimoles per litre above the pre-infusion level. Moreover, we observed that by slightly lowering the infusion rate, the onset of symptoms coincided with even more trivial blood lactate elevations—say, around one millimole per litre—and the immediate post-infusion blood lactate levels were also proportionately much lower. The bicarbonate infusions also produced no significant changes in the blood lactate levels at the onset of symptoms as would be expected from Huckabee's (1968) findings that bicarbonate infusions only lead to a reactive blood lactate elevation as a later effect. (Similarly, the rise in blood lactate levels in respiratory alkalosis only takes place after severe and prolonged overbreathing.) This means first of all that for anxiety symptoms to occur as a result of either lactate or bicarbonate infusion it is unnecessary that the blood lactate level be raised above its normal range; and secondly, it means that a raised blood lactate level cannot be

made to account for the symptoms produced by either the sodium (DL) lactate or the sodium bicarbonate infusions.

Since the symptoms produced by the infusions do not coincide with a raised blood lactate level it is merely of academic interest to know what blood lactate concentrations can directly reduce the ionized blood calcium concentration to clinically symptomatic hypocalcaemic levels. Suffice it, therefore, to say here that the blood lactate concentration would need to be at least 60 millimoles per litre (Grosz and Farmer, 1969), that is, it would need to be about fifty times higher than it actually was at the onset of symptoms and five to ten times higher than it was even at the very end of the infusion.

Although our paper has concerned itself primarily with certain technical aspects of Pitts and McClure's study one cannot ignore the fact that Pitts' (1969) lactate-anxiety theory suffers from serious weaknesses also on clinical grounds. For while it is true that anxiety neurotics have a tendency on exercise to produce higher blood lactate levels than do normal controls, the same can be said of patients who are free from anxiety neurosis but who suffer from such physical disabilities as cardiac and pulmonary disease. Moreover, unless exercised, anxiety neurotics do not as a rule have elevated blood lactate levels even when acutely anxious. They may bring about some elevation in the blood lactate level by hyperventilating, but this is also true for normal subjects. Indeed, the elevation in the blood lactate level is most likely the result of, and to compensate for, the shift in the acid-base balance of the body fluids brought about by the induced respiratory alkalosis. Finally, as we have pointed out elsewhere (Grosz and Farmer, 1969), anxiety neurosis is not typically present in patients with lactic acidaemia, that is with very marked and chronic blood lactate elevations.

Summary

Pitts and McClure proposed a new theory of anxiety neurosis based on an experiment in which they induced anxiety symptoms by infusing into their subjects a solution of sodium (DL) lactate. They claimed that the symptoms were produced by an elevated blood lactate concentration.

Because the infused sodium lactate becomes rapidly converted into sodium bicarbonate and leads to a marked metabolic alkalosis, we repeated this experiment including as control infusion of a solution of sodium bicarbonate. We found (1) that there was little difference in the major symptoms produced by the two substances, and (2) that with neither the sodium lactate nor the sodium bicarbonate infusion was the onset of symptoms associated with any significant blood lactate elevations. We concluded that neither their study nor their theory is soundly based.

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(*Received 4 May 1971*)

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Bib No. 878

Endokrynologia Polska 12, 279-89(1961)

THE EFFECT OF GLUTAMIC ACID AND OTHER ACIDIFIERS ON THE HISTOCHEMICAL PATTERN OF ENDOCRINE GLANDS OF FEMALE RABBITS (ADRENALS)

[Wplyw Kwasu Glutaminowego i Innych Substancji Zakwaszajacych na Obraz Histochemiczny Gruczolow Dokrewnych Krolic (Nadnercza)]

by

Jan Jonek

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The present article is the continuation of studies concerning the effect of acid substances on the endocrine glands of female rabbits. In earlier studies, it was found that substances such as glutamic acid, ammonium chloride, lactic acid and citric acid affect the histochemical pattern of the ovaries, increasing their hormonal activity [9]. In the ovaries, we observe a large number of rapidly-maturing and mature Graaf vesicles, completely-formed interstitial glands and yellow bodies still in the process of formation. The histochemical pattern of the ovaries presents an increased number of lipids, cholesterol and its esters, and acetalphosphatids. Fazykas observed similar morphological changes due to the effect of acid substances [2,3,4,5].

After the administration of the substances named above, phenomena of increased secretory activity are observed in the thyroid gland. The vesicles of the thyroid gland are small and do not contain colloid. The white cells, which appear in greater numbers, deserve particular attention here.

The favorable results obtained from the previous experiments were

the impetus to examine the adrenals in analogous circumstances. Since the histochemical changes observed in the ovaries and the thyroid apparently take place under the effect of increased activity of the hypophysis, its effect on the adrenals is especially interesting in such circumstances, because of the close functional connection between these two endocrine glands.

Materials and Methodology

The tests were performed during the winter on 30 virgin female rabbits aged 14 months. The weight of the rabbits was 1,500 g average. The animals were divided into groups receiving one of the following acidifiers: glutamic acid, ammonium chloride, lactic acid or citric acid. These substances were dissolved in drinkable water so that in 1 ml of the solution there were 0.09 g of pure substance. The rabbits, divided into 4 groups, received 2 ml of one of the above solutions once a day, along with 80 ml water. During the experiment, the animals were fed with dry feed (hay and oats).

In each group, some of the animals were killed and sections performed on the 12th day of solution administration, and the remainder on the 38th day. Four female rabbits of the same weight, kept under normal breeding conditions, served as controls. The animals were killed under ether narcosis. The adrenals were fixed in 10% formalin or in Susa fixative. Preparations of about 8 micron thickness were stained with eosin and hematoxylin or by means of the azan method. Some of the adrenals were tested for lipids, which were detected with Sudan IV and black Sudan.

Results

In the area of the adrenal cortex of the rabbits, 3 cell layers are found: the arcuate, the fascicular and the reticular. The arcuate layer is clearly defined, and its thickness differs according to individual cases, and is composed of individual cells only in exceptional cases (fig. 1 and 2). A detailed description of the morphological structure of the specific layers of the adrenal cortex of female rabbits is given in the previous work [9].

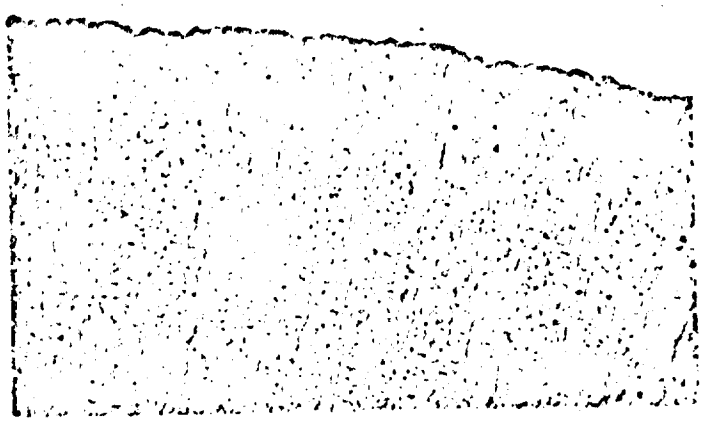

Fig. 1 Cross-section of the adrenal cortex, control group. Fixative: 10% formaldehyde. Azan stain. x 150.

The adrenal cortex of the control rabbits contains an average amount of lipids, and their distribution in the individual layers varies. The least amount of sudanophilic substances is observed in the arcuate layer. In the fascicular layer, the lipids sometimes fill the entire cytoplasm. In these cells, the sudanophilic bodies appear in the form of small or large drops. The reticular layer contains smaller amounts of lipides than the fascicular layer.

In the adrenals of rabbits receiving gluatmic acid, ammonium chloride, citric acid or lactic acid, clear signs of increased secretory activity are observed. In the morphological analysis of the functional condition

of the adrenal cortex, the following features were taken into consideration: 1) the appearance of vacuoles in the cell plasma, especially in the z. fasciculata, 2) the lipid content of specific adreno-cortical layers, 3) the increase in the cortical weight and that of the entire adrenal, 4) the degenerative changes in specific sections of the adrenal.

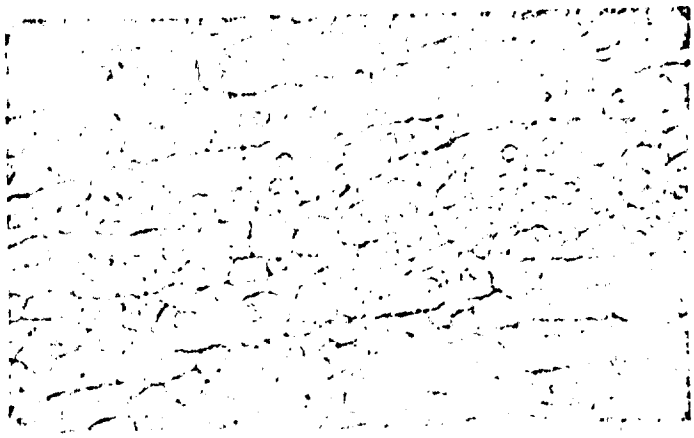

Fig. 2. Cross-section of the fasciculate zone of the adrenal cortex, control group. Fixative: formaldehyde 10%. Azan stain. x 360.

In the adrenals of the rabbits sectioned on the 12th day of the experiment, the greatest histochemical changes are observed in those animals that obtained glutamic or citric acid. In the arcuate layer, the cells are large, cylindrical and dark-colored. The cell nuclei are large, and round or oval in shape. Numerous mitotic divisions are observed in the area of this layer. Cells of a degenerative nature with a pyknotic nucleus are observed only in rare cases. In the arcuate layer, individual sudanophilic substances appearing in the form of small drops around the cell nucleus are observed (fig. 3). Vacuoles in the cell plasma are found rarely.

In the fascicular layer, numerous capillaries well filled with blood are observed. The cells are large, in places appearing to be filled with a light cytoplasm (fig. 4). In the area of the cytoplasm, numerous

vacuoles of different sizes are observed in the majority of the cells (fig. 5). Some of the cell nuclei are very dark in color, others barely delineated.

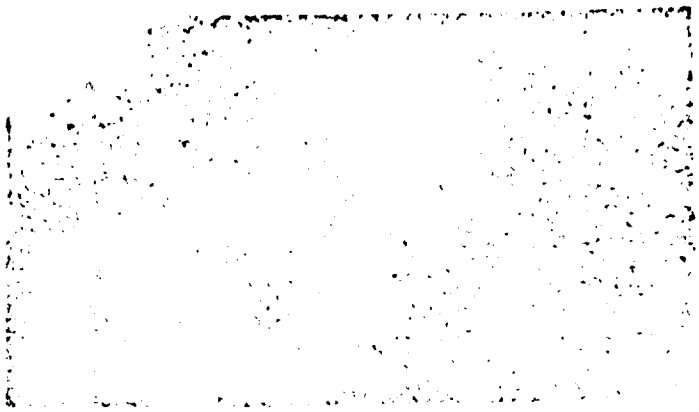

Fig. 3. Distribution of sudanophilic substances in the adrenal cortex of a female rabbit, following 12 days of ammonium chloride administration. Sudan black stain. x 120.

Fig. 4. Cross-section of adrenal cortex of female rabbit following 12 days of citric acid administration. Fixative: Susa fluid. Azan stain. x 150.

Besides the cells described above, we also found ones that were small and had no vacuoles in the area of the cytoplasm. Cells of this type appear above all in the outer area of the fascicular layer. It might be that these cells do not participate in the secretory processes, and that some of them undergo degenerative changes. The fascicular layer

is very rich in lipids, especially its inner portion. The sudanophilic bodies appear in the form of large drops of fat, filling nearly the entire cell cytoplasm (fig. 3).

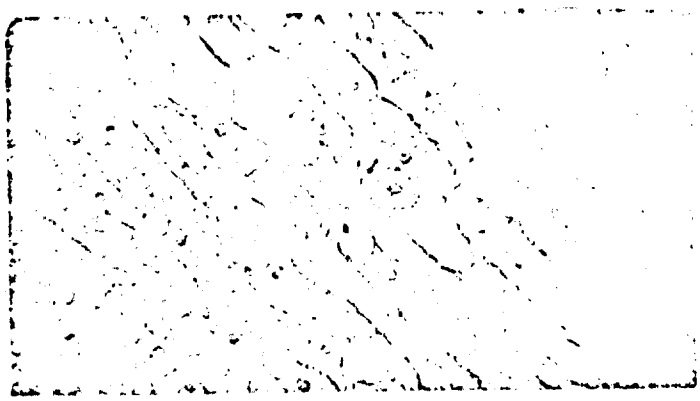

Fig. 5. A layer of the adrenal cortex of a female rabbit following 12 days of glutamic acid administration. Susa fixative. x 360.

In the area of the reticular layer, no greater differences are observed in comparison to the adrenals of the control group rabbits. The cells seem to be somewhat larger and have numerous vacuoles. Beside these cells we see small cells with pyknotic nuclei, undergoing degeneration (fig. 6). The number of lipids is less than in the fascicular layer.

Fig. 6. Cross-section of the reticular layer of the adrenal cortex of a female rabbit following 12 days of glutamic acid administration. Susa fixative. x 360.

In the adrenals of rabbits that received solutions of lactic acid and ammonium chloride, the histochemical changes are similar, but less marked.

In the experiment lasting longer, the histochemical changes are quite a bit more marked than in the short-term experiment. The adrenals of the rabbits are large and weigh considerably more than the adrenals of the controls. Where the mean weight of the adrenals of the controls was 0.39 g, the mean weight of the adrenals in the long-term experiment rose to 0.56 g. The greatest weight increase of the adrenals was noted in those animals given glutamic or citric acid. The adrenal weight of the rabbits that received lactic acid was 0.51 g, and that of those receiving ammonium chloride 0.53 g.

In this part of the experiment, the arcuate layer is clearly defined and somewhat wider than in the control group. The cells are large, cylindrical and dark-colored. The capillaries are highly developed. In the area of this layer, we observed numerous mitotic divisions.

The fascicular layer is considerably wider than that in the control group and that observed in the short-term experiment. The cells are very large, with distinct boundaries, and are lightly acid-colored (fig. 7). In the area of the cytoplasm we see numerous vacuoles of varying sizes. An especially large number of vacuoles is observed in the inner section of the fascicular layer of the adrenal cortex. The cell nuclei are large and round and alkaline-colored. Beside these cells we observe rather numerous cells of a degenerative nature. Their shape is irregular, the nuclei are small, and the chromatin is very compacted. In the area of the entire fascicular layer we observe numerous cells in the process of dividing. The capillaries are clearly formed and well filled with blood.

The reticular layer is only slightly widened. The cells are large, many-sided, with a distinct nucleus. The cytoplasm of these cells also contains vacuoles of a small diameter. Numerous degenerative cells are

also observed beside this type of cell.

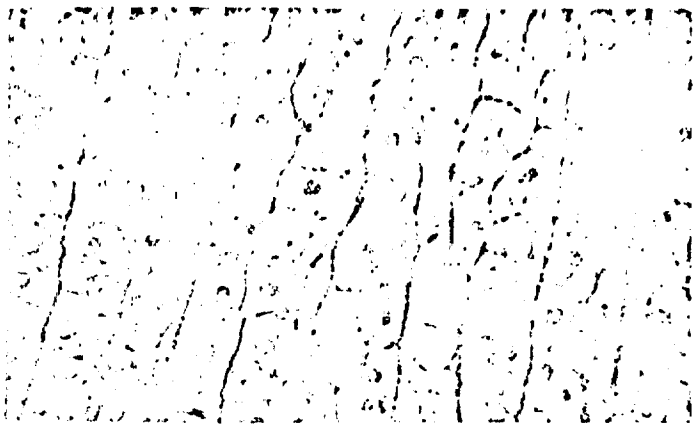

Fig. 7. Fascicular layer of the adrenal cortex of a female rabbit in the long-term experiment, receiving glutamic acid. Susa fixative. Azan stain. x 360.

The number of lipids is somewhat greater than in the adrenals observed after 12 days of acidifer administration (fig. 8). The greatest amount of lipids occurs in the inner section of the fascicular layer. Besides the cells whose cytoplasm is completely filled with sudanophilic substances, we also find ones in which the cytoplasm contains only single drops of lipids.

Fig. 8. Lipids in the adrenal cortex of a female rabbit of the long-term experiment. Sudan black stain.

The reticular layer is only slightly widened. The cells are large, many-sided, with a distinct nucleus. The cytoplasm of these cells also contains vacuoles of a small diameter. Besides this type of cell, we

also observe numerous degenerative cells. The amount of lipids in comparison to the control group and the short-term experiment is increased.

Discussion

The above-named histochemical changes indicate that the adrenal cortex of the female rabbits receiving acidifiers is found to be in a state of increased secretory activity. Changes such as the appearance of an increased amount of vacuoles in the cytoplasm or the increase in lipids after 12 days of administration of the above substances, testify to increased hormonal activity. In the extended hyperfunction of the adrenal cortex we observe, besides the above-mentioned features, an enlargement of the cells in the individual layers of the adrenal cortex and hypertrophic changes. The appearance of a greater number of degenerative cells towards the end of the experiment is a sign of the extended overstraining of the adrenals [14, 15]. It was shown that the adrenal cortex always reacts identically to various stimuli, even when non-specific factors are used [15, 16]. It does not matter whether the stimuli act on the entire system simultaneously, or whether they act only on certain organs. The important factor in the interpretation of the histochemical changes in the adrenal cortex is the strength of the stimulus used and the duration of its effect.

The histochemical changes described in this article are probably the result of the increased function of the cells of the peripheral section of the hypophysis. Acidifiers reduce the alkali reserve, which in turn stimulates the peripheral section of the hypophysis, either directly or through the hypothalamus.

Ammonium chloride (NH_4Cl) is supposed to be converted in the liver into carbamide($2\ NH_4Cl + CO_2 = NH_2Co - NH_2 + 2\ HCl + H_2O$), which is then eliminated with the urine, and the hydrochloric acid that forms during this reaction binds the basic components and thus reduces the alkali reserve

of the blood [6]. A daily dose of 0.1 - 0.2 g NH_4Cl/kg weight to humans given for 6 days causes a reduction in the alkali reserve of about 30% [13]. It can be that the remaining substances used in this experiment also underwent similar conversions in the liver.

It should be stressed that the mechanism of action of glutamic acid is not yet well-known, and the theoretical opinions of individual authors are debatable. Beside the above-described effect of the acidifiers, glutamic acid might also act directly on the cells of the adrenal cortex in its effect on the cell metabolism. Glutamic acid is an excellent amino acid of animal protein. Julesz also mentions the positive effect of ammonium chloride on the function of the adrenal cortex in humans [10]. This author observed increased 17-ketosteride secretion during administration of this substance.

Since the histochemical changes described in this article may be the result only of the direct effect of ACTH, it should be assumed that the substances used above all affected the hypophysis, either directly or through the hypothalamus. It has not yet been explained how the hypothalamus affects the the glandular portion of the hypophysis, since a direction nerve connection has been observed only between the hypothalamus and the nervous portion of the hypophysis [13].

Numerous authors observed an increase in the number of cells in the peripheral section of the hypophysis due to the effect of acidifers [11, 13, 14, 15, 16]. In this article, we observed phenomena of increased glandular activity, above all in the fascicular layer, which is the result of the direct effect of ACTH on this layer. It was shown that hormones of the adrenal cortex needed for metabolism are formed in the inner section of the fascicular layer, while the outer section of the fascicular provides a reserve section of the gland, set into action only after prolonged overstraining [7, 11, 17].

Numerous authors found morphological pictures similar to those found after using acidifiers by using ACTH [1, 10, 17]. Both the histochemical changes in the adrenals and the observations on the ovaries and the thyroid under the effect of acidifiers indicate that these substances cause an increase in the activity of endocrine glands. It can be supposed that, as a result of further studies, these substances may find some medical use in certain endocrine gland disorders.

Summary and Conclusions

Histochemical examinations were performed on the adrenal cortex of 30 female rabbits, after oral administration of glutamic acid, citric acid, lactic acid and ammonium chloride. Four female rabbits made up a control group.

On the basis of the morphological findings and the histochemical reactions, it was determined that the adrenal cortex of female rabbits receiving acidifers reveal signs of increased glandular activity. The following findings were taken into consideration in the evaluation of the function of the adrenal cortex:

1) the appearance of vacuoles in the cell plasma,

2) the amount of lipids in the adreno-cortical layers,

3) the increase in the individual layers of the adrenal cortex and the total weight of the gland,

4) degenerative changes in specific parts of the adrenal cortex.

Glutamic acid and citric acid revealed the strongest stimulative effects. The effect of lactic acid and ammonium chloride was also positive. In the long-term experiment, the histochemical changes are considerably more marked than in the short-term experiment. The greatest increase in adrenal weight was observed after administration of glutamic acid and citric acid. It was 0.56 g, as compared to the adrenal weight

of the control rabbits, which was 0.39 g.

The author believes that the changes observed take place due to the stimulation of the hypothalamic-hypophyseal system. The cells of the peripheral portion of the hypophysis secrete an increased amount of ACTH, which calls forth the morphological changes described.

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Jonek-'61
Bib No. 878

ENDOKRYNOLOGIA POLSKA
1961, XII, 279

JAN JONEK

WPŁYW KWASU GLUTAMINOWEGO I INNYCH SUBSTANCJI ZAKWASZAJĄCYCH NA OBRAZ HISTOCHEMICZNY GRUCZOŁÓW DOKREWNYCH KRÓLIC. (NADNERCZA)

Zakład Histologii i Embriologii Śląskiej A. M. w Zabrzu
b. Kierownik: prof. dr T. Pawlikowski

Niniejsza praca jest dalszym ciągiem badań dotyczących wpływu substancji kwaśnych na gruczoły dokrewne królic. We wcześniejszych doniesieniach wykazano, że takie substancje jak kwas glutaminowy, chlorek amonu, kwas mlekowy i kwas cytrynowy wpływają na obraz histochemiczny jajników, wzmagając ich czynność hormonalną [9]. W jajnikach obserwuje się dużą liczbę szybko dojrzewających i dojrzałych pęcherzyków Graafa, silnie wykształcone gruczoły śródmiąższowe oraz tworzące się ciałka żółte. Obraz histochemiczny jajników przedstawia zwiększoną ilość lipidów, cholesterolu i jego estrów oraz acetalfosfatydów. Podobne zmiany morfologiczne pod wpływem substancji kwaśnych obserwował Fazykas [2, 3, 4, 5].

Po podaniu wyżej wymienionych substancji obserwuje się w tarczycach objawy wzmożonej czynności wydzielniczej. Pęcherzyki tarczycy są małe i nie zawierają koloidu; na szczególną uwagę zasługują komórki jasne, które pojawiają się wtedy w większych ilościach.

Zachęcające wyniki w poprzednich doświadczeniach były zachętą do zbadania nadnerczy w analogicznych warunkach. Ponieważ obserwowane zmiany histochemiczne w jajnikach i tarczycy zachodzą prawdopodobnie pod wpływem wzmożonej aktywności przysadki mózgowej, jej wpływ na nadnercza jest szczególnie interesujący w takich przypadkach, ze względu na istniejący ścisły związek czynnościowy pomiędzy tymi dwoma gruczołami dokrewnymi.

MATERIAŁ I METODA

Badania przeprowadzono w okresie zimowym na 30 królicach dziewiczych w wieku 14 miesięcy. Waga królic wynosiła średnio około 1500 g. Zwierzęta podzielone na grupy otrzymywały jedną z następujących substancji zakwaszających: kwas glutaminowy, chlorek amonu, kwas mlekowy lub kwas cytrynowy. Sub-

stancje te rozpuszczano w wodzie pitnej tak, że w 1 ml roztworu znajdowało się 0,09 g substancji czystej. Królice podzielone na 4 grupy otrzymywały 1 raz dziennie po 2 ml jeden z wyżej wymienionych roztworów z dodatkiem 80 ml wody. W czasie doświadczenia zwierzęta żywiono karmą suchą (owies i siano).

W każdej grupie część zwierząt sekcjonowano w 12 dniu a część w 38 dniu stosowania poszczególnych roztworów. Za materiał kontrolny służyły 4 królice dziewicze tej samej wagi, znajdujące się w normalnych warunkach hodowli. Zwierzęta zabito w narkozie eterowej. Nadnercza utrwalano w 10% formalinie oraz w utrwalaczu Susa. Preparaty grubości około 8 mikronów barwiono eozyną i hematoksyliną oraz metodą azanową. Część nadnerczy badano na lipidy, które wykrywano sudanem IV i sudanem czarnym.

WYNIKI

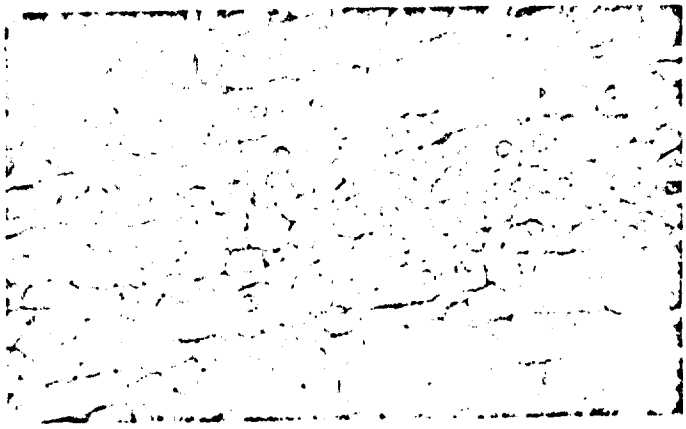
W obrębie kory nadnerczy królic wyodrębnia się 3 warstwy komórkowe: łukowatą, pasmowatą i siateczkowatą. Warstwa łukowata jest ostro zaznaczona, a grubość jej zmienna indywidualnie, tylko wyjątkowo składa się z komórek pojedyńczych (ryc. 1 i 2). Szczegółowy opis budowy morfologicznej poszczególnych warstw kory nadnerczy u królic podano w poprzedniej pracy [9].

Ryc. 1. Przekrój poprzeczny przez część korową nadnercza królicy z grupy kontrolnej. Utrwalacz: 10% formalina. Barwienie metodą azanową. Pow. około 150 x.
Fig. 1. Cross section of the adrenal cortex control. Fixation: formaldehyde; stain azan; x 150.

Część korowa nadnerczy królic kontrolnych zawiera średnią ilość lipidów, a rozmieszczenie ich w poszczególnych warstwach jest różne. Najmniej substancji sudanofilnych obserwuje się w warstwie łukowatej. W warstwie pasmowatej lipidy wypełniają niejednokrotnie całą cytoplazmę. W tych komórkach ciała sudanofilne występują w postaci

małych lub dużych kropli. Warstwa siateczkowata zawiera lipidy w mniejszych ilościach aniżeli warstwa pasmowata.

W nadnerczach królic otrzymujących kwas glutaminowy, chlorek amonu, kwas cytrynowy lub kwas mlekowy obserwuje się wyraźne cechy wzmożonej czynności wydzielniczej. W ocenie morfologicznej stanu czynnościowego kory nadnerczy brano pod uwagę następujące

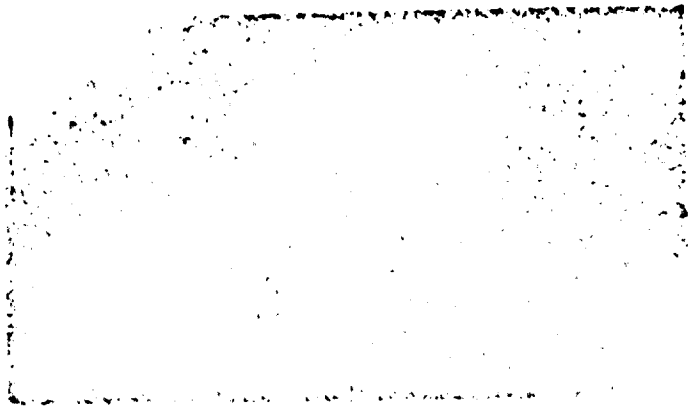


Ryc. 2. Przekrój poprzeczny przez część pasmowatą kory nadnercza królicy kontrolnej. Utrwalacz: 10% formalina. Barwienie metodą azanową. Pow. około 360 x.
Fig. 2. Cross section of the fasciculate zone in adrenal cortex of control. Fixation: formaldehyde; stain azan; x 360.

cechy: 1) występowanie wodniczek w cytoplazmie komórek, szczególnie w warstwie pasmowatej; 2) zawartość lipidów w poszczególnych warstwach kory nadnerczy; 3) przerost kory nadnerczy i ogólną wagę nadnerczy; 4) zmiany degeneracyjne w poszczególnych odcinkach nadnerczy.

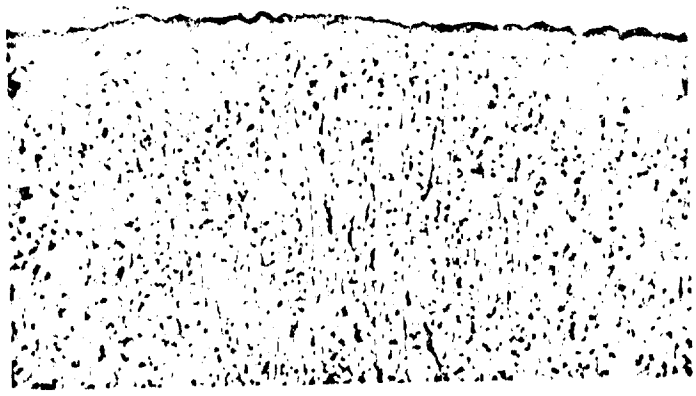
W nadnerczach królic sekcjonowanych w 12 dniu doświadczenia największe zmiany histochemiczne obserwuje się u zwierząt, które otrzymywały kwas glutaminowy lub kwas cytrynowy. W warstwie łukowatej komórki są duże, cylindryczne i barwią się silnie zasadochłonnie. Jądra komórkowe są wielkie, kształtu kulistego lub owalnego. W obrębie tej warstwy obserwuje się liczne podziały mitotyczne. Wyjątkowo obserwuje się komórki o charakterze degeneracyjnym z jądrem pyknotycznym. W warstwie łukowatej obserwuje się pojedyńczo substancje sudanofilne, które występują w postaci małych kropelek wokół jądra komórkowego (ryc. 3). Rzadko spotyka się również wakuole w cytoplazmie komórkowej.

W warstwie pasmowatej widoczne są liczne naczynia włosowate dobrze wypełnione krwią. Komórki są duże, miejscami jak gdyby napoczniałe i jasnej cytoplazmie (ryc. 4). W obrębie cytoplazmy obser-



Ryc. 3. Rozmieszczenie ciał sudanofilnych w korze nadnercza królicy po 12-dniowym podawaniu chlorku amonu. Barwienie sudanem czarnym. Pow. około 120 x.

Fig. 3. Distribution of sudanophilic substances within the adrenal cortex of female rabbit following 12 days' administration of ammonium chloride. Stain Sudan black;



Ryc. 4. Przekrój poprzeczny przez korę nadnercza królicy po 12-dniowym stosowaniu kwasu cytrynowego. Utrwalacz Susa. Barwienie metodą azanową. Pow. około 150 x.

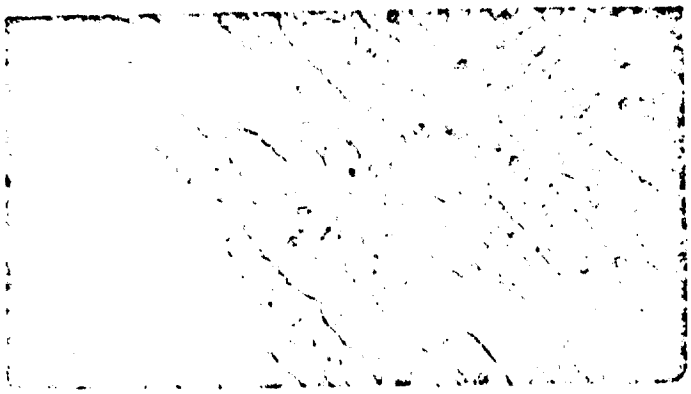
Fig. 4. Cross section of the adrenal cortex from female rabbit following 12 days' administration of citric acid. Fixation: Susa fluid; stain azan; x 150.

wuje się w większości komórek liczne wodniczki róznej wielkości (ryc. 5). Jedne jądra komórkowe barwią się silnie zasadochłonnie, inne natomiast są słabo zaznaczone.

Oprócz wyżej opisanych komórek występują i takie, które są małe i nie posiadają w obrębie cytoplazmy żadnych wodniczek. Komórki tego typu występują przede wszystkim w części zewnętrznej warstwy pasmowatej. Być może, że komórki te nie biorą udziału w procesach wydzielniczych i część z nich ulegnie zmianom degeneracyjnym. Warstwa pasmowata jest bardzo bogata w lipidy, a szczególnie jej część

wewnętrzna. Ciała sudanofilne występują w postaci dużych kropelek tłuszczu, zajmując prawie całą cytoplazmę komórki (ryc. 3).

W obrębie warstwy siateczkowatej nie obserwuje się większych różnic w porównaniu z nadnerczami królic grupy kontrolnej. Komórki wydają się być nieco większe i posiadają liczne wodniczki. Obok tych komórek widać komórki małe z pyknotycznym jądrem, ulegające degeneracji (ryc. 6). Ilość lipidów jest mniejsza aniżeli w warstwie pasmowatej.

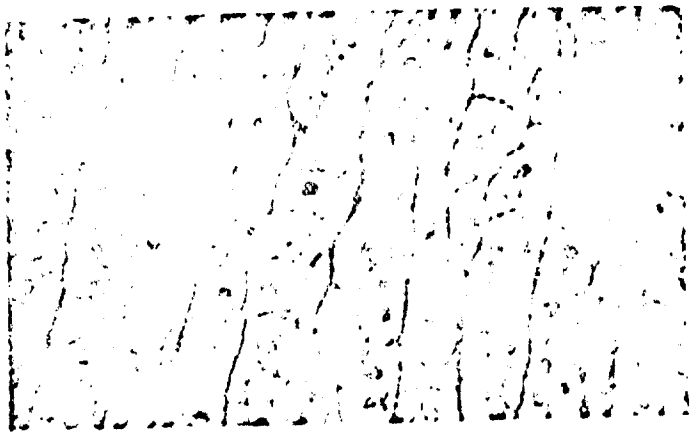


Ryc. 5. Warstwa kory nadnercza królicy po 12-dniowym zastosowaniu kwasu glutaminowego. Utrwalacz Susa. Pow. około 360 x.
Fig. 5. Adrenal cortex of female rabbit following 12 days'administration of glutamic acid. Fixation: Susa fluid; x 360.

Ryc. 6. Przekrój poprzez warstwę siateczkowatą kory nadnercza królicy po 12-dniowym podawaniu kwasu glutaminowego. Utrwalacz Susa. Pow. około 360 x.
Fig. 6. Cross section of the reticular zone in adrenal cortex of female rabbit following 12 days'administration of glutamic acid. Fixation: Susa fluid; x 360.

W nadnerczach królic, u których stosowano roztwory kwasu mlekowego i chlorku amonu zmiany histochemiczne są podobne lecz zaznaczone słabiej.

W doświadczeniu dłużej trwającym zmiany histochemiczne są o wiele silniej zaznaczone aniżeli w doświadczeniu krótkotrwałym. Nadnercza królic są duże, o wadze wiele większej w porównaniu do nadnerczy królic kontrolnych. O ile przeciętna waga nadnerczy królic kontrolnych wynosiła 0,39 g, to przeciętna waga nadnerczy w doświadczeniu długotrwałym wzrosła do 0,56 g. Największy przyrost wagi nadnerczy zanotowano u zwierząt po podaniu kwasu glutaminowego lub kwasu cytrynowego. Waga nadnerczy królic otrzymujących kwas mlekowy wynosiła 0,51 g, a otrzymujących chlorek amonu 0,53 g.



Ryc. 7. Warstwa pasmowata kory nadnercza królicy w doświadczeniu długotrwałym po kwasie glutaminowym. Utrwalacz Susa. Barwienie metodą azanową. Pow. około 360 x.

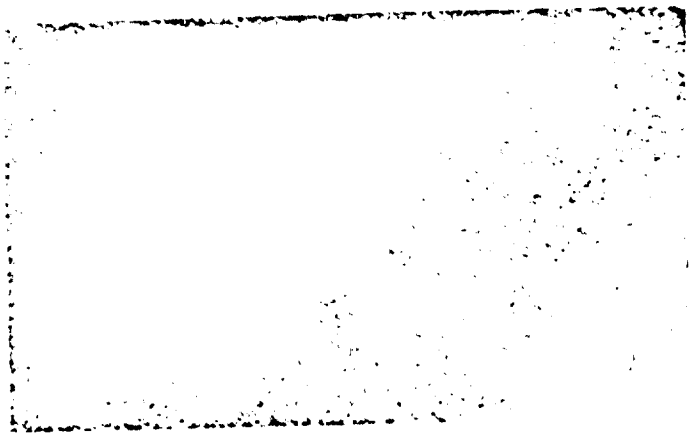
Fig. 7. Fasciculate zone in adrenal cortex of female rabbit prologedly treated with glutamic acid. Fixation: Susa fluid; stain azan; x 360.

W omawianej grupie doświadczenia warstwa łukowata jest wyraźnie zaznaczona i nieco szersza w porównaniu z grupą kontrolną. Komórki są duże, cylindryczne i barwią się silnie zasadochłonnie. Naczynia włosowate są silnie rozwinięte. W obrębie tej warstwy obserwuje się liczne podziały mitotyczne.

Warstwa pasmowata jest o wiele szersza w porównaniu do grupy kontrolnej i do nadnerczy z doświadczenia krótkotrwałego. Komórki są bardzo duże, o wyraźnych granicach i barwią się słabo kwasochłonnie (ryc. 7). W obrębie cytoplazmy występują liczne wodniczki różnej wielkości. Szczególnie dużą liczbę wodniczek obserwuje się w części wewnętrznej warstwy pasmowatej kory nadnerczy. Jądra komórkowe są duże, kuliste i barwią się silnie zasadochłonnie. Obok tych komórek obserwuje się dość licznie występujące komórki o charakterze degene-

racyjnym. Kształt ich jest nieregularny, jądra komórkowe są małe, a chromatyna ich jest silnie zbita. W obrębie całej warstwy pasmowatej obserwuje się liczne komórki w okresie podziałowym. Naczynia włosowate są silnie wykształcone i dobrze wypełnione krwią.

Warstwa siateczkowata jest tylko nieznacznie poszerzona. Komórki są duże, wieloboczne, z wyraźnym jądrem. Cytoplazma tych komórek zawiera również wodniczki małej średnicy. Obok tego typu komórek obserwuje się liczne komórki degeneracyjne.



Ryc. 8. Lipidy w korze nadnercza królicy w doświadczeniu długotrwałym. Barwienie sudanem czarnym.

Fig. 8. Lipids in adrenal cortex of female rabbit in prolonged experiment. Stain Sudan black.

Ilość lipidów jest nieco większa w porównaniu z nadnerczami po 12-dniowym stosowaniu substancji zakwaszających (ryc. 8). Najwięcej lipidów występuje w strefie wewnętrznej warstwy pasmowatej. Obok komórek, których cytoplazma jest zupełnie wypełniona substancjami sudanofilnymi występują i takie, w których cytoplazma zawiera tylko pojedyńcze kropelki lipidów.

Warstwa siateczkowata jest tylko nieznacznie poszerzona. Komórki są duże, wieloboczne, z wyraźnym jądrem. Cytoplazma tych komórek zawiera również wodniczki małej średnicy. Obok tego typu komórek obserwuje się liczne komórki degeneracyjne. Ilość lipidów w porównaniu do grupy kontrolnej i doświadczenia krótkotrwałego jest zwiększona.

DYSKUSJA

Wyżej wymienione zmiany histochemiczne wskazują na to, że kora nadnerczy królic otrzymujących substancje zakwaszające znajduje się

w okresie wzmożonej czynności wydzielniczej. Takie zmiany, jak pojawienie się zwiększonej ilości wodniczek w cytoplazmie oraz zwiększenie się lipidów po 12-dniowym stosowaniu wyżej wymienionych substancji, świadczą o wzmożonej czynności hormonalnej. W przewlekłej nadczynności kory nadnerczy obserwuje się, obok wyżej opisanych cech, przede wszystkim powiększenie się komórek w poszczególnych warstwach kory nadnerczy oraz zmiany przerostowe. Pojawienie się większej liczby komórek degeneracyjnych pod koniec doświadczenia jest oznaką przewlekłego przeciążenia nadnerczy [14, 15]. Wykazano bowiem, że kora nadnerczy reaguje na różne bodźce zawsze jednakowo, nawet wtedy jeśli zastosuje się czynniki nieswoiste [15, 16]. Jest rzeczą obojętną, czy dane bodźce działają na cały ustrój równocześnie, czy też tylko na pewne jego narządy. Ważnym czynnikiem podczas interpretacji zmian histochemicznych w korze nadnerczy jest jedynie siła zastosowanego bodźca oraz czas jego działania.

Opisane w niniejszej pracy zmiany histochemiczne są prawdopodobnie wynikiem wzmożonej czynności komórek obwodowej części przysadki mózgowej. Substancje zakwaszające zmniejszają rezerwę alkaliczną, co z kolei wpływa pobudzająco na obwodową część przysadki mózgowej, albo bezpośrednio, albo poprzez podwzgórze.

Chlorek amonu (NH_4Cl) ma być przekształcany w wątrobie na carbamid 2 NH_4Cl + CO_2 = $NH_2CO—NH_2$ + 2 HCl + H_2O), który jest następnie wydalany z moczem, zaś powstający podczas tej reakcji kwas solny wiąże związki zasadowe i w ten sposób zmniejsza rezerwę alkaliczną krwi [6]. Dawka dzienna 0,1 — 0,2 g NH_4Cl/kg wagi ciała u ludzi podawana przez 6 dni powoduje zmniejszenie rezerwy alkalicznej o około 30% [13]. Być może, że podobnym przemianom w wątrobie podlegają również pozostałe substancje stosowane w doświadczeniu niniejszym.

Należy podkreślić, że mechanizm działania kwasu glutaminowego nie jest jeszcze ostatecznie poznany, a rozważania teoretyczne poszczególnych autorów są sprzeczne. Obok wyżej opisanego działania substancji zakwaszających, kwas glutaminowy mógłby również działać bezpośrednio na komórki kory nadnerczy poprzez wpływ na przemianę materii komórek. Kwas glutaminowy jest bowiem nieodzownym aminokwasem białka zwierzęcego. O dodatnim wpływie chlorku amonu na czynność kory nadnerczy u ludzi wspomina również Julesz [10]. Autor ten obserwował podczas stosowania wymienionej substancji zwiększone wydzielanie 17-ketosterydów.

Ponieważ zmiany histochemiczne opisane w niniejszej pracy mogą by wynikiem tylko bezpośredniego działania ACTH, należy przyjąć, że sto sowane substancje zadziałały przede wszystkim na przysadkę mózgow albo bezpośrednio, albo poprzez podwzgórze. W jaki sposób podwzgórz

wpływa na część gruczołową przysadki mózgowej dotychczas nie jest wyjaśnione, ponieważ obserwowano bezpośrednie połączenia nerwowe tylko między podwzgórzem i częścią nerwową przysadki mózgowej [13].

Liczni autorzy obserwowali zwiększenie się liczby komórek w części obwodowej przysadki mózgowej pod wpływem substancji zakwaszających [11, 13, 14, 15, 16]. W niniejszej pracy obserwowano objawy wzmożonej czynności gruczołowej, przede wszystkim w warstwie pasmowatej, co jest wynikiem pośredniego działania ACTH na tę warstwę. Wykazano, że potrzebne hormony kory nadnerczy do przemiany materii wytwarzane są w części wewnętrznej warstwy pasmowatej, część zewnętrzna warstwy pasmowatej stanowi natomiast odcinek zapasowy gruczołu, uruchamiany dopiero przy obciążeniach przewlekłych [7, 11, 17].

Podobne obrazy morfologiczne do tych, jakie stwierdza się po stosowaniu substancji zakwaszających, wykazali liczni autorzy po podawaniu ACTH [1, 10, 17]. Tak zmiany histochemiczne w nadnerczach, jak i obserwacje na jajnikach i tarczycy pod wpływem substancji zakwaszających wskazują na to, że substancje te powodują zwiększenie czynności gruczołów dokrewnych. Można przypuszczać, że substancje te w wyniku dalszych badań mogą znaleźć zastosowanie lecznicze w niektórych zaburzeniach gruczołów dokrewnych.

STRESZCZENIE I WNIOSKI

Przeprowadzono histochemiczne badania kory nadnerczy u 30 królic po doustnym karmieniu ich kwasem glutaminowym, kwasem cytrynowym, kwasem mlekowym lub chlorkiem amonu. 4 królice stanowiły materiał kontrolny.

Na podstawie obrazów morfologicznych i odczynów histochemicznych stwierdzono, że kora nadnerczy królic otrzymujących substancje zakwaszające wykazuje objawy wzmożonej czynności gruczołowej. W ocenie stanu czynnościowego kory nadnerczy brano pod uwagę następujące cechy:

1) występowanie wodniczek w cytoplazmie komórek;
2) zawartość lipidów w warstwach kory nadnerczy;
3) przerost poszczególnych warstw kory nadnerczy i ogólną wagę gruczołu;
4) zmiany degeneracyjne w poszczególnych odcinkach kory nadnerczy.

Najsilniejsze działanie pobudzające wykazał kwas glutaminowy i kwas cytrynowy. Wpływ kwasu mlekowego i chlorku amonu był również dodatni. W doświadczeniu przewlekłym zmiany histochemiczne są

o wiele silniej zaznaczone aniżeli w doświadczeniu krótkotrwałym. Największy przyrost wagi nadnerczy obserwowano po kwasie glutaminowym i kwasie cytrynowym; wynosił on 0,56 g w porównaniu do wagi nadnerczy królic kontrolnych, wynoszącej 0,39 g.

Autor przypuszcza, że obserwowane zmiany zachodzą pod wpływem pobudzenia układu podwzgórzowo-przysadkowego. Komórki obwodowej części przysadki mózgowej wydzielają zwiększoną ilość ACTH, który wywołuje opisane zmiany morfologiczne.

Я. Енек

ВЛИЯНИЕ ГЛЮТАМИНОВОЙ КИСЛОТЫ И НЕКТОРЫХ ДРУГИХ ОКСИСЛЯЮЩИХ СУБСТАНЦИЙ НА ГИСТОХИМИЧЕСКУЮ КАРТИНУ ЭНДОКРИННЫХ ЖЕЛЕЗ У КРОЛИКОВ-САМОК. НАДПОЧЕЧНИКА

Проводились гистохимические исследования кортикового слоя надпочечников у 30 кроликов самок после интроэнтерального введения в пище глютаминовой кислоты, лимонной кислоты, молочной кислоты и хлорида аммония. 4 кролика составляли контрольную группу.

На основании морфологических картин и гистохимических реакций установлено, что кортиковый слой надпочечников кролиц, которым подавались оксиляющие субстанции указывает на увления подвышенной гормональной функции. В оценке функционального состояния кортикового слоя надпочечников учитывались следующие факторы:

1) появление вакуел в цитоплазме клеток
2) содержание липидов в слоях коры надпочечников
3) гипертрофия кортикового слоя надпочечников и общий вес этого органа
4) Дегенеративные изменения в отдельных драгментах кортикового слоя надпочечников.

Самое сильное возбуждающее действие проявила глютаминовая и лимонная кислоты. Влияние молочной кислоты и хлорида аммония тоже было выражено. В хроническом эксперименте гистохимические изложения выражены более ярко, чем в кратковременном эксперименте. Самый высокий пхрирост веса надпочечников наблюдали после применения глютаминовой и лимонной кислот.Вес этого органа достигая 0,55 г. в сравнении с весом надпочечников контрольных кроликов — 0,39 г.

Автор предполагает, что наблюдаемые изменения зависят от возбуждения гипоталамо-гипофизарной системы. Клетки периферической области гипофиза выделяют повышенное количество АКТГ, который вызывает описанные морфологические изменения.

J. Jonek

THE EFFECT OF GLUTAMIC ACID AND OTHER ACIDIFIERS ON THE HISTOCHEMICAL CHANGES IN THE ENDOCRINE GLANDS OF FEMALE RABBITS. (THE ADRENALS).

Histochemically detectable changes in the adrenal cortex of female rabbits after oral administration of gluatmic, citric and lactic acids or ammonium chloride were investigated.

Morphological and histochemical patterns indicated that administration of acidifiers to female rabbits resulted in an intensified activity of the adrenal cortex. Evaluation of the adreno-cortical activity was based on the following findings: 1) the presence of vacuoles in the cellular plasma 2)the contents of lipids in the adrenocortical layers; 3) increase of the individual layer and of the whole adrenal weight; 4) the presence of degenerative changes in various parts of the adrenal cortex.

Glutamic and citric acids showed the strongest stimulative effects. The effects of lactic acid and ammonium chloride were also positive. Histochemical changes were more strongly pronounced in a long-lasting experiment than in a short one. The biggest gain in adrenocortical weight was found following glutamic or citric acids administration. The average weight of an adrenal in this group was 0,56 g against 0,39 g in controls.

The author is of an opinion that the observed changes could be atributed to stimulation of the hypothalamo-hypophyseal system. The cells of the peripheral part of hypophysis secrete an increased amount of ACTH which causes the described morphological changes.

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SEMINARS IN MEDICINE

OF THE

BETH ISRAEL HOSPITAL, BOSTON

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GLUCOSE-LACTATE INTER-RELATIONS IN MAN

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THE roles of carbohydrate, lipid and protein metabolism in the fuel economy of fed, fasting and exercising man[1-3] have provided an intriguing saga in modern scientific investigation of intermediary metabolism. Lactate, a common intermediate or by-product of glycolysis that is produced in varying degrees by all tissues in the body, may exert a profound influence on the economy of glucose, since it is both an important precursor and an end product of glucose metabolism.[4] Accelerated conversion of glucose to lactate, unless balanced by a commensurate increase in lactate conversion to glucose, could result in hypoglycemia. In certain cases tumor hypoglycemia has been attributed to this mechanism.[5] The availability of lactate also could theoretically regulate glucose synthesis, since blood lactate values under physiologic conditions are less than the concentration that produces half maximum rates of gluconeogenesis.[6] At perhaps a more practical level, the association of severe and generally fatal metabolic acidosis with the accumulation or overproduction of lactic acid is a well known and difficult therapeutic problem.[7] Although in most cases impaired circulatory dynamics or tissue oxygenation or both are clearly responsible for the acidosis, in many the mechanisms are not clear. With the exception of hypoxia, relatively little is known about the factors that regulate lactate production and utilization in physiologic situations. The purpose of this presentation is to review the information that is currently available on the inter-relations of glucose and lactate metabolism in man and to discuss some situations in which this relation may be altered.

LACTATE METABOLISM

Lactate appears to be distributed in a space equivalent or slightly less than total body water.[8] It diffuses readily across cell membranes, and in contrast to pyruvate, its transport seems to be primarily passive.[9] Some controversy exists, however, over whether blood or plasma lactate concentrations are representative of those occurring intracellularly. Plasma-erythrocyte lactate concentration ratios at rest and during exercise are greater than 1.0, indicating that there is relatively little resistance to lactate transport or diffusion in this tissue.[9] In contrast, muscle lactate concentrations with exercise are consistently higher than those of blood,[10] whereas skin also appears to maintain a large intracellular-extracellular gradient (R. M. Fusaro and J. A. Johnson, unpublished data). Although the lactate space may be equivalent to total body water, such gradients indicate that its distribution may, under certain conditions, be uneven, or that the lactate pool consists of several smaller pools with differing rate constants. Thus, blood lactate concentrations may only qualitatively reflect the metabolic phenomena occurring in the tissues.

To assess the magnitude of lactate production in man, we have used isotopic dilution of ^{14}C lactate, administered by a primed-constant infusion technic. In normal subjects the lactate turnover rate was 82 mg per kilogram per hour,[11,12] or 140 g per day when extrapolated to the 70-kg reference subject. Using a comparable technic in four normal subjects, Searle and his co-workers obtained turnover values of 97 mg per kilogram per hour.[8] In addition, however, they demonstrated that identical turnover rates can be obtained with single injections of ^{14}C lactate if the isotopic decay curves are subjected to proper mathematical analysis.[13,14] Although these appear to be the only values available for lactate turnover in man, they are not appreciably different from those obtained in animals. In dogs Depocas and Forbath and their associates found lactate turnover to be 134 and 130 mg per kilogram per hour respectively,[15,16] whereas in sheep Annison and his co-workers estimated lactate turnover to be 104 mg per kilogram per hour.[17]

Questions concerning the validity of these values can be raised, on the basis of the known limitations of isotopic dilution technics and estimated in vitro rates of lactate production by various tissues. There appears to be a discrepancy between lactate production derived from projected individual tissue contributions and that found by isotopic dilution, as indicated in Table 1, which is derived from data summarized by Cahill and Owen[18] and modified to include two additional sources of lactate that had not previously been considered: gastrointestinal mucosa[19,20] and skin (R. M. Fusaro and J. A. Johnson

Table 1. Estimated Tissue Lactate Production Rates *

TISSUE	RATE mg/kg/hr
Erythrocytes	16
Brain	12
Skeletal muscle	11
Leukocytes	2
Platelets	2
Renal medulla	1
Intestinal mucosa[19,20]	5
Skin†	20
Total	69
Isotopic lactate turnover	82 ± 6 (SE; n = 11)

*Modified from Cahill.[18]
†R. M. Fusaro & J. A. Johnson, unpublished data.

Supported by the Veterans Administration Hospital, Birmingham, Ala., by grants (AM-09722-06, TO1-AM05053, HE1-1310-05) from the U.S. Public Health Service and by a grant (RR-32) from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.

unpublished data). Erythrocytes and muscle account for most of the lactate produced in vivo, with minor contributions made by brain, leukocytes and renal medulla. The estimated total available lactate that could be produced from these sources is only 46 mg per kilogram per hour, or ±60 per cent of that determined isotopically. It appears, however, that both the gastrointestinal tract and skin can theoretically be sources of lactate. Adjustments of in vitro lactate production rates of these tissues for total mass of tissue and extrapolation to the 70-kg reference subject add a minimum additional 20 to 25 mg per kilogram per hour. Thus, whereas it is reasonable to believe that isotopic technics overestimate turnover, the exact magnitude of this error cannot be quantitated, but it is unlikely that the overestimate is more than 20 per cent in the basal state.

Data concerning lactate oxidation in intact animals and man are few. Searle and his co-workers have shown that oxidation constituted 80 to 85 per cent of their observed lactate turnover rates, or approximately 80 to 85 mg per kilogram per hour.[8] Thus, lactate oxidation would account for 40 per cent of the total carbon dioxide of a 70-kg reference subject. This value, although it appears to be excessive, is similar to that obtained for glucose and is consistent with the authors' concept that ^{14}C lactate can be used to trace the flow of glucose carbon through pyruvate. By comparison, lactate oxidation constituted 43 and 30 per cent respectively of the turnover rates in dogs and sheep and accounted for 20 and 10 per cent respectively of the total carbon dioxide production.[15-17]

The identity of the biochemical precursors of lactate is of particular interest since lactate is a byproduct or intermediate of glycolysis, and glucose would be expected to make a major contribution. In man 50 to 60 per cent of the lactate turnover is derived from blood glucose,[11,12] whereas in sheep somewhat less – 40 per cent – is derived from glucose.[17] Recent studies of alanine turnover and disposition in my laboratory indicate prompt attainment of an isotopic steady state for lactate during the infusion of ^{14}C alanine, and on the basis of precursor-product specific activity ratios, it can be estimated that 20 per cent of the lactate turnover may be derived from alanine. The source of the remaining 30 per cent is still unknown at this point, but presumably represents the contributions of muscle glycogen and other amino acids.

Glucose Metabolism

In contrast to the relative paucity of data concerning lactate, there is an extensive body of information concerning glucose metabolism in intact animals and man.[21] Glucose is distributed in man in a space that is equivalent to 30 per cent of body weight, a volume that is approximately 1½ times that of the extracellular compartment and is obviously more of a conceptual than anatomic space. The glucose pool size, expressed on the basis of body weight, is 250 mg per kilogram. In the metabolic steady state, when blood glucose concentrations are constant, production and utilization are balanced, and the term turnover can be used to describe both processes. After a single injection of glucose ^{14}C, the average blood glucose specific activity half-life ($t\ ½$) is 90 minutes. The fractional rate of glucose turnover $\left(\frac{0.693}{90}\right)$ is 0.0075 per minute, and the turnover time of the glucose pool is $1.44 \times t\ ½$, or 130 minutes. The glucose turnover rate is approximately 100 to 120 mg per kilogram per hour, representing a projected glucose turnover of approximately 180 g per day for the average postabsorptive 70-kg reference subject. It is estimated that glucose oxidation to carbon dioxide accounts for 50 per cent of the glucose turnover and contributes 20 to 30 per cent of the fuel of respiration. In the postabsorptive state, estimates of daily glucose production obtained from isotopic dilution agree reasonably well with those derived from direct measurement of splanchnic glucose release, whereas in prolonged starvation marked discrepancies are noted that appear to be due to enhanced isotopic exchange.[18]

An important aspect of glucose metabolism that is also pertinent to the metabolism of lactate is the phenomenon of glucose recycling, or what is more commonly known as the Cori cycle.[4] Glucose derived from the diet or endogenous sources (glycogenolysis or gluconeogenesis or both) is delivered to peripheral tissues, where it is metabolized and in part converted to lactate. The lactate is subsequently released and transported back to the liver, where it is resynthesized into glucose, completing the cycle. When specific activity of total blood glucose is used to calculate turnover, as it is with the use of glucose-U-^{14}C, adjustments cannot be made for the amount of radioactivity returned to glucose by the process of glucose recycling, and the glucose turnover rate is underestimated to the extent that recycling has occurred. Unless specific technics are used to adjust for recycling, absolute glucose turnover rates can vary widely, without any difference in net turnover, and yet be undetected. When either glucose-1-^{14}C or glucose-6-^{14}C is used as the isotope, adjustment for recycling is possible. During the process of recycling, the carbon labeled in position 1 of glucose-1-^{14}C is randomized back into positions 1, 2, 5, 6.[22] Degradation of glucose and isolation of the 6th carbon as the formaldimedon derivative allows calculation of recycling in vivo.[22] Estimates of glucose recycling in man indicate that approximately 20 per cent of the glucose turnover participates in this cycle.[12,22,23] The marked variability of glucose recycling and its apparent lack of relation to metabolic or nutritional status has been commented upon by both Cahill[24] and Keilson.[23] It has been suggested that, since glucose converted to lactate does not undergo terminal oxidation and is returned to the liver for resynthesis into glucose, this pathway accounts for neither net production nor utilization of glucose. In the metabolic steady state the value for glucose turnover remaining after subtraction of that due to recycling should be a reasonable estimate of glucose synthesized from nonlactate sources (i.e., amino acids, glycerol, glycogen, etc.).

Glucose-Lactate Interconversion

Determination of glucose recycling measures only the portion of the metabolized glucose returned to itself, and not the overall conversion of glucose to

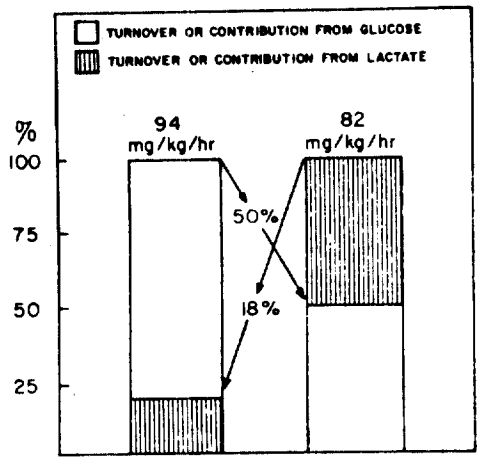

Figure 1. Glucose-Lactate Interconversion.
The column to the left represents the glucose turnover rate, the column to the right the lactate turnover rate, and the values above each column the absolute turnover rate of each of the substrates.

lactate or lactate to glucose. Although one would ideally prefer to study the interconversion of two substances simultaneously, such an analysis is practically not possible when identically labeled isotopes are used. Initially, glucose and lactate turnover and interconversion were determined in volunteers by the use of sequential administration of ^{14}C glucose and lactate on two consecutive days, although more recently this approach has been modified to include two consecutive four-hour periods on the same day. Quantitative estimates of the interconversion of glucose and lactate were derived from precursor-product specific activity ratios and their respective turnover rates. The results of these studies are summarized in Figure 1; 50 per cent of the lactate turnover in man was derived from glucose, thus accounting for 45 per cent of the glucose turnover rate. When combined, glucose recycling and conversion to lactate accounted for approximately 65 per cent of the glucose turnover. On the other hand, only 20 per cent of the lactate turnover was converted to glucose, accounting for 15 to 20 per cent of the glucose turnover. Comparable, but somewhat lower, estimates of lactate conversion to glucose were obtained by Searle in man[8] and by Depocas[15] and Annison and their co-workers[17] in animals. From these interconversion values a recycling rate of approximately 15 per cent would be expected — a value close to that found experimentally.

Starvation

When the intake of food, particularly carbohydrate, is limited, the maintenance of blood glucose homeostasis depends on hormonal readjustments that allow development of an optimum rate of gluconeogenesis and provide adequate quantities of alternate substrate for satisfaction of energy needs. The rate of glucose synthesis is determined by factors that regulate the supply of glucose precursors, as well as the activity of the rate-limiting gluconeogenic enzymes. The role of precursor availability in regulating glucose production from amino acids, particularly alanine, has been elegantly demonstrated by the studies of Felig and his associates.[24] The declining rate of splanchnic glucose release during prolonged starvation is related primarily to decreased peripheral alanine release, rather than to a decline in the activity of the gluconeogenic pathway. Similar observations have been made regarding substrate availability and gluconeogenesis in pregnancy by Metzger and his co-workers.[25] In contrast to the changes in concentration that occur with alanine and other glycogenic amino acids during prolonged starvation and with shorter periods of fasting in pregnancy, blood lactate concentrations remain unchanged.[11,24] Cahill has demonstrated that glucose recycling is not influenced by nutritional status and for that reason has concluded that the contributions of lactate and pyruvate to glucose production by liver and kidney remain constant throughout starvation. Since it is now clear that glucose is not the sole source of lactate, but that alanine and muscle glycogen are precursors as well, it cannot be assumed that the contribution of lactate to glucose synthesis is constant simply because recycling is unchanged. As is well known, blood glucose concentrations decline by 20 to 30 per cent during starvation and are accompanied by comparable decreases in the size of the glucose pool and the glucose turnover rate.[11] During starvation there is an accompanying decrease in glucose conversion to lactate that parallels the decrease in glucose turnover. Although lactate turnover also declines slightly, the conversion of lactate to glucose in starvation is increased by 50 per cent. Thus, the somewhat puzzling observation of an unchanged rate of glucose recycling in the presence of decreased glucose conversion to lactate in starvation can be explained by enhanced activity of lactate conversion to glucose. During short-term starvation, and in disagreement with the concept proposed by Cahill,[1] glucose derived from lactate increased from 23 to 35 g per day per 70-kg reference subject. The latter is, for all practical purposes, identical to the value of 40 g predicted by Owen and his associates from measurements of lactate and pyruvate extraction by liver and kidney after five weeks of starvation and the assumption that all the lactate and pyruvate was quantitatively converted to glucose.[26] It thus appears that glucose derived from lactate and pyruvate during periods of starvation of less than five to six weeks remains constant, whereas that derived from alanine declines, so that with time lactate and pyruvate become quantitatively the most important gluconeogenic precursors. The increased conversion of lactate to glucose in the presence of unchanged blood lactate concentrations is consistent with increased availability and extraction of lactate by glucose-synthesizing tissues during starvation. This is not entirely unexpected in view of the changes in glucagon secretion that occur during starvation[27] and its effects on lactate extraction and incorporation into glucose.[28]

Ethanol

Ethanol alters the interconversion of glucose and lactate in man, and both hypoglycemia and hyperlactatemia may result from its administration.[29,30] Its

ability to produce hypoglycemia is a direct result of its effect on gluconeogenesis, which is currently thought to be mediated by the changes in hepatic cytoplasmic redox that accompany its metabolic degradation.[31] Although ethanol has a profound influence on blood glucose homeostasis that is in part mediated through inhibition of gluconeogenesis, its ultimate effect on the blood glucose concentration depends upon the balance achieved between its hepatic action, which reduces glucose production,[31] and its peripheral one, which decreases glucose utilization.[32] Whereas starvation is a necessary prerequisite for the production of hypoglycemia in normal man, the role of substrate depletion in the inhibitory effects of ethanol on gluconeogenesis is less clear. Hypoglycemia does not occur with the administration of ethanol unless preceded by a fast of 48 to 72 hours,[29] but ethanol inhibits gluconeogenesis in liver tissue of both nonfasted animals and man.[31] Ethanol-induced hyperlactatemia has also been attributed to the cytoplasmic redox alterations that accompany the metabolism of ethanol and a subsequent increase in hepatic lactate production,[30] although the data supporting such a proposal are not convincing.

The isotopic technics described for studying glucose and lactate turnover have been used to investigate the effects of ethanol on the interconversion of glucose and lactate.[12,33] In these studies ethanol was administered orally as bonded whiskey at a rate of 30 to 45 ml per hour. As shown in Figure 2, neither the blood glucose nor the glucose turnover rate of briefly fasted (for 12 hours) normal subjects was altered by ethanol. The conversion of glucose to lactate by peripheral tissues was inhibited, an observation consistent with the known effects of ethanol on glucose utilization.[32] The process of glucose recycling was also dramatically reduced, owing in part to decreased glucose conversion to lactate, but primarily to decreased lactate conversion to glucose. Thus, both limbs of the Cori cycle were inhibited.

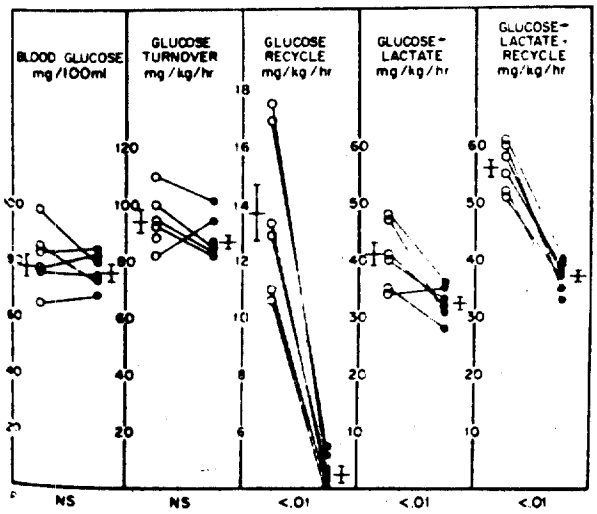

Figure 2. Effect of Ethanol on Glucose Metabolism (Reproduced from Kreisberg et Al.[12] with the Permission of the Publisher).

The open circles indicate control, the closed circles ethanol, and the bars mean ± S.E.M.

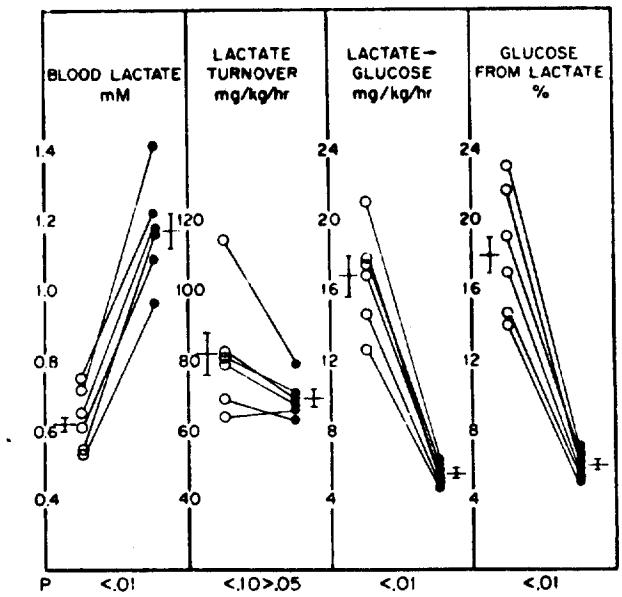

Figure 3. Effect of Ethanol on Lactate Metabolism (Reproduced from Kreisberg et Al.[12] with the Permission of the Publisher).

The open circles indicate control, the closed circles ethanol, and the bars mean ± S.E.M.

Despite a twofold rise in blood lactate concentration, as shown in Figure 3, lactate turnover tended to decline, and lactate conversion to glucose was markedly diminished. Temporally, lactate recovery in glucose was decreased within 30 minutes of the administration of ethanol, and the maximum inhibitory effect was reached within 120 minutes. Studies performed in subjects fasted for 72 hours revealed no greater inhibition of lactate incorporation into glucose by ethanol despite the occurrence of hypoglycemia. These data indicate that ethanol increases NADH in the nonstarved as well as the starved state and diverts substrate from gluconeogenesis. Thus, starvation is not a prerequisite for ethanol inhibition of gluconeogenesis, but is necessary for the development of hypoglycemia. Inhibition of lactate incorporation into glucose in nonfasted subjects is probably masked by a concomitant increase in glucogenolysis that prevents hypoglycemia. In starved subjects depletion of glycogen stores allows hypoglycemia to supervene.

In view of the proposal that ethanol-induced hyperlactatemia is due to increased lactate production, the observed decline in lactate turnover was somewhat puzzling and led to studies of the acute effect of ethanol on lactate metabolism. Conventional mathematical formulas for calculation of turnover during metabolic and isotopic steady-state conditions cannot be applied to the non-steady state, and for the studies described below, lactate inflow and outflow were calculated by the method that Steele used for glucose.[13] Figure 4 shows the effect of ethanol on lactate inflow and outflow. In the pre-ethanol period and in subjects not receiving ethanol, lactate inflow and outflow were always closely balanced. After the administration of ethanol, no changes were observed in lactate inflow (production), but a prompt inhibition of lactate out-

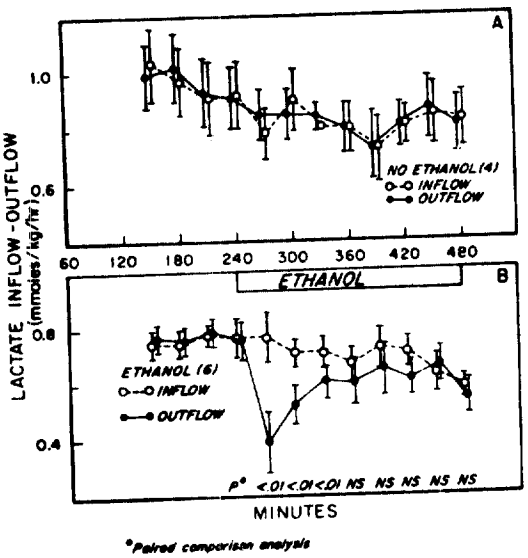

Figure 4. Effect of Ethanol on Lactate Inflow and Outflow (Reproduced from Kreisberg et Al.[34] with the Permission of the Publisher).

The bars indicate mean ± S.E.M.

flow (utilization) of 90 minutes' duration was observed. It appears that within the limits imposed by this experimental design, ethanol-induced hyperlactatemia results from decreased utilization of lactate instead of increased production. This conclusion is supported by studies indicating that ethanol decreases hepatic splanchnic lactate extraction[34] and delays lactate clearance from the blood after exercise or a lactate load in man.[35,36]

Conclusions

A dynamic relation exists between the metabolism of glucose and lactate. Exposure to ethanol and starvation are but two situations in which this relation is altered. Changes in the metabolism and interconversion of glucose and lactate, and hyperlactatemia also accompany the use of phenformin.[37] A more thorough understanding of the regulation of blood lactate concentrations requires further definition of the physiologic, pharmacologic and pathologic factors that alter glucose-lactate inter-relations. Blood lactate concentrations simply reflect the balance that exists between lactic acid production and lactate utilization. Although hyperlactatemia may be a consequence of either increased production or decreased utilization, lactic acidosis only results from the overproduction of lactic acid.

Discussion

Dr. Carl Hirsch: Do you think, then, that the increase in lactate pool that comes with ethanol ingestion results from an increase in the NADH level that shifts the equilibrium between pyruvate and lactate?

Dr. Kreisberg: Yes, but not in the way that your question implies. Certainly, ethanol alters cellular redox and cytosolic NADH concentrations. But the increase in the circulating lactate pool is not primarily due to a shift in the equilibrium between pyruvate and lactate, but to the decreased uptake of lactate by the liver as a consequence of the redox change.

A Physician: The Cori cycle must be to some extent inefficient in that it requires energy to resynthesize lactate into glucose. There is speculation, I think, that in patients with cancer, there might be an increase in recycling. Are there any measurements under such circumstances, and how much energy is lost in that cycle?

Dr. Kreisberg: Some of the initial studies of glucose recycling were done by Reichard and his associates in patients with cancer[38] specifically for the reasons that you mention. The numbers of patients studied were too small to draw any definite conclusions, but the patients with cancer tended to have higher glucose turnover and recycling rates than normal persons. If the gluconeogenic requirements were greatly increased, accelerated protein breakdown and wasting of protein stores could result. The conversion of one molecule of glucose to lactate results in a net gain of 2 molecules of ATP under strictly anaerobic conditions, but 8 molecules of ATP under aerobic conditions. The resynthesis of glucose from lactate requires 6 molecules of ATP that are furnished by the oxidation of fat in the liver.

Dr. Frank Davidoff: The amount of energy that it takes to convert lactate back to glucose that you have mentioned is based on the assumption that that process is 100 per cent efficient. It appears now that there may even be cycles within cycles, wheels within wheels, in the sense that the lactate may get halfway up to glucose and then come back down again through what is now being called a futile cycle – that is, it will go up to a triose phosphate, then be broken down again to pyruvate and then have to go up again, thereby using even more energy.

Dr. Kreisberg: The only data that I am familiar with that relate to this phenomenon are those of Williamson.[39] He has interpreted the results of his studies with ethanol and the perfused liver to indicate the existence of such a cycle at the level of phosphofructokinase and fructose diphosphatase and to attribute the inhibitory effects of ethanol on gluconeogenesis to it.

Dr. Neil Ruderman: The presence of free fatty acids is required to demonstrate the inhibitory effect of ethanol on gluconeogenesis in perfused livers. Have you done studies in fed as well as overnight-fasted man, whose liver is probably in part using the fatty acids as a fuel?

Dr. Kreisberg: We have been very interested in defining the experimental conditions in man that would allow demonstration of the stimulatory effect of ethanol on gluconeogenesis. We have done several studies in which ethanol was administered with or after a protein meal, but were unable to demonstrate, with lactate as substrate, the clinical counterpart of what Williamson demonstrated in livers perfused without free fatty acids – that is, stimulation of gluconeogenesis.[39]

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Lieberman-'30
Bib No. 1113

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STUDIES ON CALCIUM[1]

II. URINARY OUTPUT OF CALCIUM IN NORMAL INDIVIDUALS AFTER PERORAL ADMINISTRATION OF CALCIUM LACTATE AND CALCIUM GLUCONATE

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Received for publication July 7, 1930

It is of practical value to answer for a given drug two questions: How much? When? In so far as peroral administration of calcium salts is concerned, it seems to have been taken for granted that absorption is better on an empty stomach and that the equivalent of 3 to 4 grams of calcium lactate per day is about the right dose for an adult, except in cases of extreme calcium deficiency as in hypoparathyroidism when a much larger amount must be given. It seemed worth while to gather some precise data on this subject.

METHOD

Although less than 10 per cent of peroral calcium is excreted in the urine, it was assumed that the urinary calcium would, nevertheless, serve as a fairly reliable index of the rate of the blood calcium changes. Accordingly, three healthy individuals submitted to the following procedures. As a control, the individual voided hourly from 7 a.m. to 2 p.m. He ate nothing from arising to 2 p.m. He drank a glass of water (250 cc.) hourly from 8 a.m. to 1 p.m. There were no other limitations. The next day calcium lactate was given at 8 a.m. in 250 cc. of water. Everything else was maintained the same. The following day the routine was repeated except that calcium gluconate was given in 250 cc. of water. After an interval of a day, the order of calcium salts was reversed calcium gluco-

[1] Work done under grant from Sandoz Fund.

nate was given first, calcium lactate on the second day. Each subject kept a rather detailed record of pertinent signs and symptoms such as abdominal discomfort, nausea, headache, diarrhea, borborygmi, etc. At first, the subject was given 20 grams of calcium gluconate and 10 grams of calcium lactate but the subjective and objective symptoms (abdominal distress, vomiting and diarrhea) were so violent that the doses in this series were halved, 10 grams of calcium gluconate and 5 grams of calcium lactate being given. The powder was simply dissolved in a glass of water (250 cc.) and drunk. This procedure was then repeated with the important difference that the individual had his usual breakfast about 7:30 a.m. It was uniform each time consisting of two eggs, two cups of coffee, and one piece of toast. For the urinary determinations, Lyman's (1) method was used with only this difference: instead of washing through filter paper, centrifuge tubes were employed. This saved a great deal of time and was as accurate, by actual check, as the unmodified method. The volume of the specimen obtained each hour was recorded and the milligrams of CaO in each specimen determined.

By its very nature, urinary calcium cannot give as true a picture of the level of calcium in the blood as can an actual blood calcium determination. However, for obvious practical reasons, the calcium output in the urine was chosen as the index as to the rapidity and extent of absorption and excretion of the calcium ingested.

RESULTS

Several rather interesting points stand out in the data accumulated. Figure 1 shows graphically the volume of urine. It is the average of 18 determinations each on the calcium gluconate and calcium lactate with 9 controls. As can be seen, ingested calcium salts cause a very definite diuretic effect. Whereas at 2 p.m. the controls showed a volume of only 178 cc., the average volume for the gluconate salt was 360 cc.—a volume twice as great. However this action can be explained on the mere physical basis of the greater volume of water being necessary to put out a larger amount of calcium present. Of course, the individuals here

were presumably healthy so that this would have nothing to do with experiments like those of Barath and Gyurkovich (2) who showed that calcium salts cause a diuresis and a diminution of the albuminuria in nephritic edema. In these cases Blum (3) may be right in saying that the action is due to the dehydration of the blood colloids with resulting hydremia and diuresis.

Figure 2 shows the amount of CaO in milligrams that appeared in the urine during the period of observation. It will be seen that the individual puts out normally somewhere between 2 and 5 mgm. of CaO hourly. After the ingestion of the calcium salt

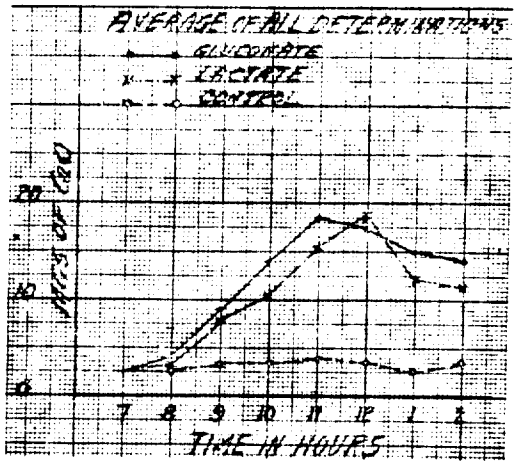

FIG. 1

there is a fairly smooth curve upwards with a maximum attained in some four hours. Then there is a beginning decrease. There is very little to choose between the two salts although a little more of the gluconate salt is recovered. However, there is a vast difference in the subjective symptoms. The gluconate was fairly well tolerated giving rise chiefly to rather annoying borborygmi and some abdominal distress. The lactate salt in equimolecular amounts gave rise to a very disagreeable headache and quite stormy bowel movements accompanied by a good deal of bowel spasm. Altogether, about 5 to 8 per cent of the calcium given was recov-

ered in the urine which is about the amount usually quoted in the literature. A much more interesting and very instructive finding is summarized in figure 3. As can be seen gluconate given on an empty stomach reaches a maximum within two hours and then begins to decrease. When given after a meal it takes longer for it to appear in any amount in the urine but six hours after ingestion the curve had not yet begun to slope downwards. As a net result much more of the calcium gluconate is utilized by the body.

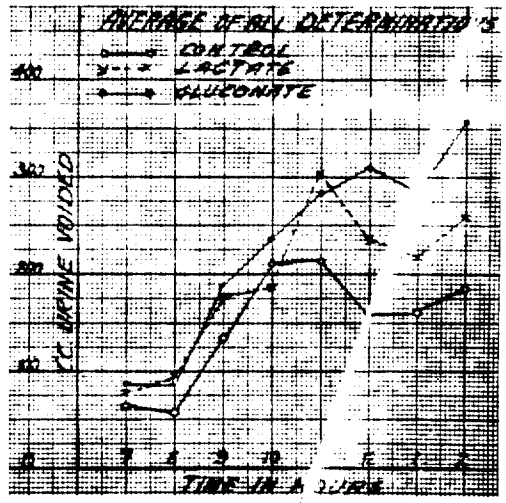

FIG. 3

Also, there was almost complete freedom from subjective symptoms. Except for the slight discomfort right after taking the material, one almost forgot that anything had been ingested. It seems, in this series at least, that there can be no question that better and longer absorption was obtained by taking the salt on a full stomach. The calcium lactate gave a very similar curve except that when given on an empty stomach the plateau of the curve was much flatter. This is probably due to the fact that the hyperperistalsis induced by the lactates caused such a rapid movement of the material through the bowel that proper absorption could not take place. At this point it seems to be worth while

repeating the statement previously made as to maximal optimal doses. The 10 grams of calcium gluconate powder and the equimolecular amount of the lactate is not only the maximal dose from the standpoint of comfort in taking the substances but is also the largest amount that can be taken without having a *decrease* in the amount of calcium ion absorbed. This apparent paradox is readily explicable when we take into consideration the fact that overdosage sets up a violent diarrhea which causes such a rapid passage through the bowels of the salts that proper absorption can not take place.

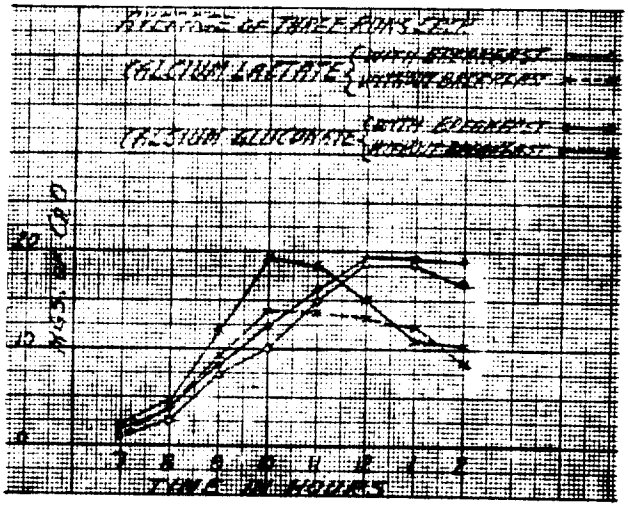

FIG. 3

CONCLUSIONS

1. Using urinary calcium as an index, the extent of calcium absorption following peroral administration of calcium gluconate and calcium lactate has been studied in three healthy individuals

2. There is a slight but very definite diuretic effect in this series.

3. There is a maximal dose beyond which the diarrheal effect begins to outweigh the size of the dose. The salt begins to pass through and out of the intestine too rapidly to be absorbed properly. The gluconate salt gives fewer subjective symptoms as compared with the lactate salt.

4. It is much better to administer the calcium salt after a meal. There is a smoother and greater absorption with a minimum of subjective distress. Also, for a given dose the physiological action of the ingested calcium persists over a more protracted period of time.

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Mason-'45
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Ind. Jour. Med. Res., 33, 2, October, 1945.

THE INFLUENCE OF BUTTER FAT IN THE ABSENCE OR PRESENCE OF CASEIN ON GROWTH IN YOUNG RATS ON A RICE DIET.

BY

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(*An Inquiry under the Indian Research Fund Association.*)

[Received for publication, March 21, 1945.]

SINCE October 1942 experiments with rats have been conducted in the Nutrition Department of the Women's Christian College (*i*) to demonstrate for educational purposes the inadequacy of diets composed mainly of rice, (*ii*) to demonstrate and test further the effects of supplements to rice diets, (*iii*) to compare South Indian cereals, and (*iv*) to explore modifications and improvements of rice diets that do not involve appreciable increase in cost. The work has benefited from the beginning from the long experience with rice diets of the Nutrition Research Laboratories, I.R.F.A., at Coonoor, and from the advice and generosity of Dr. W. R. Aykroyd.

In the course of class experiments planned to demonstrate the nutritive importance first of milk and then of some of its separate constituents with rice diets, the well-known effects of milk and of calcium lactate in accelerating growth in rats were clearly confirmed. The effect of casein, however, was more marked than reported by Aykroyd and Krishnan (1937) and although the diet was markedly deficient in total fat and contained a negligible quantity of animal fat, the addition of butter to the poor Madrasi diet not only did not aid growth but if anything adversely affected both the growth and the general condition of the animals. The same results were observed with three vegetable oils commonly used in South India, coco-nut oil, gingelly oil and ground-nut oil, as well as with shark-liver oil (unpublished data).

The following is the report of an experiment planned to test these preliminary observations, viz. the effects of these three ingredients of milk: casein, calcium and butter, singly as well as combined.

GENERAL TECHNIQUE.

The rats used are Wistar stock, and are bred in the laboratory and fed on an abundant and well-balanced diet. The litters are kept on this diet in the cage with the mother until 4 weeks' age. After two or three days of separation from the mother, during which they are still on stock diet, they are used for experiments with rice

* Grateful acknowledgment is made of grants from the Indian Research Fund Association and from the Elizabeth Thompson Science Fund, Boston, U.S.A.; also of the assistance of Miss Mary Mani, undergraduate, in the conduct of the experiment.

† The author responsible for the statistical study.

diet. The basal rice diet used in the Women's Christian College is of the following composition :—

'The poor rice diet'

	Per cent.
Parboiled milled rice	89·7
Dhal (*Cajanus indicus*)	3·0
Brinjal (*Solanum melongena*)	4·3
Green plantain	2·1
Gingelly oil (*Sesamum indicum*)	0·43
Mutton	0·26
Coco-nut	0·21

The diet contains approximately, per 100 g. uncooked foods, protein 6·5 g., fat 0·9 g., carbohydrates 74 g.; calcium 0·014 g., phosphorus 0·145 g., iron 2·32 mg.; total calories 329*.

This diet is in the main similar to the 'poor Madrasi' or 'poor rice' diet used in Coonoor with the important difference that parboiled rice is used instead of the raw rice of the Coonoor diets and that after the foods are weighed out in the proportions stated the rice and the dhal are *cooked*. The rice is *not washed* and *only as much water is used in cooking as can be absorbed*. In the process of cooking rice absorbs approximately 3¼ times its own weight of water. The cooked rice and cooked dhal are thoroughly mixed with the chopped raw ingredients and the oil, and all put once through a grinder.

It has been shown that most food factors present in rice are little affected by the degrees of heat used in cooking and that the considerable losses of minerals and vitamin B_1 and nicotinic acid during washing and cooking are recoverable in the washing and cooking water (Ranganathan, Sundararajan and Swaminathan, 1937; Aykroyd, Krishnan, Passmore and Sundararajan, 1940; Swaminathan, 1941, 1942). In this diet the rice is not washed and no cooking water is discarded so it may be considered that losses due to cooking are slight if not negligible.

The diet is given *ad lib.* but daily consumption of food is measured.

Plan of Experiment.

In order to test the individual and combined effects of casein, calcium lactate and butter on the growth in young rats fed on poor rice diet, an experiment was designed which conformed to a factorial experiment for $2 \times 2 \times 2$ levels (Fisher *et al.*, 1941, 1942, 1943). By this type of design it is possible to determine the effects of several factors on a fairly limited number of animals and yet maintain a high degree of precision.

Twenty-four young rats were distributed in eight cages, three in each cage. The three supplements were tried out in eight different ways, that is in all possible combinations. Table I gives the amount of supplements supplied, corresponding approximately to the quantities of protein, calcium and fat in 12·5 grammes of milk. One group of rats received no supplements, three groups received single supplements, three combinations of two and one group received all three supplements. By this arrangement it is possible to estimate the effect of a single supplement, e.g. butter, on the basis of 12 rats receiving butter and 12 rats not receiving butter, the two groups being treated alike except for the butter (Table II). Similarly, the rats can also be divided into two groups according to whether they have received casein or no casein, or calcium lactate or no calcium lactate. As will be shown later the interactions between the three supplements, either between pairs or between all three together, can also be ascertained by comparison between two groups of twelve rats each.

* Calculated from tables in *Health Bulletin* No. 23, Govt. of India Press, New Delhi, 1941.

TABLE I.

Results of growth experiments on rats fed on poor rice diet supplemented with casein, calcium lactate and butter.

Cage.	Supplements to the basal rice diet.	Sex.	Initial weight, g.	Average weekly gain, g.	Mean.	Relative growth rate, per cent per week.	Mean.
1.	*Nil*	F	41·5	0·39	0·57	1·52	1·820
		F	41·0	1·16		2·64	
		M	30·0	0·16		1·30	
2.	Casein, 0·4 g.	F	35·0	2·71	2·92	6·31	6·420
		M	30·0	3·44		7·33	
		M	40·0	2·61		5·62	
3.	Calcium lactate, 0·115 g. ...	F	37·0	1·72	2·67	4·60	6·160
		F	31·5	3·67		8·69	
		M	42·0	2·61		5·19	
4.	Butter, 0·5 g.	F	33·0	−0·17	−0·07	0·38	0·283
		M	46·5	−0·22		−0·24	
		M	30·5	0.17		0·71	
5.	Casein, 0·4 g. and calcium lactate, 0·115 g.	F	35·0	4·83	5·94	9·25	10·203
		M	30·5	6·94		11·97	
		M	42·0	6·06		9·39	
6.	Butter, 0·5 g. and casein, 0·4 g. ...	F	43·5	3·89	3·24	7·25	7·117
		M	32·5	3·17		7·63	
		M	34·0	2·67		6·47	
7.	Butter, 0·5 g. and calcium lactate, 0·115 g.	F	32·5	1·61	1·31	5·10	3·933
		F	32·5	0·83		3·32	
		M	42·0	1·50		3·38	
8.	Butter, 0·5 g., casein, 0·4 g. and calcium lactate, 0·115 g.	F	31·0	5·33	7·05	11·04	11·633
		F	35·0	6·22		11·62	
		M	43·5	9·61		12·24	

The rats used in this experiment were from four different stock litters. Not enough rats being available to keep a balance of sexes and litters, they were distributed at random except to ensure that the total weights of the animals per cage were approximately uniform and that each cage contained at least one animal of each sex.

The experiment lasted nine weeks.

Effects of supplements on general condition of animals.

On the poor rice diet alone the hair of the animals became loose and dry, and fell out or was chewed off by cage mates until neck and back were almost denuded. The gait and posture were affected, an effect commonly described by the workers in the laboratory as 'running on hind toes'. These are common characteristics of rats fed on this diet. The rats receiving casein appeared normal except for somewhat thinned coats. The general condition of the rats with butter was worse than that of those receiving no supplement at all: they were almost completely denuded of hair on head, neck and back; the hind legs became almost erect when standing, and the animals could rest only with the legs spread wide apart; the tails became scaly and ringed and were broken off in successive stages. Of the rats fed on single supplements the coats of those receiving calcium lactate were best.

On calcium lactate *plus* butter the coats were markedly thin; on casein and calcium lactate the coats were excellent; on casein and butter they were thinned in the first two weeks but recovered and were in good condition before the end of the experiment. The rats receiving all three supplements had excellent coats. No rats except those on the poor rice diet alone and those on butter supplement alone showed deformity of the hind legs, and only in the butter cage were the tails affected.

Method of statistical analysis.

The statistical significance of the experiment was based upon a study of the logarithmic growth curves in preference to the absolute growth curves, and the *proportionate* weight gains, expressed as the average relative growth rates per week, were submitted to statistical analysis.

(*a*) *Relative growth rates.*—The advantage of analysing relative growth rates instead of the frequently used average absolute gains is evident if it is considered that two animals with the same absolute growth rates would have grown quite differently if they started from different initial weights. Two rats from a recent experiment may be taken as an illustration of this fact, the one weighing 47 g., the other 32 g. at the beginning of the experiment. Their weights after nine weeks were 101·5 g. and 89 g. respectively, giving average weekly gains of 6·06 g. and 6·33 g. The difference is apparently quite unimportant. Against this a marked difference was found when the proportionate gains were considered. While the weight of the larger rat was doubled, that of the smaller animal was nearly trebled—unquestionably a much better performance. The true difference was borne out by the average relative growth rates which were 8·40 and 12·19 per cent respectively.

The average absolute as well as the average relative growth rates for each of the animals are given in Table I. The former, expressed in grammes, was calculated as the simple arithmetic mean of the weekly gains, whereas the latter, the relative rate, was found by determining the regression coefficient of the straight line fitted to the logarithmic growth curve ($\log_e w$). This coefficient when based upon natural logarithms indicates the rate of increase per unit of time as well as per unit of weight already attained; multiplied by 100 it expresses the percentage rate of increase per week (Fisher, 1941). A discussion of the shape of the logarithmic growth curve is given in the *Appendix*.

(*b*) *Analysis of variance.*—In Table II the relative growth rates per week of all the rats are arranged according to (*i*) different supplements, and (*ii*) initial weights, the first column showing the growth rates of the smallest animals, the third those of the largest. As already pointed out the three supplements were tried out in all possible combinations, that is in eight ways. The effect of a single supplement, e.g. butter, is estimated by comparing the mean relative growth rate of all twelve animals receiving butter with that of the remaining twelve which did not get it. As the total of the relative growth rates of the first 4 groups of rats in Table II which received no butter is 33·81 against 68·90 of the last 4 groups, the difference is −1·91; this divided by 12 gives the effect of butter as −0·11 per cent (as shown in Table IV). Each of the three interactions between the supplements, casein × butter, and the one triple-action (casein × butter × calcium lactate) is similarly analysed on the basis of all 12 animals.

Taking the former as an example, the two groups which received casein and butter simultaneously (one with calcium lactate in addition, the other without calcium lactate) *plus* the two groups receiving no casein or butter may be compared with the remaining four groups which got either casein or butter but not simultaneously. The mean relative growth rates per cent are 6·68 and 5·21 per cent respectively. The difference is 1·47 (see Table IV), and it expresses the interaction effect between butter and casein: being positive it indicates that the growth is accelerated 1·47 beyond what would be expected by the mere summing of the single effects of butter and casein.

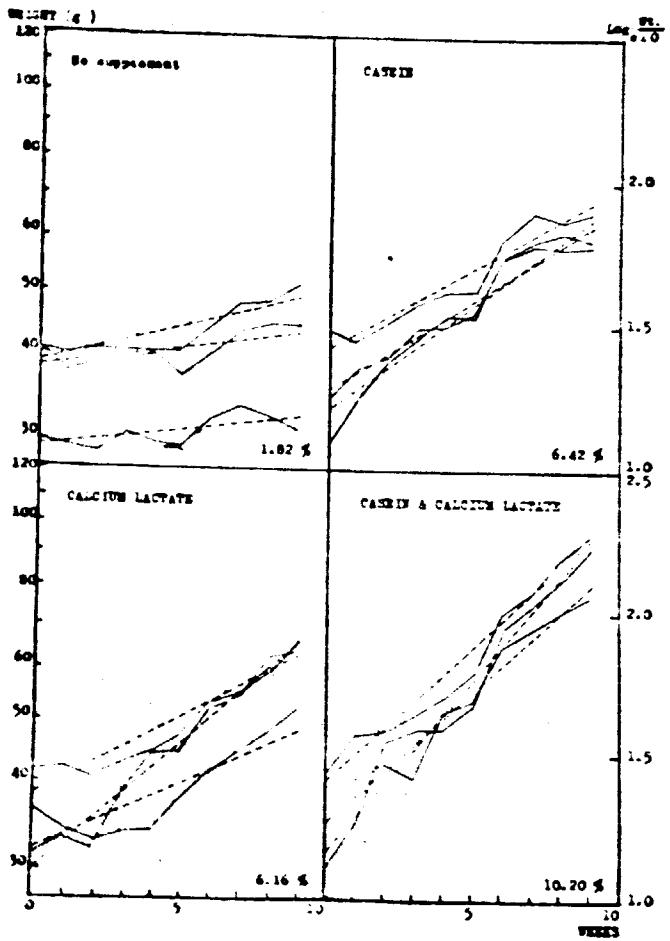

Fig. 1. Logarithmic growth curves of rats fed on a basal rice diet with supplements of casein and calcium lactate. The straight lines indicate the regression lines fitted to the curves. The percentage figures are the means of the average weekly relative growth rates.

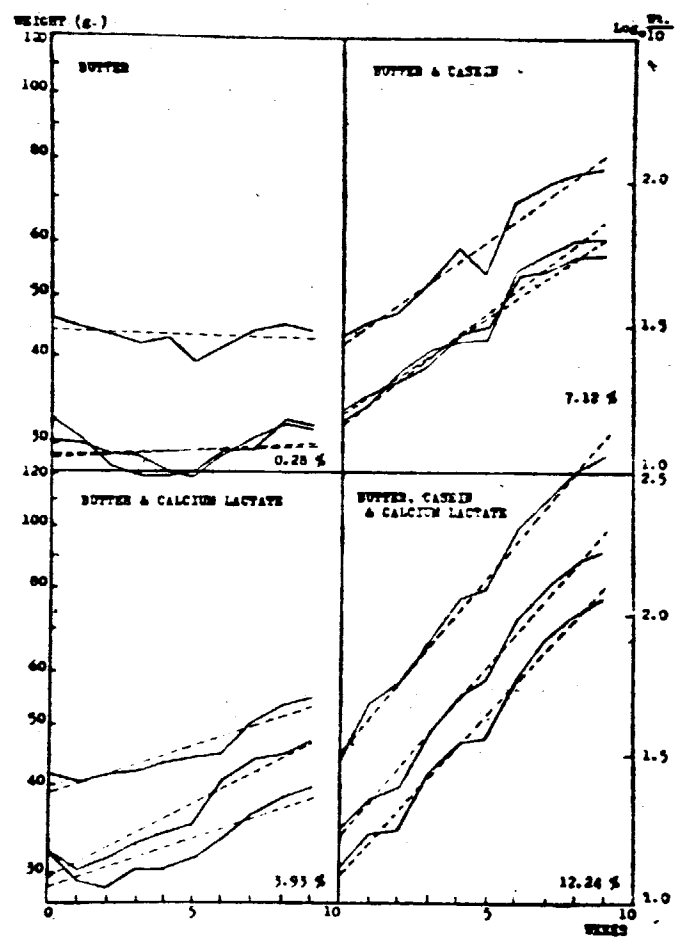

Fig. 2. Logarithmic growth curves of rats fed on a basal rice diet with a supplement of butter with or without casein and calcium lactate. The straight lines indicate the regression lines fitted to the curves. The percentage figures are the means of the average weekly relative growth rates.

TABLE II.

Relative growth rates (per cent per week) resulting from feeding rats on poor rice diet with supplements of casein, calcium lactate and butter, singly and combined.

Supplements.	(i)†	(ii)	(iii)	Total.	Mean
Nil	1·30*	2·64	1·52	5·46	1·82
Casein	7·33*	6·31	5·62	19·26	6·42
Calcium lactate	8·69	4·60	5·19*	18·48	6·16
Casein + calcium lactate	11·97	9·25*	9·39*	30·61	10·20
Butter	0·71*	0·38	−0·24*	0·85	0·28
,, + casein	7·63*	6·47*	7·25	21·35	7·12
,, + calcium lactate	5·10	3·32	3·38*	11·80	3·93
,, + casein + calcium lactate ...	11·04	11·62	12·24*	34·90	11·63
TOTAL ...	53·77	44·59	44·35	142·71	...
MEAN ...	6·72	5·57	5·54	...	5·946

* Male rats.

† In column (*i*) are entered rats with the smallest initial weights, in (*iii*) those with the largest initial weights.

The result of the analysis of variance is given in Table III :—

TABLE III.

Analysis of variance.

Items.	Sums of squares.	D.F.	M. Sq.	V.R.	Probability.
Single effects :					
Casein	201·4342	1	201·43	205·89	Less than 0·001
Calcium lactate	99·5115	1	99·51	101·71	,, 0·001
Butter	1·0045	1	1·00	1·03	0·40—0·30
Interactions :					
Casein × calcium lactate	0·0360	1	0·036	...	...
Casein × butter	13·0095	1	13·01	13·30	0·01—0·001
Calcium lactate × butter	0·0007	1	0·0007	...	...
Triple effect :					
Casein × butter × calcium lactate ...	0·7597	1	0·76	...	...
Total treatment effect	315·7561	7	...	...	...
Between initial weights	7·2111	2	3·61	3·68	0·20—0·05
Error	13·6973	14	0·9784	...	...
TOTAL	336·6645	23	...	...	..

D.F. = Degrees of freedom. M. Sq. = Mean square or variance. V.R. = Variance ratio.

Before discussing the significance of the supplementary effects, attention may be drawn to the observation that the growth rates were affected by the difference in initial weights, the smaller rats growing faster than the larger ones. This observation, though statistically not quite of significance value, is in keeping with common experience from nutrition experiments with other species of animals. Although it has no direct bearing upon the chief purpose of the present investigation, the importance of the observation lies in the possibility of increasing the efficiency of the experiment by separating from the residual sum of squares (Table III) the part contributed by the variation caused by different initial weights. By reducing the residual sum of squares on which is based the estimate of the experimental error, the latter becomes smaller and, therefore, the precision of the experiment higher. In this connection it may be added that an estimate of the experimental error on basis of the *absolute* growth rates showed it to be about twice as large as in the present analysis, corresponding to a precision about four times less.

TABLE IV.

Main effects of supplements expressed in terms of average relative growth rate per week.

Supplements.	Relative growth rate per cent rat weight.
Single effects:	
Casein	+ 5·78*
Calcium lactate	+ 4·07*
Butter	− 0·41
Interactions:	
Casein × calcium lactate	+ 0·08
Casein × butter	+ 1·47*
Calcium lactate × butter	+ 0·01
Triple-effect:	
Casein × calcium × butter ...	+ 0·36
Estimate of standard error	± 0·4038
Significance levels,	
at 5 per cent	0·87
„ 1 „	1·20
„ 0·1 „	1·67

* Highly significant.

RESULTS OF THE STATISTICAL ANALYSIS OF GROWTH RATES.

Table IV gives the numerical values of the change in relative growth rates caused by the various supplements. The result of the experiment as found by the statistical analysis may be summarized as follows.

Casein and calcium lactate caused a marked and highly significant acceleration of growth, whereas butter had a negative though not significant effect*. Of the three interactions of first order, two were small and insignificant, but the interaction between casein and butter was highly significant (+1·47 being more than three times experimental error), indicating a stimulus of the growth beyond what would be expected from a mere summing of the single effects of the two ingredients. The interaction between all three supplements was not significant.

Discussion.

The deficiencies of poor rice diets have been discussed by Aykroyd and Krishnan (*loc. cit.*) and Aykroyd, Krishnan, Passmore and Sundararajan (*loc. cit.*), who state that these diets are deficient in vitamin A, calcium and various factors in the vitamin B complex. In their experiments with rats the addition of skimmed milk powder induced a striking improvement in growth rate but it was found that much of the effect of the milk supplement could be produced by calcium lactate or calcium phosphate alone, and since it was found that casein produced a smaller improvement of the growth rate the conclusion was drawn that protein deficiency is of relatively minor importance with these diets. The addition of extra fat in the form of gingelly oil did not accelerate the growth, the observation being interpreted as 'suggesting that the rice-eater's low fat intake does not represent *per se* a serious problem'.

In the present experiment not only calcium lactate but also casein accelerated growth very markedly, the difference between the effects of casein and calcium being statistically insignificant. The most interesting findings, however, are first, that when butter was added to a rice diet, already deficient in fat, the rats were unable to utilize the fat, and not only did not grow but developed pathological symptoms more severe than, and in some respects different from those produced by the rice diet alone; secondly, that when casein was added to the butter, these pathological effects of the added fat did not appear and the beneficial effect of butter appeared as shown by acceleration of growth even greater than could be explained by the casein effect alone.

These results seem to indicate that the poor rice diet is deficient in a factor or factors essential for the normal utilization of dietary fat, and that this factor was supplied by the casein. In experiments with a basal diet so badly balanced and with such multiple deficiencies as the poor rice diet, any attempt at interpretation of results at this stage must be very tentative. That the explanation is probably not to be found in the nature of the fat supplied is indicated by the fact that gingelly oil which is rich in linoleic acid (Hoover, 1939) produced the same effect as butter (unpublished data referred to above). Of contaminants present in technical casein a calcium effect may be ruled out by the results of the present experiment with calcium lactate and butter; the possibility of the B_2-group vitamins cannot be ignored but the quantities present in the amount of casein supplied are so minute as to make this explanation unlikely. A possible factor is the amino-acid methionine supplied by the casein.

Summary.

1. The purpose of the present investigation was to examine the supplementary effects of casein, calcium lactate and butter, singly or combined, on the growth of rats fed on a basal rice diet similar to that common among the poor in South India.

2. Casein as well as calcium lactate was found to have a highly significant effect in promoting growth.

3. Butter affected both growth rate and general condition adversely.

4. Casein when added to butter counteracted the negative effect of butter and converted it into a positive effect. This interaction was highly significant. There was no significant interaction between calcium lactate and butter or between calcium lactate and casein.

* The growth on the poor rice diet alone was so slight that a negative effect great enough to be significant would have resulted in the death of the animals.

5. It is concluded that the poor rice diet is deficient in a factor or factors essential for the utilization of dietary fat and that this factor is supplied in technical casein.

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APPENDIX.

Discussion on the shape of the logarithmic growth curves.

The adoption of the average relative growth rates as basis of analysis presumes that the linear regressions by themselves were adequate expressions of the logarithmic growth curves, or in other words that the curves allowed closely a straight line. This again means that the growth rate should be constant throughout the experiment and the experiment be terminated before the growth tends to cease. As may be seen from Fig. 1 and 2 the curves did generally follow a straight course but in some cases they were slightly parabolic. In order to assess the significance of the deviations from a straight line, polynomial regression lines of second order were fitted to each logarithmic curve according to Fisher and Yates' method, and the coefficients of the terms are entered in Table V. From the coefficients to the quadratic term (column *c* of Table V) it will be seen that of the rats receiving casein as supplement showed slightly parabolic growth curves, the negative signs of the coefficients indicating an upwards convex curvature, whereas in the case of rats receiving poor rice diet only or supplements of butter, calcium lactate or both combined, the curves had a downwards convex curvature, the signs being positive, indicating that the rats to begin with lost weight but later picked up, gaining slowly. Except in the case of the rats receiving butter all the linear regression coefficients were positive and significant indicating definite gain in weight, and in the case of the rats in cages 5, 6 and 8 these coefficients alone were sufficient to indicate the shape of the curves which, therefore, truly followed the course of straight lines. In the other cases where the parabolic regression coefficients also were statistically significant, their values were so small that with a few exceptions they may be considered quite unimportant compared with the values of the linear regression coefficients. Only in case of the butter rats in cage 1 was the linear regression insignificantly but the parabolic regression significantly different from zero, which indicates that the animals just maintained their weight despite a temporary loss.

Concluding, it seems justified in the analysis to ignore the deviations from the straight regression line and rely only upon the first order regressions as expressed by the average relative growth rates.

TABLE V.

Coefficients of polynomial regression lines of the form $y = a + bx + cx^2$ fitted to the logarithmic growth curves (to base e) of rats fed on a poor rice diet with various supplements.

Cage.	Supplements.	Sex.	*a*	*b*	*c*
1.	*Nil*	F	1·373	+ 0·01524*	+ 0·00152*
		F	1·132	+ 0·02635*	+ 0·00491*
		M	1·130	+ 0·01297*	+ 0·00569

* Coefficients significantly different from zero.

TABLE V—*concld.*

Cage.	Supplements.	Sex.	*a*	*b*	*c*
2.	Casein, 0·4 g.	F	1·583	+ 0·06314*	− 0·00235*
		M	1·594	+ 0·07329*	− 0·00481*
		M	1·667	+ 0·05623*	+ 0·00125
3.	Calcium lactate, 0·115 g. ...	F	1·304	+ 0·04595*	+ 0·00894*
		F	1·487	+ 0·08692*	+ 0·00098
		M	1·540	+ 0·05189*	+ 0·00591*
4.	Butter, 0·5 g.	F	0·996	+ 0·00381	+ 0·00970*
		M	1·412	− 0·00239	+ 0·00418
		M	0·995	+ 0·00712	+ 0·01031*
5.	Casein, 0·4 g., and calcium lactate, 0·115 g.	F	1·683	+ 0·09253*	− 0·00019
		M	1·726	+ 0·11971*	− 0·00186
		M	1·790	+ 0·09387*	+ 0·00432
6.	Butter, 0·5 g., and casein, 0·4 g.	F	1·749	+ 0·07246*	+ 0·00019
		M	1·521	+ 0·07636*	− 0·00053
		M	1·385	+ 0·06473*	− 0·00106
7.	Butter, 0·5 g., and calcium lactate, 0·115 g.	F	1·279	+ 0·05099*	+ 0·00420*
		F	1·136	+ 0·03325*	+ 0·00724*
		M	1·484	+ 0·03376*	+ 0·00466*
8.	Butter, 0·5 g., casein, 0·4 g., and calcium lactate, 0·115 g.	F	1·582	+ 0·11014*	+ 0·00170
		F	1·846	+ 0·11622*	− 0·00114
		M	2·094	+ 0·12242*	− 0·00284

* Coefficients significantly different from zero.

Patologia Polska 16(1):53-5. 1965.

THE EFFECT OF CALCIUM ON THE OSMOTIC RESISTANCE OF WHITE BLOOD CELLS
(Wplyw Wapnia na Osmotyczna Opornosc Cialek Bialych)

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The osmotic resistance of white blood cells, studied by Storty et al. (4, 5), Brüsch (1) and other authors, in various diseased conditions, sometimes reveals significant fluctuations; e.g. in cases of leukemia or in inflamed conditions with accompanying leucocytosis there is generally an increase in the osmotic resistance of white blood cells, while in cases of leucopenia and pancytopenias, a drop in resistance has been found. The factors governing these fluctuations in the osmotic resistance of leucocytes could be differences in the properties of the cell membrane, differences in the biochemical structure, e.g. rich or impoverished "enzymatic equipment" or metabolic intensity, which often goes hand-in-hand with changes in the ionic composition (2).

As the studies of various authors show, an increased osmotic resistance always appears when the number of immature cells in the peripheral blood increases. Lusvarghi et al. (3) observed an increased osmotic resistance of the marrow cells compared with the leucocytes of the peripheral blood.

The question arises as to whether mature granulocytes indicate a reduced osmotic resistance due to "aging" and a reduction in their life functions (3), or whether they are cells, as can be supposed, that are best-equipped enzymatically, and are thus prepared for phagocytaric activity, whereby they would easily succumb to autolysis.

A predominance of calcium ions in relation to potassium ions, according to Kraus and Zondek, is equivalent to the excitation of the sympathetic system. The ratio of K to Ca also supposedly affects the morphology of the blood; that is, a predominance of calcium increases the leucocyte count and also intensifies their phagocytaric capacity (6). For this reason, we decided to determine the behavior of white blood cell osmotic resistance under the influence of calcium administration.

The experiments were performed on 10 white rats, weighing 105-190 g. We determined the osmotic resistance of the white blood cells by comparing their count after 13 minutes in 0.2% NaCl solution, with the count in Türk liquid. The blood for the tests was collected by pricking the tail vein, pouring the blood in turn into two mixers for white blood cells up to the 0.5 level; one of these was then filled up to 11 with Türk liquid, the other with hypotonic NaCl solution (0.2%). After precise mixing of the contents, the white blood cells were counted in a Levy chamber, using a 44 lens and 10 ocular.

After the first determinations, the rats were administered about 0.5 g calcium lactate for 24 hours every day through a stomach tube. The stomach tube was also installed in the control animals, but they received nothing through it. We studied

the osmotic resistance of the white blood cells of each animal in general twice a week.

We found the most intensive increase in resistance on the 24th day of calcium lactate administration, on the average 19.5%, which was statistically variable, as t = 3.8, P 0.01.

	A. W płynie Türka	B. W 0,2% NaCl	C. Opornych leukocytów
a. Oznaczenia wstępne	16 030	8 680	50,9%
Po Ca lact. b.	15 890	11 840	70,4%

Key:
A. in Türk liquid
B. in 0.2% Nacl
C. percentage of resistant leucocytes
a. initial determinations
b. after Ca lactate

In the control group (10 animals), after tube installation, without calcium administration, the differences in the mean percentages of resistant white corpuscles between the last determinations and the initial ones was 7.89 and was statistically invariable (t = 0.91).

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This evaluation is based on the fact that the high iron content and particulate nature of these wastes would cause almost quantative ppt. of the cadmium under the conditions necessary to ppt. the iron in solution. The technology transfer is much the same for this system as for the coke plant wastes with the same 3-5 year time period for on line operation. The economics for the carbon adsorption would be much the same as for the pre-treated coke plant wastes and for the filtration system the cost would be essentially nil since filtration would already be needed for the effluent guideline requirements.

If the industry is doing any metal plating particularly galvanizing the method of treatment would require cyanide destruction followed by the sulfide ppt of any remaining cadmium. The costs and on-line time requirements would be about the same except the on-line time could be cut to 3 yrs. Technology, however, does already exist on a full plant scale for this part of theprocess at several metal finishing plants (7).

Non-ferrous Metal Smelting and Refining

The cadmium containing wastewaters from this industry usually result from the primary efforts of recovering and refining of lead, zinc, or copper since basic production of cadmium is not normally done. For this reason the wastewaters are almost always made up of a mixture of lead, copper, zinc, and cadmium. Treatment technology to this time has been almost completely limited to high pH precipiation in a one step process. (8)(9).

The use of calcium hydroxide for ppt. of the cadmium in these wastewaters is the method of choice where sludge disposal problems are not the limiting factor. If sludge disposal is to be minimized the sulfide ppt should be substituted. As now practiced by the industry the lime ppt. without filtration or design to limit or minimize short circuiting seems to be able to achieve on effluent concentration of cadmium of about 40 μg/l(9). on the average with peaks to 110 μg/l (9). If the present effluents were to be filtered to remove the 20-40 mg/l of suspended solids they should have no trouble meeting a 40 μg/l cadmium concentration better than 25% of the time (7) (8).

Mlynarska-'65
Bib No. 1300

Patologia Polska 16(1):53-55. 1965.

MARIA MŁYNARSKA

WPŁYW WAPNIA NA OSMOTYCZNĄ OPORNOŚĆ CIAŁEK BIAŁYCH

Z Zakładu Patologii Ogólnej i Doświadczalnej w Krakowie
Kierownik: prof. dr med. B. Giędosz

Osmotyczna oporność ciałek białych badana przez Stortiego i wsp. [4, 5], ...schkego [1] i innych autorów w różnych stanach chorobowych wykazuje nieraz znaczne wahania, np. w białaczkach oraz w stanach zapalnych z towarzyszącą leukocytozą występuje przeważnie wzrost oporności osmotycznej ciałek białych, natomiast w leukopeniach i pancytopeniach stwierdzano spadek oporności. Czynnikami warunkującymi te wahania oporności osmotycznej ciałek białych mogłyby być różnice w właściwościach błony komórkowej, różnice w budowie biochemicznej, np. bogaty ...ubogi „garnitur enzymatyczny" lub nasilenie metabolizmu, które niejednokrotnie idzie w parze ze zmianami składu jonowego [2].

Jak wykazują badania różnych autorów, zwiększona oporność osmotyczna pojawia się zawsze wtedy, gdy we krwi obwodowej zwiększa się liczba komórek niedojrzałych. Lusvarghi i wsp. [3] obserwowali zwiększoną oporność osmotyczną komórek szpiku w porównaniu z ciałkami białymi krwi obwodowej.

Zachodzi pytanie, czy granulocyty dojrzałe wykazują zmniejszoną oporność osmotyczną z powodu „starzenia się" i obniżenia czynności życiowych [3], czy może są to komórki, jak można by przypuszczać, najlepiej wyposażone enzymatycznie, a więc przygotowane do czynności fagocytarnej, przy czym łatwo ulegałyby autolizie.

Przewaga jonów wapnia w stosunku do jonów potasu według Krausa Zondeka jest równoznaczna z pobudzeniem układu sympatycznego. Stosunek K do Ca ma wpływać także na morfologiczny obraz krwi, mianowicie przewaga wapnia ma zwiększać liczbę leukocytów, a także wzmagać zdolności fagocytarne [6]. Wobec tego postanowiliśmy stwierdzić, jak zmienia się oporność osmotyczna ciałek białych pod wpływem podania wapnia.

Doświadczenia przeprowadzono na 10 białych szczurach, wagi 105—...0 g. Oznaczaliśmy oporność osmotyczną ciałek białych przez porównanie ich liczby po 13 minutach przebywania w 0,2% roztworze NaCl

z liczbą obliczoną w płynie Türka. Krew do badań pobierano przez nakłucie żyły ogonowej, kolejno do dwóch mieszalników dla ciałek białych do znaku 0,5 i bezpośrednio uzupełniano do znaku 11 jeden płynem Türka, a drugi hipotonicznym roztworem NaCl (0,2%). Po dokładnym wymieszaniu zawartości mieszalnika liczono ciałka białe w komorze Levy'ego, przy użyciu obiektywu 44 i okularu 10.

Po przeprowadzeniu oznaczeń wstępnych podawano szczurom, dożołądkowo przy użyciu sondy, codziennie po około 0,5 g mleczanu wapnia przez 24 dni; kontrolnej grupie zwierząt w tym samym czasie zakładano tylko sondę. Oporność osmotyczną ciałek białych badaliśmy u każdego zwierzęcia przeważnie dwa razy w tygodniu.

Najintensywniejszy wzrost oporności wykazaliśmy w 24 dniu karmienia mleczanem wapnia, średnio o 19,5%, co było statystycznie znamienne, albowiem $t = 3,8$, $P < 0,01$.

	W płynie Türka	W 0,2% NaCl	Opornych leukocytów
Oznaczenia wstępne	16 030	8 680	50,9%
Po Ca lact.	15 890	11 840	70,4%

W grupie kontrolnej (10 zwierząt) po zakładaniu sondy, bez podawania wapnia, różnica średnich procentu opornych krwinek białych z ostatnich oznaczeń w porównaniu z wstępnymi wynosiła 7,89 i była statystycznie nieznamienna ($t = 0,91$).

М. Млынарска

ВЛИЯНИЕ КАЛЬЦИЯ НА ОСМОТИЧЕСКУЮ УСТОЙЧИВОСТЬ ЛЕЙКОЦИТОВ

Содержание

Исследована осмотическая устойчивость лейкоцитов у крыс, которым в желудок введен *Calcium lacticum* по 0,5 г в день в течение 24 дней. Самый большой рост устойчивости, в среднем на 19,5% статистически значимый обнаружен на 24 день кормления.

M. Młynarska

THE EFFECT OF CALCIUM ON THE OSMOTIC RESISTANCE OF WHITE BLOOD CELLS

Summary

Osmotic resistance of the white blood cells was studied in white rats to which *Calcium lacticum*, 0.5 g daily, was administered into the stomach during 24 days. The greatest increase in osmotic resistance, averaging 19.5% and statistically significant, was observed after 24 days of feeding calcium to the rats.

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Redakcja otrzymała: 31. XII. 1963 r.

Adres autora: Kraków, ul. Czysta 18.

Okada-'59
Bib No. 1382

Proc. Japan Acad. 35:42-46. 1959.

9. *Comparison of Tissue Deposition of Various Calcium Preparations Using Rabbit Dentin as an Indicator*

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(Comm. by T. FURUHATA, M.J.A., Jan. 12, 1959)

In the decalcified section of the dentin of rabbit there is a striated pattern around the pulp cavity, like annual rings in wood, that is stained by hematoxylin. It has been proved through time recording in hard tissues by lead acetate injection (Okada and Mimura)[1] that the layers stainable by hematoxylin are formed during the night, and those not stainable by this dye are laid down during the day.[2] It has also become almost certain from our numerous experiments that this periodic pattern is a phenomenon arising from the fact that calcium deposition in a living body varies according to the day and the night. Incisor dentin of a rabbit is being formed incessantly at the rate of ca. 25 μ per 24 hours and calcium metabolism in vivo is being recorded as a wave pattern in dentin.[3] If it were possible to compare this with a kymographion that is revolving at the rate of 25 μ per 24 hours, the method developed by the present authors for recording time by lead acetate injection would correspond to Jaquet's chronograph in recording the time correctly on dentin kymogram. It therefore becomes possible to judge the degree of calcium deposition in hard tissues and duration of such action by administration of calcium preparations and other drugs.

Materials and method. Adult male rabbits of about 2 kg in body weight were used after being housed and fed under definite conditions for 2 weeks. The periodic pattern obtained of incisor dentin is regular in a large number of cases if rabbits were kept under constant conditions, but in a small number of cases, the pattern is irregular, as in immature rabbit. The ratio of these two cases was: out of 35 cases of adult male rabbits, 31 cases (88.6%) showed regular periodic pattern and 4 cases (11.4%) showed irregular pattern. In adult female rabbits, the proportion of irregular pattern in dentin was greater than that in male rabbits. During gestation the periodic pattern in dentin tends to become light stained as the gestation period progresses, but after parturition the normal striated pattern becomes marked. At the border between these two periods, i.e. immediately after parturition, an abnormally dark stained layer, which may be termed parturition stripe, appears (Fig. 1). In young, immature rab-

but, the striated pattern of dentin is generally indistinct and there are many that show several thin stripes per day.

Fig. 1. Longitudinal section of rabbit incisor showing the change of incremental striations in dentin as the result of pregnancy and parturition (decalcified section, hematoxylin staining)
A: Period of pregnancy, B: Last period of pregnancy, C: Period of lactation, D: Parturition stripe

Calcium preparations used were calcium lactate, calcium gluconate, calcium chloride, precipitated calcium carbonate, and precipitated calcium phosphate. A single dose of 0.46 g/kg, calculated as calcium, was administered twice a day at 5 hour intervals, during the forenoon and afternoon, through a stomach tube, using 50 cc of distilled water. In order to eliminate individual variations and effect of previous administration, the preparations were administered at 4-5 day intervals to the same rabbit in optional order. For recording time on dentin, 1-2 mg/kg of lead acetate was injected into a auricular vein at the time of administration. After an experimental period of about 4 weeks, lower incisor was fixed in formaldehyde solution, hydrogen sulfide was passed through a solution of 0.2 N hydrochloric acid containing the incisor to effect decalcification, and the incisor was embedded in gelatine. The frozen section was gilt with 0.05-0.1% auric chloride solution, treated with [illegible] and stained with hematoxylin (the Boehmer [illegible]). After dehydration, the specimens were [illegible] under a microscope. The striated layers observed by hematoxylin staining after administration of calcium [illegible] were represented by [illegible], etc. according to the order of [illegible]. The width of these stained layers was measured [illegible]. Efficiency of calcium deposition from test preparation was taken as the value of this layer width divided by the width of dentin formed during 24 hours, which can be calculated from the distance between successive lead deposition lines.

Results. Efficiency of calcium deposition in rabbit dentin after oral administration of various calcium preparations is presented in the table and in Figs. 2 and 3. The most efficient deposition was from

Efficiency of tissue deposition of calcium into rabbit dentin after oral administration of various calcium preparations

Calcium preparation	No. of cases	Single dose (corresp. to 0.36 g. kg. Ca)	Efficiency of tissue deposition	
			x	m ($\alpha = 0.05$)
Lactate	5	2.51	0.740	$0.896 > m > 0.584$
Gluconate	11	4.91	0.521	$0.655 > m > 0.387$
Chloride	9	1.61	0.369	$0.447 > m > 0.291$
Carbonate (precipitd.)	8	1.14	0.433	$0.497 > m > 0.349$
Phosphate (precipitd.)	13	2.00	0.314	$0.394 > m > 0.234$

$m = x \pm \frac{Tu\sigma}{\sqrt{N}}$, m: Population mean, x: Sample mean, $\sigma = \sqrt{\frac{N}{N-1}}\,S$,

S: Standard deviation of samples

Fig. 2. Dentin of rabbit incisor (decalcified, stained with hematoxylin) showing the hypercalcified zone (deeply stained zone) laid down after the oral administration of the following calcium preparations (0.36 g. kg. Ca)

1: Calcium phosphate ($CaHPO_4$)
2: Calcium lactate
b: Time recording lead line

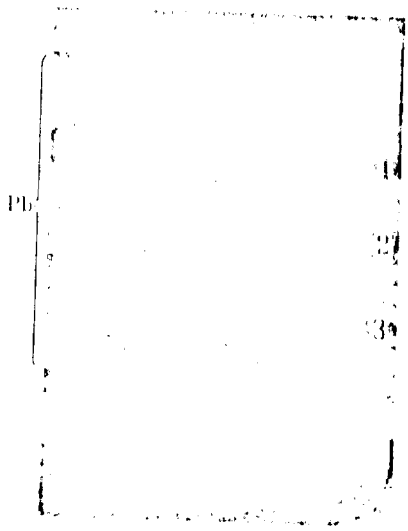

Fig. 3. Dentin of rabbit incisor (decalcified, stained with hematoxylin) showing the hypercalcified zone (deeply stained zone) laid down after the oral administration of the following calcium preparations (0.36 g. kg. Ca)

1: Calcium lactate
2: Calcium carbonate
3: Calcium phosphate ($CaHPO_4$)
Pb: Time recording lead line

calcium lactate and this was significant at the 5 per cent level of probability. This was followed by calcium gluconate, which seemed to have a significant difference from the following chloride and precipitated phosphate, but there was no significant difference among

calcium chloride, precipitated carbonate, and precipitated phosphate.

This efficiency indicates the duration of calcium deposition as represented by the width of a layer deeply stained by hematoxylin. The degree of calcium deposition, as represented by the depth of stain, was greater in water-soluble salts like the lactate, gluconate, and chloride, than the sparingly soluble salts like calcium carbonate and phosphate. In the case of calcium chloride, however, there appeared a marked non-stained layer following a deeply stained layer immediately after its administration. This tendency was observed to a lesser extent in all calcium preparations after their administration but not to such a great extent as in calcium chloride.

Discussion. Various calcium preparations are being used to promote calcium deposition in hard tissues or morbid tissues but the method of judging efficiency of deposition by increased serum calcium level and duration of this level is not the indication of the efficiency of calcium deposition. The method for measuring the potency of vitamin D (line test, X-ray method, bone-ash method) uses rachitic rat, and this can not be the measure of efficiency of calcium deposition in tissues under normal condition. This method is also extremely complicated compared to the dentin method which can judge the efficiency by a single administration. Recently, tracing of tissue deposition through radioactive ^{45}Ca has been used widely. This method is advantageous in some points but tissue cells are liable to be damaged by the radioactivity of such isotopes and presents some doubt on this kind of study. The authenticity of hematoxylin staining, which is taken as the indication of the degree of calcium deposition in the dentin method is discussed in the other report[1] and the matter will not be taken up here. The fact that the calcium deposition in dentin is the function of blood level of calcium (especially of calcium ion) and of inorganic phosphorus,[2] and that dentin is a far more sensitive indicator of calcium deposition than the bone,[3] has been discussed in a separate paper. Schour and others[4] examined the relationship between the hematoxylin-stained layer appearing in dentin after administration of vitamin D and parathyroid hormone in rats, and the level of serum calcium. He stated that the hematoxylin-stained layer in dentin could be taken as indicator of calcification. But rat dentin is less sensitive to hematoxylin staining than rabbit dentin, and furthermore, Schour did not make any time recording in hard tissues, as in the present case, and the time correspondence is not quite clear.

Summary. (1) Various calcium preparations, with a definite calcium content, were administered to rabbits with an interval of 4-5 days and the time of administration was made known by the time-recording in hard tissues by lead acetate injection (Okada-Mimura).

After the end of experimental period, decalcified and hematoxylin-stained section of the incisor dentin was prepared to measure the width of layers deeply stained by hematoxylin. The value obtained by this measurement divided by the width of dentin laid down during the 24 hours, calculated from the distance of time-recording lines formed by lead deposition, was taken as the efficiency of tissue deposition of the calcium preparation being tested. The feature of this newly devised method is that the efficiency of calcium preparations can be judged accurately and sensitively by a single administration.

(2) Oral administration of various calcium preparations in rabbits showed that the efficiency of tissue deposition was the highest in calcium lactate, followed by calcium gluconate. There was no significant difference in efficiency of administration among calcium chloride, precipitated carbonate, and precipitated phosphate.

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Am. J. Cancer 20:117-136, 1934.

Parfentjev-'34
Bib No. 1419

THE INFLUENCE OF VARIOUS PREPARATIONS OF LACTIC ACID AND SUGARS ON THE GROWTH OF TRANSPLANTED TUMORS

II. Mouse Sarcoma 180[1]

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In a previous paper (1) we discussed the effect of injections of sodium lactate and ethyl lactate on rat sarcoma 39, a tumor which tends to retrogress spontaneously in a certain percentage of cases. In this paper we report on experiments with mouse sarcoma 180, a very stable tumor with only a slight tendency toward retrogression. The effect was tested of injections not only of various preparations of lactic acid but also of different sugars. The following is a list of the preparations examined and of the number of mice which were used in the various experiments.

Sodium lactate	47	mice
Ethyl lactate alone and with other substances	143	"
Lactyl lactate acid alone and with other substances	75	"
Glycero-monolactate	95	"
Lactic acid administered orally	41	"
Underfed mice	14	"
Arabinose	114	"
Glucose	94	"
Total number of experimental mice	623	
Total number of control mice	369	
	992	

The experimental procedure in this work was the same as that applied in the experiments with rat sarcoma 39. In all cases except those in which lactic acid was given orally the lactic acid preparations and sugars were injected subcutaneously at a distance from the tumor.

In these experiments with sarcoma 180, the number of control mice, in which a piece of sarcoma was inoculated but to which lactic acid or sugar preparations were not administered, was rather large, 423.

[1] These investigations were carried out with the aid of a grant made to Dr. Leo Loeb by the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

We wish to express our gratitude to Dr. Loeb for giving us the opportunity to carry out this investigation, as well as for his valuable suggestions.

Experimental Studies

A. Experiments with Sodium Lactate

In these experiments we used altogether 155 mice; 47 being injected with lactate, and 108 serving as controls. The injected mice received daily subcutaneous injections of 0.25 c.c. of a solution of sodium lactate which contained 10 per cent of pure lactic acid. As to the method of preparation of sodium lactate, we refer to our previous paper (1).

The growth of tumors of the control mice and of the injected mice is shown in Table 1.

Table 1: *Summary of Data on Effects of Sodium Lactate on Sarcoma 180*
(Size of tumors in square centimeters)

	At beginning of experiment	First week	Second week	Third week
	Experimental Animals			
Total size of tumors in 47 animals	26.14	66.75	121.66	160.08
Average single tumors	0.56	1.42	3.20	5.00
Percentage of growth	100	253	571	895
	Control Animals			
Total size of tumors in 108 animals	66.98	181.96	280.81	299.00
Average single tumor	0.62	1.68	2.99	4.90
Percentage of growth	100	271	482	790

The injection of sodium lactate exerted apparently a very slight inhibiting effect on the growth of sarcoma 180 during the first week; during the second and third weeks no inhibition was noticeable.

Table 2: *Inhibition or Acceleration of Tumor Growth by Sodium Lactate*

	First week	Second week	Third week
Sodium lactate	−15%	+7%	+2%

B. Experiments with Ethyl Lactate

Ethyl Lactate Alone: In these experiments we used 222 white mice, 79 of which served as controls. Forty-seven of the experimental animals received injections of ethyl lactate alone, 84 were given injections of ethyl lactate combined with digitonin, and the remaining 12 received, in addition to ethyl lactate and digitonin, arabinose. As in all our previous experiments, the injections were made subcutaneously at a certain distance from the tumor.

For the sake of convenience we have in the following tables

combined the results of several smaller experiments. We followed in these series the fate of the tumors for only three weeks after the beginning of the injections. This limitation was due to the fact that the investigations were carried out during the summer and that the summer heat affected the health of the mice unfavorably.

The 79 control mice were kept under the same conditions as the experimental animals. As in our previous experiments, we started to measure the tumors of the control mice only after they had reached a size of 0.4-0.5 sq. cm. Only in one of these 79 control animals did the tumor disappear completely.

Forty-seven mice received ethyl lactate, 0.25 c.c. of a 5 per cent aqueous solution of ethyl lactate being injected subcutaneously once a day during three weeks. The injections started after the transplanted tumors had reached a size of 0.4 sq. cm. Each mouse received daily, therefore, 0.0125 gm. of ethyl lactate. Taking the average weight of the animals as about 20 gm., the dose given daily, calculated per kilo of body weight, was 0.625 gm. Such a quantity of ethyl lactate was readily tolerated, whereas larger doses caused a high rate of mortality.

Table 3 shows the summarized results of the experiments with ethyl lactate.

TABLE 3: *Summary of Data on Effects of Ethyl Lactate*

(Size of tumors in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 47 animals	18.27	50.39	102.15	176.64
Average single tumor	0.39	1.07	2.17	3.92
Percentage of growth	100.00	274.00	556.00	1005.00
	Control Animals			
Total size of tumors in 53 animals	21.25	73.03	141.81	236.62
Average single tumor	0.40	1.37	2.95	5.14
Percentage of growth	100.00	342.00	737.00	1285.00

Combination of Ethyl Lactate and Digitonin: These experiments were conducted exactly in the same way as the previous ones, with the only difference that in addition to ethyl lactate the animals received digitonin. Digitonin was added to a 5 per cent aqueous solution of ethyl lactate in the concentration of 1:50,000. Each mouse was given daily 0.005 mg. of digitonin, which was included in the dose of 0.25 c.c. of ethyl lactate. Calculated per

kilo of body weight, the amount of digitonin received daily by each mouse was 0.25 mg. The summarized results of these experiments are given in Table 4.

TABLE 4: *Summary of Data on Effects Obtained with a Combination of Ethyl Lactate and Digitonin*

(Size of tumors in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 84 animals	35.65	84.43	153.63	180.24
Average single tumor	0.42	1.00	2.04	3.33
Percentage of growth	100.00	238.00	485.00	793.00
	Control Animals			
Total size of tumors in 38 animals	15.36	51.23	99.04	149.08
Average single tumor	0.40	1.35	2.83	4.81
Percentage of growth	100.00	338.00	708.00	1203.00

Combination of Ethyl Lactate, Digitonin, and Grabinose: The method used in these experiments was the same as in the two previous experiments, except that in addition to ethyl lactate and digitonin the mice received also arabinose. Each animal was given an injection of 0.25 c.c. of a 5 per cent aqueous solution of ethyl lactate with 0.005 mg. of digitonin and 0.5 mg. of arabinose. The amount of arabinose calculated per kilo of the body weight of the animal was equal to 0.025 gm. The amounts of ethyl lactate and digitonin were the same as before. For summarized data see Table 5.

TABLE 5: *Summary of Data on Effects Obtained with a Combination of Ethyl Lactate, Digitonin, and Grabinose*

(Size of tumors in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 12 animals	6.88	15.22	24.19	32.08
Average single tumor	0.57	1.27	2.19	3.56
Percentage of growth	100.00	223.00	384.00	624.00
	Control Animals			
Total size of tumors in 15 animals	8.22	25.13	48.29	52.75
Average single tumor	0.58	1.43	3.47	5.54
Percentage of growth	100.00	246.00	600.00	955.00

Summary: Summarizing these experiments, we may state that injections of ethyl lactate alone and in combination with digitonin and arabinose have a slight inhibiting influence on the growth of sarcoma 180. If the growth of the tumors in the control animals in each of the three experiments is represented as 100, the growth of the tumors in the treated animals shows the percentage diminutions presented in Table 6.

TABLE 6. *Effect of Ethyl Lactate Alone and in Combination with Digitonin and Arabinose*

Name of compound	Inhibition of growth of tumors, in per cent		
	First week	Second week	Third week
Ethyl lactate	22%	27%	24%
Ethyl lactate and digitonin	26%	28%	31%
Ethyl lactate, digitonin, and arabinose	11%	37%	36%

In our experiments we used mice of an average body weight of 20 gm. Below are shown the changes in the weight of the mice during the course of the experiments, calculated per 20 gm. of initial weight.

Experiment with Tumor Mice

No. of experiment	Injection	Increase of body weight		
		First week	Second week	Third week
No. 1	Ethyl lactate	0.72 gm.	1.1 gm.	4.7 gm.
No. 1	Controls	1.50 gm.	2.5 gm.	6.4 gm.
No. 2	Ethyl lactate and digitonin	0	0	
No. 2	Controls	1.7 gm.	2.0 gm.	

We also injected normal mice with ethyl lactate; the results were as follows:

Experiments with Normal Mice

Injection	Increase of body weight		
	First week	Second week	Third week
Ethyl lactate	0.6 gm.	1.1 gm.	1.6 gm.
Controls	0.8 gm.	2.3 gm.	2.6 gm.

It appears, therefore, that injections of ethyl lactate cause a distinct loss of weight in mice, and we have to consider the possibility that this factor contributed to the diminished growth of the tumors, although it probably does not account entirely for this diminution.

C. Experiments with Lactyl-Lactic Acid

The lactyl-lactic acid used in these experiments was prepared by us in the following way. Market lactic acid c.p. was heated on a sand bath at 10–25 cm. pressure and at a temperature of 150–160° C. until a viscous, glassy, yellowish, waterless material representing lactyl-lactic acid resulted. One part of lactyl-lactic acid was dissolved in two parts absolute alcohol, and olive oil was added until the liquid contained 5 per cent lactyl-lactic acid. This mixture, which represents a fine and comparatively stable emulsion, was used for injection into mice. When lactyl-lactic acid was used in combination with other substances, the latter were first mixed with the alcoholic solution of lactyl-lactic acid and then olive oil was added.

In this series of experiments we used as controls 55 mice; injected with lactyl-lactic acid alone, 50 mice; injected with a mixture of lactyl-lactic acid, digitonin, and arabinose, 25 mice. In another experiment, lactyl-lactic acid was injected per rectum, 24 mice being used for this purpose. Altogether 154 mice were used. As in our previous experiments the substances were injected subcutaneously at a distance from the tumor, with the exception of the one group of animals which received injections of lactyl-lactic acid per rectum. Usually, the injections were begun six or seven days after tumor inoculation.

Three-tenths of a cubic centimeter of a 5 per cent solution of lactyl-lactic acid alone was injected daily per mouse, i.e. each mouse received 15 mg. of lactyl-lactic acid, corresponding to 0.75 gm. per kilo weight. The injections were continued for three weeks, altogether 20 injections being given. The action of lactyl-lactic acid on the growth of sarcoma 180 is shown in the following table.

TABLE 7: *Summary of Data on Effects of Lactyl-Lactic Acid Alone*
(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 50 animals	18.17	45.48	88.89	118.35
Average single tumor	0.36	0.91	1.85	2.88
Percentage of growth	100.00	252.00	514.00	800.00
	Control Animals			
Total size of tumors in 40 animals	15.16	47.88	87.76	115.86
Average single tumor	0.38	1.20	2.37	3.51
Percentage of growth	100.00	316.00	621.00	924.00

The average weight of the mice at the beginning of this experiment was 20.4 gm. The animals treated with lactyl-lactic acid gained in weight in the first week 0.7 gm. and lost in the second week 1.4 gm. In the same time the control animals gained on an average in the first week 1.5 gm., and in the second week 1.2 gm.

The experiments with injections of lactyl-lactic acid together with digitonin and arabinose were done according to the same plan as that followed in the previous series. Each mouse received daily 15 mg. of lactyl-lactic acid; 0.005 mg. of digitonin, corresponding to 0.25 mg. per kilo weight; 0.5 mg. arabinose, corresponding to 0.25 gm. per kilo weight. The results of these experiments are shown in Table 8.

TABLE 8: *Summary of Effects of Lactyl-Lactic Acid, Digitonin, and Arabinose*
(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
		Experimental Animals		
Total size of tumors in 25 animals	8.00	20.21	26.78	40.26
Average single tumor	0.32	0.81	1.27	2.37
Percentage of growth	100.00	253.00	397.00	741.00
		Control Animals		
Total size of tumors in 29 animals	9.33	29.50	60.89	98.53
Average single tumor	0.32	1.02	2.10	3.71
Percentage of growth	100.00	319.00	656.00	1159.00

TABLE 9: *Summary of Data on Effect of Lactyl-Lactic Acid Administered by Rectum*
(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
		Experimental Animals		
Total size of tumors in 25 animals	8.36	26.69	37.18	48.34
Average single tumor	.35	1.11	1.96	3.22
Percentage of growth	100.00	317.00	560.00	920.00
		Control Animals		
Total size of tumors of 29 animals	9.33	29.50	60.89	98.53
Average single tumor	.32	1.02	2.10	3.71
Percentage of growth	100.00	319.00	646.00	1159.00

The average weight of the mice at the beginning of this experiment was 18 gm. After three weeks of treatment with lactyl-lactic acid, arabinose, and digitonin the animals gained 4.8 gm. on the

average. The average gain in the same time for the control animals was 4.3 gm.

A special series of experiments was carried out in which injections of lactyl-lactic acid were made by rectum, by means of a glass capillary. Each mouse received 0.5 c.c. of a 10 per cent emulsion of lactyl-lactic acid, corresponding to 2.5 gm. per kilo weight. This emulsion was prepared as described above, but was diluted with olive oil to 10 per cent. The results of this experiment are shown in Table 9.

Summary: Summarizing these experiments, we may state that injections of lactyl-lactic acid alone, as well as in combination with digitonin and arabinose, have a moderately inhibiting influence on the growth of sarcoma 180. As in the series with ethyl lactate, the addition of arabinose increases the inhibitory effect of lactyl-lactic acid on the growth of transplanted sarcoma 180. The diminution in the growth of tumors in the treated animals is expressed in percentages in Table 10, the tumor growth of the control mice being represented as 100.

TABLE 10: *Percentage Diminution of Tumor Growth Following Injection of Lactyl-Lactic Acid*

	First week	Second week	Third week
Lactyl-lactic acid	24%	22%	18%
Lactyl-lactic acid, digitonin, arabinose	21%	40%	37%
Lactyl-lactic acid per rectum	0%	7%	13%

As to the weight of the animals during the experiments described above, it seems that injections of lactyl-lactic acid can inhibit the weight increase of young mice. The combined injections of lactyl-lactic acid with digitonin and arabinose, on the other hand, did not have an inhibiting effect on the weight. Arabinose seems, therefore, to deprive lactyl-lactic acid of its inhibiting action on the weight increase of mice.

D. Experiments with Glycero-Monolactate

Glycero-monolactate used in these experiments has been prepared by us in the following way: 92 gm. of glycerin were mixed with 90 gm. of 85 per cent market lactic acid. The mixture was heated on the boiling bath at 25 cm. vacuum during one day. On the following day the flask with the mixture was placed on the sand bath, where the temperature rose to 150–170° C.; the vacuum also was increased up to 10–12 cm. Heating in this condition was continued during several hours, until the mixture no longer gave up any trace of water. Prepared in this way, glycero-lactate can be injected subcutaneously and does not cause any necrosis.

In this series we used 60 mice as controls; 67 mice were injected with glycero-monolactate. In a special group of 47 mice, the injections were started before the tumor was transplanted. Each mouse was injected subcutaneously with 0.15 c.c. of pure glycero-monolactate. The injections were repeated almost daily for three weeks; on the whole, 20 injections were given.

The action of glycero-monolactate on the growth of sarcoma 180 is shown in the summary in Table 11.

TABLE 11: *Summary of Data on Effects of Glycero-Monolactate*
(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 67 animals	22.60	67.69	120.90	185.56
Average single tumor	.34	1.01	2.05	3.78
Percentage of growth	100.00	297.00	603.00	1111.00
	Control Animals			
Total size of tumors in 60 animals	24.45	88.23	162.82	228.37
Average single tumor	.41	1.47	3.05	4.57
Percentage of growth	100.00	359.00	741.00	1115.00

The following table shows the changes in the weights of the animals during the experiment with glycero-monolactate. In order to facilitate comparison of the different groups, the changes in weight have been calculated on the basis of a unit weight of 20 gm.

TABLE 12: *Average Increase in Weight of Controls and Animals Receiving Glycero-Monolactate*

	First week	Second week	Third week
Control animals	1.12 gm.	2.1 gm.	3.3 gm.
Experimental animals	3.2 gm.	5.6 gm.	5.4 gm.

In the special series of experiments in which preliminary injections were given, 47 mice were used: of these, 19 were controls. The remaining 28 animals received daily 0.1 c.c. of glycero-monolactate. The injections were started three weeks before the tumor was transplanted. After the tumor had been transplanted, the injections were stopped for two days; they were then begun again and continued for three weeks.

The effect of glycero-monolactate in this series of experiments is shown in the following table.

TABLE 13: *Summary of the Effects of Preliminary Injections of Glycero-monolactate*
(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
		Experimental Animals		
Total size of tumors in 28 animals	11.70	46.27	86.81	117.24
Average single tumor	.42	1.63	3.47	6.17
Percentage of growth	100.00	393.00	826.00	1470.00
		Control animals		
Total size of tumors in 19 animals	8.39	37.30	72.37	95.60
Average single tumor	.42	1.81	4.51	6.83
Percentage of growth	100.00	431.00	1070.00	1630.00

The average weight of the animals during this experiment is shown below.

TABLE 14: *Average Increase in Weight of Controls and Animals Receiving Preliminary Injections of Glycero-Monolactate*

	First week	Second week	Third week
Control animals	1.0 gm.	3.0 gm.	2.7 gm.
Experimental animals	2.8 gm.	5.6 gm.	4.1 gm.

Summarizing these experiments, we may state that the injections of glycero-monolactate have a slight inhibiting influence on the growth of sarcoma 180, but that injections of this substance made previous to the transplantation of the tumor are without effect on the growth of the latter.

It is interesting to note that the animals receiving these injections gained more in weight than the control animals.

In the following table the diminution in growth of the tumor in the treated animals is expressed in percentages, the tumor growth of the control mice being represented by 100 per cent.

TABLE 15: *Percentage Diminution of Tumor Growth Following Injections of Glycero-Monolactate*

	First week	Second week	Third week
Glycero-monolactate	31%	33%	17%
Glycero-monolactate (preliminary injection)	10%	23%	10%

E. Introduction of Lactic Acid by Mouth

In this experiment 1 c.c. of 1 per cent lactic acid solution was introduced daily per mouth into each mouse. For this purpose we used a fine graduate glass capillary covered with a rubber cap.

The capillary was pushed into the esophagus, and the lactic acid was blown up by exerting pressure on the rubber. Before use, the end of the capillary was made smooth by melting the edge in a low flame.

To check the results of administration of lactic acid, we kept a special group of underfed control mice, which received food only every other day, but always were supplied with water; they received no preparations of lactic acid.

We used in these experiments 34 mice as controls, 41 mice receiving 1 per cent lactic acid by mouth, 14 mice which were underfed.

The action of lactic acid on the growth of the tumor is shown in the following tables.

TABLE 16: *Summary of Data on Effects of 1 per cent Lactic Acid by Mouth*
(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 41 animals	14.03	48.88	105.27	117.01
Average single tumor	.34	1.19	2.77	3.90
Percentage of growth	100.00	350.00	815.00	1147.00
	Control Animals			
Total size of tumors in 34 animals	11.45	49.18	104.63	156.92
Average single tumor	.34	1.45	3.17	5.06
Percentage of growth	100.00	426.00	932.00	1490.00

TABLE 17: *Summary of Data on Effect of Underfeeding on Growth of Sarcoma 180*
(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 14 animals	3.88	10.99	22.86	36.95
Average single tumor	.28	.79	1.76	3.36
Percentage of growth	100.00	282.00	629.00	1200.00
	Control Animals			
Total size of tumors in 14 animals	4.12	16.80	45.59	63.06
Average single tumor	.36	1.20	3.26	5.26
Percentage of growth	100.00	400.00	1087.00	1753.00

Summarizing these experiments, we may state that the injections of 1 per cent lactic acid by mouth have some inhibiting influence on the growth of sarcoma 180.

The animals which received lactic acid per mouth gained less weight than the control animals. The animals of the underfed group showed a still greater loss in weight, and the retardation in tumor growth was somewhat more marked than in those animals which received lactic acid. The following table shows the change in weight of all three groups of animals.

TABLE 18: *Average Change in Weight of Animals Receiving Lactic Acid, Underfed Animals, and Controls*

	First week	Second week	Third week
Experiment with lactic acid	0	+ 1.1 gm.	+ 1.5 gm.
Control animals	+ 1.2 gm.	+ 4.7 gm.	+ 3.5 gm.
Underfed animals	— 2.7 gm.	— .9 gm.	— .7 gm.
Control animals	— .6 gm.		+ 2.7 gm.

Table 19 shows the percentage of diminution in growth of sarcoma 180 in mice which were fed with lactic acid and those which were underfed. The tumor growth of the control mice is represented as 100.

TABLE 19: *Percentage Diminution of Tumor Growth in Animals Receiving Lactic Acid and Underfed Animals*

	First week	Second week	Third week
Lactic acid	18%	13%	23%
Underfed group	34%	46%	36%

It is seen that the retardation in growth is more marked in the underfed mice than in those which received lactic acid by mouth.

TABLE 20: *Summary of Data on Effects of Glucose (Daily Injections of 0.5 c.c. of a 10 per cent Solution, each Mouse Receiving Daily 50 mg. of Glucose or 2.50 gm. per Kilo Body Weight)*

(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
		Experimental Animals		
Total size of tumors in 48 animals	17.40	60.10	157.26	215.39
Average single tumor	.36	1.25	3.35	5.40
Percentage of growth	100.00	347.00	931.00	1500.00
		Control Animals		
Total size of tumors in 38 animals	13.89	55.30	126.65	203.38
Average single tumor	.36	1.46	3.62	6.32
Percentage of growth	100.00	406.00	1006.00	1756.00

Conclusion: The injections caused perhaps a very slight inhibition of tumor growth.

F. Injections of Arabinose and Glucose

The effect of glucose, d arabinose, and l arabinose on mouse sarcoma was also studied. Altogether 295 mice were used. Of these, 87 served as controls; 114 were injected with arabinose, and 94 with glucose. The sugar was dissolved in water and injected subcutaneously daily for three consecutive weeks.

The action of sugars on the growth of sarcoma 180 is seen in Tables 20–24.

TABLE 21: *Summary of Data on Effects of Glucose (Daily Injections of 0.5 c.c. of a 20 per cent Solution, Each Mouse Receiving Daily 100 mg. of Glucose or 5 gm. Per Kilo Body Weight)*

(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 46 animals	13.22	56.10	122.86	203.62
Average single tumor	.29	1.22	2.67	4.85
Percentage of growth	100.00	421.00	921.00	1672.00
	Control Animals			
Total size of tumors in 49 animals	15.02	63.66	132.00	294.54
Average single tumor	.31	1.30	2.81	4.44
Percentage of growth	100.00	420.00	906.00	1432.00

Conclusion: No definite effect of the injections is noticeable.

TABLE 22: *Summary of Data on Effects of d Arabinose (Daily Injections of 0.25 c.c. of 4 per cent Solution of d Arabinose, Each Mouse Receiving Daily 10 mg. of d Arabinose or 0.5 gm. per Kilo Body Weight)*

(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
	Experimental Animals			
Total size of tumors in 28 animals	10.16	36.73	79.29	132.95
Average single tumor	.36	1.31	2.83	4.22
Percentage of growth	100.00	364.00	786.00	1367.00
	Control Animals			
Total size of tumors in 20 animals	7.33	32.38	59.04	98.36
Average single tumor	.37	1.62	3.10	4.88
Percentage of growth	100.00	438.00	837.00	1313.00

Conclusion: The injections caused perhaps a very slight inhibition of tumor growth.

TABLE 23: *Summary of Data on Effects of d Arabinose (Daily Injections of 0.5 c.c. of a 10 per cent Solution of d Arabinose, Each Mouse Receiving 50 mg. of d Arabinose Daily or 2.5 gm. per Kilo Body Weight)*

(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
		Experimental Animals		
Total size of tumors in 52 animals	19.27	61.25	144.55	219.86
Average single tumor	.37	1.18	2.95	5.11
Percentage of growth	100.00	319.00	797.00	1381.00
		Control Animals		
Total size of tumors in 38 animals	13.89	55.30	126.65	203.38
Average single tumor	.37	1.46	3.62	6.16
Percentage of growth	100.00	395.00	978.00	1665.00

Conclusion: There is a very slight inhibition of tumor growth.

TABLE 24: *Summary of Data on Effects of 20 per cent l Arabinose (Daily Injections of 0.5 c.c. of 20 per cent Solution l Arabinose, Each Mouse Receiving 100 mg. of l Arabinose or 5 gm. per Kilo Body Weight)*

(Size of tumor in square centimeters)

	At beginning of experiment	At end of first week	At end of second week	At end of third week
		Experimental Animals		
Total size of tumors in 34 animals	10.16	33.68	84.32	139.02
Average single tumor	.29	.99	2.48	4.63
Percentage of growth	100.00	341.00	855.00	1600.00
		Control Animals		
Total size of tumors in 29 animals	7.69	31.28	72.96	110.68
Average single tumor	.27	1.11	2.52	4.26
Percentage of growth	100.00	411.00	933.00	1578.00

Conclusion: The injections caused no inhibition of tumor growth.

The change in the weights of the animals during the experiments with sugars is shown in the Tables 25–28. In order to facilitate the comparison of the different groups of animals the calculations have been reduced to a mouse weighing 20 gm. as a standard unit.

Summary: Summarizing these experiments, we may state that the injection of sugars exerted either no effect or a very slight inhibiting effect on the growth of sarcoma 180. However in two cases, namely following injection of 20 per cent solutions of glucose and of 20 per cent solution of arabinose for a period of three weeks we observed, instead of a diminution, a slight increase in tumor growth.

TABLE 25: *Average Change in Weight with 4 per cent d Arabinose*

	First week	Second week	Third week
Experiment with 4% d arabinose	2.2 gm.	4.5 gm.	4.3 gm.
Control animals	3.1 gm.	4.7 gm.	4.4 gm.

Conclusion: There was no definite change in weight in these experiments.

TABLE 26: *Average Change in Weight with 10 per cent Glucose and 10 per cent d Arabinose*

	First week	Second week	Third week
Experiment with 10% d arabinose	1.4 gm.	2.9 gm.	4.5 gm.
Experiment with 10% glucose	1.5 gm.	3.0 gm.	6.1 gm.
Control animals	1.2 gm.	2.0 gm.	6.1 gm.

Conclusion: There is no marked change in weight in these experiments.

TABLE 27: *Average Change in Weight with 20 per cent l Arabinose*

	First week	Second week	Third week
Experiment with 20% l arabinose	2.1 gm.	4.2 gm.	6.1 gm.
Control animals	1.8 gm.	3.7 gm.	3.5 gm.

Conclusion: There is apparently a gain in weight in the mice injected with 20 per cent arabinose.

TABLE 28: *Average Change in Weight with 20 per cent Glucose*

	First week	Second week	Third week
Experiment with 20% glucose	1.9 gm.	2.7 gm.	3.6 gm.
Control animals	2.4 gm.	4.2 gm.	3.9 gm.

Conclusion: There is apparently a slight loss in weight in mice injected with 20 per cent glucose.

The following table shows the diminution in percentage of tumor growth of the experimental animals, as compared with the tumor growth of control animals.

TABLE 29: *Summary of Experiments with Sugars*

Name of compound	First week	Second week	Third week
10% d arabinose	— 19%	— 18%	— 17%
10% glucose	— 14%	— 7%	— 15%
4% d arabinose	— 13%	— 9%	— 0%
20% l arabinose	— 11%	— 6%	+ 8%
20% glucose	— 6%	— 5%	+ 9%

Under the influence of sugar injections the body weight of the animals remained the same as in the controls; but injections of 20 per cent arabinose caused a greater gain in weight than in the controls, and injections of 20 per cent glucose caused a loss in weight. How far we have in these cases to deal with causal relations and with chance happenings would need further investigation.

Discussion

Normal Growth of Sarcoma 180: For controls we used 369 mice into which the tumor was transplanted but which did not receive any special treatment. Sarcoma 180 took and continued to grow in about 95 to 98 per cent of the mice. In the 369 control mice, the tumors failed to continue to grow in only 13 animals, after the transplants had reached the size of 0.25 sq. cm. In 11 of these mice the tumors had reached the size of 0.20–0.30 sq. cm. and in 2 others they had reached the size of 0.4–0.5 sq. cm. In all of these cases the tumors gradually disappeared. Only in one additional mouse did the tumor grow for some time, reaching the size of about 1 sq. cm., and then retrogress. Hence, in only one among 369 control mice did we observe a definite retrogression of sarcoma 180; in the other 12 mice it is probable that the inoculated piece was injured at the time of inoculation. Altogether, we observed a spontaneous retrogression of sarcoma 180 in less than 0.5 per cent of the inoculated mice.

This almost complete lack of spontaneous retrogression and the high percentage of growing sarcoma among the inoculated mice show the stability of this type of tumor. Furthermore, periodic measurements of the size of the tumors of the control mice prove the great rapidity in the growth of sarcoma 180 (see Fig. 1).

We have compared above, in each series of experiments, the growth of the tumor in special groups of control mice and in mice treated with certain lactic acid preparations. In the chart we compare the growth of the tumors in *all* the control mice with that of the tumors in the different series of animals receiving certain preparations of lactic acid. We exclude from this summary, however, the mice which were used as controls in experiments with sodium lactate, since in that experiment the treatment was begun later than usual, namely at a time when the tumors had reached the size of 0.5 to 0.6 sq. cm., while usually the experiment was started when the tumor measured only 0.3 to 0.4 sq. cm. As our observations indicate, the size of the tumors at the beginning of the experiment is of great importance as far as the effectiveness of the injections is concerned.

If we compare the growth of sarcoma 180 in the controls and in animals injected with the sodium lactate, we find that the sodium lactate had perhaps a very slight inhibiting influence on the tumor growth, but only during the first week. During the second and third week the injections had no influence. On the whole, therefore, injection of sodium lactate is ineffective as far as any inhibiting action on sarcoma 180 is concerned.

Ethyl lactate injections have a somewhat more marked effect on tumor growth. They caused a diminution in the rate of tumor

growth of about 20 per cent. Injections of a mixture of ethyl lactate and digitonin were slightly more effective than those of ethyl lactate alone.

Injections of lactyl-lactic acid alone and in combination with digitonin and arabinose had a still more markedly inhibiting

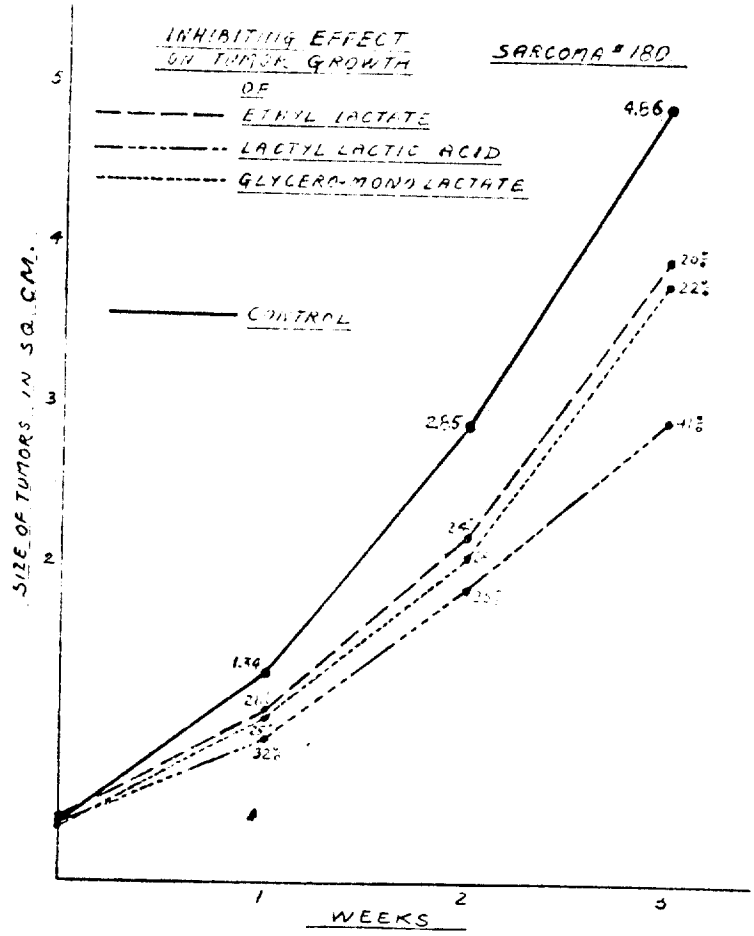

FIG. 1. INHIBITING EFFECT OF ETHYL LACTATE, LACTYL LACTIC ACID AND GLYCERO-MONOLACTATE ON GROWTH OF MOUSE SARCOMA 180
Figures represent growth of tumors in square centimeters.

influence on the growth of sarcoma 180. After three weeks the tumor growth was retarded about 41 per cent.

With glycero-monolactate two series of experiments were carried out. In one series the injections were begun when the tumors had reached the size of 0.3–0.4 sq. cm. and were continued for three weeks. In this experiment the tumors grew about 25 per cent more slowly than the tumors of the control animals. In the

second series, in which the injections were started three weeks before the tumor transplantation and continued for three weeks following transplantation, we observed a depressing action on the health of the mice, as they gained less in body weight during this period. In this series of experiments the injections of glycero-monolactate were less effective than in the first series.

The administration of lactic acid solution by mouth was also found to have a slightly inhibiting effect on the tumor growth.

The effects of the injection of various lactic acid preparations on the growth of sarcoma 180 as compared with the growth of this tumor in all the controls are shown in Figure 1.

We see, then, that certain lactic acid preparations cause a definite, although slight, inhibition of the growth of sarcoma 180. The question which we must now consider is, how far is this inhibition due to the action of these substances as such, and how far is it due to the indirect injurious effect which these substances have on the whole metabolism. We have observed that, if mice lose a great deal of weight as a result of underfeeding, the inhibition of the growth of sarcoma 180 may be greater than that produced by administration of lactic acid preparations; but we must also consider the fact that under these conditions the loss in body weight was greater than that produced by the lactic acid preparations. The majority of lactic acid preparations caused an actual or a relative loss in weight of the treated animals. The animals as a whole still gained in weight, notwithstanding the injections of lactic acid; but the weights of the mice, which indicate a gain as compared with the weights at the time of inoculation of the tumors, include the weights of the tumors, and these weights increased considerably during the progress of the experiments. It is therefore probable that many of the animals—but not all—exclusive of the tumor mass, actually lost in weight during the period of injection. Taking all the facts into consideration, we may conclude that the inhibition of growth in sarcoma 180 observed after administration of lactic acid preparations is probably due, at least in part, to the unfavorable effect of these substances on the metabolism of the bearers of the tumors. However, that the inhibiting effect is not due entirely to loss of weight caused by lactic acid is indicated by the fact that the mice injected with glycero-monolactate gained more in weight during the progress of the experiment than the control mice, although the growth of sarcoma 180 was in this case, also, diminished by about 20 per cent as compared with the control tumors.

As to the action of glucose and arabinose on the growth of sarcoma 180, these substances exert, on the whole, hardly any inhibiting effect, although in some experiments a very slight inhibition was noticeable.

If we review the entire series of experiments reported here, we find that lactic acid preparations exert an inhibiting effect on the growth of sarcoma 180, which amounts, on an average, to about 29 to 30 per cent of the growth of the same tumor in control mice, and in some cases to as much as 40 per cent. As stated, this inhibiting effect is in some cases probably to be attributed partly to the relative loss in weight caused by the lactic acid preparations. On account of the relatively slight effects which lactic acid preparations exert on the growth of sarcoma 180, significant variations of an accidental nature may occur in individual experiments, and it is therefore necessary to carry out such experiments on a large scale; this requirement, we believe, has been met in the present investigations.

In no case have we found a retrogression of sarcoma 180 due to the administration of lactic acid preparations. It was different in the case of rat sarcoma 39 (1), for in a certain percentage of the rats inoculated with that tumor we obtained retrogression. However, rat sarcoma 39 is a much more labile tumor than mouse sarcoma 180 and has a much greater tendency to spontaneous retrogression. Comparatively slight changes in the environment, which would have little effect on mouse sarcoma 180, had a relatively great effect on rat sarcoma 39. It is also probable that in the latter case the general undernourishment and ill health of a certain number of rats, induced by the injections of lactic acid preparations, were at least partly responsible for the inhibiting effect on the tumor growth.

In the literature we find a statement of B. Fischer-Wasels (3) that intravenous injections of ammonium lactate markedly inhibit tumor growth, but that subcutaneous injections of this substance or of sodium lactate are without effect. As to the action on malignant growths, of lactic acid, which, according to Warburg, is produced in relatively large amount by cancer tissue, our experiments can be used only with certain reservations, on account of the differences between the administration of lactic acid in our experiments and its spontaneous formation in the growing tumor. In the latter case small quantities of lactic acid are produced continuously and then given off into the neighboring tissue, where it presumably gains access to the circulation. In our experiments, on the contrary, a relatively large quantity is administered at one time each day, and is active for a short period, during which the organism is flooded with the substance. As Parfentjev, Suntzeff and Sokoloff have shown, the injection of lactic acid increases for some time the level of lactic acid in the blood (4). Our experiments differ, also, so far as the quantity of active substance is concerned from the experiments of Meyerhof and Lohmann (5),

in which it was shown that lactates may cause an increase in oxidation processes of cells. Different, also, were the conditions under which lactates acted when they inhibited *in vitro* the multiplication of Paramecium (6), and the conditions which prevailed in the experiments of Sládek, Parfentjev and Sokoloff (7), in which it was shown that addition of lactates to hypotonic solutions of NaCl diminished the hemolysis of erythrocytes.

Conclusions

1. We may conclude that under the conditions of our experiments various preparations of lactic acid exert a relatively slight inhibiting effect on the growth of mouse sarcoma 180. This effect was especially noticeable after subcutaneous injections of solutions of ethyl lactate, lactyl-lactic acid, and glycero-monolactate.

2. The inhibiting effect can be observed in experiments in which the weight of the treated animals is not unfavorably affected by these substances, a condition obtaining especially after the use of glycero-monolactate. This substance did not affect the weight of the mice unfavorably in our experiments. However, it is probable that in the majority of the experiments the inhibiting effect of lactic acid preparations on sarcoma 180 must be attributed in part to the unfavorable effect which these substances exert on the metabolism of the animals.

3. Injection of sugars (glucose and arabinose) either exerted no inhibiting effect on the growth of sarcoma 180 or one less than that observed after administration of lactic acid preparations.

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THE EFFECT OF FOOD PRESERVATION AGENTS ON THE SURVIVAL RATE OF RATS FOLLOWING TOTAL BODY IRRADIATION [Uber die Wirkung von Lebensmittelkonservierungsstoffen auf die Uberlebensrate von Ratten nach Ganzkörperbestrahlung]

by

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from

Strahlentherapie 139(3):362-371 (1970)

Translation from German

THE EFFECT OF FOOD PRESERVATION AGENTS ON THE SURVIVAL RATE OF RATS FOLLOWING TOTAL BODY IRRADIATION

Karl Peters and Rosemarie Peters

In a previous paper*, an attempt was made to test the influence of preservation agents which are commonly used as additives in the food industry on rats subjected to total body irradiation.

The assumption was made that even in vivo, their disinfectant properties have an effect and that in this way, the life expectancy following irradiation could be increased.

While experiments with Nipagin (p-oxybenzoic acid methyl ester) did not produce any clear information and while treatment with sodium benzoate merely showed a tendency to an increased lifespan, there was an increase in the average survival period which was possible to assure statistically following doses of hexamethylene tetramine.

In additional experiments, we used lactic acid and lactose as typical intestinal disinfectants, as well as sorbic acid as a substance with bactericidal and antimycotic action, and also as sodium phosphate, which is of significance for the carbohydrate, fat, and protein metabolism and which is used in food chemistry and a stabilizer and antioxidant.

I. Lactic Acid

Methods

Lactic acid was added to the drinking water of the experimental animals in quantities of 10 mg/ml, 5 mg/ml, 1.1 mg/ml, 0.36 mg/ml, and 0.04 mg/ml. The two strongest concentrations correspond to the therapeutic doses which have proven themselves in the treatment of acute dysentery in adults and

* Strahlentherapie 138 (1969), 724

the smallest infants [7, 29].

Drinking curves were produced from which it was possible to calculate the absolute quantity of lactic acid ingested per animal per day. Fig. 1 shows that after a preliminary treatment of 10 days, the water intake in the first days following radiation clearly recedes in all groups of animals, and that in addition, the lactic acid concentrations from 1.1 mg/ml to 10 mg/ml had an increasing inhibitory action on the liquid intake in both irradiated and non-irradiated rats.

Fig. 1. Average drinking quantity in ml per animal (m) following total body irradiation (735 R) and lactic acid treatment (1.1 mg/ml; 5 mg/ml; 10 mg/ml) ten days before irradiation and ten days after irradiation.

It can be seen from Fig. 2 that lactic acid in concentrations below 1 mg/ml (0.36mg/ml) tends to increase the water intake somewhat compared to the controls. In this experiment, the dependency of the daily drinking water consumption upon the level of the irradiated X-ray dose is also expressed.

Fig. 2. Average drinking quantity in ml per animal (m) following total body irradiation (675 to 875 R) and lactic acid treatment (0.36 mg/ml) for ten days following irradiation.

The irradiation was carried out with X-rays (220 kV, 16 mA, 0.6 mm Cu, 40 cm FHA). The dose output was 110 R/min. The doses which were administered included values ranging from 675 to 875 R.

The rats which were used in this experiment (CBI Druckrey) were raised by us.

Results

In the first experiment, lactic acid concentrations of 10 mg/ml, 5 mg/ml, and 1.1 mg/ml were tested in groups of 8 animals each. The treatment was started 10 days before the irradiation and continued up to the tenth

day following irradiation. The total body irradiation was uniform at 735 R.

It was found (see Fig. 3) that as the concentration increased, the average survival period of the animals treated with lactic acid decreased continuously compared to that of the control animals.

Fig. 3. Effect of the lactic acid (10 mg/ml, 5 mg/ml, and 1.1 mg/ml) on the average survival period in days following total body irradiation (735 R). Treatment 10 days preceding irradiation and 10 days following irradiation.

It could be seen even from the drinking water curves that lactic acid concentrations starting from 1.1 mg/ml and up have an increasing inhibitory effect on the uptake of liquid.

In the second experiment, the lactic acid concentrations were reduced to 0.36 mg/ml and 0.04 mg/ml. The treatment before the irradiation was omitted. Doses of 675, 725, 775, 825, and 875 R (see Fig. 4) were used.

Fig. 4. The effect of lactic acid (0.36 mg/ml, 0.04 mg/ml) on the average survival period in days following total body irradiation (675 to 875 R). Treatment for 10 days following irradiation.

Then, in the case of the female animals, the survival periods for the treated animals were the same as (0.04 mg/ml) or higher (0.36 mg/ml) than the controls, while in the case of the male animals, the curves intersected frequently and therefore the animals treated with lactic acid had better survival periods than the controls, at least partially. A glance at the drinking water curve (Fig. 2) shows that 0.036 per cent and 0.004 per cent lactic acid is drunk in at least the same quantity compared to pure water.

Discussion

Lactic acid is the least irritating of all organic acids.

Acute dysentery in adults can be healed with a daily quantity of one liter of a 1% solution, while in infants, even whole milk with a lactic acid content of only 0.6% has an antidiarrhetic action [29]. A simple

addition of lactic acid does a similar job [24], which has led to putting this knowledge into practice in pediatrics [3, 28]. Lactic acid has proven itself in the raising of healthy rats and pigs [34, 42]. The disinfectant action on the intestine could be one of the factors why growth is accelerated with this diet. In addition to this, there is the fact that lactic acid is a high provider of calories almost equal to grape sugar.

The antiseptic action of most acids is based on their splitting off of H ions, but this is not the only reason for lactic acid, since even the neutral salt also inhibits the growth of bacteria. In addition, bacteria toxins are rendered harmless due to the fermentation of lactic acid. For this reason, it is used in the preservation of foods. Likewise, the bacteria content of the air can be reduced by means of lactic acid mist. In addition, lactic acid has a negative chemotactic action on the lymphocytes and thereby prevents them from collecting to form pus foci. Lactic acid also has a vascular dilatating action on the coronary circulation.

Lactic acid is the structural element to which almost all carbohydrates are metabolized before the organism assimilates them again into physically suitable substances. For this reason, it is processed even in a severely damaged organism and synthesized to glycogen in the liver. For this reason, it is also recommended for rapid liver protection. Diarrhea occurs only in the case of a 7 to 8% solution. It represents a caustic agent in concentrations of 20 to 50% which attacks pathological tissue selectively. In the body, it is totally metabolized, thus avoiding an accumulation of acid radicals.

In spite of these positive properties of lactic acid, it was found that it must be used with great care in the case of rats with total body irradiation and thereby with markedly reduced general health. Concentrations which

have an absolutely curative effect in adult humans and in small children cause increasing mortality in irradiated rats. Only when the concentration is reduced to 0.036 and 0.004%, does one find hints of a positive effect. It is remarkable that 1% and 0.5% lactic acid leads to reduced desire to drink in both irradiated and non-irradiated rats, and therefore to a reduced intake of electrolytes ($CaCl_2$ and $MgSO_4$), which are normally present in water. A reduction in the electrolyte supply can even by itself lead to a reduction in the resistance to radiation [20]. Thus, the reduction in the survival period following treatment with these doses could also be a direct reaction to the reduced intake of fluid. Since the daily fluid intake at 0.036% and 0.004% lactic acid concentrations is no longer less than the water consumption of the control animals, the relationship becomes clear between the optimal water intake, the improved intake of the therapeutic agent which is related to this and the effect which is obtained with it, and explains the positive action of only the lowest lactic acid concentrations in our experiments.

II. Lactose

Methods

In order to promote a healthy intestinal flora as well as to stimulate the detoxification processes in the intestines, 5 g lactose per 100 g of food is recommended in pediatrics. Based on this, and considering the contraindication of lactose in the case of diarrhea disorders such as those which occur in animals with radiation damage after a few days, lactose was offered for drinking to animals with total body irradiation only in a 0.1% aqueous solution (= 1 mg/ml). Since the lactose animals had snouts with bloody infections even a short period after irradiation and since they

drank the solution with obvious distaste, the 0.1% solution was replaced by a 0.05% solution (0.5 mg/ml) after four days. The quantity of lactose taken by the animals per day can be seen from the drinking water curves (see Fig. 5). We found a dependency of the fluid uptake upon the level of the X-ray dose which was administered with a minimum of drinking on the third and fourth day following irradiation. The addition of lactic acid to the drinking water causes a reduction in the liquid intake in the animals irradiated with 750 and 800 R compared to the control animals irradiated to the same extent.

Fig. 5. Average drinking quantity in ml per animal (male) following total body irradiation (750 to 850 R) and lactose treatment (1 mg/ml, 0.5 mg/ml) ten days following irradiation.

X-rays of 220 kV, 16 mA, 0.5 mm Cu, and 40 cm FHA were used for the administration. The dose output was 110 R/min. The doses were between 750 and 800 R. Rats from our own stock were again used as experimental animals (Druckrey CBI).

Results

The experiments show the effect of 0.1 to 0.05% lactose solution on 18 male and 10 female rats following total body irradiation with 750, 800, and 850 R in the case of the male rats and a uniform 750 R in the case of the female rats (see Fig. 6).

Fig. 6. The effect of lactose (1 mg/ml, 0.5 mg/ml) on the average survival period in days following total body irradiation (750 to 850 R). Treatment for ten days following irradiation.

In comparison to the control animals, which had received tap water for drinking, the average survival period following 750 R was equally high in the case of the male rats treated with lactose. AT 800 R it was increased by two days, and at 850 R it was reduced by three days. In the case of the females, the lactose group showed an average survival period

reduced by four days.

Discussion

The action of the sugar depends upon its absorption speed. While monosaccharides are absorbed directly, disaccharides have to be split first. When this occurs, lactose decomposes into glucose and galactose. In children, this degradation proceeds very rapidly--in contrast to adults. It also occurs very slowly in dyspeptic conditions.

Due to its hygrophilia, lactose binds liquid and has the effect of stimulating peristalsis. For this reason, it is used by the teaspoon as a mild laxative in a 5 to 10% solution in the presence of appendicitis. At 10 to 15 g per day, it develops an uncertain laxative effect even in healthy subjects.

A stimulation of the intestinal flora can be obtained with the aid of lactose. This is particularly important in the presence of pathological intestinal bacteria which develop toxins which pass into the blood.

In addition, lactose is a good provider of calories and hardly tastes sweet [8].

In the case of the radiation-damaged intestines, there is presumably in analogy to dyspepsia, only impaired splitting and absorption of the lactic acid. Thus, suspicions could not be confirmed according to which lactose is able to exert a positive influence on the condition of rats with total body irradiation. Even at concentrations of only 1 to 0.5 mg lactose per ml drinking water, there was only a potentiation of its laxative action, and increased tendency for infections in the area of the smelt, and an increased mortality even though the animals were free of symptoms at the start of the treatment.

In analogy to the findings in the lactic acid experiments, the intake of lactose solutions is reduced so that in this case as well, the willingness to drink provides information concerning the tolerance and effect of the therapeutic agent. Presumably, lactose, like lactic acid, can be offered in only very low concentrations in order to produce positive effects on the survival period following total body irradiation.

III. Sorbic Acid

Methods

Sorbic acid is of interest due to its properties of bacteriostatic and antimycotic actions [14]. It was tolerated without causing pathological symptoms [5] in animal experiments as a 4% diet for months in dogs and rats. Due to the radiation damage and the greater sensitivity of our animals caused by this, the dose was reduced to 1.1 mg/ml, 0.36 mg/ml, and 0.02 mg/ml drinking water based on the experience of the two preceding experimental series, and was offered to groups totaling 57 males and 57 female rats for drinking. 27 males and 27 females each were used as controls.

The quantity of sorbic acid which was ingested daily by the animals can be seen from the liquid uptake per day (see Fig. 7). As we already know, this is dependent upon the level of the radiation dose, the time after the radiation, and the agent administered. While in the first experiment, the consumption of the water containing sorbic acid was below the drinking water consumption of the control animals for the greater part, in the second experiment, a clear preference for the sorbic acid solutions compared to pure water was determined.

Fig. 7. Average drinking quantity in ml per day (male) following total body irradiation (695 R) and sorbic acid treatment (1.1 mg/ml, 0.36 mg/ml) for 18 days before irradiation and 10 days after irradiation.

Doses ranging from 615 to 790 R were used (200 kV, 16 mA, 0.5 mm Cu, 40 cm FHA, dose output 110 R/min).

Rats of our cultivation were used (CBI Druckrey).

Results

The first experiment (see Fig. 8) showed the action of 0.36 mg and 0.02 mg sorbic acid per ml drinking water following the action of 615, 650, 685, 720, 755, and 790 R compared to control animals irradiated with the same doses in groups of four male and four female rats each.

In the case of the female rats treated with sorbic acid, the average survival rate is decreased compared to the control animals up to a radiation dose of 685 R; starting at 720 R, on the other hand, it is clearly above that of the control animals, the survival rate of which is markedly reduced at these doses.

In the male rats which were treated with sorbic acid, the average survival time was four days more than that of the control animals only in the case of the 0.036% group with an irradiation of 650 R. All other values were the same as or below the survival periods for the controls.

Fig. 8. Action of sorbic acid (0.36 mg/ml, 0.02 mg/ml) on the average survival period in days following total body irradiation (615 to 790 R). Treatment for ten days following irradiation.

In the second experiment (see Fig. 9), 1.1 mg and 0.36 mg sorbic acid per ml drinking water was administered for 17 days for the irradiation. The irradiation was uniform with 695 R. The treatment was continued for 10 days after the irradiation with the result that the survival times of the animals treated with sorbic acid was the same as that of the control animals. In this experiment, the drinking quantity consumption of sorbic acid solutions was noticeably heavier than the water consumption of the controls, regardless of the concentration (see Fig. 7).

Fig. 9. The action of sorbic acid (1.1 mg/ml, 0.36 mg/ml) on the average survival time in days following total body irradiation (695 R). Treatment for ten days before irradiation and ten days following radiation.

Discussion

Sorbic acid [35, 38] is an α, β unsaturated fatty acid, which occurs in its saturated form in butterfat as caproic acid. It is processed in the organism in the same way as this substance [2, 6, 43] and thus used as a source of calories [5]. Together with para-sorbic acid, it occurs in the fruit of Sorbus aucuparia.

Sorbic acid has a strong antiseptic action as a result of dissociation of H ions and also in the neutral state as a salt [14]. It is used for the prevention of bacterial infections and is also active against mold and yeasts. Its optimum pH range is at 4.5.

The action of sorbic acid is based on an inhibition of the dehydration processes of microorganisms [25]. Enzymes studies have shown that 0.112% sorbic acid inhibits the catalase activity 72 to 77% [23]. Sulfohydryl enzymes and alcohol dehydrogenase are inhibited at concentrations of 10^{-4} M. However, it is apparently impossible to inhibit the dehydrogenase enzyme system in the animal itself [26].

Sorbic acid is oxidized in feed: in the presence of carbohydrates, $CO_2 + H_2O$ are formed; when carbohydrates are absent, acetoacetate and acetone are formed [27].

The tolerance of sorbic acid in animal experiments is dependent on the concentration: a 4% diet is tolerated by dogs and rats for months without pathological symptoms or histological-anatomical changes [5]. The same is shown by a 5% diet (= 2500 mg/kg body weight) taken over 2 3/4 years; there were no recognizable pathological effects [22]. Starting with an 8% diet, a slight increase in the liver weight occurs in rats, but the liver

remains normal from a histological-pathological viewpoint [5]. In the case of a 10 to 20% diet (with sorbitan-stearate) for 2 to 4 years, growth inhibition and inability to nurse occur. The liver and kidney weights are elevated, but the mortality is not influenced [30-33].

The LD_{50} in rats is 10.5 g/kg body weight in the case of free sorbic acid . In the case of the sodium salt, it is 5.94 g/kg body weight in the case of well fed animals, and 3.65 to 4.3 g/kg body weight in the case of fasting animals.

Since the food intake of rats with total body irradiation clearly decreases and, in order to know the tolerance with some degree of certainty, we limited ourselves in the present experiments to concentrations of 1.1 mg, 0.36 mg, and 0.02 mg sorbic acid per ml drinking water. While in the first experiment following doses of 0.036% and 0.002% sorbic acid, the sorbic acid groups showed better survival periods in general only in the case of the female irradiated with relatively high doses; the other animals treated with sorbic acid, on the other hand, showed the same but largely worse survival chances than the controls; after a preliminary treatment with 0.11% and 0.036% sorbic acid, the second experiment showed no differences with respect to the survival time of the control animals and the sorbic acid animals, but it was noted that in the course of this experiment the quantity of sorbic acid which was drunk was clearly greater than the drinking water consumption of the control animals. Thus, sorbic acid does not appear to produce any clearly positive or any clearly negative action on the survival time of rats with total body irradiation when the water intake is optimal.

IV. Sodium Phosphate ($Na_2HPO_4 \cdot 2H_2O$)

Methods

Phosphoric acid and its salts are used as oxidation inhibitors and stabilizers in the preservation of foods (36, 39). On account of its important metabolic functions and its simultaneous antiseptic action [9], we were tempted to test the properties of sodium phosphate on the survival period of rats with total body irradiation as well.

In animal experiments, a 0.75% diet in rats did not show any toxic effects [1], while renal damage occurred starting with a phorphorus content of 1% and more [18, 19]. Even relatively low doses have a laxative effect in humans [12]. For that reason, we decided to use sodium phosphate in a 0.002% solution in 12 males and 16 female rats. While the females were irradiated with doses ranging from 625 to 775 R, doses of 700 to 800 R were used on the males (220 kV, 16 mA, 0.5 mm Cu, 40 cm FHA). The dose rate was 110 R/min.

Drinking water curves were made (see Fig. 10), from it was possible to compute of phosphate drunk per day. We can again see the dependence of the drinking quantity upon the intensity of the irradiation. Phosphoric acid appears to decrease the liquid intake only slightly.

Fig. 10. Average drinking quantity in ml per animal (male) following total body irradiation (700 to 800 R) and disodium phosphate treatment (0.02 mg/ml) for ten days following irradiation.

The rats used for this experiment (CBI Druckrey) were grown by us.

Results

In the case of the male and female rats treated with the 0.02% sodium phosphate solution, the average survival period following total body radiation was the same as that of the controls (see Fig. 11).

Fig. 11. The action of disodium phosphate (0.02 mg/ml) on the average survival time in days following total body radiation (700 to 800 R). Treatment for ten days following irradiation.

Discussion

Acids have a strong antiseptic action which is produced essentially by the dissociation of H ions.

In food chemistry, H_3PO_4 and its salts are used as a sequestrant, antioxidizing agent, and stabilizer in cheese, milk, fish, and meat products; in addition, it is used as a buffer, a neutralization agent, and for the acidification and aromatization of drinks and fruit products [36, 39].

Its natural occurrence as free H_3PO_4 or as sodium, potassium, or calcium salts is particularly concentrated in milk, cheese, nuts, fish, meat, fowl, eggs, and certain grains [36]. Phosphorus is also a necessary component of the human organism (approximately 450 g phosphates and phosphoric acids compounds). In this instance, it governs the most important metabolic functions such as phosphorylation in the synthesis and degradation of proteins, fats, carbohydrates, and enzymes. It is important for the muscle and liver metabolism and as a buffer substance in the blood. Through the nucleic acids of the cell nucleus, it also has a significance for the growth metabolism [9].

In all, the phosphorus and acid content of food is important. The quantity required daily in humans is 1 to 2 g per day [10]. Quantities of 2 to 4 g Na_2HPO_4 acts as a salt laxative [12]. When there is an overdosage of phosphate, excretion occurs in the feces as calcium phosphate; for this reason, there is the danger of calcium loss [37]. If there is excretion of Na_2HPO_4 in the urine following an acid-base metabolism disorder in the blood, then there is renal damage, hematuria, and bladder tenesmus [11], when the concentration is sufficient.

The earliest and most marked criterion in the toxicity of phosphates is renal damage [17, 19, 41].

Doses which do not produce an toxic effects in animal experiments are 0.75% in the diet = 375 mg/kg body weight per day [1].

The LD_{50} following oral administration in rats is 4000 mg/kg body weight $Na_4P_2P_7$ [4] or the same quantity of a mixture of 1/3 Kurrol's salt + 2/3 tetradisodium phosphate [16]. On the other hand, in the case of i.v. administration, it is as low as 18 mg/kg body weight [16]. In general, it is possible to say for a phosphorus content of the food of 1% and more that it has a nephrocalcinogenic effect [18, 21]. Further damage occurs in the form of calcification of the stomach and the aorta, loss of weight, inhibition of growth, fertility disorders, and a decrease in the life span [40].

Sodium phosphate solutions of only 0.02 mg/ml drinking water decrease the liquid intake only slightly and do not have any influence on the survival time of rats with total body irradiation.

We thank the physicists, Dr. G. Breitling, Professor, and Dr. W. Seeger for adjusting the radiation doses.

Summary

When lactic acid, lactose, sorbic acid, or sodium phosphate is added to drinking water in concentrations of 10 to 0.02 mg/ml, an increase or a decrease in the survival rates occurred depending upon the substance and concentration in the case of rats with total body irradiation. Descriptions are given of the liquid intake as a function of the dissolved substance, the age, weight, and sex of the animals, the radiation dose, and the period following the irradiation.

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Peters-'70
Bib No. 1448

Strahlentherapie 139(3):362-371. 1970.

Über die Wirkung von Lebensmittelkonservierungsstoffen auf die Überlebensrate von Ratten nach Ganzkörperbestrahlung

2. Mitteilung: I. Milchsäure, II. Milchzucker, III. Sorbinsäure, IV. Na-Phosphat

Karl Peters, Rosemarie Peters

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(Direktor: Prof. Dr. med. M. *Frommhold*)

In einer vorangegangenen Arbeit[1] wurde versucht, den Einfluß von Konservierungsstoffen, wie sie als Zusätze in der Lebensmittelindustrie geläufig sind, auf ganzkörperbestrahlte Ratten zu testen.

Es wurde vorausgesetzt, daß auch *in vivo* ihre desinfizierenden Eigenschaften zur Wirkung kommen und auf diese Weise die Lebenserwartung nach Bestrahlung gesteigert werden könnte.

Während Versuche mit Nipagin (p-oxy-Benzoesäuremethylester) keine eindeutigen Aussagen brachten und Behandlung mit Na-Benzoat lediglich die Tendenz zu einer gesteigerten Lebensdauer zeigte, kam es nach Gaben von Hexamethylentetramin zur Erhöhung der mittleren Überlebenszeit, welche statistisch zu sichern war.

[1] Strahlentherapie 138 (1969), 724.

In weiteren Versuchen verwendeten wir als typische Darmdesinfektionsmittel Milchsäure und Milchzucker, ferner Sorbinsäure als bakterizide und antimykotisch wirkende Substanz sowie Natriumphosphat, welches für den Kohlenhydrat-, Fett- und Eiweißstoffwechsel von Bedeutung ist und in der Nahrungsmittelchemie als Stabilisator und Antioxydantium gebraucht wird.

I. Milchsäure

Methodik

Milchsäure wurde in Mengen von 10 mg/ml, 5 mg/ml, 1,1 mg/ml, 0,36 mg/ml und 0,04 mg/ml dem Trinkwasser der Versuchstiere zugegeben. Die beiden stärksten Konzentrationen entsprechen den therapeutischen Dosen, die sich bei der Behandlung der akuten Dysenterie von Erwachsenen und Kleinstkindern bewährt haben [7, 29].

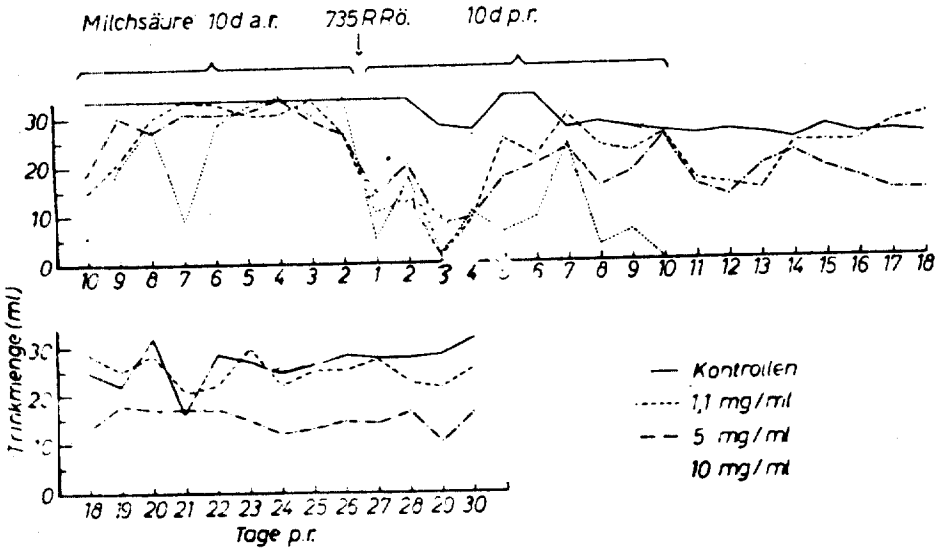

Abb. 1. Durchschnittliche Trinkmenge in ml pro Tier (♂) nach Ganzkörperbestrahlung (735 R) und Milchsäurebehandlung (1,1 mg/ml; 5 mg/ml; 10 mg/ml) zehn Tage a.r. – zehn Tage p.r.

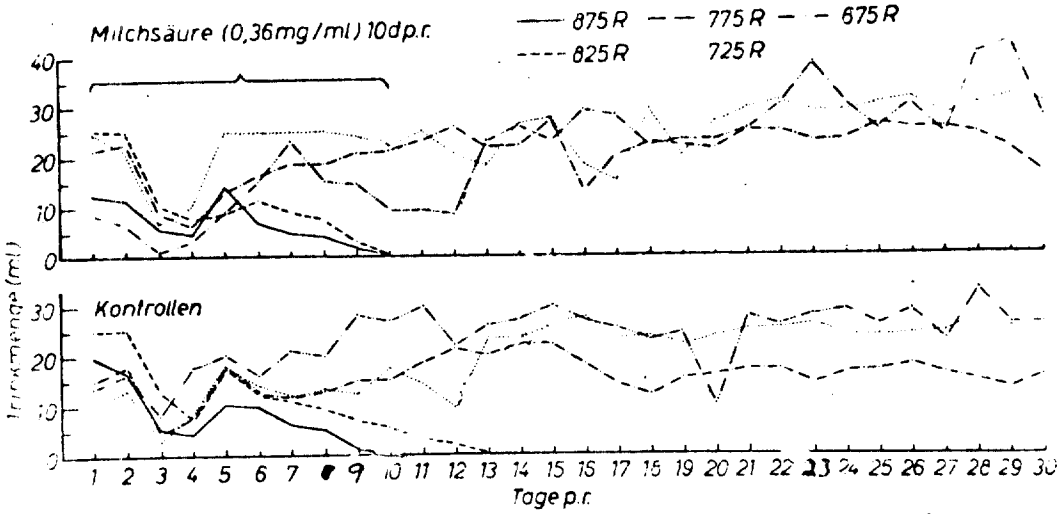

Abb. 2. Durchschnittliche Trinkmenge in ml pro Tier (°) nach Ganzkörperbestrahlung (675 bis 875 R) und Milchsäurebehandlung (0,36 mg/ml) zehn Tage p.r.

Es wurden Trinkkurven hergestellt, aus denen die absolut eingenommene Milchsäuremenge Tier Tag berechnet werden konnte. Abbildung 1 zeigt, daß nach 10tägiger Vorbehandlung die Wasseraufnahme in den ersten Tagen post rad. bei allen Tiergruppen deutlich zurückgeht, und ferner, daß Milchsäurekonzentrationen von 1,1 mg/ml bis 5 mg/ml die Flüssigkeitsaufnahme bei bestrahlten wie unbestrahlten Ratten zunehmend hemmen.

Aus Abbildung 2 geht hervor, daß Milchsäure in Konzentrationen unter 1 mg/ml (0,36 mg/ml) die Wasseraufnahme gegenüber den Kontrollen eher etwas steigert. In diesem Versuch kommt zusätzlich die Abhängigkeit des täglichen Trinkwasserverbrauchs von der Höhe der eingestrahlten Röntgendosis zum Ausdruck.

Die Bestrahlung wurde mit Röntgenstrahlen (220 kV, 16 mA, 0,5 mm Cu, 40 cm FHA) durchgeführt. Die Dosisleistung betrug 110 R/min. Die verabfolgten Dosen umfaßten Werte von 675 bis 875 R.

Die zu diesem Versuch verwendeten Ratten (CBI Dunkrey) kamen aus unserer eigenen Zucht.

Ergebnisse

In dem ersten Versuch wurden an Gruppen von je acht Tieren Milchsäurekonzentrationen von 10 mg/ml, 5 mg/ml und 1,1 mg/ml erprobt. Die Behandlung wurde zehn Tage vor der Bestrahlung begonnen und bis zum zehnten Tag nach Bestrahlung durchgeführt. Die Ganzkörperbestrahlung erfolgte einheitlich mit 735 R.

Es zeigte sich (s. Abb. 3), daß mit steigender Konzentration die mittlere Überlebenszeit der milchsäurebehandelten Tiere gegenüber der der Kontrollen zunehmend zurückging.

Bereits aus den Trinkwasserkurven war zu entnehmen, daß Milchsäurekonzentrationen von 1,1 mg/ml ab aufwärts die Flüssigkeitsaufnahme zunehmend hemmen.

In dem zweiten Versuch wurden die Milchsäurekonzentrationen auf 0,36 mg/ml und 0,04 mg/ml herabgesetzt. Die Behandlung vor der Bestrahlung entfiel. Zur Anwendung kamen Dosen von 675, 725, 775, 825 und 875 R (s. Abb. 4).

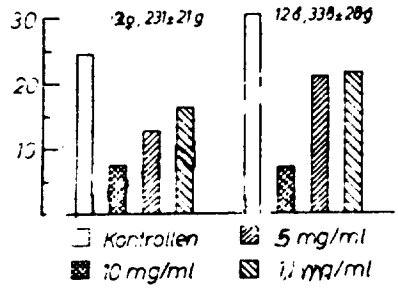

Abb. 3. Wirkung von Milchsäure (10 mg/ml, 5 mg/ml und 1,1 mg/ml) auf die mittlere Überlebenszeit in Tagen nach Ganzkörperbestrahlung (735 R). Behandlung zehn Tage a.r. + zehn Tage p.r.

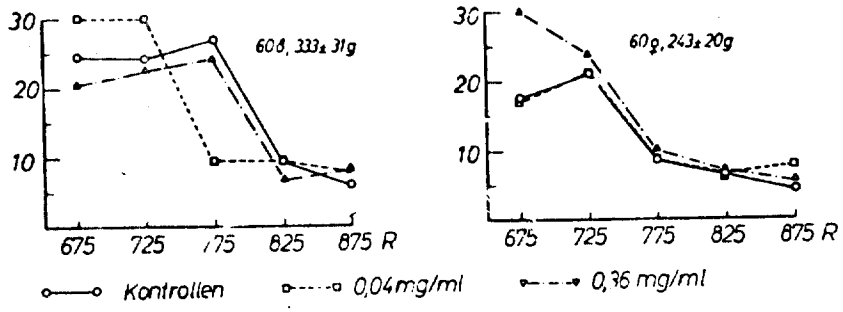

Abb. 4. Wirkung von Milchsäure (0,36 mg/ml; 0,04 mg/ml) auf die mittlere Überlebenszeit in Tagen nach Ganzkörperbestrahlung (675 bis 875 R). Behandlung zehn Tage p.r.

Jetzt lagen bei den weiblichen Tieren die Überlebenszeiten der behandelten Tiere gleichsinnig (0,04 mg/ml) oder höher (0,36 mg/ml) als die der Kontrollen, während sich bei den männlichen Tieren die Kurven mehrfach überschnitten und somit die milchsäurebehandelten Tiere wenigstens teilweise bessere Überlebenszeiten als die Kontrollen hatten. Ein Blick auf die Trinkwasserkurve (Abb. 2) zeigt, daß 0,036%ige und 0,004%ige Milchsäure reinem Wasser gegenüber in mindestens gleicher Menge getrunken wird.

Diskussion

Milchsäure ist die reizloseste aller organischen Säuren.

Die akute Dysenterie des Erwachsenen kann mit einer täglichen Menge von einem Liter einer 1%igen Lösung geheilt werden, während beim Säugling bereits eine Vollmilch mit einem Milchsäuregehalt von nur 0,6% antidiarrhoisch wirkt [29]. Ein einfacher Zusatz von Milchsäure tut schon ähnliche Dienste [24], was zur Praktizierung dieser Erkenntnisse in der Kinderklinik geführt hat [3, 28]. Selbst bei der Aufzucht von gesunden Ratten und Schweinen [34, 42] hat sich Milchsäure bewährt. Die Desinfektionswirkung auf den Darm könnte einer der Faktoren sein, warum das Wachstum mit dieser Diät beschleunigt wird. Dazu kommt, daß Milchsäure fast gleichsinnig dem Traubenzucker ein hoher Kalorienspender ist.

Die antiseptische Wirkung der meisten Säuren beruht auf ihrer Abspaltung von H-Ionen, doch trifft dies für Milchsäure nicht allein zu, da auch das neutrale Salz noch das Auskeimen von Bakterien hemmt. Außerdem werden Bakterientoxine durch Milchsäuregärung unschädlich gemacht. Sie wird daher bei der Konservierung von Lebensmitteln gebraucht. Ebenso läßt sich der Keimgehalt der Luft durch Milchsäurenebel reduzieren. Milchsäure wirkt außerdem negativ chemotaktisch auf die Lymphozyten und unterbindet somit deren Ansammlung zu Eiterherden. Gefäßerweiternd wirkt Milchsäure auch auf den Koronarkreislauf.

Milchsäure ist der Baustein, zu welchem fast alle Kohlenhydrate abgebaut werden, bevor der Organismus sie wieder zu körpereigenen Stoffen assimiliert. Aus diesem Grunde wird sie noch im schwergeschädigten Organismus verarbeitet und in der Leber zu Glykogen aufgebaut. Daher wird sie auch zum raschen Leberschutz empfohlen. Erst bei 7- bis 8%iger Lösung treten Durchfälle auf. 20- bis 50%ig stellt sie ein Ätzmittel dar, das elektiv am pathologischen Gewebe angreift. Im Körper wird sie vollständig abgebaut, wodurch eine Ansammlung von Säureradikalen vermieden wird.

Trotz dieser positiven Eigenschaften der Milchsäure zeigt sich, daß sie bei den ganzkörperbestrahlten und dadurch im Allgemeinbefinden stark reduzierten Ratten mit großer Sorgfalt dosiert werden muß. Konzentrationen, die beim erwachsenen Menschen und beim Kleinkind absolut heilend wirken, verursachen bei der bestrahlten Ratte zunehmende Sterblichkeit. Erst wenn die Konzentration auf 0,036 und 0,004% gesenkt wird, findet man Ansätze zu einer positiven Wirkung. Auffallend ist, daß 1%ige und 0,5%ige Milchsäure bei bestrahlten wie unbestrahlten Ratten zu verringerter Trinklust führt und damit zu einer verringerten Aufnahme von Elektrolyten ($CaCl_2$ und $MgSO_4$), die normalerweise im Wasser vorhanden sind. Eine Herabsetzung der Elektrolytzufuhr kann

über bereits eine Senkung der Strahlenresistenz herbeiführen [20]. Die Verkürzung der Überlebenszeit nach Behandlung mit diesen Dosen könnte also auch eine unmittelbare Reaktion auf die verminderte Flüssigkeitsaufnahme sein. Da die tägliche Flüssigkeitsaufnahme bei 0,036 und 0,004%ziger Milchsäurelösung dem Wasserverbrauch der Kontrollen nicht mehr nachsteht, wird der Zusammenhang von optimaler Wasseraufnahme, damit verbundener Aufnahmefreudigkeit des Therapeutikums und der mit ihm erzielten Wirkung deutlich und erklärt die positive Wirkung nur niederster Milchsäurekonzentrationen bei unseren Versuchen.

II. Milchzucker

Methodik

Zur Förderung einer gesunden Darmflora sowie zur Anregung von Entgiftungsprozessen im Darm werden in der Kinderheilkunde 5 g Milchzucker/100 g Nahrung empfohlen. In Anlehnung daran und in Anbetracht der Kontraindikation von Milchzucker bei Durchfallstörungen, wie sie im strahlengeschädigten Tier nach wenigen Tagen auftreten, wurde Milchzucker lediglich in einer 0,1%igen wäßrigen Lösung (= 1 mg/ml) ganzkörperbestrahlten Ratten zum Trinken angeboten. Da schon kurzfristig nach Bestrahlung die Milchzuckertiere blutig entzündete Schnauzen aufwiesen und mit offensichtlichem Widerwillen die Lösung tranken, wurde nach vier Tagen die 0,1%ige Lösung durch eine 0,05%ige ersetzt (0,5 mg/ml). Die vom Tier pro Tag aufgenommene Milchzuckermenge ist aus den Trinkwasserkurven zu entnehmen (s. Abb. 5). Es zeigte sich eine Abhängigkeit der Flüssigkeitsaufnahme von der Höhe der verabfolgten Röntgendosis mit einem Trinkminimum am dritten und vierten Tag p.r. Die Zugabe von Milchsäure zum Trinkwasser bewirkt eine Verminderung der Flüssigkeitsaufnahme der mit 750 und 800 R bestrahlten Tiere gegenüber den gleich stark bestrahlten Kontrollen.

Zur Applikation gelangten Röntgenstrahlen von 220 kV, 15 mA, 0,5 mm Cu und 40 cm FHA. Die Dosisleistung betrug 110 R/min. Die Dosen lagen zwischen 750 und 800 R. Als Versuchstiere wurden wiederum Ratten des eigenen Zuchtstammes (Druckrey CBI) verwendet.

Ergebnisse

Der Versuch zeigt an 18 männlichen und zehn weiblichen Ratten die Wirkung von 0,1- bis 0,05%iger Milchzuckerlösung nach Ganzkörperbestrahlung mit 750, 800 und 850 R bei den Männchen und einheitlich 750 R bei den Weibchen (s. Abb. 6).

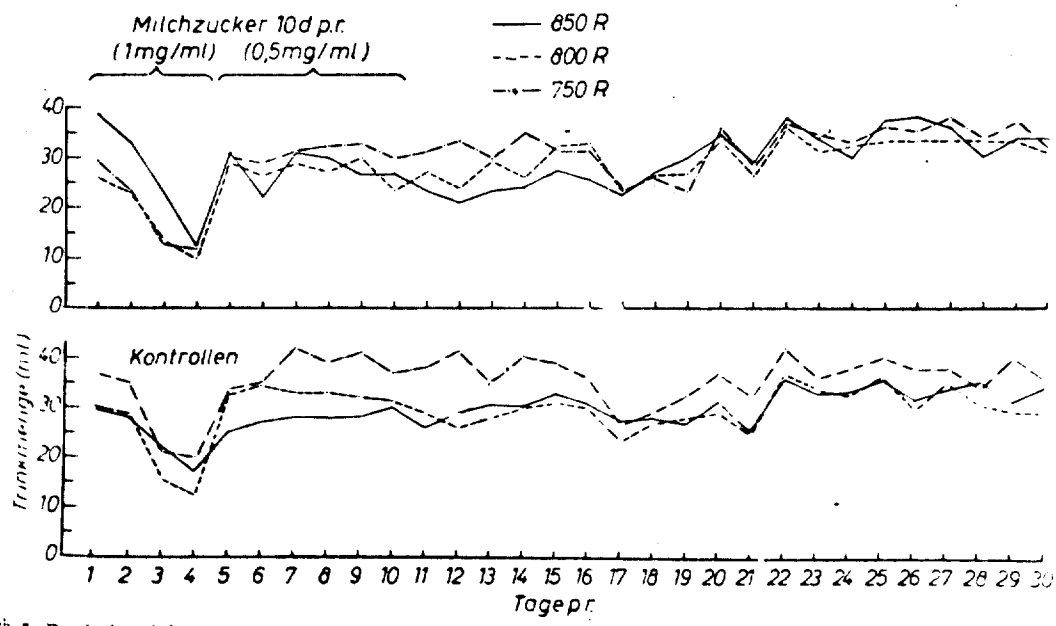

Abb. 5. Durchschnittliche Trinkmenge in ml pro Tier (♂) nach Ganzkörperbestrahlung (750 bis 850 R) und Milchzuckerbehandlung (1 mg/ml, 0,5 mg/ml) zehn Tage p.r.

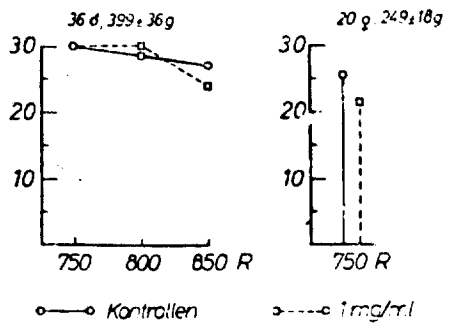

Abb. 6. Wirkung von Milchzucker (1 mg/ml, 0,5 mg/ml) auf die mittlere Überlebenszeit in Tagen nach Ganzkörperbestrahlung (750 bis 850 R). Behandlung zehn Tage p. r.

Im Vergleich zu den Kontrollen, welche Leitungswasser zum Trinken bekommen hatten, war bei den milchzuckerbehandelten Männchen die mittlere Überlebenszeit nach 750 R gleich hoch. Bei 800 R wurde sie um zwei Tage erhöht und bei 850 R um drei Tage erniedrigt. Bei den Weibchen zeigte die Milchzuckergruppe eine um vier Tage verminderte mittlere Überlebenszeit.

Diskussion

Die Wirkung der Zucker hängt von ihrer Resorptionsgeschwindigkeit ab. Während Monosaccharide unmittelbar zur Resorption kommen, müssen Disaccharide erst gespalten werden. Milchzucker zerfällt dabei in Traubenzucker und Galaktose. Beim Kind geht dieser Abbau sehr rasch vor sich – im Gegensatz zum Erwachsenen. Während dyspeptischer Zustände geschieht dies sogar sehr langsam.

Auf Grund seiner Hygrophilie bindet Milchzucker Flüssigkeit und wirkt peristaltikanregend. Deshalb wird er bei Blinddarmentzündungen in 5- bis 10%iger Lösung teelöffelweise als mildes Abführmittel gebraucht. Bei 10 bis 15 g Tag entfaltet er auch bei Gesunden eine unsichere Abführwirkung.

Mit Hilfe von Milchzucker läßt sich eine Umstimmung der Darmflora erzielen. Besonders wichtig ist dies beim Vorkommen pathologischer Darmbakterien, welche Toxine entwickeln, die ins Blut übergehen.

Außerdem ist Milchzucker ein guter Kalorienträger und schmeckt kaum noch süß [8].

Im strahlengeschädigten Darm kommt es vermutlich ähnlich der Dyspepsie nur noch zu beeinträchtigter Spaltung und Resorption der Milchzucker. So ließen sich Vermutungen nicht bestätigen, nach denen Milchzucker das Befinden ganzkörperbestrahlter Ratten positiv beeinflussen könnte. Es zeigte sich selbst noch nach Konzentrationen von nur 1 bis 0,5 mg Milchzucker/ml Trinkwasser eine Potenzierung seiner abführenden Wirkung, eine verstärkte Neigung zu Entzündungen im Schnauzenbereich und eine erhöhte Sterblichkeit, obwohl die Tiere zu Beginn der Behandlung symptomfrei waren.

Ähnlich den Befunden der Milchsäureversuche ist die Aufnahme von Milchzuckerlösungen gesenkt, so daß auch hier die Trinkfreudigkeit über Verträglichkeit und Wirkung des Therapeutikums Auskunft gibt. Vermutlich darf Milchzucker ähnlich der Milchsäure nur in sehr geringen Konzentrationen geboten werden, um positive Wirkungen auf die Überlebenszeit nach Ganzkörperbestrahlung zu erzielen.

III. Sorbinsäure

Methodik

Sorbinsäure interessiert auf Grund ihrer bakteriostatisch und antimykotisch wirkenden Eigenschaften [14]. Sie wurde im Tierversuch als 4%-Diät über Monate von Hunden und Ratten vertragen, ohne pathologische Symptome auszulösen [5]. Auf Grund der Strahlenschädigung und der dadurch bedingten stärkeren Empfindlichkeit unserer Tiere wurde nach den Erfahrungen der beiden vorhergehenden Versuchsreihen die Dosis auf 1,1 mg/ml, 0,36 mg/ml und 0,02 mg/ml Trinkwasser herabgesetzt und Gruppen von insgesamt 57 männlichen und 57 weiblichen Ratten zum Trinken angeboten. Je 27 Männchen und Weibchen dienten als Kontrollen.

Die täglich vom Tier aufgenommene Sorbinsäuremenge wird aus der Flüssigkeitsaufnahme pro Tag ersichtlich (s. Abb. 7). Diese ist, wie wir bereits wissen, abhängig von der Höhe der Strahlendosis, der Zeitdauer nach der Bestrahlung und dem verabreichten Medium. Während im ersten Versuch der Verbrauch des sorbinsäurehaltigen Wassers größtenteils unter dem Trinkwasserverbrauch der Kontrolltiere lag, war im zweiten Versuch eine deutliche Bevorzugung der Sorbinsäurelösungen gegenüber reinem Wasser festzustellen.

Zur Anwendung gelangten Dosen von 615 bis 790 R (220 kV, 16 mA, 0,5 mm Cu, 40 cm FHA, Dosisleistung 110 R/min).

Verwendet wurden Ratten des eigenen Zuchtstammes (CBI Druckrey).

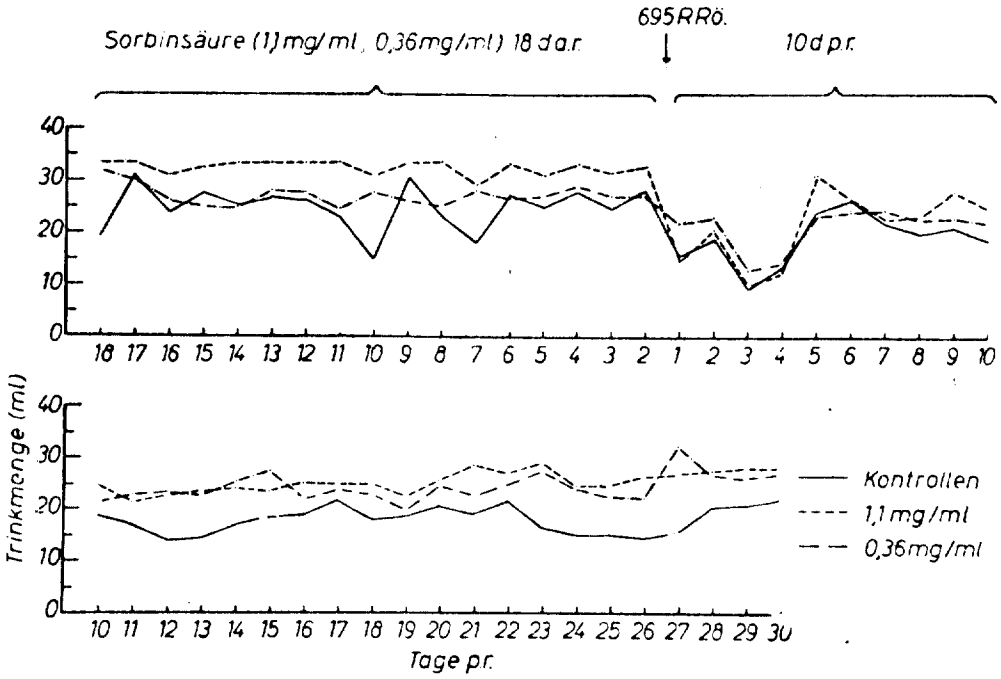

Abb. 7. Durchschnittliche Trinkmenge in ml pro Tier (♂) nach Ganzkörperbestrahlung (695 R) und Sorbinsäurebehandlung (1,1 mg/ml, 0,36 mg/ml) 18 Tage a.r. – zehn Tage p.r.

Ergebnisse

Der erste Versuch (s. Abb. 8) zeigte an Gruppen von je vier männlichen und vier weiblichen Ratten die Wirkung von 0,36 mg und 0,02 mg Sorbinsäure/ml Trinkwasser nach Einwirkung von 615, 650, 685, 720, 755 und 790 R gegenüber den mit gleichen Dosen bestrahlten Kontrolltieren.

Bei den sorbinsäurebehandelten Weibchen ist die mittlere Überlebenszeit bis zu einer Strahlendosis von 685 R gegenüber der der Kontrollen gesenkt, ab 720 R liegt sie dagegen eindeutig über der der Kontrollen, deren Überlebenszeit bei diesen Dosen stark reduziert wird.

Bei den männlichen sorbinsäurebehandelten Ratten liegt die mittlere Überlebenszeit nur bei der 0,036%-Gruppe mit einer Einstrahlung von 650 R um vier Tage über der der Kontrollen. Alle anderen Werte liegen gleichsinnig oder unter den Überlebenszeiten der Kontrollen.

In einem zweiten Versuch (s. Abb. 9) wurden 1,1 mg und 0,36 mg Sorbinsäure/ml Trinkwas-

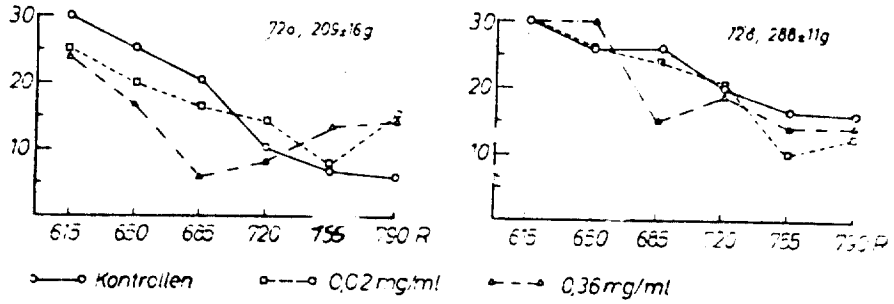

Abb. 8. Wirkung von Sorbinsäure (0,36 mg/ml, 0,02 mg/ml) auf die mittlere Überlebenszeit in Tagen nach Ganzkörperbestrahlung (615 bis 790 R). Behandlung zehn Tage p.r.

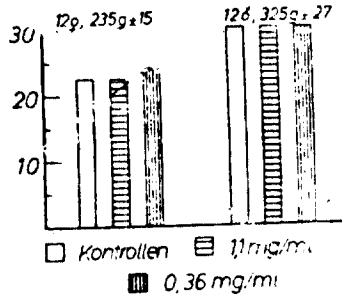

Abb. 6. Wirkung von Sorbinsäure (1,1 mg/ml, 0,36 mg/ml) auf die mittlere Überlebenszeit in Tagen nach Ganzkörperbestrahlung (695 R). Behandlung zehn Tage a.r. – zehn Tage p.r.

ser über 17 Tage vor der Bestrahlung verabreicht. Bestrahlt wurde einheitlich mit 695 R. Die Behandlung wurde noch zehn Tage nach der Bestrahlung fortgesetzt, mit dem Erfolg, daß die Überlebenszeiten der sorbinsäurebehandelten Tiere gleichsinnig denen der Kontrollen waren. — In diesem Versuch war der Trinkmengenverbrauch der Sorbinsäurelösungen, gleich welcher Konzentration, auffallend stärker als der Wasserverbrauch der Kontrollen (s. Abb. 7).

Diskussion

Sorbinsäure [35, 38] ist eine α,β-ungesättigte Fettsäure, die in gesättigter Form im Butterfett als Capronsäure vorkommt. Sie wird im Organismus in gleicher Weise verwertet wie diese [2, 6, 43] und somit als Kalorienquelle genutzt [5]. Zusammen mit Parasorbinsäure kommt sie in Früchten von Sorbus aucuparia vor.

Sorbinsäure wirkt sowohl durch die Abdissoziierung von H-Ionen wie auch in neutralem Zustand als Salz stark antiseptisch [14]. Sie wird zur Verhütung bakterieller Infektionen gebraucht und ist außerdem gegen Schimmel und Hefen wirksam. Ihr optimaler pH-Bereich liegt bei 4,5.

Die Wirkung der Sorbinsäure beruht auf einer Hemmung der Dehydrierungsvorgänge von Kleinlebewesen [25]. Enzymstudien zeigten, daß 0,112 % Sorbinsäure die Katalase-Aktivität zu 72 bis 77 % hemmt [23]. Sulfhydrylenzyme und Alkoholdehydrogenase werden bei Konzentrationen von 10^{-4} M gehemmt. Es ist aber anscheinend unmöglich, das Dehydrogenase-Enzym-System im Tier selber zu hemmen [26].

Im Futter wird Sorbinsäure oxydiert: in Gegenwart von Kohlenhydraten werden $CO_2 + H_2O$ gebildet, beim Fehlen derselben Aceto-Acetat und Aceton [27].

Die Verträglichkeit der Sorbinsäure im Tierversuch ist abhängig von der Konzentration: Eine 4-%-Diät wird von Hunden und Ratten über Monate ohne pathologische Symptome oder histologisch-anatomische Veränderungen vertragen [5]. Desgleichen zeigt eine 5-%-Diät (= 2500 mg/kg Kgw.), über 2¾ Jahre genommen, keine erkennbaren pathologischen Effekte [22]. Ab einer 8-%-Diät kommt bei Ratten eine leichte Erhöhung des Lebergewichtes vor, die Leber bleibt jedoch histologisch-pathologisch normal [5]. Bei einer 10- bis 20-%-Diät (mit Sorbitan-Stearat) über zwei bis vier Jahre treten Wachstumshemmung und Stillunfähigkeit auf. Leber- und Nierengewichte sind erhöht, die Mortalität wird nicht beeinflußt [30—33].

Die LD 50 liegt bei Ratten bei freier Sorbinsäure bei 10,5 g/kg Körpergewicht. Beim Natrium-Salz liegt sie bei gut gefütterten Tieren bei 5,94 g/kg Körpergewicht und bei Hungertieren bei 3,65 bis 4,3 g/kg Körpergewicht.

Da die Nahrungsaufnahme ganzkörperbestrahlter Ratten deutlich zurückgeht und, um die Verträglichkeit einigermaßen garantiert zu wissen, beschränkten wir uns in den vorliegenden Versuchen auf Konzentrationen von 1,1 mg, 0,36 mg und 0,02 mg Sorbinsäure/ml Trinkwasser. Während im ersten Versuch nach Gaben von 0,036%iger und 0,002%iger Sorbinsäure nur bei den mit höheren Dosen bestrahlten Weibchen die Sorbinsäure-Gruppen generell bessere Überlebenszeiten aufwiesen, die anderen sorbinsäurebehandelten Tiere dagegen gleichartige, größtenteils aber schlechtere Überlebenschancen als die Kontrollen hatten, zeigte der zweite Versuch nach einer Vorbehandlung mit 0,11%iger und 0,036%iger Sorbinsäure hinsichtlich der Überlebenszeit von Kontrollen und Sorbinsäure-Tieren keine Unterschiede. Es fiel auf, daß bei diesem Versuch die getrunkene Sorbinsäuremenge deutlich über dem Trinkwasserverbrauch der Kontrollen lag. Somit scheint Sorbinsäure bei optimaler Wasseraufnahme keine eindeutig positive, aber auch keine negative Wirkung auf die Überlebenszeit ganzkörperbestrahlter Ratten zu haben

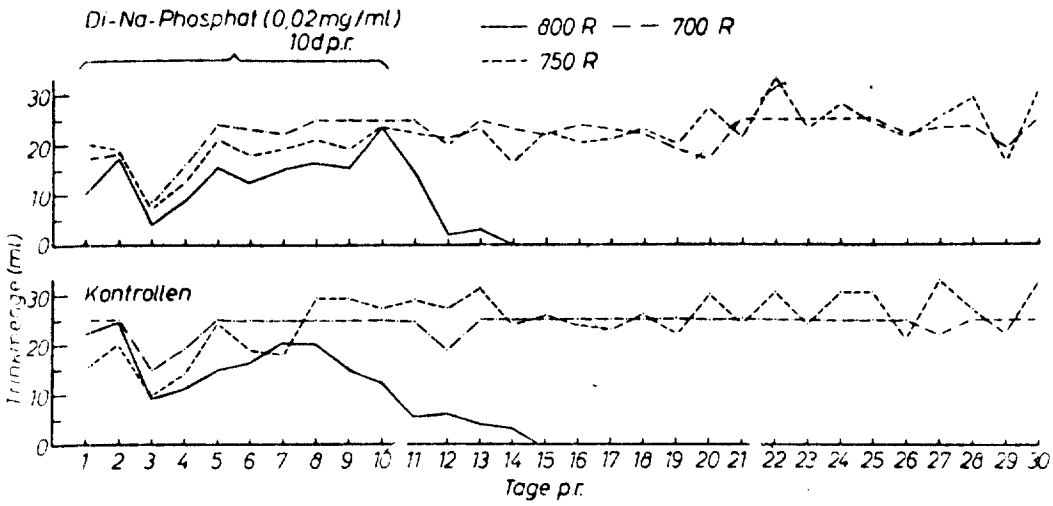

Abb. 10 Durchschnittliche Trinkmenge in ml pro Tier (°) nach Ganzkörperbestrahlung (700 bis 800 R) und Di-Na-Phosphat-Behandlung (0,02 mg/ml) zehn Tage p.r.

IV. Natriumphosphat $Na_2HPO_4 \cdot 2H_2O$

Methodik

Bei der Konservierung von Lebensmitteln werden Phosphorsäure und ihre Salze als Oxydationshemmer und Stabilisatoren gebraucht [36, 39]. Wegen seiner wichtigen Stoffwechselfunktion und der gleichzeitigen antiseptischen Wirkung [9] waren wir versucht, auch die Eigenschaften des Na-Phosphats auf die Überlebenszeit ganzkörperbestrahlter Ratten zu testen.

Im Tierversuch zeigte eine 0,75-% ige-Diät bei Ratten keine toxischen Effekte [1], während ab einem P-Gehalt von 2,0 % und mehr Nierenschäden auftreten [18, 21]. Schon relativ geringe Dosen wirken beim Menschen abführend [12]. Daher entschlossen wir uns Na-Phosphat in einer 0,002 % igen Lösung an 12 männliche und 16 weibliche Ratten zu verabreichen. Während die Weibchen mit Dosen von 625 bis 775 R bestrahlt wurden, kamen bei den Männchen 700 bis 800 R zur Anwendung (220 kV, 16 mA, 0,5 mm Cu, 40 cm FHA). Die Dosisleistung betrug 110 R/min.

Es wurden Trinkwasserkurven hergestellt (s. Abb. 10), aus denen die Menge des getrunkenen Phosphats/Tag berechnet werden kann. Wir sehen weder die Abhängigkeit der Trinkmenge von der Intensität der Bestrahlung. Phosphorsäure scheint die Flüssigkeitsaufnahme nur geringfügig zu erhöhen.

Die zu diesem Versuch verwendeten Ratten (CBI Wistar) kamen aus unserer eigenen Zucht.

Ergebnisse

Bei den mit 0,02 % iger Na-Phosphatlösung behandelten männlichen wie weiblichen Ratten lag die mittlere Überlebenszeit nach Ganzkörperbestrahlung gleichsinnig der der Kontrollen (s. Abb. 11).

Diskussion

Säuren haben starke antiseptische Wirkung, die im wesentlichen durch die Abspaltung von H-Ionen entsteht.

In der Lebensmittelchemie werden H_3PO_4 und ihre Salze als Sequestrant, Antioxydantium und Stabilisator bei Käse, Milch, Fisch- und Fleischprodukten gebraucht, außerdem als Puffer, Neutralisationsmittel und zum Ansäuern und Aromatisieren von Getränken und Früchteprodukten [36, 39].

Ihr natürliches Vorkommen als freie H_3PO_4 oder als Ka, Na oder Ca-Salze ist besonders

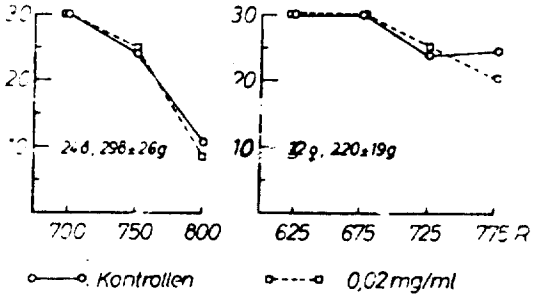

Abb. 11 Wirkung von Di-Na-Phosphat (0,02 mg/ml) auf die mittlere Überlebenszeit in Tagen nach Ganzkörperbestrahlung (700 bis 800 R). Behandlung zehn Tage p.r. ▶

konzentriert in Milch, Käse, Nüssen, Fisch, Fleisch, Geflügel, Eiern und gewissen Getreiden [30]. Phosphor ist auch ein nötiger Bestandteil des menschlichen Organismus (etwa 450 g Phosphate und Phosphorsäure-Verbindungen). Hier beherrscht er die wichtigsten Stoffwechselfunktionen wie die Phosphorylierung im Auf- und Abbau von Eiweißen, Fetten, Kohlenhydraten und Fermenten. Er ist wichtig für den Muskel und Leberstoffwechsel und als Puffersubstanz im Blut. Durch die Nukleinsäuren des Zellkerns hat er auch eine Bedeutung für den Wachstumsstoffwechsel [9].

Entscheidend ist der Phosphor- und Säuregehalt der Nahrung im Ganzen. Die täglich nötige Menge liegt beim Menschen bei 1 bis 2 g Tag [10]. Mengen von 2 bis 4 g Na_2HPO_4 wirken schon als salziges Abführmittel [12]. Bei Phosphatüberdosierung geschieht Exkretion als Ca-Phosphat in den Faeces, daher besteht Gefahr eines Ca-Verlustes [37]. Geschieht nach gestörtem Säure-Basen-Haushalt im Blut Ausscheidung von Na_2HPO_4 im Harn, so entstehen bei genügender Konzentration Nierenschäden, Hämaturie und Blasentenesmen [11].

Das früheste und stärkste Kriterium in der Toxizität von Phosphaten sind Nierenschäden [17, 19, 41].

Dosen, die im Tierversuch keine toxischen Effekte zeigen, sind 0,75% in der Diät = 375 mg/kg Körpergewicht/Tag [1].

Die LD 50 liegt nach oraler Applikation bei Ratten bei 4000 mg/kg Körpergewicht $Na_4P_2O_7$ [4] oder der gleichen Menge eines Gemisches von 1/3 Kurrols-Salz + 2/3 Tetra-Disodiumphosphat [16]. Bei i.v. Applikation liegt sie dagegen schon bei 18 mg/kg Körpergewicht [16]

Allgemein könnte man ab einem P-Gehalt der Nahrung von 1% und mehr sagen, daß er nephrokalzinogen wirkt [18, 21]. Als weitere Schäden folgen Verkalkung des Magens und der Aorta, Gewichtsverlust, Wachstumshemmung, Fertilitätsstörungen und Verkürzung der Lebenszeit [40].

Natriumphosphat-Lösungen von nur 0,02 mg/ml Trinkwasser senken die Flüssigkeitsaufnahme nur gering und haben auf die Überlebenszeit ganzkörperbestrahlter Ratten keinen Einfluß.

Den Herren Physikern Prof. Dr. G. Breitling und Dr. W. Seeg danken wir für die Einstellung der Strahlendosen.

Zusammenfassung

Zusätze von Milchsäure, Milchzucker, Sorbinsäure oder Natriumphosphat zum Trinkwasser in Konzentrationen von 10 bis 0,02 mg/ml bewirken je nach Substanz und Konzentration bei ganzkörperbestrahlten Ratten eine Abnahme oder Zunahme der Überlebensrate. Abhängigkeiten der Flüssigkeitsaufnahme von der gelösten Substanz, dem Alter, Gewicht und Geschlecht der Tiere, der Strahlendosis und der Zeitdauer nach der Bestrahlung werden beschrieben.

Summary

When lactic acid, lactose, sorbic acid or sodium-phosphate is added to the water, in concentration between 10 to 0.02 mg/ml, modifications of the survival of total body irradiated rats are observed depending on the substance and of their concentrations. Relations were noted between the quantity taken in and the kind of substance, the age, the weight and the sex of the animal, and also with the radiation dose and the time elapsed after the irradiation.

Résumé

L'adjonction d'acide lactique, de lactose, d'acide sorbique ou de phosphate de sodium à l'eau, dans des concentrations variant entre 10 à 0,02 mg/ml modifie la survie de rats après une irradiation totale. On a observé une relation entre la quantité de l'eau bue d'une part et la substance, l'age, le poids et le sexe de l'animal, et de la dose de radiations et du temps écoulé après l'irradiation d'autre part.

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Pitkin-'35
Bib No. 1470

Ann. Otol. Rhin. & Laryng. 44:842-843. 1935.

LXXXVI.

LACTIC ACID STRICTURE OF THE ESOPHAGUS.*

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CLEVELAND.

Lactic acid is commonly used by pediatricians for acidification of milk in infant feeding. It was formerly used in the treatment of various forms of dyspepsia. It has been used locally as a caustic agent in tuberculous and other ulcers, also for the purpose of dissolving diphtheritic membrane. Certain contraceptives contain lactic acid and rather extensive erosions of the vaginal mucosa and cervix have occurred from its caustic action.

In a paper entitled "The Problem of Accidental Poisoning in Children," by Dr. John Aikman, read before the Pediatric Section of the American Medical Association in 1934, lactic acid was not mentioned. One case of lactic acid poisoning was found in the literature. The patient was a woman who was being treated in a hospital for gall bladder disease by duodenal lavage with 25 per cent magnesium sulphate. A nurse by mistake injected 33 per cent lactic acid through the duodenal tube. Violent abdominal pain with vomiting of bloody mucus occurred. There was a suppression of urine and hemoglobinuria. The patient collapsed and died twelve hours after the ingestion of the lactic acid. Autopsy showed a dark red duodenal mucosa and necrotic inflammation of the jejunum. Microscopic hemorrhages were present in the mucosa of the posterior wall of the stomach.

The case I wish to report is a stricture of the esophagus following accidental administration of lactic acid.

REPORT OF A CASE.

The patient, a female infant, eleven weeks old, was being fed a formula consisting of diluted evaporated milk and corn syrup acidified with lactic acid. The patient was also receiving cod liver oil. Identical bottles were used for the lactic acid and cod liver oil. A teaspoonful of lactic acid, U. S. P. 85 per cent, was given to the patient by the mother, she believing it to be cod liver oil. The infant strangled and cried. The mother at once discovered her mistake and took the infant.

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*Read before the eighteenth annual meeting of the American Bronchoscopic Society, Toronto, June 1, 1935.

to St. Luke's Hospital, where a gastric lavage was performed. No excoriation of the oral mucosa was noted at the time.

For three weeks following the accident the patient was unable to take food without crying, coughing and regurgitating. After beginning to swallow, there was marked difficulty in respiration, with asthmatoid wheezing, which was relieved only by regurgitation. At times cyanosis was present. The patient was then seen by Dr. Fred Rittinger, who suspected esophageal obstruction. Attempted lavage failed, as the tube met an obstruction in the esophagus. A roentgenogram with barium mixture showed a stricture in the midportion of the esophagus. Esophagoscopy without anesthesia, four and one-half weeks after the accident, revealed a concentric stricture in the midportion of the esophagus admitting a 9 Fr. dilator.

As the patient was able to swallow but little, it was thought safer to do a gastrostomy. This was performed under local anesthesia by Dr. F. G. Leonard. Seventeen days after the gastrostomy, esophagoscopy was again done, and a No. 6 ureteral catheter passed into the stomach. A roentgenogram showed the catheter coiled in the stomach. The catheter was found in the stomach by the aid of a Young urethroscope and head mirror, and brought through the gastric fistula. A silk thread was tied to the catheter and drawn through the esophagus. Retrograde dilatation with Tucker dilators was done about twice a week, starting with a 12 Fr. and stepping up to a 26 Fr. during the following six weeks. The gastrostomy was then closed.

At the time of closure the patient was five and one-half months old and weighed ten pounds and two ounces. She was taking her feedings well. Six dilatations by blind bougienage, up to 30 Fr., were done during the next two months. Esophagoscopy at eleven months showed a normal lumen of the esophagus.

The child is now twenty-six months old, weighs thirty-two pounds, and seems to be in perfect health.

Since lactic acid is now more extensively used, accidents in the future may be prevented by labels cautioning against its use except in diluted form.

CARNEGIE MEDICAL BLDG.

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Roberson-'71
Bib No. 1573

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Effect of Calcium, Ascorbic Acid, Reserpine, Magnesium, and Calcium Lactate on the Performance of Two Strains of Pullets and Hens

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The high performance of commercial egg-type chickens is the result of improvements in poultry breeding, nutrition, physiology, and disease and environment control. Despite the improvements in livability, egg production, feed efficiency, and overall egg quality, two problems of great importance in the commercial egg industry are the decline in interior quality and the decline in shell quality with advancing age of the chicken. It has been estimated that, in the United States, five to seven percent of all eggs are accidentally broken before they reach the consumer (17,23). Many of these broken eggs result from thinning of the shells. Pullets in commercial laying flocks are usually replaced when interior quality and shell quality have deteriorated even though the pullets may be producing eggs at an acceptable rate. Furthermore, the practice of recycling laying chickens has increased the number of hens in commercial laying flocks. This practice may have increased the problem of low interior and exterior egg quality from older birds.

Review of Literature

Pullets begin to lay eggs with thick shells and high interior quality. As the pullets become older, the interior quality declines (15) and the egg shells become thinner. There is a point in the production cycle at which the decline in interior quality and shell quality make marketing of the eggs difficult because of broken shells and thin egg whites. The nutrition of the bird (23), environmental temperature (33), and diseases (23) also may affect interior and exterior quality of eggs.

Thornton and Moreng (28) first reported an improvement in egg shell quality from feeding ascorbic acid to laying pullets. They found the improvement more consistent when the diet contained adequate protein (29) and calcium (30). Laying chickens usually synthesize adequate ascorbic acid, but heat stress and somewhat lower temperatures may decrease synthesis or increase utilization so that the added ascorbic acid may have improved the performance of birds.

Several researchers have concluded that laying chickens do not need a dietary source of ascorbic acid (2,8,20,26) even during periods of heat stress (12,13).

Peterson (21,22) reported that the modern strains of high-producing commercial pullets require a calcium level of three to four percent of the diet. These levels are higher than the 2.25 and 2.75 percent levels of calcium recommended by the National Research Council (18,19).

Oral reserpine may afford some protection against the decline in egg production, egg weight, interior quality, and shell quality following heat stress in the laboratory (31) and under natural conditions (6,7). All researchers do not agree that reserpine improves performance of pullets during periods of heat stress (5,8,14), and the tranquilizer may even reduce performance during periods of cold stress.

Calcium lactate may improve shell thickness even in the presence of 2.75 percent calcium in the diet (3), although some researchers (11,23,26) have not shown a beneficial effect for calcium lactate. The favorable influence of calcium lactate may be the result of the lactose, which improves calcium absorption, rather than of the calcium.

Magnesium occurs in egg shell in a small but essential amount, but the National Research Council (19) does not give a minimum magnesium requirement for laying pullets. Some workers (1,30,31,36) indicate an adverse effect of supplemental magnesium on laying pullets, but recent results (9,16,25,32) indicate that rather high levels of magnesium do not affect pullet performance.

Objective of Study

Because the decline in interior and shell quality remains a pressing problem in the egg industry, the effects of calcium, ascorbic acid, reserpine, magnesium, and calcium lactate on the performance of two strains of pullets and hens were studied.

Procedures

Three experiments were conducted.

Two levels of calcium (2.25 and 4.50 percent), two levels of ascorbic acid (0 and 20 mg per pound), three levels of reserpine (0, 0.25, and 0.50 mg per pound), and two strains of layers (New Mexico Experiment Station single comb white leghorn and a commercial strain) were used in a 2 x 2 x 2 x 3 factorial arrangement of 12 dietary treatments, or 24 total treatments, in a randomized complete block design in experiments I and II. Four replicates of five pullets of each strain received each treatment in experiment I. Four replicates of five pullets and five hens of each strain received each of the treatments in experiment II.

Diets 1 and 2 contained 2.25 and

4.50 percent calcium and were made isocaloric and isonitrogenous by adjustments of the milo, soybean meal, and animal fat. The diets contained 16 percent crude protein and 900 kilocaloric productive energy per pound of diet. The supplemental calcium was added in a granular form to reduce dust and improve palatability. The ascorbic acid and reserpine were added in the prescribed amounts to the basal diets.

In experiment III, two levels of ascorbic acid (0 and 20 mg per pound) two levels of calcium lactate (0 and 0.25 percent), and two levels of magnesium (0 and 50 mg per pound) were added to the basal diet used in experiment II. The statistical design was a 2 x 2 x 2 factorial arrangement of eight treatments. The basal diet contained three percent calcium, sixteen percent crude protein, 900 kcal of productive energy per pound, and 1015 mg of magnesium per pound (table 1). Three replicates of four pullets received each of the eight treatments for the entire period. In addition, three replicates of four pullets for each treatment received the basal diet (table 1) the first half of the experimental period and the eight treatments the last half of the experiment. The experiment was begun after the pullets had been laying for three months, and it was continued through 15 months of production.

In all experiments, the pullets and hens were maintained in individual wire cages in a single-deck, back-to-back arrangement in open-type houses. Feed and water were supplied *ad libitum*.

Egg production for individual birds and feed consumption for replicate groups of birds were recorded. At two-month intervals, three eggs per layer were weighed and broken for interior quality and shell thickness determinations. Shell thickness at the equator of the egg was measured in thousandth of an inch with an Ames thickness gauge no. 25, and percentage of shell was calculated from the weights of the whole egg and of the washed, air-dried shell. The interior quality was measured in Haugh units (10).

Roughness of the shell was visually scored from 1 to 6. A score of 1 indicates a very smooth shell, and a score of 6 indicates a very rough and ridged shell.

The data were examined statistically by analysis of variance (24) and Duncan's multiple range test (4).

Results and Discussion

The commercial strain of pullets of experiment I produced more eggs ($P<0.05$) for the first three periods and for all periods combined than the New Mexico strain of pullets (table 2). In experiment II, the commercial pullets produced more eggs during period 2 and for all periods combined. The commercial strain of hens produced significantly more eggs ($P<0.05$) during period 5 than the New Mexico

Table 1. Composition of experimental diets

Ingredients	Experiment I Diet 1	Experiment I Diet 2	Experiment II
	- - - - - - percent	- - - - - -	
Milo	58.70	46.60	43.20
Yellow corn	10.00	10.00	20.00
Soybean meal, 44%	14.50	17.15	17.50
Meat and bone meal, 50%	5.00	5.00	--
Menhaden fish meal, 60%	--	--	4.00
Dehy. Alfalfa meal, 17%	5.00	5.00	3.00
Distillers dried soluble and grain, 26%	--	--	2.00
Animal fat	--	3.50	1.75
Ground limestone	3.45	9.40	6.00
Dicalcium phosphate	1.00	1.00	2.00
Salt	.25	.25	.25
Vitamin premix	2.00*	2.00*	.25**
Trace mineral premix***	.05	.05	.025
D-L Methionine	.05	.05	.05
Calculated Analyses			
Crude protein (percent)	16	16	16
Productive Energy Kcal/lb	900	900	900
Calcium (percent)	2.25	4.50	3.00
Magnesium mg/lb	--	--	1015

*Supplies per pound of diet: 2,000 IU Vitamin A, 1,000 ICU Vitamin D_3, 2.5 IU Vitamin E, .0025 mg Vitamin B_{12}, 1.5 mg riboflavin, 10 mg niacin, .075 mg folic acid, 2 mg pantothenic acid, 200 mg choline and 1 mg menadione

**Supplied per pound of diet: 4,000 IU Vitamin A, 1,000 ICU Vitamin D_3, 2.5 IU Vitamin E, .006 mg Vitamin B_{12}, 2 mg riboflavin, 12 mg niacin, 0.1 mg folic acid, 5 mg d-pantothenic acid, 400 mg choline, and 1 mg menadione in dried fish solubles, corn distillers dried grain with solubles, dried fermentation solubles, and corn distillers dried soluble

***Supplies per pound of diet: 27.2 mg manganese, 20.4 mg zinc, 9 mg iron, 1.1 mg copper and .56 mg iodine

strain of hens. The commercial strain used in these experiments was one commonly used for table-egg production by poultrymen. They produce eggs at a higher rate than the New Mexico strain of purebred white leghorns and should be used in much of the nutritional and physiological research in New Mexico.

Egg weight was significantly greater ($P<0.05$) for the commercial strain than for the New Mexico strain of pullets in experiment I during period 2 and for all periods combined, and in experiment II during periods 1, 2, 3 and for all periods combined (table 2). The egg weight of the commercial hens was greater ($P<0.05$) than that of the

Table 2. A comparison of the performance of two strains of pullets and hens

Parameter, Experiment, and Strain	Periods of 56 Days* Period 1	Period 2	Period 3	Period 4	Period 5	Mean*
Egg Production (percent)						
Experiment I (pullet)						
New Mexico	48.8 b	54.9 b	65.7 b	64.0 a	51.3 a	56.9 b
Commercial	53.9 a	62.0 a	73.0 a	62.2 a	53.9 a	62.0 a
Experiment II (pullet)						
New Mexico	59.0 a	55.8 b	62.3 a	58.1 a	50.4 a	57.2 b
Commercial	62.9 a	65.5 a	64.4 a	61.5 a	54.3 a	61.7 a
Experiment II (hen)						
New Mexico	53.1 a	46.6 a	51.1 a	48.8 a	39.6 b	47.8 a
Commercial	54.1 a	48.9 a	56.7 a	52.9 a	45.5 a	51.8 a
Egg Weight (grams)						
Experiment I (pullet)						
New Mexico	55.3 a	58.5 b	58.7 b	60.2 a	60.8 a	58.7 b
Commercial	56.6 a	60.1 a	59.9 a	60.8 a	61.4 a	59.8 a
Experiment II (pullet)						
New Mexico	57.4 b	60.9 b	60.4 b	58.5 a	58.7 a	59.2 b
Commercial	58.5 a	62.7 a	61.8 a	59.4 a	60.3 a	60.5 a
Experiment II (hen)						
New Mexico	62.8 a	63.3 b	62.3 b	59.5 b	59.0 b	61.4 b
Commercial	65.9 a	66.0 a	64.4 a	61.2 a	62.2 a	63.7 a
Haugh Score						
Experiment I (pullet)						
New Mexico	81.7 a	78.8 a	77.8 a	76.0 a	76.4 a	78.3 a
Commercial	80.4 a	78.3 b	75.8 b	75.5 a	76.3 a	77.3 a
Experiment II (pullet)						
New Mexico	80.8 a	78.8 a	75.9 a	75.7 a	74.3 a	77.1 a
Commercial	77.7 b	75.4 b	73.3 b	75.7 a	73.4 a	75.1 b
Experiment II (hen)						
New Mexico	74.1 a	71.9 a	68.6 b	71.2 a	70.7 a	71.3 a
Commercial	74.1 a	73.8 a	71.6 a	73.2 a	72.1 a	72.9 a
Shell Thickness (.001")						
Experiment I (pullet)						
New Mexico	14.2 a	14.1 a	13.1 a	13.0 a	13.1 a	13.5 a
Commercial	14.4 a	14.4 a	13.3 a	13.1 a	13.2 a	13.7 a
Experiment II (pullet)						
New Mexico	13.7 b	13.8 b	13.3 b	12.8 a	12.8 b	13.3 b
Commercial	14.0 a	14.1 a	13.7 a	12.7 a	13.1 a	13.5 a
Experiment II (hen)						
New Mexico	13.3 a	13.4 b	12.8 b	12.1 b	12.3 a	12.8 b
Commercial	13.6 a	13.7 a	13.3 a	12.6 a	12.6 a	13.2 a

*Means within experiments in each column followed by the same letter are not significantly different ($P < .05$).

Table 2. Continued

Parameter, Experiment, and Strain	Periods of 56 Days* Period 1	Period 2	Period 3	Period 4	Period 5	Mean*
Percentage of Shell						
Experiment I (pullet)						
New Mexico	9.7 a	9.6 a	9.1 a	8.8 a	8.4 a	9.1 a
Commercial	9.8 a	9.7 a	9.1 a	8.8 a	8.5 a	9.1 a
Experiment II (pullet)						
New Mexico	9.3 a	9.3 a	9.3 a	9.2 a	9.3 a	9.3 a
Commercial	9.4 a	9.2 a	9.4 a	9.1 a	9.4 a	9.3 a
Experiment II (hen)						
New Mexico	8.7 a	8.7 a	8.7 a	8.7 a	8.8 a	8.7 a
Commercial	8.7 a	8.8 a	8.8 a	8.7 a	8.8 a	8.8 a
Shell Roughness Score						
Experiment II (pullet)						
New Mexico	2.53 a	3.08 b	3.29 a	3.66 a	4.41 a	3.40 b
Commercial	2.68 a	3.35 a	3.43 a	3.81 a	4.36 a	3.53 a
Experiment II (hen)						
New Mexico	2.88 a	3.45 a	3.61 a	3.91 a	4.60 a	3.69 a
Commercial	2.99 a	3.64 a	3.73 a	4.02 a	4.60 a	3.80 a
Feed Conversion (pounds of feed per 12 eggs)						
Experiment I (pullet)						
New Mexico	6.7 a	6.3 a	5.2 a	5.3 a	6.1 a	5.9 a
Commercial	6.0 a	5.4 a	4.6 b	4.8 a	6.1 a	5.4 a
Experiment II (pullet)						
New Mexico	5.4 a	6.4 a	5.2 a	5.4 a	6.6 a	5.8 a
Commercial	4.9 a	4.7 b	4.9 a	5.0 a	5.6 a	5.0 a
Experiment II (hen)						
New Mexico	5.6 a	6.7 a	6.1 a	6.1 a	7.5 a	6.4 a
Commercial	5.5 a	6.1 a	5.2 b	5.7 a	6.3 b	5.7 b

New Mexico strain for the last three periods and for all periods combined. The difference in egg weight of the two strains of pullets is more important than the difference in egg weight of hens, because most of the eggs from hens are large enough to be classed commercially as large (57 to 64 grams).

The Haugh score of the New Mexico strain of pullets was higher ($P<0.05$) than the Haugh score of the commercial strain of pullets for periods 2 and 3 of experiment I and for periods 1, 2, 3 and all periods combined for experiment II (table 2). With hens, the Haugh score of the New Mexico strain was higher for period 3 only. The Haugh score of eggs from pullets and hens of both strains remained high throughout the production cycle, but the score declined with increasing age in the usual manner.

Shell thickness was similar for pullets of the two strains in experiment I (table 2). Shell thickness of the commercial pullets in experiment II was greater ($P<0.05$) for periods 1, 2, 3, 5 and for all periods combined. Shell thickness of commercial hens was higher for periods 1, 2, 3, 4 and for all periods combined. The superior shell quality of the commercial pullets and hens occurred even with a higher rate of egg production and a greater egg weight. Shell thickness of pullets and hens of both strains declined in the usual manner with advancing age of the bird.

The percentage of shell was not significantly ($P<0.05$) different for the two strains of pullets or hens in the two experiments (table 2). The percentage of shell and shell thickness indicate shell deposition and shell quality. The commercial strain produced eggs of superior shell quality (indicated by shell thickness and percentage of shell), but the difference between the two strains in percentage of shell was not significant ($P<0.05$).

The shells from the commercial pullets were rougher than those from the New Mexico strain of pullets for period 2 and for all periods combined (table 2). Shell roughness was similar for the New Mexico and commercial strains of hens. Pullets laid dense and smooth eggs during the first period, but the eggs became less dense and rougher in succeeding periods. The increase in shell roughness was accompanied by decreases in shell thickness and in percentage of shell with the advancing age of the chickens.

Feed conversion (pounds of feed per dozen eggs) was higher ($P<0.05$) for the New Mexico strain of pullets in period 3 of experiment I and in period 2 of experiment II (table 2). Commercial hens had a lower ($P<0.05$) feed conversion than New Mexico hens for periods 3 and 5 and for all periods combined.

Increasing the calcium level of the diet from 2.25 to 4.50 percent increased egg production of pullets in the first period of experiment I, but it did not affect egg production of pullets and hens for the other periods (table 2). The lower calcium level was adequate to support egg production under the conditions of these experiments.

Egg weight was greater ($P<0.05$) with the higher calcium level for pullets in period 5 of experiment II but not in the other periods of the two experiments (table 3). The egg weight of hens was improved by the higher calcium level of the diet for period 3 and for all periods combined.

The Haugh score of eggs from pullets and hens was higher ($P<0.05$) with the calcium level of 2.25 percent in both experiments (table 3). The reason for the lower Haugh score with the higher calcium level is not apparent, but the differences in Haugh scores were very small and may be of little practical importance.

Shell thickness of eggs was improved ($P<0.05$) for both pullets and hens with the increase in calcium level of the diet from 2.25 to 4.50 percent (table 3). The lower level of calcium was not adequate

Table 3. The effect of calcium level of the diet on the performance of pullets and hens

Parameter, Experiment, and Calcium Level	Periods of 56 Days*					
	Period 1	Period 2	Period 3	Period 4	Period 5	Mean*
Egg Production (percent)						
Experiment I (pullet)						
2.25	49.0 b	58.4 a	68.1 a	64.0 a	51.4 a	58.2 a
4.50	53.7 a	58.5 a	70.6 a	67.2 a	53.7 a	60.7 a
Experiment II (pullet)						
2.25	62.1 a	61.5 a	65.3 a	61.9 a	59.9 a	61.1 a
4.50	59.8 a	59.8 a	61.4 a	59.7 a	51.8 a	58.1 a
Experiment II (hen)						
2.25	53.4 a	47.7 a	54.6 a	52.3 a	41.4 a	49.9 a
4.50	53.8 a	47.8 a	53.2 a	49.4 a	43.8 a	49.6 a
Egg Weight (grams)						
Experiment I (pullet)						
2.25	55.6 a	59.2 a	59.1 a	60.5 a	60.9 a	59.0 a
4.50	56.4 a	59.4 a	59.6 a	60.5 a	61.3 a	59.4 a
Experiment II (pullet)						
2.25	57.6 a	61.8 a	61.2 a	58.7 a	58.8 b	59.6 a
4.50	58.3 a	61.9 a	61.0 a	59.2 a	60.2 a	60.1 a
Experiment II (hen)						
2.25	63.5 a	64.3 a	63.0 a	59.3 b	49.9 a	62.0 b
4.50	64.2 a	64.9 a	63.7 a	61.5 a	61.3 a	63.1 a
Haugh Score						
Experiment I (pullet)						
2.25	81.8 a	79.4 a	77.8 a	76.3 a	76.4 a	78.4 a
4.50	80.3 a	78.8 a	75.8 b	75.1 a	76.0 a	77.2 b
Experiment II (pullet)						
2.25	80.4 a	77.5 a	75.6 a	76.5 a	75.0 a	77.0 a
4.50	78.0 a	76.7 a	73.6 b	74.9 a	72.7 a	75.2 a
Experiment II (hen)						
2.25	75.6 a	74.5 a	71.4 a	73.9 a	72.4 a	73.6 a
4.50	72.6 b	71.3 b	66.0 b	70.4 b	70.4 b	70.7 b
Shell Thickness (.001")						
Experiment I (pullet)						
2.25	14.1 b	13.9 b	12.7 b	12.6 a	12.8 b	13.2 b
4.50	14.5 a	14.6 a	13.4 a	13.4 a	13.4 a	13.9 a
Experiment II (pullet)						
2.25	13.6 b	13.9 a	13.3 b	12.2 b	12.9 a	13.2 b
4.50	14.1 a	14.1 a	13.7 a	13.3 a	13.1 a	13.6 a
Experiment II (hen)						
2.25	13.1 a	13.5 a	12.8 b	12.0 b	12.5 a	12.8 b
4.50	13.7 a	13.6 a	13.2 a	12.7 a	12.5 a	13.1 a

*Means within experiments in each column followed by the same letter are not significantly different ($P < .05$).

Table 3. Continued

Parameter, Experiment, and Calcium Level	Periods of 56 Days *					
	Period 1	Period 2	Period 3	Period 4	Period 5	Mean*
Percentage of Shell (percent)						
Experiment I (pullet)						
2.25	9.7 a	9.5 a	8.8 b	8.5 b	8.3 b	9.0 b
4.50	9.8 a	9.8 a	9.4 a	9.0 a	8.6 a	9.3 a
Experiment II (pullet)						
2.25	9.3 a	9.3 b	9.2 b	8.7 b	9.4 a	9.1 b
4.50	9.4 a	9.4 a	9.5 a	9.6 a	9.4 a	9.5 a
Experiment II (hen)						
2.25	8.5 b	8.8 a	8.6 a	8.5 b	8.8 a	8.6 a
4.50	8.9 a	8.7 a	8.8 a	8.9 a	8.8 a	8.8 a
Shell Roughness Score						
Experiment II (pullet)						
2.25	2.61 a	3.19 a	3.33 a	3.68 a	4.24 b	3.41 b
4.50	2.60 a	3.24 a	3.40 a	3.79 a	4.54 a	3.51 a
Experiment II (hen)						
2.25	2.88 a	3.46 a	3.56 b	3.84 a	4.53 a	3.66 b
4.50	2.99 a	3.64 a	3.78 a	4.09 a	4.66 a	3.83 a
Feed Conversion (pounds of feed per 12 eggs)						
Experiment I (pullet)						
2.25	6.7 a	5.8 a	5.0 a	5.2 a	6.7 a	5.9 a
4.50	6.0 a	5.9 a	4.8 a	4.9 a	5.4 a	5.4 a
Experiment II (pullet)						
2.25	4.8 a	5.2 a	4.7 a	5.0 a	5.5 a	5.0 a
4.50	5.4 a	5.9 a	5.4 a	5.5 a	6.7 a	5.8 a
Experiment II (hen)						
2.25	5.6 a	6.6 a	5.6 a	6.1 a	7.3 a	6.2 a
4.50	5.5 a	6.1 a	5.7 a	5.7 a	6.5 a	5.9 a

to produce maximum shell deposition, although it was adequate to maintain egg production. Eggs with thicker shells due to the higher calcium level of the diet probably would not break as easily as eggs with thinner shells.

The percentage of shell, which is another measure of calcium deposition or shell quality, increased ($P<0.05$) with the increase in calcium level of the diet (table 3). A significant ($P<0.05$) strain x calcium interaction (table 4) for percent of shell for hens in experiment II revealed that the higher calcium level improved shell percent of the commercial strain of hens but did not affect shell percent of the New Mexico strain of hens. The improvement in percent of shell was accompanied by an increase in shell thickness.

The roughness of egg shells was

greater ($P<0.05$) with the higher calcium level for pullets and hens in experiment II (table 3).

With the higher calcium level, shell roughness increased even though the shell thickness and shell percentage improved. The roughness of shells increased as the pullets and hens became older, but this increase in shell roughness was associated with a decrease in shell thickness and percentage of shell. Shell roughness scores were lower for pullets than for hens. A significant ($P<0.05$) strain x calcium interaction for shell roughness of hens (all periods combined) indicated that the higher level of calcium increased the roughness of the egg shells for the New Mexico strain of hens but did not affect the roughness of egg shells of the commercial strain.

The feed conversion was not significantly affected by increasing the calcium level of the diet from 2.25 to 4.50 percent (table 3).

The principal advantage of increasing the calcium level of the diet above 2.25 percent was an improvement in shell quality. Otherwise, the increase did not greatly change the performance traits of pullets and hens.

The egg production of pullets and hens was not affected by the addition of 20 mg of ascorbic acid to the diet, except that during period 2 of experiment II, the pullets increased production with ascorbic acid supplementation.

The addition of ascorbic acid to the diet of pullets and hens did not affect egg weight, Haugh score, shell thickness, shell roughness, or pounds of feed per dozen eggs (table 5). The percentage of shell of pullets increased with the ascorbic acid addition to the diet during period 3 and for all periods combined in experiment II, but the vitamin did not affect the percentage of shell for the pullets in experiment I or for the hens in experiment II. A significant ($P<0.05$) calcium x ascorbic interaction for shell roughness indicated that the higher calcium level increased shell roughness in the presence of supplemental ascorbic acid (table 4). Ascorbic acid decreased shell roughness with 2.25 percent calcium, but it did not affect shell roughness with the higher calcium level. These results suggest that ascorbic acid may improve shell quality at sub-optimal calcium levels, but that the vitamin may be detrimental at high calcium levels.

Egg production decreased ($P<0.05$) with the addition of reserpine to the diet of pullets in periods 1 and 3 and for all periods combined in experiment I, and for periods 2 and 5 of experiment II (table 6). Reserpine decreased egg production of pullets during the early colder periods but had less effect on egg production during the later warm periods. Reserpine did not affect egg production of hens during any period.

The addition of 0.5 mg reserpine per pound to the diet increased ($P<0.05$) the pounds of feed required per dozen eggs of pullets in periods 2 and 3 and for all periods combined in experiment I (table 6). The increase in pounds of feed per dozen eggs with the higher level of

Table 4. Treatment interactions for periods combined (Experiment II)

Bird and Parameter	Interactions				
Pullet					
Shell Percent	Calcium x Reserpine		Ascorbic Acid x Reserpine		
	Reserpine mg/lb	Calcium percent 2.25	Calcium percent 4.50	Reserpine mg/lb	Ascorbic acid mg/lb 0	Ascorbic acid mg/lb 20
		B	A		B	A
	0	9.02 b	9.50 ab	0	9.08 b	9.44 a
		B	A		A	A
	0.25	9.12 ab	9.58 a	0.25	9.28 a	9.42 ab
		A	A		A	A
	0.50	9.22 a	9.34 b	0.50	9.32 a	9.24 b
Shell Roughness	Ascorbic Acid x Reserpine			Calcium x Ascorbic Acid		
	Reserpine mg/lb	Ascorbic acid mg/lb 0	Ascorbic acid mg/lb 20	Ascorbic acid mg/lb	Calcium percent 2.25	Calcium percent 4.50
		A	A		A	A
	0	3.62 a	3.40 a	0	3.48 a	3.47 a
		A	A		B	A
	0.25	3.46 b	3.52 a	20	3.33 b	3.56 a
		A	A			
	0.50	3.36 b	3.42 a			
Hen						
Shell Percent	Strain x Calcium					
	Calcium percent	Strain New Mexico	Strain Commercial			
		A	A			
	2.25	8.74 a	8.53 b			
		A	B			
	4.50	8.68 a	8.98 a			
Shell Roughness	Strain x Calcium					
	Calcium percent	Strain New Mexico	Strain Commercial			
		A	A			
	2.25	3.53 b	3.80 a			
		A	A			
	4.50	3.85 a	3.79 a			

Means within each column of each interaction followed by the same small letter are not significantly different ($P<.05$). Means within each line of each interaction followed by the same capital letter are not significantly different ($P<.05$).

reserpine was a reflection of the smaller number of eggs produced, because the diets were isocaloric and isonitrogenous. The lower level of reserpine did not affect pounds of feed per dozen eggs. The addi-

Table 5. The effect of dietary ascorbic acid on the performance of pullets and hens

Parameter, Experiment, and Ascorbic Acid (mg/lb)	Periods of 56 Days* Period 1	Period 2	Period 3	Period 4	Period 5	Mean*
Egg Production (percent)						
Experiment I (pullet)						
0	52.1 a	59.5 a	69.0 a	64.7 a	52.1 a	59.5 a
20	50.7 a	57.4 a	69.7 a	66.4 a	53.1 a	59.5 a
Experiment II (pullet)						
0	60.7 a	58.2 b	61.7 a	59.8 a	52.4 a	58.6 a
20	61.2 a	63.1 b	65.0 a	59.8 a	52.3 a	60.3 a
Experiment II (hen)						
0	55.3 a	48.3 a	55.4 a	50.8 a	42.5 a	50.4 a
20	51.9 a	47.2 a	52.4 a	50.9 a	42.6 a	49.0 a
Egg Weight (grams)						
Experiment I (pullet)						
0	56.3 a	59.2 a	59.4 a	60.6 a	61.2 a	59.4 a
20	55.6 a	59.4 a	59.3 a	60.3 a	61.0 a	59.1 a
Experiment II (pullet)						
0	57.5 a	61.7 a	61.1 a	59.1 a	59.7 a	59.8 a
20	58.4 a	62.0 a	61.1 a	58.8 a	59.2 a	59.9 a
Experiment II (hen)						
0	63.7 a	64.6 a	63.0 a	60.3 a	60.5 a	62.4 a
20	64.1 a	64.6 a	63.7 a	60.5 a	60.7 a	62.7 a
Haugh Score						
Experiment I (pullet)						
0	81.0 a	78.7 a	76.2 a	75.3 a	75.1 b	77.3 a
20	81.1 a	79.5 a	77.4 a	76.1 a	77.6 a	78.3 a
Experiment II (pullet)						
0	79.0 a	76.9 a	74.3 a	75.3 a	73.8 a	75.8 a
20	79.5 a	77.3 a	75.0 a	76.2 a	73.9 a	76.4 a
Experiment II (hen)						
0	75.0 a	73.8 a	71.4 a	73.1 a	72.7 a	73.2 a
20	73.2 a	71.9 a	68.8 a	61.2 a	70.1 a	71.0 a
Shell Thickness (.001")						
Experiment I (pullet)						
0	14.4 a	14.2 a	13.2 a	13.1 a	13.2 a	13.6 a
20	14.2 a	14.3 a	13.2 a	13.0 a	13.0 a	13.6 a
Experiment II (pullet)						
0	13.7 b	13.9 a	13.4 a	12.7 a	12.9 a	13.3 a
20	14.0 a	14.1 a	13.6 a	12.8 a	13.1 a	13.5 a
Experiment II (hen)						
0	13.4 a	13.5 a	12.9 a	12.4 a	12.3 a	12.9 a
20	13.5 a	13.6 a	13.1 a	12.3 a	12.6 a	13.0 a

* Means within experiments in each column followed by the same letter are not significantly different ($P < .05$).

Table 5. Continued

Parameter, Experiment, and Ascorbic Acid (mg/lb)	Periods of 56 Days* Period 1	Period 2	Period 3	Period 4	Period 5	Mean*
Percentage of Shell (percent)						
Experiment I (pullet)						
0	9.7 a	9.6 a	9.0 a	8.8 a	8.5 a	9.1 a
20	9.8 a	9.7 a	9.1 a	8.8 a	8.5 a	9.1 a
Experiment II (pullet)						
0	9.3 a	9.2 a	9.2 b	9.1 a	9.3 a	9.2 b
20	9.4 a	9.3 a	9.4 a	9.2 a	9.5 a	9.4 a
Experiment II (hen)						
0	8.7 a	8.7 a	8.7 a	8.7 a	8.7 a	8.7 a
20	8.7 a	8.8 a	8.8 a	8.6 a	8.9 a	8.8 a
Shell Roughness Score						
Experiment II (pullet)						
0	2.66 a	3.18 a	3.41 a	3.73 a	4.41 a	3.48 a
20	2.55 a	3.25 a	3.31 a	3.74 a	4.36 a	3.44 a
Experiment II (hen)						
0	2.99 a	3.56 a	3.74 a	3.97 a	4.66 a	3.78 a
20	2.88 a	3.54 a	3.60 a	3.96 a	4.54 a	3.70 a
Feed Conversion (pounds of feed per 12 eggs)						
Experiment I (pullet)						
0	6.5 a	5.7 a	5.0 a	5.2 a	5.9 a	5.6 a
20	6.3 a	6.0 a	4.8 a	4.9 a	6.3 a	5.6 a
Experiment II (pullet)						
0	5.4 a	5.8 a	5.2 a	5.3 a	6.0 a	5.5 a
20	4.9 b	5.3 a	4.9 a	5.1 a	6.2 a	5.3 a
Experiment II (hen)						
0	5.4 a	6.6 a	5.5 a	6.0 a	7.3 a	6.2 a
20	5.6 a	6.1 a	5.7 a	5.8 a	6.5 a	5.9 a

tion of reserpine to the diet did not affect egg weight, Haugh score, or shell thickness of eggs from pullets and hens.

A significant ($P<0.05$) calcium x reserpine interaction for percentage of shell of pullets for all periods combined in experiment II indicated that reserpine increased the shell percentage with 2.25 percent calcium in the diet, but the higher level of reserpine actually decreased the shell percentage with 4.50 percent calcium in the diet (table 4). A significant ($P<0.05$) ascorbic acid x reserpine interaction for shell percentage of pullets for all periods combined indicated that reserpine increased percentage of shell in the absence of ascorbic acid, but that the higher level of reserpine decreased shell percentage in the presence of ascorbic acid in the diet (table 4). A significant ($P<0.05$) ascorbic acid x reserpine interaction for shell roughness of pullets for

Table 6. The effect of reserpine on the performance of pullets and hens

Parameter, Experiment, and Reserpine (mg/lb)	Periods of 56 Days*					
	Period 1	Period 2	Period 3	Period 4	Period 5	Mean*
Egg Production (percent)						
Experiment I (pullet)						
0	56.2 a	61.8 a	74.4 a	68.5 a	56.6 a	63.5 a
0.25	50.1 b	58.2 a	68.3 a	65.1 a	50.9 a	58.5 b
0.50	47.8 c	55.3 a	65.3 c	63.2 a	50.3 a	56.4 c
Experiment II (pullet)						
0	63.5 a	65.0 a	66.8 a	60.8 a	54.1 a	62.0 a
0.25	59.7 a	58.7 b	61.9 a	60.6 a	55.4 a	59.2 a
0.50	59.6 a	58.3 b	61.3 a	58.1 a	47.6 b	57.0 a
Experiment II (hen)						
0	57.2 a	59.7 a	55.4 a	53.2 a	46.5 a	52.4 a
0.25	53.1 a	48.5 a	53.7 a	50.2 a	40.6 a	49.2 a
0.50	50.5 a	45.0 a	52.6 a	49.2 a	40.6 a	47.6 a
Egg Weight (grams)						
Experiment I (pullet)						
0	56.0 a	59.4 a	59.4 a	60.7 a	62.0 a	59.5 a
0.25	56.5 a	59.4 a	59.5 a	60.5 a	60.0 a	59.3 a
0.50	55.5 a	59.1 a	59.1 a	60.2 a	60.7 a	58.9 a
Experiment II (pullet)						
0	57.8 a	61.6 a	60.5 a	59.2 a	59.0 a	59.6 a
0.25	58.5 a	62.4 a	61.8 a	59.5 a	60.2 a	60.4 a
0.50	57.6 a	61.5 a	61.0 a	58.1 a	59.3 a	59.5 a
Experiment II (hen)						
0	64.5 a	63.9 a	63.3 a	60.5 a	61.2 a	62.7 a
0.25	63.3 a	65.0 a	63.5 a	60.4 a	60.8 a	62.6 a
0.50	63.7 a	64.9 a	63.3 a	60.3 a	58.9 a	62.4 a
Haugh Score						
Experiment I (pullet)						
0	80.6 a	78.9 a	77.2 a	76.1 a	76.6 a	77.9 a
0.25	81.0 a	79.6 a	76.6 a	75.4 a	76.6 a	77.9 a
0.50	81.6 a	78.8 a	76.6 a	75.7 a	75.6 a	77.7 a
Experiment II (pullet)						
0	78.3 a	75.9 a	73.4 a	75.1 a	72.8 a	75.1 a
0.25	80.1 a	78.4 a	75.3 a	75.4 a	74.1 a	76.6 a
0.50	79.3 a	77.0 a	75.2 a	76.7 a	74.7 a	76.6 a
Experiment II (hen)						
0	73.5 a	72.7 a	69.8 a	71.3 a	70.0 a	71.2 a
0.25	73.8 a	72.2 a	69.9 a	71.7 a	72.4 a	72.0 a
0.50	74.9 a	73.7 a	70.6 a	73.6 a	72.8 a	73.1 a

*Means within experiments in each column followed by the same letter are not significantly different ($P < .05$).

Table 6. Continued

Parameter, Experiment, and Reserpine (mg/lb)	Periods of 56 Days					
	Period 1	Period 2	Period 3	Period 4	Period 5	Mean
Shell Thickness (.001")						
Experiment I (pullet)						
0	14.4 a	14.2 a	13.1 a	12.8 a	12.9 a	13.5 a
0.25	14.3 a	14.4 a	13.4 a	13.2 a	13.2 a	13.7 a
0.50	14.2 a	14.1 a	13.2 a	13.1 a	13.2 a	13.6 a
Experiment II (pullet)						
0	13.7 a	13.8 a	13.3 a	12.7 a	13.0 a	13.3 a
0.25	14.0 a	14.1 a	13.8 a	12.8 a	13.1 a	13.6 a
0.50	13.8 a	14.1 a	13.4 a	12.8 a	12.8 a	13.4 a
Experiment II (hen)						
0	13.5 a	13.5 a	13.1 a	12.5 a	12.6 a	13.0 a
0.25	13.4 a	13.7 a	13.1 a	12.3 a	12.6 a	13.0 a
0.50	13.4 a	13.4 a	12.8 a	12.3 a	12.2 a	12.8 a
Percentage of Shell (percent)						
Experiment I (pullet)						
0	9.8 a	9.7 a	9.0 a	8.6 a	8.3 b	9.1 a
0.25	9.7 a	9.7 a	9.1	8.8 a	8.5 a	9.2 a
0.50	9.7 a	9.7 a	9.1 a	8.8 a	8.6 a	9.2 a
Experiment II (pullet)						
0	9.3 a	9.2 a	9.2 a	9.2 a	9.5 a	9.3 a
0.25	9.4 a	9.3 a	9.5 a	9.1 a	9.4 a	9.4 a
0.50	9.4 a	9.3 a	9.3 a	9.2 a	9.3 a	9.3 a
Experiment II (hen)						
0	8.7 a	8.8 a	8.9 a	8.7 a	9.0 a	8.8 a
0.25	8.7 a	8.8 a	8.8 a	8.6 a	8.7 a	8.7 a
0.50	8.7 a	8.6 a	8.5 a	8.6 a	8.7 a	8.6 a
Shell Roughness Score						
Experiment II (pullet)						
0	2.65 a	3.28 a	3.39 a	3.78 a	4.44 a	3.51 a
0.25	2.60 a	3.17 a	3.38 a	3.81 a	4.48 a	3.49 a
0.50	2.56 a	3.20 a	3.31 a	3.61 a	4.24 a	3.39 a
Experiment II (hen)						
0	2.90 a	3.54 a	3.64 a	3.87 a	4.50 a	3.69 a
0.25	2.93 a	3.44 a	3.70 a	4.11 a	4.68 a	3.77 a
0.50	2.97 a	3.66 a	3.67 a	3.91 a	4.62 a	3.76 a
Feed Conversion (pounds of feed per 12 eggs)						
Experiment I (pullet)						
0	5.6 b	5.2 b	5.0 a	5.0 a	5.5 a	5.3 b
0.25	6.6 a	5.9 ab	4.4 b	4.8 a	6.0 a	5.6 ab
0.50	6.8 a	6.4 a	5.2 a	5.3 a	6.8 a	6.1 a
Experiment II (pullet)						
0	4.7 a	5.2 a	4.7 a	5.1 a	5.8 a	5.1 a
0.25	5.2 a	5.5 a	5.0 a	5.0 a	5.7 a	5.2 a
0.50	5.4 a	5.9 a	5.5 a	5.5 a	7.4 a	5.9 a
Experiment II (hen)						
0	5.1 a	6.3 a	5.7 a	5.8 a	6.4 a	5.9 a
0.25	5.6 a	6.2 a	5.5 a	6.1 a	7.5 a	6.2 a
0.50	5.8 a	6.6 a	5.8 a	5.7 a	6.7 a	6.1 a

periods combined (table 4) indicated that reserpine decreased shell roughness in the absence of supplemental ascorbic acid but reserpine did not affect shell roughness in the presence of ascorbic acid.

The addition of ascorbic acid (table 7) to the diet of pullets during the latter half of the production cycle did not affect egg production, egg weight, Haugh score, shell thickness, or shell roughness. Low interior and exterior quality of eggs are more prevalent for commercial egg producers during the latter part of the production cycle, but the ascorbic acid failed to enable pullets to maintain egg quality at any higher level than pullets that had not received ascorbic acid. These results and the results for pullets in experiment I and II suggest that there is little if any advantage to adding ascorbic acid to the diet.

The addition of calcium lactate to pullet diets that already contained three percent calcium did not affect egg production, egg weight, Haugh score, or shell thickness (table 8). There was a small but significant ($P<0.05$) improvement in shell roughness with the addition of calcium lactate to the diet. Shell roughness increased with advancing age of the pullets and followed the same trend reported for experiments I and II.

The supplementation of the diet with magnesium did not affect egg production, egg weight, Haugh score, shell thickness, or shell roughness (table 9). The unsupplemented diet contained 1015 mg of magnesium per pound. This level of magnesium was adequate to maintain the performance of pullets without additional magnesium.

Table 7. The effect of ascorbic acid on the performance of pullets (Experiment III)

Ascorbic Acid (mg/lb)	Parameter	Periods of 168 Days: Period 1	Period 2	Mean
0	Egg Production	57.0 a	44.4 a	51.8 a
20	(percent)	54.8 a	41.5 a	58.7 a
0	Egg Weight	60.3 a	59.6 a	59.9 a
20	(grams)	60.7 a	60.1 a	60.4 a
0	Haugh Score	76.5 a	74.5 a	76.0 a
20		75.9 a	73.8 a	75.0 a
0	Shell Thickness	13.4 a	12.3 a	13.0 a
20	(.001")	13.4 a	12.7 a	13.1 a
0	Shell Roughness Score	2.96 a	3.36 a	3.1 a
20		2.95 a	3.32 a	3.1 a

Means within each column followed by the same letter are not significantly different ($P<.05$).

Table 8. The effect of calcium lactate on the performance of pullets (Experiment III)

Calcium Lactate (percent)	Parameter	Periods of 168 Days Period 1	Period 2	Mean
0	Egg Production (percent)	56.5 a	43.9 a	51.5 a
0.25		55.3 a	42.0 a	49.0 a
0	Egg Weight (grams)	60.4 a	60.3 a	60.2 a
0.25		60.6 a	59.4 a	60.1 a
0	Haugh Score	75.3 a	73.5 a	74.8 a
0.25		77.1 a	74.8 a	76.3 a
0	Shell Thickness (.001")	13.5 a	12.5 a	13.0 a
0.25		13.3 a	12.6 a	13.0 a
0	Shell Roughness Score	2.97 a	3.37 a	3.2 a
0.25		2.94 a	3.31 a	3.0 b

Means within each column followed by the same letter are not significantly different (P<.05).

Table 9. The effect of magnesium on the performance of pullets (Experiment III)

Magnesium (mg/lb)	Parameter	Periods of 168 Days Period 1	Period 2	Mean
0	Egg Production (percent)	57.1 a	45.0 a	51.4 a
50		54.7 a	40.9 a	49.2 a
0	Egg Weight (grams)	60.7 a	60.1 a	60.3 a
50		60.3 a	59.6 a	59.9 a
0	Haugh Score	76.8 a	74.4 a	75.8 a
50		75.6 a	73.9 a	75.3 a
0	Shell Thickness (.001")	13.4 a	12.5 a	13.1 a
50		13.4 a	12.6 a	13.0 a
0	Shell Roughness Score	2.96 a	3.42 a	3.1 a
50		2.95 a	3.26 a	3.1 a

Means within each column followed by the same letter are not significantly different (P<.05).

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Searle-'72
Bib No. 1694

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Determination of Lactate Kinetics in the Human Analysis of Data From Single Injection vs. Continuous Infusion Methods (36284)

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Serious question has been raised about the applicability of the single tracer injection method for the determination of metabolite (lactate) kinetics in the intact animal (dog) (1). Forbath *et al.* note that calculation errors may easily arise in the analysis of data comprising the terminal exponential component of the specific activity time curve, when the substrate in question undergoes very rapid turnover in the plasma. In preliminary attempts to obtain precise early data in a primed infusion study of lactate kinetics in the human, we noted that the final exponential component of the single injection lactate study was a very poor guide to the kinetics of the system. In further investigation into the relationship of the single tracer injection *vs.* primed tracer infusion of lactate in the human we have resorted to a curve-integration analysis as proposed by Steele (2) and as outlined by Tait and Burnstein (3). In the present report we present the results of data analysis from studies of lactate kinetics by both the single tracer injection and primed-tracer infusion techniques carried out in four normal individuals. These results demonstrate that comparable estimates of lactate turnover rates may be obtained by either technique.

Methods. Selection of subjects. Volunteer subjects were selected from a nondiabetic population awaiting elective surgery.

Experimental design and procedures. Experiments were begun at approximately 9:00 AM after an overnight fast of 12 hr. At zero time of each study an injection or infusion of 25 μCi of uniformly labeled ^{14}C-L-(+)-lactic acid[1] was begun according to the appropriate protocol. Following the administration of tracer substrate, blood samples were collected as depicted in Fig. 1 for the single injection tracer study, and as in Fig. 2 for the primed tracer infusion study. All blood samples were drawn rapidly without stasis into dry plastic syringes through indwelling 19-gauge scalp vein needles which had been placed in an anticubital vein and which were maintained patent with physiological saline. Immediately following collection, a measured quantity of blood (20 ml) was dispelled from the syringe to a bottle containing water and barium hydroxide. Zinc sulfate was subsequently added to each bottle in order to precipitate proteins (4). The protein-free filtrates of blood were analyzed by enzymatic methods for both glucose (5) and lactate (6). Blood glucose specific activity was determined by liquid scintillation counting after ion-exchange separation from lactic acid (7). Lactate specific activity was determined from the dimedon derivative of acetaldehyde derived from lactate (8). Expired air from each subject was monitored continuously throughout each experiment with the aid of an automated on stream breath analyzer as described by Tolbert *et al.* (9).

Results. The results of a single injection study are shown in Fig. 1. Comparison of this curve with similar data published by Forbath *et al.* (1) indicates quite similar lactate kinetics in both man and dog. Employing standard methods of graphic analysis of the final slope of the lactate curve we have in fact obtained values very similar to those obtained by Forbath *et al.* for lactate turnover. Employing a curve integral analysis which utilizes all the data of the single tracer injection study (3), we have obtained values for lactate turnover in the resting human

[1] Obtained from International Chemicals, Nuclear Division, Irvine, CA. Diluted with pyrogen free solution and sterilized by Millipore filtration.

TABLE I. Lactic Acid Kinetics in the Human. Results of Single Injection vs. Continuous Infusion Studies.

	Single injection			Continuous infusion		
Subj.	T.R.[a]	R.R.[b]	Ox.R.[c]	T.R.	R.R.	Ox.R.
P.J.	149	5.1	146	128	4.0	130
G.E.	71	4.4	47	83	11.2	68
J.P.	75	10.1	48	75	10.4	50
E.R.	107.2	4.7	85	100	10.1	104
Avg	100.5	6.1	81.5	96.5	8.9	88

[a] T.R. = Lactic acid turnover rate. [b] R.R. = Reduction of lactate to glucose. [c] Ox.R. = Oxidation of lactate to CO_2. All values: mg/kg/hr.

subject that are listed in Table I along with corresponding values obtained by primed tracer infusion techniques in the same subjects. (The values listed for single injection studies are less than would be estimated by final slope analysis and compare well to values calculated from continuous infusion studies.) Also listed in this table are rates of reductive synthesis of glucose from lactate and rates of oxidation of lactate to CO_2. These values were computed from the data shown in Figs. 1 and 2 as previously described by Searle *et al.* (10). Note in Table I that there are random variations in all the indices listed: lactate turnover rate, rate of lactate reduction to glucose, and rate of lactate oxidation to carbon dioxide. Although the numbers of subjects studied is small, we are encouraged by the fact that the averages from both methods agree quite well and that by both techniques, the sum of the values for

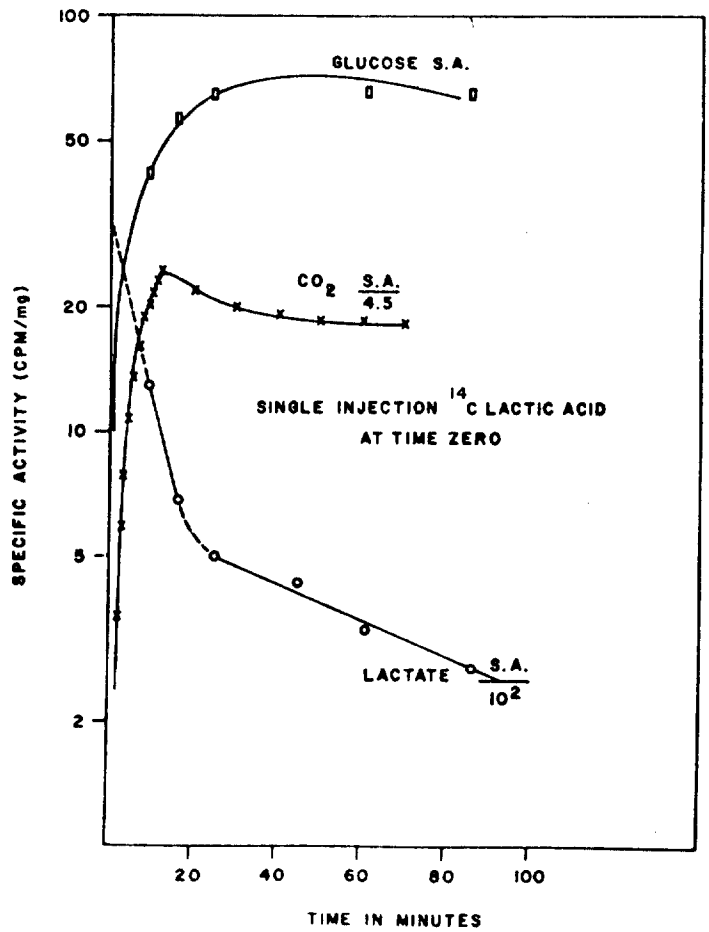

FIG. 1. Specific activity vs. time curve of lactate, glucose and expired CO_2 following a single tracer injection of 25 μCi of uniformly labeled ^{14}C-lactate.

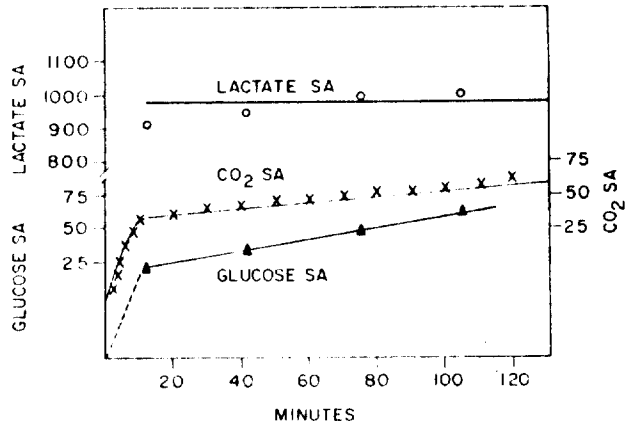

FIG. 2. Specific activity vs. time curve of lactate, glucose and expired CO_2 during the continuous infusion of 25 μCi of uniformly labeled ^{14}C-lactate.

reductive synthesis and for oxidation of lactate to CO_2, roughly equal the values for lactate turnover. From two data points taken early in primed infusion studies, estimates were made of lactate pool size (2). This information, together with lactate concentration and turnover rate, was used to produce estimates of virtual volume of distribution (space) of lactate and the turnover time of the lactate pool. These descriptive assessments of the body lactate pool are presented in Table II. A reassuring and, we feel significant, measure given in this table is the value for lactate space, expressed in % body wt. The average of these values is 49.4% of body wt. Although there are no ready references with which to compare this value of lactate space, it is reasonable to assume that a freely and rapidly diffusable molecule such as lactate would occupy a space at least equivalent to 3/4 of the body water space, as the above value indicates. Kreisberg *et al.* have arrived at similar fractional turnover rates for the lactate pool in humans starting with the assumption that lactate space would be equivalent to body water (11).

TABLE II. Lactic Acid in the Human—Pool Size, Space and Turnover Time. From continuous tracer infusion studies.

Subj.	Pool (g/kg)	Space (% body wt)	Turnover time (min)
P.J.	.035	52.5	20.0
G.E.	.028	43.2	15.5
J.P.	.023	57.0	19.0
E.R.	.032	45.6	18.4
Avg	.029	49.4	18.4

Discussion. Forbath *et al.* have rejected the single tracer injection method for estimation of lactate kinetics on the basis: that calculation of the rates of production of substances undergoing a very rapid turnover in the plasma from the terminal exponential component of the specific activity vs. time curve is subject to large errors. While this is so, it is certainly an insufficient basis for condemnation of the single-injection technique, for which computational procedures exist (3) that very adequately estimate production rates. Employing such procedures, we have demonstrated here that the single-injection technique yields values for lactate production in the human that are quite comparable to those obtained by the techniques of primed tracer infusion. In addition, we have observed that estimates of lactate reduction to glucose and oxidation to carbon dioxide are comparable for the two methods.

We suggest that the single-injection method for determining lactate turnover in humans, as we employ it, is superior to the infusion technique because it offers the following advantages : (a) The method is simple. (b) The data are obtained during a relatively short period (one hour). (c) Error due to recycling of label is relatively smaller

by the single-injection method than by the primed-infusion technique. The evidence presented here with respect to the magnitude of the various pathways of lactate metabolism is consistent with the findings of Drury and Wick (12) from studies in rabbits, and those of Annison *et al.* (13) from studies in sheep. Although they differ from those of Kreisberg *et al.* (14) in the human, with respect to CO_2 production from lactate, these authors admit that they have little confidence in their own measure of this index. In spite of this trepidation on their part, they have been led to speculate that since the lactate flux could not be completely accounted for in product pools, perhaps lactate flux was being overestimated due to a rapid equilibrium flux between lactate and pyruvate. We discount this argument on the basis of the ready accountability of carbon flux in the product pools in our study as well as in those of Drury (12) and Annison *et al.* (13). Indeed, we suggest that lactate kinetics in the human represent the flux (and perhaps total flux) of carbon through pyruvate. Using some preliminary figures we have developed for the flux of glucose to lactate (15), plus figures from past publications and other references which define glucose turnover and oxidation rates (16-18), and the figures presented in the present communication for lactate turnover and oxidation, we are able to derive approximately equal estimates of glucose oxidation given data from experiments employing either glucose or lactate as the administered labeled substrate. Thus, glucose turnover is approximately 100 mg/kg/hr with approximately 57 mg/kg/hr entering the lactate pool. lactate turnover is approximately 95 mg/kg/hr with approximately 90% being oxidized to CO_2. Note that of the 57 mg/kg/hr of glucose coming to lactate per hour is oxidized to CO_2, 90% times 57 = 51 mg/kg/hr which is the approximate rate of glucose oxidation derived from numerous studies with carbon-14 glucose (12-14).

In conclusion, we argue as do Steele (2) and Tait and Burnstein (3) that comparable information may be derived from single tracer injection and primed tracer infusion studies. Each has its own merits and shortcomings. The important fact that one should keep in mind is that whichever method is chosen, it does contain the basic kinetic information that one seeks from such experiments (if all the data are examined employing methods of mathematical analysis that are appropriate to the task at hand). With regard to the physiologic aspect of this study, we would draw the following conclusions: (a) Blood (body) lactate kinetics probably reflect the total flux of carbon through the metabolite pyruvate; (b) The primary fate of body lactate flux is not reduction to glucose in hepatic tissue, but oxidation to CO_2 probably in peripheral tissues as demonstrated by Drury *et al.* (19), and most recently in human forearm studies by Jorfeldt (20).

Summary. Comparison has been made between single injection and primed infusion methodology as a means of estimating turnover, oxidation, and reduction of lactate in the human. It is concluded that all these indices of lactate metabolism can be measured equally well with either the single injection or the primed infusion technique. The magnitude and fate of the lactate flux as determined in these studies suggest to us that lactate kinetics as measured with ^{14}C-lactate reflect the entire body flux of carbon through pyruvic acid.

This work has been supported in part by a grant from the U.S.V. Pharmaceutical Company, New York.

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Proc. Amer. Assoc. Cancer Res. 4(1):61. 1963.

Selawry-'63
Bib No.1699

240 GROWTH INHIBITION OF SARCOMA 180 BY LACTIC ACID. Oleg S. Selawry and Marilyn R. Schwartz Roswell Park Memorial Institute Buffalo 3, N.Y.

d-l-lactic acid induced reproducible, dose related inhibition of Sarcoma 180 in Ha/ICR-Swiss mice: 6-7 x 10^7 ascites tumor cells were injected subcutaneously. Groups of six mice of each sex per dose level received intraperitoneal injections of lactic acid in saline on days 1, 2, 4 and 5 after tumor implantation. Two control groups of each sex received corresponding volumes of saline. The tumors were excised and weighed on day 6.

Injection of 150 mg/kg/day caused mean tumor inhibition of 64.5% (mean weight loss 11.75%; 58% of the animals survived). Tumor inhibition of 31.5% with weight loss of 3.5% occurred following injection of 75 mg/kg of lactic acid. All animals survived. The results are significant at the p .01 level. Comparable results were obtained with l-lactic acid. Sodium and calcium lactates were ineffective at corresponding molarity.

The effect of lactic acid, pyruvic acid and related organic acids on a spectrum of newly transplanted and established tumors will be presented.

Shigehisa, T.: DIET SELECTION BY ALBINO RATS. II. SELF-SELECTION OF INORGANIC SALTS BY NORMAL ALBINO RATS. Eiyo to Shokuryo, Vol. 15, pp. pp. 377-9, 1963. Kanagawa Nutritional College.

Following the previous study (1) in which the selection of food among starch, olive oil, casein, liver oil, and yeast by animals was observed, the present study was designed to investigate the selection of organic salts by rats.

According to literatures, inorganic salt deficiency results in abnormal appetite toward salts.

Phosphorus-deprived animals begin to devour bones and corps, while iron-deprived animals nibble on nails and iron wire (2). It has been observed in both humans and animals (3-6) that the impairment of adrenal function abnormally increases their craving for table salt.

In view of these studies, a self-selection experiment was carried out in order to examine the pattern of spontaneous selection of inorganic salts by animals under normal condition and to obtain the criteria for the study of changes in dietary pattern under various conditions.

EXPERIMENTAL PROCEDURE

The subjects, the feed other than inorganic salts, care and feeding of the animals, experimental period, and feeding apparatus were the same as those in the previous study (1).

The inorganic salts offered to the animals were all 1st-grade reagents. According to Griffiths' experiment (7), 30 cc portions of aqueous 3% NaCl, 4% NaH_2PO_4, 1% K_2HPO_4, 2% calcium lactate, 1% $MgSO_4$, and 2% iron citrate solutions and distilled water were transferred to graded drinking bottles, which were placed in individual cages at random positions. The amount of intake was read as closely as to 0.1 cc at 24 hours intervals, and the content was replaced with fresh solution or water.

RESULTS AND DISCUSSION

The intake of inorganic salts was expressed in terms of mg per day. Figure 1 shows the average daily intakes of individual rats in mg throughout the experiment. The graph indicates that the animals ingested various salts in different patterns, with no specific, common tendency among the rats.

For instance, rat 1 showed the largest intake of calcium lactate among various salts, followed in rank by NaCl, K_2HPO_4, and iron citrate in that order. The intakes of NaH_2PO_4 and $MgSO_4$ were lowest.

Rat 3 took K_2HPO_4 in the largest quantity, and the intakes of NaCl,

calcium lactate, $MgSO_4$, NaH_2PO_4, and iron citrate were lower in that order.

The amount of NaCl consumed by rat 4 was extremely large, and the amounts of other salts were nearly the same.

Let us now average the intakes (mg/day) of individual rats for every 5 days (unit period) and connect the results with straight lines. Figures 2 - 4 were thus obtained. It is found that the rats selected each salt at nearly the same proportion throughout the entire period.

More specifically, as shown in Figure 2, rat 1 consumed each salt at nearly the same proportion in each period. Studying the chart more closely, the intake of NaH_2PO_4 is highly constant, and those of K_2HPO_4, calcium lactate, and $MgSO_4$, less constant, whereas the intakes of NaCl and iron citrate show relatively wide variation. Rat 3 whose selection pattern is illustrated in Figure 1 showed a wide variation in the consumption of NaH_2PO_4, whereas its iron citrate intake was relatively stable. Rat 4 (Figure 4) indicated an abnormally large intake of NaCl, but the level of consumption was constant. Thus, there was a notable individual variation in the selection of inorganic salts from rat to rat.

The consumption of individual salts by the entire animals (9 rats) is summarized below.

(1) NaCl

The daily average intake by each rat throughout the entire period varied widely from 22 to 120 mg. The fluctuation range of each rat was also wide, ± 2 - ±24.

(2) NaH_2PO_4

The individual variation was particularly notable, from 0 to 8 mg. The fluctuation within each rat was also considerable, from ±0 to ±24.

(3) K_2HPO_4

The average intake ranged from 20 to 75 mg. The individual difference was comparatively less. The variation shown by each rat was within the range of ±0.8 - ± 6, the amount of consumption being relatively constant.

(4) Calcium lactate

The individual difference was notable (26 - 122 mg), but the fluctuation shown by each animal was relatively small, within a range of ±2 - ±16.

(5) $MgSO_4$

The individual difference was most minimal, an average intake being 14 - 37 mg. The fluctuation shown by each rat was relatively great, ±1 - ±10.

(6) Iron citrate

The average value of individual rat ranged from 10 to 42 mg, and

the fluctuation range was ±1 - ±9.

CONCLUSION

The experimental results indicate that, just as inthe previous experiment with organic nutrients, the animals showed no specific pattern in the intake of organic salts, with all the animals taking organic salts in their own, different patterns.

The average values of intake by individual rats throughout the entire period (Figure 1) indicated no specific selective tendency, with a considerable individual difference. However, the average consumption of every 5 days (Figures 2,3,4) showed that the rats continued to take specific amounts of salts throughout the experiment.

As compared with the consumption of organic nutrients, the individual variation in the consumption of inorganic salts was more notable.

The author is grateful to Prof. Teruuchi of this faculty for his advice throughout this experiment.

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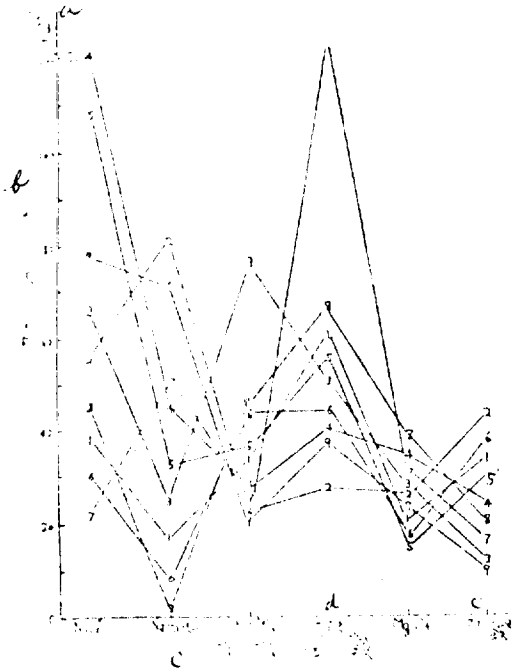


Figure 1. Average Intakes

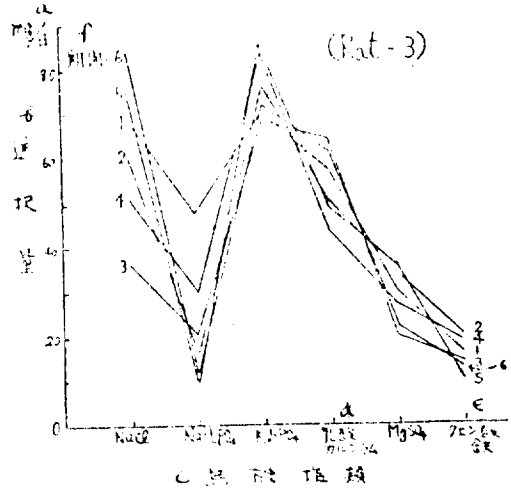


Figure 3. Selection Pattern (II)

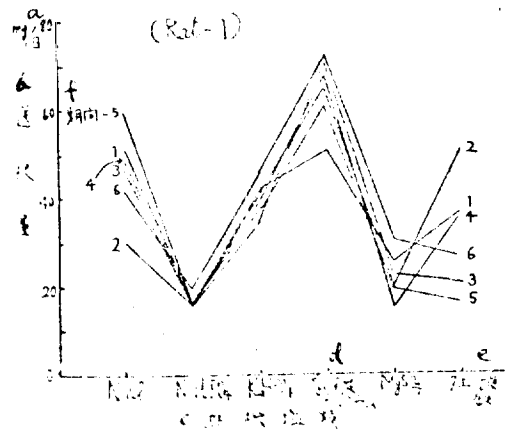


Figure 2. Selection Pattern (I)

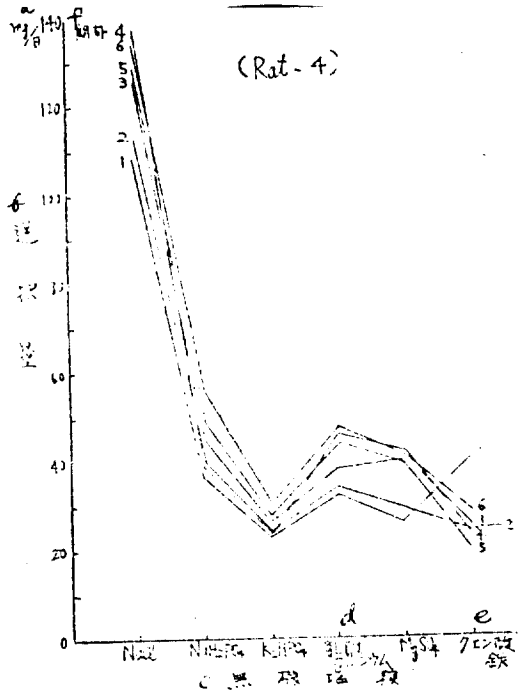


Figure 4. Selection Pattern (III)

KEY: a, mg/day; b, amount consumed; c, inorganic salts; d, calcium lactate; e, iron citrate; f, period

Shigehisa-'63
Bib No. 1720

栄養と食糧

ラッテの食物選択に関する研究(第2報)

Studies on the Diet Selection in the Albino Rat (II)

正常ラッテの無機塩類の自由選択について

Self-selections of inorganic salts in the normal albino rats

(昭和37年5月28日受理)

重久　剛
(Tsuyoshi Shigehisa)

In the self-selections of inorganic salts, selection patterns varied markedly individually as were found in organic nutrients.

A constant selection pattern was not found in the mean selections of the rats throughout the experiment, and then individual differences were noted.

However, mean selection of 5 days of individual animal showed that each animal maintained a constant selection in the individual manner throughout the experiment.

Larger fluctuations were observed among animals in th selections of inorganic salts as compared with those of organic nutrients.

前報[1]においては，動物に食物を自由に選択させた場合に，ラッテがデンプン，オリーブ油，カゼイン，肝油，酵母などのような割合で摂取するかを検討したが，本報においては，これにひき続いて，ラッテが無機塩類をどのように選択するかを実験した結果を報告する。

先人の報告によると，無機塩類の欠乏によって，その塩類に対する異常な食欲が起こることが知られている。

すなわち，リンが欠乏すると骨や死骸をさかんにむさぼり食うようになり，鉄の欠乏した動物は，釘や鉄線を舐るようになる[2]。また副腎機能が障害されると，食塩に対する欲求が異常に高まることが，人や動物で観察されている[3)4)5)6)]。

今回は，これらの報告を考慮し，自然の状態における動物の無機塩類選択の様相を観察するとともに，種々の条件によって，この食塩類がどのように影響されるかを検討する[illegible]ために，前報にひき続いて無機塩類についての自由選択実験を行なった。

実験方法

被験動物，無機塩類以外の飼料，動物の取り扱い，実験期間，および[illegible]などは，前報[1]と全く同様である。

[illegible]無機塩類は，すべて試薬一級品を用い，Griffiths[7]の実体に従って，3%NaCl，4%NaH_2PO_4，1%K_2HPO_4，2%乳酸カルシウム，1%$MgSO_4$，2%[illegible]ン酸鉄の各水溶液および蒸溜水を，各々30ccずつ[illegible]き給水ビンに入れ，毎回ランダムに位置を変えて[illegible]謝ケージの上方から与え，24時間ごとに摂取量を0.1[illegible]まで読みとり毎回新しく入れかえた。

結果と考察

測定の結果は，各無機塩類の摂取量を，1日[illegible]mg数で表わし，各ラッテについて，摂取状況の全[illegible]中の平均値を示せば，第1図の如くで，各ラッテは[illegible]ぞれ各塩類を特異な割合で摂取しており，各ラッテ[illegible]通した摂取の割合などは見出し得なかった。

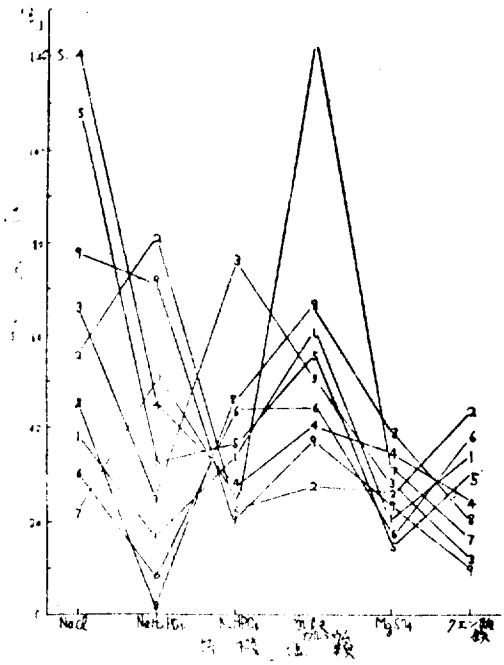
例えば，ラッテ・1についてみれば，乳酸カルシ[illegible]の摂取量が最も多く，NaCl，K_2HPO_4，クエン酸[illegible]れに次ぎ，NaH_2PO_4，$MgSO_4$が最も少ない。

これに対して，ラッテ・3は，K_2HPO_4の摂取[illegible]も多く，NaClがこれに次ぎ，つづいて乳酸カル[illegible]ム，$MgSO_4$，NaH_2PO_4，クエン酸鉄の順で減少[illegible]る。

またラッテ・4では，NaClの摂取量が極めて多[illegible]その他はほぼ同様の摂取量を示している。

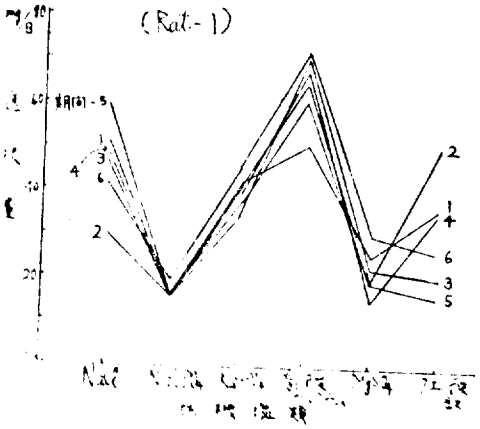
しかるに，各ラッテの摂取量(mg/日)を5日[illegible]

第 1 図 平均摂取量



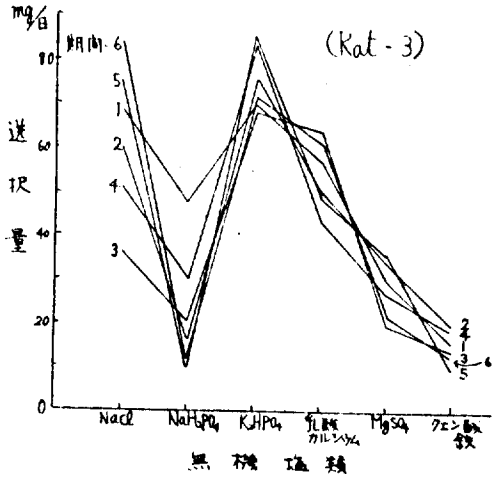
これを1期間とする）に平均して，各塩類の摂取量をそれぞれ直線で結ぶと，第2，3，4図の如くで，ラッテは各期ごとにほぼ同様の割合で各塩類を選択しることがみとめられる。

第 2 図 選択形態（I）

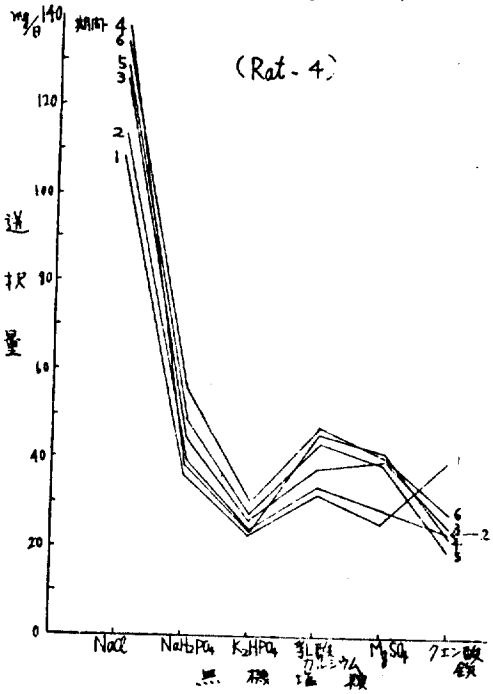


ないが，第2図に示す如く，ラッテ・1は，各期間ほぼ同様の割合で各塩類を選択していることがみとめられる。しかしこれを詳細に検討すると，NaH_2PO_4，$MgSO_4$は極めて一定しており，K_2HPO_4，乳酸カルシウム，$MgSO_4$がこれに準ずるのに対し，NaCl，クエン酸鉄摂取量は比較的大きく変動している。しかるに第3図に示すラッテ・3では，NaH_2PO_4が大きく変動しているのに対し，クエン酸鉄は比較的一定した選択量を

第 3 図 選択形態（II）



第 4 図 選択形態（III）



示している。また第4図に示すラッテ・4では，NaClの摂取量が異常に多いにもかかわらず，ほぼ一定量を選択しているなど，ここにも各ラッテに，それぞれ著しい個体差が認められる。

次に各塩類について全被験動物（9匹）の摂取状況をまとめた。

(1) NaCl

各ラッテの全期間を通じての1日平均摂取量は，22～120mg で個体差が大きく，各ラッテの変動範囲も，±2～±24で比較的大きい。

(2) NaH_2PO_4

個体差が特に著しく，0〜80mgで，各ラッテ自身の変動も大きく，±0〜±24の範囲にある。

(3) K_2HPO_4

各ラッテの平均摂取量は，20〜75mgで，他の塩類に比べて個体差が少なく，また各ラッテ自身の変動も，±0.8〜±6で，ほぼ一定の摂取を続けている。

(4) 乳酸カルシウム

個体差は大きく，26〜122mgの範囲にあるが，変動は比較的小さく，±2〜±16の範囲にある。

(5) $MgSO_4$

個体差は最も小さく各ラッテの平均値は14〜37mgであるが，各ラッテ自身の変動は，±1〜±10で，比較的大きい変動を示している。

(6) クエン酸鉄

各個体の平均値は，10〜42mgの範囲にあり，変動は±1〜±9であった。

総　　括

以上の結果は，無機塩類の摂取量も，有機栄養素の場合（前報）とほぼ同様に，各被験動物は，それぞれ非常に異なった一定の選択形態を示すことを明らかにしている。

すなわち，全期間を通じて各ラッテの摂取量をそれぞれ平均した値（第1図）には，ほとんど一定の選択形態は認められず，著しい個体差を示すが，各ラッテの摂取量を，それぞれ5日ごとに平均した値（第2，[illegible]図）は，全期間を通じて各ラッテが，それぞれ，[illegible]定の摂取量を維持し続けたことを示している。

しかし有機栄養素の場合に比較すれば，無機塩[illegible]取量には，同一の個体についても，変動が大きい[illegible]認められる。

終りに臨み，御指導を賜った本学の照内淳也教授，[illegible]謝の意を表します。

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Am. J. Dis. Child., 69, 1945.

Trainer-'45
Bib No.1887

ESOPHAGEAL STENOSIS DUE TO LACTIC ACID

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PORTLAND, ORE.

The recent report of Young and Smith [1] has called attention to the possible caustic effect of lactic acid. These writers reported 3 deaths due to the action of this substance in feeding formulas and supported their observations with data derived from experiments with rabbits. The possibility that severe reactions may occur in infants as a result of errors in preparation of feeding formulas seems to have been generally overlooked in recent years, despite the widespread use of lactic acid. While Young and Smith were unable to find mention of this substance in any of the standard textbooks on toxicology, we found it briefly discussed under both "Caustic Acids" and "Organic Acids" in Sollmann's "Manual of Pharmacology." [2]

We have recently encountered a case exhibiting the chronic effects of accidental swallowing of this substance. So far as we have been able to ascertain, the late sequelae of lactic acid in the alimentary tract have never been recorded. A bibliographic search brought to light one reference worthy of being added to the literature cited by Young and Smith. It is especially interesting because it appears to be the first record of extensive use of lactic acid by clinicians as a therapeutic agent. Eckerbom [3] reviewed a presentation of the subject by Dr. Jelinek at the Doctoren-Collegium in Vienna, Nov. 9, 1885, together with a discussion by Bum, Mosetig-Moorhof, Wienlechner and several others who had made use of this substance. Jelinek's description of the action of lactic acid is interesting.

When fresh mucous membrane is painted with lactic acid there immediately appear marked reddening, and often even thickening, of the epithelium, but there is never pain or formation of a crust. If, however, pathologically changed mucosa is treated, an intense burning pain occurs, and a corrosion body is formed. The more watery and edematous the swelling, the more pronounced is this reaction, and the more painful the painted part may be. If it is located in the larynx, hoarseness and cramp of the glottis follow if the solution applied is as strong as 20:100. However, the reaction is gradually reduced in degree under the treatment described, and eventually concentrated lactic acid can be used, even in the larynx.[3a]

Bum described the use of lactic acid–silica paste, with which ". . . clearly pathologic, spongelike, easily bleeding granulations change to grayish black, easily removed, porridge-like masses; after this change the wounds appear as if scraped out with a sharp instrument. Their walls appear reddish, feel firm and do not bleed even if roughly handled." Mosetig-Moorhof used up to "1 syringe of 50 per cent solution" in treating subcutaneous epithelioma and obtained "good results." Up to that time these men had used lactic acid as a therapeutic agent as follows: Jelinek had used it in the treatment of laryngeal tuberculosis; gangrenous, granular and hypertrophic pharyngitides; ozena, and scrofulous and hypertrophic rhinitis; Bum, in the treatment of localized tuberculosis of the skin and of the lymph glands with ulceration and fistulas; and Mosetig-Moorhof, in the treatment of lupus, papilloma, epithelioma and carcinoma.

In the German pharmacopeia of Eckerbom's day (1886) lactic acid is described as *Acideum lactecium,* a colored, inodorous, clear and somewhat thick-flowing fluid, not vaporizing at ordinary temperatures. A current description of lactic acid is found in the "Pharmacopeia of the United States" [4] and the "British Pharmacopeia." [5] In the former it is listed as "a mixture of $HC_3H_5O_3$ and lactic anhydride equivalent to a total of not less than 85 per cent and not more than 90 per cent of $HC_3H_6O_3$." Lange's "Handbook of Chemistry" [6] gives the dissociation constant of lactic acid as 1.55×10^{-4}. A 10 per cent solution is roughly equivalent to a one and one-tenth molar solution. Calculated by the usual formula the p_H of such a solution is 1.88. However, the ordinary method of calculation is not strictly applicable when the molar strength is as high as in the present instance; so the p_H values of a series of dilutions of lactic acid

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1. Young, E. G., and Smith, R. P.: Lactic Acid, a Corrosive Poison. J. A. M. A. **125**:1179-1181 (Aug. 26) 1944.

2. Sollmann, T.: A Manual of Pharmacology, ed. 6, Philadelphia, W. B. Saunders Company, 1942.

3. Eckerbom: Lactic Acid as a Caustic, Eira **10**: 15-17, 1886.

3a. Prof. Olof Larsell provided a translation from the Swedish.

4. Lactic Acid, in Pharmacopeia of the United States XII, Easton, Pa., Mack Printing Co., 1942, p. 23.

5. Lactic Acid, in British Pharmacopeia, London, Constable & Co., Ltd., 1932, p. 26.

6. Lange, N. A.: Handbook of Chemistry, ed. 3, Sandusky, Ohio, Handbook Publishers, Inc., 1939.

U. S. P. were determined electrometrically by one of us (J. B. T.). Similar dilutions in reconstituted evaporated milk, such as is used in preparation of feeding formulas, indicated that a 20 per cent solution has a p_H sufficiently low to act as a direct caustic agent, while an aqueous solution of only 10 per cent has that property (fig. 1).

These data emphasize the fact that the purely chemical concept of lactic acid as a weak acid should not be carried over to physiologic thinking. They indicate the basis of its caustic activity, which, taken in conjunction with the case to be reported here and the observations of Young and Smith, makes it evident that lactic acid is a potentially dangerous substance.

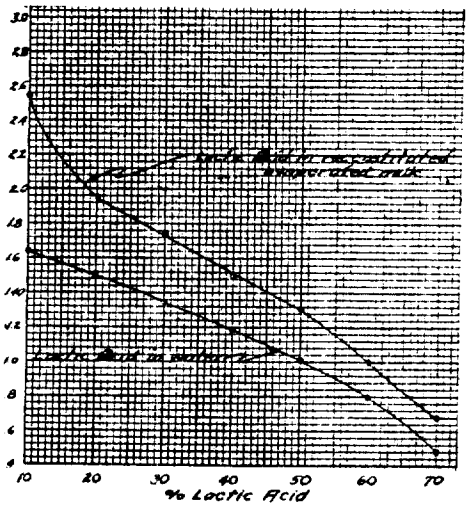

Fig. 1.—Chart showing the p_H of various solutions of lactic acid U. S. P. in reconstituted evaporated milk and in water.

REPORT OF A CASE

History.—J. H., a month old boy weighing 6.7 Kg., entered Doernbecher Memorial Hospital for Children, Portland, Ore., on Aug. 26, 1944 with a history of abrupt onset of inability to swallow, with regurgitation of his feeding formula into the nose and mouth for the previous twenty-four hours. His history revealed that at 2 weeks of age the infant received by error a teaspoon of lactic acid of full strength (U. S. P. 87.5 per cent), instead of elixir of phenobarbital. Ingestion of the acid resulted in visible burns in the oral cavity, with stasis of secretions in the pharynx. Because of burns of the mouth the child required hospitalization for ten days. Convalescence was complicated by an attack of measles followed by otitis media, which persisted for a month. Stasis in the pharynx required postural drainage before the child could be fed, and even then there were gagging and regurgitation. At 3 months of age he could tolerate full feedings of the formula but could not swallow solid food which was added to them. The patient was otherwise asymptomatic until he reached 5 months of age, when inability to swallow suddenly developed. This condition lasted five hours and was relieved by vomiting a small piece of paper. Otherwise the infant did well until the final episode, when he again could not swallow feedings and regurgitated through the nose and mouth. The parents stated that the baby appeared hungry, was afebrile and was in full control of his extremities.

Physical Examination.—Physical examination revealed an irritable, mildly dehydrated 7 month old boy, who did not appear to be in acute distress. His temperature was 102.4 F. The pharynx was injected and was filled with frothy mucus. The infant gagged when offered water and regurgitated it through the nose and mouth. The chest was resonant throughout; expansion was free and equal; numerous resonating rales of all sizes were heard throughout the pulmonary fields, associated with rough breath sounds. No neuromuscular irregularities were noted. Hematologic examination revealed a white cell count of 8,000, with polymorphonuclear leukocytes 68 per cent, staff forms 2 per cent, small lymphocytes 27 per cent and monocytes 3 per cent. A fluoroscopic examination made while the child swallowed barium sulfate revealed an obstruction characteristic of stricture at the level of the tracheal bifurcation. Esophagoscopic examination showed an old, scarlike stricture, which had a small slit opening.

Diagnosis, Treatment and Course.—The impression was that the infant had an esophageal stricture due to an old corrosive burn, complicated by aspiration pneumonia. The child was given sulfonamide compounds, fluids administered parenterally and supportive treatment but failed to respond; he died on his second day in the hospital, August 27.

REPORT OF NECROPSY

Gross Examination.—Only the pertinent observations need be described. The body was 65 cm. in length and apparently well nourished. The anterior fontanel was open, the posterior closed. The mucosa of the oral cavity was smooth, pink and glistening. There were no visible teeth. The subcutaneous fat was from 2 to 5 mm. thick anteriorly.

The opened esophagus (fig. 2) exhibited a stricture just below the level of the thyroid cartilage. The lumen had a circumference of 2 cm. above the stricture; it narrowed to a little less than 1 cm. at the stricture and broadened to 2 cm. below it. A second stricture was located 2.4 cm. inferiorly; at this point the opening again narrowed to approximately 1 cm. A small wad of folded paper was removed from the region between the strictures. The paper was moist but still somewhat firm. A perforation of the esophagus 2 mm. in diameter was present midway between the strictures and appeared to extend posteriorly into the mediastinum. There was a green discoloration of the mediastinal tissues above and below the perforation for a distance of approximately 5 mm. Below the second constriction the circumference of the esophagus broadened to 2.4 cm.; it was 3 cm. at the cardiac orifice.

The mucosa of the trachea and the main bronchi was pink, with a red exudate on its surface. The upper lobe of the left lung was crepitant; the lower lobe was firm and red posteriorly and crepitant and pink anteriorly. The right lung was pink both externally and on the cut surface, and numerous petechiae were scattered over the external surface. The gastric mucosa had a few small areas of focal hyperemia but was otherwise without gross change.

After thorough fixation the esophagus was sectioned. Sections in the longitudinal direction showed a distinct whitish line 2 to 3 mm. thick in the presumptive mucosal layer at the level of the stricture. Elsewhere

the mucosa was less than 1 mm. thick. In the location of the submucosa all along this area a yellow to brown line was to be seen. The muscularis was not well defined macroscopically. A transverse cut that included the small opening in the mucosa previously mentioned readily explains the longitudinal ridge that showed so plainly from the inside of the opened esophagus. The perforation extended at an angle through the wall for a total distance of 2 cm. in the longitudinal direction, with a breadth of 1 cm. in the transverse direction. The outer part of the esophageal wall was soft, mushy and blackish.

Microscopic Examination.—Esophagus: Sections of blocks cut longitudinally at, above and below the point of constriction disclosed a transitional stratified squamous epithelium that was not appreciably thicker than normal or irregular in outline save for a dipping into

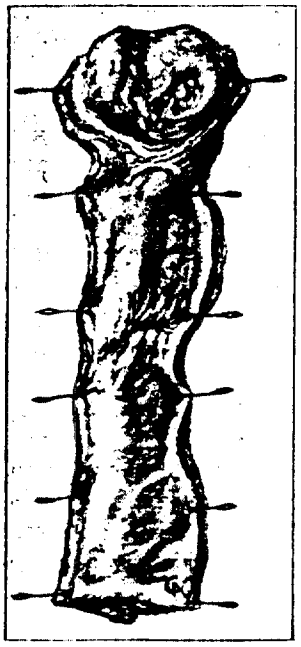

Fig. 2.—Halftone drawing of the esophagus, natural size. The upper stricture is the more pronounced. Between the strictures is a prominent ridge, over which is a small defect leading into the esophageal wall. Inflammation and necrosis are responsible for the ridging.

folds. The various layers normal to this type of mucosa were represented in the proper order. At one point a microscopic-sized denuded area interrupted the squamous surface. The connective tissue here showed evidence of necrosis or digestion, but there was no inflammation. There was no way of knowing whether or not this condition was the result of the digestive action of vomitus. The submucosa was considerably thickened, especially where the greatest stenosis existed. Furthermore, the density of the tissue here and its abundant collagen contrasted with the looseness of the normal structure of the submucosa. Rather deep down was a small, elongated focus of hemorrhage. There was also an increase of connective tissue in the muscularis, where the tissue was seen in the form of distinct streaks. One section passed through the small point of perforation of the mucosa. Here was seen not only necrosis of the mucosa and the submucosa but also well defined, acute, exudative inflammation, which spread into the submucosa and the muscularis. Most of the wandering cells were neutrophilic granulocytes, the remainder being monocytes and lymphocytes.

Lungs: The bronchi showed numerous areas containing both neutrophils and desquamated epithelium. Some air sacs were likewise filled with granulocytes. In both bronchi and air sacs there was occasional granular bluish material, such as one associates with vomitus, together with masses of yellowish pigment which might have been bile. It seems likely, therefore, that the bronchitis and the pneumonia had an aspiratory basis. Atelectasis and hyperemia completed the picture.

COMMENT

The facts of the present case require little elaboration. The clinical history was exceptionally clearcut and was in accordance with the observations at the postmortem examination, which was conducted within an hour after the patient's death. There was a history of sudden onset of caustic poisoning, with esophageal stenosis which developed steadily and was made suddenly acute by lodgment of a foreign body (paper wad) between two strictures. This incident may or may not have been associated with the existence of the small area of perforation. The presence of the foreign body, in all probability, led to the final episode of regurgitation, aspiration pneumonia, and asphyxia.

One may say, then, that poisoning with lactic acid initiates a series of events which are disabling at best; at worst, as in this case, the poisoning is an essential contributory cause of death. The present case differed from the cases of acute poisoning by lactic acid which have already been cited, but the pathologic process was undoubtedly the same in all. The baby's survival of the immediate effect of the caustic is ascribable to the fact that spasm and regurgitation of the bulk of the acid took place almost immediately, so that little if any acid reached the stomach or bowel and was absorbed.

SUMMARY AND CONCLUSIONS

An infant had caustic burns of the mouth and pharynx because of ingestion of concentrated lactic acid. His case differed from those previously reported in that the patient lived long enough for healing and esophageal stricture to take place.

The caustic effects of lactic acid described by Jelinek and Bum in 1885 are essentially similar to the changes observed in the present case.

The thesis of Young and Smith, that concentrated lactic acid should be considered a caustic poison and that care should be exercised in labeling and dispensing this substance, is strongly supported by the facts of the present case.

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Roczniki Panstwowego Zaktadu Hig. 3:273-292. 1952.

Wysokinska-'52
Bib No. 2035

A COMPARISON OF THE EFFECTS OF LACTIC AND ACETIC ACID ON THE RAT ORGANISM
(Porównanie Dzialania Kwasu Mlekowego i Octowego na Organizm Szczura)

by

Zofia Wysokinska

From the S.G.G. Institue of Physiology of Human Nutrition in Warsaw

After the appearance of 50% lactic acid on the market as a consumption article to substitute for vinegar, the question arose as to the nature of its physiological effect and the amounts in which it can be used without any danger to the health. For this reason, I decided to concern myself with this problem by examining the effect of this acid on the animal organism. I used white rats for the experiments. The selection of the rats as experimental animals was prompted by the fact that the framework of a large part of nutritional studies has been worked out on the basis of tests on rats and forms a good basis for laying out the fundamentals of economical human nutrition. I therefore assumed that they would react in a manner most similar to man upon introduction of appropriate doses of lactic acid.

Despite the fact that in Germany, especially during the last war, lactic acid was prescribed for human consumption, the literature I was able to find on the subject of its physiological action is rather limited. On the other hand, many

articles are dedicated to the question of transformation of lactic acid under physiological and pathological conditions. The question of appearance of lactic acid in relaxed and in fatigued muscles is widely treated. Peters and Slyke (1), citing a series of authors, thoroughly discuss conversion of lactic acid in the muscles. Guli and Novikowa (2), on the basis of experiments with rabbits fed with an acid diet, an alkaline diet and a neutral diet, confirm that the lactic acid level in the blood, the muscles and the liver was lower, and the efficiency of the muscles greater in those animals fed the acid diet. The question of conversion of lactic acid into glycogen is also touched upon in several articles. C.F. Cori and G.T. Cori (3) say that in rats glycogen forms more easily from d-lactates than l-lactates, pointing out that l-lactic acid is used 4 times less than its d-form. Meyerhof and Lohman (4) obtained similar results when they studied the synthesis of glycogen in isolated mammal tissues. Grant (5) also studied the formation of glycogen in cats after introduction of ammonium lactate through the upper visceral artery. The differences in assimilability of various food compounds after introduction of acids was determined in pigs by Hansen (6), in ruminants by Liebseher (7), and in poultry by Burckhardt (8). More recent works (9, 10, 11), discussing the differences lactic acid undergoes in the organism, are based on tests performed with lactic acid containing radioactive carbon in the carboxyl group or in position alpha or beta. The authors explain how much radioactive carbon was contained in the formed glycogen, and how much in the eliminated carbon dioxide in relation to the location of the radioactive carbon.

The chief object of the above studies was to clarify the changes that lactic acid undergoes in the organism. However, I found very few experimental studies, in the literature available to me, performed with the goal of testing the effect of lactic acid on the organism. Hermann (12) discusses experiments on rabbits he intravenously fed acids, including lactic and acetic acid. The author underlines the paradoxical alkalizing effect of acetic acid and other in contradiction to the acidifying effect of lactic, tartaric acid, etc. Dobrowolskaja-Zawadskaja (13) tested the effect of lactic acid, and especially soda and lime lactates on mice. She confirmed the appearance of certain external phenomena, such as a reduction in activeness, respiratory retardation and a hindrance to rallying, including cataleptic muscle tightening. Collazo (14) and Parfentiew (15) both indicate about 6 g per kg of animal weight as a sodium lactate dose that is not harmful to rabbits. For the amount of lactic acid found in the blood after lactate administration, these same authors indicate very highly differing figures.

The effect of various compounds, among others lactic and acetic acid, on the alkaline reserves of the blood was studied by Markees and Menczer (16). They used rabbits as experimental animals, administrating the acids orally. They determined no marked reduction in the alkali reserves, though the amount of CO_2 decreased, but they emphasize that this could have been within the limits of normal changes. To other rabbits they administered glucose, and then acids. These acids, with the exception of lactic, caused, under these conditions, a definite reduction in the alkali reserves. The authors point out that the above acids, administered in the form of a salt, do

not reduce the amount of CO_2. Fasold (17, 18) describes an increase alkalinity in the urine of children who received racemic lactic acid, as well as the effect of large doses of this acid on children suffering from manifest tetanus.

My own experiments, which I intended to perform by examining the effect of lactic acid, initially were only supposed to consist of observations of changes occurring in the blood. I therefore planned to determine the amount of hemoglobin, red corpuscles and carbon dioxide content and pH of the blood.

In wanting to determine the dose of lactic acid that I should administer to the rats, I had first to find out what amounts would be toxic, and how much could be given with no danger. I then decided to determine the pH of the urine and, after beginning experiment II, I also decided to examine changes in the metabolism. At the time I was performing the tests, I also had the intention of checking and monitoring the weight and overall condition of the animals. For purposes of comparison, I decided to perform analogous experiments with acetic acid.

Methods

I determined the hydrogen ion concentration in the total blood potentiometrically, using a hydroquinhydrone electrode, which offers better results in the presence of protein than does the quinhydrone electrode (19). As a comparison electrode, I used a saturated mercurous chloride micro-electrode, constructed specially in such a way as to be suitable for measurements in minimal amounts of fluid. I performed the calculations according to the formula: $pH = \frac{V + 0.4529 + 0.00002\ t}{0.00019832\ T}$

The measurements of the blood pH had to be performed very quickly, for the reason that rat blood coagulates quite rapidly. For the purpose of delaying solidification, I performed the measurements in tiny vessels with a rounded bottom, lined with paraffin (fig. 1).

The tests on the CO_2 content in the blood could not be performed in the Van Slyke apparatus, since I only had 2-3 drops of material at one time to examine. I therefore used the micro method, determining the CO_2 in the Scholander-Roughton analyzer (20). This method is little known in Poland; therefore, I describe it in some detail. The method is based essentially on isolating the CO_2 from the blood with the help of monobasic sodium phosphate, simultaneously creating a vacuum. After isolation of the CO_2, the gas content in the apparatus is determined. Then, the isolated carbon dioxide is absorbed in 10% NaOH. From the difference in content, the amount of CO_2 is calculated in relation to the amount of blood collected, taking into account a correction for pressure and temperature. Also needed for the determinations, besides the apparatus itself, are: 1) capillary pipettes, 2) a wooden or metal stopper, 4-5 cm in length, with a rubber tip for closing the capillary apparatus, 3) a metal plate 1.5 cm x 5.5 cm, bent in the shape of a "U", 4) syringes through which the fluid can most easily be introduced into the apparatus. Reagents: 1) distilled water, free of CO_2, 2) capryl alcohol, 3) saturated $NaH_2PO_4.H_2O$ solution, 4) 10% NaOH, 5) glycerine, 6) heparin.

The lower section of the Scholander apparatus (fig. 2) is in the form of a syringe with a glass piston about 8 cm long.

The syringe is elongated into a calibrated capillary tube, broadened on top in the manner of a cup (fig. 2 and 3C). This cup serves to introduce the fluids into the apparatus. The apparatus fits vertically into the arm of the tripod. After the piston is smeared with glycerine, the cup of the apparatus is filled with water, which is drawn into the bottom portion of the apparatus by means of partial withdrawal of the syringe piston. Then the water is forced back into the cup. The pur-

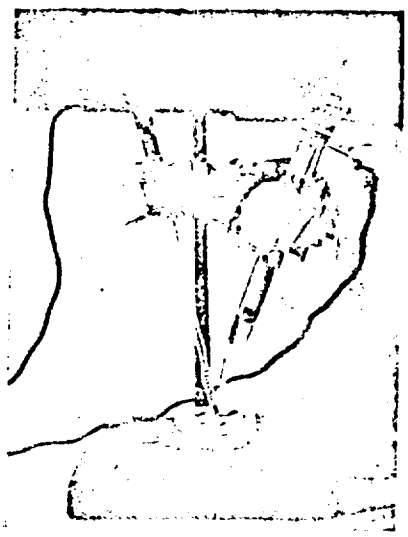

Fig. 1

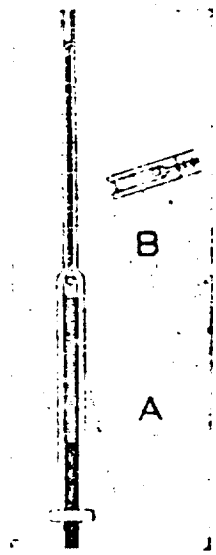

Fig. 2

pose of this is to force air out of the apparatus. The same water from the cup is again let into the syringe, setting its upper level in the capillary at 1-2 mm below the bottom of the cup, thus creating a bubble of air in the top part of the capillary; this bubble will henceforth separate the water from the blood. The blood, first collected into a small capillary pipette rinsed with a heparin solution and then dried, is drawn directly into the apparatus by further pulling out the piston and pressing the pipette rather hard against the bottom of the cup (fig. 3 A, B). After the blood is introduced into the

capillary, its amount is read off, expressed in the b scale. Then 1-2 graduations of capryl alcohol is introduced as an anti-foam agent, displacing the air bubble that has formed between the blood and the alcohol. Now, the cup is filled up to the line with the saturated NaH_2PO_4 solution, which is introduced further by means of further withdrawal of the piston, setting the upper level of the solution at a distance of about 2 mm below the bottom of the cup. The capillary is sealed with the stopper, equipped with a rubber tip and moistened with phosphate solution. The stopper should be pressed in hard with the index finger of the left hand (fig. 4A). With the capillary thus closed, the arm with the apparatus is set up at a slant, and with the right hand, the syringe piston is pulled out, creating a partial vaccum in the apparatus. With the help of the metal plate, placed between the head of the piston and the bottom of the syringe, the piston is kept in withdrawn position (fig. 4B). After the apparatus is removed from the tripod, it

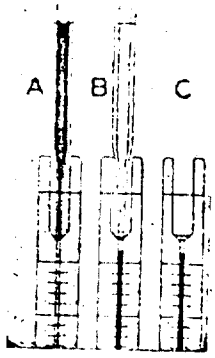

Fig. 3

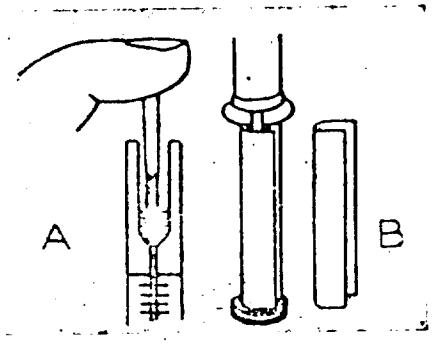

Fig. 4

is shaken for 2 min, the stopper of the capillary being kept closed. Then follows the CO_2 isolation. By removing the supporting plate, the piston is slowed down, and is now pulled

into the depths of the syringe, until pressure is equalized. After checking to see whether there is a drop of phosphate, serving as a "cork", in the cup, the stopper is removed and the apparatus is placed in water for ½ minute, for the purpose of equalizing the temperature.

After the apparatus is dried and again placed vertically in the arm of the tripod, the upper level of the gas bubble is set at the zero point on the capillary scale, and its volume V_1 is read off. Next, the cup is filled with 10% NaOH, which is drawn into the syringe. A few turns of the apparatus facilitate complete CO_2 absorption. After renewed introduction of the gas bubble into the capillary, the volume V_2 is read off.

The calculations are performed according to the formula:

$$\text{percentual volume of } CO_2 = (V_1 - V_2) \times f \times \frac{100}{b}$$

V_1 -- gas bubble volume before CO_2 absorption

V_2 -- gas bubble volume after CO_2 absorption

f -- correction for pressure and temperature

b -- amount of blood expressed by the number of gradations in the capillary.

I determined the metabolism in a simple apparatus constructed in my own laboratory. The rat, placed in a small desiccator, breathed air free of CO_2 and H_2O (prewashing), circulated, if possible, with the same speed by means of a hydraulic pump. The metabolic products were absorbed in absorption apparata, filled with calcium chloride and soda lime.

I determined the amount of hemoglobin in a "Hellige" hemoglobinometer, in which 100% corresponds to 17 g Hb in 100 ml blood.

I determined the red corpuscles by means of the usual clinical method (21).

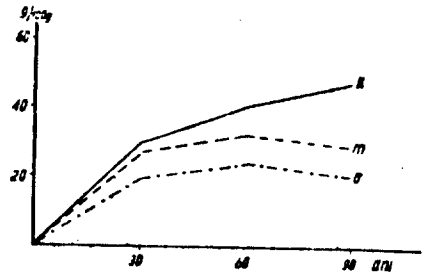

Fig. 5
Experiment I. Mean weight changes in the rats.

The blood needed for the determinations was obtained by cutting off a piece of the rat's tail. The rat urine was collected in metabolic cages.

Experimental Portion

The total number of rats on which the experiments were performed was 238. From this number, I used 130 rats for preliminary tests and for determining the lethal dose, while 93 were used for the main experiments. The remainder -- 15 females -- I set aside for breeding.

The animals were fed with the so-called basic diet*), containing the following components:

wild oats	22%	kitchen salt	1%
wheat	20%	$CaCO_3$	0.5%
rye	20%	margarine	3%
casein	3.5%	cod liver oil	1%
powdered milk	8%	meat or fish meal	15%
		yeast	6%

*) This diet was worked out at the Nutritional Hygiene Department of the National Hygiene Institute and has been used here for several years for animal breeding.

The amount of fodder eaten by the animals was not controlled closely. The rats ate as much as they wished. On the average, the amount of fodder eaten by one rat over a period of 24 hours was 10-15 g.

The preliminary tests, intended to determine the lethal dose, were performed by introducing a tube directly into the stomach, through which lactic or acetic acid were force-fed in various amounts and concentrations. I used commercial fermenting 50% lactic acid or chemically pure glatial acetic acid, suitably diluted. I began the tests by giving six-week old males (weighing 200-220 g) 50% acid solutions. Ten rats received lactic acid, ten others acetic.

The volume of the dose the first day was 0.25 ml, or about 0.625 g pure acid per kg animal weight. The next day, the amount of acid was increased to 0.5 ml. Daily increase in the doses by 0.25 ml made possible a single administration of 4.5 ml of 50% lactic acid, which amounted on the average to 11. 25 g/kg weight. Two rats died after receiving 3 ml. A similar administration of 50% acetic acid made it possible to reach a single dose of 2.5 ml.

The administration of the above amounts of acid had a very significant effect on reduction in the animals' weight. The rats that received the lactic acid lost 15% in the course of a week, while those that received acetic acid lost up to 20% of their initial weight. After a single administration of large acid doses, I found no changes in the CO_2 content and the pH of the blood. On the other hand, the pH of the urine decreased considerably. The pH value was an average of 5.72 among the

"M" rats, and 5.53 among the "O" rats. The above figures are the means of measurements made of the urine of 5 rats of each group 3 hours after force-feeding of the acids.

Autopsies of the animals that died after acetic acid administration revealed thickening of the stomach and duodenal mucosa, as well as congestion in the entire digestive tract. In those animals given lactic acid, I was able to observe intensive congestion of the liver, as well as much-loosened gastric and duodenal mucous membrane. This membrane could be scraped off easily with a blunt instrument. In light of these facts, I used more highly diluted acids in further experiments.

I performed three main experiments. In each experiment, I had three groups of animals. The rats of one group received lactic acid daily. For brevity, I designated this group with the letter "M". The rats of the second group received the same amounts of acetic acid (designated by the letter "O"). The third group served as controls, "K". Each of the experiments was conducted for 90 days.

In the first experiment, considered a trial, I had 19 ten-week males. The rats of the first group -- "M" -- numbering eight, daily were force-fed 3 ml 10% lactic acid. Group "O" also consisted of eight rats, which received the same amount of acetic acid in the same way. The three remaining rats were used as controls, "T".

The administration of the fluids through the tube is called "force-feeding" in the following.

Four "O" rats died on the first day of the experiment. During the first week, two "M" rats and one "O" rat died. The

following week, another "M" rat and another "O" rat died.

The initial weight of the "M" group rats was 174-229 g, that of the "O" group 190-2[illegible] g, and that of the "K" group 185-242 g. The animals were weighed each week. The weight changes per 100 g animal weight in three 30-day periods were:

A. Grupa	B. I okres 30-dniowy g/100 g			C. II okres 30-dniowy g/100 g			D. III okres 30-dniowy g/100 g		
	a od	b do	c średn.	a od	b do	c średn.	a od	b do	c średn.
„M"	23,6	30,3	26,9	1,2	12,9	5,8	—6,3	+1,4	—2,9
„O"	16,7	22,7	19,7	1,4	8,2	4,8	—1,6	—5,7	—3,7
„K"	24,0	39,2	29,6	8,1	11,8	11,1	+4,6	+8,5	+6,5

Key:

A. group
B. first 30-day period
C. second 30-day period
D. third 30-day period
a. from b. to c. mean

The mean increase in the period of 90 days was 30.3 in the "M" group, 20.8 in the "O" group, and 47.2 g/100 g weight in the "K" group (fig. 5).

The amount of hemoglobin of the "M" group rats at the beginning of the experiment was from 91 to 101%, with a mean of 94.1. In group "O" it was 96 to 100%, with a mean of 98.2. In the "K" group it was 97 to 103, with a mean of 99.3. After a passage of ninety days, the Hb content fell among the "M" rats to a mean of 84%. The mean decrease was 10.8% of the initial amount. In the "O" group, the Hb level decreased in this same period by 17.9%. In the "K" group, I noticed no changes in the amount of hemoglobin, while individual differences varied from -1.0 to +2%, with a mean of 0.7%.

The number of red corpuscles in 1 mm^3 at the beginning of

the experiment was from 7,980,000 to 9,270,000 in group "M", 8,960,000 to 9,100,000 in group "O", and 8,980,000 to 9,310,000 in group "K". After ninety days, the mean decrease in group "M" was 13.3%, 23.3% in group "O". Among the rats of group "K", I scarcely found any differences in the red corpuscle count.

The animals from this experiment had no determined amount of CO_2 in the blood before the acid administration began. I did not determine the CO_2 until near the end of the experiment. The mean amount of CO_2 in group "M" was 37.46%, in group "O" 36.65%, in group "K" 46.20%.

The pH measurements for the blood taken on the first day of the experiment revealed the following means:

in group "M" -- 7.41
in group "O" -- 7.42
in group "K" -- 7.41

On the thirtieth day, the blood pH measured 15-30 minutes after force-feeding was:

group "M" -- 7.40
group "O" -- 7.42
group "K" -- 7.39

Measurements taken 1-2 hours after force-feeding differed on the average from the others by -0.01 pH in group "M", -0.06 in group "O" and +0.01 in group "K". I performed analogous measurements on the ninetieth day of the experiment.

The differences there in group "M" were on the average -0.04, in group "O" -0.03 and 0.06 pH. In group "K", the mean difference was -0.01.

I determined the urine pH 1-2 hours after force-feeding, and a second time 3-4 hours after force-feeding. On the nine-

tieth day of the experiment, the mean differences in these two measurements were -0.50 in group "M", -0.63 in group "O" and -0.14 pH in group "T".

Among the animals of specific groups there were certain differences in external appearance. Above all, the rats of group "O" differed in dimensions, as their weight increase was small. The rats of this group also bled from time to time and had bloody spots near their mouths and noses. The pelt was bristly and took on a yellowish color.

In the last stage of the experiment, these rats were active and restless, and their skin was cooler; upon listening to their chest cavities, ruckling and whistling could be detected. I did not notice these phenomena in group "M".

I performed the second experiment on 63 rats. The age of the rats varied from 80 to 83 days. I separated the rats into three groups, as in the first experiment. I put them in cages holding 6-9 each. Each cage also held rats from each group. Essentially, the course of the experiments was the same as in experiment I. The animals of groups "M" and "O" were force-fed the same amounts of acid and in the same concentrations as described above. However, I did make certain changes. Most important, I force-fed the control rats distilled water, in order to eliminate any possible effect of the actual force-feeding on the rats of groups "M" and "O". Then I increased the number of determinations of the CO_2 content in the blood and pH measurements of the blood and urine. Beyond this, I examined the metabolism and performed autopsies of the rats after the experiment was completed.

During the first week of acid administration, 5 rats from group "M" died. The following week, another three died. During the entire experimental period, 16 rats from group "O" died. On the other hand, no rats from group "K" died in the entire course of the force-feeding.

In the first thirty days of the experiment, the mean weight increases per 100 g of the rats' weight were as follows:

	"M"	"O"	"K"
males	24.1 g	16.4 g	32.9 g
females	17.6 g	6.5 g	21.8 g

The following comparison shows the data concerning weight changes over the next thirty days:

	"M"	"O"	"K"
males	5.1 g/100 g	2.3 g/100 g	13.0 g/100 g
females	4.7 g/100 g	1.5 g/100 g	5.4 g/100 g

In the last thirty-day period, the weight changes in group "M" varied from -13.6 to +6%. In group "O", the males lost 5.4 and 7.6 g/100 g weight. In this same period, the rats of group "K" lost on the average 5.5 g for the males and 3.4 g per 100 g weight for the females.

The mean differences in the amount of hemoglobin after ninety days, during which the animals were force-fed lactic acid, acetic acid or water, were the following percentages:

group	"M"	"O"	"K"
males	-12.3	-18.1	+1.1
females	-13.3	-17.7	+0.9

In the end stage, the blood of the rats of group "M" contained 80-87% Hb. One rat had 79% Hb. In group "O", the Hb content was 78, 79 and 82%.

The mean red corpuscle count in 1 mm^3 before the beginning of the experiment was about 9,220,000. Deviations from the mean were rather considerable. From 63 rats, four had more than 10,000,000/mm^3, 2 rats had less than 7,000,000 red corpuscles. The number of red corpuscles in the remainder varied from 8,000,000 to 9,800,000/mm^3. After ninety days of the experiment, the number of red corpuscles fell to 7,240,000-8,240,00/mm^3 in group "M". The mean decrease was 11.8%. In group "O", the decrease was greater and was 32.5% on the average among the females, and 27.2% among the males. The number of red corpuscles in the end stage of the experiment was less than 7,000,000/mm^3. Determining the number of red corpuscles of rats of group "M" in the beginning and end stage revealed no substantial changes, with 2 exceptions, when the red corpuscle count of one rat increased by 16.7%, and decreased in another case by 8.3%. Among the remaining rats, the variations in a ninety-day period were from -2 to +5.4%.

The percentual volume of carbon dioxide in the blood during the first determination, performed at the beginning of the experimental period, was 33.3 to 50%, being less than 40% only among 15 rats, and above 45% among 22. On the thirtieth day, I performed two determinations. The first took place before force-feeding, the second 1-2 hours after force-feeding. I confirmed no differences among the results obtained. In the end stage of the experiment, I determined the percentage of CO_2 5 times a day, every 2-3 hours. I performed the first determination before force-feeding on the same day, and about 24 hours after force-feeding of the previous day. I performed the last

determinations 6-7 hours after force-feeding on the same day. In group "M", the decrease in the amount of CO_2 in the blood appeared 0.5-1 hours after force-feeding. The amount of CO_2 decreased by 7-10%. In 4 rats, I noted no clear reduction in the amount of CO_2. Within 2-3 hours there was either an equalization, or the amount even rose above the initial value. The maximum amount of CO_2 generally appeared around 4-5 hours after force-feeding. Within 6-7 hours after force-feeding, the amount decreased, but generally still exceeding the initial value. Analogous determinations among the rats of group "O" on the thirtieth day revealed a mean difference of about 5%. The results of determinations performed 5 times a day every 2-3 hours on the ninetieth day yielded the lowest values for CO_2 in 0.5-1 hour after acid administration. An equalization of the amount of CO_2 in the blood occurred slowly. The level did not even out until after 6-7 hours. I noted no appearance of a maximum here. The CO_2 volume decreased by 10.6, 9.2% among the males and 8.6, 4.1 and 3.9% among the females. In group "T" I noticed slight variations in the CO_2 amount, but they appeared only irregularly. (Fig. 6).

The blood pH determined at the same time had a certain tendency to decrease ½ to 1 hour after force-feeding in group "M". At the same time, in group "O" the pH decrease was insignificant. In this group, the tendency of a minimum to appear showed itself 2-3 hours after force-feeding, while at the same time the pH of the blood in group "M", had completely equalized. The differences in group "M" were -0.04 pH on the average, -0.05 pH in group "O", and 0.02 pH in group "T" (fig. 7).

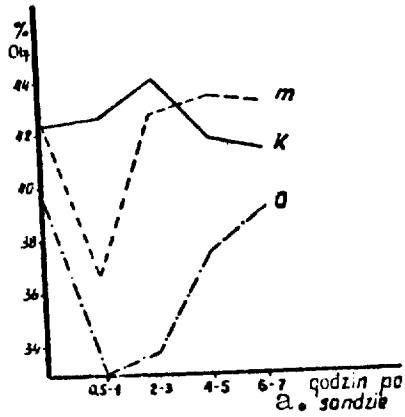

Fig. 6

Experiment II. Mean changes in CO_2 amount on the ninetieth day.
a. hours after force-feeding

The measurements I made on the thirtieth day of the experiment indicated an insignificant reduction in the urine pH of the rats of groups "M" and "O" 1-2 hours after force-feeding. The increase in the number of measurements in the course of one day in the end stage of the experiment made it possible to observe large variations in the urine pH. Among the rats of group "M", on the morning before acid administration, the urine revealed an alkaline reaction, and in some rats the pH approached 7.45. The alkalinity of the urine persisted for more than 24 hours, if another dose was not given. On the other hand, after force-feeding, the alkalinity decreased slightly after 1-2 hours. The next measurement, taken 3-4 hours after force-feeding, revealed an acid reaction of the urine. In the later stages, there was again a rise in the pH. Analogous measurements among the rats of group "O" did not reveal an alkaline reaction. The lowest pH occurred 4-5 hours after feeding. In subsequent measurements, the urine pH rose progressively, but I never found

values greater than 0.59 in this group (Fig. 8).

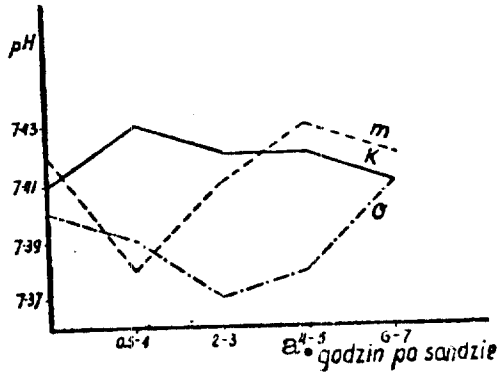

Fig. 7

Experiment II. Mean changes in blood pH on 90th day.
a) hours after force feeding

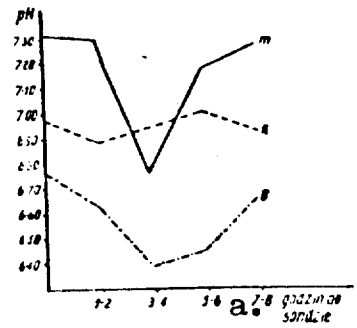

Fig. 8

Experiment II. Mean changes in urine pH on 90th day.
a) hours after force feeding

I performed determinations of the amount of oxygen collected and the amount of eliminated metabolic products among 15 rats of all three groups. The examination lasted 15 minutes. The rats were first put on a fast for 24 hours. I tried to perform these tests on all the rats at the same time of day and in the same amount of time after force-feeding. This time was about 2 hours.

A. Grupa	B. g O_2/100 g wagi/godz. a. od – do	b. średnio	C. Spółczynnik oddechowy a. od – do	b. średnio
„M"	0,199—0,240	0,217	1,003—1,023	1,009
„O"	0,184—0,198	0,191	1,007—1,037	1,021
„K"	0,166—0,190	0,179	0,666—0,786	0,723

Key:
A. group
B. g O_2/100 g weight/hour
C. respiratory coefficient
a. from-to b. mean

As can be seen from the table, the respiratory coefficients

in groups "M" and "O" are nearly identical, significantly higher than in group "K". On the other hand, the oxygen consumption, calculated in terms of 100 g weight, rose quite a bit more in group "M" than in group "O".

In this experiment also, the animals belonging to specific groups varied in appearance. The "K" rats retained their liveliness and normal appearance. In group "O", as previously, there were changes in the fur and respiration. The "M" rats also had accelerated respiration, louder than normal. Autopsies of the animals performed after 90 days of acid administration revealed certain differences in comparison with the control animals. In the "M" group, the liver was swollen and had a dark-purple cast. The amount of fat in the subcutaneous tissue and that surrounding the internal organs was much less than in the case of the controls. The mucosa of the stomach and duodenum could be scraped away easily using a blunt instrument. The rats of group "O" had hardly any fat at all, not even in the area of the kidneys, where there was very little. In the swollen and congested gastric and duodenal mucosa I noticed bloody extravasations. The liver was likewise swollen and of a deep red color, and poor density. The liver and kidneys of the rats of groups "M" and "O" differed in weight from these same organs among the "K" rats; in groups "M" and "O", these organs were heavier than in group "K".

For the third experiment I used 11 nine-week old males. I divided the animals into groups as above. The rats of groups "M" and "O" received 10% lactic or acetic acid in the afternoon, but not through force-feeding as previously, but rather mixed

with their feed. I mixed 4 ml with 20 g meal, which, assuming an average consumption of 15 g per rat, in effect introduced about 3 ml acid into the organism; i.e. as much as in the previous tests.

The curve of the weight changes in the rats of this experiment was of a nature similar to that of the two preceding ones. Group "K" revealed the maximum increases. The mean hemoglobin drop was: 6.3% in group "M" and 8.3% in group "O". The red corpuscle count dropped 11.6% on the average in group "M", and 18.9% in group "O".

The curves of the changes in the blood and urine pH and the blood CO_2 volume are similar in groups "M" and "O". Changes in the amount of CO_2 in the blood were slight. A slight increse occurred in the morning hours. I noticed the lowest values during the evening. The differences in the blood pH did not exceed 0.02, reaching the maximum during the morning. On the other hand, the urine pH reached its maximum during the afternoon hours. An alkaline urine reaction appeared at this same time also among the rats of group "O".

The animals of the separate groups did not differ in external appearance; only the breathing of the rats of group "O" was whistling. Autopsies of the animals did not show any evident changes. In the same manner, there were no defined differences in weight of specific internal organs among groups "M", "O" and "K".

At the time I was beginning the experiment, I became interested in the question as to whether acid administration to females during gestation and lactation would have an effect on the pH of the milk. In order to determine this, I set aside

15 females for reproduction. I gave five of them normal doses of lactic acid, five acetic acid, and the remainder water. Two "M" mothers and three "O" mothers died before bearing the young. The rest bore their young normally. I measured the milk pH twice: 10 and 20 days after birth. I obtained the milk by squeezing and massaging the teats; the milk was collected into capillary tubes. In both measurements, the milk pH did not reveal any differences among the separate groups; it had a weak alkaline reaction (pH = 7.2). On the other hand, the urine of the young, nursed by "M" and "O" mothers, had a lower pH (6.70 and 6.52) in comparison with the young of mothers from group "K" (7.06).

Comparison of Results

A comparison of the results obtained from the separate experiments leads to the supposition that the effect of lactic acid and acetic acid on the organism is similar.

The drop in weight increase in comparison with group "K" occurred among the rats of groups "M" and "O" in all the experiments. The weight increases among group "O" were the lowest.

A. Grupa	B. Doświadczenie		
	I. g/100 g	II. g/100 g	III. g/100 g
„M"	30,35	31,94	64,00
„O"	20,78	20,10	44,91
„K"	53,15	58,2	95,5

Key:
A. group
B. experiment

The rat mortality in the course of the experiment was higher in group "O" than in group "M".

From the above we see than acetic acid had a greater effect than lactic acid, especially when force fed. On the other hand, administration of the acids mixed with the feed did not cause a single death in any of the groups. However, tests on determining the lethal dose revealed that acetic acid is more lethal than lactic acid.

Changes in the amount of hemoglobin and red corpuscles were very evident among the rats of "M" and "O" in all the experiments. The greatest decrease was found in group "O" among those rats that were force fed the acid; it was lower among those administered the acids mixed with their feed. These changes could not have been caused the collection of blood for the separate determinations, as the rats of group "K" showed no defined differences, and blood collection was identical in all the groups.

A. Grupa	B. Hemoglobina. Różnica %			C. Krwinki czerwone. Różnica %		
	D. Doświadczenie			D. Doświadczenie		
	I	II	III	I	II	III
„M"	— 10,76	— 12,4	— 6,3	— 13,3	— 12,8	— 11,6
„O"	— 17,91	— 18,1	— 8,3	— 23,9	— 27,2	— 18,9
„K"	+ 0,67	+ 1,1	— 0,3	+ 0,1	+ 2,1	— 0,07

Key:
A. group
B. hemoglobin. % difference
C. red corpuscles. % difference
D. experiment

In experiment II, the differences occurring in the blood pH of groups "M" and "O" as compared to group "K" were not great, but in both the first groups I was able to notice a clear tendency of this value to decrease in rather constant periods, depending upon the time of acid administration. As can be seen from the graphs, which were made on a large scale,

the blood pH increased after 0.5-1 hours after force-feeding among the rats of group "M". The mean difference was -0.04 pH. Equalization took place very rapidly, since in the next measurement the pH is equal to or higher than the initial value. In group "O" I determined the pH drop later, 2-3 hours after force-feeding. However, it lasted longer; I did not notice equalization until 6-7 hours after administration of this acid. In group "K", the differences were smaller and did not appear regularly, which can be seen easily from figure 7.

In experiment II, as seen from the graphs, the drop in the CO_2 amount among the rats of groups "M" and "O" was clearly related to the time of acid administration. In both groups, the minimum appeared 0.5-1 hour after force-feeding. Equalization was rapid in group "M", slower in group "O".

The results of urine pH determinations among the rats of groups "M" and "O" are similar in all the experiments as concerns the decrease in this value after acid administration. However, a confirmation of urine alkalinity of the rats of group "M" did not occur in experiment II until a greater number of measurements were taken. However, I did not achieve an alkaline reaction of the urine of the group "O" rats; on the other hand, in experiment III I noticed an increase in the urine pH in this group as well during the morning and afternoon hours.

In the results of the determinations of CO_2 amount, blood and urine pH, we can see a certain relation in groups "M" and "O". The decrease and increase in specific values in the blood is related to the time that passed from the moment of acid administration; on the other hand, analogous changes taking place

in the urine pH are shifted by a few hours (fig. 9, 10, 11).

All the data obtained from the separate experiments indicate that, though both acids affected the rats similarly, acetic acid nonetheless called forth longer-lasting changes, though they were not always more intensive. The CO_2 amount and the blood pH of the rats of group "M" returned to the norm more quickly, which can be explained by the fact that the organism is well-disposed toward utilization or elimination of lactic acid as a solid product of the transformations taking place in the system; acetic acid, on the other hand, submits to metabolic processes with greater difficulty, especially in the case of repeated administration of doses.

Large variations in the pH values in the urine, especially in group "M", would seem to indicate an initial elimination of excess acid through the kidneys, and then neutralization of the acid by alkalis, including produced ammonia. The evident increase in the urine alkalinity after the passage of 24 hours from acid administration may occur because the organism accustomed to acid continues to be well-disposed to its neutralization.

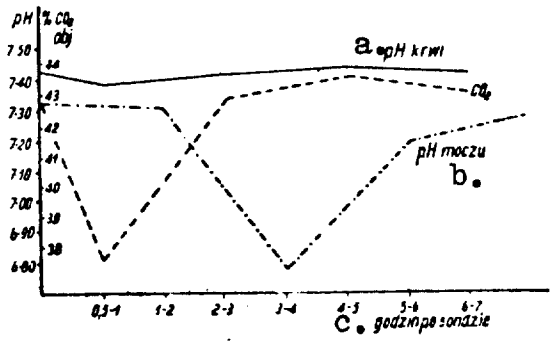

Fig. 9

Experiment II. Mean changes in the CO_2 amount and changes in the blood and urine pH of group M on the 90th day

a. blood pH b. urine pH c. hours after force-feeding

The increase in the respiratory coefficient among "O" rats with the same amount of conxumed oxygen as in group "M" in-

dicates the elimination of an increased amount of carbon dioxide during respiration; the carbon dioxide supposedly comes from the blood bicarbonates decomposed by the acid. The same can apparently be said in discussing group "M", where the respiratory coefficient is likewise large, though there is a simultaneously greater consumption of oxygen. We thus see that among the rats of group "M", within about 2 hours after acid administration, there occurs a rapid burning of the carbohydrates, especially lactic acid, as the coefficients of these compounds are similar.

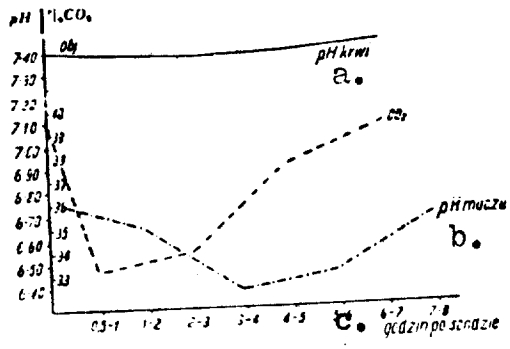

Fig. 10

Experiment II. Mean changes in CO_2 amount and changes in blood and urine pH in group O on the 90th day

a, b, c as in fig. 9

The increase in the weight of the liver and kidneys in groups "M" and "O" as compared to group "K" is founded in the greater congestion of these organs, and as concerns the liver, also in the increase in the amount of glycogen or fat deposited.

Judging from the results obtained in the experiments, the effect of lactic and acetic acid on the rat organism could be considered harmful. However, taking into account the size of the doses, quite large as they reached about 1.5 g pure acid per kg of weight, and the administration of these amounts for three months, which is about 10% of a rat's life, it can be

assumed that neither of the acids exerts a harmful effect when used normally. A confirmation of this may be found in the fact that harmful phenomena appeared acutely only among rats that received the acid directly to their stomachs; among those, on the other hand, for whom the acids were mixed with the feed, the negative effects appeared only in much milder form.

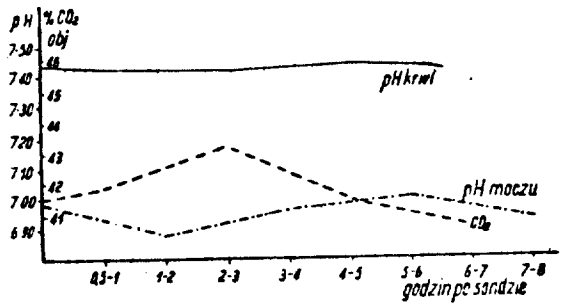

Fig. 11

Experiment II. Mean changes in CO_2 amount and changes in blood and urine pH of group K on the ninetieth day a,b,c as above.

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Otrzymano dn. 23 marca 1951 r.

Wysokinska-'52
Bib No. 2035

Roczniki Panstwowego Zaktadu Hig. 3:273-292. 1952.

ZOFIA WYSOKIŃSKA

PORÓWNANIE DZIAŁANIA KWASU MLEKOWEGO I OCTOWEGO NA ORGANIZM SZCZURA

Z Zakładu Fizjologii Żywienia Człowieka S. G. G. W. w Warszawie

Po ukazaniu się na rynku 50%-owego kwasu mlekowego jako artykułu konsumpcyjnego, zastępującego ocet, wysunęło się zagadnienie, jakie jest jego działanie fizjologiczne i w jakich ilościach można go używać bez szkody dla zdrowia. Wobec tego postanowiłam zająć się tą sprawą badając wpływ tego kwasu na organizm zwierzęcy. Do doświadczeń użyłam białych szczurów. Wybór ich jako zwierząt doświadczalnych nasunął się dlatego, że zręby dużej części nauki żywienia są opracowane głównie na podstawie badań na szczurach i stanowią dobrą bazę do wytyczania zasad racjonalnego żywienia ludzi. Przypuszczałam więc, że będą one reagowały najbardziej podobnie do człowieka również na wprowadzenie odpowiednich dawek kwasu mlekowego.

Mimo że w Niemczech kwas mlekowy był zalecany szczególnie podczas ostatniej wojny do konsumpcji dla ludzi, piśmiennictwo, jakie znalazłam na temat jego działania fizjologicznego, jest dość skromne. Natomiast bardzo dużo prac jest poświęconych przemianom kwasu mlekowego w warunkach fizjologicznych i patologicznych. Szeroko opracowana jest sprawa występowania kwasu mlekowego w mięśniach wypoczętych i zmęczonych. *Peters i Slyke* (1) cytując szereg autorów bardzo obszernie omawiają przemiany kwasu mlekowego w mięśniach. *Guli i Novikowa* (2) po doświadczeniach na królikach karmionych dietą zakwaszającą, alkalizującą albo obojętną stwierdzają, że poziom kwasu mlekowego krwi, mięśni i wątroby był niższy, a wydolność mięśni była większa u tych zwierząt, które otrzymywały dietę zakwaszającą. Zagadnienie przechodzenia kwasu mlekowego w glikogen również jest poruszane w wielu pracach. *C. F. Cori i G. T. Cori* (3) podają, że u szczurów glikogen tworzy się łatwiej z d-mleczanów niż z l-mleczanów, nadmieniając, że l-kwas mlekowy jest użytkowany 4 razy mniej niż jego d-forma. Podobne wyniki otrzymali *Meyerhof i Lohman* (4) badając syntezę glikogenu w wyizolowanych tkankach ssaków. Również

Grant (5) badał tworzenie się glikogenu u kotów po wprowadzeniu mleczanu amonu przez górną żyłę trzewiową. Różnice przyswajalności różnych składników pokarmowych po podawaniu kwasów określali u świnek *Hansen* (6), u zwierząt przeżuwających *Liebscher* (7) oraz u drobiu *Burckhardt* (8). Nowsze prace (9, 10, 11), omawiające przemiany, jakim ulega kwas mlekowy w organizmie, są oparte o badania z kwasem mlekowym zawierającym węgiel radioaktywny w grupie karboksylowej lub w pozycji α lub β. Autorowie podają, ile radioaktywnego węgla zawierał wytworzony glikogen, a ile wydalony dwutlenek węgla w zależności od położenia węgla radioaktywnego.

Prace powyższe miały głównie na celu wyświetlenie, jakim przemianom ulega kwas mlekowy w organizmie. Natomiast prac doświadczalnych, prowadzonych w celu zbadania działania kwasu mlekowego na organizm, w dostępnym mi piśmiennictwie znalazłam niewiele. *Hermann* (12) opisuje doświadczenia na królikach, którym podawał dożylnie różne kwasy, między innymi mlekowy i octowy. Autor podkreśla paradoksalny alkalizujący wpływ kwasu octowego i innych w przeciwstawieniu do zakwaszającego wpływu kwasu mlekowego, winowego itp. Wpływ kwasu mlekowego, a właściwie mleczanów sodu i wapnia na myszy badała *Dobrowolskaja-Zawadskaja* (13). Stwierdziła ona występowanie pewnych objawów zewnętrznych, jak zmniejszenie ruchliwości, zwolnienie oddychania oraz zaburzenia [illegible] i kataleptyczne napięcie mięśni. Jako dawkę mleczanu sodowego n. szkodliwą dla królików *Collazo* (14) oraz *Parfentiew* (15) zgodnie podają ilość około 6 g na kg wagi zwierzęcia. Dla ilości kwasu mlekowego znajdującego się w krwi po podaniu mleczanów autorowie ci podają liczby bardzo różniące się między sobą.

Wpływ różnych związków, między innymi i kwasu mlekowego oraz octowego, na rezerwy alkaliczne krwi, badali *Markees i Menczer* (16). Jako zwierząt doświadczalnych użyli oni królików, którym doustnie podawali kwasy. Autorowie nie stwierdzili wyraźnego zmniejszenia rezerw alkalicznych, chociaż ilość CO_2 zmniejszała się, ale podkreślają, że mogło to być w granicach normalnych zmian. Innym królikom podawali oni glukozę, a następnie kwasy. Kwasy te z wyjątkiem mlekowego powodowały w tym przypadku wyraźne zmniejszenie rezerw alkalicznych. Autorowie nadmieniają, że kwasy powyższe podawane w postaci soli nie zmniejszają ilości CO_2. *Fasold* (17, 18) podaje zwiększenie alkaliczności moczu u dzieci, które dostawały racemiczny kwas mlekowy, jak również wpływ dużych dawek tegoż kwasu na dziecko cierpiące na jawną tężyczkę.

Doświadczenia własne, które zamierzałam prowadzić badając działanie kwasu mlekowego, miały początkowo pójść tylko w kierunku obserwacji zmian zachodzących we krwi. Projektowałam więc oznaczanie ilości hemo-

globiny, krwinek czerwonych oraz zawartości dwutlenku węgla i pH krwi.

Chcąc ustalić dawkę kwasu mlekowego, którą miałam podawać szczurom, musiałam się zorientować, jakie ilości będą dla nich toksyczne, a jakie można podawać bez niebezpieczeństwa. Następnie postanowiłam oznaczać pH moczu oraz, już po rozpoczęciu doświadczenia II, zdecydowałam się również na badanie przemiany materii. W czasie wykonywania prac miałam również zamiar kontrolować wagę i ogólny wygląd zwierząt. Dla porównania postanowiłam prowadzić analogiczne doświadczenia z kwasem octowym.

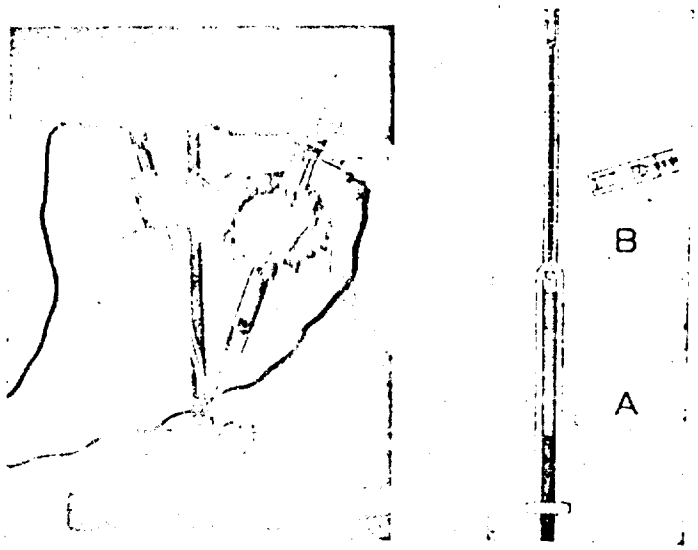
Metody

Stężenie jonów wodorowych oznaczałam potencjometrycznie w pełnej krwi stosując elektrodę hydrochinhydronową, która w obecności białka daje lepsze wyniki niż chinhydronowa (19). Jako elektrodę porównawczą używałam nasyconą mikro elektrodę kalomelową, specjalnie skonstruowaną, tak aby nadawała się do oznaczeń w minimalnych ilościach płynów. Obliczenia przeprowadzałam wg wzoru: $pH = \frac{V + 0.4529 + 0.00002\,t}{0.00019832\,T}$

Pomiary pH krwi trzeba było wykonywać bardzo prędko, między innymi z tego względu, że krew szczurów krzepnie dość szybko. W celu opóźnienia krzepnięcia oznaczenia wykonywałam w maleńkich naczyńkach o dnie kulistym, wylanym parafiną (ryc. 1).

Badania zawartości CO_2 w krwi nie można było wykonać w aparacie Van Slyke'a, gdyż jednorazowo miałam do dyspozycji jedynie 2—3 krople materiału do badań. Zastosowałam więc metodę mikro, oznaczając CO_2 w analizatorze Schelander-Roughton (20). Metoda ta jest mało znana w Polsce, podaję więc jej opis dość szczegółowo. Zasada metody polega na wydzielaniu CO_2 z krwi za pomocą jednosodowego fosforanu przy jednoczesnym wytworzeniu próżni. Po wydzieleniu się CO_2 odczytuje się objętość gazów zawartych w aparacie. Następnie pochłania się wydzielony dwutlenek węgla w 10%-owym NaOH. Z różnicy objętości oblicza się, uwzględniając poprawkę na ciśnienie i temperaturę, jego ilość w stosunku do objętości pobranej krwi. Do oznaczeń oprócz samego aparatu są potrzebne jeszcze: 1) kapilarne pipetki, 2) zatyczka drewniana lub metalowa długi 4—5 cm, opatrzona zakończeniem gumowym do zamknięcia kapilary aparatu; 3) blaszka metalowa o wymiarach 1,5 cm × 5,5 cm, zgięta w kształcie litery „U"; 4) strzykawki, przez które najłatwiej wprowadzić płyny do aparatu. Odczynniki: 1) woda destylowana wolna od CO_2, 2) alkohol kaprylowy, 3) nasycony roztwór $NaH_2PO_4 \cdot H_2O$, 4) 10%-owy NaOH, 5) gliceryna, 6) heparyna.

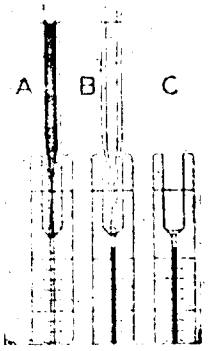
Aparat Scholandera (ryc. 2) w dolnej części ma kształt strzykawki z tłokiem szklanym około 8 cm długości. Strzykawka jest wydłużona w kalibrowaną rurkę kapilarną, rozszerzoną u góry w rodzaj kubeczka (ryc. 2 i 3C). Kubeczek ten służy do wprowadzenia płynów do aparatu. Aparat umieszcza się pionowo w łapie statywu. Po posmarowaniu tłoka gliceryną kubeczek aparatu napełnia się wodą, którą wciąga się do dolnej części aparatu przez częściowe wysunięcie tłoka strzykawki. Następnie wodę prze-



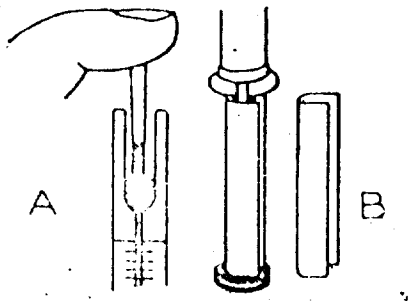
Ryc. 1 Ryc. 2

pycha się z powrotem do kubka. Czynność ta ma na celu usunięcie powietrza z aparatu. Tę samą wodę z kubka wciąga się ponownie do strzykawki ustalając jej górny poziom w kapilarze na 1–2 mm poniżej dna kubka, tworząc w ten sposób w górze kapilary pęcherzyk powietrza, który będzie następnie oddzielać wodę od krwi. Krew, pobraną uprzednio do małej kapilarnej pipetki przepłukanej roztworem heparyny, a następnie wysuszonej, wciąga się bezpośrednio do aparatu wysuwając dalej tłok strzykawki i dość silnie przyciskając pipetkę do dna kubka (ryc. 3 A, B). Po wprowadzeniu krwi do kapilary odczytuje się jej objętość wyrażoną w podziałkach b. Następnie wprowadza się alkohol kaprylowy w ilości 1–2 podziałek, jako środek zapobiegający pienieniu, usuwając ewentualny pęcherzyk powietrza powstały między krwią a alkoholem. Teraz kubek napełnia się do kreski nasyconym roztworem $NaHPO_4$ i wprowadza przez dalsze wysuwanie tłoka do

aparatu, ustalając górny poziom w odległości około 2 mm poniżej dna kubka. Zatyczką, opatrzoną gumowym zakończeniem i zmoczoną roztworem fosforanu, szczelnie zamyka się kapilarę. Zatyczkę należy silnie dociskać wskazującym palcem lewej ręki (ryc. 4A). Przy zamkniętej kapilarze łapę z aparatem ustawia się ukośnie i prawą ręką wysuwa się tłok strzykawki tworząc częściową próżnię w aparacie. Za pomocą blaszki metalowej, włożonej między główkę tłoka a dół strzykawki, zatrzymuje się tłok w pozycji wysunię-



Ryc. 3



Ryc. 4

tej (ryc. 4B). Po zdjęciu aparatu ze statywu wstrząsa się nim 2 min. przy ciągle zamkniętej zatyczką kapilarze. Następuje wydzielanie się CO_2. Przez usunięcie blaszki podtrzymującej zwalnia się tłok, który teraz zostaje wciągnięty w głąb strzykawki do momentu wyrównania ciśnienia. Po sprawdzeniu, czy w kubku jest kropla fosforanu, która posłuży za „korek", usuwa się zatyczkę i aparat wkłada się na 1/2 minuty do wody w celu wyrównania temperatury.

Po osuszeniu i ponownym pionowym zamocowaniu aparatu na statywie górny poziom pęcherzyka gazu ustawia się na podziałce zerowej kapilary i odczytuje się jego objętość V_1. Następnie kubek napełnia się 10%-owym NaOH, który wciąga się do strzykawki. Kilka ruchów obrotowych wykonanych aparatem ułatwia całkowite pochłonięcie CO_2. Po ponownym wprowadzeniu pęcherzyka gazowego do kapilary odczytuje się objętość V_2.

Obliczenia wykonuje się wg wzoru:

procentowa objętość CO_2 $(V_1 - V_2) \cdot f \cdot \frac{100}{b}$

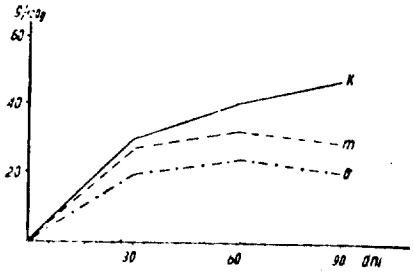
V_1 — objętość pęcherzyka gazów przed pochłonięciem CO_2

V_2 — objętość pęcherzyka gazów po pochłonięciu CO_2

f — poprawka na ciśnienie i temperaturę

b — ilość krwi wyrażona liczbą podziałek w kapilarze

Przemianę materii oznaczałam w prostym aparacie skonstruowanym we własnym zakresie. Szczur, umieszczony w małym eksykatorze, oddychał powietrzem wolnym od CO_2 i H_2O (płuczki wstępne), przeciąganym możliwie z tą samą szybkością pompą wodną. Produkty przemiany materii były



Ryc. 5
Doświadczenie I. Średnie zmiany wagowe u szczurów

absorbowane w aparatach pochłaniających, napełnionych chlorkiem wapnia i wapnem sodowanym.

Ilość hemoglobiny oznaczałam w hemoglobinometrze „Hellige", w którym 100% odpowiada 17 g Hb w 100 ml krwi.

Krwinki czerwone obliczałam zwykłą metodą kliniczną (21).

Krew potrzebną do oznaczeń pobierałam obcinając szczurowi kawałek ogona. Mocz szczurów zbierałam w klatkach metabolicznych.

Część doświadczalna

Ogólna liczba szczurów, na których robiłam doświadczenia, wynosiła 238 sztuk. Z liczby tej 130 sztuk użyłam do badań wstępnych i do określania dawki śmiertelnej, a 93 sztuki do doświadczeń głównych. Resztę — 15 samic — nastawiłam na rozmnażanie.

Zwierzęta były karmione tzw. dietą podstawową*) zawierającą następujące składniki:

owies	— 22%	sól kuchenna	— 1%
pszenica	— 20%	$CaCO_3$	— 0.5%
żyto	— 20%	margaryna	— 3%
kazeina	— 3.5%	tran	— 1%
mleko proszk.	— 8%	mączka mięs. lub rybna	— 15%
		drożdże	— 6%

*) Dieta ta została opracowana w Dziale Higieny Żywienia Państwowego Zakładu Higieny i jest tu używana do hodowli szczurów od kilku lat.

Ilość zjedzonego przez szczura pożywienia nie była ściśle określana. — Szczury jadły, ile chciały. Średnio ilość zjedzonego pokarmu przez jednego szczura w ciągu 24 godzin wynosiła 10—15 g.

Badania wstępne, mające na celu ustalenie dawki śmiertelnej, przeprowadzałam przez podawanie sondą bezpośrednio do żołądka kwasu mlekowego lub octowego w różnych ilościach i stężeniach. Używałam handlowego fermentacyjnego 50%-owego kwasu mlekowego oraz chemicznie czystego lodowatego kwasu octowego, odpowiednio rozcieńczonych. Próby rozpoczęłam od podawania sześciotygodniowym samcom (wagi 200—220 g) pięćdziesięcioprocentowych roztworów kwasów. Dziesięć szczurów otrzymało kwas mlekowy, dziesięć innych — octowy.

Objętość dawki w pierwszym dniu wynosiła 0,25 ml, czyli około 0,625 g czystego kwasu na kg wagi zwierzęcia. W następnym dniu ilość kwasów zwiększyłam do 0,5 ml. Codzienne zwiększanie dawki o 0,25 ml pozwoliło na jednorazowe podanie 4,5 ml 50%-owego kwasu mlekowego, co średnio wynosiło 11,25 g/kg wagi. Dwa szczury padły po otrzymaniu 3 ml. Analogiczne podawanie 50%-owego kwasu octowego dało możność osiągnięcia jednorazowej dawki 2,5 ml.

Podawanie powyższych ilości kwasów bardzo silnie wpływało na obniżenie wagi zwierząt. Szczury otrzymujące kwas mlekowy traciły w ciągu tygodnia do 15%, a te, które dostawały kwas octowy, do 20% wagi początkowej. Po jednorazowym podaniu dużych dawek kwasów nie stwierdziłam żadnych zmian w zawartości CO_2 i pH krwi. Natomiast pH moczu obniżało się bardzo znacznie. Wartość pH wynosiła średnio 5,72 u szczurów „M" oraz 5,53 u szczurów „O". Liczby powyższe są średnimi z pomiarów dokonanych w moczu 5 szczurów każdej grupy w 3 godz. po podaniu kwasów przez sondę.

Sekcje zwierząt, które padły po podaniu kwasu octowego, wykazywały zgrubienie śluzówki żołądka i dwunastnicy oraz przekrwienia w całym przewodzie pokarmowym. U zwierząt, którym dawałam kwas mlekowy, można było zauważyć silne przekrwienie wątroby oraz łatwe odstawanie błony śluzowej żołądka i dwunastnicy. Błona ta dawała się zeskrobywać tępym narzędziem. Wobec tego w dalszych doświadczeniach używałam kwasy bardziej rozcieńczone.

Wykonałam trzy główne doświadczenia. W każdym doświadczeniu miałam trzy grupy zwierząt. Szczury jednej grupy codziennie otrzymywały kwas mlekowy. Grupę tę oznaczyłam dla krótkości literą „M". Szczury drugiej grupy otrzymywały takie same ilości kwasu octowego (w skrócie są nazwane literą „O"). Trzecią grupę zostawiłam dla kontroli — „K". Każde z doświadczeń prowadziłam przez okres 90 dni.

W pierwszym doświadczeniu, traktowanym jako próbne, miałam 19 dziesięciotygodniowych samców. Szczury grupy pierwszej — „M", w liczbie ośmiu, otrzymywały codziennie przez sondę 3 ml 10%-owego kwasu mlekowego. Grupa „O" składała się również z ośmiu szczurów, którym dawałam w ten sam sposób i w tej samej ilości kwas octowy. Trzy pozostałe szczury zostawiłam jako kontrolne — „K".

Podawanie płynów przez sondę nazywam w dalszym ciągu „podawaniem sondy" lub jeszcze krócej „sondą".

Cztery szczury „O" padły w pierwszym dniu doświadczenia. W pierwszym tygodniu padły dwa szczury grupy „M" i jeden grupy „O". W następnym tygodniu padł znowu jeden szczur „M" i jeden „O".

Początkowa waga szczurów w grupie „M" wynosiła 175—229 g, w grupie „O" — 190—225 g, w grupie „K" — 185—242 g. Zwierzęta były ważone co tydzień. Zmiany wagowe przypadające na 100 g wagi zwierzęcia w trzech 30-dniowych okresach wynosiły:

Grupa	I okres 30-dniowy g/100 g			II okres 30-dniowy g/100 g			III okres 30-dniowy g/100 g		
	od	do	średn.	od	do	średn.	od	do	średn.
„M"	23,6	30,3	26,9	1,2	12,9	5,8	—6,3	+1,4	—2,9
„O"	16,7	22,7	19,7	1,4	8,2	4,8	—1,6	—5,7	—3,7
„K"	24,0	39,2	29,6	8,1	11,8	11,1	+4,6	+8,5	+6,5

Średni przyrost w okresie 90 dni wynosił w grupie „M" — 30,3, w grupie „O" — 20,8 i w grupie „K" — 47,2 g/100 g wagi (ryc. 5 zamieszczona na str. 278).

Ilość hemoglobiny u szczurów grupy „M" wynosiła na początku doświadczenia od 91 do 101%, średnio 94,1. W grupie „O" — 96 do 100%, średnio 98,2. W grupie „K" — 97 do 103, średnio 99,3. Po upływie dziewięćdziesięciu dni zawartość Hb spadła u szczurów grupy „M" do ilości średniej 84%. Średni ubytek wynosił 10,8% w stosunku do ilości początkowej. W grupie „O" poziom Hb obniżył się w tym samym okresie o 17,9%. W grupie „K" zmian w ilości Hb nie spostrzegłam, różnice indywidualne wahały się od — 1,0 do + 2%. Średnio + 0,7%.

Liczba krwinek czerwonych w 1 mm³ na początku doświadczenia wynosiła od 7.980.000 — 9.270.000 w grupie „M", 8.960.000 — 9.100.000 w grupie „O" i 8.980.000 — 9.310.000 w grupie „K". Po dziewięćdziesięciu dniach średni ubytek w grupie „M" osiągnął 13,3%, w grupie „O" — 23,3%.

U szczurów grupy „K" nie stwierdziłam prawie żadnych różnic w liczbie krwinek czerwonych.

Zwierzęta z tego doświadczenia nie miały oznaczonej zawartości CO_2 w krwi przed rozpoczęciem podawania kwasów. Oznaczenie to wykonałam dopiero pod koniec doświadczenia. Średnia ilość CO_2 w grupie „M" wynosiła 37,46%, w grupie „O" 36,65%, w grupie „K" 46,20%.

Pomiary pH krwi wykonane w pierwszym dniu doświadczenia wykazywały następujące średnie:

w grupie „M" — 7,41
w grupie „O" — 7,42
w grupie „K" — 7,41

W trzydziestym dniu pH krwi mierzone po upływie 15—30 minut po podaniu sondy wynosiło:

grupa „M" — 7,40
grupa „O" — 7,42
grupa „K" — 7,39

Pomiary wykonane w 1—2 godz. po sondzie różniły się średnio od poprzednich o — 0,01 pH w grupie „M", — 0,06 w grupie „O" i + 0,01 w grupie „K". Analogiczne pomiary wykonałam w dziewięćdziesiątym dniu doświadczenia.

Różnice między nimi w grupie „M" wynosiły średnio — 0,04, w grupie „O" — 0,03 i 0,06 pH. W grupie „K" średnia różnica wynosiła — 0,01.

pH moczu oznaczałam w czasie 1—2 godz. po podaniu sondy i po raz drugi w 3—4 godz. po sondzie. W dziewięćdziesiątym dniu doświadczenia średnie różnice w tych dwóch pomiarach wynosiły — 0,50 w grupie „M", — 0,63 w grupie „O" i — 0,14 pH w grupie „K".

U zwierząt poszczególnych grup występowały pewne różnice w wyglądzie zewnętrznym. Przede wszystkim szczury grupy „O" różniły się wymiarami, gdyż przyrost ich wagi był mały. Następnie szczury tej grupy od czasu do czasu krwawiły i miały wtedy plamy krwawe koło pyszczka i nosa. Sierść stawała się najeziona i przybierała barwę żółtawą.

W ostatnim etapie doświadczenia szczury te były ruchliwe, a skóra ich stawała się chłodniejsza; przy osłuchiwaniu klatki piersiowej słychać było rzężenie i świsty. Objawów tych nie spostrzegłam w grupie „M".

Drugie doświadczenie wykonałam na 63 szczurach. Wiek szczurów wahał się od 80 do 83 dni. Szczury podzieliłam na trzy grupy, tak jak w doświadczeniu pierwszym. Umieściłam je w klatkach po 6—9 szt. W każdej klatce były szczury ze wszystkich grup. Zasadniczo kierunek badań był taki sam,

jak w doświadczeniu I. Zwierzęta grup „M" i „O" otrzymywały przez sondę takie same ilości kwasów i w takim samym stężeniu co poprzednie. Poczyniłam jednak pewne modyfikacje. Przede wszystkim szczurom grupy kontrolnej podawałam przez sondę wodę destylowaną, by wyeliminować ewentualny wpływ samego faktu podawania sondy na szczury grupy „M" i „O". Następnie zwiększyłam liczbę oznaczeń zawartości CO_2 w krwi, pomiarów pH krwi i moczu. Ponadto badałam przemianę materii oraz wykonywałam sekcje szczurów po ukończonym doświadczeniu.

W pierwszym tygodniu podawania kwasów padło 5 szczurów z grupy „M". W następnym tygodniu padły jeszcze trzy. W grupie „O" padło w ciągu całego okresu doświadczalnego 16 zwierząt. Natomiast z grupy „K" przez cały czas podawania sondy nie padł żaden szczur.

W pierwszych trzydziestu dniach doświadczenia średnie przyrosty na 100 g wagi szczura były następujące:

	„M"	„O"	„K"
samce	24,1 g	16,4 g	32,9 g
samice	17,6 g	6,5 g	21,8 g

Po następnych trzydziestu dniach dane dotyczące średnich zmian wagowych przedstawia załączone zestawienie:

	„M"	„O"	„K"
samce	5,1 g/100 g	2,3 g/100 g	13,0 g/100 g
samice	4,7 g/100 g	1,5 g/100 g	5,1 g/100 g

W ostatnim okresie trzydziestodniowym zmiany wagowe w grupie „M" wahały się od −13,5 do +6%. W grupie „O" samcom ubyło 5,4 i 7,6g/100 g wagi. Ubytek wagi wystąpił u wszystkich samic: 1,6, 3,6 i 4,3 g/100 g wagi. Szczurom grupy „K" w tym samym okresie przybyło na wadze średnio: samcom 5,5 g i samicom 3,4 g na 100 g wagi zwierzęcia.

Średnie różnice w ilości hemoglobiny po upływie dziewięćdziesięciu dni, podczas których podawano zwierzętom przez sondę kwas mlekowy, octowy lub wodę, wynosiły procentowo:

dla grupy	„M"	„O"	„K"
samce	−12,3	−18,1	+1,1
samice	−13,3	−17,7	+0,9

W okresie końcowym krew szczurów grupy „M" zawierała 80—87% Hb. Jeden szczur miał 79% Hb. W grupie „O" ilość Hb wynosiła 78, 79 i 82%.

Średnia liczba krwinek czerwonych w 1 mm³ przed rozpoczęciem doświadczenia była około 9.220.000. Odchylenia od średniej były dość znaczne. Spośród 63 szczurów cztery miały powyżej 10.000.000/mm³, 2 szczury po-

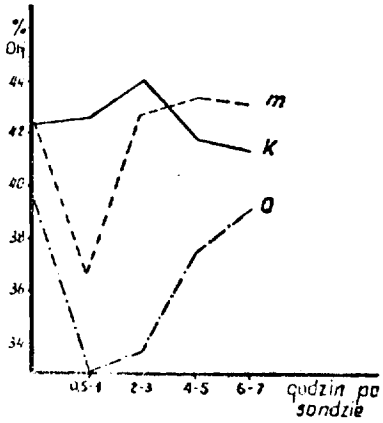
niżej 7.000.000 krwinek czerwonych. Liczba krwinek czerwonych u reszty wahała się od 8.000.000 do 9.800.000/mm³. Po upływie dziewięćdziesięciu dni doświadczenia liczba krwinek czerwonych spadła w grupie „M" do 7.240.000 — 8.240.000/mm³. Średni ubytek wynosił 11,8%. W grupie „O" ubytek był większy i wynosił średnio u samic 32,5%, u samców 27,2%. Liczba krwinek czerwonych w końcowym okresie doświadczenia była poniżej 7.000.000/mm³. Oznaczanie liczby krwinek czerwonych u szczurów grupy „K" w okresie początkowym i końcowym nie wykazywało zasadniczo różnic z wyjątkiem 2 przypadków, kiedy liczba krwinek czerwonych u jednego szczura wzrosła o 16,7%, a u innego spadła o 8,3%. U pozostałych szczurów wahania w okresie dziewięćdziesięciodniowym wynosiły od —2 do +5,4%.

Procentowa zawartość dwutlenku węgla w krwi podczas pierwszego oznaczenia, wykonanego na początku okresu doświadczalnego, wynosiła 33,3 do 50% z tym, że tylko u 15 szczurów wartość ta była poniżej 40%, a u 22 powyżej 45%. W trzydziestym dniu wykonałam dwa oznaczenia. Pierwsze miało miejsce przed sondą, drugie w 1 — 2 godz. po sondzie. Różnic w otrzymanych wynikach nie stwierdziłam. W końcowym okresie doświadczenia procentową zawartość CO_2 w krwi oznaczałam w ciągu dnia pięciokrotnie co 2—3 godziny. Pierwsze oznaczenie wykonywałam przed podaniem sondy w dniu bieżącym, a około 24 godz. po sondzie podanej w dniu poprzednim. Ostatnie oznaczenia wykonywałam w 6—7 godz. po podaniu sondy w dniu bieżącym. W grupie „M" zmniejszenie ilości CO_2 w krwi występowało w czasie 0,5—1 godz. po podaniu sondy. Ilość CO_2 zmniejszała się o 7—10%. U 4 szczurów nie zauważyłam wyraźnego obniżenia się ilości CO_2. W 2—3 godz. następowało albo wyrównanie, albo nawet ilość jego wzrastała powyżej wartości początkowej. Maksimum ilości CO_2 wypadało przeważnie na 4—5 godz. po sondzie. W 6—7 godz. po sondzie ilość malała, przewyższając jednak najczęściej wartość początkową. Oznaczenia analogiczne u szczurów grupy „O" w trzydziestym dniu wykazywały średnią różnicę około 5%. Wyniki oznaczeń wykonanych 5-krotnie co 2—3 godz. w dziewięćdziesiątym dniu dały najniższe wartości dla ilości CO_2 w 0.5—1 godz. po podaniu kwasu. Wyrównanie ilości CO_2 w krwi następowało powoli. Poziom wyrównywał się dopiero po upływie 6—7 godz. Nie zauważyłam tutaj występowania maksimum. Zawartość CO_2 zmniejszała się o 10,6, 9,2 u samców oraz 8,6, 4,1, 3,9% u samic. W grupie „K" spostrzegłam nieznaczne wahania w ilości CO_2, ale występowały one nieregularnie (ryc. 6).

pH krwi oznaczane w analogicznym czasie miały pewną tendencję do obniżania się w 1/2—1 godz. po podaniu sondy w grupie „M". W tym samym czasie w grupie „O" obniżenie pH było nieznaczne. W grupie tej

tendencje do ustalania się minimum przypadały na 2—3 godz. po sondzie, podczas gdy w tym samym czasie pH krwi w grupie „M" było już całkowicie wyrównane. Różnice w grupie „M" wynosiły średnio — 0,04 pH, w grupie „O" — 0,05 pH, podczas gdy w grupie „K" średnio 0,02 pH (ryc. 7).

Pomiary, jakie wykonałam w trzydziestym dniu doświadczenia, wskazywały na nieznaczne obniżenie się pH moczu u szczurów grupy „M" i „O" w 1—2 godz. po sondzie. Zwiększenie liczby pomiarów w ciągu jednego dnia



Ryc. 6
Doświadczenie II. Średnie zmiany ilości CO_2 w 30 dniu

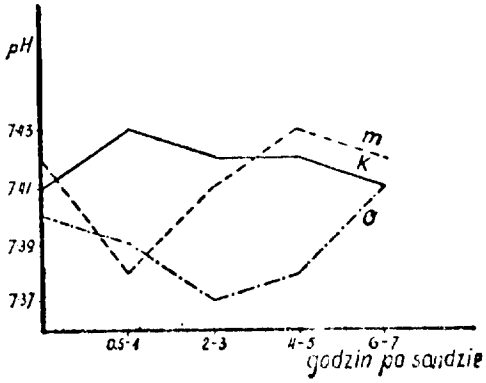
w końcowym okresie doświadczenia pozwoliło zaobserwować duże wahania w pH moczu. U szczurów grupy „M" mocz rano przed podaniem kwasu w dniu badania wykazywał reakcję alkaliczną, pH dochodziło u niektórych szczurów do 7,45. Alkaliczność moczu utrzymywała się powyżej 24 godzin, jeżeli następna dawka kwasu nie została wprowadzona. Natomiast po podaniu sondy w 1—2 godz. alkaliczność nieznacznie malała. Następny pomiar wykonany w 3—4 godz. po sondzie wykazywał kwaśny odczyn moczu. W późniejszych etapach znowu następował wzrost pH. U szczurów grupy „O" analogiczne pomiary nie ujawniały odczynu alkalicznego. Najniższe pH przypadało na 4—5 godz. po sondzie. W następnych pomiarach pH moczu stopniowo wzrastało, nie stwierdziłam jednak w tej grupie wartości wyższych niż 6,89 (ryc. 8).

Oznaczenie ilości pobranego tlenu oraz wydalonych produktów przemiany materii wykonywałam u 15 szczurów wszystkich trzech grup. Badanie trwało 15 minut. Szczury były uprzednio głodzone przez 24 godzin. Próby

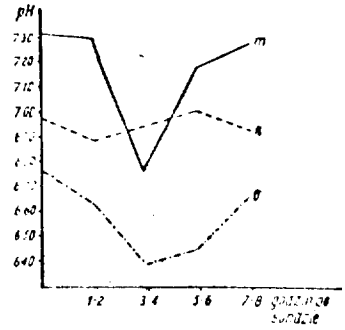
te usiłowałam przeprowadzać u wszystkich szczurów o tej samej porze dnia oraz w takim samym czasie po sondzie. Czas ten wynosił około 2 godzin.

Grupa	g O_2/100 g wagi/godz.		Spółczynnik oddechowy	
	od — do	średnio	od — do	średnio
„M"	0,199—0,240	0,217	1,003—1,023	1,009
„O"	0,184—0,198	0,191	1,007—1,037	1,021
„K"	0,166—0,190	0,179	0,666—0,786	0,723

Jak widać z załączonej tabeli, spółczynniki oddechowe w grupie „M" i „O" są prawie identyczne, znacznie wyższe niż w grupie „K". Natomiast zażycie tlenu w przeliczeniu na 100 g wagi wzrosło w grupie „M" o wiele więcej niż w grupie „O".



Ryc. 7
Doświadczenie II. Średnie zmiany pH krwi w 90 dniu



Ryc. 8
Doświadczenie II. Średnie zmiany pH moczu w 90 dniu

I w tym doświadczeniu zwierzęta należące do poszczególnych grup różniły się wyglądem. Szczury „K" zachowały żywotność i normalny wygląd. W grupie „O", tak jak i poprzednio, wystąpiły zmiany sierści i oddechu. Szczury „M" miały także oddech przyśpieszony i głośniejszy. Sekcje zwierząt wykonane po 90 dniach podawania kwasów wykazały pewne różnice w porównaniu ze zwierzętami kontrolnymi. W grupie „M" wątroba była powiększona i miała odcień ciemnowiśniowy. Ilość tłuszczu w tkance podskórnej i otaczającej narządy wewnętrzne była dużo mniejsza niż u kontrolnych. Śluzówka żołądka i dwunastnicy łatwo dawała się usunąć za pomocą tępego narzędzia. Szczury grupy „O" tłuszczu nie miały prawie

wcale, nawet w okolicy nerek było go bardzo mało. W zgrubiałej i przekrwionej śluzówce żołądka i dwunastnicy spostrzegłam wybroczyny krwawe. Wątroba była również powiększona, barwy silnie ciemnoczerwonej, małej spoistości. Wątroba i nerki u szczurów grupy „M" i „O" różniły się ciężarem od narządów tych u szczurów „K"; w grupie „M" i „O" narządy te były cięższe niż w grupie „K".

Do trzeciego doświadczenia użyłam 11 dziewięciotygodniowych samców. Zwierzęta podzieliłam na grupy jak poprzednio. Szczury grupy „M" i „O" otrzymywały w godzinach południowych 10%-owy kwas mlekowy lub octowy nie przez sondę jak poprzednio, ale dodany do pożywienia. Mieszałam 1 ml z 20 g paszy, co przy spożyciu średnio przez szczura 15 g pożywienia dawało w efekcie wprowadzenie do organizmu około 3 ml kwasu, tzn. tyle, ile w doświadczeniach poprzednich.

Krzywa zmian wagowych szczurów tego doświadczenia miała charakter zbliżony do dwóch poprzednich. Maksymalne przyrosty wykazywała grupa „K". Ubytek hemoglobiny wynosił średnio: w grupie „M" 6,3% i „O" 8,3%. Liczba krwinek czerwonych spadła średnio w grupie „M" o 11,6%, w grupie „O" o 18,9%.

Krzywe zmian zachodzących w pH krwi, moczu oraz w ilości CO_2 krwi mają charakter analogiczny w grupie „M" i „O". Zmiany w ilości CO_2 we krwi były niewielkie. Lekka zwyżka występowała w godzinach rannych. Najniższe wartości zauważyłam w godzinach wieczorowych. Różnice pH krwi nie przekraczały 0,02 uzyskując swoje maksimum w godzinach rannych. Natomiast pH moczu osiągało swoje maksimum w godzinach południowych. Odczyn alkaliczny moczu występował o tej porze również u szczurów „O".

Wyglądem zewnętrznym zwierzęta poszczególnych grup nie różniły się od siebie, jedynie oddech szczurów grupy „O" był świszczący. Sekcje zwierząt nie wykazywały żadnych widocznych zmian. Nie było również wyraźnych różnic w wadze poszczególnych narządów wewnętrznych w grupach „M", „O" i „K".

W czasie prowadzenia doświadczenia zainteresowałam się tym, czy podawanie kwasu samicom w czasie ciąży i laktacji wpłynie na pH mleka. W celu sprawdzenia tego nastawiłam 15 samic na rozmnażanie. Pięciu z nich dawałam normalne dawki kwasu mlekowego, pięciu innym kwas octowy, pozostałym wodę. Dwie matki „M" i trzy „O" padły przed urodzeniem młodych. Pozostałe urodziły młode normalnie. pH mleka mierzyłam dwukrotnie: 10 i 20 dnia po porodzie. Mleko otrzymywałam uciskając i masując sutki, a zbierałam je do rurek kapilarnych. pH mleka w obydwu pomiarach nie wykazywało różnic między poszczególnymi grupami; miało ono odczyn

słabo alkaliczny (pH = 7,2). Natomiast mocz młodych, karmionych przez matki „M" i „O", miał pH niższe (6,70 i 6,52) w porównaniu z młodymi od matek z grupy „K" (7,06).

Zestawienie wyników

Zestawienie wyników, otrzymanych z poszczególnych doświadczeń, nasuwa przypuszczenie, że działanie kwasu mlekowego i octowego na organizm jest podobne.

Zmniejszenie przyrostu wagi w porównaniu z grupą „K" występowało we wszystkich doświadczeniach u szczurów grupy „M" i „O". Przyrosty wagi grupy „O" były najniższe.

Grupa	Doświadczenie		
	I. g / 100 g	II. g / 100 g	III. g / 100 g
„M"	30,35	31,94	64,00
„O"	20,78	20,10	44,91
„K"	53,15	58,2	95,5

Śmiertelność szczurów w czasie prowadzenia doświadczenia była wyższa w grupie „O" niż w „M".

Z powyższego wynika, że kwas octowy działał silniej niż mlekowy, zwłaszcza gdy był podawany sondą. Podawanie natomiast kwasów z pożywieniem nie spowodowało ani jednego zgonu w żadnej z grup. Próby nad ustaleniem dawki śmiertelnej wykazały jednak, że kwas octowy jest bardziej toksyczny niż mlekowy.

Zmiany w ilości hemoglobiny i krwinek czerwonych wystąpiły bardzo wyraźnie u szczurów „M" i „O" we wszystkich doświadczeniach. Ubytek największy był w grupie „O" u tych szczurów, które otrzymywały kwas przez sondę; mniejszy zaś u tych, którym mieszano go z pożywieniem. Zmiany te nie mogły być spowodowane pobieraniem krwi do poszczególnych oznaczeń, gdyż szczury „K" nie wykazują wyraźnych różnic, a pobieranie krwi we wszystkich grupach było identyczne.

Grupa	Hemoglobina. Różnica %			Krwinki czerwone. Różnica %		
	Doświadczenie			Doświadczenie		
	I	II	III	I	II	III
„M"	— 10,76	— 12,4	— 6,3	— 13,3	— 12,8	— 11,6
„O"	— 17,91	— 18,1	— 8,3	— 23,9	— 27,2	— 18,9
„K"	+ 0,67	+ 1,1	— 0,3	+ 0,1	+ 2,1	— 0,07

W doświadczeniu II różnice, zachodzące w pH krwi grup „M" i „O" w porównaniu z grupą „K", nie były duże, ale w dwóch pierwszych grupach dawała się zauważyć wyraźna tendencja do obniżenia się tej wartości w dość stałych okresach uzależnionych od czasu podawania kwasów. Jak widać z wykresów, które są zrobione w bardzo dużej skali, pH krwi obniżało się u szczurów grupy „M" w 0,5—1 godz. po podaniu sondy. Różnica wynosiła średnio —0,04 pH. Wyrównanie następowało bardzo szybko, gdyż już w następnym pomiarze pH jest równe lub wyższe od początkowego. W grupie „O" obniżenie pH stwierdziłam później, bo dopiero w 2—3 godziny po sondzie. Utrzymywało się ono natomiast dłużej; wyrównanie zauważyłam dopiero w 6—7 godzin po podaniu tego kwasu. W grupie „K" różnice były mniejsze i nie występowały regularnie, co dobrze widać na rycinie 7.

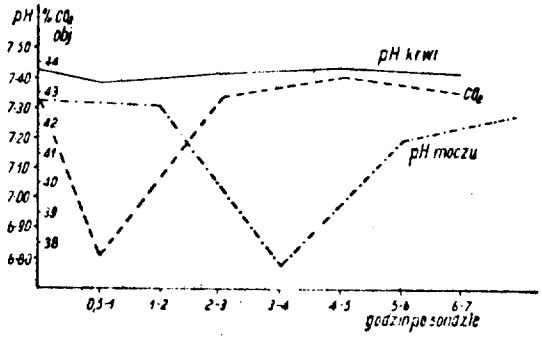
W doświadczeniu II, jak widać z wykresów, zmniejszanie się ilości CO_2 u szczurów grupy „M" i „O" było wyraźnie uzależnione od czasu podania kwasów. W obydwóch grupach minimum występowało w 0,5—1 godz. po sondzie. Wyrównanie w grupie „M" było szybkie, w grupie „O" wolniejsze.

Wyniki oznaczania pH moczu u szczurów „M" i „O" we wszystkich doświadczeniach są podobne, jeżeli chodzi o zmniejszanie się tej wartości po podaniu kwasów. Stwierdzenie jednak alkaliczności moczu szczurów „M" nastąpiło dopiero w doświadczeniu II przy zwiększonej liczbie pomiarów. Nie uchwyciłam jednak wtedy odczynu alkalicznego w moczu szczurów grupy „O"; natomiast w III doświadczeniu zauważyłam w godzinach rannych i południowych również i w tej grupie zwiększenie pH moczu.

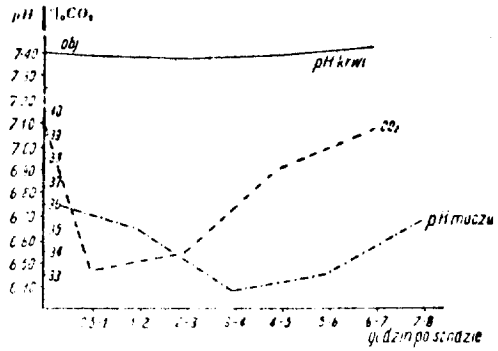
W wynikach oznaczeń ilości CO_2, pH krwi i moczu widać pewną zależność w grupach „M" i „O". Obniżanie i zwiększanie się poszczególnych wartości w krwi jest uzależnione od czasu, jaki upłynął od momentu podania kwasów; analogiczne zmiany natomiast występujące w pH moczu są przesunięte o parę godzin (ryc. 9, 10, 11).

Wszystkie dane otrzymane z poszczególnych doświadczeń wskazują na to, że chociaż obydwa kwasy działały na szczury podobnie, to jednak kwas octowy wywoływał zmiany trwające dłużej, chociaż nie zawsze głębsze. Zawartość CO_2 i pH krwi szczurów grupy „M" szybciej wracała do normy, co można tłumaczyć tym, że organizm jest nastawiony na zużytkowanie lub usuwanie kwasu mlekowego, jako stałego produktu przemian zachodzących w ustroju; kwas octowy natomiast może trudniej poddaje się procesom metabolicznym, zwłaszcza przy ciągłym ponawianiu dawek.

Duże wahania w wartościach pH w moczu, zwłaszcza u grupy „M", wskazywałyby na początkowe wydalanie przez nerki nadmiaru kwasu, a następnie zobojętnienie go alkaliami względnie wyprodukowanym amo-



Ryc. 9
Doświadczenie II. Średnie zmiany ilości CO_2 i zmiany pH krwi i moczu grupy M w 90 dniu



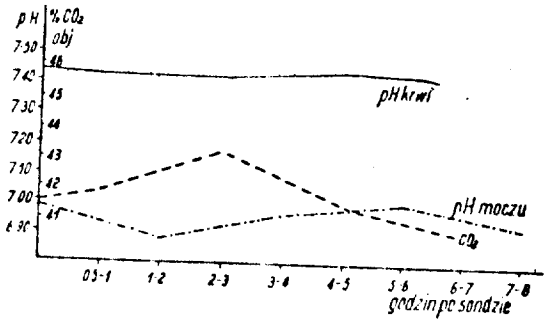
Ryc. 10
Doświadczenie II. Średnie zmiany ilości CO_2 i zmiany pH krwi i moczu grupy O w 90 dniu

niakiem. Wyraźne zwiększenie alkaliczności moczu po upływie 24 godzin, od podania kwasu następuje, być może, dlatego, że organizm przyzwyczajony do kwasu jest nastawiony w dalszym ciągu na jego neutralizację.

Zwiększenie spółczynnika oddechowego u szczurów „O" przy prawie tej samej ilości zużytego tlenu co w grupie „K" wskazuje na wydalanie podczas oddychania zwiększonej ilości dwutlenku węgla, który przypuszczalnie pochodzi z rozłożonych kwasem dwuwęglanów krwi. To samo można prawdopodobnie powiedzieć omawiając grupę „M", gdzie współczynnik oddechowy jest również duży, ale jednocześnie występuje większe zużycie tlenu. Widać więc, że u szczurów grupy „M" w około 2 godz. po podaniu kwasu następuje szybkie spalanie węglowodanów względnie kwasu mlekowego, gdyż spółczynniki tych związków są zbliżone.

Zwiększenie wagi wątroby i nerek w grupach „M" i „O" w porównaniu z grupą „K" ma swoje uzasadnienie w większym przekrwieniu tych narządów, a jeżeli chodzi o wątrobę, to także i w zwiększeniu ilości odkładanego glikogenu lub tłuszczu.

Sądząc z wyników otrzymanych w doświadczeniach działanie kwasu mlekowego i octowego na organizm szczurów można by uważać za ujemne. Biorąc jednak pod uwagę duże dawki, bo wynoszące około 1,5 g czystego



Ryc. 11
Doświadczenie II. Średnie zmiany ilości CO_2 i zmiany pH krwi i moczu grupy K w 90 dniu

kwasu na kg wagi, i podawanie tych ilości przez trzy miesiące, co u szczura stanowi prawie 10% jego życia, należy przypuszczać, że obydwa kwasy nie wywierają ujemnego wpływu przy normalnym użyciu. Potwierdzenie tego można znaleźć w tym, że ujemne objawy występowały ostro tylko u szczurów otrzymujących kwasy bezpośrednio do żołądka; u tych natomiast, którym kwasy mieszano z pożywieniem, objawy ujemne występowały w dużo łagodniejszej postaci.

З. Высокиньска

СРАВНЕНИЕ ДЕЙСТВИЯ МОЛОЧНОЙ И УКСУСНОЙ КИСЛОТ НА ОРГАНИЗМ КРЫС

Содержание

Автор исследовал действие молочной и уксусной кислот, вводимых per os белым крысам. Установлено, что токеническая доза составляет 5 гр молочной кислоты и 2,5 гр уксусной кислоты на 1 кг веса.

При постепенном увеличении дозы толерацция доходила до 11,25 гр. молочной кислоты и 7,25 гр. уксусной кислоты.

В хронических опытах (90 дней) крысам вводили ежедневно при помощи зонда по 3 мл 10%-ой молочной или уксусной кислоты. Было обнаружено большое падение содержания гемоглобина и эритроцитов, особенно у крыс из группы уксусной кислоты.

Изменения pH крови и мочи а также количества CO_2 иллюстрирует сводка:

		До введения кислот	0,5—1 час после введения	2—3 часа после введения	4—5 часов после введения	6—7 часов после введения
CO_2 об'емный %	Группа М	42,2	37,0	47,6	42,6	42,2
	„ У	39,0	33,7	34,6	38,6	40,4
	„ К	43,5	42,9	44,4	42,2	42,1
pH крови	Группа М	7,41	7,37	7,41	7,43	7,42
	„ У	7,42	7,41	7,38	7,39	7,42
	„ К	7,42	7,42	7,42	7,41	7,42
pH мочи	Группа М	7,27	7,26	6,78	7,15	7,23
	„ У	6,85	6,70	6,48	6,57	6,76
	„ К	6,98	6,89	6,88	6,99	6,83

Дыхательный коэффициент, определяемый через 2 часа после введения кислот, был значительно выше у крыс, получавших кислоты, чем у контрольных.

Увеличение же потребления кислорода проявили только крысы из группы молочной кислоты. Крысы из группы уксусной кислоты обнаружили минимальный прирост веса, а в конечном периоде даже падение веса.

Выводы: Полученные результаты позволяют считать, что действие молочной и уксусной кислот являются аналогичным, при чем изменения наступающие под влиянием уксусной кислоты являются более длительными, хотя и не всегда более глубокими.

Z. Wysokińska

LA COMPARAISON ENTRE L'ACTION DE L'ACIDE LACTIQUE ET CELLE DE L'ACIDE ACETIQUE SUR L'ORGANISME DU RAT.

Résumé

On a examiné l'action de l'acide lactique et celle de l'acide acétique administrés par voie buccale aux rats blancs et l'on a pu établir la dose toxique qui est de 5 g pour l'acide lactique et de 2,5 g pour l'acide acétique par 1 kg du poids de l'animal, mais en élevant progressivement la dose on arrive à 11,25 g pour l'acide lactique et à 7,25 g pour l'acide acétique.

Dans des expériences de longue durée (90 jours) l'on administrait journellement aux rats à l'aide d'une sonde buccale 3 ml d'acide lactique ou acétique à 10% et l'on a constaté une grande baisse tant du Hb que du nombre de globules rouges du sang, surtout chez les animaux qui recevaient de l'acide acétique.

Les variations du pH du sang et de l'urine ainsi que de la quantité du CO_2 présente le tableau suivant (Remarque: M — groupe à l'acide lactique, O — groupe à l'acide acétique, K — groupe de contrôle):

	Avant l'administration des acides	Après l'administration des acides			
		0,5 à 1 hr	2 3 hrs	4 5 hrs	6 7 hrs
CO_2, en vol. gr. M	42,2	37,0	41,6	42,6	42,2
„ O	39,0	33,7	31,6	38,6	40,4
„ K	43,5	42,9	44,4	42,2	42,1
pH du sang gr. M	7,41	7,37	7,41	7,43	7,42
„ O	7,42	7,41	7,38	7,39	7,42
„ K	7,42	7,42	7,42	7,41	7,42
pH de l'urine gr. M	7,27	7,26	6,78	7,15	7,23
„ O	6,85	6,70	6,48	6,57	6,76
„ K	6,98	6,89	6,88	6,99	6,83

Le coefficient de respiration mesuré 2 heures après l'administration des acides était beaucoup plus élevé chez les rats qui recevaient des acides que chez les rats de contrôle. L'augmentation d'absorption d'oxygène avait lieu seulement chez des rats qui recevaient de l'acide lactique. Des rats du groupe de l'acide acétique avaient une augmentation du pouls plus faible et pendant la période finale de l'expérience accusaient même une baisse.

Les résultats obtenus permettaient de conclure que l'action de deux acides est analogue, mais les changements qui se produisaient sous l'action de l'acide acétique sont plus durables, toutefois sans être plus profonds.

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Otrzymano dn. 23 marca 1951 r.

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Young-'44
~~Bib No.~~ 2050

LACTIC ACID: A CORROSIVE POISON

REPORT OF THREE FATAL CASES WITH EXPERIMENTAL CONFIRMATION

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The common occurrence and physiologic role of lactic acid have tended to establish the general opinion that this substance is not to be considered as poisonous. Lactic acid is not mentioned in any of the standard textbooks on toxicology. We have been able to find only one reference to it as a cause of death in a human being. Leschke[1] cites a case described by Fühner[2] of a woman aged 27 who died in twelve hours after the administration by duodenal tube of 100 cc. of a 33 per cent aqueous solution of lactic acid in error for magnesium sulfate. This resulted in dyspnea, vomiting of blood and mucus, a rapid feeble pulse, hemoglobinuria and cyanosis. The autopsy revealed a dark red patch of erosion in the duodenum. Both duodenum and jejunum showed hemorrhagic infiltration of all coats and necrosing inflammation. Leschke remarks that "lactic acid has a corrosive action exactly like that of any other acid of corresponding hydrogen ion concentration." The dissociation constant of lactic acid (K_a) is usually given as 1.38×10^{-4} at 25 C.[3] This makes it comparable with formic acid at 2.14×10^{-4}, long considered a poisonous corrosive and almost ten times stronger than acetic acid, which has also been so classified when in high concentration.

From the experimental point of view there is confirmatory evidence. Dreyfus[4] has shown that the rectal administration of 40 cc. of a 3 per cent solution of acetic, butyric, lactic or tartaric acids to rabbits caused death in a few hours. Pike, Osnato and Notkin[5] injected lactic acid in doses of 0.07 to 0.1 Gm. per pound of body weight into cats to produce convulsions, which were later followed by death with respiratory failure and pneumatorrhexia. Lastly, Fürth and Engel[6] found that lactic acid given orally to rabbits in doses of 0.6 to 1.6 Gm. per kilogram of body weight caused death, and they quote Parnas as showing that 2.8 to 3.6 Gm. per kilogram could be given intravenously. They state that mice can withstand about the same dosage as rabbits but that rats are more resistant. In rats and mice alkalosis induced by sodium bicarbonate did not alter the effect.

Recently we were called on to investigate the cause of death of 3 premature infants. They were all being fed the same lactic acid milk mixture on the basis of the following formulas, and all ingredients had been used previously for the same purpose: (1) evaporated milk, water and acid sugar solution, equal parts; (2) acid sugar solution: water, 1 pint, corn syrup, 1 tablespoon, lactic acid (concentrated) 1 teaspoon.

REPORT OF CASES

CASE 1.—*Clinical History.*—Baby H., a very small white girl aged 35 days, weighing 3.85 pounds (1,746 Gm.), was found dying three quarters of an hour after receiving the lactic acid milk mixture by gavage. She was one month premature and weighed 3.38 pounds (1,533 Gm.) at birth.

Autopsy Findings.—The autopsy was performed within a few hours of death.

General Appearances: There were no excoriations on the face, mouth or lips.

Lungs: The right upper lobe showed minute peribronchial hemorrhages with pus exuding from the bronchioles. Microscopic examination revealed this to be a typical capillary bronchitis. Areas of emphysema, atelectasis and acute congestion were also present in the lung. The left lung showed only a little acute congestion.

Esophagus: There was a small black necrotic, eroded area at the cardiac end, extending into the stomach, which was confirmed histologically.

Heart: The heart was normal except for some cloudy swelling of the myocardium and a little terminal dilatation of the right side.

Peritoneal Sac: The peritoneal sac contained some blackish fluid stomach contents.

Stomach: This had perforated, and its blackish, mucous contents had exuded. Its wall was black, hemorrhagic, necrotic and extremely friable and the seat of an acute gangrenous gastritis.

Intestine: Except for a slight injection of the jejunum, the intestine showed no gross or microscopic change. The contents were not blood stained.

Mesentery and Omentum: These showed white necrotic areas on a black gangrenous background.

Pancreas: Gross examination revealed some blackening and superficial necrosis externally, but only some congestion was visible on microscopic examination.

Liver: There were areas of superficial hemorrhage and acute congestion with cloudy swelling of the liver cells, confirmed histologically. The under aspect of the left lobe was blackened from the action of sulfide.

Spleen: It was very soft, toxic and congested.

Adrenals: Except for some superficial discoloration of the left gland, they were normal.

Kidneys: The kidneys showed cloudy swelling and some congestion of the glomeruli but no nephritis.

Comment: The capillary bronchitis present in the right lung would appear to have been present before the poisoning in view of the short time which elapsed between the feeding with the lactic acid milk mixture and death.

Pathologic Diagnosis: Acute gangrenous gastritis, a result of corrosive poisoning; capillary bronchitis of the right lung; toxemia.

CASE 2.—*Clinical History.*—Baby C., a small white girl aged 37 days and weighing 4.33 pounds (1,965 Gm.), was found in a collapsed, pale, distressed condition with a feeble rapid pulse. She died three and one-fourth hours after receiving the lactic acid milk mixture by gavage. Although two months premature, she was otherwise healthy and weighed 3.03 pounds (1,581 Gm.) at birth. Oxygen and carbon dioxide were given without avail.

Autopsy Findings.—The postmortem examination was performed within four hours of death.

General Appearances: No excoriations were visible on the face, lips or mouth.

From the Departments of Biochemistry and Pathology, Dalhousie University.

Dr. F. V. Woodbury, medical coroner, referred the cases to us. Mr. J. A. H. Rice assisted with some of the experiments. Dr. G. B. Wiswell supplied the clinical data.

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Lungs: Both lungs showed acute congestion and there were some petechiae in the upper lobes. No evidence of pneumonic change, either gross or microscopic, was detected.

Heart: Except for one small petechial hemorrhage on its surface near the base of the left ventricle and some cloudy swelling in the myocardium, the heart was otherwise normal.

Peritoneal Sac: It contained some blackish stomach contents.

Esophagus and Stomach: There was a black gangrenous area in the esophagus ½ inch from the cardiac end of the stomach. The whole wall of the stomach was black and extremely friable and exhibited an intensely acute gangrenous gastritis with a small area of early erosion and perforation.

Intestine: The duodenum, jejunum and ileum all showed an acute enteritis, but the large bowel was relatively free, with nothing abnormal in the sigmoid or rectum.

Mesentery of the Small Intestine and Omentum: White fatty areas were visible on a black gangrenous background.

Mesenteric Lymph Nodes: They were somewhat enlarged from a simple acute lymphadenitis.

Liver: The liver showed pronounced cloudy swelling and acute congestion.

Spleen: It was very soft, congested and toxic.

Kidneys: Both kidneys were the seat of cloudy swelling and acute congestion, and some petechial hemorrhages were seen at the upper pole of the left. No nephritis was present.

Pathologic Diagnosis: Acute gangrenous gastritis with perforation and acute enteroenteritis, suggestive of corrosive poisoning; toxemia.

CASE 3.—*Clinical History.*—Baby M., a small white boy aged 17 days and weighing 4.16 pounds (1,887 Gm.), was under observation for a longer time than the 2 previous infants as fifteen and one-half hours had elapsed between the feeding of the lactic acid milk mixture by savage and death. The baby was born at seven and one-half months of term and was thus six weeks premature. The birth weight was 3.81 pounds (1,72[illegible] Gm.).

The main symptoms were those of pallor, collapse and a feeble, rapid pulse. There were no convulsions or vomiting, and no staining of the diapers was noted, such as would have been expected with a hemoglobinuria. Up to the time of the onset of the symptoms of poisoning the infant was healthy and progressing favorably. Nikethamide and vitamin K were given without result.

Autopsy Findings.—The autopsy was performed two hours after death.

General Appearances: There were no excoriations of the face, lips or mouth.

Lungs: The lungs showed only acute congestion and some petechial hemorrhages. No signs of capillary bronchitis or of pneumonia were present.

Heart: The myocardium showed cloudy swelling but there was no other abnormal change seen.

Esophagus: The last 2 inches of its lower end were necrotic and gangrenous.

Stomach: As in the previous cases, the wall was blackened and hemorrhagic and was the seat of an acute hemorrhagic and gangrenous gastritis. No perforation, however, had occurred. The contents had a black color.

Intestine: Both the small and the large intestine were inflamed and the seat of acute enteroenteritis which extended down to the sigmoid colon.

Mesenteric Glands: They were enlarged and showed an acute lymphadenitis.

Liver: There was a pronounced cloudy swelling, with degeneration and acute congestion with small scattered areas of hemorrhage. Although a slight infection in the cells in the fatty areas was visible, no true toxic necrosis could be identified on microscopic examination.

Spleen: It was slightly enlarged, soft, toxic and intensely congested, with hemorrhagic areas through the pulp.

Kidneys: Both showed considerable cloudy swelling with some necrosis of the secreting tubules, diffuse congestion of the blood vessels and some generalized swelling of the glomeruli with congestion of their capillaries. The condition present had the character of an acute catarrhal nephritis or nephrosis. No accumulations of hemoglobin were visible in the collecting tubules.

Urinary Bladder: This was normal and contained practically no urine.

Pathologic Diagnosis: Acute gangrenous gastritis and acute enteroenteritis with acute nephrosis or catarrhal nephritis, a result of corrosive poisoning; toxemia.

CASE 4.—The same lactic acid milk mixture was also given by bottle to a 10 day old full term infant, but after one mouthful which caused choking and reddening of the face, the baby spit it out and refused to take more.

Dr. Wiswell informs us that the baby developed an infected throat and suffered from a little bronchial irritation with wheezing for a day or two but made a complete recovery. Refusal to take the mixture evidently was the means of saving its life.

CHEMICAL EXAMINATION

Unfortunately, the acid milk mixture fed to the babies was thrown away immediately after the first death was discovered and was therefore not available for analysis. Furthermore, the amount of the mixture taken by the babies was not exactly known, but it was probably about 1 ounce. The various ingredients were, however, examined qualitatively and the hydrogen ion concentration determined either colorimetrically or electrometrically by a Beckman p_H meter. All conformed to their labeled contents. The corn syrup was acid, with p_H at 4.03, and the evaporated milk at p_H 6.7. The lactic acid possessed a specific gravity of 1.2, corresponding to the U. S. P. syrupy variety at 85 per cent with a p_H less than 1.

The stomach contents, together with peritoneal fluid from case 1 measured 25 cc. of a dark brown mucous liquid without curds and with a slightly sour odor. The p_H was 2.5. The p_H of the stomach contents in case 2 was 3.0 and in case 3 about 5. A determination of the content of lactic acid in the stomach contents in case 1 was carried out according to the technic of clarification of Gerty T. Cori[7] and of estimation of Friedmann and Kendall[8] with the apparatus of West.[9] The concentration was 0.19 per cent lactic acid, or a total of 41 mg. in the whole specimen.

EXPERIMENTS WITH RABBITS

The experimental feeding of the milk mixture with an increased amount of lactic acid was next tried on adult rabbits. The formula used was made up of equal parts of the original evaporated milk, water and the concentrated lactic acid (85 per cent). This mixture was rather disagreeably acid to taste and had a p_H 2.1. It was 34 per cent (w/v) lactic acid. The casein curd which formed initially dissolved as the hydrogen ion concentration passed to the acid side of the isoelectric point.

RABBIT 1.—One ounce, i. e. approximately the amount of the feeding estimated by the nurse, of this mixture was given by stomach tube to a 6 pound rabbit which had not been fed for about twelve hours. The animal was soon obviously distressed

7. Cori, G. T.: The Influence of Insulin and Epinephrine on the Lactic Acid Content of Blood and Tissues, J. Biol. Chem. 63: 55[illegible] 1[illegible] 5.

8. Friedmann, T. E., and Kendall, A. I.: The Determination of Lactic Acid, J. Biol. Chem. 82: 73-83, 1929.

9. West, E. S.: An Improved Lactic Acid Apparatus, J. Biol. Chem. 92: 485-48[illegible], 1931.

and the respiration became labored at 64 per minute in one hour. The rabbit died suddenly in convulsions within two hours. There was no vomiting, defecation or urination throughout the period. This would indicate death following a dose of 10.2 Gm., or about 1.7 Gm. per kilogram, i. e. 0.77 Gm. per pound for this rabbit. This dosage is of the same order of magnitude as the minimum lethal dose recorded by Fürth and Engel[9] at 0.6 to 1.6 Gm. per kilogram. At autopsy the stomach was found greatly distended with fluid containing mucus, blood and much fecal material. The fundic portion of the wall was very thin and near to perforation. The mucosa showed an intense, hemorrhagic, almost gangrenous, acute gastritis. The duodenum was soft and congested, and the rest of the small intestine was essentially empty. The jejunum showed some hemorrhages into its mucosa in the upper portion. The spleen was congested, rather soft and toxic, with some hemorrhages into the pulp. The kidney showed only some congestion and cloudy swelling. The lungs were edematous, congested and hemorrhagic but showed no evidence of capillary bronchitis or bronchopneumonia. The urine was scanty (2.5 cc.), very turbid with amorphous phosphates and distinctly alkaline (p_H 8.8). There was no protein or sugar present. The pathologic diagnosis of cause of death was acute hemorrhagic gastroenteritis. This is a striking confirmation of the suspected cause of death of the 3 babies, as the clinical and pathologic pictures were so similar.

RABBIT 2.—Another animal, weighing 5 pounds, was fed by stomach tube 30 cc. of a mixture of 2 parts whole milk to 1 part lactic acid (U. S. P. 85 per cent). This solution had a p_H of 1.58. The rabbit had been starved for sixteen hours previously. It was our purpose to study the effect on the alkali reserve of the blood in this animal and to determine the rate of absorption of the lactic acid. The respiratory rate was very rapid throughout most of the period, beginning at 150 per minute, rising to 240 in one hour and falling to about 200 until shortly before death, when the deep, slow Kussmaul respiration set in. The animal died in convulsions six hours after the administration of the lactic acid.

The alkali reserve of the blood was measured periodically in the Van Slyke apparatus[10] on oxalated plasma. Prior to the experiment it was 44 volumes of carbon dioxide per hundred cubic centimeters of plasma. At thirty minutes it was 38, and just prior to death 33.

The findings at autopsy performed immediately after death were essentially as recorded previously. The stomach contained 144 Gm. of a semifluid brownish mass with some characteristic fecal pellets. It had a slightly sour odor. The p_H was 3.30. The concentration of lactic acid was 1.91 per cent (w/v) and the total in the stomach contents was 2.75 Gm. As the amount originally administered was 10.1 Gm., there thus remained only 27.5 per cent unabsorbed after about six hours. This figure is in agreement with the determinations of Cori[11] on the rate of absorption of 12 to 15 per cent sodium lactate from the intestine of the rat. She found 25.8 per cent absorbed in the first hour, 44.9 per cent in the second, 59.1 per cent in the third and 69.7 per cent in the fourth. Our figure was 72.5 per cent in six hours. The concentration of lactic acid in the original solution, from which the feeding mixture was prepared, was determined after boiling for thirty minutes with excess of normal sodium hydroxide to hydrolyze the lactone and titrating the excess with normal hydrochloric acid. The concentration was 84.2 per cent.

RABBIT 3.—A similar experiment was carried out with another rabbit, except that the animal was allowed to swallow about 20 cc. of the acid milk mixture naturally. Measurement of the alkali reserve showed values of 67 at the start of the experiment, 55 after three hours and 43 after eight hours. The animal died during the following night, in approximately forty hours.

RABBIT 4.—A similar experiment on another animal fed 30 cc. of the acid milk mixture by stomach tube resulted in death with convulsions in ten minutes. There was no vomiting, but a blood stained froth was visible at the external nares and the lungs were noticeably edematous. In this case the stomach was so eroded that it ruptured on removal from the abdominal cavity. There were about 100 cc. of contents present.

COMMENT

From these observations and experiments it would seem obvious that lactic acid must be considered as a corrosive poison. The explanation of the deaths of the 3 babies is probably to be found in the administration of an acid milk mixture containing too high a concentration of lactic acid. This resulted in hemorrhage and erosion of the gastric and duodenal mucosa such that death resulted in toxemia from severe gastroenteritis. The interval prior to death was forty-five minutes in case 1, three and one-fourth hours in case 2 and fifteen and one-half hours in case 3. The rabbits died in ten minutes, two hours, six hours and about forty hours after identical doses. From the experiments with rabbits it does not appear as if the resulting acidemia was a major factor. That it has an effect, however, is apparent from the reduction in the alkali reserve of the blood. This aspect has been studied by Cohen,[12] who showed that intravenous injection of lactic acid at 0.045 Gm. per kilogram caused increased respiratory rhythm both in rate and amplitude but that the response was not specific for lactic acid. Rodler[13] has also shown that lactic acid milk containing 6 Gm. per liter tended to produce an acidosis as measured by increased urinary acidity and decreased excretion of phosphate.

In comparison with the recognized toxicology of acetic acid it is not surprising that lactic acid, a much stronger acid, should also be considered as a corrosive poison. Due care should therefore be exercised in the labeling and dispensing of this reagent so commonly used in the feeding of infants.

SUMMARY

The deaths of 3 premature infants have been investigated, both pathologically and chemically, and found to be due to an acute hemorrhagic and gangrenous gastritis. The deaths occurred in 0.75, 4.25 and 15.5 hours following the administration of an acid milk mixture containing an excess of lactic acid. The 2 babies who were larger and lived longer also showed an acute enterocolitis, and the 1 who lived for 15.5 hours had, in addition, an acute catarrhal nephritis or nephrosis.

After administration by mouth of a lactic acid milk mixture containing 10.1 Gm. in 30 cc. to rabbits, death followed in ten minutes and two, six and forty hours in 4 animals. The symptoms and findings were identical with those of the infants and death was attributed to acute hemorrhagic gastritis. There was a moderate degree of acidosis.

Lactic acid must therefore be regarded as a corrosive poison, and due care must be exercised in its use as an infant food.

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