M O N O G R A P H

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A D I P I C A C I D

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ADIPIC ACID

Table of Contents

Summary	1
Chemical Information	4
Biological Data	
Acute Toxicity	8
Short Term Studies	10
Long Term Studies	14
Special Studies	16
Biochemical Aspects	18
Bibliography	24
Documents	29

ADIPIC ACID

Summary

The use of adipic acid in the food industry has prompted investigations into its biological and biochemical characteristics.

The metabolism of adipic acid has been investigated by several workers. Rusoff et al. (55) stated that orally administered adipic acid (approximately 250 mg/kg) is almost completely absorbed by the rat. Earlier studies by Enders (14) lend support to this statement. The absorbed adipic acid is primarily excreted in the urine unchanged or in the breath as CO_2 (14, 18, 40, 55). Tests by Rusoff et al. (55) indicate that adipic acid is metabolized by beta-oxidation in much the same manner as fatty acids. Metabolic products found in the urine after feeding ^{14}C-labeled adipic acid have been identified as urea, glutamic acid, lactic acid, beta-ketoadipic acid and citric acid (55). Following repeated feedings of adipic acid to rats, Enders (14) observed the urinary excretion of adipic acid to drop to zero within 24 hours after the last feeding (5 days for rabbits). Whole-body analysis of rats sacrificed 24 or 72 hours after the last adipic acid feeding disclosed no accumulation of adipic acid in the body tissues (14). In a similar study, Rusoff et al. (55) found some slight accumulation of adipic acid, primarily in the liver and kidneys. Studies by Lang and Bartsch (34) indicate that the degree of excretion of adipic acid is independent of the dosage and that the rat does not develop a capacity to convert increased amounts of the substance.

Stohr (60) and Rusoff et al. (55) found no significant increase in liver glycogen formation following oral administration of adipic acid to rats. Mori (40) reported that urinary oxalic acid excretion was increased following subcutaneous administration of adipic acid to rabbits. Kabelitz (29), however, found no change in urinary oxalic acid excretion following administration of adipic acid to humans by intestinal tube.

To date, investigations into the biological characteristics of adipic acid have been limited to laboratory animals, primarily the rat.

In a test of the teratogenicity of adipic acid, 5 groups (24-31 animals/group) each of pregnant mice, rats, and hamsters were given, respectively, graduated doses of up to 263, 288, and 205 mg adipic acid/kg body weight for 10, 10, and 5 consecutive days during gestation (19). Detailed examination of all dams and fetuses revealed no clearly discernible effects on nidation or on maternal or fetal survival (19).

The exposure of 2 male and 2 female rats to adipic acid dust at a concentration of 126 mu-g/l of air for 6-hour periods daily 5 days per week for a total of 15 exposures was observed by Gage (20) to induce no signs of toxicity.

The acute toxicity of adipic acid has been tested by various routes in mice, rats and rabbits. A value of 1900 mg/kg was established by Horn et al. (28) as an LD_{50} for adipic acid following oral administration to 39 adult male albino mice as a 6% suspension in 0.5% methyl cellulose. The i.v. injection of a 2% aqueous solution of adipic acid at a rate of 0.01 ml per second to 39 mice was found by Horn et al. (28) to yield an LD_{50} of 680 mg/kg. Intraperitoneal injections of 600 and 900 mg/kg as a 3% aqueous solution were found by Horn et al. (28) to be lethal to mice.

Through the i.p. injection of a 3% aqueous solution of adipic acid to 21 male albino rats, Horn et al. (28) arrived at an LD_{50} of 275 mg/kg.

Enders (14) reported that the average lethal dose of oral adipic acid for rabbits lies between 2.43 and 4.86 g/kg, the lower dose producing only minor effects. Rose et al. (54) found that adipic acid (approximately 1-2 g/kg) is mildly nephropathic to rabbits when administered subcutaneously. Harding and Nicholson (25) contend that Na adipate (unspecified amount) appears innocuous to rabbits when administered intramuscularly.

A number of studies have been conducted on the short-term toxicity of adipic acid to rats, the most notable of which is the series of experiments conducted by Lang and Bartsch (34). They found that adipic acid fed to 72 female rats in daily doses of 0-40 mg for 4 weeks produced no change in the growth curve or in other outward behavior (34). Of 69 male rats fed 0-800 mg adipic acid/day for 5 weeks only those receiving the highest level (800 mg/day) exhibited any symptoms of toxicity - poor growth, heavy diarrhea and unkempt appearance during the first 2-3 weeks of feeding (34). In their third experiment, male and female rats given 400 mg adipic acid/day for 33 weeks showed no ill effects, while rats receiving 800 mg/day manifested effects similar to those just mentioned (34). Several pregnant females were included in the group receiving 400 mg/day and it was found that they were able to bear and nurse young (34). Lang and Bartsch (34) also treated rats with 0-400 mg adipic acid/day for 19 weeks in a protein-deficient diet and found that the toxicity of adipic acid is greater under these circumstances - the rats receiving 400 mg/day exhibited significant and prolonged growth inhibition.

In a 90-day feeding test in which 60 albino rats were given a basal diet supplemented with 0, 0.1, 1.0, or 5.0% adipic acid, the only adverse effect produced by the treatment was a marked retardation of growth among the animals receiving 5.0% adipic acid (42). A study in which Na citrate, Na adipate and citric acid were fed to groups of albino Carworth Farms rats for 14 weeks showed that all three of these regimens caused retardation of growth (42). When rats from the groups receiving citric acid and sodium adipate were switched to an untreated basal diet after the 14-week feeding period, the rats responded with rapid weight gain during the ensuing weeks (42). In another study it was found that adipic acid produces no evidence of pathology in mature rats or guinea pigs when administered orally as powder, as an alcoholic solution, or in the form of sodium adipate (42). However, doses between 638 and 1332 mg/kg/day given to immature rats for 9 weeks (5 days per week) produced significantly greater weight losses in those rats than in control rats fed equimolar

doses of sodium acetate (42). In a study by Enders (14) five 60-80 g rats given 0.243 g adipic acid by stomach tube daily for 4 weeks exhibited no significant effects of the treatment on growth rate. Three 300-g adult rats given 0.72 g adipic acid by stomach tube daily for 4 weeks exhibited no symptoms of toxicity (14).

The only long-term study available on adipic acid is a feeding study by Horn et al. (28) in which a total of 169 albino Carworth Farms rats were maintained for two years on either a basal diet or the basal diet containing either adipic acid (0.1, 1, 3 and 5%) or citric acid (3 and 5%). During the rapid growth period of the studies, weight gains for the rats receiving 3 or 5% adipic or citric acid were significantly less than the controls, while growth in the other groups appeared normal (28). No other evidence of toxicity was discerned (28).

ADIPIC ACID

Chemical Information

I. Nomenclature

A. Common Names

1. Adipic Acid
2. Hexanedioic Acid

B. Chemical Names

1. 1,4-Butanedicarboxylic acid

C. Trade Names

None

D. Chemical Abstracts No.

000124-04-9

II. Empirical Formula

$C_6H_{10}O_4$

III. Structural Formula

$$\begin{array}{l} CH_2CH_2COOH \\ | \\ CH_2CH_2COOH \end{array}$$

IV. Molecular Weight

146.14

V. Specifications

Food Chemicals Codex

Assay	Not less than 99.6% and not more than the equivalent of 101.0% of $C_6H_{10}O_4$
Melting Range	Between 151.5 degrees and 154 degrees
Limits of Impurities	
Arsenic (as As)	Not more than 3 parts per million (0.0003%)
Heavy metals (as Pb)	Not more than 10 parts per million (0.001%)
Residue on ignition	Not more than 0.002%
Water	Not more than 0.2%

VI. Description

A. General Characteristics

Adipic acid is a white, crystalline, odorless solid with a slightly acid taste.

B. Physical Properties

Adipic acid is characterized by the following typical data: density, real (solid), 1.366 g/cm^3, apparent (crystals), 0.635 g/cm^3 (39.6 lg/ft^3); mp, 152.1 degrees C; bp at 760 mm Hg, 330.5 degrees C (with decomposition), at 100 mm Hg, 265.1 degrees C, and at 10 mm Hg, 205.5 degrees C; flash point (Cleveland open cup), 210 degrees C; fire point (Cleveland open cup), 232 degrees C; viscosity (of melt) at 160 degrees C, 4.54 cps, and at 193 degrees C, 2.64 cps. The pH values for water solutions of adipic acid at 25 degrees C are as follows:

Adipic acid, wt %	0.1	0.2	0.4	0.6	1.2	2.5
pH	3.2	3.1	3.0	2.9	2.8	2.7

Adipic acid is slightly soluble in water and very soluble in methanol. The temperature-solubility characteristics in various solvents are shown in Figure 1. The solubility of adipic acid can be increased appreciably by using binary mixtures of water with methanol, ethanol, or acetone. Table 1 gives solubilities in mixtures of water with acetone and ethanol.

Table 1. Solubility of Adipic Acid in Binary Mixtures at 40°C

Weight percent of solvent in water	Grams of adipic acid per 100 grams of mixture	
	Acetone	Ethanol
0	5.2	5.2
20	15.0	13.7
40	28.2	25.9
60	35.9	34.0
80	32.4	33.5
100	8.7	22.4

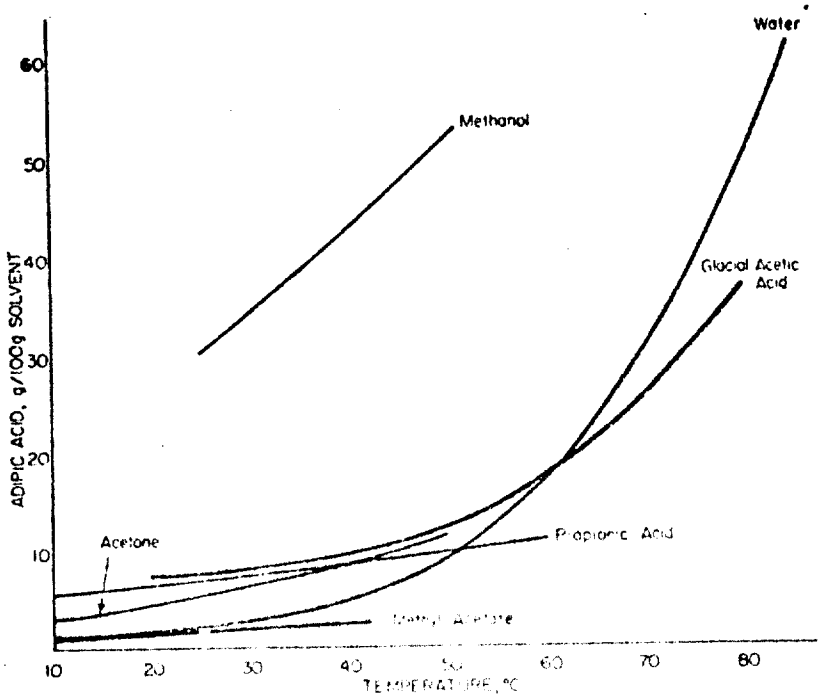

Fig. 1. Solubility of adipic acid.

C. Stability

Adipic acid is relatively thermally stable under a nitrogen blanket; heating for 15 hours at 232 degrees C is required to develop a marked discoloration. Accompanying the decomposition, significant amounts of water, carbon dioxide, and cyclopentanone, and some valeric acid, C_4H_9COOH, are formed. At 300 degrees C decomposition to cyclopentanone is nearly quantitative. The presence of compounds such as calcium oxide and basic barium salts markedly accelerates the reaction. Cyclopentanone can be conveniently prepared by heating a mixture of barium hydroxide and adipic acid at 285-295 degrees C; yields of 75-85% are obtained. Holding adipic acid for four minutes at its boiling point results in the formation of as much as 7% of polymeric adipic anhydride. The mechanism of the thermal decomposition of adipic acid and its esters is believed to involve the formation of cyclopentanone-2-carboxylic acid (or its ester) as the first step, followed by decarboxylation (59).

Adipic acid is particularly stable toward oxidation. Under manufacturing conditions, there is very little attack on adipic acid by air oxidation, even though it is heated under pressure to temperatures as high as 275 degrees F (59).

VII. Analytical Methods

The presence of adipic acid in a material is readily determined by identification of one or more of its derivatives, which are easily prepared by standard procedures. The most common derivatives of adipic acid are as follows:

Derivative	*Melting point, °C* (50)
amide	220
anilide	240
p-toluidide	241
p-nitrobenzyl ester	105
p-bromophenacyl ester	154
p-phenylphenacyl ester	148

The adipic acid content of a material may be precisely determined by a number of methods. If it is the only acid present, as might be the case in a powdered gelatin dessert, electrometric titration with sodium hydroxide is a satisfactory procedure. When other acids or interfering compounds are present, a method utilizing isotope dilution of the sample with radio-labeled (carbon-14) adipic acid may be used. After addition of a known amount of labeled acid to the sample in solution, adipic acid is recovered from the solution by crystallization and is purified. Because the labeled acid has the same solubility behavior as the normal acid, the measured radioactivity of the recovered material then indicates the extent to which the added acid has become diluted with normal acid and, hence, the percentage of adipic acid in the original sample. This method is considered to be absolute. Other methods that have been successfully employed include partition chromatography with a silicic acid column and infrared absorption of the sodium salt (59).

VIII. Occurrence

A. Plants

Adipic acid has been found in beet juice.

B. Animals

No Information Available

C. Synthetics

It is produced commercially by nitric acid oxidation of cyclohexanol or a mixture of cyclohexanol and cyclohexanone. The former is obtained by reduction of phenol, while the latter is produced by oxidizing cyclohexane.

D. Natural Inorganic Sources

No Information Available

ADIPIC ACID

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage mg/kg Body Weight	Measurement	Ref.
Adipic Acid	Mice	39 M	p.o.	1900	LD_{50}	28
Adipic Acid	Mice	39	i.v.	680	LD_{50}	28
Adipic Acid	Rats	21 M	i.p.	275	LD_{50}	28

Mice

Using a total of 39 adult male albino mice, a value of 1900 mg/kg was established by Horn et al. as an LD_{50} for adipic acid when administered orally as a 6% suspension in 0.5% methyl cellulose. Autopsy of the animals that died showed distention of the stomach and small intestine, with a spastic concentration of the cecum. Irritation and hemorrhage of the intestines were noted. Initial mortality developed overnight and deaths continued throughout the first week, after which the survivors appeared normal (28).

The intravenous injection of adipic acid to a total of 39 mice at various dosage levels, administered as a 2% aqueous solution at a rate of 0.01 ml per second, was found by Horn et al. to yield an LD_{50} of 680 mg/kg. The acid caused immediate, convulsive death, probably due to acute acidosis as the pH of the solution was 3.08. Autopsy showed hemorrhagic lungs but no other gross pathology. In survivors recovery was apparently complete and there were no latent deaths (28).

Horn et al. gave a few mice lethal doses (600 and 900 mg/kg) of a 3% aqueous solution of adipic acid intraperitoneally. The mice showed depression immediately and, at autopsy, the intestines appeared irritated and the lungs appeared hemorrhagic (28).

Rats

In an effort to evaluate the acute toxicity of adipic acid, 21 male albino rats were given, by Horn et al., a 3% aqueous solution of adipic acid intraperitoneally. An LD_{50} of 275 mg/kg was determined. Mortality occurred during the first 5 days, the animals showing hemorrhagic lungs and irritation of the intestines. The survivors, sacrificed 1 week after administration, showed extensive irritation and adhesions of the visceral organs (28).

Rabbits

Enders found that the average lethal dose of adipic acid lies between

2.43 and 4.86 g/kg for rabbits when administered orally. The lower dose is not lethal and causes some slight discomfort to the animals; the rabbits sit inactive, eat little, have a distended stomach, and suffer from more or less intense diarrhea. Within 24 hours all of these symptoms nearly disappear. At the higher dosage the animals die 10-30 hours after feeding. Autopsy shows that the entire intestine is swollen and filled with masses of brown liquid. A microscopic examination of the liver and kidneys reveals marked venous obstruction in these organs (14).

When adipic acid is administered to rabbits i.v., the only effect produced by a dose of 2.43 g/kg is polyuria, due to which the animals often lose up to 20% of their weight within 8 hours (14).

The data detailed in Table 1 was cited by Rose et al. as typical of their experiments on the nephropathic action of adipic acid. Their studies indicate that adipic acid is mildly nephropathic when administered subcutaneously to rabbits (54).

TABLE 1

Adipic acid

Rabbit 16, male, 2160 grams

DATE	ADIPIC ACID SPIKED	PHTHALEIN RECOVERED	BLOOD					NOTES, ETC.
			Non-protein nitrogen	Urea N	Creatinine	Sugar	NaCl	
	grams	in cent	mgm.	mgm.	mgm.	per cent	per cent	
December 5								No food; water ad lib.
December 6		75*	45.5	22.2	1.4	0.114	0.47	10:00 a.m., 6 cc. blood. 10:20 a.m., renal test
December 6	2.0							7:00 p.m., acid 8 injected subcutaneously as sodium salt in 30 cc. of water
December 7		79†	58.3	33.3	1.7	0.127	0.45	9:30 a.m., 6 cc. blood. 10:00 a.m., renal test
December 7	2.0							6:30 p.m., acid 8 injected subcutaneously as sodium salt in 20 cc. of water
December 8		79‡	49.0	29.6	1.7			10:30 a.m., 7 cc. blood. 10:40 a.m., renal test
December 9	4.0							10:00 a.m., acid 8 injected subcutaneously as sodium salt in 30 cc. of water
December 9		20§						2:30 p.m., renal test
December 10		81¶	42.6	23.3	1.6	0.137	0.48	10:00 a.m., 7 cc. blood. 2:00 p.m., renal test. Experiment discontinued

*First period, 3 cc. urine containing 44 per cent dye; second period, 3 cc. urine containing 31 per cent dye. Total 75 per cent.

†First period, 6 cc. urine containing 70 per cent dye; second period, 2 cc. urine containing 9 per cent dye. Total 79 per cent.

‡First period, 3 cc. urine containing 57 per cent dye; second period, 3 cc. urine containing 22 per cent dye. Total 79 per cent.

§First period, 12 cc. urine containing 11 per cent dye; second period, 10 cc. urine containing 9 per cent dye. Total 20 per cent.

¶First period, 8 cc. urine containing 73 per cent dye; second period, 17 cc. urine containing 8 per cent dye. Total 81 per cent.

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, %
ADIPIC ACID NAS 0003 FEMA 2011	01	BAKED GOODS(R)	*	.00384	.01337
	04	FATS OILS(R)	*	.27000	********
	07	FROZN DAIRY(R)	*	.00030	.00040
	10	MEAT PRODS(R)	*	.26700	.26700
	11	POULTRY(R)	*	.01500	.01500
	15	CONDM RELSH(R)	*	1.40063	1.40875
	20	GELATIN PUD(R)	5	.34009	.39975
	22	SNACK FOODS(R)	*	.05000	.18636
	23	BEV TYPE I(R)	*	.00400	.00450
	24	BEV TYPE II(R)	*	.00400	********
	27	GRAVIES(R)	*	.07500	.10000
	28	IMIT DAIRY(R)	*	.42500	********
	34	INS CCF TEA(R)	*	.00067	.00400

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
ADIPIC ACID NAS 0003 FEMA 2011	6/ 9	2,000,550	5,073,550	5,091,757	7	11,213	5,102,970

VI. Consumer Exposure

Adipic acid is employed as a leavening acidulant in baking powders and as the acidulant in powdered concentrates for fruit-flavored drinks and bottled beverages. It may also be used for improving the melting characteristics and texture of processed cheese and cheese spreads, as an agent for increasing the shipping quality of products containing egg white and as a gel-inducing agent in imitation jams and jellies. It can be used in the canning of vegetables, as an acidulant in candies and flavoring extracts, as a sequestrant in edible oils, and as an acidulant in throat lozenges. Its propyl ester adds a soysauce flavor to foods. Combined with sodium metaphosphate, it was been used in the preservation of sausages and other meats.

The following tables were compiled from data collected during a GRAS survey, NAS/NRC in 1972. Data from the Market Research Corporation of America (MRCA), on the frequency of eating the substance, produced the food consumption values, and the mean portion sizes in each food category were taken from USDA data.

Table 2 gives usage levels found in regular foods. Table 11 shows annual poundage data for the years 1960 and 1970. Table 13 reports the possible daily intakes of various food categories and the age groupings represented.

to recover 50% of the administered dose from the urine and concluded that the remaining 50% had been decomposed (18).

In studies in which rats accustomed and unaccustomed to adipic acid consumption were fed 400 or 800 mg/day for 14 days, Lang and Bartsch determined that the degree of excretion of adipic acid was approximately equal in all cases, indicating that the degree of excretion is independent of the dosage and that the rat does not develop a capacity to convert increased amounts of the substance (34).

Through the analysis of urine which was collected from 300-g rats receiving 0.73 g adipic acid by stomach tube daily for 4 weeks Enders found that 60-70% of the administered dose was excreted daily. Excretion of the adipic acid dropped to zero within the first 24 hours after the last feeding. Whole-body analysis of these rats and of young, growing rats which had received 0.243 g adipic acid/day for 4 weeks (the rats were sacrificed 72 hours after the last feeding) disclosed no accumulation of adipic acid in the body tissues (14).

In an effort to determine the extent of excretion of adipic acid, Enders treated rabbits by stomach tube on two successive days with 2.43 g adipic acid/kg. Collection and analysis of the urine for 6 days starting on the day of the first treatment revealed that an average of 57% of the administered dose was excreted unchanged in the urine. The excretion of adipic acid reached a maximum on the second day and dropped to zero on the fifth. In additional tests, the excretion of adipic acid was found to be somewhat faster and more complete when the acid was administered i.v. (14).

Following s.c. injection of 0.8-2.0 g of adipic acid (neutralized with sodium carbonate) into rabbits weighing 2.7-3.45 kg, Mori found that an average of 61% of the injected dose was excreted unchanged in the urine within 24 hours after injection. Urinary oxalic acid excretion was observed to increase following the administration of adipic acid (40).

Kabelitz reported that the administration of 30 g of adipic acid to each of two human subjects by intestinal tube did not affect their urinary oxalic acid excretion (29).

IV. Effects on Enzymes and Other Biochemical Parameters

No Information Available

V. Drug Interaction

No Information Available

Biochemical Aspects

I. Breakdown

No Information Available

II. Absorption - Distribution

See next section

III. Metabolism and Excretion

Rusoff et al. investigated the metabolism of adipic acid through tests in which fasting male albino Carworth Farms rats, weighing 150-250 g, were given a single dose of 50 mg of radioactive adipic acid by stomach tube. At this concentration the adipic acid was almost completely absorbed and up to 70% of the administered dose was excreted in the breath, as $^{14}CO_2$, during the 24 hours following administration. Radioactive metabolic products identified as urea, glutamic acid, lactic acid, beta-ketoadipic acid and citric acid, as well as adipic acid, were found in the urine (55).

Rusoff et al. stated that the presence of beta-ketoadipic acid provides some evidence that adipic acid is metabolized by beta-oxidation in much the same fashion as fatty acids. Additional evidence supporting this conclusion is provided by further tests in which succinate appeared in the urine of rats fed with radioactive adipic acid and injected with malonic acid. Evidence that acetate is a metabolite of adipic acid is provided by the presence of radioactive acetyl-gamma-phenyl-alpha-aminobutyric acid in the urine of rats after feeding gamma-phenyl-alpha-aminobutyric acid and ^{14}C-labeled adipic acid. The isolation from the urine, following feeding tests with radioactive CO_2, of traces of some of the same metabolic products as found after adipic acid feeding indicates that some of the metabolic products found in the urine are not direct degradation products of adipic acid, e.g., urea, but contain radioactive carbon derived via CO_2 from adipic acid (55).

Rusoff et al. found that the tissues from the sacrificed rats showed very little residual radioactivity. Of all the tissues examined, the highest activity appeared in the liver and kidney. Although negligible amounts of glycogen were isolated from the livers, the glycogen did not appear to be radioactive. However, when glycogen formation in the liver was encouraged by the oral administration of glucose with radioactive adipic acid, a high concentration of glycogen was isolated which was radioactive (55).

The feeding of 0.2-0.3 g adipic acid to fasted 110-130 g male white or dappled rats was found by Stohr to produce no significant increase in liver glycogen (60).

Following subcutaneous injection of 10 g of sodium adipate into each of 4 young dogs in doses of 0.25 g twice daily, Flaschentrager was able

Five groups of 25-27 virgin adult female golden hamsters from an outbred strain were mated and then given oral doses of 0.0, 2.0, 9.5, 44.0, or 205.0 mg of adipic acid/kg body weight daily from the 6th day of gestation through the 10th day. On Day 14 all animals were subjected to Caesarean section and observations and measurements were performed as in the preceeding studies. The oral administration of up to 205 mg of adipic acid/kg body weight to pregnant hamsters for 5 consecutive days was observed to have no clearly discernible effect on nidation or on maternal or fetal survival (19).

Inhalation

The subacute inhalation toxicity of adipic acid was investigated by Gage through tests using 2 male and 2 female Alderley Park specific-pathogen-free rats, with an average weight of 200 g. The rats were exposed to adipic acid dust at a concentration of 126 mu-g/l of air for 6-hour periods daily 5 days per week for a total of 15 exposures. No signs of toxicity were observed among the rats. Blood tests gave normal values and autopsy revealed all organs to be normal (20).

Table 9. Incidence of Tumors and/or Lung Pathology

Male Group	Deaths				Sacrificed	
	Lung pathology	Tumors	Other causes	Total deaths	Lung pathology	Tumors
Control	7	3	3	12	4	1
Adipic						
0.1%	3	2	3	7	7	2
1%	1	2	2	5	7	2
3%	3	..	1	4	3	..
5%	..	4	1	5	4	..
Citric						
3%	1	2	3	6	1	..
5%	1	2	1	4	4	1

During the rapid growth period of the studies, weight gains for the rats receiving 3 or 5% adipic or citric acid were significantly less than the controls; however, there was no significant difference among those four experimental groups. Growth of the other groups was comparable to that of the respective controls. There was no evidence of gross or microscopic pathology or increased incidence of tumors or lung pathology associated with the feeding of either acid. The length of survival of the various groups did not differ significantly from the controls (28).

IV. Special Studies

Teratogenicity

To test the teratogenicity of adipic acid, five groups of 25-31 virgin adult female albino CD-1 outbred mice were mated and then given oral doses of 0, 2.6, 12.0, 56.0, or 263.0 mg of adipic acid/kg body weight daily from the 6th day of gestation through the 15th day. The animals were observed for appearance and behavior and their body weights monitored throughout the experiment. On Day 17 all dams were subjected to Caesarean section under surgical anethesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality. All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each liter underwent detailed visceral examinations employing 10 X magnification. The remaining two-thirds were cleared in KOH, strained with alizarin red S dye and examined for skeletal defects. The results of the tests indicate that the oral administration of up to 263 mg of adipic acid/kg body weight to pregnant mice for 10 consecutive days has no clearly discernible effect on nidation or on maternal or fetal survival (19).

In a similar study, 5 groups of 24-25 virgin adult female albino rats (Wistar derived stock) were mated and then given oral doses of 0.0, 2.9, 13.0, 62.0, or 288.0 mg of adipic acid/kg body weight daily from the 6th day of gestation through the 15th day. On Day 20 all dams were subjected to Caesarean section and observations and measurements were performed as in the aforementioned study. The oral administration of up to 288 mg of adipic acid/kg body weight to pregnant rats for 10 consecutive days was found to have no clearly discernible effect on nidation or on maternal or fetal survival (19).

observations were made of the general appearance and condition of each animal. Whenever possible, gross autopsy was performed on those animals that died during the course of the experiment (28).

After 2 years on the respective diets, the surviving rats were weighed, sacrificed by a blow on the head, and examined for gross and microscopic pathology. The brain, thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, and testes of approximately half of each group of males were weighed. The kidneys, spleen, liver, and heart of each female were weighed. Microscopic examination of the following tissues were done on a representative number of animals of each group: thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, small intestine, large intestine, pancreas, bone marrow, testes or ovaries, and uterus. The results of the feeding studies are summarized in Tables 7-9 (28).

Table 7. Summary of Average Body Weights of Albino Rats

(Controls received the basal diet. Other animals received the basal diet containing the indicated percentage of the adipic acid or citric acid)

	Average Body Weight in Grams								
	Males							Females	
		Adipic Acid				Citric Acid			
Week	Control	0.1%	1%	3%	5%	3%	5%	Control	Adipic acid, 1%
0	59	61	63	61	57	62	61	49	48
8	269	280	265	224	182	239	225	178	175
16	325	333	320	276	233	298	278	222	213
24	361	374	354	309	264	329	320	242	233
32	377	391	376	329	291	328	339	257	249
40	397	407	401	357	314	370	361	279	263
48	423	433	421	372	322	393	377	275	270
56	428	447	436	380	336	400	388	286	277
64	426	455	436	385	339	407	401	295	284
72	407	447	431	385	336	400	389	301	288
80	408	441	430	383	349	411	391	313	301
88	413	448	432	398	344	411	389	309	303
96	432	424	436	396	354	409	393	318	308
104	440	417	437	400	360	417	397	321	304

Table 8. Summary of Data for Albino Rats Receiving Basal Laboratory Diet or Basal Diet of Adipic or Citric Acid for 2 Years

(Per cent of survival based on length of survival as well as number of animals)

Level	Sex	No. of Rats		Av. Body Weight, G.		Food Consumed, G., Av./Rat/Day	Compound Consumed, Mg., Av./Rat/Day	Survival, %
		Start	Finish	Initial	Final			
Control	M	20	8	59	440	16.8		82.5
	F	10	8	49	321	14.2		98.9
Adipic acid								
0.1%	M	20	13	61	417	17.0	17.0	87.7
1%	M	20	15	63	437	17.5	175	94.7
	F	19	17	48	304	15.8	158	96.3
3%	M	20	16	61	400	16.8	505	94.5
5%	M	20	15	57	360	15.8	814	97.2
Citric acid								
3%	M	20	14	62	417	17.1	512	92.6
5%	M	20	16	61	397	15.7	784	95.0

In a study by Enders, 5 young rats, weighing 60-80 g, were given by stomach tube 0.243 g of adipic acid daily for 4 weeks. A similar group of rats was maintained as a control. The determination of body weights at the end of the test period revealed no significant differences between the treated rats and the controls (14).

Three 300 g adult rats were fed by stomach tube 0.73 g adipic acid daily for 4 weeks. The weight of the rats remained constant and they appeared healthy throughout the test period. The animals were sacrificed at the end of the test period and examined without finding any adverse effects (14).

Guinea Pigs

See page 12, paragraph 4.

III. Long-Term Studies

Rats

In a two-year feeding study by Horn et al., albino rats of the Carworth Farms strain were placed on either a basal diet or the basal diet containing either adipic acid or citric acid, as follows (28):

Group	Males	Females
Basal laboratory diet used as control	20	10
Basal diet containing 0.1% adipic acid	20	0
Basal diet containing 1% adipic acid	20	19
Basal diet containing 3% adipic acid	20	0
Basal diet containing 5% adipic acid	20	0
Basal diet containing 3% citric acid	20	0
Basal diet containing 5% citric acid	20	0

The body weights and food consumption of all rats were recorded at weekly intervals during the course of the study. In addition, weekly

TABLE 6

Expt. No.	Animals Used: Type	Animals Used: No.	Material Used	Dosage	No. Doses	Duration	Deaths	Pathology, Etc.
1	Rats (mature)	4	Adipic A. *	100 mg. (310-386 mg/K)	25	5 weeks	1 **	None *Adipic acid was given as 20% sol. in 95% ethyl alcohol. **Death from pneumonia
	Rats (mature)	4	Adipic A. *	200 mg. (610-922 mg/K)	25	5 weeks		
2	Guinea Pigs	5	Adipic A. *	400 mg. (682-942 mg/K)	5	1 week		None
				600 mg. (1032-1739 mg/K)	25	5 weeks		*Adipic acid solid given in capsules.
3	Rats (Immature)	10	Na adipate*	199 mg. (638-1332 mg/K)	44	9 weeks	4**	None **Deaths all due to infection
		10	Na acetate*	284 mg. (873-2202 mg/K)	44	9 weeks	2**	*Na adipate and Na acetate given in equimolecular doses in aqueous solution.

Histological examination of the rats used by Lang and Bartsch revealed nothing remarkable in the case of the animals receiving less than 400 mg adipic acid per day. The histological findings in the case of the animals treated with higher doses were independent of the level of protein intake. Slight histological alterations were discovered in the liver and kidneys and marked changes were exhibited in the intestinal mucosa, offering the picture of a chronic inflammatory condition. The absorption epithelium was higher than normal, the cells appeared elongated and richer in plasma; the nuclei were very voluminous and situated toward the base of the cell. The border of the epithelium toward the base was unclear and the border along the tunica propria was bumpy. The absorption border of the epithelium was markedly swollen. The number of goblet cells was increased. There was an intense effort at regeneration, evident from the numerous mitoses in the crypts. The lymphatic tissue in the tunica propria was significantly increased (34).

In a 90-day feeding test on adipic acid, groups of 10 male albino rats were given a basal diet supplemented with 0, 0.1, 1.0 or 5.0% adipic acid and similar groups of females were given 0 or 1.0% adipic acid. Monitoring of the body weights of the animals indicated that 0.1% and 1.0% levels of adipic acid added to the diet of either male or female rats does not exert a significant influence on either the survival or the body weight of rats. However, the male rats receiving 5.0% adipic acid exhibited a marked retardation of growth during the entire feeding period. Since this effect was not associated with an alteration of food intake and there was no marked gross pathology at sacrifice, the researchers concluded that the growth retardation was associated with impairment of food utilization. No histological examination of tissues was done (42).

Four groups of young male albino rats (60 g) of the Carworth Farms strain were used in a study of subacute feeding of Na citrate, Na adipate, and citric acid. Three of the groups received a basic laboratory diet supplemented with 5% Na adipate (10 rats), Na citrate (10 rats) or citric acid (5 rats), while the fourth group was maintained on the basic diet as a control (5 rats). During the 14-week feeding period there were no deaths; however, retardation of growth, as compared to the controls, was observed in all of the treated groups. At the end of the 14-week feeding period, 5 of the rats receiving citric acid and 5 receiving Na adipate were placed on control food for a period of 8 weeks, while the remaining rats were sacrificed and subjected to postmortem examination. The rats placed on the basic diet responded with rapid weight gain during the 8-week period, after which they too were sacrificed. All of the rats appeared healthy at the time of sacrifice and there was no evidence of gross pathology at autopsy (42).

Adipic acid, administered orally for up to 5 weeks as powder, as an alcoholic solution, or in the form of sodium adipate, was found to produce no gross pathology in mature rats or guinea pigs (see Table 6). However, doses between 638 and 1332 mg/kg/day of sodium adipate given to immature rats for 9 weeks (5 days per week) produced significantly greater weight losses in these rats than in control rats fed equimolar doses of sodium acetate (42).

In the third experiment, both male and female rats were given 0, 400, or 800 mg adipic acid/day for a period of 33 weeks. No adverse effects were observed among the rats receiving 400 mg/day. Several pregnant females were included in this group and it was found that the adipic acid consumption had no effect on them, i.e., they bore litters and were capable of suckling them. The animals receiving the higher dosage exhibited significantly lower weight gains and higher mortality than the controls. As in the previous experiment, these rats suffered from heavy diarrhea, appeared unkempt, and were generally apathetic during the first 3 weeks of the study. These rats recovered gradually and after 33 weeks reached the same weight as the animals receiving 400 mg adipic acid/day (34).

Table 4. Weight gain and mortality of rats that were fed with adipic acid for 33 weeks

Daily adipic acid dose in mg	Initial weight g	Weight after 8 weeks	Weight after 33 weeks	No. of animals	No. of dead animals
0	74	207 ± 35	-----	11	4
400	74	183 ± 25	325 ± 25	9	4
800	73	154 ± 24	320 ± 31	4 (1)	10

In order to allow the toxic effects of adipic acid to manifest themselves more strongly, Lang and Bartsch treated rats with adipic acid in a protein-deficient diet. The diet consisted only of crushed wheat supplemented with cod-liver oil and had a protein concentration of 11%. The results of the study, as presented in Table 5, show that the toxicity of adipic acid is greater in the case of insufficient protein intake than in the case of optimal nutrition. In contrast to the previous experiments, the rats in this study fed with 400 mg adipic acid per day exhibited a significant inhibition of growth compared to the controls. The inhibition of growth was still manifest after 19 weeks; the rapid recovery observed previously did not occur here. Other than the inhibition of growth, the animals revealed no outer remarkable signs. The blood picture of the rats was normal, except for a mild anemia of all the groups (34).

Table 5. Weight gain and mortality of rats that were fed adipic acid in a protein-deficient diet (11% protein)

Daily adipic acid dose mg	Initial weight g	Weight after 6 weeks g	Weight after 19 weeks g	No. of dead animals
0	54	102 ± 16 (10)	200 ± 28 (5)	2
50	54	103 ± 9 (10)	179 ± 32 (7)	0
100	53	94 ± 14 (10)	172 ± 39 (5)	1
200	54	104 ± 13 (8)	182 ± 33 (5)	2
400	55	79 ± 14 (10)	144 ± 26 (5)	2

Following i.m. injection of Na adipate (unspecified amount) in rabbits, Harding and Nicholson found slight local swelling with a marked necrotic area and, in 2 of the 13 animals, mild renal irritation. The authors concluded that Na adipate appears innocuous when administered in this manner (25).

II. Short-Term Studies

Rats

In an investigation of the short-term toxicity of adipic acid, Lang and Bartsch conducted a series of experiments in which a total of over 200 rats were fed adipic acid at levels of up to 800 mg/day for periods of up to 33 weeks (34).

In their preliminary experiment (see Table 2), adipic acid fed to female rats in daily doses of 0-40 mg for 4 weeks produced no change in the growth curve or in other outward behavior (34).

Table 2. Weight gain of rats that were fed with adipic acid for 4 weeks.

Daily adipic acid dose mg	Weight gain g	Number of experimental animals
0	55 ± 13*	17
10	52 ± 14	18
20	62 ± 11	20
40	68 ± 16	17

Average deviation $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$

In the second experiment, male rats were fed 0-800 mg adipic acid/day for 5 weeks (see Table 3). The weight gains of the rats fed 200 and 400 mg/day were not significantly different from those of the control animals, while the growth of the animals receiving 800 mg/day was significantly decreased. The animals receiving 800 mg/day suffered from heavy diarrhea and appeared unkempt during the first 2-3 weeks but recovered during the fourth and fifth weeks (34).

Table 3. Weight gain of rats that were fed with adipic acid for 5 weeks.

Daily Adipic Acid dose in g	Initial Weight g	End Weight g	Number of Animals
0	49 ± 7	154 ± 20	18
200	52 ± 7	156 ± 26	18
400	44 ± 5	139 ± 15	18
800	47 ± 7	100 ± 11	15

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
ADIPIC ACID NAS 0003	01 BAKED GOODS(R)	*	0-5 MO.	.130560	.172800	.454560
			6-11 MO.	.975360	1.989120	3.395960
			12-23 MO.	2.092600	3.448320	7.266960
			2-65+ YR.	5.268480	7.826920	18.343640
ADIPIC ACID NAS 0003	04 FATS OILS(R)	*	0-5 MO.	1.350000	1.350000	**********
			6-11 MO.	7.560000	20.250000	**********
			12-23 MO.	17.010000	32.400000	**********
			2-65+ YR.	47.250000	85.320000	**********
ADIPIC ACID NAS 0003	07 FROZN DAIRY(R)	*	0-5 MO.	.003000	.012300	.004000
			6-11 MO.	.028500	.079200	.030000
			12-23 MO.	.043200	.101400	.057600
			2-65+ YR.	.076800	.155100	.102400
ADIPIC ACID NAS 0003	10 MEAT PRODS(R)	*	0-5 MO.	2.937000	7.740000	2.937000
			6-11 MO.	55.269000	146.988000	55.269000
			12-23 MO.	80.634000	138.573000	80.634000
			2-65+ YR.	209.328000	347.367000	209.328000
ADIPIC ACID NAS 0003	11 POULTRY(R)	*	0-5 MO.	.075000	.345000	.075000
			6-11 MO.	.585000	1.980000	.585000
			12-23 MO.	.990000	2.760000	.990000
			2-65+ YR.	1.935000	4.920000	1.935000

yielded the normal ether values.

These results, which speak against retention and for decomposition of the non-excreted dicarboxylic acids, are of special significance when they are related to the total amounts administered in the four experimental groups. For example, in the growth experiments, 6.8 g adipic acid, 8.8 g azelaic acid and 9.4 g sebacic acid were fed to each animal in 28 days. Of this, about 70% were excreted with the urine. If we assume retention, then, for example, the animals fed with adipic acid would have had to have stored more than 2 g in their bodies. As we were able in the above described model tests to detect amounts of 50 mg dissolved adipic acid in the rat body, such a large amount could not have passed by us undetected. Also, the detection of decomposition products of higher di-acids after feeding of these acids (6), and the test results of Emmrich and Emmrich-Glaser (13) after feeding with tetradecandicarboxylic acid speak against storage of administered dicarboxylic acids.

Discussion of the Experimental Results

All three examined dicarboxylic acids are only very slightly toxic. If we convert the lethal rabbit dose for men, we find that only amounts of 250 g or more would have a lethal effect on a normal test person. Cumulation can be determined only for adipic acid, and then only in the case of the rabbit. The harmful effect is based on the slow absorption of this substance and the consequent phenomena in the intestine, therefore not on absorptive effect. Since the speed of excretion is faster than that of absorption in the intestine, it is not very probable that any absorptive effects appear at all.

Azelaic and sebacic acid lead to cramps in the rabbit when given in very high doses. Furthermore, it is striking that rats do not manifest the cumulation phenomena observed in rabbits after adipic acid feeding.

Retention of the dicarboxylic acids fed to the animals and not excreted does not occur, for the dicarboxylic acids we fed could not be detected in unbound form. However, there still remains the possibility of an irreversible sedimentation of protein or bone substance. However, this is not very probable.

Summary

1. The toxicity of adipic acid, azelaic acid and sebacic acid was determined by establishing the single lethal dose in the rabbit and the effect of feeding to rats over several weeks. It was shown that these dicarboxylic acids have a very slight toxic effect.

2. During this feeding, the excretion of the dicarboxylic acids was examined. In the case of rabbits, adipic acid was excreted slowly, azelaic and sebacic acids more rapidly. This is due to varying speed of absorption in the intestine. The rats excrete all three acids with the same rapidity.

3. After intravenous injection to the rabit, the speed of excretion of adipic acid is the same as that of the other acids.

4. In examining the bodies of rats that had been fed high doses of dicarboxylic acids for 4 weeks, no dicarboxylic acids could be

intake. In spite of this, we have devoted an additional examination to this question of possible retention.

Retention

We examined the bodies of rats that had been killed after the end of the growth experiments, 72 hours after the last dicarboxylic acid feeding. The same was done with the adult rats that had been fed for 4 weeks with dicarboxylic acids. In both cases, the animals were not killed until the dicarboxylic acid excretion in the urine had stopped.

The dead rats were cut up in toto with a meat-cutting machine, and the sludge thus obtained was boiled for one hour with ten times the amount of 98% alcohol. The liquid thus obtained contained about 10% water and could dissolve both dicarboxylic acids and free acids, as well as salts, at about 70°. In the case of retention, the unbound acids would have to pass into this liquid. The aqueous alcohol was acidified and the alcohol removed by means of vacuum distillation. The watery residue was first shaken out with petroleum ether. In this way, the fat extracted by the alcohol was removed and isolated after evaporation of the petroleum ether. The watery liquid was then treated like urine and its ether-soluble remainder determined in the usual manner. In order to exclude the assumption that the di-acids are incorporated into the fat molecules in the case of retention, the separated fat was saponified with alcoholic potassium hydroxide, and the soap solution obtained was acidified. After this, it was shaken out with ether, the ether was evaporated and the residue titrated as usual.

In preliminary tests, in this way, normal, non-pretreated rats were examined and the average value of the ether-soluble remainder in their bodies determined. This value, obtained from the individual values of 8 rats, was 0.16 milliequivalent per 100 g rat. Furthermore, normal rats were injected intraperitoneally with each of the three dicarboxylic acids in the form of salts and acids, the animals were killed, and the ether-soluble remainder in their bodies determined. In this way, for example, 100 mg injected adipic acid yielded a remainder of 2/3. The value of the ether-soluble remainder was clearly risen, and thus it was proven that our method was capable of detecting retained dicarboxylic acids dissolved in the body.

Examination of the fat of normal rats revealed that upon its splitting, as is to be expected, no dicarboxylic acids appear. Titration of the residue after evaporating the ether yielded the values of 0.02-0.04 milliequivalents, normal for the ether we used.

The values of the ether-soluble remainder, that were obtained upon processing the rats fed with dicarboxylic acids, agree completely with those of normal rats. In the case of rats that were fed for 4 weeks with adipic acid, they are 0.18 milliequivalents, for rats fed with azelaic acid 0.14 milliequivalents, and for rats fed with sebacic acid 0.19 milliequivalents, in contrast to the normal value of 0.16 milliequivalents. Each value represents the mean of 6 individual values. The minor deviations lie within the limits of natural dispersion. Even in the case of fat splitting, no di-acids could ever be detected; titration of the ether residue obtained in the above manner

is true for both adipic and azelaic and sebacic acid feeding. The daily excreted amounts hover between 60 and 70%. This behavior denies any damage to the rat organism from the dicarboxylic acids, and agrees with the results described in the section on toxicity. Especially, there is no cumulation and no longer excretion after the last feeding.

Table 7. Excretion of dicarboxylic acids in the case of a four-week feeding to rats.

(Excretion in milliequivalent)

A. Versuchstag	B. Ratte 1 und 2: Verfütterung von 0,73 g Adipinsäure pro Tier		C. Ratte 3 und 4: Verfütterung von 0,94 g Azelainsäure pro Tier		D. Ratte 5 und 6: Verfütterung von 1,01 g Sebacinsäure pro Tier	
1	0,51	0,34	0,46	0,48	0,48	0,58
2	0,39	0,39	0,38	0,45	0,51	0,57
3	0,47	0,28	0,36	0,55	0,52	0,45
4	0,43	0,33	0,48	0,38	0,49	0,60
5	5,28	5,18	6,90	6,60	5,80	6,22
6	5,27	5,36	6,44	6,67	5,72	6,11
7	5,41	5,40	6,42	6,82	6,00	6,05
8	5,33	5,35	6,37	6,81	6,05	6,08
12	5,73	5,62	6,47	6,98	5,82	6,18
13	5,61	5,68	6,53	6,72	6,17	6,41
14	5,75	5,54	6,37	6,74	6,07	6,51
19	5,65	5,26	6,91	7,19	6,18	6,35
22	5,34	5,37	6,86	7,22	6,03	6,28
23	5,77	5,19	6,96	6,99	6,02	6,42
26	5,61	5,56	6,92	6,95	6,14	6,54
29	5,54	5,32	6,81	7,13	6,18	6,52
31	5,41	5,51	6,56	6,67	6,01	6,04
32	5,55	5,37	6,91	6,87	5,89	6,25
33	0,48	0,32	0,45	0,44	0,48	0,56
34	0,42	0,38	0,40	0,55	0,52	0,46
1. Durchschnittswerte der normalen Vorperiode:						
	0,45	0,34	0,42	0,47	0,50	0,58
2. Durchschnittswerte während der Verfütterung:						
	5,50	5,41	6,67	6,88	6,01	6,27
3. Durchschnittswerte der Ausscheidung in g während der Verfütterung:						
	0,49	0,49	0,62	0,63	0,62	0,64
4. Durchschnittswerte der Ausscheidung in % der pro Tag verfütterten Dosis:						
	67	67	66	67	61	64

Key:
A. day of experiment
B. rats 1 and 2: feeding with 0.73 g adipic acid per animal
C. rats 3 and 4: feeding with 0.94 g azelaic acid per animal
D. rats 5 and 6: feeding with 1.01 g sebacic acid per animal
1. mean values of the normal preliminary period
2. mean values during feeding
3. mean values of excretion in g during feeding
4. mean values of excretion in % of daily administered dose

The non-excreted 30% of the total administered dose of dicarboxylic acids could have been either burned or stored. The last possibility is not very probably, for then we would have to expect that the stored dicarboxylic acids would be excreted after discontinuation of

first 24 hours after the second administration. The total excreted amount after intravenous injection of adipic acid was only slightly higher than in the case of oral administration.

In one case, a rabbit survived the oral administration of the lethal dose of 2/30 equivalent weight, i.e. 4.86 g per kg animal (adipic acid); the animal lived for 24 hours after feeding. During this time, 2.05 g adipic acid were excreted in the urine. From the result of intravenous injection of this substance it follows that about 2/3 of the amount actually arrived in the bloodstream is excreted. Since in this case, according to our conventional calculation, 2.05 g were excreted, about 3 g must have been absorbed; that is only about 21% of the amount introduced in this case -- 14.1 g adipic acid. However, it follows further from the experiments with intravenous injection, that even more than 3 g of the adipic acid arrived in the bloodstream have no harmful effect. Thus, from this observation of the excretion conditions, we find confirmation of the view expressed above, that the death of the rabbits poisoned with orally administered adipic acid is not the result of the substance arrived in the body, i.e. in the metabolism, but can rather be attributed to the amount of adipic acid remaining in the intestine, which draws the water from the body.

After injection into the veins of the ear, the ears became inflamed and partly necrotic. However, this is unspecific and can be attributed to the great concentration of the injected solutions.

The behavior of dicarboxylic acid excretion upon long-term administration was examined in rats. The general course of the experiment has already been described above. Two adult rats each of 300 g weight were fed daily with adipic acid, azelaic acid and sebacic acid over a period of four weeks. The rats received 1/100 equivalent weight of the acids in question per animal. During the four weeks, the dicarboxylic acid content in the urine was determined four times in the first week, three times for the next two weeks, and four times in the last week; it was also determined in four days of a normal preliminary period and for two days following the last feeding. The urine was collected for 24 hours, separately for each rat, filled to 250 ccm with wash water, and then 50 ccm of this amount were examined in the conventional manner for dicarboxylic acid content. Table 7 gives the values for each rat of the ether-soluble remainder in milliequivalents in the four days of the normal preliminary period, then, from the fifth to the thirty-second feeding day, the values during feeding, and finally the values of the first two days on which the animals were not fed any more. The last two lines show average values of the dicarboxylic acid excreted in 24 hours, in g and in %.

In observing Table 7, we notice first that in the case of rats, after oral administration of adipic acid, the same high percentage of adipic acid is excreted on the first day, as is the case with the two other dicarboxylic acids. Also, the milliequivalent values of the ether-soluble remainder in the urine do not rise on the second day of feeding for any of the three dicarboxylic acids. Besides this, excretion of all three dicarboxylic acids has finished within the first 24 hours after the last feeding. From beginning to end of the four-week experiment, excretion remains within the natural limits and is constant within the dispersion determined by the experimental technology; this

Table 6 gives a survey of the results obtained; here, the amounts excreted every 24 hours are given in percentage of the single daily doses administered. Furthermore, the averages of the daily and total amounts of excretion are calculated in %.

Table 6. Excretion of dicarboxylic acids in a short-term experiment (Excretion in % of the administered single dose).

A Versuch	B. Adipinsäure				C. Azelainsäure				D. Sebazinsäure			
Nr.	1. Tag	2. Tag	3. Tag	4. Tag	1. Tag	2. Tag	3. Tag	4. Tag	1. Tag	2. Tag	3. Tag	4. Tag
1	16	46	34	10	62	60	20	0	59	85	3	0
2	15	45	41	6	65	67	20	0	57	80	2	0
3	20	57	27	13	68	66	18	0	59	87	6	0
4	30	70	22	0	62	63	22	0	58	78	3	0
Durchschnitt:	20	55	31	7	64	64	20	0	58	83	4	0
1. Durchschnittlich ausgeschiedene Gesamtmenge in % der zugeführten Gesamtdosis:		57				74				72		

Key:
A. experiment no.
B. adipic acid
C. azelaic acid
D. sebacic acid Tag = day Durchschnitt = average
1. average excreted total amount in % of the total dose administered

In both tables 5 and 6, the first day is the day on which the feeding took place for the first time. In thecase of all three dicarboxylic acids, excretion ends on the fifth day of the experiment, after 2 days of administration. The total excretion is between 50 and 80% of the amounts administered, thus within the limits found by other researchers as well. The excretion was observed every day during and after feeding.

Thus it was found that azelaic acid was excreted up to 64% as early as the first day, that its excretion does not increase on the second day of feeding, and that after the last administration of the acid, it stops within 48 hours. On the other hand, adipic acid excretion reaches its maximum on the second day of feeding, i.e. when the effect of the second administration is added to that of the first feeding. It is then 55%, in contrast to 20% of the first day. Besides this, it usually does not end until the third day after the last feeding. Sebacic acid assumes the middle position. As in the case of azelaic acid, its excretion is nearly 60% of the administered single dose on the first day; on the third day after the last feeding, excretion has already dropped to zero. On the other hand, as is the case with adipic acid, excretion still increases on the second day.

The cause of the especially delayed excretion of adipic acid can probably be found in the slow absorption of this substance, which we had also observed in the abnormal fluid content of the intestine after feeding. We were able to prove that this deviate behavior of adipic acid is in fact based on delayed absorption, and not on delayed excretion, by injecting the same amount of adipic acid intravenously in two experiments. We used rabbits whose excretion of this dicarboxylic acid after feeding had already been observed. In the case of intravenous injection, one had excreted 59% on the first day, the other 71%. (The percentage figures are a percent of the administered single dose). Furthermore, excretion was finished within the

Table 4. Feeding of adipic acid. Rabbit no. 3. 2.5 kg.

A.	B.	C.	D.	
Versuchstag	Adipinsäure-zufuhr in g	Ätherlöslicher Rest im Harn (in Milliäquivalent)	Ausgeschiedene Adipinsäure a. in g	b. in % der verfütterten Einzeldosis
1	0	9,20	0	0
2	0	10,40	0	0
3	0	10,31	0	0
4	0	8,69	0	0
5	0	9,85	0	0
6	0	9,93	0	0
7	6,08	20,00	1,00	16
8	6,08	38,72	2,82	46
9	0	30,74	2,05	34
10	0	16,00	0,61	10
11	0	9,61	0	0
12	0	10,04	0	0

Key:
A. day of experiment
B. adipic acid intake in g
C. ether-soluble remainder in urine (in milliequivalent)
D. excreted adipic acid
   a. in g
   b. in % of the orally administered single dose

Normal average of the ether-soluble remainder in the urine during the first to sixth day: 9.73 milliequivalent.

A total of 6.48 g adipic acid (67 milliequivalent), i.e. 53% of the total administered amount, were excreted.

Four such experiments each were conducted for adipic acid, azelaic acid and sebacic acid. The amounts fed and excreted are shown in g in Table 5.

Table 5. Administered and excreted amounts of dicarboxylic acid in a short-term experiment

A.		B.	C.	D.			
a. Kaninchen Nr.	b. Gewicht in kg	Säure	Dosis 2 mal in g	Ausscheidung in g 1. Tag	2. Tag	3. Tag	4. Tag
3	2,5	1. Adipinsäure	6,08	1,00	2,82	2,05	0,61
4	2,3		5,59	0,84	2,53	2,28	0,35
5	2,2		5,35	1,09	3,03	1,44	0,68
6	2,0		4,86	1,45	3,39	1,09	0,00
9	1,9	2. Azelainsäure	5,96	3,70	3,55	1,17	0,00
10	1,9		5,96	3,88	3,99	1,19	0,00
11	2,2		6,90	4,72	4,56	1,25	0,00
12	2,1		6,58	4,08	4,14	1,45	0,00
13	2,0	3. Sebazinsäure	6,74	3,98	5,70	0,20	0,00
14	2,1		7,07	4,03	5,66	0,14	0,00
15	2,4		8,08	4,78	7,04	0,50	0,00
16	2,1		7,07	4,13	5,54	0,21	0,00

Key:
A. rabbit
   a. no.
   b. weight in kg
B. acid
C. dose twice in g
D. excretion in g Tag = day
1. adipic acid
2. azelaic acid
3. sebacic acid

Table 3. Weight gain of young rats upon feeding of dicarboxylic acids.

A.			B.			C.			D.		
Gruppe I Verfütterung von Adipinsäure 0,243 g Tier/Tag			Gruppe II Verfütterung von Azelainsäure 0,314 g/Tier Tag			Gruppe III Verfütterung von Sebazinsäure 0,337 g Tier/Tag			Gruppe IV Kontrollen: Verfütterung von Wasser 2,0 ccm Tier/Tag		
Nr.	1. Tag	27. Tag	Nr.	1. Tag	27. Tag	Nr.	1. Tag	27. Tag	Nr.	1. Tag	27. Tag
1	75	110	1	65	105	1	70	110	1	65	100
2	75	105	2	60	105	2	75	125	2	80	120
3	60	100	3	80	110	3	75	120	3	70	105
4	80	115	4	70	105	3	70	105	4	70	110
5	70	100	5	70	110	5	60	90	5	75	110

Key:
A. Group I; feeding of adipic acid, 0.243 g per animal/day
B. Group II; feeding of azelaic acid, 0.314 g per animal/day
C. Group III; feeding of sebacic acid, 0.337 g per animal/day
D. Group IV; controls: feeding of water, 2.0 ccm per animal/day

Average weight gains:
Group I: 34 g Group II; 38 g Group III: 40 g Group IV: 37 g

Since ~~only~~ young, still growing animals were not harmed by fedding of dicarboxylic acids, we used adult rats in the experiments, with 3 animals weighing about 300 g for each of the three dicarboxylic acids. This experiment, too, was conducted for 4 weeks. The dosage was the same as in the case of the growth experiments: the first group of rats received 0.73 g adipic acid per animal and per day, the other two groups received 0.94 g azelaic acid or 1.01 g sebacic acid per animal and per day in 5-7 ccm water through stomach tube.

The weight of these adult rats remained constant throughout the entire time,; the animals appeared healthy to the very end of the experiment. Their general behavior did not differ from that of normal rats. At the end of the feeding period, the rats were killed and the remainder nitrogen content of the blood determined. It was normal for all the rats, between 20 and 40 mg%. There were no functional kidney damages, in correspondence with the findings of Rose and others (14), and in contrast to the phenomena observed after administration of glutaric acid.

Excretion

In order to complete the toxicity experiments described above, we examined the excretion of the dicarboxylic acids.

In further short-term experiments, we fed rabbits for two successive days with 1/30 the equivalent weight of each of the dicarboxylic acids in question; i.e. 2.43 g adipic acid, 3.14 g azelaic acid, and 3.37 g sebacic acid per kg animal. Thus, we selected the largest possible compatible amounts that were not yet lethal for the animals, according to the previoustoxicity experiments. We determined the ether-soluble remainder of the urine separately for each day, over a normal preliminary period of 5-6 days, for the 2 days of feeding, and the 4 days following the last feeding. Table 4 shows the course of such an experiment with adipic acid.

1/30 and 2/30 equivalent weight per kg animal for all three dicarboxylic acids. The first dose, i.e. 2.43 g adipic acid, 3.14 g azelaic acid and 3.37 g sebacic acid per kg rabbit was not lethal in all three cases. In the case of azelaic acid and sebacic acid feeding, no side effects appeared. Oral adipic acid administration caused some slight discomfort to the animals: the rabbits sit inactive in their cages, eat little and have a distended stomach. When the abdomen is knocked, a distinct gurgling is heard. The animals suffer from more or less intense diarrhea. After 24 hours, all these symptoms have nearly disappeared. If the adipic acid dose is doubled to 4.86 g per kg rabbit, the animals die 10-30 hours after feeding. Autopsy shows that the entire intestine is swollen, and filled with masses of brown liquid. A microscopic examination of the liver and kidneys reveals marked venous obstruction in these organs.

The entire intoxication picture suggests an inhibition of absorption, a passage of fluid into the digestive tract, as the basis of the mechanism of the conducting elements of the bitter salt series.

After administration of the corresponding amounts of azelai and sebacic acids -- 6.28 and 6.74 g per kg rabbit, i.e. 2/30 the equivalent weight -- these symptoms and phenomena are not detected. However, in this case too, death takes place about 12-18 hours after feeding; however, the intoxication picture is completely different. Soon after feeding, the animals lie on their sides, some have clonic cramps, the extremities shake, and there is nystagmus. Autopsy shows no pathological findings in the stomach-intestinal tract. These intoxication phenomena suggest an injury to the central nervous system.

If the dicarboxylic acids are not given orally, but rather intravenously, then 1/30 of the equivalent weight of the adipic acid per kg rabbit is tolerated without side effects. There is only a [illegible]ria, due to which the animals often lose up to 20% of their weight within 8 hours. Injection of azelaic and sebacic acid leads to the death of the animals, usualy still during the injection, as soon as about 1/45 of the equivalent weight is injected. Here there is cessation of breathing, while the heart still continues to beat. This, too, suggests an injury to the central nervous system.

Since the orally and intravenously administered amounts of fluid were relatively large, -- up to more than 84 ccm in the case of sebacic acid -- 100 cmm physiological saline solution were administered orally and intravenously for control purposes; no harmful effects were manifested.

The effect of longer administration was examined on young and adult rats. As a dose we selected the largest amount that can be given by stomach tube, taking into consideration the amount of solution, without fear of purely mechanical effects. This dose was about 1/30 the equivalent weight of each dicarboxylic acid per kg rat. The amounts fed in g are seen in Table 3. The amounts of liquid used are between 1.25 and 2.50 ccm per rat and per day. The experiment lasted for 4 weeks. The controls received an equal amount of water during this time. In the case of the young rats, no effect on weight gain was observed, in comparison with the controls (see Table 3). No difference could be determined in other areas either.

Key to table 1:

A. value found
B. actual value (by multiplying with the corresponding factor)
1. adipic acid
2. azelaic acid
3. sebacic acid

(The gram and percentage values of the following tables are always the converted mean values of a repeat determination.)

In examining the urine after feeding dicarboxylic acids, care was taken that the di-acid quantities that were expected in the single portions of the urine used for chemical determination, were of the above dimensions. This was achieved by using more or less wash water.

As suggested by Verkade (11), in dosing, we always used equivalent amounts, so that the three dicarboxylic acids that we had selected could be compared without difficulty. Thus, for example, to one animal group we gave 2.43 g adipic acid, 3.14 g azelaic to the next, and 3.37 g sebacic acid to the third, i.e. in each case, 1/30 of the equivalent weight per kg. (Equivalent weights: adipic acid: 73 g, azelaic acid: 94 g, and sebacic acid: 101 g).

The dicarboxylic acids were obtained as pure substances from the firm of Schuchardt and dissolved in sodium carbonate solution. More details on the solutions can be found in Table 2. The solutions used for the rabbits were acid. Since our rats had an acid urine, the dicarboxylic acid solutions were completely neutralized for feeding to the rats, in order to prevent a precipitation of the excreted acids in the urine. However, controls showed that this was not necessary. In using acid dicarboxylic acid solutions in feeding experiments with rats, the same excreted amounts were found.

Table 2. Dicarboxylic acid solutions used

	A. Versuche an Kaninchen		B. Versuche an Ratten	
	a. Konzentration %	b. Anteil der neutralisierten Disäuren in %	a. Konzentration %	b. Anteil der neutralisierten Disäuren in %
1. Adipinsäure	20	75	20	100
2. Azelainsäure	18	75	14	100
3. Sebazinsäure	13	75	14	100

Key:

A. rabbit experiments
a. concentration
b. proportion of neutralized di-acids in %

B. rat experiments
a. concentration
b. proportion of neutralized di-acids in%

1. adipic acid 2. azelaic acid 3. sebacic acid

Since azelaic acid and sebacic acid solutions of these concentrations precipitate at room temperature (Table 2), they were always heated to 40° before processing.

Toxicity

Lethal doses for one oral administration to rabbits were between

error due to incomplete draining of the bladder at the end of a 24-hour collecting period was as small as possible, as the urine was in this way less concentrated with respect to the excreted dicarboxylic acids.

The acids we selected for feeding were adipic acid, azelaic acid and sebacic acid. Adipic acid has the formula $COOH-(CH_2)_4-COOH$. It is used in the production of baking powder and lemonade as a replacement for tartaric acid or citric acid, as well as in the production of the artifical fiber "nylon silk" (16). Azelaic acid has the formula $COOH-(CH_2)_7-COOH$, sebacic acid the formula $COOH-(CH_2)_8-COOH$. Thus, we selected a short-membered di-acid with an even carbohydrate count and two dicarboxylic acids of moderate length carbohydrate chain, one with an uneven and one with an even carbohydrate count. We did not use dicarboxylic acids with longer carbohydrate chains because of their easy combustibility (13).

The chemical determination of these dicarboxylic acids in the urine is done according to the method proposed by Emmrich and Höhne (12). The principle consists in the following: 50 ccm urine are acidified and shaken out with ether, boiled for an hour with double the normal caustic soda, and then again acidified and subjected to steam distillation. In this way, first all the ether-soluble substances are found, the hippuric acid destroyed, and the volatile acids removed. Then the residue is shaken out with petroleum ether and then with ether to remove the fatty acids. The ether is evaporated, the residue dissolved in water and titrated.

Before beginning a feeding, the average of the ether-soluble remains of the 24-hour urine was determined for at least 4 days of a normal preliminary period. Calculation of the excreted amounts after feeding was done according to the process of Emmrich and Höhne (12), by subtracting the average thus obtained from the titration value of the experiment day, and multiplying the difference with the equivalent weight of the acids in question. The error that decomposition products of the administered dicarboxylic acids add to the actual values was taken into consideration.

In order to be able correctly to convert the titration values in the urine, increased after feeding of the dicarboxylic acids, into weight amounts of excreted dicarboxylic acids, we performed the following preliminary tests. We determined the blank value of ether-soluble acids of a normal urine, and then, in the same urine, the value of the ether-soluble remainder after foregoing administration of 30, 90, and 150 mg dicarboxylic acids. The titration values thus obtained were converted into mg of the corresponding acids (see above) and related to the weighed and administered amounts. For example, of 90 mg sebacic acid, 81 mg were regularly recovered. In this way we obtained conversion factors of 4/3 for adipic acid, 20/19 for azelaic acid and [illegible]/9 for sebacic acid. Three examples are shown in Table 1.

Table 1

A.	B. Gefundener Wert mg	C. Wirklicher Wert (durch Multiplikation mit dem entsprechenden Faktor erhalten) mg
1. Adipinsäure	171	228
2. Azelainsäure	171	180
3. Sebazinsäure	171	190

The resorption of dicarboxylic acids -- e.g. azelaic acid -- is complete (7). Even in the case of diarrhea, there are no dicarboxylic acids in the feces (13).

Emmrich and Emmrich-Glaser also treat the question of retention of the dicarboxylic acids in their work (13). These researchers daily fed 0.5 g tetradecandicarboxlyic acid for 8 days to two adult rats, and then saponified the animal bodies. No retained dicarboxylic acids could be found.

Rose and Harding and Nicholson (14) found tubular nephritis after administration of glutaric acid. Fodera (15) -- cited by Frankel (15) -- comparatively examined low fatty acids and the corresponding dicarboxylic acids. He ascribed a lowering in toxicity to the introduction of the second carboxyl group. Verkade et al. (11) witnessed the appearance of local edemas after subcutaneous injection of di-acids.

In sum, from previous research we see only a slight toxicity of the dicarboxylic acids having more than five carbohydrate atoms, though partly considerably large doses were used. For example, Emmrich and Höhne (12) administered 10 g sebacic acid orally per day. In spite of this, the question of the toxicity of the dicarboxylic acids has aroused new interest, as after the feeding of synthetically produced fats, an increase in dicarboxylic acid excretion is not prevented. These synthetically produced fats consist of glycerides of fatty acids of even and uneven carbohydrate count. On the other hand, Verkade (3) warns against oils and fats that cause a marked di-aciduria upon feeding.

We therefore set ourselves the task of determining the toxicity of individually selected dicarboxylic acids, and testing their cumulation. Since in the course of the experiments it was found that the acids examined had only slight specific effects; their excretion and retention in the animal body were then examined.

Methodology

As experimental animals we used rabbits and rats. The substances to be tested were dosed precisely by means of application with the stomach tube. In this way, we avoided the disadvantage of the solutions to be administered being thrown away with the food remains. For the same reasons, we rejected experiments with dogs, since they will refuse food containing unusual components, and are also inclined to vomiting, especially after stomach sounding. Furthermore, with rabbits it was more easily possible to inject intravenously the relatively large amounts of solution -- up to 100 ccm -- for purposes of comparison. We rejected subcutaneous injection, as this was not recommended due to the local stimulative effect of the high doses we used. In the case of the long-term experiments, the use of rats was most advantageous in terms of managing with small substance quantities and simplifying the examination of the animal body for retained dicarboxylic acids; this would have been more difficult with larger animals.

The animals' diet was kept completely constant throughout the experimental period. The rabbits received grass or white beets. The rats received a bread consisting of 60% cornmeal, 30% dry milk, 8% dry yeast and 2% cod liver oil. Common salt was also added to this bread, to stimulate extensive drinking, so that the greatest possible amount of urine could be obtained. In this way, we guaranteed that the

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PHYSIOLOGICAL COMPATIBILITY AND EXCRETION OF DICARBOXYLIC ACIDS

by

Alfred Enders

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According to experiments by Verkade and his collaborators (1), fatty acids are decomposed in the organism not only through β-oxidation, but also through ω-oxidation. The end products of this ω-oxidation appear in the urine as dicarboxylic acids. Thus, after feeding of the triglyceride tricaprin, sebacic acid and adipic acid are found in the urine; after administration of triundecylin, pimelic and azelaic acid can be detected in the urine (2). These dicarboxylic acids appearing in the urine are, especially from a physiologico-chemical point of view, the points of origin of numerous new investigations. In this new research, adipic acid, azelaic acid and sebacic acid, among others, were fed to people or dogs in amounts up to 20 g per day, usually in the form of their water-soluble sodium salts (4-7). The total urine was collected during the feeding period and examined quantitatively for the di-acids and their decomposition products. In the urine, 30-75% of the administered dicarboxylic acids could be found again. In the case of subcutaneous injection, elimination did not change significantly (8, 9, 10). Mori (10) injected rabbits subcutaneously with adipic acid in the form of a sodium salt -- up to 20 g -- and found about 50% of the adipic acid in the urine. Verkade et al. (11) were the first to be able to compare the excretion of two dicarboxylic acids by means of subcutaneous injections of equimolar amounts of adipic and sebacic acid.

Emmrich and Höhne (12) fed several test persons azelaic acid in amounts increasing daily --0.5 to 10 g -- and continuously checked the ether-soluble remainder in 24-hour urine. Of each dose the same percentage, namely about 68% on the average, was excreted. However, in this experimental set-up it was unavoidable that that part of the excretion amount that is excreted in the twenty-fifth to forty-eighth hour after feeding, adds to the excretion amount that belongs to the dose fed on the following days. (See Emmrich and Höhne table (12)). In the case of experiments with sebacic acid, Flaschenträger and Bernhard (4) found, on the other hand, that percentage-wise, the smaller the amounts administered, the less is excreted. In general, the dicarboxylic acids with a higher carbohydrate count appear to be burned more easily (Verkade (6, 11); Emmrich (13)).

* 60. Stoehr, R. 1938. On the question of the formation of glycogen from dicarboxylic acids. Klin. Wochenschelft 17(47):1663-1664.

61. Supran, M. K., J. J. Powers, P. V. Rao, T. P. Dornselfer, and P. H. King. 1966. Comparison of different organic acids for the acidification of canned pimientos. Food Technol. 20(2): 117-122.

62. Weitzel, G. 1942. Metabolic studies of adipic acid. Ber. Verhandl. Saechs. Akad. Wiss. Leipzig. Math-phys. Klasse 93:9-18.

63. Yufera, E. P., J. Sanchez, and J. Alberola. 1965. Detection of adulteration in citrus juices. III. Identifications of novolatile acids in orange juices from the United States. Rev. Agroquim. Tecnol. Alimentos. 5(1):121-124.

64. Zagrodzki, S., and K. Szwajcowska. 1966. Determination of organic acids present in beet sugar factory juices. Zeszytyy Probl. Postepow Nauk Rolniczych No. 62b:175-177.

65. Anon. 1929. Adipic acid and its derivatives. Rev. Produits Chim. 32:73-75.

66. Anon. 1970. Acidulants-the search for sours. Canadian Food Industries 41(11):42-43.

45. NV Lijm-en Gelatinefabriek'Delft'. 1970. Method for preparing a dry gelatin product and method for using this for a gelatin pudding powder. Netherlands Patent Application 6,809,670.

46. Ough, C. S. 1963. Sensory examination of four organic acids added to wine. J. Food Sci. 28(1):101-106.

47. Parareda, J. S., J. Alberola, and I. Garcia. 1964. Detection of adulterations in citrus juices. II. Identification of acids in orange varieties. Rev. Agroquim. Tecnol. Alimentos 4(3):371-374.

48. Pintauro, N. D., and B. J. Hall. 1962. Gelatin dessert containing adipic aicd. U.S. Pat. 3,067,036.

49. Polya, E., and B. A. Lister. 1965. An acidulent for gelatin jelly desserts. U.S. Pat. 3,218,176.

50. Polya, E., and B. A. Lister. 1968. Gelatin jelly desserts. Canadian Patent 802,834.

51. Pray, L. W., and J. J. Powers. 1966. Acidification of canned tomatoes. Food Technol. 20(1):87-91.

52. Primo, E., A. Casas, J. Alberola, M. Martinez, and M. P. Cornejo. 1969. Detection of adulteration in citrus juices. XV. Identification of carboxylic acids present in orange juice, commerical sucrose and citric acid by gas-liquid (GLC) and thin layer chromatography (TLC). Revista de Agroquimica y Tecnologia de Alimentos 9(3):415-422.

53. Raida, H. 1923. Calcium movement through neutral salts. Ztschr. F. D. Ges. Exp. Medizin 37:266-273.

* 54. Rose, W. C., C. J. Weber, R. C. Corley, and R. W. Jackson. 1975. The nephropathic action of the dicarboxylic acids and their derivatives. III. Acids of six to nine carbons. J. Pharmacol. 25:59-64.

* 55. Rusoff, I. I., R. R. Baldwin, F. J. Dominques, C. Monder, W. J. Ohan, and R. Thiessen, Jr. 1960. Intermediary metabolism of adipic acid. Toxicol. and Applied Pharmacol. 2:316-330.

56. Schrauth, W. 1929. The technical significance of adipic acid and its derivatives. Chem. Ztg. 53:41-43.

57. Shustanova, L. A., and A. L. Markman. 1968. Oil from Muretia transitoria seeds. Khim. Prir. Soedin. 4(5):313-314.

58. Simola, P. E., and T. Kosunen. 1938. The excretion of citric acid by rats after administration of various organic acids. Suomen Kemistilehti 11B:22-23.

* 59. Standish, W. L., and S. V. Abrams. Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed. 1:405-421. E. I. du Pont de Nemours and Co., Inc.

30. Kazinik, E. M., N. V. Novorusskaya, L. M. L'vovich, and G. A. Gudkova. 1969. Gas-liquid chromatography of high-boiling aliphatic dicarboxylic acids. Zh. Anal. Khim. 24(10):1592-1594.

31. Khomenko, A. N., I. A. Goncharova, and A. G. Stradomskaya. 1969. Chromatographic determination of novolatile organic acids dissolved in natural waters. Gidrokhim. Mater. 50:96-101.

32. Kiyokawa, K. 1951. Effects of several fatty acids and salts upon renal gaseous metabolism, blood flow, and urinary output. Folia Pharm. Japon. 47:32-49.

33. Koch, K-H. 1969. Method for eliminating and/or changing tannin, taste and odour substances in green coffee. West German Pat. Application 1,492,744.

* 34. Lang, K., and A. R. Bartsch. 1953. Adipic, metabolism and tolerance for adipic acid. Biochem. Ztschr. 323:462-468.

35. Miller, A., and J. K. Rocks. 1966. High bloom strength algin gel. U.S. Pat. 3,266,906.

36. Mitchell, W. A., and K. S. Roani. 1966. Effervescent beverage powders. U.S. Pat. 3,241,977.

37. Miyazaki, S., Y. Suhara, and T. Kobayashi. 1969. Separation of aliphatic dibasic acids by thin-layer chromatography. J. Chromatogr. 39(1):88-90.

38. Morgan, J. F., S. Tolnai, and G. F. Townsend. 1960. In vitro antitumor activity of fatty acids. II. Saturated dicarboxylic acids. Can. J. Biochem. Physiol. 38:597-603.

39. Mori, S., and T. Takeuchi. 1970. Thin-layer chromatography of diamines, dicarboxylic acids, and omega-amino acids. Application to the analysis of copolyamides. J. Chromatogr. 47(2):224-231.

* 40. Mori, Y. 1918. The decomposition of muconic and adipic acids in the animal body. J. of Biol. Chem. 35:341-351.

41. Mountney, G. J., and J. O'Malley. 1965. Acids as poultry meat preservatives. Poultry Sci. 44(2):582-586.

* 42. NAS/NRC Questionnaire. 1943. The toxicity of adipic acid. Adipic acid, 7-safety information.

43. Neame, K. D. 1965. Effect of acidic (dicarboxylic) alpha-amino acids on uptake of L-histidine by intestinal mucosa, testis, spleen and kidney in vitro: a comparison with effect in brain. J. Physiol. 181:114-123.

44. Nesse, J. H. von, and D. O. Stephens. 1969. Free-flowing, cold-water-soluble drink mixes. West German Patent Application 1,517,076.

15. Eventova, M. S., and E. N. Safonova. 1962. (Identification) of aliphatic dibasic acids and cyclopentanone. Vestn. Mosk. Univ., Ser. II, Khim. 17(1):68-72.

16. Fauconnier, B. 1955. Development of influenza virus and pH of the allantoic fluid of embryonated eggs. IV. Inhibiting action of acid substances. Ann. Inst. Pasteur 89:101-110.

17. Ferraz, P., and M. E. Almeida Relvas. 1961. Organic acids in biological fluids. I. Differential colorations as a function of their concentration. J. Chromatog. 6:505-513.

* 18. Flaschentraeger, B. 1927. Contribution to the knowledge of fat metabolism. Ztschr. F. Physiol. Ch. 159:297-308.

* 19. Food and Drug Research Laboratories, Inc. 1973. Teratologic evaluation of F.D.A. 71-50. (Adipic Acid) in mice, rats, and hamsters. Food and Drug Administration CA-272, Rockville, Md.

* 20. Gage, J. C. 1970. The subacute inhalation toxicity of 109 industrial chemicals. Brit. J. Industr. Med. 27:1-18.

21. Gepshtain, A., and A. Lifshitz. 1970. Organic acids of orange juice. Lebensmittel-Wissenschaft und Technologie 3(6)115-117.

22. Gross, D. 1959. High-voltage paper electrophoresis of organic acids and determination of migration rates. Chem. Ind. 1219-1220p.

23. Haarhoff, K. N. 1969. Use of multivariate nonlinear regression anlaysis in fitting enzyme kinetic models. Empirical study of the inhibition of aspartate aminotransferase by dicarboxylic acid substrate analogs. J. Theor. Biol. 22(1):117-150.

24. Haardtl, H. 1962. Fungicidal properties of oils and solid fillers. Deut. Apotheker-Ztg. 102:447-448.

* 25. Harding, V. J., and T. F. Nicholson. 1931. The nephropathic action of dicarboxylic acids on rabbits. J. Pharmacol. 42:373-381.

26. Hodge, H. C., et al. 1966. Tests on mice for evaluating carcinogenicity. Toxic Appl. Pharmacol. 9:583-596.

27. Hohls, H. W. 1963. Influence of malic, malonic, adipic, and aspartic acids on the thermal energy of growing chicks. Arch. Gefluegelk. 27(3):235-258.

* 28. Horn, H. J., E. G. Holland, and L. W. Hazleton. 1957. Safety of adipic acid as compared with citric and tartaric acid. J. Agric. Food Chem. 5(10):759-761.

* 29. Kabelitz, G. 1943. Research on the effect of the diacidogenic fatty acids, C6 to C11, their glycerides and some food fats on oxalic acid excretion. Klin. Wochenschrift 22(26/27):439-441.

ADIPIC ACID

Bibliography

1. Becker, E. 1954. Paper chromatographic detection of water-soluble organic acids in foods. Z. Lenensm.-Untersuch. u. -Forsch. 98: 249-257.

2. Bernhard, K., and M. Andreae. 1937. Metabolism tests with decorbonic acids. Hoppe-Seyler's Z. Physiol. Chem. 245:103-106.

3. Boehme, H., and H. Opfer. 1953. Detection of adipic acid. Z. Lebensm.-Untersuch. u. -Forsch. 97:173-177.

4. Breckwoldt, R. G. 1970. Artificially sweetened beverages and mixes. U.S. 3,510,310.

5. Brehme, Th. 1949. Adipic acid for the preparation of acidified formulas for babies. Deutsche Med. Wochenschr. 74(15):474.

6. Brogioni, M., and G. Bosi. 1966. Minor components in natural butter. Gas chromatographic analysis. Olearia 20(3-4):80-81.

7. Campbell, A. D. 1962. Irish moss food product. U.S. Pat. 3,031,308.

8. Canic, V. D., and N. Perisic-Janjic. 1970. Separation of organic acids by thin-layer chromatography on starch. Tehnika 25(2):330-332.

9. Caughey, W. S., J. D. Smiley, and L. Hellerman. 1957. L-glutamic acid dehydrogenase: Structural requirements for substrate competition: Effect of thyroxine. J. Biol. Chem. 224(1):591-607.

10. De Lindemann, L. 1970. Separation of mono- and dibasic fatty acids by gas chromatography. J. Chromatogr. 51(2):297-300.

11. Dowden, B. F., and H. J. Bennett. 1965. Toxicity of selected chemicals to certain animals. J. Water Pollution Control Federation 37(9):1308-1316.

12. Drews, E. 1954. Detection of organic acids and preservatives by means of paper chromatography. Getreide u. Mehl 3:85-88, 90-91. Food Sci. Abstr. 26:595.

13. Edson, N. L. 1936. Ketogonesis-antiketogenesis. III. Metabolism of aldehydes and dicarbonic acids. Biochemical J. 30:1855-1861.

* 14. Enders, A. 1941. Physiological compatibility and excretion of dicarboxylic acids. Arch. Exptl. Path. Pharmakol. 197:706-709.

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
ADIPIC ACID NAS 0003	15	CONDM RELSH(R)	*	0-5 MO.	**********	1.400000	**********
				6-11 MO.	11.250540	30.813660	11.270000
				12-23 MO.	39.217640	106.447880	39.445000
				2-65+ YR.	123.255440	296.933560	123.970000
ADIPIC ACID NAS 0003	20	GELATIN PUD(R)	5	0-5 MO.	6.501000	9.192430	7.995000
				6-11 MO.	43.531520	131.954920	51.168000
				12-23 MO.	46.932420	114.270240	55.165500
				2-65+ YR.	69.378360	178.547250	81.549000
ADIPIC ACID NAS 0003	22	SNACK FOODS(R)	*	0-5 MO.	**********	.050000	**********
				6-11 MO.	.200000	.550000	.745540
				12-23 MO.	.550000	1.550000	2.049360
				2-65+ YR.	.650000	1.850000	2.422680
ADIPIC ACID NAS 0003	23	BEV TYPE I(R)	*	0-5 MO.	.096000	.144000	.108000
				6-11 MO.	.908000	3.108000	1.021500
				12-23 MO.	2.162000	6.500000	2.439000
				2-65+ YR.	4.160000	11.108000	4.680000
ADIPIC ACID NAS 0003	24	BEV TYPE II(R)	*	0-5 MO.	.000000	.000000	**********
				6-11 MO.	**********	.004000	**********
				12-23 MO.	**********	.008000	**********
				2-65+ YR.	1.300000	3.776000	**********
ADIPIC ACID NAS 0003	27	GRAVIES(R)	*	0-5 MO.	.075000	.225000	.100000
				6-11 MO.	1.050000	2.925000	1.400000
				12-23 MO.	2.700000	7.650000	3.600000
				2-65+ YR.	6.225000	15.975000	8.300000
ADIPIC ACID NAS 0003	28	IMIT DAIRY(R)	*	0-5 MO.	.000000	.000000	**********
				6-11 MO.	5.950000	9.775000	**********
				12-23 MO.	3.400000	14.450000	**********
				2-65+ YR.	3.825000	6.375000	**********
ADIPIC ACID NAS 0003	34	INS COF TEA(R)	*	0-5 MO.	.001340	.022110	.008000
				6-11 MO.	.035510	.087770	.212000
				12-23 MO.	.041540	.150080	.248000
				2-65+ YR.	.811370	1.737980	4.844000
ADIPIC ACID NAS 0003		ALL CATEGORIES ********** ********** **********	17	0-5 MO.	11.469700	20.647270	11.601580
				6-11 MO.	127.297920	352.502070	128.104920
				12-23 MO.	195.775600	428.306920	191.915710
				2-65+ YR.	473.463450	961.920810	455.474720

Versuchen mit erwachsenen Ratten mit nur je 3 Tieren im Gewicht von etwa 300 g für jede der 3 Dicarbonsäuren. Auch dieser Versuch wurde 4 Wochen lang durchgeführt. Die Dosierung war so wie bei den Wachstumsversuchen: die erste Gruppe von 3 Ratten erhielt 0,73 g Adipinsäure pro Tier und Tag, die anderen beiden Gruppen erhielten 0,94 g Azelainsäure bzw. 1,01 g Sebazinsäure pro Tier und Tag in 5—7 ccm Wasser mit der Schlundsonde verabreicht.

Das Gewicht dieser erwachsenen Ratten blieb während der ganzen Zeit konstant, die Tiere sahen bis zum Schluß des Versuches gesund aus. Ihr allgemeines Verhalten unterschied sich in nichts von dem normaler Ratten. Am Schluß der Verfütterungsperiode wurden die Ratten getötet und der Reststickstoffgehalt des Blutes bestimmt. Er war bei allen Ratten normal und bewegte sich zwischen 20 und 40 mg%. Funktionelle Nierenstörungen fehlten, entsprechend den von Rose u. a. (14) erhobenen Befunden, im Gegensatz zu den nach Glutarsäureverabreichung beobachteten Erscheinungen.

Ausscheidungsverhältnisse.

Zur Ergänzung der bisher beschriebenen Toxizitätsversuche untersuchten wir die Ausscheidung der Dicarbonsäuren.

In weiteren kurzfristigen Versuchen verfütterten wir an Kaninchen an zwei aufeinanderfolgenden Tagen je $\frac{1}{50}$ Äquivalentgewicht der betreffenden Dicarbonsäure, d. h. 2,43 g Adipinsäure, 3,14 g Azelainsäure und 3,37 g Sebazinsäure pro kg Tier. Wir wählten also möglichst große, noch verträgliche Mengen, die nach den vorhergegangenen Toxizitäts-

Tabelle 4. Verfütterung von Adipinsäure. Kaninchen Nr. 3. 2,5 kg.

Versuchstag	Adipinsäure-zufuhr in g	Ätherlöslicher Rest im Harn (in Milliäquivalent)	Ausgeschiedene Adipinsäure	
			in g	in % der verfütterten Einzeldosis
1	0	9,20	0	0
2	0	10,40	0	0
3	0	10,31	0	0
4	0	8,69	0	0
5	0	9,85	0	0
6	0	9,93	0	0
7	6,08	20,00	1,00	16
8	6,08	38,72	2,82	46
9	0	30,74	2,05	34
10	0	16,00	0,61	10
11	0	9,61	0	0
12	0	10,04	0	0

Normaler Durchschnitt des ätherlöslichen Restes im Harn während des 1. bis 3. Tages: 9,73 Milliäquivalent.

Im ganzen wurden 6,48 g Adipinsäure (67 Milliäquivalent), d. h. 53% der gesamten zugeführten Menge wieder ausgeschieden.

findet sich am Magen-Darmkanal kein pathologischer Befund. Diese Vergiftungserscheinungen weisen auf eine zentral-nervöse Schädigung hin.

Gibt man die Dicarbonsäuren nicht per os, sondern intravenös, dann wird $^1/_{30}$ des Äquivalentgewichtes der Adipinsäure pro kg Kaninchen ohne Nebenerscheinungen überstanden. Es zeigt sich nur eine Polyurie, bei der die Tiere oft bis 20% ihres Gewichtes innerhalb 8 Stunden verlieren. Infusion von Azelain- und Sebazinsäure führt zum Tode der Versuchstiere, meistens, noch während der Infusion, sobald etwa $^1/_{45}$ des Äquivalentgewichtes injiziert ist. Dabei zeigt sich ein Atemstillstand, während das Herz noch weiter schlägt. Auch dieses weist auf eine Schädigung des Zentralnervensystems hin.

Da die oral und intravenös zugeführten Flüssigkeitsmengen relativ groß waren – im Falle der Sebazinsäure bis über 84 ccm – wurden zur Kontrolle 100 ccm physiologischer Kochsalzlösung oral und intravenös gegeben, ohne daß sich schädigende Wirkungen gezeigt hätten.

Der Einfluß längerer Darreichungen wurde an jungen und an erwachsenen Ratten untersucht. Als Dosis wählten wir die größte Menge, die man unter Berücksichtigung der Lösungsmenge mit der Schlundsonde geben kann, ohne rein mechanische Einwirkungen fürchten zu müssen. Diese Dosis war etwa $^1/_{30}$ Äquivalentgewicht jeder Dicarbonsäure pro kg Ratte. Die verfütterten Mengen in g gehen aus Tabelle 3 hervor. Die verwendeten Flüssigkeitsmengen bewegen sich zwischen 1,25 und 2,50 ccm pro Ratte und Tag. Der Versuch wurde 4 Wochen lang durchgeführt. Die Kontrollen erhielten während dieser Zeit eine gleiche Menge Wasser. Bei den jungen Ratten wurde kein Einfluß auf die Gewichtszunahme gegenüber den Kontrolltieren beobachtet (siehe Tabelle 3). Auch im sonstigen Verhalten ließ sich kein Unterschied feststellen.

Tabelle 3. Gewichtszunahme junger Ratten bei Verfütterung von Dicarbonsäuren.

Gruppe I Verfütterung von Adipinsäure 0,243 g Tier/Tag			Gruppe II Verfütterung von Azelainsäure 0,314 g Tier/Tag			Gruppe III Verfütterung von Sebazinsäure 0,327 g Tier/Tag			Gruppe IV Kontrollen: Verfütterung von Wasser 2,0 ccm Tier/Tag		
Nr.	1. Tag	27. Tag	Nr.	1. Tag	27. Tag	Nr.	1. Tag	27. Tag	Nr.	1. Tag	27. Tag
1	75	110	1	65	105	1	70	110	1	65	100
2	75	105	2	60	105	2	75	125	2	80	120
3	60	100	3	80	110	3	75	120	3	70	105
4	80	115	4	70	105	3	70	105	4	70	110
5	70	100	5	70	110	5	60	90	5	75	110

Durchschnittliche Gewichtszunahmen:

Gruppe I: 34 g | Gruppe II: 38 g | Gruppe III: 40 g | Gruppe IV: 37 g

Da selbst junge, noch wachsende Tiere durch die Verfütterung von Dicarbonsäuren nicht geschädigt worden waren, begnügten wir uns bei den

Säuren im Urin vorzubeugen. Kontrollen zeigten jedoch, daß dies nicht nötig gewesen wäre. Bei Verwendung saurer Dicarbonsäurelösungen in Verfütterungsversuchen an Ratten wurden dieselben Ausscheidungsmengen gefunden.

Tabelle 2. Verwendete Dicarbonsäurelösungen.

	Versuche an Kaninchen		Versuche an Ratten	
	Konzentration %	Anteil der neutralisierten Disäuren in %	Konzentration %	Anteil der neutralisierten Disäuren in %
Adipinsäure	20	75	20	100
Azelainsäure	13	75	14	100
Sebazinsäure	13	75	14	100

Da Azelainsäure- und Sebazinsäurelösungen dieser Konzentration (Tabelle 2) bei Zimmertemperatur ausfallen, wurden sie vor der Verabreichung stets auf 40° erwärmt.

Toxizität.

Die letalen Dosen bei einmaliger oraler Darreichung liegen bei Kaninchen bei allen drei Dicarbonsäuren zwischen $^1/_{30}$ und $^2/_{30}$ Äquivalentgewicht pro kg Tier. Erstere Dosis, d. h. 2,43 g Adipinsäure, 3,14 g Azelainsäure oder 3,37 g Sebazinsäure pro kg Kaninchen war in allen drei Fällen nicht letal. Bei der Verfütterung von Azelainsäure und Sebazinsäure zeigten sich dabei keine Nebenerscheinungen. Adipinsäureverabreichung per os bewirkte leichtes Unwohlsein der Tiere; die Kaninchen sitzen teilnahmslos im Käfig, fressen wenig und haben einen aufgetriebenen Bauch. Bei Beklopfen des Abdomens hört man ein deutliches Gluckern. Die Tiere leiden an mehr oder weniger starken Durchfällen. Schon nach 24 Stunden sind alle diese Erscheinungen weitgehend abgeklungen. Verdoppelt man die Adipinsäuredosis auf 4,86 g pro kg Kaninchen, dann sterben die Tiere 10—30 Stunden nach der Verfütterung. Bei der Obduktion sieht man, daß der ganze Darm gebläht und mit Massen brauner Flüssigkeit angefüllt ist. Bei der mikroskopischen Untersuchung von Leber und Niere liebenen an diesen Organen ausgeprägte venöse Stauungserscheinungen nachweisen.

Das ganze Vergiftungsbild weist auf eine Resorptionshemmung bzw. auf einen Übertritt von Flüssigkeit in den Verdauungskanal, wie sie dem Mechanismus der Abführmittel aus der Bittersalzreihe zugrunde liegt.

Nach Verabreichung der entsprechenden Mengen Azelain- und Sebazinsäure — 6,28 und 6,74 g pro kg Kaninchen, d. h. $^2/_{30}$ des Äquivalentgewichtes — finden sich diese Erscheinungen nicht. Auch hier tritt allerdings der Tod der Versuchstiere etwa 12—18 Stunden nach der Verfütterung ein, jedoch ist das Vergiftungsbild ein völlig anderes. Die Tiere nehmen bald nach der Verfütterung Seitenlage ein, einige zeigen klonische Krämpfe, Zittern der Extremitäten und Nystagmus. Bei der Obduktion

Fehler, daß Abbauprodukte der verfütterten Dicarbonsäuren sich zu den wirklichen Werten addierten, wurde in Kauf genommen.

Um die nach Verfütterung der Dicarbonsäuren erhöhten Titrationswerte im Urin sicher in Gewichtsmengen ausgeschiedener Dicarbonsäuren umrechnen zu können, stellten wir folgende Vorversuche an. Wir bestimmten den Leerwert ätherlöslicher Säuren eines normalen Urins und danach im selben Urin den Wert des ätherlöslichen Restes nach vorheriger Zugabe von 30, 90 und 150 mg der Dicarbonsäuren. Die so erhaltenen Titrationswerte wurden in mg der entsprechenden Säure umgerechnet (siehe oben) und zu den abgewogenen und hinzugefügten Mengen in Beziehung gesetzt. Es wurden z. B. von 90 mg Sebazinsäure regelmäßig 81 mg wieder aufgefunden. Auf diese Art wurden Umrechnungsfaktoren erhalten, die für Adipinsäure $^{4}/_{3}$, für Azelainsäure $^{20}/_{10}$ und für Sebazinsäure $^{10}/_{9}$ betrugen. Drei Beispiele stehen in Tabelle 1.

Tabelle 1.

	Gefundener Wert mg	Wirklicher Wert (durch Multiplikation mit dem entsprechenden Faktor erhalten) mg
Adipinsäure	171	228
Azelainsäure	171	180
Sebazinsäure	171	190

(Die Gramm- und Prozentwerte der folgenden Tabellen sind stets die umgerechneten Mittelwerte einer Doppelbestimmung.)

Bei der Untersuchung des Harns nach Verfütterung von Dicarbonsäuren wurde darauf geachtet, daß die Disäuremengen, die in den zur chemischen Bestimmung verwandten Einzelportionen des Harns zu erwarten waren, sich in den obigen Größenordnungen hielten. Dies wurde durch Verwendung von mehr oder weniger Spülwasser erreicht.

Wir verwandten nach Verkade (11) bei der Dosierung stets äquivalente Mengen, um die drei Dicarbonsäuren, die wir ausgewählt hatten, einwandfrei vergleichen zu können. So gaben wir z. B. bei einer Tiergruppe 2,43 g Adipinsäure, bei der zweiten 3,14 g Azelainsäure, bei der dritten 3,37 g Sebazinsäure pro kg, d. h. in jedem Falle $^{1}/_{30}$ des Äquivalentgewichtes pro kg. (Äquivalentgewichte: Adipinsäure: 73 g, Azelainsäure: 94 g und Sebazinsäure: 101 g.)

Die Dicarbonsäuren wurden als Reinsubstanzen von der Firma Schuchardt bezogen und in Natriumcarbonatlösung gelöst. Näheres über die Lösungsverhältnisse ist aus Tabelle 2 zu ersehen. Die bei den Kaninchen verwendeten Lösungen waren sauer. Da unsere Ratten einen sauren Harn aufwiesen, wurden die Dicarbonsäurelösungen bei Verfütterung an Ratten völlig neutralisiert, um einem Ausfallen der ausgeschiedenen

cutanen Injektion sahen wir von vornherein ab, da diese sich wegen der lokalen Reizwirkung der von uns verwendeten hohen Dosen nicht empfahl. Bei den langfristigen Versuchen bot die Verwendung von Ratten den Vorteil, mit geringen Substanzmengen auszukommen sowie die Untersuchung des Tierkörpers auf retinierte Dicarbonsäure einfacher zu gestalten, als dies bei größeren Tieren möglich gewesen wäre.

Die Kost der Tiere wurde während der Versuchszeit völlig konstant gehalten. Die Kaninchen erhielten Gras oder weiße Rüben. Die Ratten erhielten ein aus 60% Maismehl, 30% Trockenmilch, 8% Trockenhefe und 2% Lebertran zusammengesetztes Brot. Diesem Brot wurde außerdem Kochsalz zugesetzt, um ein reichliches Trinken und damit eine möglichst große Urinmenge zu erzielen. Wir erreichten dadurch, daß der Fehler durch unvollständige Entleerung der Blase am Schluß einer 24stündigen Sammelperiode so gering als möglich wurde, da der Harn auf diese Art bezüglich der ausgeschiedenen Dicarbonsäuren weniger konzentriert war.

Die von uns zur Verfütterung ausgewählten Säuren sind Adipinsäure, Azelainsäure und Sebazinsäure. Adipinsäure hat die Formel $COOH\ (CH_2)_4\ COOH$. Sie findet Verwendung bei der Herstellung von Backpulver und Limonade als Ersatz von Weinsäure bzw. Zitronensäure, sowie bei der Produktion der Kunstfaser „Nylonseide" (16). Azelainsäure hat die Formel $COOH\ (CH_2)_7\ COOH$. Sebazinsäure entspricht der Formel $COOH\ (CH_2)_8\ COOH$. Wir haben also eine kurzgliedrige Disäure von gerader Kohlenstoffzahl gewählt und zwei Dicarbonsäuren mittlerer Länge der Kohlenstoffkette, davon eine mit ungerader und eine mit gerader Kohlenstoffzahl. Von der Einbeziehung von Dicarbonsäuren noch längerer Kohlenstoffketten wurde angesichts deren guter Verbrennbarkeit (13) abgesehen.

Die chemische Bestimmung dieser Dicarbonsäuren im Urin erfolgte nach der von Emmrich und Höhne angegebenen Methode (12). Das Prinzip besteht in folgendem: 50 ccm Harn werden angesäuert und mit Äther ausgeschüttelt, eine Stunde mit doppelt normaler Natronlauge gekocht und danach wieder angesäuert und einer Wasserdampfdestillation unterworfen. Dadurch werden erst sämtliche ätherlöslichen Substanzen aufgenommen, die Hippursäure zerstört, und die flüchtigen Säuren entfernt. Dann wird der Rückstand zur Entfernung der Fettsäuren mit Petroläther und dann mit Äther ausgeschüttelt. Der Äther wird abgedunstet, der Rückstand in Wasser gelöst und titriert.

Vor Beginn einer Verfütterung wurde stets der Durchschnitt des ätherlöslichen Restes des 24-Stunden-Urins an mindestens 4 Tagen einer normalen Vorperiode bestimmt. Die Berechnung der ausgeschiedenen Mengen nach der Verfütterung erfolgte entsprechend dem Vorgehen von Emmrich und Höhne (12), indem wir den so gewonnenen Durchschnitt von dem Titrationswert des Versuchstages subtrahierten und die Differenz mit dem Äquivalentgewicht der betreffenden Säuren multiplizierten. Der

gemeinen scheinen die Dicarbonsäuren mit höherer Kohlenstoffzahl leichter verbrannt zu werden [Verkade (6, 11); Emmrich (13)].

Die Resorption der Dicarbonsäuren — z. B. von Azelainsäure — ist vollständig (7). Auch bei Durchfall finden sich keine Dicarbonsäuren im Kot (13).

In der Arbeit von Emmrich und Emmrich-Glaser (13) wird auch zur Frage der Retention der Dicarbonsäuren Stellung genommen. Diese Forscher verfütterten täglich 0,5 g Tetradekandicarbonsäure 8 Tage lang an zwei erwachsene Ratten und verseiften anschließend die Tierkörper. Retinierte Dicarbonsäuren waren in diesen nicht nachzuweisen.

Rose sowie Harding und Nicholson (14) fanden nach Verabreichung von Glutarsäure eine tubuläre Nephritis. Fodera (15) — zit. nach Fränkel (15) — untersuchte vergleichend niedere Fettsäuren und die entsprechenden Dicarbonsäuren. Er schrieb dem Eintritt der zweiten Carboxylgruppe eine Herabsetzung der Toxizität zu. Verkade und Mitarbeiter (11) sahen nach subcutaner Injektion von Disäuren lokale Ödeme auftreten.

Im ganzen folgt aus den bisherigen Arbeiten eine geringe Giftigkeit der Dicarbonsäuren, die mehr als fünf Kohlenstoffatome besitzen, wurden doch teilweise recht erhebliche Dosen verwandt; z. B. verfütterten Emmrich und Höhne (12) 10 g Sebazinsäure pro Tag. Trotzdem gewann die Frage der Toxizität der Dicarbonsäuren erneutes Interesse, da nach Verfütterung synthetisch hergestellter Fette eine Vermehrung der Dicarbonsäureausscheidung nicht ausgeschlossen ist. Diese synthetisch hergestellten Fette bestehen aus Glyceriden der Fettsäuren gerader und ungerader Kohlenstoffzahl. Auf der anderen Seite warnt Verkade (3) vor Ölen und Fetten, die bei Verfütterung eine starke Diacidurie verursachen.

Wir stellten uns daher die Aufgabe, die Toxizität einzelner ausgewählter Dicarbonsäuren festzustellen und ihre Kumulation zu prüfen. Da sich im Verlauf der Versuche ergab, daß die untersuchten Säuren nur geringe spezifische Wirkungen aufwiesen, wurden außerdem ihre Ausscheidungsverhältnisse und ihre Retention im Tierkörper untersucht.

Methodik.

Als Versuchstiere wählten wir Kaninchen und Ratten. Bei diesen Versuchstieren wurden die zu untersuchenden Substanzen durch Applikation mit der Schlundsonde genau dosiert. Wir vermieden dadurch den Nachteil, daß bei Vermengung in die Nahrung die zu verabreichenden Lösungen mit den Futterresten verschleudert wurden. Aus denselben Gründen sahen wir von Versuchen mit Hunden ab, da diese leicht das Futter, welches ungewohnte Bestandteile enthält, ganz ablehnen und außerdem, besonders nach Schlundsondierung, zu Erbrechen neigen. Ferner war es bei Kaninchen leichter möglich, die relativ großen Lösungsmengen — bis zu 100 ccm — zu Vergleichszwecken intravenös zu spritzen. Von einer sub-

Enders-'41
Bib No. 14

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Verträglichkeit und Ausscheidungsverhältnisse von Dicarbonsäuren.

Von
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(Eingegangen am 9. April 1941.)

Nach Versuchen von Verkade und seinen Mitarbeitern (1) werden die Fettsäuren im Organismus außer durch β-Oxydation auch durch ω-Oxydation abgebaut. Die Endprodukte dieser ω-Oxydation erscheinen im Urin als Dicarbonsäuren. So findet sich nach Verfütterung des Triglycerides Tricaprin im Harn Sebazinsäure und Adipinsäure, nach Verabreichung von Triundecylin sind im Urin Pimelin- und Azelainsäure nachzuweisen (2). Diese im Harn erscheinenden Dicarbonsäuren sind, vorwiegend von physiologisch-chemischer Seite, der Ausgangspunkt zahlreicher, neuer Untersuchungen. Dabei wurden — unter anderen — Adipinsäure, Azelainsäure und Sebazinsäure meist in Form ihrer wasserlöslichen Natriumsalze an Menschen oder an Hunde in Mengen bis zu 20 g pro Tag verfüttert (4—7). Der gesamte Harn wurde während der Verfütterungsperiode gesammelt und quantitativ auf die verfütterten Disäuren und ihre Abbauprodukte untersucht. Im Harn konnten 30—75% der verabreichten Dicarbonsäuren wieder nachgewiesen werden. Bei subcutaner Injektion änderte sich die Ausscheidung nicht wesentlich (8, 9, 10). Mori (10) injizierte bei Kaninchen subcutan Adipinsäure als Natriumsalz — bis zu 2,0 g — und fand etwa 50% der Adipinsäure im Urin wieder. Verkade und Mitarbeiter (11) konnten als erste durch subcutane Injektion äquimolarer Mengen von Adipin- und Sebazinsäure die Ausscheidungsverhältnisse zweier Dicarbonsäuren einwandfrei vergleichen.

Emmrich und Höhne (12) verfütterten an menschlichen Versuchspersonen von Tag zu Tag steigende Mengen von Azelainsäure — 0,5 bis 10 g — und bestimmten fortlaufend den ätherlöslichen Rest im 24-Stunden-Urin. Von jeder Dosis wurde derselbe Prozentsatz, nämlich durchschnittlich etwa 68%, ausgeschieden. Bei dieser Versuchsanordnung war es allerdings nicht zu vermeiden, daß sich der Teil der Ausscheidungsmenge, der erfahrungsgemäß erst in der 25.—48. Stunde nach einer Verfütterung ausgeschieden wird, zu der Ausscheidungsmenge, die zu der am folgenden Tage verfütterten Dosis gehört, addiert. [Siehe Tabelle von Emmrich und Höhne (12)]. Bei Versuchen mit Sebazinsäure fanden im Gegensatz dazu Flaschenträger und Bernhard (4), daß prozentual um so weniger ausgeschieden wird, je geringer die zugeführten Mengen sind. Im all-

found. From this we can conclude that the remainder not excreted in the urine is burned in the metabolism.

Literature

1) Verkade, P. E., u. J. van der Lee: Hoppe-Seylers Z. **215**, 225 (1933). — 2) Verkade, P. E., u. J. van der Lee: Ebenda **227**, 213, 1934. — 3) Verkade, P. E., u. J. van der Lee: Ebenda **225**, 230 (1934). — 4) Flaschenträger, B., u. K. Bernhard: Ebenda **238**, 221 (1936). — 5) Bernhard, K., u. M. Andrae: Ebenda **245**, 103 (1937). — 6) Verkade, P. E., J. van der Lee u. A. J. van Alphen: Ebenda **237**, 186 (1935); **250**, 47 (1937). — 7) Smith, G.: J. biol. Chem. (Am.) **103**, 531 (1933). — 8) Flaschenträger, B.: Hoppe-Seylers Z. **159**, 297 (1926). — 9) Baer u. Blum: Hofmeisters Beitr. z. chem. Physiol. **11**, 101 (1908). — 10) Mori: J. biol. Chem. (Am.) **35**, 341 (1918). — 11) Verkade, P. E., J. van der Lee u. A. J. van Alphen: Hoppe-Seylers Z. **252**, 163 (1938). — 12) Emmrich, R., u. E. Hohne: Ber. d. mathemat.-physikal. Kl. d. sächsischen Akademie zu Leipzig, XCII, 15. I. 1940. — 13) Emmrich, R., u. J. Emmrich-Glaser: Hoppe-Seylers Z. **266**, 183 (1940). — 14) Rose: Chem. Zbl. **1924**, S. 2410; **1925**, S. 669; Harding, V., u. T. F. Nicholson: Ber. Physiol. usw. **64**, 599 (1932). — 15) Fränkel: Arzneimittelsynthese, S. 102. Berlin, Verlag Jul. Springer, 1919; Fodera: Arch. d. Farmacol. **1894**, S. 417. — 16) Karrer, P.: Lehrbuch der organischen Chemie, S. 295. Leipzig, G. Thieme, 1940.

versuchen für die Versuchstiere noch nicht tödlich waren. Wir bestimmten einzeln für jeden Tag den ätherlöslichen Rest des Urins, und zwar in einer normalen Vorperiode von 5–6 Tagen, an den 2 Tagen der Verfütterung sowie an den der letzten Verfütterung folgenden 4 Tagen. Tabelle 4 zeigt den Verlauf eines solchen Versuchs mit Adipinsäure.

Je 4 solcher Versuche wurden mit Adipinsäure, Azelainsäure und Sebazinsäure ausgeführt. Die dabei verfütterten und wieder ausgeschiedenen Mengen in g sind aus Tabelle 5 zu ersehen.

Tabelle 5. Verfütterte und wieder ausgeschiedene Dicarbonsäuremengen im kurzfristigen Versuch.

Kaninchen		Säure	Dosis 2 mal in g	Ausscheidung in g			
Nr.	Gewicht in kg			1. Tag	2. Tag	3. Tag	4. Tag
3	2,5	Adipinsäure	6,08	1,00	2,82	2,05	0,61
4	2,3		5,59	0,84	2,53	2,28	0,35
5	2,2		5,35	1,09	3,03	1,44	0,68
6	2,0		4,86	1,45	3,39	1,09	0,00
9	1,9	Azelainsäure	5,96	3,70	3,55	1,17	0,00
10	1,9		5,96	3,88	3,99	1,19	0,00
11	2,2		6,90	4,72	4,56	1,25	0,00
12	2,1		6,58	4,08	4,14	1,45	0,00
13	2,0	Sebazinsäure	6,74	3,98	5,70	0,20	0,00
14	2,1		7,07	4,03	5,66	0,14	0,00
15	2,4		8,08	4,78	7,04	0,50	0,00
16	2,1		7,07	4,13	5,54	0,21	0,00

Tabelle 6 gibt eine Übersicht der erhaltenen Resultate; hier sind die in je 24 Stunden ausgeschiedenen Mengen in Prozent der verfütterten einzelnen Tagesdosen aufgeführt. Ferner sind die Durchschnitte der Tages- und Gesamtausscheidungsmengen in % berechnet.

Tabelle 6. Ausscheidung von Dicarbonsäuren im kurzfristigen Versuch.
(Ausscheidung in % der verfütterten Einzeldosis.)

Versuch	Adipinsäure				Azelainsäure				Sebazinsäure			
Nr.	1. Tag	2. Tag	3. Tag	4. Tag	1. Tag	2. Tag	3. Tag	4. Tag	1. Tag	2. Tag	3. Tag	4. Tag
1	16	46	34	10	62	60	20	0	59	85	3	0
2	15	45	41	6	65	67	20	0	57	80	2	0
3	20	57	27	13	68	66	18	0	59	87	6	0
4	30	70	22	0	62	63	22	0	58	78	3	0
Durchschnitt:	20	55	31	7	64	64	20	0	58	83	4	0
Durchschnittlich ausgeschiedene Gesamtmenge in % der zugeführten Gesamtdosis:		57				74				72		

dings noch die Möglichkeit einer irreversiblen Ablagerung an Eiweiß- oder Knochensubstanz. Dies ist aber unwahrscheinlich.

Zusammenfassung.

1. Die Toxizität von Adipinsäure, Azelainsäure und Sebazinsäure wurde durch Feststellung der einmaligen letalen Dosis am Kaninchen und der Wirkung mehrwöchentlicher Verfütterung an der Ratte bestimmt. Es zeigte sich, daß diese Dicarbonsäuren eine sehr geringe Giftwirkung besitzen.

2. Während dieser Verfütterung wurde die Ausscheidung der Dicarbonsäuren untersucht. Bei Kaninchen wurde Adipinsäure langsam, Sebazin- und Azelainsäure schneller ausgeschieden. Dies beruht auf verschieden schneller Resorption aus dem Darm. Die Ratte scheidet alle drei Säuren gleich schnell aus.

3. Nach intravenöser Injektion am Kaninchen ist die Ausscheidungsgeschwindigkeit der Adipinsäure wie die der anderen beiden Säuren.

4. Bei der Untersuchung der Körper von Ratten, die 4 Wochen lang mit hohen Dosen Dicarbonsäuren gefüttert worden waren, konnten keine Dicarbonsäuren wieder aufgefunden werden. Daraus ist zu schließen, daß der nicht in den Harn ausgeschiedene Rest im Stoffwechsel verbrannt wird.

Literatur.

1) Verkade, P. E., u. J. van der Lee: Hoppe-Seylers Z. **215**, 225 (1933). — 2) Verkade, P. E., u. J. van der Lee: Ebenda **227**, 213, 1934. — 3) Verkade, P. E., u. J. van der Lee: Ebenda **225**, 230 (1934). — 4) Flaschenträger, B., u. K. Bernhard: Ebenda **238**, 221 (1936). — 5) Bernhard, K., u. M. Andrae: Ebenda **245**, 193 (1937). — 6) Verkade, P. E., J. van der Lee u. A. J. van Alphen: Ebenda **237**, 186 (1935); **250**, 47 (1937). — 7) Smith, G.: J. biol. Chem. (Am.) **103**, 531 (1933). — 8) Flaschenträger, B.: Hoppe-Seylers Z. **159**, 297 (1926). — 9) Baer u. Blum: Hofmeisters Beitr. z. chem. Physiol. **11**, 101 (1908). — 10) Mori: J. biol. Chem. (Am.) **35**, 341 (1918). — 11) Verkade, P. E., J. van der Lee u. A. J. van Alphen: Hoppe-Seylers Z. **252**, 163 (1938). — 12) Emmrich, R., u. E. Hühne: Ber. d. mathemat.-physikal. Kl. d. sächsischen Akademie zu Leipzig, XCII, 15. I. 1940. — 13) Emmrich, R., u. J. Emmrich-Glaser: Hoppe-Seylers Z. **266**, 183 (1940). — 14) Rose: Chem. Ztl. 1924, S. 2410; **1925**, S. 669; Harding, V., u. T. F. Nicholson: Ber. Physiol. u. w. 64, 599 (1932). — 15) Fränkel: Arzneimittelsynthese, S. 102. Berlin, Verlag Jul. Springer, 1919; Federa: Arch. d. Farmacol. **1894**, S. 417. — 16) Karrer, P.: Lehrbuch der organischen Chemie, S. 295. Leipzig, G. Thieme, 1940.

von normalen Ratten völlig überein. Sie betragen bei Ratten, die 4 Wochen mit Adipinsäure gefüttert wurden, 0.18 Milliäquivalent, bei den mit Azelainsäure gefütterten Tiere 0.14 Milliäquivalent und bei den mit Sebazinsäure gefütterten Tieren 0,19 Milliäquivalent gegenüber dem Normalwert von 0.16 Milliäquivalent. Jeder Wert stellt den Durchschnitt von 6 Einzelwerten dar. Die geringfügigen Abweichungen liegen im Bereich der natürlichen Streuung. Auch bei der Fettspaltung konnten in keinem Falle Disäuren nachgewiesen werden; die Titration des auf obige Weise erhaltenen Ätherrückstandes ergab die normalen Ätherwerte.

Diese Ergebnisse, die gegen eine Retention und für einen Abbau der nicht ausgeschiedenen Dicarbonsäuren sprechen, erhalten ein besonderes Gewicht, wenn man die in den 4 Versuchsgruppen verabreichten Gesamtmengen dazu in Beziehung setzt. Es wurden z. B. in den Wachstumsversuchen in 28 Tagen 6,8 g Adipinsäure, 8,8 g Azelainsäure und 9,4 g Sebazinsäure pro Tier verfüttert. Davon wurden etwa 70% mit dem Urin ausgeschieden. Nimmt man eine Retention an, so hätten z. B. die mit Adipinsäure gefütterten Ratten über 2 g in ihrem Körper gespeichert haben müssen. Da es uns in den oben geschilderten Modellversuchen gelang, selbst Mengen von 50 mg im Rattenkörper gelöste Adipinsäure nachzuweisen, hätte uns eine derart große Menge keinesfalls entgehen können. Auch der Nachweis von Abbauprodukten höherer Disäuren nach deren Verfütterung (6) sowie die Versuchsergebnisse von Emmrich und Emmrich-Glaser (13) nach Verfütterung von Tetradekandicarbonsäure sprechen gegen eine Speicherung von verfütterten Dicarbonsäuren.

Diskussion der Versuchsergebnisse.

Alle drei untersuchten Dicarbonsäuren sind sehr wenig giftig. Rechnet man die letale Kaninchendosis auf den Menschen um, so ergibt sich, daß erst Mengen von über 250 g bei einer normalen Versuchsperson tödlich wirken würden. Eine Kumulation ist nur bei der Adipinsäure und da auch nur beim Kaninchen festzustellen. Die schädliche Wirkung beruht bei dieser Substanz auf ihrer langsamen Resorption und den dadurch bedingten Erscheinungen im Darm, also nicht auf resorptiver Wirkung. Da die Geschwindigkeit der Ausscheidung schneller ist, als die der Resorption aus dem Darm, ist es wenig wahrscheinlich, daß überhaupt resorptive Wirkungen auftreten.

Azelain- und Sebazinsäure führen beim Kaninchen in sehr hohen Dosen zu Krämpfen. — Weiterhin ist auffällig, daß Ratten die bei Kaninchen beobachteten Kumulationserscheinungen nach Adipinsäureverfütterung nicht zeigen.

Eine Retention der verfütterten und nicht wieder ausgeschiedenen Dicarbonsäuren findet nicht statt, denn die von uns verfütterten Dicarbonsäuren waren in ungebundener Form nicht nachzuweisen. Es bliebe aller-

Retention.

Wir untersuchten Körper von Ratten, die nach Beendigung der Wachstumsversuche, 72 Stunden nach der letzten Dicarbonsäure-Verfütterung, getötet worden waren. Ebenso wurde mit den erwachsenen Ratten verfahren, die 4 Wochen lang mit Dicarbonsäure gefüttert worden waren. In beiden Fällen wurden die Tiere erst dann getötet, als die Dicarbonsäureausscheidung in den Harn bereits beendet war.

Die getöteten Ratten wurden in toto mit einer Fleischmaschine zerkleinert und der so erhaltene Brei eine Stunde mit der 10fachen Menge 98%igem Alkohol gekocht. Die entstandene Flüssigkeit enthielt etwa 10% Wasser und vermochte Dicarbonsäuren sowohl als freie Säuren wie als Salze bei 70° in genügender Menge zu lösen. Im Falle einer Retention hätten die nicht gebundenen Säuren in diese Flüssigkeit übergehen müssen. Der wässerige Alkohol wurde angesäuert und der Alkohol durch Vakuumdestillation entfernt. Der wässerige Rückstand wurde zuerst mit Petroläther ausgeschüttelt. Hierdurch wurde das durch den Alkohol extrahierte Fett entfernt und nach Abdunsten des Petroläthers isoliert gewonnen. Die wässerige Flüssigkeit wurde danach wie Urin behandelt und in üblicher Weise ihr ätherlöslicher Rückstand bestimmt. Um die Annahme auszuschließen, daß die Disäuren im Falle einer Retention in die Fettmoleküle eingebaut seien, wurde das gesondert gewonnene Fett mit alkoholischer Kalilauge verseift und die erhaltene Seifenlösung angesäuert. Durch Ausschütteln mit Petroläther wurden zuerst die Fettsäuren entfernt. Danach wurde mit Äther ausgeschüttelt, der Äther abgedunstet und der Rückstand wie üblich titriert.

In Vorversuchen wurden auf diese Art normale, unvorbehandelte Ratten untersucht und der durchschnittliche Wert des ätherlöslichen Restes ihres Körpers bestimmt. Dieser Wert, aus den Einzelwerten von 8 Ratten gewonnen, betrug 0,16 Milliäquivalent pro 100 g Ratte. Ferner wurden normalen Ratten intraperitoneal jede der drei Dicarbonsäuren als Salze und als Säuren gespritzt, die Tiere anschließend getötet und der ätherlösliche Rest ihres Körpers bestimmt. Auf diese Art konnten z. B. von 100 mg eingespritzter Adipinsäure durchschnittlich $^2/_3$ wiedergewonnen werden. Der Wert des ätherlöslichen Restes war deutlich gestiegen und es war damit bewiesen, daß unsere Methode imstande war, retinierte, in Körper gelöste Dicarbonsäuren nachzuweisen.

Die Untersuchung des Fettes normaler Ratten ergab, daß bei seiner Spaltung, wie nicht anders zu erwarten, keine Dicarbonsäuren auftreten. Die Titrierung des Rückstandes nach Abdunsten des Äthers ergab die für den von uns benutzten Äther üblichen Werte von 0,02—0,04 Milliäquivalent.

Die Werte des ätherlöslichen Restes, die bei der Verarbeitung der mit Dicarbonsäure gefütterten Ratten erhalten wurden, stimmen mit den

Tabelle 7. Ausscheidung von Dicarbonsäuren bei 4 Wochen langer Verfütterung an Ratten.

(Ausscheidung in Milliäquivalent.)

Versuchstag	Ratte 1 und 2: Verfütterung von 0,73 g Adipinsäure pro Tier		Ratte 3 und 4: Verfütterung von 0,94 g Azelainsäure pro Tier		Ratte 5 und 6: Verfütterung von 1,01 g Sebazinsäure pro Tier	
1	0,51	0,34	0,46	0,48	0,48	0,58
2	0,39	0,39	0,38	0,45	0,51	0,47
3	0,47	0,28	0,36	0,55	0,52	0,45
4	0,43	0,33	0,48	0,38	0,49	0,60
5	5,28	5,18	6,90	6,60	5,80	6,22
6	5,27	5,36	6,44	6,67	5,72	6,11
7	5,11	5,40	6,42	6,82	6,00	6,05
8	5,33	5,35	6,37	6,81	6,05	6,08
12	5,73	5,62	6,47	6,98	5,82	6,18
13	5,61	5,68	6,53	6,72	6,17	6,41
14	5,75	5,54	6,37	6,74	6,07	6,51
19	5,65	5,26	6,91	7,19	6,18	6,35
22	5,34	5,37	6,86	7,22	6,03	6,28
23	5,77	5,19	6,96	6,99	6,02	6,42
26	5,61	5,56	6,92	6,95	6,14	6,54
29	5,54	5,32	6,81	7,13	6,18	6,52
31	5,41	5,51	6,56	6,67	6,01	6,04
32	5,55	5,37	6,91	6,87	5,89	6,25
33	0,48	0,32	0,45	0,44	0,48	0,56
34	0,42	0,38	0,40	0,55	0,52	0,46
	Durchschnittswerte der normalen Vorperiode:					
	0,45	0,34	0,42	0,47	0,50	0,53
	Durchschnittswerte während der Verfütterung:					
	5,50	5,41	6,67	6,88	6,01	6,27
	Durchschnittswerte der Ausscheidung in g während der Verfütterung:					
	0,49	0,49	0,62	0,63	0,62	0,64
	Durchschnittswerte der Ausscheidung in % der pro Tag verfütterten Dosis:					
	67	67	66	67	61	64

über Toxizität geschilderten Ergebnissen. Insbesondere findet sich keine Kumulation und keine längere Ausscheidung nach der letzten Verfütterung.

Die nicht ausgeschiedenen 30% der gesamten verfütterten Dicarbonsäuremengen können entweder verbrannt oder gespeichert worden sein. Letztere Möglichkeit besaß von vornherein wenig Wahrscheinlichkeit, denn man hätte dann erwarten müssen, daß die gespeicherten Dicarbonsäuren nach Absetzen der Zufuhr doch noch zur Ausscheidung gekommen wären. Trotzdem haben wir aber dieser Frage einer eventuellen Retention zusätzliche Untersuchungen gewidmet.

mit intravenöser Infusion folgt aber ferner, daß sogar mehr als 3 g der in die Blutbahn gelangten Adipinsäure keine schädigende Wirkung besitzen. Somit ergibt sich aus dieser Betrachtung der Ausscheidungsverhältnisse eine Bestätigung der oben von uns geäußerten Ansicht, daß der Tod der mit Adipinsäure per os vergifteten Kaninchen keine Folge der in den Körper, d. h. der in den Stoffwechsel gelangten Substanz ist, sondern auf die im Darm zurückgebliebene Menge Adipinsäure zurückzuführen ist, die ihrerseits das Wasser aus dem Körper an sich zieht.

Nach der Injektion in die Ohrvenen entzündeten sich jeweils die Ohren und nekrotisierten oft teilweise. Dies ist jedoch unspezifisch und auf die starke Konzentration der eingespritzten Lösungen zurückzuführen.

Das Verhalten der Dicarbonsäureausscheidung bei längerer Verabreichung wurde an Ratten untersucht. Der allgemeine Verlauf des Versuchs ist bereits oben geschildert worden. Je zwei erwachsene Ratten von 300 g Gewicht wurden 4 Wochen lang täglich mit Adipinsäure, Azelainsäure und Sebazinsäure gefüttert. Die Ratten erhielten $^1/_{100}$ Äquivalentgewicht der betreffenden Säure pro Tier. Während der 4 Wochen wurde der Dicarbonsäuregehalt im Harn in der ersten Woche 4mal, in den folgenden zwei Wochen je 3mal, in der letzten Woche wieder 4mal bestimmt, außerdem noch an 4 Tagen einer normalen Vorperiode und an den zwei der letzten Verfütterung folgenden Tagen. Der Harn wurde für jede Ratte getrennt während 24 Stunden gesammelt, mit Spülwasser auf 250 ccm aufgefüllt und von dieser Menge 50 ccm wie üblich auf Dicarbonsäuregehalt untersucht. In Tabelle 7 stehen für jede Ratte die Werte des ätherlöslichen Restes in Milliäquivalent in der normalen Vorperiode von 4 Tagen, danach vom 5.—32. Versuchstag — die Werte während der Verfütterung und zum Schluß die Werte der ersten beiden Tage, an denen die Tiere nichts mehr verfüttert erhielten. Die letzten zwei Zeilen zeigen Durchschnittswerte der in 24 Stunden ausgeschiedenen Dicarbonsäure in g und %.

Bei Betrachtung der Tabelle 7 fällt zuerst auf, daß bei Ratten nach der oralen Verabreichung von Adipinsäure sofort am 1. Tage derselbe hohe Prozentsatz Adipinsäure ausgeschieden wird, wie bei den anderen beiden Dicarbonsäuren. Auch steigen die Milliäquivalentwerte des ätherlöslichen Restes im Harn am 2. Tage der Verfütterung bei keiner der drei Dicarbonsäuren weiter an. Außerdem ist bei jeder der drei Dicarbonsäuren die Ausscheidung bereits in den ersten 24 Stunden nach der letzten Verfütterung beendet. All dies steht im Gegensatz zu den Befunden beim Kaninchen. Von Anfang bis Ende des vierwöchentlichen Versuches bleibt die Ausscheidung sowohl bei Adipin- wie auch bei Azelain- und Sebazinsäureverfütterung im Rahmen der natürlichen und der durch die Versuchstechnik bedingten Streuung konstant. Die täglich ausgeschiedenen Mengen schwanken nur zwischen 60 und 70%. Dieses Verhalten spricht gegen eine Schädigung des Rattenorganismus durch die von uns verfütterten Dicarbonsäuren und steht in Übereinstimmung mit dem im Abschnitt

In Tabelle 5 und 6 ist als 1. Tag jeweils der Tag gerechnet, an dem zum ersten Male eine Verfütterung stattfindet. Bei allen drei Dicarbonsäuren ist nach zweitägiger Zufuhr am 5. Versuchstag die Ausscheidung beendet. Die Gesamtausscheidung bewegt sich zwischen 50 und 80% der zugeführten Mengen, also in den auch von anderen Untersuchern gefundenen Grenzen. Weiter führte die Betrachtung der Ausscheidung an den einzelnen Tagen während und nach der Verfütterung.

Dabei erwies sich, daß Azelainsäure bereits am 1. Tage zu etwa 64% ausgeschieden wurde, daß ihre Ausscheidung auch am 2. Tage der Verfütterung nicht weiter ansteigt und daß sie nach der letzten Verabreichung der Säure innerhalb 48 Stunden beendet ist. Dagegen erreicht die Adipinsäureausscheidung ihr Maximum erst am 2. Tage der Verfütterung, d. h. dann, wenn sich die Wirkung der 2. Zufuhr zu der der 1. Verfütterung addiert. Sie beträgt dann 55%, gegenüber 20% des 1. Versuchstages. Außerdem ist sie meist erst mit dem 3. Tage nach der letzten Verfütterung beendet. Die Sebazinsäure nimmt eine Mittelstellung ein. Wie bei der Azelainsäure beträgt ihre Ausscheidung am 1. Versuchstag beinahe 60% der verfütterten Einzeldosis, auch ist ihre Ausscheidung bereits am 3. Tage nach der letzten Verfütterung auf 0 gesunken. Dagegen steigt, wie bei der Adipinsäure, die Ausscheidung am 2. Tage noch an.

Die Ursache der besonders verzögerten Ausscheidung der Adipinsäure ist wohl in der langsamen Resorption dieses Stoffes zu suchen, die wir ja auch an dem abnormen Flüssigkeitsgehalt des Darmes nach der Verfütterung beobachtet hatten. Daß dies abweichende Verhalten der Adipinsäure tatsächlich auf verzögerter Resorption und nicht auf verzögerter Ausscheidung beruht, konnten wir nachweisen, indem wir dieselbe Menge Adipinsäure in zwei Versuchen intravenös spritzten. Wir verwendeten dabei Kaninchen, bei denen die Ausscheidung dieser Dicarbonsäure nach Verfütterung bereits untersucht worden war. Bei intravenöser Injektion wurde bereits am 1. Tage 59% bei dem einen, 71% bei dem anderen Kaninchen ausgeschieden. (Die Prozentzahlen verstehen sich auch hier wieder als Prozent der verabreichten Einzeldosis.) Ferner war die Ausscheidung bereits in den ersten 24 Stunden nach der zweiten Verabreichung beendet. — Die Gesamtausscheidungsmenge nach intravenöser Injektion von Adipinsäure lag nur wenig über der bei oraler Verabreichung.

In einem Falle überlebte das Kaninchen nach oraler Verabreichung der letalen Dosis von $^2/_{30}$ Äquivalentgewicht, d. h. 4,86 g Adipinsäure pro kg Tier, noch die ersten 24 Stunden nach der Verfütterung. Im Urin wurden während dieser Zeit 2,05 g Adipinsäure ausgeschieden. Aus dem Ergebnis der intravenösen Injektion dieses Stoffes folgt, daß von der wirklich in die Blutbahn gelangten Menge etwa $^2/_3$ ausgeschieden wird. Da in diesem Falle nach unserer üblichen Berechnung 2,05 g ausgeschieden waren, müssen also etwa 3 g resorbiert worden sein, das sind nur 21%, der in diesem Falle eingeführten Menge von 14,1 g Adipinsäure. Aus den Versuchen

625 ccm hot water, filter, and acidify with 24.6 ccm (n = 1.98, 2 mol.) acetic acid. After standing in ice, we syphon off, wash with water, squeeze out for tint, and dry over the water bath for constancy. Yield is 8.8 g or 90%. Melting point 125°, sintering at 120°. After one recrystallization from hot water (10.26 g in 250 ccm) we obtain snow white needles (9.6 g or 94%) with a constant melting point of 126.5°, sintering at 123°C.

For analysis we dried to constancy in a vacuum with P_2O_5.

4.602 mg substance yielded 10.050 mg CO_2 and 3.850 mg H_2O.
4.810 " " " 10.520 " CO_2 " 4.070 " H_2O.
3.155 " " " 0.188 ccm N at 759 mm and 17°.
3.630 " " " 0.215 ccm N at 764 mm and 18°.

$C_{10}H_{19}O_3N$ (201 mol. weight)

Est.	C 59.7%	H 9.45%	N 6.96%
Found	C 59.54, 59.64	H 9.36, 9.47	N 7.01, 6.97.

The crystals are optically positive, partly inclined, partly disappearing in longitudinal direction, according to the surface formation. Very often, we see cigar-shaped single crystals beside the star-shaped prisms.

Sebamic acid is nearly insoluble in ether, in contrast to sebacic acid, from which it can therefore easily be separated. In other organic solvents, except for benzene, petroleum ether and chloroform, it is easily soluble.

Behavior of sebamic acid in relation to acetic acid. 0.3 g sebamic acid are boiled with 15 ccm acetic acid (n = 1.98) for three hours in a reflux cooler. After cooling, 250.3 mg or 83.5% are recovered. Melting point: 120° sintering. 125 -126° definitely melted. Mixed melting point with pure sebamic acid is 125-126°C; with sebacic acid sintering at 112°, melted at 116-120°C. Sebamic acid is resistant to acetic acid.

Behavior of sebamic acid in relation to hydrochloric acid. 0.300 g sebamic acid are boiled with 15 ccm diluted hydrochloric acid (2 n) for 3 hours at reflux. When it stands, boric acid-like flakes crystallize: 280 mg, or 93%. Melting point up to 128° unchanged, at 129° there is beginning sintering, at 131° like thawing ice, at 133-134° melted clear. Mixed melting point with sebacic acid as above. Mixed melting point with sebamic acid: sintering at 115-116°, melted at 117°! Sample soluble in ether. Cooking with diluted hydrochloric acid disintegrates sebamic acid quantitatively.

Behavior of sebamic acid in relation to soda. 0.3001 g sebamic acid are boiled with 15 ccm soda (2 n) for 2 hours at reflux. After cooling, acidification with acetic acid and syphoning at room temperature yields 250 mg or 83%. Melting point at 110° shows sintering, clear melting at 120°. Mixed melting point with sebamic acid: sintering at 115°, clear melting at 124°. No depression! Boiled with about 60 ccm ether, filtered. The ether insoluble matter now sinters at 120° and becomes clear at 125 to 126°. The ether-soluble crystals sinter at 120° and become clear at 126-127°. Their mixed melting point with sebacic acid: sintering at 124°, melted at 128°. Melting point with sebamic acid: sintering at 115°, melted at 120°!

Boiling with soda attacks sebamic acid only slightly.

II. Experiments with Sebamic Acid

a) Sebamic acid ethylester $CO(NH_2) \cdot (CH_2)_8 \cdot COOC_2H_5$

25 g sebacic acid monoethylester (melting point 37°) according to Grün and Wirth were heated with 60 g thionyl chloride for one half hour in a water bath with reflux, then the remaining thionyl chloride is separated into a vacuum. The yield of crude, brownish-colored chloride is 27.0 g or 100%. The chloride is then carefully poured into 100 ccm ice-cooled concentrated ammonia. The temperature rose to 25°C. The precipitated sebamic acid ethyl is mixed with about 500 ccm ether, washed neutrally and dried with sodium sulfate. The ether left behind 18.4 g or 87.5%. Melting point 70°C.

Recrystallization. 17.8 g are dissolved brown in 70 ccm ether at reflux, another 10 ccm ether are added, as upon cooling, everything freezes into a crystal sludge. From this, 11.6 g are precipitated white with 100 ccm petroleum ether; this is equal to 65%. Melting point is 70-71°. From water, in which the amide ester is about 1:700 soluble in heat, there come snow white, optically positive, fine needles tapering in longitudinal direction, and having a boric acid-like appearance. Melting point 70-71°. In methanol 1:2 and in ethanol very soluble. For analysis, dried in a vacuum with phosphorous pentoxide.

4.905 mg substance (from ether-petroleum-eth.) yielded 11.300 mg CO_2, 4.430 mg H_2O.
4.380 mg substance (from water) yielded 10.100 mg CO_2, 3.990 mg H_2O.
3.600 mg " yielded 0.194 ccm N at 759 mm and 18°.
4.020 " " " 0.214 " N " 764 " " 18°.

$C_{12}H_{23}O_3N$ (229.19 molecular weight)

Est.	C 62.83%	H 10.11%	N 6.31%
Found	C 62.84, 62.88	" 10.10, 10.20	" 6.31, 6.27

Sebamic acid, $COOH(CH_2)_8 \cdot CONH_2$

Preliminary experiments had shown that the baryum salt of sebacic acid, the acid ester, and sebamic acid is insoluble in methanol. Sebacin diamide is resistant to boiling methanol barium hydroxide solution (n/1). Diethylester and also sebamic acid ethyl yield a crystalline precipitate only after standing in the cold (2 hours and 20 minutes). Thus, it had to be possible to saponify the ester group upon careful treatment of the amino group, as the reaction product immediately precipitated from the system.

11.65 g sebamic acid ethyl were dissolved in 20 ccm absolute methanol and mixed with 61 ccm (0.835 n) methanolic barium hydroxide (½ mol.). After 1½ hours a crystal sludge forms. However, the conversion is not complete until this is heated, after 15 hours of standing, for five minutes in a water bath, until the reaction has become neutral. After cooling, we syphon off, wash with methanol and dry over the water bath. Yield of sebamic acid barium is 13.05 g, or 96% of the theoretical.

To isolate sebamic acid, we dissolve 13 g of the barium salt in

the brown extracts, 5 g of unaltered adipic acid with a melting point of 150°C and the same mixture melting point could be obtained. From the remains, only a few more milligrams crystallized in the end. The acid, like suberic and sebacic acid, can be washed well with acetylester. Thus 50% of the adipic acid is decomposed.

b) Suberic acid

10 g of pure suberic acid (Merck) were introduced in the same manner as the adipic acid, and the urine processed in the same way. The dog weighed 4.37 kg and received 0.5 g twice daily. 1.5 g of crude allantoin crystallized from the concentrated urine extracted with ether. 0.6 g of pure allantoin were obtained upon recrystallization from water. Here about 50 mg of brown masses were obtained as a water-insoluble residue, which yielded 8.9 mg of not quite pure uric acid after treatment with glacial acetic acid and reprecipitation with caustic soda and hydrochloric acid. All the uric acid reactions according to Hoppe-Seyler-Thierfelder were positive. Also, the characteristic crystal form of uric acid (Behrens-Kley, microchemical analysis) from diluted acetic acid was observed under the polarization microscope. However, the ultimate analysis yielded no indisputable proof.

4.360 mg substance yielded 7.690 mg CO_2, 1.500 mg H_2O, 0.034 mg residue.

2.145 mg " " 0.340 ccm N at 756 mm, 20°.

Est. for uric acid	C 35.69%	H 2.38%	N 33.33%
" " kynuric acid	" 63.5%	" 3.7 %	" 7.4 %
Found	" 48.09%	" 3.85%	" 18.32%

From this it results that the uric acid was not pure. I succeeded in recognizing kynuric in what remained by its Ba salt, which is very characteristic under the microscope. Thus, uric acid is determined in the case of the dog fed exclusively with a rice diet, with which the nitrogen minimum is nearly attained, and it would be interesting to verify whether uric acid, also in a case of nitrogen minimum, is a normal metabolic end product of an ordinary dog, not just the dalmatian.

6 g, or 60%, unaltered suberic acid were found in the ether extracts. The melting point and the mixed melting point were 130°C.

c) Sebacic acid

10 g of sebacic acid were injected subcutaneously to a 21.2 kg dog, after a three-day preliminary period, in two daily doses of 0.5 g, as a sodium salt. The urine of fourteen days was processed as above. Here, too, the allantoin (3.3g) had small black lugs dispersed throughout it (0.3 g); these often collected into dumbbell and grapelike formations. They definitely contain kynuric acid (Ba salt). Precise testing for uric acid is in process. The appearance of the lugs clearly resembles the spherulites that are obtained when the urine of a dog fed extensively with meat is acidified according to the method of Meissner and Shepard.

0.15 g, or 61%, unaltered sebacic acid were separated from the ether extracts. The melting point and mixed melting point were 132°C.

it was best to test the amino group. W. Dieter[24] tested the splitting capacity of yeast in relation to amino acids, and determined that pure yeast does not attack asparagin, acetamide and some aromatic amides as long as it does not rise. In the animal body, the amino acids that are foreign to the body are in many cases not very easily oxidizable[25]. Phenylacetic acid and mandelic acid amide are attacked only slightly (unpublished observation of Thomas). While malamide is hard to burn off, succinimide is burned off nearly completely. Of the semi-amides, oxaminic acid moves to the urine partly unaltered; only asparagin and glutamin are decomposed easily and completely.

There are no other biological observations on semi-amides.

We therefore made sebamic acid. Rowney[26] and Kraut[27] indicate that they obtained it by letting the diethylester stand with ammonia and distilling ammonia salt. They presented no details. Etaix[28] mentions only the melting point of 170°C. We were not able to obtain the acid with a melting point of 170°C. Perhaps Etaix was dealing with a mixture of di- and monoamide. Sebamic acid melts at 126-127°. We obtained a goodly amount by converting the diethylester of sebacic acid into the monoethylester according to the method of A. Grün and Th. Wirth[29], obtained the corresponding chloride with thionyl chloride, and converted this into the amide ester with ammonia. Partial hydrolysis was accomplished with methylalcoholic baryum hydroxide. Before we injected the sebamic acid, we studied its resistance to acids and alkalis. It can be re-obtained in goodly quantities from urine. Processing of the experimental urine yielded only 10% crude and 5% pure. Sebacic acid was not found. Thus, sebamic acid, in contrast to the dicarboxylic acid, had been nearly completely decomposed.

Experimental Part

I. Injection Experiment with Dicarboxylic Acid

a) Adipic acid

10 g of pure adipic acid were injected under the skin of 4 young dogs*) (7.35, 5.00, 6.3, 5.2 kg) in the form of a sodium salt, in 2 daily doses of 0.25 g each, once in the morning and once in the evening. After 5 days of this, the urine was collected for another 3 days. The dogs were fed only with rice cooked with salt and water. The preliminary period lasted 3 days. The urine was concentrated by evaporation together with the cage wash water.

Beforehand, I determined that in the case of an hour-long steam distillation of the three dicarbonic acids used here, no trace was transferred into the distillate.

The evaporated urine (about 150 ccm) was extracted with ether in soxhlet until crystals of kynuric acid, which is insoluble in ether, were precipitated onto the flask walls (5 days). The individual ether extracts were processed partly separately, partly together. From

*) Between the fifth and sixth days of injection, one animal perished from subacute, yellow liver atrophy. There is no connection between the introduction of adipic acid and the cause of death, as the other three animals remained perfectly healthy.

at the same time is noteworthy. In the fall of 1922, at our request, Dr. Andersen of St. George's Hospital in Leipzig tested the absorbability of adipic acid on men and dogs, since it was of industrial interest to know whether adipic acid can successfully replace the more expensive citric acid.

Dr.E. Andersen's Feeding Experiment with Adipic Acid

	A. Adipinsäure			B. Oxalsäure im Harn	
	a. verfüttert insgesamt pro Tag		b. ausgeschieden im Harn	c. Vorperiode	d. Versuch
1 Hund (10 kg)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2 Andersen	[illegible]	[illegible]	[illegible]	—	[illegible]
3 Patientin	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4 Bact. coli	5 verändert Adipinsäure nicht				

Key:
A. adipic acid
B. oxalic acid in the urine
a. fed; total per day
b. eliminated in the urine
c. preliminary period
d. experiment

1. dog (10 kg)
2. Andersen
3. female patient
4. bact. coli
5. does not convert adipic acid

According to these results, the dog attacks adipic acid much better than humans. No adipic acid was found in the feces of the dog. In our experiment, 42% adipic acid was found.

After the conclusion of my tests, Gregg-Smith[22] reports in a short note that pelargonic acid fed to a dog is completely burned off, while azelaic acid is eliminated up to as much as 40%. Thus he refutes the view of Leathes (1908), contending that the biological fatty acid decomposition deposits the unsaturated group of higher fatty acids that are prepared by nature. If this were the case, then even oleic acid would have to act antiketogenically, as it would have to split into azelaic and pelargonic acid. Most experiments so far have been performed only with fats, i.e. glycerides. In this connection, it must be pointed out that in the case of exclusive feeding of pure butyric and oleic acid to a pig, [23], surprisingly no acetonuria and no acidosis appeared, in spite of minimum N.

Our experiments with suberic and sebacic acid confirm the experiments of Baer and Blum. 60 or 40% is eliminated in unaltered form.

The next assumption is that the second carobxyl group impedes the decomposition of the long normal chains. We therefore attempted to lock the COOH group biologically while maintaining the chemical acid nature. Because they split easily, the ester, anhydride and chloride groups are not suitable for this. Nitrils are toxic. Thus,

only in the case of the low acids. Hans Müller[9] obtained lactic acid from fumaric acid by means of yeast fermentation. Juda Hirsch Quastel[10] was able to separate pyruvic acid and acetic acid from succinic acid and fumaric acid after fermenting with Bac. pyocyaneus. Recently, Momose Goro[11] believes to have obtained acetone with malonic acid in two cases of bleeding a liver, and he formulates decomposition through acetaldehydealdol. Baer and Blum[12], however, found no effect of malonic acid, succinic acid and pyrotartaric acid on the acetone bodies, sugar and N excretion in phloridzine dogs. However, in their liver bleeding experiments, Baer and Blum[13] and Friedmann[14] were able to determine, using branched dicarboxylic acids as a characteristic, that the COOH group binds very strongly, in contrast to the methyl group. It is very striking that of the higher polybasic acids[15], except for glutaric acid and citric acid, only a few have thus far been isolated as components of plants and animals. Recently H. Wieland and Alles[16] found suberic acid incorporated as suberylarginine in bufotoxin, the poison of common toads (Bufo vulgaris). In Japanese and carnauba wax the high dicarboxylic acids C_{20}, C_{25} are present, according to Schaal[17]. With the exception of oxalic acid, the dicarboxylic acids are very slightly or not at all toxic. Then, with increasing methylene count, toxicity even decreases[18]. However, as W.Rose[19] recently determined in his research on the effect of dicarboxylic acids and their derivatives on the kidneys, glutaric acid produces a tubular nephritis with erosion of the glomeruli. Its Ca salt is not a cause, as it is more water-soluble than the non-toxic form. The higher acids, adipic, pimelic, suberic, azelaic acid, on the other hand, damage the kidney only slightly. Rose believes, since the acids with an even carbohydrate count are as non-toxic as those with an uneven carbohydrate count, that they are not decomposed on the path of β-oxidation, as otherwise the more toxic glutaric acid would have to appear as an intermediate product. If this view is correct, then in a metabolic experiment, no adipic acid could be found from suberic acid, and no suberic acid from sebacic acid. At the same time, we were interested in the question of whether β-oxidation might not attack both completely equal carboxyl groups at the same time, and the decomposition in the case of the dicarboxylic acids takes place in two places. Baer and Blum[20] injected phlorizine diabetic dogs subcutaneously with large daily doses (7-10g) in order to study sugar elimination and acidosis. Nitrogen, sugar, acetone and oxybutyric acid elimination decreased with the C_6 -C_7 acids and C_8 - C atoms. Azelaic and sebacic acid decreased only acidosis and otherwise had no effect. The cause did not lie in a varying gradual combustibility. Processing of the urine from the day of injection yielded 12% adpic acid, 47% pimelic acid, 62 and 56% suberic acid, 50% azelaic acid and 45 and 13.6% sebacic acid as unaltered acids. However, the low combustibility of the acids was not yet proved in these injection experiments, as the organism was overwhelmed at once with very large amounts. However, as the authors themselves pointed out, more acids could be eliminated in unaltered form in the following days.

Ten years later, Mori[21] studied adipic acid in the rabbit with respect to the decomposition of muconic acid, and found 61.3% in the urine. The increase in oxalic acid (3-4 times) that occurred

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INFORMATION ON THE METABOLISM OF FATS
VI.

ACTION OF DICARBOXYLIC ACIDS AND SEBAMIC ACIDS IN THE ANIMAL ORGANISM

by

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In the study of fatty acid decomposition, only a few intermediate products have been identified so far. In defense of the theory of β-oxidation, Dakin[1], answering Friedmann[2], determined the presence of β-oxypropionic acid and acetophenone in the animal organism during his research on the fate of phenylpropionic acid in the animal organism. In the β-oxidation of succinic acid, Batelli and Stern[3] observed inactive malic acid. Einbeck[4] then broke the dehydration process down further to fumaric acid, and hydratization to malic acid.

The search for other easily accessible models of higher fatty acids led us to the dicarboxylic acids. It is notable that Baer and Blum[5] had found succinic acid in the urine of phlorizine-diabetic dogs after injecting them with large doses of glutaric acid. Even though the amount was quite small and only qualitatively determined, it was still plausible to assume that succinic acid could be a derivative of glutaric acid, since it is not otherwise found in dog urine. The data of Meisner[6] (1866) concerning the abundance of succinic acid in dog urine were not confirmed by Salkowski[7] (1871) and Lango[8] (1877). In the light of what we have presented so far, this is not quite comprehensible, as glutaric acid would have to be converted into malonic acid. The lower normal dicarboxylic acids up to C_5 are easily burned in the animal body, with the exception of oxalic acid; the same is true of the corresponding oxydicarboxylic acids, with the exception of tartaric acid. The separation of a carboxyl group from dicarboxylic acids is definitely observed, but

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10. Juda H. Quastel, Biochemical. Jl. Bd. 18, S. 365 (1924); Rona Ber. Bd. 27, S. 201 (1924).
11. Momose Goro, Jl. of Biol. Chem. Bd. 4, S. 441 (1925); Rona Ber. Bd. 33, S. 370 (1925).
12. Baer und Blum, Hofm. Beiträge Bd. 10, S. 93 (1907). Siehe auch Magnus-Levy, Die Acetonkörper in Ergebn. d. Inn. Med. und Kind.-Heilk. Bd. 1, S. 387 (1908) und Geelmuyden, Ergeb. d. Phys. v. Asher-Spiro Bd. 22, S. 196 (1923).
13. Baer und Blum, Arch. für exp. Pathol. Bd. 55, S. 89 (1906); Bd. 58, S. 92 (1907).
14. Friedmann, Hofm. Beiträge Bd. 11, S. 368 u. 373 (1908).
15. Hoppe-Seyler-Thierfelder, Hdb. d. Phys.-Chem. Analyse 1909, S. 96.
16. Wieland und Alles, Chem. Ber. Bd. 55, II, S. 1796 (1922).
17. Schaal, Chem. Ber. Bd. 40, S. 4787 (1907).
18. Fodera, Arch. d. Pharmakol. 1894, S. 417 und Fränkel, Arzneimittelsynthese Bd. 1, S. 102. 133.
19. W. C. Rose. I und II: Chem. Zbl. 1924, II, S. 2410; Rona Ber. Bd. 30, S. 457 (1925); III und IV: Chem. Zbl. 1925, II, S. 669; Rona Ber. Bd. 31, S. 602 u. 603 (1925).
20. Baer und Blum, Hofm. Beiträge Bd. 11, S. 101 (1908).
21. Yoshitane Mori, Jl. of Biol. Chem. Bd. 35, S. 341 (1918).
22. Gregg und Smith, Jl. of Biol. Chem. Bd. 67, S. XXVII (1926).
23. Lueg und Flaschenträger, Klin. Wochenschr. Heft 15, S. [illegible] (1925).
24. W. Dieter, Diese Zs. Bd. 120, S. 281 (1922).
25. Heffter, Ergebn. d. Phys. v. Asher-Spiro, Bd. 4, S. 226 (1905) und Porges, a. a. O. Bd. 10, S. 35 ff. (1910); Oppenheimer, Fermente Bd. 2, S. 779 (1925).
26. Rowney, Liebigs Ann. der Chem. Bd. 82, S. 123 (1852); Bd. [illegible], S. 719.
27. Kraut, Jl. für prakt. Chem. 1863, S. 358.
28. Etaix, A. ch. [4] Bd. 9, S. 465 (1866).
29. A. Grün und Th. Wirth, Chem. Ber. Bd. 55, II, S. 2215 (1922).

säure: bei 124° sintern, bei 128° geschmolzen. Schmelzpunkt mit Sebamidsäure bei 115° sintern, bei 120° geschmolzen!

Kochen mit Soda greift die Sebamidsäure nur wenig an.

Tierversuch.

Wie in den früheren Versuchen wurden einem Hund (8,9 kg) nach zweitägiger Vorperiode 10 g Sebamidsäure als Natriumsalz in zwei täglichen Dosen von je 0,5 g subkutan gespritzt. Der Harn wurde mit einem Gemisch von Benzol und Chloroform (2,5 : 1, spez. Gew. = etwa 1) desinfiziert. Nach 6 Tagen Nachperiode wurde der filtrierte Harn, der ganz schwach lackmus, nicht kongosauer reagierte, im Vakuum bei 60° eingedampft. Es bleiben etwa 87 g Rückstand, der mit 20 ccm Eisessig in 200 ccm Wasser angesäuert wird. Nach kurzer Zeit fallen Krystalle, die sich beim Stehen in Eis auf 5,4 g vermehren. Sie bestehen zu 42% aus reinem Allantoin, 0,4 g Sebamidsäure und anorganischen Salzen. Die wässrige Harnlösung wurde nun viermal je 24, und am Schluß 48 Stunden mit Äther extrahiert. Die gelösten Mengen 1,3, 0,8, 0,4, 0,3, 0,4 g nehmen rasch ab. Zur besseren Aufarbeitung werden sie vereint und daraus 1 g unveränderte Sebamidsäure erhalten. Die Spaltung zu Sebacinsäure ist also nicht erfolgt. Die Endlaugen liefern nach dreistündigem Kochen mit Salzsäure und erschöpfender Ätherextraktion keine Sebacinsäure.

Die Sebamidsäure ist praktisch quantitativ abgebaut worden.

Literatur.

1. Dakin, Hofm. Beiträge Bd. 11, S. 401 (1908).
2. Friedmann, Hofm. Beiträge Bd. 11, S. 151 (1908).
3. Battelli und Stern, Biochem. Z. Bd. 31, S. 475 (1911).
4. Einbeck, Diss. Zs. Bd. 90, S. 301 (1914); Biochem. Zs. Bd. 95, S. 296 (1919).
5. Baer und Blum, Hofm. Beiträge Bd. 10, S. 80 (1905).
6. Meissner, Z. f. d. Med. 3, Bd. 24, S. 97 (1865), Meissner und Shepard, Untersuchungen über das Entstehen der Hippursäure im Organismus, Hannover 1866.
7. Salkowski, Pflüg. Arch. Bd. 2, S. 267 (1869), Bd. 4, S. 95 (1871).
8. Longo, Diss. Bd. 1, S. 213 (1877).
9. H. Müller, Hofm. Chem. act. Bd., S. 163, 259 (1902).

Die Krystalle sind optisch positiv, in Längsrichtung zum Teil schief, zum Teil gerade auslöschend, je nach Flächenausbildung. Sehr oft sieht man neben den zu Sternen angeordneten Prismen Einzelkrystalle von Zigarrenform.

Die Sebamidsäure ist in Äther nahezu unlöslich im Gegensatz zur Sebacinsäure, von der sie dadurch gut getrennt werden kann. In den sonstigen organischen Lösungsmitteln außer Benzol, Petroläther, Chloroform ist sie gut löslich.

Verhalten der Sebamidsäure gegen Essigsäure. 0,3 g Sebamidsäure werden mit 15 ccm Essigsäure (n = 1,95) 3 Stunden am Rückflußkühler gekocht. Nach dem Abkühlen werden 250,3 mg = 83,5% wiedererhalten. Schmelzp.: 120° sintern, 125—126° klar geschmolzen. Mischschmelzpunkt mit reiner Sebamidsäure 125—126° C; mit Sebacinsäure: sintern 112°, geschmolzen 116—120° C. Gegen Essigsäure ist Sebamidsäure beständig.

Verhalten der Sebamidsäure gegen Salzsäure. 0,300 g Sebamidsäure werden mit 15 ccm verdünnter Salzsäure (2 n) 3 Stunden am Rückfluß gekocht. Beim Stehen krystallisieren kornähnliche Schuppen: 280 mg = 93%. Schmelzpunkt bis 128° unverändert, bei 129° beginnende Sinterung, bei 131° wie tauendes Eis, bei 133—134° klar geschmolzen. Mischschmelzpunkt mit Sebacinsäure genau wie vorher. Mischschmelzpunkt mit Sebamidsäure. Sinterung bei 115—116°, geschmolzen bei 117°! Probe in Äther löslich. Kochen mit verdünnter Salzsäure zerstört Sebamidsäure quantitativ.

Verhalten von Sebamidsäure gegen Soda. 0,3001 g Sebamidsäure werden mit 15 ccm Soda (2 n) 2 Stunden am Rückfluß gekocht. Nach dem Erkalten wurde mit Essigsäure angesäuert und abgesaugt. Bei Zimmertemperatur 250 mg = 83%. Schmelzpunkt bei 110° sintern, bei 120° klar. Mischschmelzpunkt mit Sebamidsäure: bei 115° sintern, bei 124° klar. Keine Depression! Mit etwa 60 ccm Äther gekocht, filtriert. Ätherunlösliches sintert jetzt bei 120° und wird klar bei 125 bis 126°. Die ätherlöslichen Krystalle sintern bei 120° und werden klar bei 126—127°. Ihr Mischschmelzpunkt mit Sebacin-

Sebamidsäure, $COOH(CH_2)_8 \cdot CONH_2$.

Vorversuche hatten gezeigt, daß sowohl das Bariumsalz der Sebacin-, des sauren Esters und der Sebamidsäure unlöslich in Methanol ist. Das Sebacindiamid ist beständig gegen kochende methanolische Barytlauge (n/1). Diäthylester und auch sebamidsaures Äthyl geben in der Kälte erst nach einigem Stehen (2 Stunden und 20 Minuten) eine krystalline Fällung. Es mußte also gelingen, unter Schonung der Amidgruppe die Estergruppe zu verseifen, da das Reaktionsprodukt sofort aus dem System ausfiel.

11,65 g sebamidsaures Äthyl wurden in 20 ccm absolutem Methanol gelöst und mit 61 ccm (0,835 n) methanolischem Bariumhydroxyd ($^1/_2$ Mol.) versetzt. Nach $1^1/_2$ Stunden ist bereits ein Krystallbrei entstanden. Die Umsetzung ist jedoch erst vollständig, wenn man nach 15 Stunden Stehen noch kurz (5 Min.) auf dem Wasserbad erwärmt, bis die Reaktion neutral geworden ist. Nach dem Abkühlen saugt man ab, wäscht mit Methanol nach und trocknet auf dem Wasserbad. Ausbeute an sebamidsaurem Barium 13,05 g = 96 % der Theorie.

Zur Isolierung der Sebamidsäure löst man 13 g des Ba-Salzes in 652 ccm heißem Wasser, filtriert und säuert mit 24,6 ccm (n = 1,98, 2 Mol.) Essigsäure an. Nach Stehen im Eis wird abgesaugt, mit Wasser gewaschen, auf Ton abgepreßt und auf dem Wasserbad zur Konstanz getrocknet. Ausbeute 8,8 g = 90 %. Schmelzp. 125°, Sinterung bei 120°. Nach einmaligem Umkrystallisieren aus heißem Wasser (10,26 g in 250 ccm) erhält man schneeweiße Nadeln (9,6 g = 94 %) mit einem konstanten Schmelzpunkt von 126,5°, Sinterung 123° C.

Zur Analyse wurde über P_2O_5 im Vakuum zur Konstanz getrocknet.

4,602 mg Substanz gaben 10,050 mg CO_2 und 3,850 mg H_2O.
4,810 „ „ „ 10,520 „ CO_2 „ 4,070 „ H_2O.
3,155 „ „ „ 0,188 ccm N bei 759 mm und 17°.
3,630 „ „ „ 0,215 ccm N „ 761 mm „ 18°.

$C_{10}H_{19}O_3N$ (201 Mol.-Gew.)

Ber.	C 59,7 %	H 9,45 %	N 6,96 %
Gef.	„ 59,54, 59,64	„ 9,36, 9,47	„ 7,01, 6,97.

wenn man nach Meissner und Shepard den Harn eines mit Fleisch reichlich gefütterten Hundes ansäuert.

Aus den Ätherextrakten wurde 6,15 g = 61 % unveränderte Sebacinsäure isoliert. Schmelzpunkt und Mischschmelzpunkt 132° C.

II. Versuche mit Sebamidsäure.

a) Sebamidsäureäthylester $CO(NH_2)\cdot(CH_2)_8\cdot COOC_2H_5$.

25 g Sebacinsäure-mono-äthylester (Schmelzp. 37°) nach Grün und Wirth werden mit 60 g Thionylchlorid ½ Stunde auf dem Wasserbad am Rückfluß erhitzt und dann im Vakuum das restliche Thionylchlorid verjagt. Ausbeute an rohem, etwas braun gefärbten Chlorid 27,0 g = 100 %. Das Chlorid wird dann vorsichtig in 100 ccm eisgekühltes konz. Ammoniak eingegossen. Temperatur stieg auf 25° C. Das ausgefallene Sebamidsäureäthyl wird in etwa 500 ccm Äther aufgenommen neutral gewaschen und mit Natriumsulfat getrocknet. Der Äther hinterließ 18,4 g = 87,5 %. Schmelzp. 70° C.

Umkrystallisieren. 17,8 g werden in 70 ccm Äther am Rückfluß braun gelöst, 10 ccm Äther noch zugegeben, da beim Abkühlen alles zu einem Krystallbrei erstarrt. Mit 100 ccm Petroläther werden 11,6 g daraus weiß gefällt, d. i. 65 Proc. Schmelzp. 70—71°. Aus Wasser, in dem der Amidester in der Hitze etwa 1:700 löslich ist, kommen fast quantitativ schneeweiße, optisch positive, in Längsrichtung auslöschende feine Nadeln, von borsäureähnlichem Aussehen. Schmelzpunkt 70—71°. In Methanol 1:2 und in Äthanol gut löslich. Zur Analyse wird über Phosphorpentoxyd im Vakuum getrocknet.

4,905 mg Substanz (aus Äther-Petrol-Ä.) gaben 11,300 mg CO_2, 4,430 mg H_2O.
4,880 mg Substanz (aus Wasser) gaben 10,100 mg CO_2, 3,990 mg H_2O.
3,600 mg „ gaben 0,194 ccm N bei 759 mm und 18°.
4,020 „ „ „ 0,214 „ N „ 764 „ „ 18°.

$C_{12}H_{23}O_3N$ (229,19 Mol.-Gew.)

Ber.	C 62,83 %	H 10,11 %	N 6,31 %
Gef.	„ 62,84, 62,88	„ 10,10, 10,20	„ 6,31, 6,27.

Rückstand wurden dabei etwa 50 mg brauner Massen erhalten, die nach Behandeln mit Eisessig und Umfällen mit Natronlauge und Salzsäure 8,9 mg noch nicht ganz reine Harnsäure gaben. Sämtliche Harnsäurereaktionen nach Hoppe-Seyler-Thierfelder waren positiv. Ebenso wurde die charakteristische Krystallform der Harnsäure (Behrens-Kley, Mikrochemische Analyse) aus verdünnter Essigsäure im Polarisationsmikroskop beobachtet. Die Elementaranalyse lieferte jedoch keinen eindeutigen Beweis.

4,360 mg Substanz gaben 7,690 mg CO_2, 1,500 mg H_2O, 0,034 mg Rückstand.

2,145 mg „ „ 0,340 ccm N bei 756 mm, 20°.

Ber. für Harnsäure	C 35,69 %	H 2,38 %	N 33,33 %
„ „ Kynurensäure	„ 63,5	„ 3,7	„ 7,4
Gef.	„ 48,09	„ 8,85	„ 18,32

Daraus folgt, daß die Harnsäure nicht rein war. Es gelang mir, aus dem verbliebenen Rest mit Sicherheit die Kynurensäure durch ihr unter dem Mikroskop sehr charakteristisches Ba-Salz zu erkennen. Damit ist die Harnsäure beim Hund bei ausschließlicher Reiskost, mit der das Stickstoffminimum nahezu erreicht wird, festgestellt, und es wäre interessant nachzuprüfen, ob die Harnsäure auch im Stickstoffminimum ein normales Stoffwechselendprodukt des gewöhnlichen, nicht bloß des Dalmatinerhundes ist.

An unveränderter Korksäure wurden in den Ätherauszügen 6 g wiedergefunden = 60 %. Schmelzpunkt und Mischschmelzpunkt 130° C.

c) Sebacinsäure.

10 g Sebacinsäure werden einem 21,2 kg schweren Hund nach dreitägiger Vorperiode in täglichen Dosen von zweimal 0,5 g als Natriumsalz unter die Haut gespritzt. Der Harn von 14 Tagen wird wie früher verarbeitet. Auch hier ist das Allantoin (3,3 g) durchsetzt von kleinen schwarzen Warzen (0,3 g), die sich oft zu hantel- und traubenförmigen Gebilden zusammenlegen. Sie enthalten sicher Kynurensäure (Ba-Salz). Die genaue Prüfung auf Harnsäure ist im Gang. Das Aussehen der Warzen erinnert deutlich an die Sphärolithe, die man erhält,

Versuchsteil.

I. Injektionsversuch mit Dicarbonsäuren.

a) Adipinsäure.

10 g reine Adipinsäure wurden 4 jungen Hunden*) (7,35, 5,00, 6,3, 5,2 kg) als Natriumsalz in täglichen Dosen von 2 mal je 0,25 g morgens und abends unter die Haut gespritzt. Nach diesen 5 Tagen wurde der Harn noch 3 Tage gesammelt. Als Futter diente nur mit Salz und Wasser gekochter Reis. Die Vorperiode dauerte 3 Tage. Der gesamte Harn wurde gemeinsam mit den Käfigspülwässern kongosauer eingedampft.

Vorher überzeugte ich mich, daß auch bei einer 1 stündigen Wasserdampfdestillation von den hier verwandten drei Dicarbonsäuren keine Spur mit in das Destillat überging.

Der eingedampfte Harn (etwa 150 ccm) wurde im Soxhlet mit Äther so lange (5 Tage) extrahiert, bis Krystalle von der in Äther unlöslichen Kynurensäure sich an der Kolbenwand absetzten. Die einzelnen Ätherauszüge wurden zum Teil getrennt, zum Teil gemeinsam verarbeitet. Aus den braunen Extrakten konnten 5 g unveränderte Adipinsäure mit einem Schmelzpunkt von 150° und einem ebensolchen Mischschmelzpunkt gewonnen werden. Aus den Laugen krystallisierten am Schluß nur mehr Milligramme aus. Mit Essigester läßt sich die Säure, wie auch die Kork- und Sebacinsäure gut reinigen. 50% der Adipinsäure werden demnach abgebaut.

b) Korksäure.

10 g reine Korksäure (Merck) werden wie die Adipinsäure zugeführt und der Harn in gleicher Weise aufgearbeitet. Der Hund wog 4,37 kg und erhielt täglich zweimal 0,5 g. Aus dem mit Äther erschöpften konz. Harn krystallisierten 1,5 g rohes Allantoin. Beim Umkrystallisieren aus Wasser wurden 0,6 g reines Allantoin erhalten. Als in Wasser unlöslicher

*) Ein Tier ging vom 5. auf 6. Injektionstag an subakuter, gelber Leberatrophie zugrunde. Ein Zusammenhang der Todesursache mit der Einverleibung von Adipinsäure besteht nicht, da die anderen 3 Tiere vollkommen gesund blieben.

Wir versuchten daher die eine COOH-Gruppe biologisch zu verschließen unter Aufrechterhaltung der chemischen Säurenatur. Die Ester-, Anhydrid- und Chloridgruppe ist dazu wegen der leichten Aufspaltbarkeit nicht geeignet. Nitrile sind giftig. Es lag daher nahe, die Amide zu versuchen. W. Dieter[24]) hat das Spaltungsvermögen der Hefe gegenüber Säureamiden untersucht und festgestellt, daß Reinzuchthefe Asparagin, Acetamid und einige aromatische Amide nicht angreift, solange sie nicht wächst. Im Tierkörper sind die körperfremden Säureamide in vielen Fällen[25]) nicht leicht oxydabel. Phenylessigsäure- und Mandelsäureamid werden nur wenig angegriffen (unveröffentlichte Beobachtung von Thomas). Während Malamid schwer, wird Succinimid nahezu völlig verbrannt. Von den Halbamiden geht Oxaminsäure zum Teil unverändert in den Harn über, nur Asparagin und Glutamin werden leicht und völlig zerstört.

Weitere biologische Beobachtungen an Halbamiden liegen nicht vor.

Wir haben uns daher die Sebamidsäure hergestellt. Rowney[26]) und Kraut[27]) geben an, sie durch Stehenlassen des Diäthylesters mit Ammoniak und durch Destillation des Ammonsalzes erhalten zu haben. Nähere Angaben fehlen. Etaix[28]) beschreibt sie kurz mit dem Schmelzp. 170° C. Wir konnten die Säure mit dem Schmelzp. 170° C nicht erhalten. Vielleicht hat Etaix ein Gemisch von Di- und Monamid in Händen gehabt. Die Sebamidsäure schmilzt bei 126—127°. Wir erhielten sie in guter Ausbeute, indem wir den Diäthylester der Sebacinsäure nach A. Grün und Th. Wirth[29]) in den Monoäthylester überführten, mit Thionylchlorid das entsprechende Chlorid gewannen und dieses mit Ammoniak zum Amidester umsetzten. Die partielle Hydrolyse gelang mit methylalkoholischem Bariumhydroxyd. Bevor wir die Sebamidsäure injizierten, studierten wir ihre Beständigkeit gegen Säuren und Alkalien. Sie läßt sich aus Harn mit guter Ausbeute wiedergewinnen. Die Aufarbeitung des Versuchsharns lieferte nur 10% roh und 5% rein wieder. Sebacinsäure wurde nicht gefunden. Die Sebamidsäure war also im Gegensatz zur Dicarbonsäure fast völlig abgebaut worden.

gewerblichen Interesse war, ob die Adipinsäure die teure Citronensäure ohne Schaden ersetzen könne.

Fütterungsversuch mit Adipinsäure von Dr. E. Andersen.

	Adipinsäure			Oxalsäure im Harn	
	verfüttert		ausgeschieden im Harn	Vorperiode	Versuch
	insgesamt	pro Tag			
Hund (10 kg)	20	2	8,8 %	7,9 mg	11,3 mg
	10	2	10,1 „	8,2 „	14,0 „
Andersen	25	5	55,0 „	—	20
Patientin	35	5	72,5 „	25 „	85
Bact. coli	verändert Adipinsäure nicht				

Nach diesen Ergebnissen greift der Hund die Adipinsäure viel besser an als der Mensch. Im Kot des Hundes wurde keine Adipinsäure gefunden. Nach unserem Versuch waren 12 % der Adipinsäure wiedergefunden.

Nach Abschluß meiner Versuche teilt Gregg-Smith[22] in einer kurzen Notiz mit, daß Pelargonsäure an einen Hund verfüttert, völlig verbrannt wird, während Azelainsäure zu 10 % wieder ausgeschieden wird. Er widerlegt damit die Ansicht von Leathes (1908), daß der biologische Fettsäureabbau den von der Natur vorgerichteten ungesättigten Graupen der Fettsäuren ansetzt. Wenn dies der Fall wäre, dann müßte ja auch die Ölsäure antiketogen wirken, da sie in Azelainsäure und Pelargonsäure zerfallen müßte. Die meisten Versuche sind bisher nur mit Fetten d. h. Glyceriden angestellt worden. Es muß in diesem Zusammenhang darauf hingewiesen werden, daß bei einseitiger Fütterung von reiner Butter- und Ölsäure beim Schweine[23] überraschenderweise keine Acetonurie und keine Acidosis trotz N-Minimum auftrat.

Unsere Versuche mit Kork- und Sebacinsäure bestätigen die Versuche von Baer und Blum. 60 bzw. 40 % wurden unverändert ausgeschieden.

Das Nächstliegende ist anzunehmen, daß die zweite Carboxylgruppe den Abbau der langen normalen Ketten verhindert

rali hervor. Ihr Ca-Salz kommt als Ursache nicht in Frage, da es wasserlöslicher als das der ungiftigen Form ist. Die höheren Säuren, Adipin-, Pimelin-, Kork-, Azelainsäure, schädigen demgegenüber die Niere nur sehr wenig. Rose glaubt nun, da die Säuren mit gerader wie die mit ungerader Kohlenstoffzahl gleich ungiftig sind, daß sie nicht auf dem Wege der β-Oxydation abgebaut werden, da sonst die giftigere Glutarsäure als intermediäres Produkt auftreten müßte. Wenn diese Ansicht richtig ist, dann dürfte man im Stoffwechsel weder aus Korksäure keine Adipin- und aus Sebacinsäure keine Korksäure finden. Zugleich interessierte uns die Frage, ob nicht die β-Oxydation an den zwei völlig gleichen Carboxylgruppen zu gleicher Zeit angreift und der Abbau bei den Dicarbonsäuren sich an zwei Stellen vollzieht. Von Baer und Blumenthal[1]) wurden zum Studium der Zuckerausscheidung und der Acidose phlorizindiabetischen Hunden große Tagesdosen (7—10 g) subcutan verabfolgt. Stickstoff-, Zucker-, Aceton- und Oxybuttersäureausscheidung verminderte sich mit den Säuren C_7—C_9 und C_8—C-Atomen. Azelain- und Sebacinsäure senkten nur die Acidose und waren sonst ohne Wirkung. Die Ursache lag nicht in einer verschiedenen graduellen Verbrennbarkeit. Die Aufarbeitung des Harns vom Injektionstag lieferte von der Adipinsäure 12%, Pimelinsäure 47%, Korksäure 62 und 56%, Azelainsäure 50% und Sebacinsäure 45 und 13,6% an unveränderten Säuren. Die schwere Verbrennbarkeit der Säuren ist jedoch mit diesen Injektionsversuchen nicht bewiesen, da ja der Organismus mit sehr großen Mengen auf einmal überschwemmt worden war. Es konnte aber, worauf Verfasser selbst hinweisen, auch in den folgenden Tagen noch weitere Säuren unverändert ausgeschieden werden.

10 Jahre später hat Morita[1]) die Adipinsäure am Kaninchen im Hinblick auf den Abbau der Muconsäure untersucht und sie zu 61,3% im Harn wiedergefunden. Bemerkenswert ist die dabei auftretende (3—4fache) Vermehrung der Oxalsäure. Im Herbst 1922 hat auf unsere Veranlassung Dr. Andersen im Krankenhaus St. Georg in Leipzig die Resorbierbarkeit der Adipinsäure an Menschen und Hunden geprüft, da es von

reichliche Vorkommen von Bernsteinsäure im Hundeharn sind von Salkowski[5]) (1871) und Lango[6]) (1877) nicht bestätigt worden. Dies wäre auch mit unseren bisherigen Vorstellungen nicht recht verständlich, da die Glutarsäure ja in die Malonsäure übergehen müßte. Die niederen normalen Dicarbonsäuren bis C_5 werden im Tierkörper sehr leicht verbrannt, mit Ausnahme der Oxalsäure, ebenso auch die entsprechenden Oxydicarbonsäuren mit Ausnahme der Weinsäure. Die Ablösung einer Carboxylgruppe aus Dicarbonsäuren ist mit Sicherheit beobachtet, aber nur bei den niederen körpervertrauten. Hans Müller[8]) erhielt aus Fumarsäure durch Gärung mit Hefe Milchsäure. Juda Hirsch Quastel[10]) konnte aus Bernsteinsäure und Fumarsäure nach Vergären mit Bac. pyocyaneus Brenztraubensäure und Essigsäure isolieren. Neuerdings glaubt Momose Goro[11]) von 9 Versuchen mit Malonsäure bei der Leberdurchblutung in zwei Fällen Aceton erhalten zu haben und formuliert den Abbau über Acetaldehyd-Aldol. Baer und Blum[12]) aber fanden am Phloridzinhund gerade für Malon-, Bernstein- und Brenzweinsäure keine Beeinflussung der Acetonkörper, Zucker- und N Ausscheidung. Baer und Blum[13]) und Friedmann[14]) jedenfalls konnten bei ihren Leberdurchblutungsversuchen mit verzweigten Dicarbonsäuren gerade als Charakteristikum feststellen, daß die COOH-Gruppe sehr fest haftet gegenüber der Methylgruppe. Es ist sehr auffallend, daß von den höheren mehrbasischen Säuren[15]) außer der Glutarsäure und der Citronensäure nur ganz wenige abnormale Bestandteile von Pflanze und Tier bis jetzt isoliert wurden. H. Wieland und Alles[16]) haben kürzlich die Korksäure als Suberylarginin im Bufotoxin, dem Giftstoff der einheimischen Kröte (Bufo vulgaris), eingebaut gefunden. Im Japan- und Carnaubawachs sind nach Schaal[17]) hohe Dicarbonsäuren C_{20}, C_{25} vorhanden. Die Dicarbonsäuren sind außer der Oxalsäure wenig oder gar nicht giftig. Mit zunehmender Methylenzahl nimmt die Giftigkeit auch noch ab.[18]) Die Glutarsäure mit allerdings, wie W. Rose[19]) in seinen Untersuchungen über die Nierenwirkung von Dicarbonsäuren und ihrer Derivate jüngst festgestellt hat, eine tubuläre Nephritis mit Verödung der Glome-

Flaschentraeger-'26
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Beiträge zur Kenntnis des Fettstoffwechsels.

VI.

Verhalten von Dicarbonsäuren und Sebamidsäure im Tierkörper.

Von

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(Der Redaktion zugegangen am 11. August 1926.)

Beim Studium des Fettsäureabbaues sind bisher nur wenig Zwischenprodukte gefaßt worden. Dakin[1]) stellte zur Verteidigung der Theorie von der β-Oxydation gegen Friedmann[2]) bei der Untersuchung des Schicksals der Phenylpropionsäure im Tierkörper β-Oxypropionsäure und Acetophenon fest. Batelli und Stern[3]) beobachteten bei der β-Oxydation der Bernsteinsäure inaktive Apfelsäure. Einbeck[4]) hat dann den Vorgang in Dehydrierung zur Fumarsäure und Hydratation zur Apfelsäure aufgelöst.

Die Suche nach weiteren leicht zugänglichen Modellen höherer Fettsäuren führte uns zu den Dicarbonsäuren. Es fällt auf, daß Baer und Blum[5]) bei der Injektion von größeren Dosen Glutarsäure an phlorizindiabetischen Hunden Bernsteinsäure im Harn gefunden hatten. Wenn auch die Menge nur ganz klein und nur (qualitativ) sichergestellt war, so lag doch die Vermutung nahe, daß die Bernsteinsäure ein Abbauprodukt der Glutarsäure sein konnte, da sie im Hundeharn sonst nicht gefunden wird. Die Angaben von Meissner[6]) (1866) über das

*) Ausgeführt mit Mitteln der Notgemeinschaft der Deutschen Wissenschaft.

20. Baer und Blum, Hofm. Beiträge Bd. 11, S. 101 (1908).
21. Yoshitane Mori, Jl. of Biol. Chem. Bd. 35, S. 341 (1918).
22. Gregg und Smith, Jl. of Biol. Chem. Bd. 67, S. XXVII (1926).
23. Lueg und Flaschenträger, Klin. Wochenschr. Heft 15, S. 696 (1925).
24. W. Dieter, Diese Zs. Bd. 120, S. 281 (1922).
25. Heffter, Ergebn. d. Phys. v. Asher-Spiro, Bd. 4, S. 226 (1905) und Porges, a. a. O. Bd. 10, S. 35ff. (1910); Oppenheimer, Fermente Bd. 2, S. 779 (1925).
26. Rowney, Liebigs Ann. der Chem. Bd. 82, S. 123 (1852); Beilstein II, S. 719.
27. Kraut, Jl. für prakt. Chem. 1863, S. 358.
28. Etaix, A. ch. [7] Bd. 9, S. 405 (1896).
29. A. Grün und Th. Wirth, Chem. Ber. Bd. 55, II, S. 2215 (1922).

Animal Experiment

As in the earlier experiments, a dog (8.9 kg) was injected subcutaneously, after a two-day preliminary period, with 10 g sebamic acid in the form of a sodium salt, in two daily doses of 0.5 g each. The urine was disinfected with a mixture of benzene and chloroform (2.5:1, specific weight about 1). After 6 days of a post-period, the filtered urine, which reacted very weakly in litmus, and was not congo acidic, was concentrated by evaporation at 60° in a vacuum. About 87 g residue remained and were acidified with 20 ccm acetic acid in 200 ccm water. After a short time, crystals that increase to about 5.4 g upon standing in ice are precipitated. They consist, up to 42%, of pure allantoin, 0.4 g sebamic acid and inorganic salts. The watery urine lye was now extracted 4 times for 24 hours, and finally for 48 hours with ether. The dissolved amounts of 1.8, 0.8, 0.4, 0.3 and 0.4 g rapidly decrease. For better processing, they are combined and from this, 1 g of unaltered sebamic acid is obtained. Thus, splitting to sebacic acid does not take place. The discard solution yielded no sebacic acid after three hours of boiling with hydrochloric acid and depleting ether extraction.

Sebamic acid was, practically speaking, quantitatively decomposed.

Literature

1. Dakin, Hofm. Beiträge Bd. 11, S. 404 (1908).
2. Friedmann, Hofm. Beiträge Bd. 11, S. 151 (1908).
3. Battelli und Stern, Biochem. Zs. Bd. 31, S. 478 (1911).
4. Einbeck, Diese Zs. Bd. 90, S. 301 (1914); Biochem. Zs. Bd. 95, S. 296 (1919).
5. Baer und Blum, Hofm. Beiträge Bd. 10, S. 80 (1907).
6. Meissner, Zs. rat. Med. 3, Bd. 24, S. 97 (1865); Meissner und Shepard, Untersuchungen über das Entstehen der Hippursäure im Organismus, Hannover 1866.
7. Salkowski, Pflügers Arch. Bd. 2, S. 367 (1869); Bd. 4, S. 95 (1871).
8. Longo, Diese Zs. Bd. 1, S. 213 (1877/78).
9. H. Müller, Helv. chim. act. Bd. 5, S. 163, 239 (1922).
10. Juda H. Quastel, Biochemical. Jl. Bd. 18, S. 365 (1924); Rona Ber. Bd. 27, S. 201 (1924).
11. Momose Goro, Jl. of Biol. Chem. Bd. 4, S. 441 (1925); Rona Ber. Bd. 33, S. 370 (1925).
12. Baer und Blum, Hofm. Beiträge Bd. 10, S. 93 (1907). Siehe auch Magnus-Levy, Die Acetonkörper in Ergebn. d. Inn. Med. und Kind.-Heilk. Bd. 1, S. 387 (1908) und Geelmuyden, Ergeb. d. Phys. v. Asher-Spiro Bd. 22, S. 196 (1923).
13. Baer und Blum, Arch. für exp. Pathol. Bd. 55, S. 89 (1906); Bd. 56, S. 92 (1907).
14. Friedmaun, Hofm. Beiträge Bd. 11, S. 368 u. 373 (1908).
15. Hoppe-Seyler-Thierfelder, Hdb. d. Phys.-Chem. Analyse 1925, S. 96.
16. Wieland und Alles, Chem. Ber. Bd. 55, II, S. 1796 (1922).
17. Schaal, Chem. Ber. Bd. 40, S. 4787 (1907).
18. Fodera, Arch. d Pharmakol. 1894, S. 417 und Fränkel, Arzneimittelsynthese Bd. 1, S. 102, 133.
19. W. C. Rose, I und II: Chem. Zbl. 1924, II, S. 2410; Rona Ber. Bd. 30, S. 457 (1925); III und IV: Chem. Zbl. 1925, II, S. 669; Rona Ber. Bd. 31, S. 602 u. 608 (1925).

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 210

Material FDA 71-50

Dose 205.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date January 19, 1973

Laboratory No. 1362 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 2091	P	11	11	11		[illegible]	5		1.68	
G 2092	NP	0	0						----	
G 2093	NP	0	0						----	
G 2094	P	13	12	12		3	9		1.65	
G 2095	P	14	14	13		6	7	1	1.57	
G 2096	P	10	10	10		5	5		1.86	
G 2097	P	12	12	12		6	6		1.86	
G 2098	P	11	11	11		5	6		1.85	
G 2099	P	11	11	11		4	7		1.99	
G 2100	P	14	13	13		5	8		1.70	
G 2101	P	12	12	12		3	9		1.80	
G 2102	P	14	14	14		5	9		1.82	
G 2103	P	10	12	10		6	[illegible]	1	1.80	
G 2104	P	12	12	11		5	6	1	1.58	
G 2105	P	10	9	9		6	3		1.91	
G 2106	P	14	12	11		6	5	[illegible]	1.66	
G 2107	P	12	14					14	----	
G 2108										Not Assigned
G 2109	NP	8	8						----	
G 2110	P	13	13	12		[illegible]	[illegible]	1	1.64	
G 2111	P	14	14	13				1	1.66	
G 2112	P	12	[illegible]	12					1.89	
G 2113	NP	--**	[illegible]						----	
G 2114	P	12	14	12		[illegible]	8		1.63	
G 2115	P	12	12	12		5	7		1.83	
G 2116	P	19	15	15		7	[illegible]		1.43	
G 2117	P	16	14	14		[illegible]	[illegible]		1.65	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 209

Material FDA 71-50

Dose 44.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date January 19, 1973

Laboratory No. 1362 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 2061	P	12	12	11		[illegible]	5	2	1.85	
G 2062	P	14	14	14		9	5		1.59	
G 2063	P	10	11	10		2	8		1.62	
G 2064	P	14	14	14		5	9		1.46	
G 2065	NP	0	0						----	
G 2066	P	11	11	11		[illegible]	8		1.74	
G 2067	P	13	12	12		3	9		1.76	
G 2068	P	12	12	12		6	6		1.65	
G 2069	P	13	12	12		3	9		1.63	
G 2070	P	17	15	15		9	6		1.60	
G 2071	NP	7	0						----	
G 2072	P	12	12	11		3	8	1	1.68	
G 2073	P	17	17	17		9	[illegible]		1.61	
G 2074	P	12	12	12		8	4		1.90	
G 2075	P	13	12	12		5	7		1.67	
G 2076	P	14	14	14		5	9		1.58	
G 2077	P	15	15	15		[illegible]	[illegible]		1.55	
G 2078	P	13	14	13		[illegible]	11	1	1.69	
G 2079	P	12	1[illegible]	10		[illegible]	5	1	1.68	
G 2080	P	15	15	15		[illegible]	6		1.90	
G 2081	P	12	1[illegible]	10		[illegible]	6	1	1.66	
G 2[illegible]	P	12	[illegible]	12		[illegible]	[illegible]		1.85	
G 20[illegible]	P	13	[illegible]	13		[illegible]	7		1.68	
G 2084	P	13	13	13		7	6		1.76	
G 2085	P	13	13	13		[illegible]	9		1.90	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 208

Material FDA 71-50

Dose 9.5 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date January 19, 1973

Laboratory No. 1362 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 2031	P	12	11	11		7	4		1.62	
G 2032	P	10	10	10		4	6		1.74	
G 2033	P	10	9	9		3	6		1.61	
G 2034	P	11	11	11		7	4		1.74	
G 2035	P	14	14	14		4	10		1.73	
G 2036	P	11	11	11		4	7		1.52	
G 2037	NP	4	0						----	
G 2038	P	18	18	18		11	7		1.81	
G 2039	P	11	11	11		7	4		1.70	
G 2040	NP	2	0						----	
G 2041	P	11	12	11	1	2	9		1.56	
G 2042	P	15	14	14		8	6		1.78	
G 2043	P	12	12	12		7	5		1.81	
G 2044	P	10	10	10		3	7		1.83	
G 2045	NP	8	0						----	
G 2046	NP	4	0						----	
G 2047	P	15	16	15		6	9	1	1.67	
G 2048	P	13	13	13		6	7		1.75	
G 2049	NP	5	0						----	
G 2050	P	13	13	11		7	4	2	1.73	
G 2051	P	12	12	12		7	5		1.77	
G 2052	P	9	--	--		--	--		----	Natural Birth Da
G 2053	P	15	14	14		6	8		1.77	
G 2054	P	11	11	11		5	6		1.79	
G 2055										Not Assigned
G 2056	P	24	20	19		6	13	1	1.87	
G 2057	P	14	11	11		6	5		1.69	
G 2058	P	18	14	14		4	10		1.79	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Appendix II

Reproduction Data in Hamsters (Individual)

Group 207

Material FDA 71-50

Dose 2.0 mg/kg

Date January 19, 1973

Laboratory No. 1362 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 2001	P	9	9	9		4	5		----	Natural Birth Day
G 2002	P	13	13	13		6	7		1.77	
G 2003	NP	6	0						----	
G 2004	P	11	10	10		5	5		1.70	
G 2005	P	10	9	8	1	2	6		1.55	
G 2006	P	14	14	14		4	10		1.77	
G 2007	P	14	15	13		2	11	2	1.44	
G 2008	P	10	10	9		3	6	1	1.74	
G 2009	NP	0	0						----	
G 2010	P	15	13	13		8	5		1.86	
G 2011	P	14	14	14		5	9		1.92	
G 2012	P	15	15	15		4	11		1.94	
G 2013	P	14	14	14		8	6		----	Natural Birth Day
G 2014	P	13	13	13		8	5		1.90	
G 2015	P	13	12	12		5	7		1.73	
G 2016	NP	--**	0						----	
G 2017	P	12	12	12		8	4		1.97	
G 2018	P	16	16	16		7	9		1.51	
G 2019	P	15	14	13		3	10	1	1.79	
G 2020	P	13	13	13		9	4		1.83	
G 2021	NP	11	0						----	
G 2022	P	13	13	13		3	10		1.73	
G 2023	P	12	12	12		8	4		1.60	
G 2024	P	15	15	14		7	7	1	1.65	
G 2025	P	14	13	13		8	5		1.76	

* P = Pregnant; NP = Not Pregnant

** Not Readable

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 202

Material Aspirin

Dose 250.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date January 19, 1973

Laboratory No. 1362

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 2601	P	12	12	12		6	6		1.76	
A 2602	P	11	11	11		4	7		1.46	
A 2603	P	11	10	10		4	6		1.76	
A 2604	P	11	12	10		7	3	2	1.70	
A 2605	P	14	15	13		5	8	2	1.87	
A 2606	P	10	10	9		3	6	1	1.86	
A 2607	P	11	12	10		4	6	2	1.86	
A 2608	P	10	11	9		1	8	2	1.53	
A 2609	P	16	15	15		6	9		1.82	
A 2610	P	16	16	16		4	12		1.75	
A 2611	P	14	16	14		3	11	2	1.96	
A 2612	P	5	3	3		0	3		1.78	
A 2613	P	15	14	14		3	11		1.86	
A 2614	P	14	15	13		6	7	2	1.79	
A 2615	P	13	14	11		6	5	3	1.76	
A 2616	P	12	12	12		6	6		1.57	
A 2617	P	14	13	13		9	4		1.74	
A 2618	P	12	12	12		4	8		1.86	
A 2619	P	13	12	12		5	7		1.69	
A 2620	P	12	11	11		1	10		1.68	
A 2621	NP	11	0						----	
A 2622	P	--**	10	9		4	5	1	1.79	
A 2623	P	11	11	11		4	7		1.90	
A 2624	P	10	10	10		4	6		1.78	
A 2625	P	13	13	13		6	7		1.54	

* P = Pregnant; NP = Not Pregnant

** Not Read

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 201

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Hamsters (Individual)

Date January 19, 1973

Laboratory No. 1362

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 2601	P	14	13	13		6	7		1.84	
S 2602	NP	9	0						----	
S 2603	P	14	13	13		7	6		1.69	
S 2604	P	10	10	10		8	2		1.75	
S 2605	P	15	16	16		8	8		1.51	
S 2606	P	11	11	11		3	8		1.39	
S 2607	P	12	13	9		2	7	4	1.79	
S 2608	P	14	13	13		3	10		1.88	
S 2609	P	15	15	15		7	8		1.85	
S 2610	P	14	13	13		6	7		1.59	
S 2611	P	16	15	15		3	12		1.56	
S 2612	P	12	12	11		2	9	1	1.89	
S 2613	P	10	11	9		1	8	2	1.56	
S 2614	P	15	14	14		8	6		1.61	
S 2615	P	13	12	12		7	5		1.72	
S 2616	P	11	11	11		3	8		1.76	
S 2617	P	13	13	13		5	8		1.68	
S 2618	P	12	13	10		4	6	3	1.56	
S 2619	P	13	13	13		4	9		1.76	
S 2620	P	15	15	15		[illegible]	11		1.68	
S 2621	P	16	15	14		6	8	1	1.78	
S 2622	P	14	14	14		1	13		1.67	
S 2623	P	14	13	13		9	4		1.78	
S 2624	P	13	13	13		5	8		1.73	
S 2625	P	14	13	13		6	7		1.77	

* P = Pregnant; NP = Not Pregnant

sites, live and dead fetuses were recorded. All live pups were weighed and the genital tract of each dam was examined for any anatomical abnormalities.

All fetuses were examined grossly for the presence of external congenital defects and one-third of each litter underwent detailed visceral examination under 10X magnification. The remaining two-thirds of the pups were cleared in potassium hydroxide, stained with alizarin red dye, and examined for the presence of skeletal abnormalities.

Appendix I

Teratology Study in Hamsters

Virgin adult female golden hamsters from an outbred strain were individually housed in mesh bottom cages in temperature and humidity controlled quarters with free access to food and fresh tap water at all times. They were mated (1 to 1) with mature males and the appearance of motile sperm in the vaginal smear was considered as Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 10 of gestation, the indicated dose levels of the test material were administered by oral intubation. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≦ 250	1	≦ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 8, 10, and 14 of the gestation period. All animals were observed daily for appearance and behavior with particular attention to food consumption in order to better recognize any abnormalities resulting from anorexic effects in the pregnant animal.

On Day 14, all animals were subjected to Caesarian section under deep anesthesia and the numbers of implantation sites, resorption

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210

Date January 19, 1973

Table 4

Species Hamsters

Laboratory No. 1362 g

Average Body Weights*

Group	Material	Dose Level	Day 0	Day 6	Day 8	Day 10	Day 14**
		mg/kg	g				
201	Sham	0.0	109.2	114.1	118.8	130.2	153.1 (24)
202	Aspirin***	250.0	104.5	110.1	117.6	124.9	145.2 (24)
207	FDA 71-50	2.0	102.2	108.8	112.7	123.9	148.1 (19)
208	FDA 71-50	9.5	109.9	114.6	118.2	128.5	151.0 (21)
209	FDA 71-50	44.0	104.0	108.9	112.4	122.0	142.7 (23)
210	FDA 71-50	205.0	102.8	107.1	111.0	121.0	140.8 (22)

* Of pregnant dams

** Number of surviving dams in parentheses (c.f. Table 1)

*** Positive control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210 Date January 19, 1973

Material FDA 71-50 Laboratory No. 1362 g

Table 3-a

Summary of Soft Tissue Abnormalities

(Hamsters)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
201	Sham	0.0	S 2618	9	Meningoencephalocele
208	FDA 71-50	9.5	G 2033	1	Meningoencephalocele

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210 Laboratory No. 1362 g

Table 3

Material FDA 71-50 Date January 19, 1973

Summary of Skeletal Findings*
(Hamsters)

Findings / Group No.:	201	202	207	208	209	210
Dose (mg/kg):	Sham	Aspirin**	2.0	9.5	44.0	205.0
Live Fetuses Examined (at term)	211/24	188/24	166/19	183/21	199/23	173/21
Sternebrae						
Incomplete oss.	76/21	77/22	53/18	62/21	108/23	49/16
Scrambled						
Bipartite	39/14	33/18	22/13	30/16	13/11	25/16
Fused						
Extra				1/1		
Missing	44/16	31/12	25/12	29/13	38/14	23/10
Other						
Ribs						
Incomplete oss.						
Fused/split		1/1				1/1
Wavy						
Less than 12	1/1					
More than 13	43/17	36/13	32/13	32/15	32/15	48/18
Other						
Vertebrae						
Incomplete oss.	11/8	6/5	2/2	3/3	4/3	9/5
Scrambled						
Fused						
Extra ctrs. oss.				1/1		
Scoliosis				1/1	1/1	
Tail defects						
Other						
Skull						
Incomplete closure	1/1					1/1
Missing				1/1		
Craniostosis						
Other						
Extremities						
Incomplete oss.	62/20	44/15	20/10	13/7	44/16	30/9
Missing						
Extra						
Miscellaneous						
Hyoid; missing	4/4	1/1	1/1		7/4	3/3
Hyoid; reduced	5/4	6/4	5/4	2/2	9/6	5/2
Pelvic bones; missing						2/2

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group : 201 & 202; 207 through 210

Material : FDA 71-50

Table 2
Reproduction Data
(Hamsters)

Date : January 19, 1973

Laboratory No. : 1362 g

Group :	201	202	207	208	209	210
Dose (mg/kg) :	Sham	Aspirin**	2.0	9.5	44.0	205.0
Pregnancies						
Total No.	24	24	21	22	23	22
Died or Aborted (before Day 14)	0	0	2	1	0	0
To term (on Day 14)	24	24	19	21	23	22
Corpora Lutea						
Total No.	329	291	292	312	309	286
Average/dam mated	13.2	12.1	12.2	11.6	12.4	11.4
Live Litters						
Total No.*	24	24	19	21	23	21
Implant Sites						
Total No.	314	290	246	267	298	271
Average/dam*	13.1	12.1	13.0	12.7	13.0	12.3
Resorptions						
Total No.*	11	17	5	4	6	21
Dams with 1 or more sites resorbed	5	9	4	3	5	7
Dams with all sites resorbed	--	--	--	--	--	1
Per cent partial resorptions	20.8	37.5	21.1	14.3	21.7	31.8
Per cent complete resorptions	--	--	--	--	--	4.55
Live Fetuses						
Total No.	303	273	240	262	291	250
Average/dam*	12.6	11.4	12.6	12.5	12.7	11.4
Sex ratio (M/F)	0.64	0.63	0.78	0.85	0.72	0.84
Dead Fetuses						
Total No.*	--	--	1	1	1	--
Dams with 1 or more dead	--	--	1	1	1	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	--	--	5.26	4.76	4.35	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	1.70	1.75	1.75	1.73	1.70	1.75

* Includes only those dams examined at term.

** Positive Control : 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 201 & 202; 207 through 210

Material: FDA 71-50

Table 1

Fate Summary
(Hamsters)

Date: January 19, 1973

Laboratory No.: 1362 g

Group	Material	Dose ** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
201	Sham	0.0	25	24	25	24
202	Aspirin*	250.0	25	24	25	24
207	FDA 71-50	2.0	25	21	23	19
208	FDA 71-50	9.5	27	22	26	21
209	FDA 71-50	44.0	25	23	25	23
210	FDA 71-50	205.0	26	22	26	22

* Positive Control: 250.0 mg/kg

** Administered as a water solution (See Appendix I)

1) Includes all dams examined at term

Food and Drug Research Laboratories
INCORPORATED

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date February 26, 1973

Laboratory No. 1362 g
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-50 (Adipic Acid)

Examination Requested: Teratologic evaluation of FDA 71-50 in hamsters

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 205 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the twenty-first of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 210

Material FDA 71-50

Dose 288.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date January 19, 1973

Laboratory No. 1361 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 1091										Not Assigned
G 1092	P	8	8	8		3	5		3.85	
G 1093	P	16	15	15		8	7		3.90	
G 1094	P	12	11	11		6	5		3.95	
G 1095	P	11	11	11		6	5		3.94	
G 1096	P	14	14	14		6	8		4.02	
G 1097	P	10	7	7		4	3		4.05	
G 1098	NP	7	0						----	
G 1099	P	15	15	15		4	11		3.82	
G 1100	P	13	13	13		8	5		3.88	
G 1101	P	11	11	11		3	8		3.70	
G 1102	P	17	15	15		8	7		4.28	
G 1103	P	10	10	10		5	5		4.29	
G 1104	P	13	12	12		8	4		3.92	
G 1105	P	10	10	10		4	6		5.60	
G 1106	NP	6	0						----	
G 1107	P	16	14	14		9	5		3.78	
G 1108	NP	12	0						----	
G 1109	P	--**	11	11		6	5		3.76	
G 1110	P	15	9	9		4	5		4.09	
G 1111	P	12	11	11		4	7		3.94	
G 1112	P	12	12	11		7	4	1	3.67	
G 1113	NP	5	0						----	
G 1114	P	11	11	10		6	4	1	4.04	
G 1115	P	7	10	5		2	3	5	3.23	

* P = Pregnant; NP = Not Pregnant

** Ovary Missing

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Appendix II

Reproduction Data in Rats (Individual)

Group 209

Material FDA 71-50

Dose 62.0 mg/kg

Date January 19, 1973

Laboratory No. 1361 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 1061	P	12	11	11		5	6		4.13	
G 1062	P	8	5	5		2	3		4.21	
G 1063	P	11	10	10		4	6		3.84	
G 1064	P	14	13	13		[illegible]	7		3.86	
G 1065	P	12	12	12		4	8		4.11	
G 1066	P	7	7	7		1	6		3.79	
G 1067	P	16	16	16		6	10		3.61	
G 1068	P	12	12	12		6	6		3.80	
G 1069	NP	7	0						----	
G 1070	P	15	15	15		12	3		3.90	
G 1071	P	12	11	11		5	6		4.02	
G 1072	P	14	12	12		5	7		3.65	
G 1073	P	14	14	14		8	6		4.04	
G 1074	P	13	13	13		10	3		4.10	
G 1075	P	14	12	12		5	7		3.77	
G 1076	NP	0	0						----	
G 1077	P	14	13	13		6	7		3.83	
G 1078	P	8	8	8		3	5		4.14	
G 1079	P	9	1	1		0	1		5.25	
G 1080	NP	4	0						----	
G 1081	P	14	14	14		[illegible]	6		4.03	
G 1082	P	14	13	13		[illegible]	6		4.28	
G 1083	P	14	13	13		10	3		4.04	
G 1084	P	12	12	12		6	6		3.95	
G 1085	P	9	8	8		5	3		3.77	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 208

Material FDA 71-50

Dose 13.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date January 19, 1973

Laboratory No. 1361 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 1031	P	13	13	13		6	7		4.39	
G 1032	P	12	11	10	1	3	7		3.88	
G 1033	P	14	14	14		5	9		3.40	
G 1034	P	10	10	10		4	6		3.67	
G 1035	P	14	12	12		5	7		3.56	
G 1036	P	13	13	13		8	5		3.62	
G 1037	P	11	6	6		2	4		3.73	
G 1038	P	9	9	9		6	3		3.86	
G 1039	P	9	9	9		3	6		4.50	
G 1040	P	11	11	11		6	5		4.03	
G 1041	P	10	10	10		7	3		4.35	
F 1042	P	10	9	8		6	2	1	4.14	
G 1043	P	13	13	12	1	4	8		3.65	
G 1044	P	13	12	12		6	6		3.26	
G 1045	P	15	13	13		7	6		3.67	
G 1046	NP	10	0						----	
G 1047	P	17	6	6		2	4		4.32	
G 1048	P	12	12	9	1	5	4	2	3.99	
G 1049	P	13	9	9		3	6		3.78	
G 1050	P	13	10	10		5	5		3.37	
G 1051	P	11	9	9		5	4		3.83	
G 1052	P	11	10	10		3	7		3.41	
G 1053	P	13	11	11		5	6		4.03	
G 1054	P	13	11	11		4	7		3.76	
G 1055	P	13	11	11		7	4		3.69	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 207

Material FDA 71-50

Dose 2.9 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date January 19, 1973

Laboratory No. 1361 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 1001	P	11	11	11		6	5		3.77	
G 1002	P	11	11	11		6	5		4.03	
G 1003	P	14	13	13		5	8		3.76	
G 1004	P	13	12	12		5	7		3.84	
G 1005	P	11	13	7		6	1	6	3.48	
G 1006	P	15	15	15		7	8		3.80	
G 1007	NP	13	0						----	
G 1008	P	12	12	12		6	6		3.97	
G 1009	P	15	14	14		5	9		4.14	
G 1010	P	12	11	11		9	2		3.65	
G 1011	P	10	10	10		4	6		3.63	
G 1012	P	10	7	7		5	2		4.04	
G 1013	P	10	9	9		4	5		5.18	
G 1014	P	6	5	5		1	4		3.73	
G 1015	P	16	12	12		9	3		3.98	
G 1016	P	12	12	12		7	5		3.98	
G 1017	P	15	12	12		4	8		4.08	
G 1018	P	14	12	12		8	4		3.84	
G 1019	P	15	12	12		4	8		3.85	
G 1020	NP	9	0						----	
G 1021	P	13	10	10		6	4		3.70	
G 1022	P	13	12	12		5	7		3.91	
G 1023	P	14	11	11		6	5		3.90	
G 1024	P	14	13	13		4	9		3.73	
G 1025	P	16	11	11		5	6		3.45	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 202

Material Aspirin

Dose 250.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date January 19, 1973

Laboratory No. 1361

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 1601	P	16	16	16		8	8		3.00	
A 1602	P	12	7		2			5	----	
A 1603	P	10	8	8		5	3		2.11	
A 1604	NP	15	0						----	
A 1605	P	13	13	13		4	9		2.98	
A 1606	NP	0	0						----	
A 1607	P	4	12					12	----	
A 1608	P	8	5	5		3	2		2.71	
A 1609	P	12	12	12		5	7		2.54	
A 1610	P	7	7	7		2	5		2.79	
A 1611	P	11	10	10		4	6		1.57	
A 1612	P	10	10	10		4	6		2.89	
A 1613	P	9	9	9		3	6		2.90	
A 1614	P	12	13	12		5	7	1	2.05	
A 1615										Not Assigned
A 1616	P	14	13	13		4	9		3.28	
A 1617	P	--**	11					11	----	Aborted Day 1!
A 1618	NP	9	0						----	
A 1619	P	--***	5		3			2	----	
A 1620	NP	4	0						----	
A 1621	P	8	10					10	----	
A 1622	P	14	11	11		6	5		2.20	
A 1623	P	13	12	3		3	0	9	2.16	
A 1624	P	13	11	3		3	0	8	1.93	
A 1625	P	9	11	9		6	3	2	2.12	
A 1626	P	10	12	8	1	4	4	3	1.87	
A 1627	P	9	10					10	----	
A 1628	P	10	8	1	1	1	0	6	2.77	
A 1629	P	12	13					13	----	

* P = Pregnant; NP = Not Pregnant

** Ovary Missing

*** Not Read

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 201

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Rats (Individual)

Date January 19, 1973

Laboratory No. 1361

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 1601	P	9	8	8		3	5		4.03	
S 1602	P	16	15	15		3	12		3.74	
S 1603	NP	4	0						----	
S 1604	P	11	12	11		4	7	1	3.76	
S 1605	NP	10	0						----	
S 1606	NP	8	0						----	
S 1607	P	13	12	12		6	6		3.55	
S 1608	P	11	11	11		6	5		3.84	
S 1609	P	13	13	13		5	8		3.63	
S 1610	P	12	11	11		5	6		3.75	
S 1611	P	10	10	10		3	7		3.88	
S 1612	P	13	11	11		6	5		3.44	
S 1613	P	14	14	13	1	8	5		3.66	
S 1614	NP	8	0						----	
S 1615	P	12	12	12		7	5		3.95	
S 1616	P	16	12	12		4	8		4.28	
S 1617	NP	7	0						----	
S 1618	P	14	11	11		6	5		3.85	
S 1619	P	14	11	11		6	5		3.81	
S 1620	P	12	11	11		4	7		3.58	
S 1621	P	14	11	10		4	6	1	3.61	
S 1622	P	15	11	11		5	6		3.64	
S 1623	P	14	13	13		7	6		5.61	
S 1624	P	12	11	11		7	4		3.74	
S 1625	P	10	7	7		4	3		4.15	

* P = Pregnant; NP = Not Pregnant

was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

Appendix I

Teratology Study in Rats

Virgin adult female albino rats (Wistar derived stock) were individually housed in mesh bottom cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubations. The controls were sham treated with the vehicle at a level equivalent to the group receiving the highest test dose. The test material was prepared and doses calculated according to the following table:

Dosage (mg/kg)	Dose (ml/kg)	Concentration (mg/ml)
≦ 250	1	≦ 250
251 - 500	2	125 - 250
501 - 750	3	133 - 250
751 - 1000	4	187 - 250
1001 - 1250	5	200 - 250
1251 - 1500	6	208 - 250
1501 - 1600	6.4	235 - 250

Body weights were recorded on Days 0, 6, 11, 15, and 20 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 20 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210

Date January 19, 1973

Table 4

Species Rats

Laboratory No. 1361 g

Average Body Weights*

Group	Material	Dose Level	Day 0	6	11	15	20**
		mg/kg	g				
201	Sham	0.0	215	240	253	276	344 (20)
202	Aspirin***	250.0	215	238	249	264	303 (23)
207	FDA 71-50	2.9	213	235	251	272	336 (23)
208	FDA 71-50	13.0	214	235	247	271	332 (24)
209	FDA 71-50	62.0	214	237	253	274	340 (22)
210	FDA 71-50	288.0	217	239	253	273	343 (20)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210 Date January 19, 1973

Material FDA 71-50 Laboratory No. 1361 g

Table 3-a

Summary of Soft Tissue Abnormalities

(Rats)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description
202	Aspirin*	250.0	A 1614	4	Encephalomyelocele
				1	Encephalomyelocele; umbilical hernia
			A 1619	1	Encephalomyelocele
			A 1625	1	Meningoencephalocele
				1	Encephalomyelocele
			A 1626	1	Encephalomyelocele; umbilical hernia
				2	Meningoencephalocele
			A 1628	1	Encephalomyelocele; umbilical hernia
208	FDA 71-50	13.0	G 1032	1	Meningoencephalocele

* Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210 Laboratory No. 1361 g

Table 3

Material FDA 71-50 Date January 19, 1973

Summary of Skeletal Findings*
(Rats)

Findings / Group No.: / Dose (mg/kg):	201 Sham	202 Aspirin**	207 2.9	208 13.0	209 62.0	210 288.0
Live Fetuses Examined (at term)	159/20	100/16[a]	176/23	175/24	171/22	157/20
Sternebrae						
Incomplete oss.	66/19	80/16	46/18	60/13	61/17	44/15
Scrambled						
Bipartite	1/1	7/5	1/1	1/1	1/1	1/1
Fused		1/1				
Extra						
Missing	17/8	85/16	20/9	4/3	7/2	9/5
Other						
Ribs						
Incomplete oss.						
Fused/split		10/5				
Wavy	16/7	45/14	12/5	26/1[illegible]	12/7	29/10
Less than 12						
More than 13	1/1	81/13	6/3	1/1	5/3	
Other						
Vertebrae						
Incomplete oss.	20/10	92/16	19/8	25/9	12/7	[illegible]/8
Scrambled		1/1				
Fused						
Extra ctrs. oss.						
Scoliosis		1/1				
Tail defects						
Other						
Skull						
Incomplete closure	21/11	43/15	25/12	35/10	23/11	26/11
Missing		9/3				
Craniostosis						
Other						
Extremities						
Incomplete oss.		4/3				
Missing						
Extra						
Miscellaneous						
Hyoid; missing	19/8	52/15	14/9	26/12	23/10	19/11
Hyoid; reduced	3/3	7/5	18/8	24/10	18/12	24/11

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 250.0 mg/kg

a) One litter lost in processing

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 201 & 202; 207 through 210

Date: January 19, 1973

Material: FDA 71-50

Laboratory No.: 1361 g

Table 2
Reproduction Data
(Rats)

Group:	201	202	207	208	209	210
Dose (mg/kg):	Sham	Aspirin**	2.9	13.0	62.0	288.0
Pregnancies						
Total No.	20	24	23	24	22	20
Died or Aborted (before Day 20)	0	1	0	0	0	0
To term (on Day 20)	20	23	23	24	22	20
Corpora Lutea						
Total No.	292	264	314	303	279	263
Average/dam mated	11.7	10.2	12.6	12.1	11.2	11.4
Live Litters						
Total No.*	20	17	23	24	22	20
Implant Sites						
Total No.	227	238	260	254	245	230
Average/dam*	11.4	10.4	11.3	10.6	11.1	11.5
Resorptions						
Total No.*	2	81	6	3	--	7
Dams with 1 or more sites resorbed	2	12	1	2	--	3
Dams with all sites resorbed	--	4	--	--	--	--
Per cent partial resorptions	10.0	52.2	4.35	8.33	--	15.0
Per cent complete resorptions	--	17.4	--	--	--	--
Live Fetuses						
Total No.	224	150	254	248	245	223
Average/dam*	11.2	6.52	11.0	10.3	11.1	11.2
Sex ratio (M/F)	0.85	0.88	1.00	0.89	1.02	0.99
Dead Fetuses						
Total No.*	1	7	--	3	--	--
Dams with 1 or more dead	1	4	--	3	--	--
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	5.00	17.4	--	12.5	--	--
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	3.88	2.46	3.89	3.83	4.01	3.99

* Includes only those dams examined at term.

** Positive Control: 250.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 201 & 202; 207 through 210

Date: January 19, 1973

Table 1

Material : FDA 71-50

Laboratory No. : 1361 g

Fate Summary
(Rats)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
201	Sham	0.0	25	20	25	20
202	Aspirin*	250.0	28	24	27	23
207	FDA 71-50	2.9	25	23	25	23
208	FDA 71-50	13.0	25	24	25	24
209	FDA 71-50	62.0	25	22	25	22
210	FDA 71-50	288.0	24	20	24	20

* Positive Control : 250.0 mg/kg
** Administered as a water solution (See Appendix I)
1) Includes all dams examined at term

Food and Drug Research Laboratories
INCORPORATED

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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date February 26, 1973

Laboratory No. 1361 g
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-50 (Adipic Acid)

Examination Requested: Teratologic evaluation of FDA 71-50 in rats

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 288 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the twenty-first of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

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FOOD AND DRUG RESEARCH LABORATORIES, INC.

Appendix II

Reproduction Data in Mice (Individual)

Group 210

Material FDA 71-50

Dose 263.0 mg/kg

Date January 19, 1973

Laboratory No. 1360 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
Continued.										
G 0118	P	14	13	13		8	5		0.80	
G 0119	P	12	10	9		4	5	1	0.93	
G 0120	NP	0	0						----	
G 0121	NP	0	0						----	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Appendix II

Reproduction Data in Mice (Individual)

Group 210

Material FDA 71-50

Dose 263.0 mg/kg

Date January 19, 1973

Laboratory No. 1360 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 0091	P	16	2	2		1	1		1.04	
G 0092	P	15	16	16		10	6		0.84	
G 0093	P	14	6	4	1	2	2	1	0.81	
G 0094	P	14	13	12	1	1	11		0.77	
G 0095	P	18	15	15		4	11		0.84	
G 0096	P	13	10	10		7	3		0.88	
G 0097	NP	3	0						----	
G 0098	P	15	13	12	1	6	6		0.71	
G 0099	P	12	13	12		5	7	1	0.87	
G 0100	P	19	14	14		10	4		0.80	
G 0101	P	15	13	13		7	6		0.83	
G 0102	P	13	12	12		7	5		0.78	
G 0103	NP	13	0						----	
G 0104	NP	0	0						----	
G 0105	NP	12	0						----	
G 0106	P	11	11	10	1	6	4		0.68	
G 0107	P	13	14	13		5	8	1	0.90	
G 0108	P	12	12	12		6	6		0.89	
G 0109	P	15	12	1		1	0	11	0.55	
G 0110	NP	14	0						----	
G 0111	P	14	11	11		8	3		0.81	
G 0112	P	14	13	12	1	4	8		0.51	
G 0113	NP	0	0						----	
G 0114	NP	14	0						----	
G 0115	NP	12	0						----	
G 0116	NP	14	0						----	
G 0117	P	13	12	11		4	7	1	0.71	

Continued on next page.

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 209

Material FDA 71-50

Dose 56.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date January 19, 1973

Laboratory No. 1360 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 0061	P	10	11	11		7	3		1.02	
G 0062	P	14	13	13		9	4		0.94	
G 0063	P	17	12	12		4	8		0.90	
G 0064	P	16	13	13		4	9		0.75	
G 0065	P	14	14	13		10	3	1	0.77	
G 0066	P	16	15	14		6	8	1	0.87	
G 0067	P	7	4	3		1	2	1	0.85	
G 0068	NP	11	0						----	
G 0069	P	14	13	13		7	6		0.86	
G 0070	P	18	15	15		7	8		0.91	
G 0071	P	14	13	13		8	5		0.78	
G 0072	P	11	9	9		5	4		0.85	
G 0073	P	16	12	11	1	8	3		0.96	
G 0074	P	13	13	12		5	7	1	0.80	
G 0075	P	13	13	11		6	5	2	0.95	
G 0076	P	16	17	16		10	6	1	0.86	
G 0077	P	12	10	9	1	4	5		0.94	
G 0078	P	16	15	14		11	3	1	0.86	
G 0079	P	7	2	1		0	1	1	0.64	
G 0080	P	12	12	12		9	3		0.83	
G 0081	P	11	12	11		7	4	1	0.85	
G 0082	P	12	11	11		6	5		0.80	
G 0083	P	14	14	14		4	10		1.10	
G 0084	P	16	15	15		9	6		0.93	
G 0085	P	12	11	9		4	5	2	0.78	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 208

Material FDA 71-50

Dose 12.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date January 19, 1973

Laboratory No. 1360 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 0031	P	14	12	11		3	8	1	0.83	
G 0032	P	14	13	13		8	5		0.93	
G 0033	P	12	11	11		4	7		0.81	
G 0034	P	--**	10	10		5	5		1.02	
G 0035	P	15	11	10	1	1	9		0.69	
G 0036	P	--**	10	10		5	5		0.84	
G 0037	P	13	12	12		7	5		0.95	
G 0038	P	17	16	14		7	7	2	0.90	
G 0039	P	14	12	12		7	5		0.93	
G 0040	P	15	15	15		7	8		0.97	
G 0041	P	15	8	8		2	6		0.81	
G 0042	P	12	12	12		4	8		0.89	
G 0043	P	11	10	10		2	8		0.94	
G 0044	NP	4	0						----	
G 0045	P	12	7	7		3	4		1.10	
G 0046	P	11	7	7		2	5		1.04	
G 0047	P	15	13	13		4	9		0.87	
G 0048	P	11	8	5		1	4	3	0.88	
G 0049	P	12	9	9		4	5		0.92	
G 0050	P	13	9					9	----	
G 0051	NP	9	0						----	
G 0052	P	10	9	6		2	4	3	0.95	
G 0053	P	12	10	9		3	6	1	0.80	
G 0054	P	15	13	11		6	5	2	0.83	
G 0055	P	17	15	14		6	8	1	0.92	

* P = Pregnant; NP = Not Pregnant

** Ovary Missing

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 207

Material FDA 71-50

Dose 2.6 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date January 19, 1973

Laboratory No. 1360 g

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
G 0001	P	13	8	8		0	8		1.05	
G 0002	P	14	14	14		6	8		0.81	
G 0003	P	16	14	12		4	8	2	0.96	
G 0004	P	13	13	13		4	9		1.01	
G 0005	P	12	12	12		3	9		0.91	
G 0006	P	12	15	12		7	5	3	0.88	
G 0007	P	15	16	15		9	6	1	0.98	
G 0008	P	13	12	11		6	5	1	0.88	
G 0009	P	12	11	10		3	7	1	0.89	
G 0010	P	10	10	10		5	5		1.12	
G 0011	P	12	5	5		2	3		1.16	
G 0012	NP	12	0						----	
G 0013	P	9	8					8	----	
G 0014	P	11	10	10		3	7		0.84	
G 0015	NP	10	0						----	
G 0016	P	12	12	12		3	9		0.80	
G 0017	P	12	11	9		1	8	2	0.75	
G 0018	NP	9	0						----	
G 0019	P	15	12	12		3	9		0.83	
G 0020	P	16	16	16		1	15		0.79	
G 0021	P	15	14	12		4	8	2	0.75	
G 0022	P	11	11	5	1	2	3	5	0.90	
G 0023	NP	13	0						----	
G 0024	P	15	12	10	1	6	4	1	0.76	
G 0025	P	13	14	13		6	7	1	0.87	

* P = Pregnant; NP = Not Pregnant

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Date January 19, 19[illegible]

Appendix II

Laboratory No. 136[illegible]

Group 202

Material Aspirin

Dose 150.0 mg/kg

Reproduction Data in Mice (Individual)

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
A 0601	NP	10	0						----	
A 0602	P	--**	11	10		4	6	1	0.79	
A 0603	NP	0	0						----	
A 0604	NP	11	0						----	
A 0605	NP	15	0						----	
A 0606	P	10	11	10		4	6	1	1.04	
A 0607	P	15	11	11		5	6		0.82	
A 0608	P	14	11					11	----	
A 0609	P	12	11	11		3	8		0.70	
A 0610	P	15	13	13		6	7		0.83	
A 0611	P	9	5	3		3	0	2	1.17	
A 0612	P	13	10	10		4	6		0.85	
A 0613	P	10	9	9		5	4		0.75	
A 0614	P	14	13	13		6	7		0.87	
A 0615	P	10	9	8		4	4	1	0.62	
A 0616	P	12	12	11		7	4	1	0.78	
A 0617	P	14	7	7		6	1		1.10	
A 0618	P	13	9	9		5	4		0.90	
A 0619	NP	12	0						----	
A 0620	NP	9	0						----	
A 0621	P	14	11	10		2	8	1	0.88	
A 0622	P	12	10	9		6	3	1	0.90	
A 0623	NP	12	0						----	
A 0624	P	13	13	12		6	6	1	0.72	
A 0625	P	13	11	11		5	6		0.84	
A 0626	NP	11	0						----	
A 0627	NP	0	0						----	
A 0628	P	14	12					12	----	
A 0629	P	10	9	9		3	6		0.78	
A 0630	P	14	2	2		1	1		0.70	

* P = Pregnant; NP = Not Pregnant

** Ovary Missing

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group 201

Material Sham

Dose 0.0 mg/kg

Appendix II

Reproduction Data in Mice (Individual)

Date January 19, 1973

Laboratory No. 1360

Dam No.	Fate*	Corpora Lutea	Implant Sites	Fetuses Alive	Fetuses Dead	Sex M	Sex F	Resorption Sites	Average Fetus Weight (g)	Remarks
S 0601	P	16	13	12		7	5	1	0.89	
S 0602	NP	4	0						----	
S 0603	P	17	15	15		8	7		0.96	
S 0604	P	14	13	11	1	6	5	1	0.75	
S 0605	P	14	13	13		5	8		0.92	
S 0606	P	14	13	13		7	6		0.82	
S 0607	P	12	7	6	1	3	3		0.88	
S 0608	P	15	12	12		4	8		0.92	
S 0609	P	12	9	8		3	5	1	0.94	
S 0610	NP	4	0						----	
S 0611	P	17	13	12		8	4	1	0.92	
S 0612	P	13	11	11		7	4		0.85	
S 0613	P	14	12	12		7	5		1.00	
S 0614	P	11	12	10	1	5	5	1	0.85	
S 0615	P	15	6	6		3	3		1.01	
S 0616	NP	7	0						----	
S 0617	P	14	13	12		6	6	1	0.79	
S 0618	P	13	10	10		6	4		0.70	
S 0619	P	14	13	13		7	6		0.86	
S 0620	P	12	11	11		8	3		0.85	
S 0621	NP	0	0						----	
S 0622	P	13	11	10	1	5	5		0.77	
S 0623	P	15	9	9		3	6		0.89	
S 0624	P	13	14	12		5	7	2	0.89	
S 0625	P	14	12	11		4	7	1	0.74	

* P = Pregnant; NP = Not Pregnant

Appendix I

Teratology Study in Mice

Virgin adult female albino CD-1 outbred mice were gang-housed in disposable plastic cages in temperature and humidity-controlled quarters with free access to food and fresh tap water. They were mated with young adult males, and observation of the vaginal sperm plug was considered Day 0 of gestation. Beginning on Day 6 and continuing daily through Day 15 of gestation, the females were dosed with the indicated dosages by oral intubation; the controls were sham treated.

Body weights were recorded on Days 0, 6, 11, 15, and 17 of gestation. All animals were observed daily for appearance and behavior with particular attention to food consumption and weight, in order to rule out any abnormalities which may have occurred as a result of anorexic effects in the pregnant female animal.

On Day 17 all dams were subjected to Caesarean section under surgical anesthesia, and the numbers of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were also recorded. The urogenital tract of each dam was examined in detail for anatomical normality.

All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations employing 10X magnification. The remaining two-thirds were cleared in potassium hydroxide (KOH), stained with alizarin red S dye and examined for skeletal defects.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210

Date January 19, 1973

Table 4

Species Mice

Laboratory No. 1360 g

Average Body Weights*

Group	Material	Dose Level	Day 0	6	11	15	17**
		mg/kg	g				
201	Sham	0.0	31.2	34.2	37.7	45.2	51.6 (21)
202	Aspirin***	150.0	28.6	31.7	32.8	40.0	43.5 (21)
207	FDA 71-50	2.6	31.0	33.5	36.0	44.5	49.3 (21)
208	FDA 71-50	12.0	29.3	31.4	34.3	42.6	47.8 (23)
209	FDA 71-50	56.0	31.7	34.8	37.5	44.9	50.3 (24)
210	FDA 71-50	263.0	32.9	35.5	34.9	40.9	45.4 (20)

* Of pregnant dams
** Number of surviving dams in parentheses (c.f. Table 1)
*** Positive control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210 Date January 19, 1973

Material FDA 71-50 Laboratory No. 1360 g

Table 3-a

Summary of Soft Tissue Abnormalities

(Mice)

Group	Material	Dose Level mg/kg	Dam	Number of Pups	Description

None Observed

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups 201 & 202; 207 through 210 Laboratory No. 1360 g

Table 3

Material FDA 71-50 Date January 19, 1973

Summary of Skeletal Findings*
(Mice)

Findings / Group No.:	201	202	207	208	209	210
Dose (mg/kg):	Sham	Aspirin**	2.6	12.0	56.0	263.0
Live Fetuses Examined (at term)	158/21	126/19	152/20	161/22	192/24	149/20
Sternebrae						
Incomplete oss.	95/20	47/13	71/19	91/20	129/23	116/19
Scrambled						
Bipartite	3/3	3/3		5/5	5/5	4/3
Fused						
Extra			1/1			
Missing	20/7	24/9	18/9	23/9	18/8	36/10
Other						
Ribs						
Incomplete oss.						
Fused/split						
Wavy		1/1				
Less than 12						
More than 13	18/9	18/10	43/14	17/10	24/12	17/7
Other						
Vertebrae						
Incomplete oss.	8/3	16/8	3/2		2/2	14/5
Scrambled						
Fused						
Extra ctrs. oss.						
Scoliosis						
Tail defects						
Other						
Skull						
Incomplete closure	2/1					
Missing						
Craniostosis						
Other						
Extremities						
Incomplete oss.	8/3	17/8	3/3	9/5	1/1	13/5
Missing						
Extra						
Miscellaneous						
Hyoid; missing	57/16	36/14	41/15	50/14	45/16	44/14
Hyoid; reduced	23/13	18/9	21/12	35/16	41/16	31/15
Pelvic bones; incomplete				2/1		1/1

* Numerator=Number of fetuses affected; Denominator=Number of litters affected.

** Positive control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Group: 201 & 202; 207 through 210

Material: FDA 71-50

Table 2
Reproduction Data
(Mice)

Date: January 19, 1973

Laboratory No.: 1360 g

Group:	201	202	207	208	209	210
Dose (mg/kg):	Sham	Aspirin**	2.6	12.0	56.0	263.0
Pregnancies						
Total No.	21	21	21	23	24	20
Died or Aborted (before Day 17)	0	0	0	0	0	0
To term (on Day 17)	21	21	21	23	24	20
Corpora Lutea						
Total No.	307	331	315	293	332	364
Average/dam mated	12.3	11.4	12.6	12.7	13.3	11.7
Live Litters						
Total No.*	21	19	20	22	24	20
Implant Sites						
Total No.	242	210	250	252	289	235
Average/dam*	11.5	10.0	11.9	11.0	12.0	11.8
Resorptions						
Total No.*	9	32	27	22	12	16
Dams with 1 or more sites resorbed	8	10	11	8	10	6
Dams with all sites resorbed	--	2	1	1	--	--
Per cent partial resorptions	38.1	47.6	52.4	34.8	41.7	30.0
Per cent complete resorptions	--	9.52	4.76	4.35	--	--
Live Fetuses						
Total No.	229	178	221	229	275	214
Average/dam*	10.9	8.48	10.5	9.96	11.5	10.7
Sex ratio (M/F)	1.04	0.91	0.55	0.68	1.23	0.98
Dead Fetuses						
Total No.*	4	--	2	1	2	5
Dams with 1 or more dead	4	--	2	1	2	5
Dams with all dead	--	--	--	--	--	--
Per cent partial dead	19.1	--	9.52	4.35	8.33	25.0
Per cent all dead	--	--	--	--	--	--
Average Fetus Weight, g	0.87	0.84	0.90	0.90	0.87	0.80

* Includes only those dams examined at term.

** Positive Control: 150.0 mg/kg

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Groups: 201 & 202; 207 through 210

Date: January 19, 1973

Material : FDA 71-50

Laboratory No. : 1360 g

Table 1

Fate Summary
(Mice)

Group	Material	Dose** mg/kg	Total		Surviving at Term	
			Mated	Pregnant	Total	Pregnant[1]
201	Sham	0.0	25	21	25	21
202	Aspirin*	150.0	30	21	30	21
207	FDA 71-50	2.6	25	21	25	21
208	FDA 71-50	12.0	25	23	25	23
209	FDA 71-50	56.0	25	24	25	24
210	FDA 71-50	263.0	31	20	31	20

* Positive Control : 150.0 mg/kg

** Administered as a water solution (10 ml per kg of body weight)

1) Includes all dams examined at term

Food and Drug Research Laboratories
INCORPORATED

Maurice Avenue at 58th Street
Maspeth, New York 11378
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FINAL REPORT

Submitted to: DHEW/Public Health Service
Food and Drug Administration CA-272
5600 Fishers Lane-Room 5C-13
Rockville, Maryland 20852

Date February 26, 1973

Laboratory No. 1360 g
Contract No. FDA 71-260

Sample: Fine white crystalline material

Marking: FDA 71-50 (Adipic Acid)

Examination Requested: Teratologic evaluation of FDA 71-50 in mice.

Procedure: See Appendix I

Results: See Tables 1 through 4 and Appendix II

Conclusion: Subject to reexamination in the light of later findings, the following is concluded:

"The administration of up to 263 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Comment: Attention is called to the fact that this is the twenty-first of a series of reports which will be issued in accordance with the terms of the contract cited above. Eventually, a total of at least 42 compounds will have been tested in 21 pairs; each pair being run concurrently against one sham-treated control and one positive control group. Because of the inherent variability of biological data of the type dealt with here, the accumulation and pooling of sequential sets of control values will greatly enhance the statistical value of the findings and the ultimate reliability of the test results.

FOOD AND DRUG RESEARCH LABORATORIES, INC.

Kenneth Morgareidge

Kenneth Morgareidge, Ph.D.
Vice President

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Generation of the test atmospheres

The air used for the atmospheres was filtered, dried to a relative humidity of less than 10%, and supplied at a line pressure of 1 atm ($1{\cdot}013 \times 10^5$ Nm^{-2}). In the results section, the methods used to prepare the atmospheres are indicated by letters in parentheses which refer to the following list. The letter is followed by any information relating to the particular experiment.

A A nearly saturated vapour obtained by passing air through a liquid contained in a bubbler with a sintered glass air-distributor disc. The volume of the liquid was usually 10-20 ml and, if the size of the sample available permitted, it was replaced daily. Unless otherwise stated, the bubbler was maintained in a water-bath at room temperature, about 20°C.

B A nearly saturated vapour obtained by passing air through a column of a granular solid. If the sample supplied was a fine powder, it was dispersed on the surface of granular kieselguhr.

C An atmosphere prepared by methods A or B and diluted to a known extent with clean air.

D A vapour concentration by injecting a liquid at a known rate into a metered stream of air by means of a controlled fluid-feed atomizer (Gage, 1953). For concentrations much less than 100 ppm a solution of the liquid in a toxicologically inert solvent was used. For very volatile liquids the syringe was cooled in an ice-water bath.

E A metered stream of a gas or vapour from a cylinder was diluted with a metered stream of air. The diluted gas was passed through a jet to produce efficient mixing by turbulence.

F A gas or vapour contained in a large polyethylene bag at atmospheric pressure was introduced into a metered air stream at a known rate by means of a peristaltic pump (Watson Marlow).

G A powdered solid injected into a metered air stream at a known rate by the apparatus described by Byers and Gage (1961).

H A condensed fume prepared by passing an aerosol obtained by methods D or G through a well-insulated cylindrical electric furnace located in the central tube of the exposure chamber. The furnace was heated to a temperature sufficiently high to volatilize the substance, which on cooling condensed to a fume.

I A method was devised for the continuous generation of methyl nitrite, which is too unstable to be isolated and stored. A solution of hydrochloric acid in methanol was injected at a known rate on to a mixture of equal parts of sodium nitrite and anhydrous sodium sulphate. In the presence of excess methanol the rate of addition of hydrochloric acid defined the rate of liberation of methyl nitrite. The vapour diffused through a sintered glass plate into a metered air stream.

Measurement of concentration

The nearly saturated concentration prepared by methods A and B was estimated by weighing the sample before and after the day's run, and relating the weight loss to the volume of air passing. This concentration, expressed in milligrammes per litre, was converted to parts per million on the assumption that the sample was pure. Both of these estimates are only approximate; this applies particularly to materials of low volatility and to impure samples when the more volatile fraction may evaporate first. For these reasons method D was usually preferred for such materials.

With the other methods the concentrations have usually been those calculated from the known rate of introduction of the substance into the air stream. In some experiments the concentration was checked by direct analysis of the atmosphere and was found to be within 10% of the expected value. A direct determination was always made when the method could not give a reliable indication of concentration, as with aerosols or organic peroxides. Concentrations determined by analysis are indicated by a superscript letter which refers to the following list.

a Gas-liquid chromatography. A Pye 104 instrument with a flame ionization detector was used. The column was 5 ft by [illegible] in filled with 60-80 mesh Celite coated with SE30. The temperature of the column was maintained at about 20% below the boiling point of the liquid. A sample of the atmosphere was injected directly into the N_2 carrier gas by means of a gas sampling valve (0·5-10 ml).

b Peroxides by iodometry. A measured volume of the air was passed through a 1% w/v aqueous potassium iodide solution, and the liberated iodine was measured absorptiometrically at 425 nm.

c Peroxides by oxidation of ferrous thiocyanate. A 50-ml sample of the air was collected in a large glass syringe containing 10 ml ferrous thiocyanate reagent (100 ml 0·5% w/v ammonium thiocyanate, 1 ml 6 N sulphuric acid, 0·1 g ferrous ammonium sulphate, 100 ml water prepared freshly each day). The syringe was shaken for 5 minutes and the ferric thiocyanate concentration was [illegible] absorptiometrically at 460 nm.

d Titration. A measured volume of the air was passed through water (sulphur dichloride) or 10% v/v aqueous ethanol (adipic acid), and the absorbed acid was titrated with sodium hydroxide solution.

e Methyl nitrite. A measured volume of the air was passed through 10 ml reagent (0·1 N chlorobenzene, 10 ml N HCl, acetic acid to 1 litre); 2 ml 1% aqueous *N*-(1-naphthyl)ethylenediamine dihydrochloride was added, and after 15 minutes the absorbance was measured at 550 nm.

Design of the experiments

Alderley Park specific-pathogen-free rats with an average weight of 200 g were used in most of these experiments. They were maintained in the exposure chamber for periods of up to 6 hours, and between repeated daily exposures they were returned to their cages where food and water were freely available. In the initial experiments the concentrations were selected to produce, if possible, acute effects after short exposures. Thereafter the exposure period was extended and the concentration lowered until the animals could survive 6-hour exposures, five days a week, for up to four weeks. With liquids, the vapour pressure limited the range of concentrations which could be tested in these acute and subacute experiments. The rats were weighed each morning, and their conditions and behaviour were recorded throughout the exposure period. Urine was collected overnight

2-t-Butoxyethanol, 5
s-Butylamine, 9
t-Butyl peracetate, 11
t-Butyl peroxypivalate, 11
n-Butyraldehyde, 6
Cetostearyl methacrylate, 8
Chloroacetonitrile, 10
6-Chlorododecafluorohexylsulphur pentafluoride, 14
2-Chloroethylsulphur pentafluoride, 13
1-Chloronaphthalene (technical), 8
4-Chloro-octafluorobutylsulphur pentafluoride, 13
Chloropentafluorobenzene, 7
1-Chloropropan-2-ol, 5
2-Chloropropane, 6
2-Chlorotetrafluoroethylsulphur pentafluoride, 13
Cumene α-hydroperoxide, 11
N-2-Cyanoethylaniline, 10
Decahydronaphthalene, 5
1,6-Diaminohexane, 9
Dichlorobutenes (mixed isomers), 7
1,1-Dichloroethene, 7
1,3-Dichlorotetrafluorobenzene, 7
α- Dicyclopentadiene, 4
1-Diethylaminopentan-2-one, 10
Diethylenetriamine, 10
00′-Diethyl phosphorochloridothio[illegible] 12
Dimethoxymethane, 5
2-Dimethylaminoethyl methacrylate, 8
3,6-Dimethyl-1,2-benzisoxazole, 11
Dimethyl carbonate, 9
Dimethyl disulphide, 13
3,5-Dimethylmorpholine, [illegible]
Dinonylamine, 9
Diphenyldimethoxysilane, 12
Dipropionyl peroxide, 11
Di-s-butylamine, 9
Divinyl disulphide, 13
Ditolyl disulphide, 13
Ethyl chloroformate, 9
Ethyl 3-chlorophenylformimidate, 14
2-Ethylhexyl acrylate, 8
2-Ethylhexyl methacrylate, 8
2-Ethyl-2-hydroxymethylpropane-1, 3-diol, 5
Ethyl t-butyl peroxyoxalate, 11
N-Formylpiperidine, 10
Glycol dimethacrylate, 8
Hexachlorobutadiene, 7
Hexafluorobenzene, 7
N,N-Hexamethylenedipamide, 14
2-Hydroxyethyl methacrylate, 8
2-Hydroxypropyl methacrylate, 8
4-Hydroxytetrahydropyran, 11
Iron pentacarbonyl, 14
Isobutoxyethene, 5
Isobutyraldehyde, 6
Iso-octanol, 5
2-Isopropoxyethanol, 5
Isopropyl chloroformate, 9
Lauryl mercaptan, 13
Lauryl methacrylate, 8
Methacrylic acid, 6
2-Methoxy-3,4-dihydropyran, 11
Methoxyethene, 5
2-Methylbenzoxazole, 10
2-Methylbuta-1,3-diene, 4
Methyl chloroformate, 8
2-Methyl-1,3-dioxolan, 10
Methyl isothiocyanate, 8
Methyl nitrite, 9
Methyl salicylate, 8
2-Methylthiazole, 10
Nonylamine, 9
Octyl methacrylate, 8
Pentachloropyridine, 10
Phosphorus tri-isocyanate, 12
Propionaldehyde, 6
n -Propyl cyanide, 10
N-Propylethylideneamine, 14
Silicon tetrafluoride, 12
Silicon tetraisocyanate, 12
Sulphur chloride pentafluoride, 13
Sulphur dichloride, 13
3a,4,7,7a-Tetrahydro- 4,7-methanoindene, 4
Tetramethylsilane, 12
Tributylamine, 9
Tributyl phospite, 12
1,2,4-Trichlorobenzene, 7
Trichloromethylsulphenyl chloride, 13
1,3,5-Trichlorotrifluorobenzene, 7
Trimethoxyboroxine, 14
1, 2, 4-Trimethylbenzene, 5
Trinonylamine, 9
Trispentafluoroethyldimethanol, 5
1, 1, 1-Trishydroxymethylpropane bicyclic phosphite, 12
Vinyl acetate, 8
Vinylsulphur pentafluoride, 13

Methods and materials

Samples investigated

The samples submitted by ICI Divisions were prepared in either the Research Department experimental plant, or were taken from full-scale production. The size of the sample available determined to some extent the scale of the experimental work. Few of the samples could be described as pure chemicals; they were not fractionated before use as the toxicological properties of the materials as supplied were relevant to these investigations. Where the sample was known to contain a considerable admixture of other components, this information is included in the Results section.

Design of exposure chambers

In all of these experiments the animals have been exposed to dynamic atmospheres, that is, to atmospheres continuously generated and passed through the exposure chamber. The design of exposure chamber has varied with the number of animals involved and with the nature of the substance under investigation. For groups of four or fewer rats a glass desiccator, containing wire mesh partitions to separate the animals, was used. Larger numbers, up to eight rats, were exposed in the chamber described elsewhere (Gage, 1959); usually the inner Perspex chamber of that design was replaced by a glass cylinder, 30 cm diameter and 25 cm high. For atmospheres containing particulate matter a chamber with a more pyramidal top (Gage, 1968) was used.

Gage- 70
Bib No. 20

Brith. J. of Indust. Med. 27:1-18. 1970.
Brit. J. Industr. Med., 1970, 27, 1-18

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The subacute inhalation toxicity of 109 industrial chemicals

J. C. GAGE
Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories, Alderley Park, Macclesfield, Cheshire

Gage, J. C. (1970). *Brit. J. industr. Med.*, 27, 1–18. **The subacute inhalation toxicity of 109 industrial chemicals.** The inhalation toxicity of 109 substances has been studied by exposing experimental animals to known concentrations in air for periods of about three weeks. The toxic properties of these substances are reviewed in relation to the effects of similar compounds on animals and on man. Provisional operational limits are suggested to assist in the design of new plant and in the establishment of codes for safe manufacturing practice.

Most serious occupational diseases arising from exposure to chemicals are caused by an attack on, or absorption through, the respiratory tract. On occasion such effects can be predicted from oral or parenteral administration of the chemical to experimental animals, but, in general, if a substance presents an inhalation risk, it is preferable to undertake a direct investigation by exposing animals to known concentrations in air. This survey covers the work on inhalation toxicity which has been undertaken over a period of 20 years in a laboratory engaged in the study of the toxic properties of chemicals used in industry.

All of the samples investigated for inhalation toxicity over this period were submitted by the manufacturing divisions of ICI Ltd.; they averaged about 30 a year. Not all of these have been included in this survey. Some were of too indeterminate a composition, such as still residues; some were proprietary products whose formulation was uncertain; for some the investigation was never completed for a variety of reasons; for a few, publication has been restricted for commercial or other considerations.

The aim of these investigations has been to provide information to aid in plant design and in the establishment of safety precautions to prevent occupational disease when the materials are produced or used in manufacturing operations. The experimental results have led to a decision on whether the compound may be handled without special precautions other than those demanded by sound manufacturing practice, whether exhaust ventilation should be installed or whether other features in plant design are required to prevent excessive exposure. The results have also enabled a prediction of the effects likely to be encountered in man from brief or repeated over-exposure, and have provided guidance on treatment to a works medical officer confronted with an accident or with a failure to apply the recommended safety precautions.

The compounds investigated are indexed below in alphabetical order.

1-Acetyl-γ-butyrolactone, 10
Acrylic acid, 6
Acrylyl chloride, 14
Adipic acid, 6
2-Aminobutan-1-ol, 10
2-Aminomethyl-3, 4-dihydropyran, 11
Bis-2-chloro-1-methylethyl ether, 6
Bis-2-ethoxyethyl ether, 6
2,2-Bis-*p*-hydroxyphenylpropane, 14
Bis-2-methoxyethyl ether, 6
Bis-3-methylbutyl peroxydicarbonate 11
Bispentafluorosulphur oxide, 13
(2-Bromoethoxy) benzene, 7
5-Bromopentan-2-one, 6

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UNTERSUCHUNGEN ÜBER DEN EINFLUSS DER DIACIDOGENEN FETTSÄUREN, C_6 BIS C_{11}, IHRER GLYCERIDE UND EINIGER NAHRUNGSFETTE AUF DIE OXALSÄUREAUSSCHEIDUNG BEIM MENSCHEN.

Von

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Durch die Arbeiten von VERKADE und LEE sowie von FLASCHENTRÄGER und Mitarbeitern ist außer der β-Oxydation der Fettsäuren (KNOOP) noch ein anderer Oxydationstyp, die sog. ω-Oxydation, bekannt geworden. Dieser Oxydationsform unterliegen vorwiegend die grad- und ungradzahligen Fettsäuren mit einer Kohlenstoffatomzahl zwischen 6—12. Bei der ω-Oxydation entstehen im intermediären Stoffwechsel Dicarbonsäuren, die, soweit sie einer weiteren Oxydation unterliegen, β-oxydiert werden. Ein Teil der entstehenden Dicarbonsäuren entgeht dem weiteren Abbau im intermediären Stoffwechsel und kommt im Urin zur Ausscheidung. Diese Dicarbonsäuren haben die gleiche C-Atomzahl wie die verfütterte Fettsäure oder wie die um 2 und 4 Kohlenstoffatome ärmere Säure. Die stärkste Diacidurie bewirken die Fettsäuren C_{10} und C_{11} (VERKADE).

Die Menge der im Urin zur Ausscheidung gelangenden Dicarbonsäuren ist im Vergleich zur verfütterten Fettsäuremenge gering. VERKADE konnte z. B. nach peroraler Applikation von 480 g Triundecylin (C_{11}) im Urin 19 g Nonandicarbonsäure (C_{11}), 0,37 g Azelainsäure (C_9) und 0,35 g Pimelinsäure (C^7) isolieren. Auch nach Untersuchungen von FLASCHENTRÄGER sowie neuerdings von EMMRICH werden die Dicarbonsäuren mit kürzerer C-Atomkette in wesentlich geringerer Menge ausgeschieden. Nach VERKADE ist die Adipinsäure (C_6) die niedrigste Dicarbonsäure, die als Stoffwechselprodukt der ω-Oxydation der Fettsäuren im Urin auftritt. Neuere Untersuchungen von HANSON, der Versuche am Menschen mit einem Fett durchführte, das in einem hohen Prozentsatz ungradzahlige Fettsäuren enthielt, ergaben auch eine erhebliche Ausscheidung von Bernsteinsäure im Urin, die vermutlich ebenfalls als Abbauprodukt der ω-Oxydation entstanden ist. VERKADE konnte dagegen bei seinen Versuchen keinen Anstieg der Bernsteinsäurefraktion im Urin feststellen.

Theoretisch ist gut vorstellbar, daß auch Oxalsäure als Dicarbonsäure mit niedrigster C-Atomzahl durch die ω-Oxydation in einem geringen Prozentsatz entstehen kann. In diesem Sinne sprechen auch Versuche von MORI an Kaninchen. Er beobachtete nach der Verfütterung von Adipinsäure (C_6) eine 3—4mal so hohe Ausscheidung von Oxalsäure im Urin. OHKAWA konnte jedoch diese Befunde bei einer späteren Nachuntersuchung nicht bestätigen. ANDERSEN, der auf Veranlassung von FLASCHENTRÄGER, Adipinsäure an Menschen und Hunden verfütterte, fand ebenfalls wie MORI eine wesentliche Vermehrung der Oxalsäure im Urin (3—4 bzw. 1,5 bis 3,5fach).

Bei der Aufarbeitung des Urins auf Dicarbonsäuren nach den Angaben von VERKADE wie auch von FLASCHENTRÄGER

A. Fettsäureglycerid		B. Ausgeschiedene Oxalsäuremengen: 1. Versuchsperson a. mg	2. Versuchsperson b. mg
1. Vorversuch:	1. Tag 3.	16,9	13,2
	2. „	15,5	11,3
2. Versuch: C_6	1. „	20,8	13,6
	2. „	16,7	12,6
	3. „	16,3	13,1
Vorversuch: Mittelwert 4.		16,2	12,3
Versuch: Mittelwert		17,9	13,1
Vorversuch:	1. Tag	15,4	10,6
	2. „	10,0	12,6
Versuch: C_7	1. „	11,4	14,2
	2. „	15,4	17,4
	3. „	13,2	14,8
Vorversuch: Mittelwert		12,7	11,6
Versuch: Mittelwert		13,3	15,5
Vorversuch:	1. Tag	12,9	8,9
	2. „	12,1	10,2
Versuch: C_8	1. „	12,2	9,5
	2. „	11,6	10,6
	3. „	12,4	9,1
Vorversuch: Mittelwert		12,5	9,6
Versuch: Mittelwert		12,1	9,7
Vorversuch:	1. Tag	12,6	17,2
	2. „	7,5	15,1
Versuch: C_9	1. „	11,6	10,2
	2. „	10,9	8,8
	3. „	8,7	12,6
Vorversuch: Mittelwert		10,1	16,2
Versuch: Mittelwert		10,4	10,5
Vorversuch:	1. Tag	9,0	14,6
	2. „	11,2	12,3
Versuch: C_{10}	1. „	22,1	8,8
	2. „	10,0	12,0
	3. „	11,7	10,7
Vorversuch: Mittelwert		10,1	13,5
Versuch: Mittelwert		14,6	10,5
Vorversuch:	1. Tag	9,3	13,5
	2. „	12,7	12,0
Versuch: C_{11}	1. „	7,9	10,9
	2. „	11,2	14,9
	3. „	12,5	10,3
Vorversuch: Mittelwert		11,0	13,1
Versuch: Mittelwert		10,5	12,0

Key:

A. fatty acid glyceride
B. excfeted amounts of oxalic acid
a. first test person
b. second test person
1. preliminary test
2. experiment
3. day
4. mean value

In the case of these tests as well, we could determine no effect of the various fats on the oxalic acid fraction could be determined. We can therefore omit the exposition of the individual analysis values. Likewise, we could determine no decrease in the amount of oxalic acid excreted upon higher fat intake. The decrease in oxalic acid excretion after large fat doses observed by other researchers is probably due to the thus caused decreased intake of other foods (vegetables, bread, meat, etc.) during abundant fat intake.

Summary: Oxalic acid excretion in man is not affected by the intake of diacidogenic fatty acids or their glycerides. The ordinary food fats, which usually contain a very small percentage of diacidogenic fatty acids, also cause no increase or decrease in oxalic acid excretion.

Literature

Literatur: ANDERSEN, Hoppe-Seylers Z. 159, 297 (1927). — DE SAYDRO, Pathologica (Genova) 6, 231 (1914). — EMMRICH u. EMMRICH-GLASER, Hoppe-Seylers Z. 266, 183 (1940). — EMMRICH u. NERZ, Hoppe-Seylers Z. 266, 174 (1940). — FLASCHENTRÄGER, Helvet. chim. Acta 18, 962 (1935). — FLASCHENTRÄGER u. BERNHARD, Hoppe-Seylers Z. 238, 221 (1936). — FÜRBRINGER, Dtsch. Arch. klin. Med. 143, 1876. — HANSON, Ernährung 6, 273 (1941). — HOCK, Ernährung 6, 278 (1941). — KLEMPERER u. TRITSCHLER, Z. klin. Med. 44, 337 (1901). — LÜTHJE, Z. klin. Med. 35, 271 (1893). — MACLEAN u. SALKOWSKI, Hoppe-Seylers Z. 60, 20 (1909). — MERZ u. MAUGERI, Hoppe-Seylers Z. 201, 31 (1931). — MORI, J. of biol. Chem. 35, 341 (1918). — OIKAWA, Jap. J. med. Sci., Trans II Biochem. 4, 77 (1938). — PICCININI u. LOMBARDI, Riforma med. 41, 726 (1925); 42, 867 (1926). — RAUBITSCHEK, Prag. med. Wschr. 1910, 283. — SALKOWSKI, Berl. klin. Wschr. 1900, Nr 20. — STRADOMSKY, Virchows Arch. 163, 404 (1901). — VERKADE u. a., Hoppe-Seylers Z. 225, 230 (1934); 237, 186 (1935); 227, 213 (1934). — WĘGRZYNOWSKI, Hoppe-Seylers Z. 83, 112 (1913).

with other methods. According to Wegrzynowski, the absolute values are also quite precise with this method. Merz and Maugeri do consider the ether extraction of the oxalic acid to be very incomplete. We attempted to restrict this error by intensively extracting the extraction product with sufficient acidification with hydrochloric acid, in order to limit the dissociation of the oxalic acid as much as possible. In some control determinations, no more oxalic acid could be found in the already de-etherized extract.

The production of the triglycerides of the fatty acids was accomplished a 12-hour boiling of the fatty acid-glycerine mixture in a reflux cooler in the presence of zinc as a catalyst. The substance obtained was washed out with a great deal of water and several times with a weak bicarbonate solution, in order to remove free glycerine, fatty acids, mono- and diglycerides.

The examinations were performed in the following manner: 2 healthy persons each received a standard diet for 4 days before the beginning of the experiment. The composition of this diet was as poor as possible in oxalic acid-containing foods and was extensively constant. This diet was also given during the 3 days of the experiment. Two days before the beginning of the experiment, the amount of oxalic acid excreted daily was determined. On the morning of the first experimental day, each test person received 50 g of the fatty acid or the glyceride through an intestinal tube. The urine was collected on the test days and for the following two days, and the oxalic acid was determined quantitatively. In the case of some sensitive test persons, vomiting or diarrhea appeared shortly after the fatty acid intake. These experiments were then repeated on other, less sensitive test persons. Only those experiments were evaluated, in which no stomach-intestine disorders appeared.

The results of the tests uniformly revealed that the oxalic acid fraction undergoes no changes as a result of administration of larger amounts of diacidogenic fatty acids or their glycerides, other than changes that lie within the individual range of variations or errors. In order to conserve space, the following table shows only the test results of the glyceride group of experiments. The analysis values of the tests with the free fatty acids generally correspond with these test results.

In the case of the constant diet, the oxalic acid excretion for most of the test persons was very near the average. Only in the case of a few test persons did the values vary outside the average range. These variations are probably determined by the individually different intestinal flora (oxalic acid-forming bacteria).

In the second test series, 2 test persons each received an additional 150 g butter, olive oil, pig lard or margarine along with the standard diet daily for 5 days, with an intermediate period of 2 days each between the individual test sections. Unfortunately, due to extraneous (time) circumstances, the 4 fats could not be given to the same test persons. Only 2 fats each were examined for the same test persons. Before the beginning of the experiment, the participants received a standard diet with the same amount of a mixed fat (butter and margarine) for 4 days. After this, only the corresponding test fat was given. The oxalic acid determination was done on the last two days of the preliminary test and on 4 days of the actual experiment.

a later investigation. Andersen, who at Flaschentrager's request fed men and dogs with adipic acid, found, like Mori, a significant increase in oxalic acid in the urine (3-4 or 1.5 to 3.5 times).

In the processing of the urine for dicarboxylic acids according to the data of Verkade and Flaschentrager, who extracted the acidified urine with ether and later crystallized the dicarboxylic acids in fractions out of the ether extract with organic solvents, the oxalic acid is lost, or can be obtained from the remaining oily residue of the extraction product only in small percentage.

Thus, in order to clarify this matter, it seemed important to us to process the oxalic acid fraction of the urine specially. This research is not only theoretically interesting, but also medically. Even if the usual food fats contain no or only a few diacidogenic fatty acids, coconut oil, for example, and to a lesser degree some plant oils, contain fatty acids with a C-atom count of 6, 8, 10, 12 and 14, which cause a slight diaciduria (Verkade). Thus recently Hock reported on a fat that contains abundant amounts of uneven count fatty acids with moderate C-atom count and that, according to Hanson, leads to an excretion of dicarboxylic acids in the urine in metabolic experiments on men. Thus, if the oxalic acid excretion in the urine were increased by the ω-oxidation of fatty acids, then the usefulness of a fat containing diacidogenic fatty acids as food would be limited considerably, or would be prohibited entirely.

There have been numerous detailed investigations concerning the metabolism of oxalic acid. The upper limit of the amount of oxalic acid excreted daily is 20 mg (Fürbinger). The exogenic oxalic acid portion comes from the oxalates of the food, or forms in the stomach-intestinal tract as a result of the activity of microorganisms (Bact. oxalatigenum, Bact. oxalagenes)from potatoes, vegetables, cereals, etc. (de Sandro, Piccinni and Lombard). In the case of a diet rich in fat, less oxalic acid is supposedly excreted in the urine than in the case of a diet rich in meat and carbohydrates (Klemperer, Lüthje, Mills, Raubitscheck, Stradomsky and others). Besides this, oxalic acid arising from the intermediate metabolism, formed endogenically, is also excreted in the urine. It supposedly originates from the collagenic substances (connective tissue), but above all from glycocoll and creatinine (Fürbinger, Klemperer and Tritschler, Lüthje, and others).

Our experiments were performed in two series. In the first experimental series, the fatty acids C_6-C_{11}, known to be diacidogenic, were examined as free fatty acids, and in a second group, the glycerine esters of the same fatty acids were examined individually for their effect on the oxalic acid fraction in men. In the second experimental series, the ordinary food fats (butter, pig lard, margarine and olive oil) were administered to men in large amounts and the oxalic acid excretion determined.

The quantitative determination of oxalic acid was done according to the method of MacLean and Salkowski. Processing for series examinations is actually somewhat protracted, but the relative comparative values agree well in the case of this method. Besides this, a larger quantity of urine (0.5 l) is processed, so that the determination errors are much smaller than they are in comparison

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RESEARCH ON THE EFFECT OF THE DIACIDOGENIC FATTY ACIDS, C_6 to C_{11}, THEIR GLYCERIDES AND SOME FOOD FATS ON OXALIC ACID EXCRETION IN MAN

by

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The works of Verkade and Lee, as well as Flaschentrager and collaborators have revealed, besides the β-oxidation of fatty acids (Knoop), yet another type of oxidation, the so-called ω-oxidation. This form of oxidation affects above all the even and uneven count fatty acids with a carbon atom count between 6 and 12. In ω-oxidation, dicarboxylic acids appear in the intermediate metabolism that are β-oxidized, insofar as they do undergo any further oxidation. Part of the appearing dicarboxylic acids does not go through further decomposition in the intermediate metabolism, and is excreted in the urine. These dicarboxylic acids have the same C-atom count as the fatty acids administered orally, or as the acids with 2 to 4 carbon atoms less. Fatty acids C_{10} and C_{11} cause the most marked diaciduria. (Verkade)

The amount of dicarboxylic acids excreted in the urine is small in comparison with the amount of fatty acids administered orally. For example, after oral administration of 480 g triundecylin (C_{11}), Verkade was able to isolate 19 g nonandicarboxylic acid (C_{11}), 0.37 g azelaic acid (C_9) and 0.35 g pimelic acid (C^7) in the urine. Likewise, according to research by Flaschentrager and recently Emmrich, the dicarboxylic acids with shorter C-atom chains are excreted in significantly smaller amounts. According to Verkade, adipic acid (C_7) is the lowest dicarboxylic acid that appears in the urine as a metabolic product of the ω-oxidation of fatty acids. New research by Hanson, who conducted experiments with men, using a fat that contained uneven count fatty acids in high percentage, also revealed a considerable excretion of succinic acid in the urine; this acid supposedly also formed as a decomposition product of ω-oxidation. On the other hand, in his experiments, Verkade could determine no increase in the succinic acid fraction in the urine.

Theoretically it can easily be imagined that oxalic acid, too, as a dicarboxylic acid with the lowest C-atom count can appear in small percentage through ω-oxidation. This is supported by mori's experiments with rabbits. After feeding of adipic acid (C_6), he observed 3 to 4 times greater an excretion of oxalic acid in the urine. However, Oikawa was not able to confirm these findings in

(4) Flaschenträger, B., *Z. physiol. Chem.* **159,** 297 (1926).
(5) Foulger, J. H., Haskell Laboratory of Industrial Toxicology, unpublished report, 1943.
(6) Gruber, C. M., Jr., Halbeisen, W. A., *J. Pharmacol. Exptl. Therap.* **94,** 65 (1948).
(7) Hanson, H., *Ernahrung* **6,** 273 (1941); *Chem. Zentr.* **1,** 2153 (1942).
(8) Harding, V. J., Nicholson, T. F., *J. Pharmacol.* **42,** 373 (1931).
(9) Litchfield, J. T., Jr., Wilcoxon, F., *J. Pharmacol. Exptl. Therap.* **96,** 99 (1949).
(10) Rose, W. C., *Ibid.*, **24,** 123 (1924).
(11) *Ibid.*, p. 147.
(12) Rose, W. C., Weber, C. J., Corley, R. C., Jackson, R. J., *J. Pharmacol.* **25,** 59 (1925).
(13) Simola, P. E., Kosunen, T., *Suomen Kemistilehti* **11B,** 22 (1938).
(14) Weitzel, C., *Ber. Verhandl. sachs. Akad. Wiss. Leipzig, Math. phys. Kl.* **93,** 9 (1942); *Chem. Zentr.* **2,** 556 (1942).

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Males. The average body weights for the male rats are tabulated in Table II for each 8-week interval. Throughout the entire 2-year study, the 0.1 and 1% adipic acid groups were comparable with the control groups. During the rapid growth period, the weight gains of the 3 and 5% adipic acid and the 3 and 5% citric acid groups were significantly less than the control groups; however, there was no significant difference among these four test groups. Throughout the latter half of the study, the average body weights of the various test groups were not remarkable—although the 5% adipic acid group was consistently the lowest.

Table III presents a summary of food and compound consumed and survival data for the entire 2-year feeding period. There was only a slight, but consistent, reduction in food consumption by the 5% adipic acid and 5% citric acid groups. Other test groups were comparable to the control group. The per cent survival for each test group was better than the control group.

Autopsy data for the male animals that died during the course of the 2-year feeding program and for the sacrificed rats were analyzed for incidence of tumors and/or lung pathology. To be included in the following table, a tumor must have presented gross evidence of being a new growth.

Male Group	Deaths: Lung pathology	Deaths: Tumors	Deaths: Other causes	Deaths: Total deaths	Sacrificed: Lung pathology	Sacrificed: Tumors
Control	7	3	3	12	4	1
Adipic						
0.1%	3	2	3	7	7	2
1%	1	2	2	5	7	2
3%	3	..	1	4	3	..
5%	..	4	1	5	4	..
Citric						
3%	1	2	3	6	1	..
5%	1	2	1	4	4	1

These findings appear not to be related to the compounds under study as an equivalent incidence was observed in the controls.

Throughout the study, especially the final 6 months, the following signs were observed among all the groups, including the controls: wheezing, blood-tinged crust about the noses and eyes, and body sores. The incidence of these findings did not appear to be significantly different among the groups although a lower incidence of signs indicative of respiratory infection and body sores occurred in the 5% adipic acid group.

When the surviving males were sacrificed at the end of the 2-year period, there was no significant gross pathology that could be related to either compound at least as frequently in the controls as in the experimental animals. There was no significant difference in organ weights of the experimental groups *vs.* the controls.

Microscopic examination of thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, bone marrow, and large and small intestines revealed these tissues to be within normal limits in all groups of male rats.

Females. The average body weights for the female rats are tabulated in Table II for each 8-week interval while food and compound consumption, together with survival data, are presented in Table III. There was no significant difference between the body weight gains or food consumption for the two groups.

In the last 6 months, the animals exhibited signs normally associated with advancing senility in rats. There was an equal incidence of blood-tinged crust about the eyes and noses, unthriftiness, and body sores in both groups. A few control and experimental animals had alopecia, and one experimental rat appeared to develop a middle ear infection during the 102nd week. The average weight of the kidneys, spleen, liver, and heart, together with organ-to-body weight ratios, appeared to be within normal limits.

One experimental and two control animals died during the final 6 months. All three exhibited diarrhea, respiratory infection, and loss of weight prior to death. Upon autopsy, one control rat and one experimental rat were found to have tumors, while the other control animal had a granular liver and dark red apexes on both lungs.

When the surviving animals were sacrificed at the end of the 2-year period, there was no significant gross pathology that could be related to ingestion of the compound. There was an equal incidence of mottled, granular livers with peripheral thickening in both the control and experimental animals. Two of the surviving control rats and one experimental animal had ovarian tumors; ovarian cysts were noted in both control and experimental rats.

The results of the above experiments indicate that adipic acid is significantly less toxic than tartaric or citric acid following intravenous administration to mice. The doses were calculated as milligrams per kg. and as millimoles per kg. The action of these three acids appears comparable.

No direct comparison of the intravenous toxicity of citric acid in these tests and those reported by Gruber and Halbeisen (*5*) is possible because they used a faster rate of injection. The LD_{50} values of 1.06 millimoles per kg. obtained in this experiment is midway between the LD_{50} and the LD_{100} of Gruber and Halbeisen.

Single oral administrations of an almost saturated solution (*3%*) of adipic acid did not cause appreciable mortality in tolerable volumes. With a 6% suspension, the LD_{50} approximated 2 grams per kg. Comparable values for citric and tartaric acids are not available.

Following intraperitoneal administration to rats, adipic acid appears to be more toxic than citric (*6*). The intraperitoneal administration of adipic acid resulted in extensive irritation and adhesion of visceral organs.

During the rapid growth period of the 2-year feeding studies, weight gains for the male rats receiving 3 or 5% adipic or citric acid was significantly less than the male controls; however, there was no significant difference among these four experimental groups. Growth for other groups—0.1 and 1.0% male and 1.0% female—was comparable to that of the respective controls. There was no evidence of gross pathology associated with the feeding of either acid. There was no significant difference in survival among the various groups from the controls. The incidence of lung pathology, tumors, or soft testes was observed at least as frequently in the controls. The organ-to-body weight ratios appeared to be within normal range. The results of microscopic examination appeared to be within normal limits for the representative tissues studied.

Comparison of the chronic feeding of adipic acid with citric acid (herein reported) and also with tartaric acid in an equivalent study (*3*) indicates that adipic acid is comparable with citric and tartaric acids.

Literature Cited

(1) Corley, R. C., Rose, W. C., *J. Pharmacol.* **27**, 165 (1926).

(2) Enders, A., *Arch. exptl. Pathol. Pharmakol.* **197**, 706 (1941); *Chem. Zentr.* **1**, 2554 (1942).

(3) Fitzhugh, O. G., Nelson, A. A., *J. Am. Pharm. Assoc., Sc. Ed.* **36**(7), 217 (1947).

Table I. Acute Toxicity of Adipic, Citric, or Tartaric Acid to Male Albino Mice or Rats

(Dosages are as the acid. Values are the number of animals dead per number of animals tested)

Dose, Mg./Kg.	Adipic: Oral[a], mice	Adipic: Intraperitoneal[b], rats	Adipic: Intravenous[c], mice	Citric, Intravenous[c], Mice	Tartaric, Intravenous[c], Mice
175				2/13	
200		1/7		6/13	0/3
225				10/13	
250				3/3	
300		4/7			
350		6/7			
400					0/2
450					1/13
475					2/13
500					9/13
650			4/13		
675			7/13		
700			8/13		
1500	3/13				
2000	8/13				
2500	9/13				
LD_{50}, mg./kg.	1900	275	680	203	485
Confidence limits, mg./kg.	1640–2200	193–392	653–708	190–217	462–509
LD_{50}, millimoles/kg.	13.0	1.88	4.65	1.07	3.23

[a] 6% suspension in 0.5% methyl cellulose.
[b] 3% aqueous solution.
[c] 2% aqueous solution.

Table II. Summary of Average Body Weights of Albino Rats

(Controls received the basal diet. Other animals received the basal diet containing the indicated percentage of the adipic acid or citric acid)

	Average Body Weight in Grams								
	Males							Females	
		Adipic Acid				Citric Acid			Adipic acid,
Week	Control	0.1%	1%	3%	5%	3%	5%	Control	1%
0	59	61	63	61	57	62	61	49	48
8	269	280	265	224	182	239	225	178	175
16	325	333	320	276	233	298	278	222	213
24	361	374	354	309	264	329	320	242	233
32	377	391	376	329	291	328	339	257	249
40	397	407	401	357	314	370	361	279	263
48	423	433	421	372	322	393	377	275	270
56	428	447	436	380	336	400	388	286	277
64	426	455	436	385	339	407	401	295	284
72	407	447	431	385	336	400	389	301	288
80	408	441	430	383	349	411	391	313	301
88	413	448	432	398	344	411	389	309	303
96	432	424	436	396	354	409	393	318	308
104	440	417	437	400	360	417	397	321	304

Table III. Summary of Data for Albino Rats Receiving Basal Laboratory Diet or Basal Diet of Adipic or Citric Acid for 2 Years

(Per cent of survival based on length of survival as well as number of animals)

Level	Sex	No. of Rats: Start	No. of Rats: Finish	Av. Body Weight, G.: Initial	Av. Body Weight, G.: Final	Food Consumed, G., Av./Rat/Day	Compound Consumed, Mg., Av./Rat/Day	Survival, %
Control	M	20	8	59	440	16.8		82.5
	F	10	8	49	321	14.2		98.9
Adipic acid								
0.1%	M	20	13	61	417	17.0	17.0	87.7
1%	M	20	15	63	437	17.5	175	94.7
	F	19	17	48	304	15.8	158	96.3
3%	M	20	16	61	400	16.8	505	94.5
5%	M	20	15	57	360	15.8	814	97.2
Citric acid								
3%	M	20	14	62	417	17.1	512	92.6
5%	M	20	16	61	397	15.7	784	95.0

values of 680, 203, and 485 mg. per kg. respectively (4.65, 1.04, and 3.23 millimoles per kg.). The results of these experiments are presented in Table I. These acids caused immediate, convulsive deaths, probably due to acidosis as the pH of the solutions was 3.05, 2.50, and 2.53, respectively. Autopsy showed hemorrhagic lungs but no other gross pathology. In survivors, recovery was apparently complete and there were no latent deaths. Statistical analysis was done by the method of Litchfield and Wilcoxon (*9*).

Chronic Feeding. Young male and female albino rats of the Carworth Farms strain, having approximate mean initial weights of 60 and 50 grams, respectively, were selected at random for use in these studies. All of the rats were housed individually in cages with wire mesh floors elevated above the droppings. The animals had free access to food and water at all times.

Groups of rats were placed on either the basal laboratory diet or the basal diet containing either adipic acid or citric acid, as follows:

Group	Males	Females
Basal laboratory diet used as control	20	10
Basal diet containing 0.1% adipic acid	20	0
Basal diet containing 1% adipic acid	20	19
Basal diet containing 3% adipic acid	20	0
Basal diet containing 5% adipic acid	20	0
Basal diet containing 3% citric acid	20	0
Basal diet containing 5% citric acid	20	0

The body weights and food consumption of all rats were recorded at weekly intervals during the course of the study. In addition, weekly observations were made of the general appearance and condition of each animal. Whenever possible, gross autopsy was performed on those animals that died during the course of the experiment.

After 2 years on the respective diets, the surviving rats were weighed, sacrificed by a blow on the head, and examined for gross and microscopic pathology. The brain, thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, and testes of approximately half of each group of males were weighed. The kidneys, spleen, liver, and heart of each female were weighed. Microscopic examination of the following tissues were done on a representative number of animals of each group: thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, small intestine, large intestine, pancreas, bone marrow, testes or ovaries, and uterus.

Safety of Adipic Acid as Compared with Citric and Tartaric Acid

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Increased usage of adipic acid as a food additive has prompted the comparison of it with citric and tartaric acids. Acute and chronic administration to laboratory animals has shown that adipic acid is comparable to these acids and is a safe food additive.

ADIPIC ACID (1,4-butanedicarboxylic acid), citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid), and tartaric acid (1,2-hydroxy-1,2-ethanedicarboxylic acid) are straight chain organic acids of 6, 5, and 4 carbon atoms, respectively. Adipic acid has no substituted groups, citric acid has both a hydroxy and carboxylic group substituted on the second carbon, while tartaric acid has a substituted hydroxy group on both the first and second carbons. Of these acids, only adipic is nonhygroscopic. Because of the increased interest in their use as food additives, the following work was undertaken.

Review of Available Literature

Rose (*10, 11*) is responsible for several investigations in the course of which he found that, following subcutaneous administration, adipic acid was mildly irritating to the kidneys, while glutaric acid (1,3-propanedicarboxylic acid) was nephrotoxic. In 1925, Rose and coworkers (*12*) concluded from their investigations that none of the higher homologs were irritating to the kidneys. Corley and Rose (*1*) examined 19 different acids for nephrotoxicity but found that only three—tartaric, mucic, and glutaric—exerted pronounced toxicity. Mucic acid is tetrahydroxyadipic acid. These authors concluded that: The number of carbon atoms, per se, present in a dicarboxylic acid has no relation to its toxicity; the introduction of a hydroxy or ketonic group on the first carbon of glutaric acid destroys its nephrotoxic effects; and the introduction of a hydroxy or ketonic group on the first carbon of adipic acid does not influence the toxicity.

Somewhat later, Harding and Nicholson (*8*) directed studies toward evaluating this apparent discrepancy in toxicity in acids which possessed rather similar properties. Their studies indicated that following either subcutaneous or intramuscular administration, glutaric acid was readily absorbed with a minimum of local reaction, whereas adipic acid caused marked local reaction at the site of injection and higher homologs of the series could be definitely identified as retained crystals at the site. Based on these observations, at least a portion of the difference in renal toxicity might be due to poor absorption of adipic acid and the higher homologs; their work confirmed the nephrotoxicity of glutaric acid. However, following subcutaneous administration, Flaschenträger (*4*) recovered approximately 50% of administered adipic acid in the urine.

In 1942, Enders (*2*) reported that adipic acid, azelaic acid (1,7-heptane dicarboxylic acid), and sebacic acid (1,8-octanedicarboxylic acid) are only slightly toxic when given in large single oral doses to rabbits, or when fed daily to rats over a long period of time. Excretion of these acids in the urine of rats showed adipic acid to be more slowly excreted than the others.

Simola and Kosunen (*13*) fed the sodium salts of a series of organic acids in single doses to adult rats and analyzed the urine for increased citric and ketonic acid excretion. All of the acids studied increased both the citric and ketonic acids, but the increase was slight with adipic acid.

Both Hanson (*7*) and Weitzel (*14*), in their studies on the urinary recovery of orally administered adipic acid to humans, concluded that decomposition took place in the body with small amounts being more completely catabolized than large amounts.

An unpublished report by Foulger (*5*) is of particular interest from a practical standpoint as it presents the results following the repeated administration of relatively large doses of adipic acid. Immature rats failed to gain weight properly when given 638 to 1332 mg. per kg. Fitzhugh and Nelson (*3*) reported 2-year rat feeding experiments on several acids, including tartaric acid, which they found was not toxic in concentrations up to 1.2% of the diet.

More recently, Gruber and Halbeisen (*6*) reported that, following intraperitoneal administration to rats, adipic acid appears to be more toxic than citric. They reported deaths from citric acid up to 1 week but could not associate this with postmortem findings. The intraperitoneal administration of adipic acid resulted in extensive irritation and adhesion of visceral organs. A rapid intravenous injection of citric acid in mice resulted in an LD_{50} of 0.22 millimoles per kg. When the injection was made at the rate of 1.5 millimoles of acid per minute until the animals died, the average LD_{100} was 2.08 millimoles per kg.

Experimental

Acute Oral Administration. Male albino mice were used in this study. A 3% aqueous solution of adipic acid, kept at body temperature, was tried but proved impractical as sufficiently large doses to determine an LD_{50} could not be administered. Therefore, a 6% suspension of adipic acid in 0.5% methyl cellulose was administered orally, resulting in an LD_{50} of 1900 mg. per kg. or 13.0 millimoles per kg. (Table I). Autopsy of the animals that died showed distention of the stomach and small intestine, with a spastic concentration of the caecum. Irritation and hemorrhage of the intestines were noted. Initial mortality developed overnight and deaths continued throughout the first week, after which survivors appeared normal. All animals were sacrificed after 10 days.

Acute Intraperitoneal Administration. A few mice were given lethal doses (600 and 900 mg. per kg.) of a 3% aqueous solution of adipic acid intraperitoneally. These mice showed depression immediately and, at autopsy, the intestines appeared irritated and the lungs appeared hemorrhagic.

Male albino rats were given a 3% aqueous solution of adipic acid intraperitoneally (Table I). Mortality occurred during the first 5 days. The LD_{50} was 275 mg. per kg. (1.88 millimoles per kg.). Animals that succumbed showed hemorrhagic lungs and irritation of the intestines. The survivors, sacrificed 1 week after administration, showed extensive irritation and adhesions of the visceral organs.

Acute Intravenous Administration. Intravenous injection to mice at various dosage levels, at a rate of 0.01 ml. per second, with 2% solutions of adipic,

[1] Deceased.

Sodium suberate: Kidneys. Rabbit 5064. A few areas of fibrous connective tissue proliferation and lymphocyte infiltration were seen around a few of the glomeruli and the surrounding tubules. Remainder of kidney, normal.

Rabbit 656 and 657. No abnormalities noted.

Site of injection: Rabbit 5064. There was a raised nodule at the site of injection with capillary engorgement. The surrounding muscle was entirely destroyed, and was replaced by a necrotic mass infiltrated with a number of polymorphonuclear leukocytes. Surrounding the area of necrosis was an area of inflammatory reaction, in which the muscle cells had disappeared and been replaced by young granulation tissue, heavily infiltrated by an endothelial cells and polymorphonuclear leukocytes. Scattered through the area of necrosis in the endothelial cells, and between many of the less severely damaged muscle fibers were numerous fine granules which stained a muddy red with Scharlach R. (Granules of suberic acid embedded in gelatin and stained with Scharlach R had the same muddy red appearance.)

Rabbit 656 and 657. As rabbit 5064 except that rabbit 657 showed an abscess of about 3 mm. diameter at inoculation point. The abscess was sterile.

REFERENCES

(1) Rose, W. C.: Jour. Pharmacol. and Exper. Therap., 1924, xxiv, 123; 147.
Rose, W. C., Weber, C. J., Corley, R. C., and Jackson, R. W.: Jour. Pharmacol. and Exper. Therap., 1925, xxv, 59.

(2) Umeda, N., and Ringer, A. I.: Proc. Soc. Exper. Biol. and Med., 1916, xiv, 33.

(3) Wilenko, G. G.: Deut. Med. Woch., 1908, xxxvi, 1897.

c. Results. The examination of the urine for albumin and casts will be found reported in table 1. Table 2 shows the non-protein nitrogen determinations on the same animals. Urea determinations were also made on the blood samples. The results of these determinations ran parallel to the non-protein nitrogen results and are not reported for the sake of brevity.

d. Autopsy reports:

Controls: Kidneys. Rabbit 150. No gross or microscopic abnormality.

Rabbit 97. No gross or microscopic abnormality.

Rabbit 495. Glomeruli normal. A few cells in some of the convoluted tubules showed slight granular degeneration.

Sodium succinate: Kidneys. Rabbit 597. A few tubules showed cells with swelling and coarse granulation.

Site of injection: Rabbit 597. Gross examination; slight engorgement of blood vessels but no swelling. Microscopic examination showed slight haemorrhage with leukocytosis between some of the muscle fibres.

Sodium glutarate: Kidneys. Rabbit 151. Normal in gross, a few convoluted tubules showed granular degeneration.

Rabbit 165. Convoluted tubules showed marked granular degeneration. Cytoplasm swollen, coarsely granular and intensely eosinophilic. Some of the cells were disintegrating, and being desquamated into lumina of tubules. Glomeruli appeared normal.

Rabbit 497. Convoluted tubules showed very extensive necrosis with marked calcareous deposits throughout. Glomeruli appeared normal.

Site of injection: Rabbit 414. Bland necrosis of muscle fibers extending for short distance around site of injection. Slight infiltration of polymorphonuclear leukocytes in area.

Rabbit 639. Slight engorgement of blood vessels. No swelling.

Sodium adipate: Kidneys. Rabbit 628. Normal.

Site of injection: Rabbit 628. Marked diffuse swelling. Blood vessels markedly engorged with marked necrotic area infiltrated by endothelial cells and leukocytes.

Rabbit 412. Marked swelling and distinct reddening of tissues. Marked necrotic area infiltrated by endothelial cells and leukocytes.

Rabbit 522. Similar to above.

observed a temporary nephrotoxic action of this acid. The amount which reaches the kidneys, however, must be small, and we certainly attribute this partly to its localization at the site of injection and partly, as Rose suggests, to its possible rapid oxidation.

We feel then that the unique position of glutaric acid as a nephrotoxic agent to the rabbit is rather apparent than real; that the dicarboxylic acids as a series are all fundamentally nephrotoxic, but that when administered intramuscularly or subcutaneously, localizing reactions, increasing as one ascends the homologous series limit the amount which reaches the kidney.

EXPERIMENTAL

The description of our experimental procedure is as follows:

a. Dicarboxylic acids and sodium salts:

Succinic acid: Eastman Kodak Company pure product, recrystallized twice from water.

Glutaric acid: Eastman Kodak Company pure product, recrystallized twice from xylene.

Adipic acid: Eastman Kodak Company pure product, recrystallized twice from 95 per cent alcohol.

Suberic acid: Schuchardt pure product, recrystallized three times from distilled water.

Sodium salts: The sodium salts were made by adding to the acid the quantity of 2N NaOH required to produce a di-sodium salt. 2.2 grams of sodium glutarate were used in each case and the other acids were used so that the same proportion of their gram molecular weight was injected.

b. Animals. Young male rabbits fresh from the breeder were used. In no case were they kept in the stock cages a week before using; generally only three days elapsed. This minimised the occurrence of spontaneous nephritis among our animals. Urine samples were obtained by catheter; blood samples were taken by cardio-puncture.

One day's preliminary fasting preceded the injection of the sodium salt, which fasting was continued throughout the period of observation.

might interfere with the exhibition of their nephrotoxic action, one instinctively turns to the physical properties. The higher members of the series resembles the higher members of the fatty acids, becoming insoluble in water, and soluble in fat solvents; their sodium salts resemble soaps rather than simple solutions in water. The cytolitic action of soaps is well known, and it consequently became a possibility that any action produced by the higher sodium dicarboxylates might be locally confined, and the failure to observe nephropathic action due to the non-absorption of the acid ion. We, consequently, examined the site of injection. In the case of suberic acid our supposition evidently turned out to be correct. In all 3 animals examined there was an area of necrosis and intense inflammation. In one of them a definite sterile abscess was present. In all of them were to be observed on microscopic examination, fine granules scattered throughout the necrotic area, sometimes scattered between the muscle cells and in some places being engulfed by endothelial cells. These granules stained red with Scarlach R. Under the circumstances, as suberic acid adsorbs Scarlach R, the granules would appear to be suberic acid, or as is probable in a necrotic area—calcium suberate. The suberic acid has evidently not been absorbed, and its intensely necrotic action has been locally confined. The sites of injection after sodium succinate or sodium glutarate showed no such reaction. Such slight reaction as was present might be well ascribed to the local damage produced by the needle itself and the pressure of the entering solution on the surrounding cells.

The injection of sodium adipate produced a slight local swelling with a marked necrotic area. Neither the necrosis nor the inflammation was as marked as after sodium suberate but it was much more evident than after sodium succinate or sodium glutarate. It is not possible in the case of this acid to observe local evidence of its retention as in the case of suberic acid, but the presence of a necrosis and an inflammatory reaction is taken as an attempt to produce localization of the injury if possible. Undoubtedly, in some cases, a portion of the adipic acid is absorbed and reaches the kidneys. We have stated that Rose

so toxic an agent as the latter and in general we believe our results show it to be perhaps, an even less toxic agent than Rose imagined. The resistance of the animals evidently varied, however, and any difference detectable between our results and those of Rose may readily be ascribed to that cause.

We can also support Rose in his observations on the apparent harmlessness of sodium adipate. Here again our results might lead to the conclusion that this acid was even less toxic than Rose had supposed. Rose claimed a mild nephrotoxic action; our results showed only a mild renal irritation in 2 animals out of 13. In any case it is evident that judged by results on the renal tubular epithelium, glutaric acid appears sharply differentiated from adipic acid. As suberic acid in our hands also showed no severe nephrotoxic action in 4 animals, Rose's general conclusion that glutaric acid is different from its higher homologues appears confirmed.

The evidence by which glutaric acid is separated from its lower homologues, however, does not appear so conclusive. Our own results with succinic acid show that the difference between that acid and glutaric acid is one of degree, and not one of kind. That Rose failed to find any nephrotoxic acid in sodium succinate is due, most probably, to his reliance on blood analyses alone. That malonic acid showed no action on the kidney is not surprising. The instability of sodium malonate is such as to render it unlikely that much of the injected material reached the kidney in that form. It would be almost equivalent to giving an injection of sodium acetate. The position occupied by glutaric acid might then appear as a point in a progressive increase in nephrotoxic properties, rather than as a unique example, and the failure to observe similar properties in the higher members, might be due to the development of new properties with increasing molecular weight. Such a view is strengthened by Rose's claim that malic acid is slightly nephrotoxic, raising the non-protein nitrogen and decreasing the phenolsulphonephthalein excretion and thus linking the mild renal irritant—succinic acid with the more intensely nephropathic agent—tartaric acid.

In search of properties of the higher dicarboxylic acids which

TABLE 2

Showing non-protein nitrogen in rabbits after intramuscular injection of sodium salt of dicarboxylic acid

SODIUM SALTS	NON-PROTEIN NITROGEN						RABBIT NUMBER
	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	
Controls	—	—	22	48	50	48	150
	37	32	40	44	39		97
	—	46	62	58			495
	27	37	62	76	60	60	477
Succinic acid	27	43	47	42	47	52	476
	26	35	64	48	53	50	478
	—	65	60	65	67		490
	27	63	65	50			320
	—	25	49	57	50		579
Glutaric acid	—	49	45	75	73		151
	—	27	37	42	41		414
	37	46	67	63			639
	—	42	82	64	51	71	496
	27	44	42	43			415
	—	46	75	60	50	54	148
	—	45	80	62	46	57	147
	32	50	98	86			165
	—	60	66	72	88		494
	27	46	113	70	57	64	149
	44	103	112	122	133		497
Adipic acid	—	39	39	40			5065
	—	39	32	32			5171
	—	35	41	39			5175
	25	48	48	49			416
	29	48	48	48			417
	—	27	29	38	40		626
	—	30	37	46	46		627
	20	26	40	46	55		640
	25	22	33	39	45		646
	—	25	32	37	37		412
	—	23	35	38	39		522
	—	25	33	41	44		628
Suberic acid	—	31	38	40			5194
	—	30	35	39	45		5064
	—	33	37	32			656
	—	33	43	44	—		657

TABLE 1

Showing presence or absence of albuminuria and casts in rabbits after intramuscular injection of sodium salt of dicarboxylic acid

Na salts	Albuminuria						Casts when present days shown in parentheses	Rabbit number
	First day	Second day	Third day	Fourth day	Fifth day	Sixth day		
Control	–	–	–	–	–	–	None	150
	–	–	–	–	–		None	97
	–	–	–	–			None	495
	–	–	–	++	+++		Present (4, 5)	477
Succinic acid	–	–	+	++	++	–	Present (3, 4)	476
	–	+	++	++	+	+	Present (3, 4, 5)	478
	–	+	+	+	+		Present (3, 4, 5)	490
	–	–	–	–			None	320
	–	+	+	+			Present (2, 3, 4)	579
Glutaric acid	–	–	–	–	–	–	None	151
	–	–	–	–	–	–	None	414
	–	–	+	+			Present (3, 4)	639
	–	–	+	+	+	+	Present (3, 4, 5, 6)	496
	–	+	+	+			Present (2, 3, 4)	415
	–	–	+	+	+	–	Present (3, 4, 5, 6)	148
	–	+	+	+	+	–	Present (2, 3, 4, 5)	147
	–	+	++	++			Present (3, 4)	165
	–	–	++	++	++		Present (3, 4, 5)	494
	–	+	+++	++	++		Abundant (3, 4, 5)	149
	–	+++	+++	+++	*	†	Abundant (2, 3, 4)	497
Adipic acid	–	–	–	–			None	5065
	–	–	+	+			Present (4)	5171
	–	–	–	–			None	5175
	–	–	–	–			None	416
	–	–	–	–			None	417
	–	–	–	–	–	–	None	626
	–	–	–	–	–	–	None	627
	–	–	+	+	+	+	Present (4, 5)	540
	–	–	–	–	–	–	None	646
	–	–	–	–	–	–	None	412
	–	–	–	–	–	–	None	522
	–	–	–	–	–	–	None	628
Suberic acid	–	+	–	–			None	5194
	–	–	–	–	–	–	None	5064
	–	–	–	–			Present (3, 4)	656
	–	–	+++	+++			Abundant (3, 4)	657

* Anuria.
† Death.

nephropathic action of oxalic and tartaric acids was due to their precipitation as relatively insoluble calcium salts in the kidney tubules.

So sharp a divorcement between chemical constitution and physiological activity invited re-investigation. We, consequently, re-examined the action of succinic, glutaric, adipic and suberic acids on the rabbit. The sodium salt was administered by intramuscular injection, rather than subcutaneous. Due precautions were observed on the sterility of the solutions injected, and on the condition of the rabbit previous to the experiment in order to minimize the risk of nephritis occurring from causes other than the dicarboxylic acid.

The experimental details are given in the latter portion of the paper. The effect of the injection on the presence or absence of albumin and casts, and on the non-protein nitrogen is shown in tables 1 and 2 respectively. We also performed post-mortem examinations.

The general results can be recorded as follows:

a. Sodium succinate acts as a mild renal irritant, producing albuminuria and cylindruria in the majority of rabbits. Its effect, however, is not so marked as to produce rises in the non-protein nitrogen sufficiently great to differentiate these animals from the control group.

b. Sodium glutarate is also a renal irritant, though a few animals appear resistant to its action. A few, however, appear much more susceptible and in these animals one observes a rise in the non-protein nitrogen sometimes followed by recession but sometimes the pathological process continues to anuria and death.

c. Sodium adipate appears innocuous. There is no more albuminuria than could be accounted for by the occurrence of the "spontaneous nephritis" in rabbits. The non-protein nitrogen results appear at an almost lower level than in the control animals.

d. Sodium suberate showed renal irritation in one animal out of three, without any rise in the non-protein nitrogen.

The results with sodium glutarate confirm those of Wilenko (3) and Rose. It behaves as a moderately strong nephrotoxic agent, producing a tubular degenerative nephritis similar to that produced by sodium tartarate. It would appear, however, not to be

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THE NEPHROPATHIC ACTION OF DICARBOXYLIC ACIDS ON RABBITS

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Some four to six years ago Rose and his collaborators (1) studied the action of the series of dicarboxylic acids, from malonic to azelaic, on the kidney of the rabbit. The acids were given by subcutaneous injection as a solution of the sodium salt. The effect on the kidney was gauged by the increase in the non-protein nitrogen of the blood and by the decrease in the output of phenolsulphonephthalein. Their results showed that glutaric acid occupied an anomalous position in the homologous series of dicarboxylic acids. The two acids immediately below it in the series—malonic and succinic—possessed no action on the kidney, while those immediately above—adipic, pimelic, suberic and azelaic—though producing a temporary interference with renal excretion, did not compare with glutaric acid in intensity of toxic action. "The data serve to emphasize the fact . . . . that glutaric acid manifests an unique behavior in the animal organism. Except for oxalic acid it is decidedly the most toxic member of the homologous series."

Such a position claimed for glutaric acid as a nephrotoxic agent, seems totally unwarranted by its ordinary chemical behaviour. By no series of chemical observations is it sharply differentiated from its immediate neighbours. It appears closely related to succinic acid on the one hand, and to adipic acid on the other. Rose himself showed that its calcium salt showed no relationship in solubility to calcium oxalate, but resembled calcium succinate and calcium adipate. This he did, because of the hypothesis of Umeda and Ringer (2) who stated that the

TABLE 1 (continued)

Trichloromethylsulphenyl chloride ..	0·1
Sulphur chloride pentafluoride ..	0·5
Bispentafluorosulphur oxide	0·5
Dimethyl disulphide	5
Divinyl disulphide	2
Vinyl sulphur pentafluoride	20
2-Chloroethyl sulphur pentafluoride	20
2-Chlorotetrafluoroethyl sulphur pentafluoride	20
4-Chlorooctafluorobutyl sulphur pentafluoride	100
6-Chlorododecafluorohexyl sulphur pentafluoride	250
Acrylyl chloride	0·1
Ethyl 3-chlorophenylformimidate ..	5
Ethylidene propylimine	2
Iron pentacarbonyl	2

TABLE 2

SOVIET RECOMMENDED MAXIMAL ALLOWABLE CONCENTRATIONS

	ppm	
Isoprene	17	Korbakova and Fedorova (1964)
Cyclopentadiene	2	Uloyan (1965)
Methacrylic acid	0·003	Stulova, Rumyantseva, and Ivanova (1962)
Butyraldehyde	0·3	Korbakova (1964)
Diaminohexane	0·0002	Kulakov (1967)
Cumene hydroperoxide	0·001	Solomin (1966)

References

American Conference of Governmental Industrial Hygienists (1966). Documentation of threshold limit values.

—— (1968). Threshold limit values for air-borne contaminants.

American Petroleum Institute (1948). API Toxicological Reviews, β,β'-Dichloroethyl ether.

Bättig, K., Grandjean, E., Rossi, L., and Rickenbacher, J. (1958). Toxikologische Untersuchungen über Trimethylbenzol. *Arch. Gewerbepath. Gewerbehyg.*, 16, 555-566.

Byers, P. D., and Gage, J. C. (1961). The toxicity of precipitated silica. *Brit. J. Industr. Med.*, 18, 295-302.

Carpenter, C. P., Pozzani, U. C., Weil, C. S., Nair, J. H., Keck, G. A., and Smyth, H. F. Jr. (1956). The toxicity of butyl cellosolve solvent. *Arch. Industr. Hlth*, 14, 114-131.

Carpenter, C. P., Smyth, H. F. Jr., and Pozzani, U. C. (1949). The assay of acute vapor toxicity, and the grading and interpretation of results on 96 chemical compounds. *J. Industr. Hyg.*, 31, 343-346.

Floyd, E. P., and Stokinger, H. E. (1958). Toxicity studies of certain organic peroxides and hydroperoxides. *Amer. Industr. Hyg. Ass. J.*, 19, 205-212.

Gage, J. C. (1953). A controlled fluid-feed atomizer. *J. sci. Instrum.*, 30, 25.

—— (1959). The toxicity of epichlorohydrin vapour. *Brit. J. Industr. Med.*, 16, 11-14.

—— (1961). Toxicity of paraquat and diquat aerosols generated by a size-selective cyclone: effect of particle size distribution. *Ibid.*, 18, 304-314.

Garmer, N. L., and Leigh, J. M. (1967). Some effects of hexafluorobenzene in cats. *Brit. J. Pharmacol.*, 31, 345-350.

Goldblatt, M. W., and Chisman, W. E. (1944). Toxic effects of ethylene chlorohydrin. *Brit. J. Industr. Med.*, 1, 207-223.

Hemker, H. C. (1962). Lipid solubility as a factor influencing the activity of uncoupling phenols. *Biochim. Biophys. Acta*, 53, 46.

Kapkaev, A. (1957). Characteristics of Halowax as an industrial poison. Quoted from *Chem. Abstr.*, 1958, 52, 14001a.

Korbakova, A. I. (1964). Standard levels of new industrial chemicals in the air of work premises. Quoted from *Chem. Abstr.*, 1964, 61, 16694b.

——, and Fedorova, V. I. (1964). Toxicology of isoprene. Quoted from *Chem. Abstr.*, 1965, 63, 17017d.

Kulakov, A. E. (1967). Permissible concentration of hexamethylenediamine in the air in populated areas. Quoted from *Chem. Abstr.*, 1968, 69, p.1221, abstr. no. 12744h.

Medved, L. I., and Kagan, J. S. (1966). Toxicology. *Ann. Rev. Pharmacol.*, 6, 293-308.

Prendergast, J. A., Jones, R. A., Jenkins, L. J., and Siegel, J. (1967). Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoro-methane and 1,1-dichloroethylene. *Toxicol. appl. Pharmacol.*, 10, 270-289.

Rylova, M. L. (1953). Toxicity of 1,1-ethane dichloride. Quoted from *Chem. Abstr.*, 1953, 47, 11599i.

Sim, V. M., and Pattle, R. E. (1957). Effect of possible smog irritants on human subjects. *J. Amer. med. Ass.*, 165, 1908-1913.

Skog, E. (1950). A toxicological investigation of lower aliphatic aldehydes. I. Toxicity of formaldehyde, acetaldehyde, propionaldehyde and butyraldehyde; as well as of acrolein and crotonaldehyde. *Acta pharmacol. (Kbh.)*, 6, 299-318.

Smyth, H. F., Carpenter, C. P. and Weil, C. S. (1951). Range-finding toxicity data: list IV. *Arch. Industr. Hyg.*, 4, 119-122.

Solomin, G. I. (1964). Maximum permissible concentration of isopropylbenzene and its hydroperoxide in the atmosphere. Quoted from *Chem. Abstr.*, 1964, 68, 13785f.

—— (1966). Hygienic substantiation of the daily average maximum permissible concentration of isopropylbenzene and its hydroperoxide in the atmosphere. Quoted from *Chem. Abstr.*, 1966, 68, 4523d.

Stulova, E. A., Rumyantseva, E. P., and Ivanova, A. G. (1962). Influence of methacrylic acid on the health of workers. Quoted from *Chem. Abstr.*, 1964, 61, Column 8808.

Uloyan, S. M. (1965). Permissible concentration of butyraldehyde in the air of industrial areas. Quoted from *Chem. Abstr.*, 1966, 65, 4524b.

Werner, H. W., Nawrocki, C. Z., Mitchell, J. L., Miller, J. W., and von Oettingen, W. F. (1945). Effects of repeated exposures of rats to vapors of monoethyl ethylene glycol ether. *J. Industr. Hyg.*, 26, 374-379.

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highest concentration producing no toxic effects in animals by the application of a 'safety factor', which has varied according to the effects seen at the next highest concentration. In some cases the limit has been influenced by the established threshold limit values of analogous substances, and any information on human exposure has been taken into consideration. With materials with a strong odour, the limit has been set at the expected tolerable level.

Not all of the substances studied have proved to be of commercial interest. Those which have been introduced into manufacturing processes have been handled under proper supervision and there is no indication that the provisional operational limit has been set too high.

Comparison with Soviet limits

None of the substances studied has yet been considered by the ACGIH Committee on Threshold Limit Values (1968), but several have been the subject of investigations on animals and on man in the Soviet Union, which have led to the recommended maximal allowable concentrations in Table 2. The differences between the limits in Tables 1 and 2 are too large to be ignored. The Soviet animal experiments are usually of several months' duration, but it is unlikely that this plays an important part as there is often a wide divergence between the ACGIH threshold limit values and Soviet maximal allowable concentrations, even when the exposure periods are similar. The Soviet results cannot be passed over by doubting the toxicological significance of studies on nervous system function by techniques such as the conditioned reflex, negative induction or electroencephalography. It is true that these methods are extensively used and that there is little information in the English language dealing with the experimental details and the interpretation of the results; the review by Medved and Kagan (1966) indicates that many of the original publications are in journals inaccessible outside the Soviet Union. It is more disturbing that the Soviet investigators appear to have far more sensitive indices of early haematological changes and of organ damage. In the few Russian papers available to the author in translation, there is little indication how the statistical significance of differences between the test animals and a control group under identical conditions has been established. There is no doubt of the need for closer collaboration between toxicologists inside and outside the Soviet Union.

I wish to acknowledge the skilled assistance which I have received over the years, particularly from Mr. Z. S. Berczy, Mr. C. A. Manley, and Mr. R. A. Riley. Pathological reports have been prepared by Dr. J. G. S. Crabbe, Dr. E. Weston Hurst, Dr. T. F. McElligott, and Dr. D. M. Conning.

TABLE 1

PROVISIONAL OPERATIONAL LIMITS

(Figures are ppm unless otherwise stated)

Isoprene	20
Dicyclopentadiene	25
Decalin	100
1,2,4-Trimethylbenzene	50
2-Isopropoxyethanol	10
2-t-Butoxyethanol	10
Chloropropanol	0·2
Tris(pentafluoroethyl)methanol	15
Dichlorodi-isopropyl ether	100
Diethyleneglycol dimethyl ether	400
Methyl vinyl ether	100
Isobutyl vinyl ether	1000
Dimethoxymethane	200
Propionaldehyde	200
n-Butyraldehyde	100
Isobutyraldehyde	10
5-Bromopentan-2-one	50
2-Chloropropane	25
1,1-Dichloroethene	0·1
Dichlorobutenes	1
Hexachlorobutadiene	25
1,2,4-Trichlorobenzene	100
Hexafluorobenzene	100
Chloropentafluorobenzene	25
1,3,5-Trichlorotrifluorobenzene	20
Acrylic acid	20
Methacrylic acid	10
Methyl nitrite	100
Dimethyl carbonate	50
Vinyl acetate	1
Methyl isothiocyanate	50
2-Ethylhexyl acrylate	25
2-Ethylhexyl methacrylate	1
Methyl chloroformate	1
Ethyl chloroformate	2
Isopropyl chloroformate	75
s-Butylamine	25
Di-s-butylamine	10
Tributylamine	10
Nonylamine	25
Aminobutanol	25
1,6-Diaminohexane	25
Diethylaminopentan-2-one	50
Propyl cyanide	5
Chloroacetonitrile	100
2-Methoxy-2,3-dihydropyran	5
2-Aminomethyl-3,4-dihydropyran	50
2-Methylbenzoxazole	50
N-Formylpiperidine	10
2-Methylthiazole	25
3,5-Dimethylmorpholine	50
2-Methyl-1,3-dioxolane	20
t-Butyl peroxypivalate	25
Ethyl t-butyl peroxyoxalate	2
Dipropionyl peroxide	10 mg/m³
Diiso-amyl peroxydicarbonate	10
Cumene hydroperoxide	0·05
t-Butyl peracetate	0·1
Trimethylolpropane phosphite	2
Phosphorus triisocyanate	3
Silicon tetrafluoride	20
Silicon tetraisocyanate	250
Tetramethylsilane	5 (as HCl)
Sulphur dichloride	

Organic peroxides These compounds were irritant to the eyes and respiratory tract, presumably because of their high reactivity, but it is not possible to relate their toxicity to their reactivity with iodide solution. The results obtained with cumene hydroperoxide may be compared with those of Floyd and Stokinger (1958), who found the LC_{50} (4 hours) to be 220 ppm in rats. Soviet claims (Solomin, 1964, 1966) suggest that the concentration of this compound must be reduced to 0·001 ppm to avoid effects on animals and on man.

Organic phosphorus compounds Trishydroxymethylpropane phosphite has shown an unexpectedly high toxicity: it is one of the most toxic compounds handled in this laboratory. It is fairly readily hydrolysed to yield dihydroxybutylphosphonic acid, which is of low toxicity by oral or parenteral administration. Its marked action on the central nervous system is probably due to its having sufficient stability to penetrate cell membranes as a non-ionized molecule, and its ultimate action may be due to its hydrolysis product or a reaction *in situ*.

Diethyl phosphorchloridothionate is primarily an irritant, presumably due to the reactive chlorine atom, but it is also a weak *in vivo* inhibitor of cholinesterase.

Silicon compounds The toxicity of silicon tetrafluoride is probably due to hydrogen fluoride released by hydrolysis. Similarly, the irritant action of silicon tetraisocyanate may be due to isocyanic acid. The stable silane derivatives are of low toxicity.

Sulphur compounds This group contains members which, presumably because of their high reactivity, are powerful lung irritants. Sulphur chloride pentafluoride, bispentafluorosulphur monoxide, and trichloro-methylsulphenyl chloride are at least as toxic as phosgene. The toxicity of sulphur dichloride is probably due to hydrolysis to hydrogen chloride. Vinylsulphur pentafluoride and divinyl disulphide have, in addition to their irritant action, a toxic effect on the liver or kidneys.

Miscellaneous Iron pentacarbonyl is a lung irritant, it affects the central nervous system and causes liver and kidney damage. A measurement of blood carboxyhaemoglobin would provide no guide to intoxication.

The value of inhalation experiments

Most of the substances which have been tested in these inhalation experiments have also been studied in these laboratories for oral and parenteral toxicity and for their effects on the skin and eyes. The systemic effects elicited after oral or intraperitoneal administration, or by percutaneous absorption, give, in general, a qualitative indication of the systemic effects obtained by inhalation studies, but, because of the differences in rates of absorption and metabolic transformations, there is little useful quantitative information.

Inhalation experiments are of special value for the study of those compounds which have an immediate or delayed irritant action on the lungs, for the intensity of these effects cannot be predicted with any certainty by other routes of administration. A survey of the results gives a strong indication that these effects on the lung are associated with the chemical reactivity of the molecule, particularly if the onset of severe symptoms is delayed. It seems probable that there is an initial modification of cell membranes, as postulated for the action of phosgene and ketene, followed by permeability changes leading to oedema and haemorrhage.

After short exposures to high concentrations of lung irritants, the effects seen at histological examination of lung tissue indicate that death can be attributed to an interference with gas exchange. After more prolonged exposure to lower concentrations, the cause of death is less certain, for although lung changes may be apparent, they are sometimes insufficient to account for the lethal action. Moreover, at still lower concentrations the animals may be in poor condition with a diminished weight increase, without any trace of damage being detectable in the lungs. It seems likely that exposure to irritant gases and vapours gives rise to stress which is responsible for the marginal toxic effects, and it is possible that the occasional observation of a diminution in the size of the thymus may be in some way connected with such stress effects.

Provisional operational limits

Subacute inhalation experiments lasting approximately three weeks cannot be regarded as an adequate basis for the establishment of threshold limit values which will define safe working concentrations under all conditions, although a study of the origins of the list published by the ACGIH (1966) shows that some of their values have been derived from more tenuous evidence. Nevertheless, the results obtained in these investigations permit an assessment of the toxic hazard which is of value to the chemical engineer in the design of plant, or which can form the basis of a code of safety precautions, provided that those exposed are under adequate medical supervision. The limiting concentrations derived from these experiments may be termed provisional operational limits to distinguish them from threshold limit values, which should preferably be based on more extended experiments on a variety of species, supported by evidence from human exposure.

The limits in Table 1 have been derived from the

or its metabolite (Carpenter, Pozzani, Weil, Nair, Keck, and Smyth, 1956) increases the fragility of aged red cells, and that the effect disappears when the average age is reduced by haemopoiesis, returning only when the normal age distribution is restored. Isopropoxyethanol shows the same effect but to a lesser extent. Perfluoro-triethylcarbinol is a powerful uncoupler of oxidative phosphorylation, an effect which has been confirmed by *in vitro* studies on mitochondria in which a dissociation between oxygen uptake and the phosphorylation of ADP was observed which was quantitatively and qualitatively similar to that from dinitrophenol (Gage, unpublished work). This compound must be one of the simplest to show this effect; it conforms to the requirements of an uncoupler (Hemker, 1962) in that it is acidic due to the influence of the fluorine atoms on the hydroxyl group, and it is lipid-soluble in its non-ionized form. Chloropropanol is irritant but it does not show the central effects of chloroethanol (Goldblatt and Chiesman, 1944).

Ethers All the ethers had the central depressant action of diethyl ether, with the exception of dichlorodiisopropyl ether, which showed irritant properties, like its ethyl homologue (American Petroleum Institute, 1948).

Aldehydes and ketones The aldehydes have anaesthetic properties, with no marked irritant action. According to Skog (1950), the LC_{50} (30 minutes) of butyraldehyde to rats is 6% v/v. Sim and Pattle (1957) state that groups of men exposed to concentrations of butyraldehyde and isobutyraldehyde greater than 200 ppm for 30 minutes experienced no irritation, but some nausea with isobutyraldehyde. Soviet investigations on man (Uloyan, 1965) claim that a variety of effects are produced at 3 ppm.

Acids A comparison of the results with acrylic and methacrylic acids demonstrates the reduction in irritant action by the introduction of a methyl group, an effect observed with the methyl esters which show a 10-fold difference in American Conference of Governmental Industrial Hygienists (ACGIH, 1968) threshold limit values. The low toxicity of methacrylic acid in animals is in conflict with Soviet claims (Stulova, Rumyantseva, and Ivanova, 1962) that concentrations down to 6 ppm produce marginal changes in the function of the nervous system in man.

Chlorinated hydrocarbons The aliphatic chlorinated hydrocarbons demonstrated the liver and kidney damage characteristic of some members of this series. The two unsaturated 4-carbon compounds, dichlorobutene and hexachlorobutadiene, are highly toxic; both are capable of producing severe kidney damage, but with dichlorobutene lung irritation predominates and the renal effect has been observed only after percutaneous absorption (Ferguson, unpublished work). Smyth, Carpenter, and Weil (1951) found that exposure of rats for 4 hours to 62 ppm dichlorobutene (isomer unspecified) killed 2/6. The chlorinated ethylenes, on the other hand, are of relatively low toxicity. The results with 1,1-dichloroethene are extended by the observations of Prendergast, Jones, Jenkins, and Siegel (1967), who found no clear toxic manifestations apart from a retarded weight increase in a variety of species exposed to 100 ppm 5 hours/day for six weeks, while some liver damage was found after continuous exposures 24 hours/day to 47 ppm for 90 days. Rylova (1953) states that 25 ppm is irritant to man.

Pentafluorobenzene is a narcotic; Garmer and Leigh (1967) have shown that the anaesthetic concentration for cats is 1·5-2·5% v/v. As the fluorine atoms are replaced by chlorine, the anaesthetic action remains but a cytotoxic action appears, together with an effect on porphyrin metabolism.

The results with chloronaphthalene are in contradiction to Soviet claims (Kapkaev, 1957) that concentrations in the region of 1 ppm cause liver damage with a variety of blood changes and a hyperacidic gastritis.

Esters The unsaturated esters of saturated carboxylic acids are typically of low toxicity, high concentrations producing irritation and narcosis. With the exception of vinyl acetate, none of the esters of this type which have been examined was sufficiently volatile to show these effects. Dimethyl carbonate rapidly hydrolyses so its toxicity may be taken to be that of methanol. The high toxicity of the chloroformates is due to their great chemical reactivity; presumably like phosgene they modify cell membranes to produce permeability changes. Methyl nitrite produced methaemoglobinaemia *in vivo* at a rate similar to that of sodium nitrite; if its action is due to inorganic nitrite then its hydrolysis *in vivo* must be very rapid.

Amines The amines examined showed irritant and central stimulant effects; these increased with the degree of substitution, but the higher members have too low a volatility to present a significant vapour hazard. The results with diaminohexane are in contradiction to Soviet claims (Kulakov, 1967) that changes in the blood cells and in motor responses are seen in rats at 0·04 mg/m³.

Nitriles The compounds tested were primarily irritant, and there is no clear indication that any of the effects observed were due to liberated cyanide.

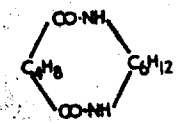
Heterocyclics There are no notable common features in this group.

500 ppm (D): 3F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal (slight lung congestion?)

6-Chlorododecafluorohexylsulphur pentafluoride
liq. b.p. 143°C $Cl(CF_2)_6 \cdot SF_5$
2500 ppm (D): 4F rats: 2 × 5-hr exposures: no weight gain: autopsy, organs normal
500 ppm (D): 4F rats: 13 × 6-hr exposures: no toxic signs: autopsy, organs normal

Miscellaneous

NN-Hexamethyleneadipamide
solid, m.p. 250°C
Fume 18 mg/litre (H): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: blood and urine tests normal: autopsy (histol.) liver cell vacuolation and necrosis



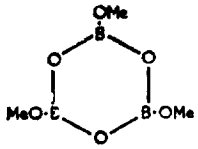
Fume 15 mg/litre (H): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Acrylyl chloride $CH_2{:}CH{\cdot}CO{\cdot}Cl$
[propenoyl chloride]
liq. b.p. 73°C
100 ppm (D): 4M rats: 1 × 2-hr exposure: lethargy, respiratory difficulty, autopsy, lung oedema
25 ppm (D, xylene): 4F rats: 1 × 4-hr exposure: eye irritation, respiratory difficulty, incoordination, 1 died: autopsy, lungs distended and oedematous, (histol.) lung emphysema and oedema
5 ppm (D, acetone): 4F rats: 5 × 5-hr exposures: eye irritation, respiratory difficulty, lethargy, low rectal temperature, weight loss, 3 rats died on 3rd day: autopsy (histol.) pneumonia
2·5 ppm (D, acetone): 4M 4F rats: 3 × 6-hr exposures: weight loss, low rectal temperature, 1 died: autopsy, lungs distended, (histol.) lung oedema and inflammation
1 ppm (D, acetone): 4M 4F rats: 15 × 5-hr exposures: no toxic signs: autopsy, organs normal

2,2-Bis-*p*-hydroxyphenylpropane $Me_2C(C_6H_4{\cdot}OH{-}p)_2$
solid, m.p. 152-156°C
Saturated (H): 4M rats: 5 × 6-hr exposures: no toxic signs: autopsy, organs normal

Ethyl *p*-chlorophenylformimidate $3{\cdot}ClC_6H_4{\cdot}N{:}CH{\cdot}OEt$
liq. b.p. 120°C (10 mm)
Saturated (A) [0·26 mg/litre, 35 ppm]: 3M rats: 9 × 6-hr exposures: eye and nose irritation, lethargy, respiratory difficulty, weight loss: autopsy, lungs discoloured, (histol.) lungs—areas of consolidation and collapse with peribronchiolar lymphocytic reaction
22 ppm (D, ethanol): 6M rats: 11 × 6-hr exposures: slight lethargy and respiratory difficulty: autopsy, organs normal

Trimethoxyboroxine
liq. decomp.
Saturated (A) [3 mg/litre, 600 ppm]: 3 M4F rats: 9 × 6-hr exposures: slight lethargy: autops, organs normal



N-Propylethylideneamine $CH_3{\cdot}CH{:}N{\cdot}C_3H_7$
liq. b.p. 74°C
250 ppm (D): 4M 4F rats: 1 × 5-hr exposure: eye and nose irritation, respiratory difficulty (M more affected), poor condition: autopsy (histol.) increased macrophages in lungs
100 ppm (D): 4M 4F rats: 6 × 6-hr exposures: nose irritation, respiratory difficulty, lethargy, weight loss, 1 died, blood and urine tests normal: autopsy (histol.) increased macrophages in lungs
10 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs apart from retarded weight gain, blood and urine tests normal: autopsy, organs normal
5 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Iron pentacarbonyl $Fe(CO)_5$
liq. b.p. 103°C
33 ppm (D, pet. ether): 4M 4F rats: 1 × 5·5-hr exposure: lethargy, respiratory difficulty, 4% carboxyhaemoglobin, 3 dead next day: autopsy (histol.) lung oedema and congestion
15 ppm (D, pet. ether): 4M 4F rats: 2 × 5·5-hr exposures: lethargy, respiratory difficulty, 0·2-0·4% carboxyhaemoglobin, 4 dead 3-4 days later: autopsy (histol.) lung oedema and congestion
7 ppm (D, pet. ether): 4M 4F rats: 18 × 5·5-hr exposures: no toxic signs: autopsy, organs normal

Discussion

Review of toxicological properties

Hydrocarbons None of the compounds examined showed any effects on the blood cells; the results with trimethylbenzene did not confirm the rather doubtful evidence that this compound has such an effect in man (Bättig, Grandjean, Rossi, and Rickenbacher, 1958). Soviet work (Korbakova, 1964) claims that low concentrations of cyclopentadiene and dicyclopentadiene can affect the blood as well as the lung and the functioning of the nervous system. Other Soviet work casts doubt on the low toxicity of isoprene, indicating marginal organ damage after prolonged exposure to 67-200 ppm (Korbakova and Pudorpva, 1964).

Alcohols The alcohols examined show interesting features. *t*-Butoxyethanol had the characteristic haemolytic action of *n*-butoxyethanol described by Werner, Nawrocki, Mitchell, Miller, and von Oettingen (1943), and Carpenter, Smyth, and Pozzani (1949). The results strongly suggest that the alcohol

sures: 4M 4F rats: no toxic signs: autopsy, organs normal

Trichloromethylsulphenyl chloride $Cl_3C{\cdot}SCl$
liq. b.p. 148-9°C
100 ppm (D): 4M rats: 1 × 1-hr exposure: severe respiratory difficulty, all died: autopsy (histol.) lung oedema
10 ppm (D, acetone): 4M rats: 1 × 6-hr exposure: lethargy, respiratory difficulty, 3 died later: autopsy (histol.) lung oedema
2 ppm (D, acetone): 4M rats: 20 × 6-hr exposures: initial respiratory difficulty: autopsy, lungs congested
0·5 ppm (D, acetone): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Dimethyl disulphide $MeS{\cdot}SMe$
liq. b.p. 112°C
250 ppm (D): 2M 2F rats: 13 × 6-hr exposures: lethargy, respiratory difficulty, low weight gain: autopsy, organs congested
100 ppm (D): 2M 2F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Dixylyl disulphide (solution in mineral oil contained about 40% with other sulphides) $[Me_2C_6H_3.S]_2$
Saturated [3·5 μg/litre]: 4F rats: 15 × 7-hr exposures: no toxic signs: autopsy, organs normal

Divinyl disulphide $[CH_2{:}CH{\cdot}S]_2$
liq. b.p. 86°C
480 ppm (D): 2M 2F rats: 1 × 4·5-hr exposure: eye and nose irritation, respiratory difficulty, weight loss, 1 rat died later: autopsy (histol.) livers congested with necrosis and fibrosis
130 ppm (D, acetone): 2M 2F rats: 4 × 5-hr exposures: intense lachrymation, nose irritation, lethargy, weight loss: autopsy, organs normal
18 ppm (D, acetone): 2M 2F rats: 15 × 6-hr exposures: initial nose and eye irritation, lethargy, poor condition, retarded weight gain, blood and urine tests normal: autopsy, organs normal
6 ppm (D, acetone): 2M 2F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Sulphur chloride pentafluoride $SClF_5$
vapour b.p. −20°C
100 ppm (F): 2M rats: 1 × 1-hr exposure: severe respiratory difficulty, both died: autopsy, lungs swollen and dark, (histol.) lungs—oedema and haemorrhage, liver and kidneys—congestion
20 ppm (F): 2M 2F rats: 1 × 5-hr exposure: respiratory difficulty: autopsy (histol.) lungs—oedema and congestion, liver and kidneys—congestion
5 ppm (F): 4M 4F rats: 3 × 5-hr exposures: respiratory difficulty, weight loss: autopsy (histol.) lungs—congestion and oedema
1 ppm (F): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Bispentafluorosulphur oxide $S_2F_{10}O$
liq. b.p. 29°C
1000 ppm (D, cooled): 4F rats: 1 × 2-hr exposure: respiratory difficulty, narcosis, cyanosis, froth at nose, all died: autopsy, severe lung oedema
100 ppm (D, cooled): 4F rats: 1 × 3·5-hr exposure: respiratory difficulty, narcosis, cyanosis, all died: autopsy, severe lung oedema
20 ppm (D, pet. ether): 4F rats: 1 × 6-hr exposure: no toxic signs during exposure, 2 died later: autopsy (histol.) marked perivascular and peribronchiolar lung oedema, acute bronchitis
10 ppm (D, pet. ether): 4M 4F rats: 20 × 6-hr exposures: lethargy, respiratory difficulty, weight gain retarded: autopsy (histol.) lung oedema, liver, kidneys and spleen congested
5 ppm (D, pet. ether): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Lauryl mercaptan $C_{12}H_{25}{\cdot}SH$
[dodecanethiol]
liq. b.p. 144-148°C (95 mm)
Saturated (A): 2M 2F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Vinylsulphur pentafluoride $CH_2{:}CH\ SF_5$
liq. b.p. 41°C
800 ppm (D, cooled): 2M 2F rats: 1 × 5-hr exposure: respiratory difficulty, lethargy, incoordination, 1 died: autopsy (histol.) livers—inflammation and fatty infiltration, kidneys—tubules dilated with degeneration
200 ppm (D, cooled): 2M 2F rats: 5 × 6-hr exposures: respiratory difficulty, lethargy weight loss, 1 died: autopsy (histol.) lungs—congestion and inflammation, livers—congestion and fatty infiltration, kidneys—tubules dilated with some degeneration
50 ppm (D, cooled): 4M 4F rats: 19 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-Chloroethylsulphur pentafluoride $Cl{\cdot}CH_2{\cdot}CH_2{\cdot}SF_5$
liq. b.p. 92°C
200 ppm (D): 4M rats: 1 × 2-hr exposure: tremors and convulsions, 1 died: autopsy, organs normal
50 ppm (D): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-Chlorotetrafluoroethylsulphur pentafluoride $Cl{\cdot}CF_2{\cdot}CF_2{\cdot}SF_5$
liq. b.p. 46·5°C
5000 ppm (D, cooled): 2M 2F rats: 1 × 3-hr exposure: respiratory difficulty leading to gasping and convulsions, all died during or soon after exposure: autopsy (histol.) lungs congested and oedematous, liver congested
1000 ppm (D, cooled): 2M 2F rats: 1 × 6-hr exposure: respiratory difficulty, 3 died after exposure: autopsy (histol.) lungs oedematous
200 ppm (D, cooled): 2M 2F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

4-Chloro-octafluorobutyl sulphur pentafluoride $Cl(CF_2)_4SF_5$
liq. b.p. 99°C
5000 ppm (D): 4M rats: 1 × 5-hr exposure: no toxic signs: autopsy, organs normal

Saturated (A) [30 ppm[b]]: 2M 2F rats: 1 × 4-hr exposure: nose irritation, respiratory difficulty: autopsy, lung oedema

1·6 ppm[b] (D, ethyl acetate): 2M 2F rats: 8 × 6-hr exposures: nose irritation, respiratory difficulty, lethargy, weight loss

0·7 ppm[b] (D, ethyl acetate): 2M 2F rats: 20 × 6-hr exposures: lethargy, diminished weight increase, nose irritation

0·15 ppm[b] (D, ethyl acetate): 2M 2F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Organic phosphorus compounds

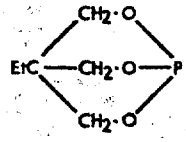
Tributyl phosphite $P(OBu)_3$

liq. b.p. 125-127°C (15 mm)

Saturated (A) [2·3 mg/litres, 220 ppm]: 2M 2F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy, organs normal

1,1,1-Trishydroxymethylpropane bicyclic phosphite

solid m.p. 56°C



Saturated (C): 4M 4F rats: 1 × 1-hr exposure: tremors, convulsions, all died during or soon after exposure: autopsy, liver, adrenals and kidneys congested, lungs pale with petechial haemorrhage

10 ppm (D, pet. ether): 4M 4F rats: 1 × 4-hr exposure: tremors, convulsions, all died: autopsy, organs normal

5 ppm (D, pet. ether): 4M 4F rats: 2 × 6-hr exposures: rapid breathing, tremors and convulsions, all died

2·5 ppm (D, pet. ether): 4M 4F rats: 5 × 6-hr exposures: tremors on 3rd day, weight loss, 1 died: autopsy, organs normal

1 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: slight transient head tremors on 4th day, retarded weight gain, blood and urine tests normal: autopsy, organs normal

0·5 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: reduced weight gain (F): autopsy, organs normal

0·25 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Phosphorus tri-isocyanate $P(NCO)_3$

liq. b.p. 168°C

Supplied as solid polymer and depolymerized by heating at 80°C and 25 mm pressure.

Saturated (A) [6 mg/litre, 900 ppm]: 2M 2F rats: 1 × 1-hr exposure: nose and eye irritation, respiratory difficulty, autopsy, organs normal

500 ppm (D, ethyl acetate): 6F rats: 2 × 5-hr exposures: eye and nose irritation, respiratory difficulty, weight loss: autopsy, organs normal

OO'-Diethyl phosphorochloridothionate $(EtO)_2PS\text{-}Cl$

liq. b.p. 80-90°C (18 mm)

104 ppm (D): 4M rats: 2 × 4-hr exposures: nose and eye irritation, salivation, respiratory difficulty, autopsy, lungs swollen, gut distended with gas, (histol.) lung emphysema

20 ppm (D): 4M rats: 14 × 5-hr exposures: severe nose irritation, respiratory difficulty, weight loss: autopsy (histol.) lungs—alveolar thickening and areas of collapse, degeneration of kidney tubular cortex

5 ppm (D, chloroform): 4M rats: 14 × 5-hr exposures: slight nose irritation: autopsy (histol.) lungs—slight thickening of alveolar walls

Silicon compounds

Silicon tetrafluoride SiF_4

compressed gas (b.p. —95°C)

1000 ppm (E): 4F rats: 1 × 20-min exposure: severe nose and eye irritation, respiratory difficulty, lethargy: autopsy, organs normal

300 ppm (F): 4F rats: 3 × 4·5-hr exposures: nose and eye irritation, respiratory difficulty, progressive deterioration of condition, 1 died: autopsy, lungs distended, (histol.) lung congestion and emphysema, liver congested, degeneration of kidney cortical tubules

60 ppm (F): 4F rats: 14 × 6-hr exposures: lethargy, nose irritation, weight gain retarded: autopsy, organs normal

15 ppm (F): 3M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Tetramethylsilane Me_4Si

liq. b.p. 26·5°C

8000 ppm (D, cooled): 4M rats: 1 × 6-hr exposure: no toxic signs: autopsy, organs normal

1600 ppm (D, cooled): 4M rats: 15 × 6-hr exposures: lethargy: autopsy, organs congested

Diphenyldimethoxysilane $(C_6H_5)_2Si(OMe)_2$

liq. non-volatile 140°C (2 mm)

Saturated (A): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Silicon tetraisocyanate $Si(NCO)_4$

solid-liq. m.p. 26°C, b.p. 186°C

Saturated (A, 30°C) [1·6 mg/litre, 200 ppm]: 4F rats: 6 × 5-hr exposures: eye and nose irritation, respiratory difficulty, no weight gain: autopsy, lungs and kidneys congested

50 ppm (D, ethyl acetate): 4M 4F rats: 20 × 5-hr exposures: no toxic signs: autopsy, organs normal

Sulphur compounds

Sulphur dichloride S_2Cl_2

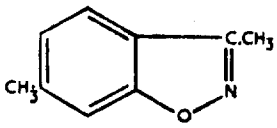
liq. b.p. 59°C [approx. 75% pure, contained 20% SCl_4]

340 mg/m[3] [a] (D, cooled): 4M 4F rats: 8 × 6-hr exposures: limp, eye and nose irritation, respiratory difficulty, weight loss: autopsy, organs normal

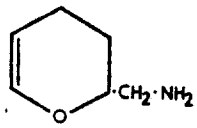
100 mg/m[3] [a] (D, cooled): 4M 4F rats: 15 × 6-hr exposures: eye and nose irritation, respiratory difficulty, lethargy: autopsy, organs normal

33 mg/m[3] [a] (D, pet. ether, cooled): 15 × 6-hr expo-

3,6-Dimethyl-1,2-benzisoxazole
liq. b.p. 70°C (0·5 mm)
Saturated (A) [0·35 mg/litre, 60 ppm]: no toxic signs, blood and urine tests normal: autopsy, organs normal

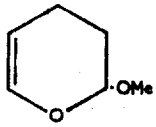


2-Aminomethyl-3,4-dihydropyran
liq. b.p. 74°C



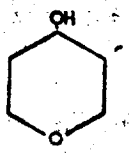
250 ppm (D): 4M 4F rats: 1 × 5-hr exposure: eye and nose irritation, respiratory difficulty (M more affected), poor condition: autopsy (histol.) excess macrophages in lungs
100 ppm (D): 4M 4F rats: 6 × 6-hr exposures: nose irritation, lethargy, weight loss, blood and urine tests normal, 1M died: autopsy (histol.) excess macrophages in lungs
10 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: retarded weight gain, blood and urine tests normal: autopsy, organs normal
5 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs. autopsy, organs normal

2-Methoxy-3,4-dihydropyran
liq. b.p. 127°C

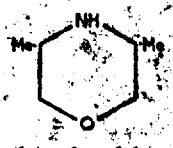


1000 ppm (D): 2M 2F rats: 15 × 6-hr exposures lethargy, weight loss, blood and urine tests normal: autopsy, organs normal
350 ppm (D): 4M 4F rats: 15 × 6-hr exposures: unresponsive: autopsy, organs normal
250 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

4-Hydroxytetrahydropyran
liq. b.p. 196°C
Saturated (A): 6F rats: 6 × 6-hr exposures: slight restlessness: autopsy, organs normal



3,5-Dimethylmorpholine
liq. b.p. 143-5°C

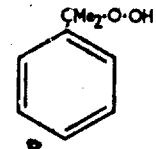


750 ppm (D): 4M 4F rats: 15 × 6-hr exposures: nose irritation, respiratory difficulty, lethargy, weight loss, urine tests normal, blood—reduced leucocyte count with increased polymorphs in females, anaemia and reticulocytosis: autopsy (histol.) hyperplasia of reticuloendothelial cells in spleen and of lymphoid tissue in lungs
250 ppm (D): 4M 4F rats: 15 × 6-hr exposures: diminished weight increase (F): autopsy, organs normal
50 ppm (D, water): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Peroxy compounds

Ethyl t-butyl peroxyoxalate (30·8% w/w in white spirit) EtO·CO·CO·O·O·CMe$_3$
Saturated (A) [1 ppm^b]: 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Cumene α-hydroperoxide (41·5% w/w in cumene) [isopropylbenzene hydroperoxide]
Saturated (A) [50 ppm^c]: 2F rats: 3 × 4-hr exposures: incoordination, tremor, narcosis, 1 died: autopsy (histol.) lungs congested, kidneys congested



31·5 ppm^c (D, ethanol): 6F rats: 7 × 5-hr exposures: salivation, respiratory difficulty, tremors, hyperaemia of ears and tail, weight loss: autopsy (histol.) lungs—emphysema and thickening of alveolar walls
16 ppm^c (D, ethanol): 6F rats: 12 × 4·5-hr exposures: salivation, nose irritation: autopsy, organs normal

Dipropionyl peroxide (22·7% w/w in white spirit) $(C_2H_5·CO·O)_2$
Saturated (A): 3M rats: 1 × 1·5-hr exposure; nose and eye irritation, respiratory difficulty, all dead 1 hr later: autopsy, lungs haemorrhagic
100 ppm^b (D): 2M 2F rats: 2 × 5-hr exposures: nose and eye irritation, respiratory difficulty, lethargy, weight loss, 1 died 2 days later: autopsy, organs normal
30 ppm^b (D): 2M 2F rats: 4 × 5-hr exposures: nose irritation: autopsy, organs normal
10 ppm^b (D, pet. ether): 4M 4F rats: 19 × 5-hr exposures: lethargy, retarded weight gain: autopsy, organs normal
7 ppm^b (D, pet. ether): 4M 4F rats: 14 × 5-hr exposures: no toxic signs: autopsy, organs normal

t-Butyl peroxypivalate (33·3% w/w in white spirit) Me$_3$C·CO·O·O·CMe$_3$
200 ppm (D): 2M 2F rats: 1 × 5-hr exposure: nose irritation, respiratory difficulty, lethargy, weight loss: autopsy, organs normal
50 ppm (D): 2M 2F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Bis-3-methylbutyl peroxydicarbonate (20% w/w in white spirit) $(C_5H_{11}·O·CO·O)_2$
Saturated (A) [1·7 ppm^b] 2 × 6-hr exposures: no toxic signs, apart from slight nose irritation attributable to white spirit: autopsy, organs normal
Mist 140 mg/m^{3b} (D, white spirit): 4F rats: 3 × 4-hr exposures: nose and eye irritation, respiratory difficulty, weight loss: autopsy (histol.) pneumonia
Mist 44 mg/m^{3b} (D, white spirit): 4M rats: 3 × 5-hr exposures: eye and nose irritation, respiratory difficulty, lethargy: autopsy (histol.) lungs—thickened alveolar walls, peribronchiolar leucocytic reaction

t-Butyl peracetate (50% w/w in dimethyl phthalate) CH$_3$·CO·O·O·CMe$_3$

Fume (H) 1 mg/litre: 4M 4F rats: 15 × 6-hr exposures: no toxic signs, (histol.) organs normal

Diethylenetriamine $NH_2(CH_2)_2NH(CH_2)_2NH_2$
[bis-2-aminoethylamine]
liq. b.p. 208°C
Saturated (A) [0·55 mg/litre, 130 ppm]: 2M 2F rats: 15 × 6-hr exposures: no toxic signs (hair coarsened): autopsy, organs normal

2-Aminobutan-1-ol $CH_3{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CH_2OH$
liq. b.p. 178°C
Saturated (A) [0·3 mg/litre, 85 ppm]: 2M 2F rats: 15 × 6-hr exposures: increased white cell count, high blood urea, urine tests normal: autopsy, organs normal
50 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy, organs normal

1-Diethylaminopentan-2-one $CH_3{\cdot}(CH_2)_2{\cdot}CO{\cdot}CH_2{\cdot}NEt_2$
liq. b.p. 100° (23 mm)
Saturated (A): 3M rats: 10 × 6-hr exposures: eye irritation, salivation, no weight gain: autopsy (histol.) slight thickening of alveolar walls
78 ppm (D): 7M rats: 14 × 6-hr exposures: slight nasal irritation: autopsy (histol.) slight thickening of alveolar walls

Nitriles

n-Propyl cyanide $C_3H_7{\cdot}CN$
[1-cyanopropane]
liq. b.p. 116-7°C
Saturated (A) [*ca* 2% v/v]: 4M rats: 1 × 1-hr exposure: eye and nose irritation, respiratory difficulty, coma, all died
2000 ppm (D): 4M 4F rats: 1 × 4-hr exposure: respiratory difficulty, weight loss, lethargy, convulsions, rectal temp. <32°C: autopsy, lungs congested
400 ppm (D): 4M 4F rats: 2 × 5·5-hr exposures: lethargy, weight loss, low body temperature, gasping, 1 died: autopsy, organs normal
200 ppm (D): 4M 4F rats: 20 × 6-hr exposures: no toxic signs, daily urinary thiocyanate, 6 μg/rat (normal 0·02 μg): autopsy, organs normal

Chloroacetonitrile $ClCH_2{\cdot}CN$
[chloromethyl cyanide]
liq. b.p. 124°C
Saturated (A) [34 mg/litre, 1% v/v]: 2M 2F rats: 1 × 30-min exposure: acute lachrymation, all died
300 ppm (D): 2M 2F rats: 3 × 5-hr exposures: respiratory difficulty, lachrymation, incoordination, low body temperature, weight loss: autopsy, organs congested
80 ppm (D): 4M 4F rats: 6 × 6-hr exposures: slight respiratory difficulty, lethargy, low weight gain: autopsy, lungs congested, (histol.) congestion of kidney, liver and spleen
20 ppm (D, isopropanol): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, slight kidney congestion

N-2-Cyanoethylaniline $PhNH{\cdot}(CH_2)_2{\cdot}CN$
solid, m.p. 50-51°C
Saturated (C) [0·07 mg/litre, 12 ppm], 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Heterocyclics

1-Acetyl-γ-butyrolactone $AcCH_2{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2$
liq. b.p. *ca* 125°C (13 mm)
Saturated (A): 3M rats: 10 × 7-hr exposures: no toxic signs: autopsy, organs normal

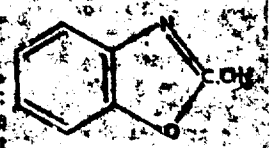
2-Methyl-1,3-dioxolan $MeCH{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}O$
liq. b.p. 78-81°C
500 ppm (D): 4M 4F rats: 15 × 6-hr exposures: retarded weight gain in females, blood and urine tests normal: autopsy, organs normal
250 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

N-Formylpiperidine $C_5H_{10}N{\cdot}CHO$
liq. b.p. 220°C
Saturated (A) [0·74 mg/litre, 150 ppm]: 2M 2F rats: 12 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-Methylthiazole $MeC{:}N{\cdot}CH{:}CH{\cdot}S$
liq. b.p. 128°C
100 ppm (D): 4M 4F rats: 15 × 6-hr exposures: eye and nose irritation, retarded weight gain, lethargy, blood and urine tests normal: autopsy, organs normal
35 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: lethargy: autopsy, organs normal
25 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Pentachloropyridine C_5NCl_5
solid, m.p. 123-125°C
Saturated (B) [0·01 mg/litre, 1 ppm]: 2M 2F rats: 15 × 6-hr exposures: no toxic signs, urine tests normal: autopsy, organs normal

2-Methylbenzoxazole
liq. b.p. 200°C
Saturated (A) [1·9 mg/litre, 340 ppm]: 2M 2F rats: 15 × 6-hr exposures: eye irritation, narcosis, weight loss: autopsy, organs normal



188 ppm[a] (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy (histol.) slight lung inflammation
60 ppm[a] (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

and collapse and some oedema and haemorrhage in lungs, kidneys congested

5 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: nose irritation, lethargy: autopsy, organs normal

1 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Ethyl chloroformate — Cl·CO·OEt
[ethyl chloromethanoate]
liq. b.p. 95°C

20 ppm (D, pet. ether): 4M 4F rats: 10 × 6-hr exposures: nose irritation, respiratory difficulty, poor condition, weight loss: autopsy, lungs distended, (histol.) lung haemorrhage

5 ppm (D, pet. ether): 4M 4F rats: 20 × 6-hr exposures: retarded weight increase: autopsy, organs normal

1 ppm (D, pet. ether): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Isopropyl chloroformate — Cl·CO·OPri
[isopropyl chloromethanoate]
liq. 103-105°C (decomp.)

200 ppm (D): 4M 4F rats: 1 × 5-hr exposure: 2M rats: dyspnoea, both died later

50 ppm (D): 4M 4F rats: 11 × 6-hr exposures: respiratory difficulty, weight loss, 1 died: autopsy (histol.) lung haemorrhage

20 ppm (D, isopropanol): 4M 4F rats: 20 × 6-hr exposures: nasal irritation: autopsy, organs normal

5 ppm (D, isopropanol): 4M 4F rats: no toxic signs: autopsy, organs normal

Methyl nitrite — $CH_3·O·NO$
vapour (b.p. −12°C)

250 ppm[e] (I): 4M 4F rats: 1 × 4-hr exposure: rats gasping and pale, 7 died

110 ppm[e] (I): 4M 4F rats: 13 × 6-hr exposures: good condition though pale, methaemoglobin rose to 30-40% of total Hb by end of each exposure with recovery overnight: autopsy, organs normal

84 ppm[e] (I): 4M 4F rats: 15 × 6-hr exposures: good condition, methaemoglobin 10%: autopsy, organs normal

25 ppm[e] (I): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

35 ppm[e] (I): 1F cat (3·4 kg): 1 × 6-hr exposure: behaviour normal, methaemoglobin 6%

Dimethyl carbonate — Me_2CO_3
liq. b.p. 90·2°C

Saturated (A) [5000 ppm, 20 mg/litre]: 2M 2F rats: 1 × 6-hr exposure: eye irritation, salivation, respiratory difficulty, incoordination, rapid recovery after exposure: autopsy, organs normal

1000 ppm (D): 2M 2F rates: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Amino compounds

s-Butylamine — $CH_3·CH_2·CHMe·NH_2$
[2-aminobutane]
liq. b.p. 62°C

Saturated (A) [280 mg/litre, 10% v/v]: 7 M rats: 1 × 5-hr exposure: intense eye and nose irritation respiratory difficulty, convulsions, all died: autopsy, cornea opaque and white, (histol.) organs normal

233 ppm (D): 7M rats: 13 × 6·5-hr exposures: discomfort, lethargy, retarded weight gain: autopsy, organs normal

Di-s-butylamine — $(CH_3·CH_2·CHMe)_2NH$
[bis-(2-methylpropyl)amine]
liq. b.p. 135°C

Saturated (A) [50 mg/litre, 1% v/v]: 4M 4F rats: 19 × 6·5-hr exposures: restlessness, initial tremors, incoordination, no weight gain: autopsy, organs normal

Tributylamine — Bu_3N
liq.

120 ppm (D): 4M 4F rats: 19 × 6-hr exposures: nose irritation, restlessness, incoordination and tremors, no weight gain: autopsy, organs normal

62 ppm (D, pet, ether): 4M 4F rats: 19 × 6-hr exposures: lethargy, no weight gain: autopsy, organs normal

29 ppm (D, pet. ether): 4M 4F rats: 19 × 6-hr exposures: slight lethargy: autopsy, organs normal

Nonylamine — $CMe_3·CH_2·CHMe·(CH_2)_2·NH_2$
[1-amino-3,5,5-trimethylhexane]
liq. b.p. 176°C

Saturated (A) [2 mg/litre, 340 ppm]: 3F rats: 1 × 35-min exposure: nose and eye irritation, salivation, tremors: autopsy, organs normal

66 ppm (D): 6F rats: 6 × 6-hr exposures: nose and eye irritation, tremors: autopsy, organs normal

16·5 ppm (D, pet. ether): 6F rats: 10 × 6-hr exposures: no toxic signs: autopsy, organs normal

Dinonylamine — $[CMe_3·CH_2·CHMe·(CH_2)_2]_2NH$
[bis-(3,5,5-trimethylhexyl)amine]
liq. b.p. 172°C (20 mm)

Saturated (A) [0·16 mg/litre, 15 ppm]: 3F rats: 14 × 6-hr exposures: slight restlessness: autopsy, organs normal

Trinonylamine — $[CMe_3·CH_2·CHMe·(CH_2)_2]_3N$
[tris-(3,5,5-trimethylhexyl)amine]
liq. b.p. 216°C (20 mm)

Saturated (0·08 mg/litre, 7 ppm): 3F rats: 8 × 6-hr exposures: no toxic signs: autopsy, no organ damage

1,6-Diaminohexane — $NH_2(CH_2)_6NH_2$
[hexamethylenediamine]
liq. b.p. 200°C (supplied as 90% aqueous solution)

Fume (H) 10 mg/litre: 4M 4F rats: 2 × 6-hr exposures: nose irritation, respiratory difficulty, lethargy, 1M 1F died: autopsy, lungs congested, (histol.) peribronchiolar inflammation, areas of haemorrhage and oedema in lungs, vacuolation of kidney tubules

Fume (H) 5 mg/litre: 4M 4F rats: 11 × 6-hr exposures: nose and lung irritation, little weight gain, lethargy, poor condition, 1 died, urine and blood tests normal: autopsy, petechial haemorrhage in lungs, (histol.) lung inflammation

1-Chloronaphthalene (technical) $C_{10}H_7Cl$
liq. b.p. 250-280°C
Saturated (A) [0·25 mg/litre, 37 ppm]: 3F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Esters

Vinyl acetate $CH_3{\cdot}CO{\cdot}O{\cdot}CH{:}CH_2$
liq. b.p. 72°C
Saturated (A): 4M 4F rats: 5 min: rapid anaesthesia, all died
2000 ppm (D): 4M 4F rats: 15 × 6-hr exposures: eye and nose irritation, respiratory difficulty, poor condition, low weight gain: autopsy (histol.) excess macrophages in lungs
630 ppm (D): 4M 4F rats: 15 × 6-hr exposures: low weight gain (F): autopsy, organs normal
250 ppm (D): 4M 4F rats: 15 × 6-hr exposures: low weight gain (F), blood and urine tests normal: autopsy, organs normal
100 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Methyl salicylate $C_6H_4(OH)$-2-CO_2Me
[methyl 2-hydroxybenzoate]
liq. b.p. 223°C
Saturated (A) [700 mg/m^3, 120 ppm]: 4F rats: 20 × 7-hr exposures: no toxic signs: autopsy, organs normal

Methyl isothiocyanate $CH_3{\cdot}NCO$
liq.
10 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: lethargy, low weight gain (F), blood and urine tests normal: autopsy, thymus small, (histol.) organs normal
2·5 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-Ethylhexyl acrylate
[2-ethylhexyl propenoate]
$CH_2{:}CH{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CHEt{\cdot}[CH_2]_3{\cdot}CH_3$
liq. b.p. 215-219°C
Saturated (A) [1 mg/litre, 130 ppm]: 2M 2F rats: 13 × 6-hr exposures: initial weight loss, lethargy, slight respiratory difficulty, blood and urine tests normal: autopsy, organs normal
50 ppm (D, ethanol): 2M 2F rats: no toxic signs: autopsy, organs normal

2-Ethylhexyl methacrylate
[2-ethylhexyl 2-methylpropenoate]
$CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CHEt{\cdot}[CH_2]_3{\cdot}CH_3$
liq. b.p. 224°C
Saturated (A) [0·15 mg/litre, 60 ppm], 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy (histol.) increased cellularity in lungs
25 ppm (D, ethanol): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Octyl methacrylate $CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}C_8H_{17}$
[octyl 2-methylpropenoate]
liq. b.p. 112°C (10 mm)
Saturated (A): 2M 2F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Lauryl methacrylate $CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}C_{12}H_{25}$
[dodecyl 2-methylpropenoate]
(contains 25% C_{14} ester)
liq. b.p. 170°C (10 mm), 205°C (50 mm)
Saturated (A): 2M 2F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Cetostearyl methacrylate
[cetyl to stearyl 2-methylpropenoate]
$CH_2{:}CMe{\cdot}CO{\cdot}OR$ [R = $C_{16}H_{33}$ to $C_{18}H_{37}$]
solid, m.p. 20°C
Saturated (B): 2M 2F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-Dimethylaminoethyl methacrylate
[2-dimethylaminoethyl 2-methylpropenoate]
$CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NMe_2$
liq. b.p. 187°C
Mist (?) 250 ppm (D): 4M 4F rats: 15 × 6-hr exposures: nose and eye irritation, rapid breathing, weight gain low and irregular, blood and urine tests normal: autopsy, organs normal
100 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-Hydroxyethyl methacrylate
[2-hydroxyethyl 2-methylpropenoate]
$CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2OH$
liq.
Saturated (A) [0·5 mg/litre, 90 ppm]: 4M 4F rats: 15 × 6-hr exposures: erratic weight gain (F): autopsy, organs normal

2-Hydroxypropyl methacrylate
[2-hydroxypropyl 2-methylpropenoate 80%]
$CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH(OH){\cdot}CH_3$
[2-hydroxy-1-methylethyl 2-methylpropenoate 20%]
$CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}CHMe{\cdot}CH_2OH$
liq. b.p. 79°C (5 mm)
Saturated (A) [0·5 mg/litre, 90 ppm]: 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Glycol dimethacrylate $(CH_2{:}CMe{\cdot}CO{\cdot}O{\cdot}CH_2)_2$
[ethylene bis-(2-methylpropenoate)]
liq. b.p. 120°C (20 mm)
Saturated (A) [1 mg/litre, 120 ppm]: 3F rats: 13 × 6-hr exposures: slight lethargy: autopsy (histol.) alveolar walls thickened, peribronchiolar lymphocytic reaction

Methyl chloroformate $Cl{\cdot}CO{\cdot}OMe$
[methyl chloroformethanoate]
liq. b.p. 72°C
20 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: nose irritation, respiratory difficulty, lethargy, poor condition, weight loss: autopsy, lungs distended and haemorrhagic, (histol.) areas of consolidation

no toxic signs: autopsy (histol.) liver—extensive vacuolation and necrosis
250 ppm (D): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

1,1-Dichloroethene $CH_2:CCl_2$
[vinylidene chloride]
liq. b.p. 31·9°C
500 ppm (D): 4m 4F rats: 20 × 6-hr exposures: nose irritation, retarded weight gain: autopsy (histol.) liver cell degeneration
200 ppm (D): 4M 4F rats: 20 × 6-hr exposures: slight nose irritation: autopsy, organs normal

Dichlorobutenes (mixed isomers)
1. $CH_2:CH{\cdot}CHCl{\cdot}CH_2Cl$
2 and 3. *cis* and *trans*-$CH_2Cl{\cdot}CH:CH{\cdot}CH_2Cl$
[1. 37·3% 3,4-dichlorobut-1-ene 2. 17% *cis*-1,4-dichlorobut-2-ene 3. 45·7% *trans*-1,4-dichlorobut-2-ene]
liq. b.p. 123-158°C
18 ppm (D, pet. ether): 4M 4F rats: 8 × 6-hr exposures: initial lachrymation, lethargy, respiratory difficulty, low rectal temp., progressive weight loss, blood and urine tests normal: autopsy, emaciated, lungs haemorrhagic, thymus atrophied, (histol.) lungs emphysematous with areas of haemorrhage and oedema
6 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: initial weight loss and lethargy, later normal: autopsy, thymus slight atrophy
2-3 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Hexachlorobutadiene $Cl_2C:CCl{\cdot}CCl:CCl_2$
liq.
250 ppm (D): 4M 4F rats: 2 × 4-hr exposures: eye and nose irritation, respiratory difficulty, females affected more than males, apparent recovery after exposure: autopsy (histol.) degeneration of middle renal proximal tubules and of adrenal cortex
100 ppm (D): 4M 4F rats: 12 × 6-hr exposures: eye and nose irritation, respiratory difficulty, poor condition, weight loss, slight anaemia in females, urine tests normal, 2 females died: autopsy, kidneys pale and enlarged, adrenals enlarged, degeneration of renal cortical tubules with epithelial regeneration
25 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: poor condition, diminished weight gain in females, respiratory difficulty, blood and urine tests normal: autopsy, kidneys pale and enlarged, (histol.) damage to renal proximal tubules
10 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: retarded weight gain in females: autopsy, organs normal
5 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

(2-Bromoethoxy)benzene $PhO{\cdot}CH_2{\cdot}CH_2Br$
[2-phenoxyethyl bromide]
solid, m.p. 30°C, b.p. *ca* 145°C (50 mm)
Saturated (A, 37°C) [0·4 mg/litre, 80 ppm]: 3F rats: 13 × 5·5-hr exposures: discomfort, lethargy, initial weight loss: autopsy, organs normal

1,2,4-Trichlorobenzene (up to 20% 1,2,3-trichlorobenzene) $C_6H_3Cl_3$
liq. b.p. 206-225°C
Saturated (A) [2·5 mg/litre, 200 ppm]: 2M 2F rats: 15 × 6-hr exposures: lethargy, retarded weight gain: autopsy, organs normal
70 ppm (D): 2M 2F rats: 15 × 6-hr exposures: initial lachrymation, lethargy, retarded weight gain: autopsy, organs normal
20 ppm (D, ethanol): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Hexafluorobenzene C_6F_6
liq. b.p. 80°C
1000 ppm (D): 4M 4F rats: 6 × 6-hr exposures: no weight gain (F): autopsy (histol.) lungs—macrophages, spleen—reactive hyperplasia
500 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no porphyrinuria, blood and urine tests normal, weight gain retarded (F): autopsy, organs normal
250 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Chloropentafluorobenzene C_6F_5Cl
solid, m.p. 18·1°C, b.p. 116°C
1000 ppm (D): 4M 4F rats: 4 × 6-hr exposures: lethargy, incoordination, no porphyrinuria: autopsy, organs normal
500 ppm (D): 4M 4F rats: 15 × 6-hr exposures: unresponsive, no porphyrinuria: autopsy, organs normal
50 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

1,3-Dichlorotetrafluorobenzene (contains about 5% 1,2-isomer) C_6F_4-1,3-Cl_2
liq. b.p. 156°C
Saturated (A) [30 mg/litre, 3000 ppm]: 2M 2F rats: 1 × 30-min exposure: eye irritation, nasal discharge, respiratory difficulty, light narcosis
1000 ppm (D): 4M 4F rats: 4 × 6-hr exposures: light narcosis with recovery overnight, increased urinary coproporphyrin (M), porphobilinogen (M and F): autopsy (histol.) damage to kidney tubules
500 ppm (D): 4M 4F rats: 15 × 6-hr exposures: light narcosis with recovery overnight: weight gain retarded, blood and urine tests normal: autopsy (histol.) slight kidney tubular lesions
100 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

1,3,5-Trichlorotrifluorobenzene C_6F_3-1,3,5-Cl_3
solid, m.p. 50-60°C
Saturated (B) [3·6 mg/litre, 380 ppm]: 2M 2F rats: 2 × 6-hr exposures: nose irritation, respiratory difficulty, narcosis, males died, increased urinary protein and porphobilinogen, high blood urea: autopsy (histol.) liver—focal necrosis and centrilobular vacuolation with fatty changes, kidney—tubular necrosis
83 ppm (C): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy, livers enlarged

initial lachrymation, low weight gain, blood tests normal: autopsy (histol.) slight lung inflammation
500 ppm (D): 4M 4F rats: 15 × 6-hr exposures: low weight gain (f), blood and urine tests normal: autopsy, organs normal
250 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy, organs normal

Bis-2-methoxyethyl ether
[diethyleneglycol dimethyl ether, diglyme]
$MeO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}OMe$
liq. b.p. 159°C
600 ppm[a] (D): 4M 4F rats: 15 × 6-hr exposures: irregular weight gain, blood and urine tests normal: autopsy, thymus atrophied, adrenals congested
200 ppm[a] (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy, organs normal

Bis-2-ethoxyethyl ether $EtO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}OEt$
[diethylene glycol diethyl ether, diethyl carbitol]
liq. b.p. 188°C
Saturated (A) [2·6 mg/litre 400 ppm]: 4M rats: 17 × 7-hr exposures: restlessness: autopsy, organs normal

Bis-2-chloro-1-methylethylether $(ClCH_2{\cdot}CHMe)_2O$
liq. b.p. 187°C
700 ppm (D): 2M 2F rats: 1 × 5-hr exposure: nose and eye irritation, respiratory difficulty, 2 died later: autopsy (histol.) congestion of liver and kidneys
350 ppm (D): 4M 4F rats: 8 × 5-hr exposures: lethargy, respiratory difficulty, retarded weight gain: autopsy (histol.) congestion of liver and kidneys
70 ppm (D): 4M 4F rats: 20 × 6-hr exposures: lethargy, weight gain retarded: autopsy, organs normal
20 ppm (D, ethanol): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Aldehydes and ketones

Propionaldehyde $CH_3{\cdot}CH_2{\cdot}CHO$
[propanal]
liq. b.p. 46-50°C
Saturated (A): 2M 2F rats: 1 × 30-min exposure: anaesthetized, all died
1500 ppm (D): 4M 4F rats: 5 × 6-hr exposures: no weight gain: autopsy (histol.) liver cell vacuolation
50 ppm (D): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

n-Butyraldehyde $CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CHO$
[butanal]
liq. b.p. 75°C
1000 ppm (D): 3M 4F rats: 12 × 6-hr exposures: no toxic signs: autopsy, organs normal

Isobutyraldehyde (92%) $Me_2CH{\cdot}CHO$
[isobutanal]
liq. b.p. 64°C
1000 ppm (D): 4M 4F rats: 12 × 6-hr exposures: slight nose irritation: autopsy, organs normal

5-Bromopentan-2-one $BrCH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}CH_3$
liq. b.p. 80°C (13 mm)
Saturated (A): 3M rats: 9 × 7-hr exposures: eye irritation, salivation, slight narcosis, respiratory difficulty, slight convulsions, rectal temp. <35°C: autopsy, organs congested, lungs haemorrhagic, (histol.) thickening of alveolar walls
Mist (?) 19 ppm [D]: 7M rats: 15 × 6-hr exposures: slight lethargy, no organ damage

Acids

Acrylic acid $CH_2{:}CH{\cdot}CO_2H$
[propenoic acid]
liq. b.p. 142°C
Saturated (A) [19 mg/litre, 6000 ppm]: 2M 2F rats: 1 × 5-hr exposure: nose and eye irritation, respiratory difficulty, unresponsive, 1 died: autopsy (histol.) lung haemorrhage, liver and kidney tubules–degenerative changes
1500 ppm (D) 4M 4F rats: 4 × 6-hr exposures: nasal discharge, lethargy, weight loss: autopsy (histol.) kidneys congested
300 ppm (D): 4M 4F rats: 20 × 6-hr exposures: some nose irritation, lethargy, retarded weight gain: autopsy, organs normal
80 ppm (D): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Methacrylic acid $CH_2{:}CMe{\cdot}CO_2H$
[2-methylpropenoic acid]
liq. b.p. 161°C
Saturated [4·5 mg/litre, 1300 ppm]: 2M 2F rats: 5 × 5-hr exposures: nose and eye irritation, weight loss, blood and urine tests normal: autopsy, organs normal
300 ppm (D): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal (slight renal congestion?)

Adipic acid $HO_2C{\cdot}[CH_2]_4{\cdot}CO_2H$
[hexanedioic acid]
solid, m.p. 151°C
Dust 125 mg/litre (G): 2M 2F rats: 15 × 6-hr exposures: no toxic signs, blood tests normal: autopsy, organs normal

Chlorinated hydrocarbons

2-Chloropropane $CH_3{\cdot}CHCl{\cdot}CH_3$
[isopropyl chloride]
liq. b.p. 36·5°C
1000 ppm (D): 4M 4F rats: 20 × 6-hr exposures:

1,2,4-Trimethylbenzene $1,2,4\text{-}(CH_3)_3C_6H_3$
[pseudocumene]
liq. b.p. 169°C
Saturated (A) [10 mg/litre, 2000 ppm]: 4M 4F rats: 12 × 6-hr exposures: nose and eye irritation, respiratory difficulty, lethargy, tremors, low weigh increase, blood tests normal: autopsy, organs normal
1000 ppm (D), 4M 4F rats: 15 × 6-hr exposures: initial slight eye and nose irritation, blood tests normal: autopsy, organs normal

Decahydronaphthalene $C_{10}H_{18}$
[decalin]
liq. b.p. 183-196°C
1000 ppm (D): 4M 4F rats: 1 × 4-hr exposure: tremors, convulsions, 3 deaths: autopsy, lungs congested
200 ppm (D): 4M 4F rats: 20 × 6-hr exposures: no toxic signs: autopsy, organs normal

Alcohols

Iso-octanol (mixture of branched chain alcohols, approx. $C_8H_{17}OH$)
liq. b.p. 185-9°C
Saturated (A) [1 mg/litre, 180 ppm]: 3F rats: 13 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-Isopropoxyethanol $Me_2CH{\cdot}O{\cdot}CH_2{\cdot}CH_2OH$
[isopropyl glycol ether]
liq. b.p. 140-144°C (90%)
1000 ppm (D): 4M 4F rats: 15 × 6-hr exposures: initial nasal irritation, lethargy, haemoglobinuria, porphyrinuria, Hb low, (4th day) MCHC low, reticulocytosis, later blood and urine normal: autopsy, organs appeared normal, (histol.) lungs congested
300 ppm (D): 4M 4F rats: 15 × 6-hr exposures: slight transient fall in Hb and MCHC (3rd day): autopsy (histol.) lungs congested
100 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

2-t-Butoxyethanol $Me_3C{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}OH$
[t-butyl glycol ether]
liq. b.p. 152°C
Saturated (A) [13 mg/litre, 2400 ppm]: 4M 4F rats: 1 × 5-hr exposure: comatose, haematuria, Hb 35-50% normal, all died 1-2 days later
250 ppm (D): 4M 4F rats: 4 × 6-hr exposures: initial haemaglobinuria and lethargy, low Hb and MCHC, weight loss
100 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, urine and blood tests normal apart from increased red cell osmotic fragility; autopsy, organs normal
50 ppm (D): 4M 4F rats: 15 × 6-hr exposures: as 100 ppm experiment
20 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood normal: autopsy, organs normal

Tris(pentafluoroethyl)methanol $(C_2F_5)_3C{\cdot}OH$
[perfluorotriethylcarbinol]
liq. b.p. 105°C
100 ppm (D): 4M 4F rats: 1 × 1-hr exposure: gasping respiration, nasal discharge, 7 rats died, rapid rigor
25 ppm (D, pet. ether): 4M 4F rats: 2 × 6-hr exposures: gasping, nasal discharge, 1 died: autopsy, lungs haemorrhagic
10 ppm (D, pet. ether): 4M 4F rats: 4 × 6-hr exposures: increased water consumption, rectal temp. 39·5°C, nasal discharge, rapid breathing, prostrate, blood tests normal, 2 died: autopsy, lungs haemorrhagic
2·5 ppm (D, pet. ether): 4M F4 rats: 15 × 6-hr exposures: increased water consumption, prostrate, blood and urine tests normal, weight increase normal: autopsy, organs normal
1·0 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

1-Chloropropan-2-ol $MeCH(OH){\cdot}CH_2Cl$
[propylene chlorohydrin]
liq. b.p. 133°C
1000 ppm (D): 2M 2F rats: 2 × 6-hr exposures: lethargy after 1st exposure, 3 days later given 2nd exposure, 1 died: autopsy. lungs oedematous and congested, liver pale, (histol.) lungs interstitial inflammatory exudate, liver cells swollen and vacuolated with nuclear degeneration
250 ppm[a] (D): 2M 2F rats: 15 × 6-hr exposures: lethargy, irregular weight gain, blood and urine tests normal: autopsy (histol.) lungs congested with perivascular oedema
100 ppm (D): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy (histol.) lungs congested with perivascular oedema
30 ppm[a] (D, ethanol): 4M 4F rats: 14 × 6-hr exposures: no toxic signs: organs normal

2-Ethyl-2-hydroxymethylpropane-1, 3-diol
[Trishydroxymethylpropane] $C_2H_5{\cdot}C(CH_2OH)_3$
solid m.p. 58°C, b.p. *ca.* 150°C (0·2-0·4 mm)
Saturated (A, 70°C) [20 μg/litre, 3·5 ppm]: 2M 2F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Ethers

Dimethoxymethane $(MeO)_2CH_2$
[methylal]
liq. b.p. 41-2°C
4000 ppm (D): 4F rats: 8 × 5-hr exposures; no toxic signs: autopsy, organs normal

Methoxyethene $MeO{\cdot}CH{:}CH_2$
[methyl vinyl ether]
vapour b.p. 5·5°C (supplied in cylinder)
2000 ppm (F): 4M 4F rats: 15 × 6-hr exposures: no toxic signs, blood and urine tests normal: autopsy, organs normal

Isobutoxyethene $Me_2CH{\cdot}CH_2{\cdot}O{\cdot}CH{:}CH_2$
[isobutyl vinyl ether]
liq. b.p. 83°C
Saturated (A): 2M 2F rats: 1 × 15-min exposure: rapid anaesthesia, all died
1000 ppm (D): 4M 4F rats: 15 × 6-hr exposures:

after the last exposure day for biochemical tests. The animals were left overnight with food and drink. On the following day the rats were anaesthetized with halothane and partially exsanguinated by heart puncture for haematological tests. After a gross examination of the organs, the lungs were inflated with formol-saline and immersed in the same fixative. The following organs were also taken for microscopical examination after fixation in formol-corrosive: lungs, liver, kidneys, spleen, and adrenals; and occasionally heart, jejunum, ileum, and thymus.

If at any stage effects were observed which could be attributed to the exposure, the experiment was repeated with progressively lower concentrations until a concentration was reached which was without effects on the animals. At intervals of about two months, batches of control rats were maintained in a chamber for the exposure period, in order to check the characteristics of the colony.

Results

Presentation of experimental results

Where practicable, the chemical name used is that recommended by the International Union of Pure and Applied Chemistry (IUPAC) classification. This is followed in brackets by any other name in common usage. Where the IUPAC name is little used, as with methacrylic acid, the widely used name appears first, followed by the systematic name. Any information on purity is included. A structural formula is given. The physical state and the melting or boiling points (where known) are stated.

Details of the experiments undertaken and the results obtained are presented according to the following scheme:

Atmospheric concentration and method of generation; number, sex, and species of animals; number and duration of exposures; clinical observations; autopsy.

The following annotations explain the conventions used in this presentation.

Atmosphere. The measured or calculated concentration followed in parenthesis by a letter indicating the method of generating the atmosphere and any special details. A superscript letter indicates the method used for determining the concentration. These letters refer to procedures detailed in the Methods section. Unless otherwise stated, the concentrations were dilutions of a vapour or a gas, with saturated atmospheres, the concentration in brackets is that estimated from the weight loss, unless otherwise indicated.

Clinical observations. The following descriptive terms have been used: *nose irritation*, sneezing progressing with increasing severity to a nasal discharge and a bloody exudate; *eye irritation*, eyes closed, progressing to lachrymation; *respiratory difficulty*, rapid shallow breathing progressing to laboured and slow breathing; *lethargy*, less than normal activity and a lower response to noise; *hypersensitive* or *unresponsive*, an increased or diminished reaction, respectively, to noise and handling; *incoordination*, unsteady movements, staggering gait; *no toxic signs* implies that the animals remained in good condition. In most experiments blood and urine tests were made at the lower concentrations, and any abnormal results are indicated. The urine tests included specific gravity, *p*H, reducing sugars, bilirubin, and protein. The blood tests included haemoglobin (Hb) concentration, packed cell volume, mean corpuscular Hb content, a white and differential cell count, a platelet count, clotting function, and the concentration of urea sodium, and potassium. Control tests for the haematological examination were made on the group of animals before exposure. *Autopsy*—no comment on the gross pathology indicates that the organs appeared normal. Comment after (*histol.*) indicates effects seen on microscopical examination of the tissues. *Organs normal* indicates that these examinations revealed no changes which could be attributed to the treatment.

Hydrocarbons

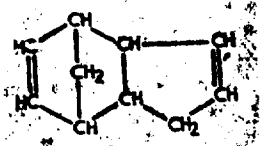
2-Methylbuta-1,3-diene [isoprene] $CH_2{:}CH{\cdot}CMe{:}CH_2$

liq. b.p. 34°C

6000 ppm (D, cooled): 2M 2F rats: 6 × 6-hr exposures: no toxic signs: autopsy, lungs slightly congested, (histol.) organs normal

1670 ppm (D, cooled): 2M 2F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

3a,4,7,7a-Tetrahydro-4,7-methanoindene [α-Dicyclopentadiene]



solid m.p. 25-30°C, b.p. 170° (decomp.)

2000 ppm (D, pet. ether): 2M 2F rats: 1 × 1-hr exposure: eye and nose irritation, dyspnoea, narcosis, 1 died later: autopsy (histol.) lungs, liver and kidneys congested

1000 ppm (D, pet. ether): 2M 2F rats: 1 × 4-hr exposure: eye and nose irritation, dyspnoea, muscular incoordination, tremors, hypersensitivity, all died later: autopsy, lungs congested, (histol.) lungs, liver, and kidneys congested

250 ppm (D, pet. ether): 2M 2F rats: 10 × 6-hr exposures: 1 died after 2nd exposure, survivors lost weight, nose irritation, dyspnoea, lethargic, tremors, hypersensitive, blood tests normal: autopsy, organs normal

100 ppm (D, pet. ether): 4M 4F rats: 15 × 6-hr exposures: no toxic signs: autopsy, organs normal

Vermutlich sind diese Schwankungen mitbedingt durch die individuell verschiedene Darmflora (oxalsäurebildende Bakterien).

In der zweiten Versuchsserie erhielten je 2 Versuchspersonen für 5 Tage mit einem Zwischenraum von je 2 Tagen zwischen den einzelnen Versuchsabschnitten zu der Standardkost täglich 150 g Butter, Olivenöl, Schweineschmalz oder Margarine*. Leider konnten aus äußeren (zeitlichen) Gründen die 4 Fette nicht an den gleichen Versuchspersonen gegeben werden. Es wurden nur jeweils 2 Fette an den gleichen Versuchspersonen untersucht. Vor Versuchsbeginn erhielten die Versuchsteilnehmer für 4 Tage eine Standardkost mit der gleichen Menge eines Mischfettes (Butter + Margarine). Danach wurde ausschließlich das entsprechende Versuchsfett gegeben. Die Oxalsäurebestimmung erfolgte an den beiden letzten Tagen des Vorversuches und an 4 Tagen des eigentlichen Versuchs.

Auch bei diesen Versuchen konnte ebenfalls keine Beeinflussung der Oxalsäurefraktion durch die verschiedenen Fette festgestellt werden. Auf die Anführung der einzelnen Analysenwerte kann deshalb verzichtet werden. Ebenfalls konnte keine Verminderung der ausgeschiedenen Oxalsäuremenge bei erhöhter Fettzufuhr beobachtet werden. Die von anderen Untersuchern beobachtete Herabsetzung der Oxalsäureausscheidung bei großen Fettgaben beruht wohl vorwiegend auf der dadurch bedingten verminderten Aufnahme anderer Nahrungsstoffe (Gemüse, Brot, Fleisch u. a.) bei reichlicher Fettzufuhr.

Zusammenfassung: Die Oxalatsäureausscheidung beim Menschen wird durch die Zufuhr diacidogener Fettsäuren bzw. ihrer Glyceride nicht beeinflußt. Die üblichen Nahrungsfette, die vielfach in einem ganz geringen Prozentsatz diacidogene Fettsäuren enthalten, bewirken ebenfalls keine Erhöhung bzw. Verminderung der Oxalsäureausscheidung.

Literatur: ANDERSEN, Hoppe-Seylers Z. 159, 297 (1927). — LE SAYDRO, Pathologica (Genova) 6, 231 (1914). — EMMRICH u. EMMRICH-GLASER, Hoppe-Seylers Z. 266, 183 (1940). — EMMRICH u. NEBE, Hoppe-Seylers Z. 266, 174 (1940). — FLASCHENTRÄGER, Helvet. chim. Acta 18, 962 (1935). — FLASCHENTRÄGER u. BERNHARD, Hoppe-Seylers Z. 238, 221 (1936). — FÜRBRINGER, Dtsch. Arch. klin. Med. 143, 1876. — HANSON, Ernährung 6, 273 (1941). — HOCK, Ernährung 6, 278 (1941). — KLEMPERER u. TRITSCHLER, Z. klin. Med. 44, 337 (1901). — LÜTHJE, Z. klin. Med. 35, 271 (1893). — MACLEAN u. SALKOWSKI, Hoppe-Seylers Z. 60, 20 (1909). — MERZ u. MAUGERI, Hoppe-Seylers Z. 201, 31 (1931). — MORI, J. of biol. Chem. 35, 341 (1918). — OIKAWA, Jap. J. med. Sci., Trans II Biochem. 4, 77 (1938). — PICCININI u. LOMBARDI, Riforma med. 41, 726 (1925); 42, 867 (1926). — RAUBITSCHEK, Prag. med. Wschr. 1910, 283. — SALKOWSKI, Berl. klin. Wschr. 1900, Nr 20. — STRADOMSKY, Virchows Arch. 163, 404 (1901). — VERKADE u. a., Hoppe-Seylers Z. 225, 250 (1934); 237, 186 (1935); 227, 213 (1934). — WEGRZYNOWSKI, Hoppe-Seylers Z. 83, 112 (1913).

die den angesäuerten Urin mit Äther extrahieren und später aus dem Ätherextrakt die Dicarbonsäuren mit organischen Lösungsmitteln fraktioniert auskristallisieren, geht die Oxalsäure verloren oder kann nur in einem ganz geringen Prozentsatz aus den verbleibenden schmierigen Rückständen des Extraktionsproduktes gewonnen werden.

Es erschien uns daher zur Klärung dieser Frage wichtig, speziell die Oxalsäurefraktion des Urins aufzuarbeiten. Dieser Untersuchung kommt nicht nur ein theoretisches, sondern auch ein medizinisch-praktisches Interesse zu. Wenn auch die üblichen Nahrungsfette keine oder nur wenige diacidogene Fettsäuren enthalten, so enthält z. B. Cocosfett und im geringen Maße auch einige Pflanzenfette Fettsäuren mit einer C-Atomzahl von 6, 8, 10, 12 und 14, die eine geringe Diacidurie bewirken (VERKADE). So hat neuerdings HOCK über ein Fett berichtet, das reichlich ungradzahlige Fettsäuren von mittlerer C-Atomzahl enthält und das nach HANSON im Stoffwechselversuch beim Menschen zu einer Ausscheidung von Dicarbonsäuren im Urin führt. Würde also die Oxalsäureausscheidung im Urin durch die ω-Oxydation der Fettsäuren erhöht werden, so wäre die Verwendbarkeit eines Fettes für die Ernährung, das diacidogene Fettsäuren enthält, erheblich eingeschränkt bzw. wäre sie ganz abzulehnen.

Über den Oxalsäurestoffwechsel liegen sehr zahlreiche und eingehende Untersuchungen vor. Die obere Grenze der täglich im Urin zur Ausscheidung kommenden Oxalsäuremenge liegt bei 20 mg (FÜRBRINGER). Der exogene Oxalsäureanteil entstammt den Oxalaten der Nahrung oder entsteht im Magen-Darmkanal durch die Tätigkeit der Mikroorganismen (Bact. oxalatigenum, Bact. oxalagenes) aus Kartoffeln, Leguminosen, Cerealien usw. (DE SANDRO, PICCININI und LOMBARDI). Bei fettreicher Kost soll weniger Oxalsäure im Urin ausgeschieden werden als bei fleisch- und kohlehydratreicher Kost (KLEMPERER, LÜTHJE, MILLS, RAUBITSCHEK, STRADOMSKY u. a.). Daneben kommt auch im Urin endogen entstandene Oxalsäure zur Ausscheidung, die dem intermediären Stoffwechsel entstammt. Sie soll den leimgebenden Substanzen (Bindegewebe), vor allem aber dem Glykokoll und Kreatinin, entstammen (FÜRBRINGER, KLEMPERER und TRITSCHLER, LÜTHJE u. a.).

Unsere Versuche wurden in 2 Versuchsserien durchgeführt. In der ersten Versuchsreihe wurden die als diacidogen bekannten Fettsäuren, C_6—C_{11}, als freie Fettsäuren und in einer zweiten Untersuchungsreihe die Glycerinester der gleichen Fettsäuren einzeln auf ihren Einfluß auf die Oxalsäurefraktion am Menschen untersucht. In der zweiten Versuchsserie wurden die üblichen Nahrungsfette (Butter, Schweineschmalz, Margarine und Olivenöl) in großer Menge am Menschen verabreicht und die Oxalatsäureausscheidung ebenfalls bestimmt.

Die quantitative Bestimmung der Oxalsäure erfolgte nach der Methode von MACLEAN und SALKOWSKI. Die Aufarbeitung ist zwar für Reihenuntersuchungen etwas langwierig, aber die relativen Vergleichswerte stimmen bei dieser Methode recht gut überein. Außerdem wird ein größeres Uringquantum (0,5 l) aufgearbeitet, so daß die Bestimmungsfehler im Vergleich zu anderen Methoden wesentlich geringere sind. Nach WEGRZYNOWSKI sind auch die absoluten Werte nach dieser Methode recht genau. MERZ und MAUGERI halten zwar die Ätherextraktion der Oxalsäure für sehr unvollkommen. Wir versuchten diesen Fehler dadurch einzuschränken, daß das Extraktionsgut bei ausreichender Säuerung mit Salzsäure, um die Dissoziation der Oxalsäure weitgehendst zurückzudrängen, intensiv extrahiert wurde. In einigen Kontrollbestimmungen konnte in dem bereits entätherten Extrakt keine weitere Oxalsäure mehr gefunden werden.

Die Herstellung der Triglyceride der Fettsäuren erfolgte durch 12stündiges Kochen der Fettsäure-Glycerin-Mischung am Rückflußkühler in Gegenwart von Zink als Katalysator. Die gewonnene Substanz wurde mit reichlich Wasser und mehrfach mit schwacher Bicarbonatlösung ausgewaschen, um freies Glycerin, Fettsäuren, Mono- und Diglyceride möglichst zu entfernen.

Die Untersuchungen wurden in folgender Weise durchgeführt: Jeweils 2 gesunde Versuchspersonen erhielten ab 4 Tage vor Versuchsbeginn eine Standardkost, die ihrer Zusammensetzung nach möglichst arm an oxalsäurehaltigen Nahrungsstoffen war und eine weitgehend konstante Zusammensetzung aufwies. Diese Kost wurde auch während der 3tägigen Versuchszeit gegeben. 2 Tage vor Versuchsbeginn wurde der täglich ausgeschiedene Oxalsäuremenge bestimmt. Am Morgen des 1. Versuchstages erhielt jede Versuchsperson mittels der Duodenalsonde 50 g der Fettsäure oder des Glycerids appliziert. Der Urin wurde am Versuchstage und an den 2 folgenden Tagen gesammelt und die Oxalsäure quantitativ bestimmt. Bei einigen empfindlichen Versuchspersonen trat kurze Zeit nach der Fettsäuregabe Erbrechen oder Durchfälle auf. Diese Versuche wurden dann an anderen weniger empfindlichen Versuchspersonen wiederholt. Es wurden nur die Versuche gewertet, wo sich keine Magen-Darmstörungen einstellten.

Die Resultate der durchgeführten Versuche ergaben eindeutig, daß die Oxalsäurefraktion durch die Zufuhr größerer Mengen diacidogener Fettsäuren oder ihrer Glyceride keine Veränderung erfährt, die außerhalb der individuellen Schwankungsbreite bzw. Fehlerbreite liegt. Aus Gründen der Platzersparnis werden im folgenden nur die Versuchsergebnisse der Untersuchungsreihe mit den Glyceriden angeführt. Die Analysenwerte der Versuche mit den freien Fettsäuren entsprechen sinngemäß diesen Versuchsergebnissen.

Fettsäureglycerid		Ausgeschiedene Oxalsäuremengen 1. Versuchsperson mg	2. Versuchsperson mg
Vorversuch:	1. Tag	16,9	13,2
	2. „	15,5	11,3
Versuch: C_6	1. „	20,8	13,6
	2. „	16,7	12,6
	3. „	16,3	13,1
Vorversuch: Mittelwert		16,2	12,5
Versuch: Mittelwert		17,9	13,1
Vorversuch:	1. Tag	15,4	10,6
	2. „	10,0	12,6
Versuch: C_7	1. „	11,4	14,2
	2. „	15,4	17,4
	3. „	13,2	14,8
Vorversuch: Mittelwert		12,7	11,6
Versuch: Mittelwert		13,3	15,5
Vorversuch:	1. Tag	12,9	8,9
	2. „	12,1	10,2
Versuch: C_8	1. „	12,2	9,5
	2. „	11,6	10,0
	3. „	12,4	9,1
Vorversuch: Mittelwert		12,5	9,6
Versuch: Mittelwert		12,1	9,7
Vorversuch:	1. Tag	12,6	17,2
	2. „	7,5	15,1
Versuch: C_9	1. „	11,6	10,2
	2. „	10,9	8,8
	3. „	8,7	12,6
Vorversuch: Mittelwert		10,1	10,2
Versuch: Mittelwert		10,4	10,5
Vorversuch:	1. Tag	9,0	[illegible]
	2. „	11,2	[illegible]
Versuch: C_{10}	1. „	22,1	8,8
	2. „	10,0	12,0
	3. „	11,7	10,7
Vorversuch: Mittelwert		10,1	[illegible]
Versuch: Mittelwert		14,0	[illegible]
Vorversuch:	1. Tag	9,2	[illegible]
	2. „	12,7	[illegible]
Versuch: C_{11}	1. „	7,9	[illegible]
	2. „	11,2	[illegible]
	3. „	12,[illegible]	[illegible]
Vorversuch: Mittelwert		11,0	[illegible]
Versuch: Mittelwert		10,5	[illegible]

Bei der fast konstanten Zusammensetzung der Kost stellte sich die Oxalsäureausscheidung bei der Mehrzahl der Versuchspersonen auf einen Mittelwert ein. Nur bei einigen Versuchspersonen schwankten die Werte innerhalb der mittleren Breite.

Bib No. 54

J. Pharmacol. 25:59-64. 1925.

THE NEPHROPATHIC ACTION OF THE DICARBOXYLIC ACIDS AND THEIR DERIVATIVES

III. ACIDS OF SIX TO NINE CARBONS[1]

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Having shown in previous communications (6) (7), that glutaric acid is severely nephropathic when injected subcutaneously as its sodium salt, and that malonic and succinic acids, under similar circumstances, are non-toxic, it appeared of interest to test the effects of some of the higher members of the oxalic acid series. It seemed possible that such studies might throw light on the mode of oxidation of dicarboxylic acids. If the latter, like the fatty acids, were burned by β-oxidation, pimelic (C_7-) and azelaic (C_9-) acids should yield glutaric acid as an intermediate metabolic product, while adipic (C_6-) and suberic (C_8-) acids should lead to the production of succinic acid. Inasmuch as glutaric acid is oxidized with difficulty in the organism of the rabbit, and is distinctly detrimental to the kidneys, its production in metabolism would doubtless occasion severe renal injury. Under such circumstances, higher dicarboxylic acids containing odd numbers of carbons should manifest more pronounced nephropathic effects than those containing even numbers of carbons. With these considerations in mind, experiments were carried out with adipic, pimelic, suberic, and azelaic acids. The results of blood analyses and of the Geraghty-Rowntree (3) renal-function tests were taken as criteria of nephropathicity. Fasting rabbits were used as the experimental animals, and the methods of

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ATTACHMENT C

Company Code

ADIPIC ACID

0	0	0	3

7- SAFETY INFORMATION

7b- Unpublished Studies

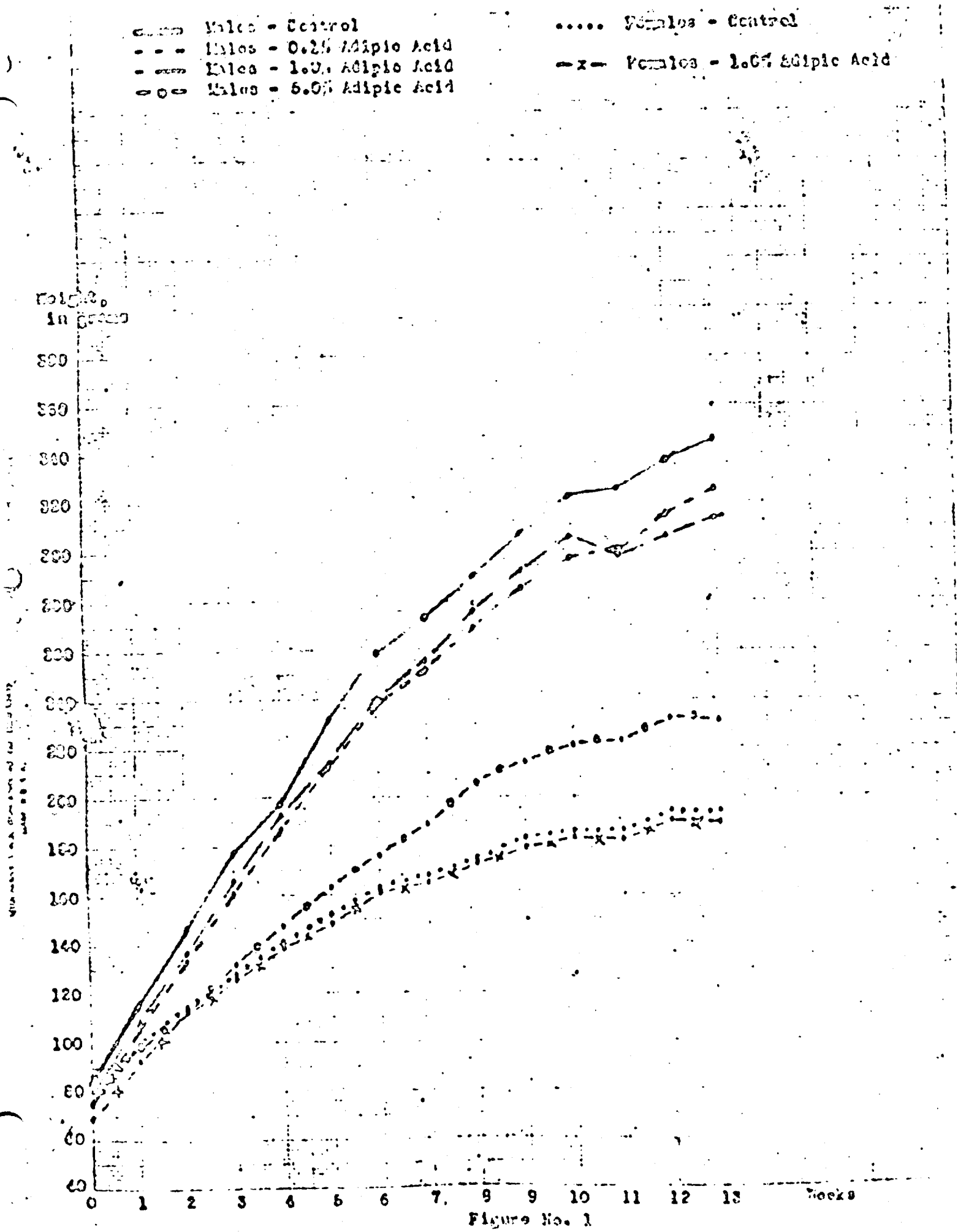
Males - Control
Males - 0.25% Adipic Acid
Males - 1.0% Adipic Acid
Males - 5.0% Adipic Acid
Females - Control
Females - 1.0% Adipic Acid
Weeks

Figure No. 1

Table No. 3 - (continued). Summary of autopsy findings for male and female albino rats which had received for approximately 90 days the basic laboratory diet, and the basic diet to which was added 0.1%, 1.0%, or 5.0% adipic acid.

RAT NUMBER	AUTOPSY FINDINGS
MALES - 5.0% ADIPIC ACID (continued)	
768	Died after 41 days Gastrointestinal tract: distention Peritoneum: irritated and hemorrhagic Bloody nasal discharge
769	Liver: parasytic
772	Liver: parasytic
773	Liver: parasytic
FEMALES - 1.0% ADIPIC ACID	
755	Adrenals: appeared enlarged
760	Liver: parasytic
763	Liver: parasytic
764	Liver: parasytic

Table No. 3 - Summary of autopsy findings for male and female albino rats which had received for approximately 90 days the basic laboratory diet, and the basic diet to which was added 0.1%, 1.0%, or 5.0% adipic acid.

RAT NUMBER	AUTOPSY FINDINGS
MALES - CONTROL	
715	Liver: parasytic
717	Liver: parasytic
723	Died after 31 days Lungs: consolidated Intestines: irritated and distended
MALES - 0.1% ADIPIC ACID	
735	Liver: parasytic
742	Liver: parasytic
MALES - 1.0% ADIPIC ACID	
745	Testes: one atrophied
748	Liver: parasytic
749	Liver: parasytic
750	Liver: parasytic Spleen: enlarged (weight, 2.0 grams) Abdominal wall: hard, tumorous mass adhered
753	Died after 34 days Pleural cavity: bloody fluid Stomach, large and small intestines: marked distention Scrotal sac: Bloody fluid Liver: pale Kidneys: soft and mushy Testes: pinkish-colored, veins did not show
MALES - 5.0% ADIPIC ACID	
766	Thyroid: appeared small
767	Thyroid: appeared small

for male and female albino rats receiving the basic laboratory diet, and the basic diet to which was added 0.1%, 1.0%, or 5.0% adipic acid.

COMPOUND	NUMBER OF RATS		AVERAGE BODY WEIGHT		RAT DAYS		
	Start	Finish	Start gm.	Finish gm.	Theoretical	Actual	% of Survival
MALES							
Control	10	9	84	346	910	850	93
0.1% Adipic Acid	10	10	76	325	930	930	100
1.0% Adipic Acid	10	9	81	313	920	862	94
5.0% Adipic Acid	10	9	84	231	910	860	95
FEMALES							
Control	10	10	75	194	920	920	100
1.0% Adipic Acid	10	10	69	189	920	920	100

	TOTAL FOOD AND ADIPIC ACID CONSUMED	FOOD CONSUMED		ADIPIC ACID CONSUMED	
	gm.	Total gm.	Av/Rat/Day gm.	Total gm.	Av/Rat/Day gm.
MALES					
Control		15057	18.64*		
0.1% Adipic Acid	18618	18599.38	20.00	18.62	0.02
1.0% Adipic Acid	16396	16232.04	18.83	163.96	0.19
5.0% Adipic Acid	18066	17162.70	19.96	903.30	1.05
FEMALES					
Controls		14281	16.68**		
1.0% Adipic Acid	16189	16027.11	17.42	161.89	0.18

* Based on 808 actual rat days since a portion of the food consumption for the final week was not determined.

** Based on 856 actual rat days since a portion of the food consumption for the final week was not determined.

Table No. 1 - Summary of average weekly body weights and food consumption, in grams, of male and female albino rats receiving the basic laboratory diet, and the basic diet to which was added 0.1%, 1.0%, or 5.0% adipic acid.

AVERAGE BODY WEIGHTS

Week Number	MALES Control	MALES 0.1% Adipic Acid	MALES 1.0% Adipic Acid	MALES 5.0% Adipic Acid	FEMALES Control	FEMALES 1.0% Adipic Acid
0	84	76	81	84	75	69
1	116	105	108	98	99	93
2	146	133	137	112	115	113
3	177	161	166	132	128	126
4	197	187	193	147	141	139
5	231	212	214	163	152	148
6	259	238	240	176	163	161
7	273	252	256	189	168	165
8	290	269	276	206	175	173
9	307	285	292	214	183	179
10	322	297	306	221	186	183
11	325	301	298	223	186	182
12	337	315	306	232	194	190
13	346	325	313	231	194	189

AVERAGE FOOD CONSULPTION

1	105	103	98	107	95	130
2	115	120	113	142	113	134
3	122	130	117	139	139	123
4	118	135	132	144	130	129
5	133	145	146	161	133	149
6	138	150	149	163	122	130
7	140	150	147	148	130	117
8	141	142	152	160	109	114
9	144	147	149	151	111	116
10	143	151	153	159	111	118
11	135	144	122	151	99	105
12	140	154	131	148	112	122
13	146	192	163	156	122	133

might influence food digestion and absorption. Histopathological examination of the various tissues may shed additional light upon this question.

Experimental:

The results of these experiments are summarized in the attached tables. Table No. 1 presents the weekly average body weight and food consumption for the various groups. The body weights are presented graphically in Figure No. 1. Table No. 2 summarizes the survival, body weight, total food and adipic acid consumed. Table No. 3 lists the grossly observable autopsy findings.

From these data it is apparent that 0.1% and 1.0% levels of adipic acid added to the diet of either male or female rats does not exert a significant influence on either the survival or the body weight. On the other hand, the male rats receiving 5.0% of adipic acid in the diet exhibited a marked retardation of growth during the entire interval. Since this is not associated with an alteration of food intake and there was no marked gross pathology at sacrifice, it is assumed that the growth retardation is associated with food utilization.

The design of these experiments does not provide a clue to the exact mechanism responsible for growth retardation at the 5.0% level. Since, however, the animals maintained a continuous weight increase over the 90-day period, presented a healthy, thrifty appearance, ate an average amount of food and showed no gross lesions, it does not appear that the mechanism is one of direct or specific toxicity. The acute toxicity experiments indicated that large doses of adipic acid solution were acutely irritating to the gastrointestinal tract. Since irritation was not grossly apparent at the conclusion of the feeding experiments although the average daily consumption per rat was approximately 1.0 gram, it is entirely possible that the low pH and high acid consumption

Company Code

ADIPIC ACID

7- SAFETY INFORMATION

UNPUBLISHED STUDIES

0	0	0	3

Date: March 14, 1950

Material: Adipic Acid

Lot No:

Subject: Subacute Feeding

A report dated January 2, 1950 presented data on acute toxicity of adipic acid by various routes of administration, with certain comparable data for citric and tartaric acids. The following report summarizes the data from 90-day subacute feeding tests on adipic acid added to the diet of male and female albino rats. A group of 10 animals received one of the following diets:

Males

Control
0.1% Adipic Acid
1.0% Adipic Acid
5.0% Adipic Acid

Females

Control
1.0% Adipic Acid

The basic diet consisted of specially ground Ken-L dog meal. The animals were individually housed and provided with water at all times. At weekly intervals each animal was weighed and the amount of food consumed was determined. At the end of the subacute feeding period the animals were sacrificed by chloroform inhalation and gross autopsies were performed. Representative tissues were preserved for histopathological examination.

TABLE 1

Expt. No.	Animals Used: Type	Animals Used: No.	Material Used	Dosage	No. Doses	Duration	Deaths	Pathology, Etc.
1	Rats (mature)	4	Adipic A. *	100 mg. (310-386 mg/K)	25	5 weeks	1 **	None *Adipic acid was given as 20% sol. in 95% ethyl alcohol. **Death from pneumonia.
	Rats (mature)	4	Adipic A. *	200 mg. (610-922 mg/K)	25	5 weeks		
2	Guinea Pigs	5	Adipic A. *	400 mg. (682-942 mg/K)	5	1 week		None *Adipic acid solid given in capsules.
				600 mg. (1032-1739 mg/K)	25	5 weeks		
3	Rats (Immature)	10	Na adipate*	199 mg. (638-1332 mg/K)	44	9 weeks	4**	None **Deaths all due to infection *Na adipate and Na acetate given in equimolecular doses in aqueous solution.
		10	Na acetate*	284 mg. (873-2202 mg/K)	44	9 weeks	2**	

There was a significantly greater incidence of weight loss in the animals treated with sodium adipate than in those treated with sodium acetate, both during the weekly period of treatment and during the week end of rest. The adipate group thus more frequently lost weight during the treatment period and showed a lower ability to recover during the week end of rest.

as
6.29.43

The Toxicity of Adipic Acid

Preliminary tests of the toxicity of adipic acid were planned to find, if possible, a lethal dosage of the material by mouth. White rats and guinea pigs were used. The experiments conducted are summarized briefly in Table 1.

Comparison of Growth Rate of Animals in Experiment 3.

Since adipic acid is not very soluble in water, and sodium adipate is soluble, control animals in experiment 3 were given a dosage of sodium acetate in equimolecular proportions to the dosage of sodium adipate given the test animals, as control of the possible action of the sodium ion.

During each week of study, the animals were fed adipate or acetate on five days (Monday through Friday), but no treatment was given on Saturday or Sunday. The rats were weighed on Monday morning and Friday afternoon, and from records of these weighings the average daily weight change for each rat was calculated for each weekly period of treatment, and each week end of no treatment. Data from the control group given sodium acetate was used to calculate control mean and standard deviation of daily weight changes during the treatment and non-treatment periods. A weight increase less than the mean minus twice the standard deviation was considered abnormal. (Abnormal weight changes were actually all weight losses.) The number of such abnormal weight losses was then counted for the sodium adipate group and compared with the number expected by chance alone. (In a very large number of observations weight changes equal to or less than the mean minus twice the standard deviation should not occur by chance alone in more than 2.5% of observations. In actual experiments, of course, a statistically large number of observations is not attainable, but for the actual experimental groups the expected chance occurrence of such abnormal weight losses is calculated.) The value

$$\frac{\text{Observed No. Abnormal Wt. Changes}}{\text{No. Abn. Wt. Changes Expected by Chance}}$$

if greater than 1, indicates a significant difference between the two groups. This value calculated for the sodium adipate group of rats for the period of study, January 25 to March 15, on the basis of weight changes in the sodium acetate treated rats was

Monday to Friday (treatment period)	1.95
Saturday and Sunday (rest period)	1.61

The Toxicity of Adipic Acid

Summary

Adipic acid, given by mouth as powder, as an alcoholic solution, or in the form of sodium adipate, over a relatively short period of time, produced no pathology in rats or guinea pigs. However, doses between 638 and 1332 mg. kilo given to immature rats on 44 occasions over 9 weeks produced significantly greater weight losses in these rats than in control rats fed equimolecular doses of sodium acetate.

If adipic acid and its salts are to be used as components of any form of food, more extensive study must be made to determine its actual physiological action and the reason for the failure of treated rats to gain weight.

NAS/NRC Questionnaire

June 29, 1943

ATTACHMENT C

Company Code

ADIPIC ACID

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7- SAFETY INFORMATION

7b- Unpublished Studies

The Toxicity of Adipic Acid

of adipic acid, which so far is not regarded as an intermediary product of physiological metabolism.

The administration of adipic acid is always followed by the increase of oxalic acid in the urine, whereas this increase is not detected in the experiment with muconic acid.

The investigations concerning the decomposition of adipic acid in the surviving liver are not yet completed. The results will be published later.

BIBLIOGRAPHY.

Bode, F., *Ann. Chem. u. Pharm.*, 1864, cxxxii, 95.
Hensel, M., and Riesser, O., *Z. physiol. Chem.*, 1913, lxxxviii, 38.
Jaffé, M., *Z. physiol. Chem.*, 1909, lxii, 58.
Limpricht, H., *Ann. Chem. u. Pharm.*, 1873, clxv, 253.
Markownikoff, W., *Ann. Chem. u. Pharm.*, 1898, cccii, 34.

over muconic acid. But my results to the contrary make Jaffé's assumption untenable. We are obliged to assume that the benzene is converted to but a slight extent if at all to muconic acid in the animal body.

TABLE IX.

Muconic Acid.

Date.	Rabbit.	Acid given.	Acid in urine.		Remarks.
		gm.	*gm.*	*per cent*	
Mar. 9–10	A	0.8	0.5904	73.08	Injected at one time.
Apr. 30–May 1	B	0.8	0.5840	73.00	" " " "
May 9–10	"	0.8	0.5928	74.10	" " four times.
" 11–12	C	0.8	0.5716	71.45	" " " "
" 15–16	"			72.91	Average.
		0.8	0.3490	43.63	Introduced in stomach.

TABLE X.

Adipic Acid.

Date.	Rabbit.	Acid given.	Acid in urine.		Remarks.
		gm.	*gm.*	*per cent*	
June 11–12	D	2.0	1.1035	55.18	Injected at one time.
" 21–22	"	2.0	0.9746	48.73	" " " "
" 29–30	"	2.0	1.0968	54.84	" " " "
July 6–7	"	0.8	0.4368	54.60	" " four times.
" 9–10	"	0.8	0.4798	59.86	" " one time.
" 12–13	E	2.0	1.1868	9.34	" " " "
" 20–21	"	0.8	0.5104	63.80	" " four times.
Feb. 13–14	A	0.8	0.5826	72.83	" " one time.
Mar. 25–26	F	0.8	0.5624	70.30	" " " "
May 16–17	B	0.8	0.5890	73.63	" " " "
Average				61.31	

The physiological relations of muconic acid or any of its derivatives, especially their relation to aromatic amino-acids might be decided through further investigations. In this paper I wish only to call attention to the fact that the decomposition of muconic acid itself in the animal organism occurs no more readily than that

TABLE VII.

Rabbit F, Weight about 2,830 Gm.

Date.	Adipic acid given.	Adipic acid in urine	Oxalic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*gm.*	*cc.*			
Mar. 24			0.0053	182	1.018	Slightly acid.	
" 25	0.8						Adipic acid injected subcutaneously at once in the form of its sodium salt.
" 26		0.5624	0.0064	165	1.025	Neutral.	
" 27			0.0058	170	1.018	Slightly acid.	
" 28			0.0055	152	1.020	" "	

TABLE VIII.

Rabbit B, Weight about 2,750 Gm.

Date.	Adipic acid given.	Adipic acid in urine.	Oxalic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*gm.*	*cc.*			
May 15			0.0072	270	1.013	Slightly alkaline.	
" 16	0.8						Adipic acid injected subcutaneously at once in the form of its sodium salt.
" 17		0.5890	0.0077	253	1.015	" "	
" 18			0.0055	255	1.014	" "	
" 19			0.0067	255	1.014	" "	

tered acid appeared again in the urine. For the sake of clearness, these results are collected in Tables IX and X.

Finding that muconic acid administered to a rabbit was almost completely oxidized in the body so that only 1 per cent of it appeared unchanged in the urine, Jaffé assumed that, if benzene is injected into a rabbit, 25 to 30 per cent of it may be decomposed

TABLE V.

Rabbit E, Weight about 3,450 Gm.

Date.	Adipic acid given.	Adipic acid in urine.	Oxalic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*gm.*	*cc.*			
June 11			0.0040	198	1.012	Slightly alkaline.	
" 12	2.0						Adipic acid injected subcutaneously at once in the form of its sodium salt.
" 13		1.1868	0.0144	122	1.028	" "	
" 14			0.0047	175	1.014	" "	
" 15			0.0045	175	1.014	" "	
" 20	0.8						Adipic acid injected subcutaneously at four times as before.
" 21		0.5104	0.0072	247	1.021	" "	
" 22			0.0053	180	1.012	" "	
" 23			0.0051	168	1.013	" "	

TABLE VI.

Rabbit A, Weight about 3,450 Gm.

Date.	Adipic acid given.	Adipic acid in urine.	Oxalic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*gm.*	*cc.*			
Feb. 11			0.0048	167	1.020	Slightly acid.	
" 12			0.0058	182	1.016	Neutral.	
" 13	0.8						Adipic acid injected subcutaneously at once in the form of its sodium salt.
" 14		0.5826	0.0056	182	1.018	Slightly acid.	
" 15			0.0048	190	1.015	Neutral.	
			0.0042	184	1.014	Slightly acid.	

TABLE IV.

Rabbit D, Weight about 2,700 Gm.

Date.	Adipic acid given.	Adipic acid in urine.	Oxalic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*gm.*	*cc.*			
June 10			0.0048	180	1.012	Slightly alkaline.	
" 11	2.0						Adipic acid injected subcutaneously at once in the form of its sodium salt.
" 12		1.1035	0.0130	146	1.025	" "	
" 13			0.0060	198	1.012	" "	
" 14			0.0042	180	1.011	" "	
" 21	2.0						Same as for June 11.
" 22		0.9746	0.0190	125	1.025	Slightly acid.	
" 23			0.0042	215	1.013	Slightly alkaline.	
" 24			0.0064	192	1.013	" "	
" 29	2.0						Same as for June 11.
" 30		1.0968	0.0189	127	1.025	Slightly acid.	
July 1			0.0042	213	1.013	Slightly alkaline.	
" 2			0.0051	167	1.017	" "	
" 6	0.8						Adipic acid was dissolved in 20 cc., neutralized by sodium carbonate, divided into four portions, each portion injected simultaneously at intervals of 3 hrs.
" 7		0.4368		325	1.015	Slightly acid.	The urine was collected during 36 hrs. after the first injection, and adipic acid in it was estimated.
" 10	0.8						Adipic acid injected subcutaneously at once in the form of its sodium salt.
" 11		0.4789	0.0084	135	1.020	Slightly alkaline.	
" 12			0.0077	137	1.018	" "	
" 13			0.0046	133	1.012	" "	

All specimens of adipic acid obtained from the urine were combined, recrystallized, and identified by analysis.

0.2058 gm. substance (dried at 105–110°C.) gave 0.3712 gm. CO_2 and 0.1305 gm. H_2O.

	Calculated for $C_6H_{10}O_4$: *per cent*	Found: *per cent*
C	49.29	49.19
H	6.90	7.10

TABLE III.

Rabbit C, Weight about 3,400 Gm.

Date.	Muconic acid given.	Muconic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*cc.*			
May 11	0.8					
" 12		0.5716	114	1.022	Slightly alkaline.	
" 13, 12 hrs. later.		Trace.	184	1.015	" "	
" 14, 24 hrs. later.		Not present.	125	1.016	" "	
" 15	0.8	0.340			" "	Muconic acid in form of its sodium salt was dissolved in 20 cc. of water and given by stomach tube.
" 16		Not present.	230	1.015	" "	
" 17			157	1.015	" "	

In every experiment with muconic or adipic acid I have estimated the oxalic acid in the feces of the 24 hours before and after the injection of one acid or the other, but found no difference in any two specimens (data omitted).

As the tables show, my conclusions, contrary to those of Jaffé, indicate that muconic acid was for the greater part eliminated unchanged in the urine. The result obtained with adipic acid was similar to that of Kotake, and the greater part of the adminis-

TABLE II.

Rabbit B, Weight about 2,750 Gm.

Date.	Muconic acid given.	Muconic acid in urine.	Oxalic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*gm.*	*cc.*			
Apr. 30	0.8						Muconic acid injected subcutaneously at once in the form of the sodium salt.
May 1		0.5840	0.0069	210	1.020	Slightly acid.	
" 2			0.0067	265	1.014	Slightly alkaline.	
" 7–8			0.0143	345	1.016	" "	Oxalic acid estimated in urine collected during 36 hrs.
" 9	0.8						Muconic acid dissolved in 20 cc. of water, neutralized by sodium carbonate, divided into four portions, and each portion injected subcutaneously at intervals of 3 hrs.
" 9–10		0.5928	0.0146	145	1.025	Slightly acid.	Urine of the 24 hrs. after the first injection and urine of the following 12 hrs. separately collected; muconic acid was estimated in each specimen. Oxalic acid estimated at one time in all the urine of the 36 hrs.
		Trace.		132	1.015	Neutral.	
" 10–11			0.0148	240	1.015	Slightly alkaline.	
				115	1.020	" "	

The mother liquor, which reacted alkaline on account of the presence of ammonia, was evaporated on the water bath to about 50 cc., and after cooling strongly acidified by means of hydrochloric acid, and was extracted fifteen times with ether. The ether was distilled to about 50 cc. and then decanted into a beaker and allowed to stand in the air. Muconic or adipic acid, eliminated unchanged in the urine, crystallized out. This was carefully purified and weighed.

All results obtained are summed up in Tables I to VIII.

TABLE I.

Rabbit A, Weight about 3,450 Gm.

Date.	Muconic acid given.	Muconic acid in urine.	Oxalic acid in urine.	Urine.	Specific gravity.	Reaction.	Remarks.
	gm.	*gm.*	*gm.*	*cc.*			
Nov. 3			0.0084	218	1.013	Slightly alkaline.	
" 4			0.0088	195	1.015	" "	
" 5	0.8						Muconic acid injected subcutaneously in the form of the sodium salt.
" 6			0.0085	218	1.015	Slightly acid.	
" 24	0.8						Same as for Nov. 5.
" 25			0.0087	194	1.019	" "	
Mar. 9	0.8						Same as for Nov. 5.
" 10		0.5904	0.0040	153	1.024	Neutral.	

All specimens of muconic acid obtained from the urine crystallized out in prisms of a brownish color. They were dissolved together in water with sodium carbonate, decolorized with animal charcoal, and then thoroughly acidified with sulfuric acid whereupon muconic acid separated out. It was filtered off, recrystallized from alcohol (80 per cent), and identified by analysis.

0.2351 gm. substance (dried at 100°C. in vacuum) gave 0.4356 gm. CO_2 and 0.0935 gm. H_2O.

	Calculated for $C_6H_6O_4$: *per cent*	Found: *per cent*
C	50.70	50.53
H	4.22	4.45

adipic—in the animal body should be simultaneously and exactly repeated, so that both results may be accurately compared. Following this suggestion of Kotake's, I undertook to repeat the experiments of Jaffé and of Kotake.

EXPERIMENTAL.

Muconic and adipic acids used in the experiments were synthetically prepared in the following ways.

Mucic acid obtained by the action upon milk sugar was, at first, converted into dichloromuconic acid by means of phosphorus pentachloride, according to the description of Bode, and then into hydromuconic acid. Finally, the latter acid was changed into muconic acid over dibromomuconic acid according to Limpricht and Marquardt.

Adipic acid was prepared according to Markownikoff by the action of nitric acid upon cyclohexane which was obtained through the reduction of benzene according to Sabatier and Senderens.

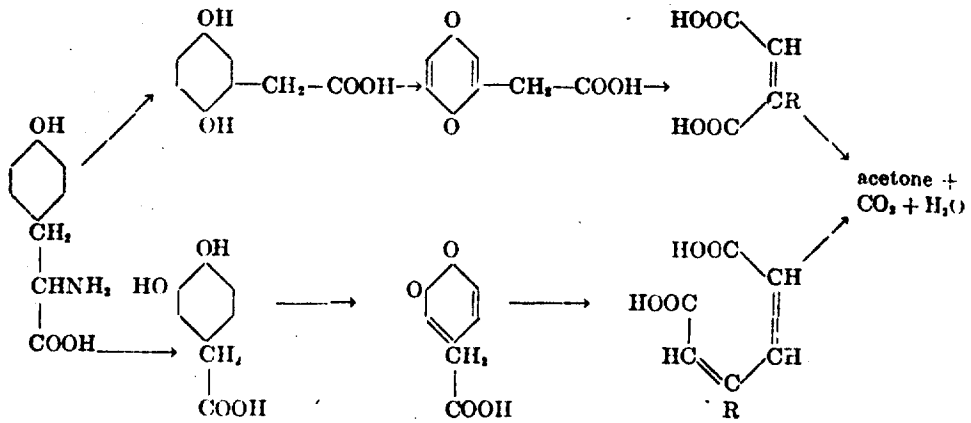
The experiments were carried out on rabbits fed with okara (a residue obtained from bean curds). The animals were subcutaneously injected with muconic and adipic acids, previously neutralized by means of sodium carbonate. Each specimen of the urine collected during the following 24 hours was examined.

It may be probable that adipic acid is eventually oxidized into oxalic acid, this oxidation taking place according to the β-oxidation of Knoop.

$$\begin{array}{c} COOH - CH_2 - CH_2 - CH_2 - CH_2 - COOH \\ \downarrow \\ (COOH - CH_2 - CH_2 - COOH) \\ \downarrow \\ COOH - COOH \end{array}$$

Recently Hensel and Riesser found that the surviving liver forms acetone bodies from muconic acid.

Consequently in my experiments with these acids I intended, on the one hand, to estimate the rate of their vital cleavage and on the other, to investigate the increase of oxalic acid in the urine, and further to examine the formation of acetone bodies from adipic acid in the surviving liver. The quantitative determination was carried out by the process of Autenrieth and Barth, the acid being isolated in the form of calcium salt and then converted into calcium oxide.



Previously in the laboratory of Jaffé, Kotake carried out a feeding experiment with tyrosine. The urine of a rabbit was examined for muconic acid or any of its derivatives, with negative result. Later Kotake, working on investigations of the fate of bivalent fatty acids in the animal body, found that adipic acid was with difficulty subjected to animal combustion and that a greater part of the acid administered was eliminated unchanged in the urine.

In chemical constitution muconic acid differs from adipic acid only in that the former possesses two double linkages, while the latter is a saturated compound having the following formulas.

$$\underset{\text{(muconic acid)}}{COOH - CH = CH - CH = CH - COOH}$$

$$\underset{\text{(adipic acid)}}{COOH - CH_2 - CH_2 - CH_2 - CH_2 - COOH}$$

It is well known that there is considerable difference between saturated and unsaturated compounds in their behavior towards many chemical reagents. But so far this difference has not been verified in animal oxidation in which the combustibility of substances is chiefly decided by other physiological relations. Consequently the easy combustibility of muconic acid in the animal body contrary to the behavior of adipic acid, appears to indicate an important physiological meaning. It is therefore desirable that the investigations as to the fate of both acids—muconic and

Mori-10
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THE DECOMPOSITION OF MUCONIC AND ADIPIC ACIDS IN THE ANIMAL BODY.

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Some aromatic substances readily undergo combustion in the animal organism with the splitting of the benzene ring. It is well known that aromatic amino-acids derived from protein hydrolysis, tyrosine and phenylalanine, are completely oxidized in the body. Most investigators believe that these amino-acids are decomposed over homogentisic acid. In fact the latter is easily burned in the animal body and capable of yielding acetone bodies in the perfusion experiment with a surviving liver. However, the manner in which homogentisic acid is converted into acetone bodies is unknown.

Jaffé discovered an unsaturated acid, namely, muconic acid, in the urine of dogs and rabbits to which benzene had been given, and by that fact demonstrated for the first time the demolition of the benzene nucleus.

At that time Jaffé found that his acid was readily combustible in the animal body, so that when injected into a rabbit to the amount of 2 gm., only 1 per cent was eliminated unchanged in the urine. According to the result of Jaffé's experiment, muconic acid or any of its derivatives might be regarded as intermediary products during the process of the vital cleavage of tyrosine and phenylalanine. Fromherz and Hermanns believe that these last two acids decompose through a dual path as follows:

400 mg ergeben sich geringe histologische Veränderungen an Leber und Niere und schwerere an der Darmschleimhaut. Störungen des Wachstums oder sonstige äußerlich erkennbare Symptome werden bei dieser Dosierung nicht gefunden. Tagesdosen von 800 mg bewirken Durchfälle, Veränderungen des Haarkleides und des Verhaltens der Tiere sowie signifikante Verzögerungen des Wachstums. Abgesehen von den Befunden am Darm sind die histologisch nachweisbaren Organveränderungen gering.

Bei einer eiweißarmen Diät mit 11% Protein wirken schon 400 mg Adipinsäure täglich wachstumshemmend.

Trotz einer beträchtlichen täglichen Ausscheidung von Adipinsäure im Harn weist die Niere keine schweren anatomischen Veränderungen auf.

Der Umfang der Ausscheidung von Adipinsäure ist bei an Adipinsäure gewöhnten und ungewöhnten Ratten gleich. Der Organismus erlahmt nicht die Fähigkeit, vermehrte Mengen der Substanz umzusetzen.

Die durch die Verfütterung größerer Adipinsäuredosen bedingten Schäden betreffen in erster Linie die Darmschleimhaut.

Literatur.

[1] Baer u. Blum: Hofmeisters Beiträge **11**, 101 (1908). — [2] Mori, Y.: J. of Biol. Chem. **35**, 341 (1918). — [3] Flaschenträger, B.: Hoppe Seylers Z. **159**, 297 (1926). — [4] Bernhard, K., u. M. Andreae: Hoppe Seylers Z. **245**, 103 (1937). — [5] Verkade, P. E., J. van der Lee u. A. S. van Alphen: Hoppe Seylers Z. **250**, 47 (1937). — [6] Verkade, P. E., J. van der Lee u. A. S. van Alphen: Hoppe Seylers Z. **252**, 163 (1938). — [7] Weitzel, G.: Ber. Math. Phys. Klasse Sächs. Akad. Wiss. (Leipzig) **93**, 9 (1941). — [8] Lang, K., u. K. H.Ässler: Biochem. Z. **323**, 456 (1953).

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Ratten bezüglich der Ausscheidung der Substanz im Harn. Der Organismus gewinnt offensichtlich durch eine längere Verabreichung der Substanz nicht die Fähigkeit, die Substanz besser im Stoffwechsel umzusetzen, etwa durch eine adaptive Enzymbildung bedingt. Weiterhin ist zu ersehen, daß die Höhe der Ausscheidung — jedenfalls in dem untersuchten Bereiche — unabhängig von der Dosis ist. Wie auch frühere Untersucher [7] finden wir ebenfalls starke individuelle Verschiedenheiten in der Ausnutzung der Adipinsäure.

Tabelle 5. *Bilanzversuche an an Adipinsäure gewöhnten und nicht gewöhnten Ratten.* Die Tiere wurden 14 Tage hindurch mit täglich 400 bzw. 800 mg Adipinsäure gefüttert. Die verfütterte Gesamtdosis betrug somit 5,6 bzw. 11,2 g.

Verfütterte Gesamtdosis g	Gewöhnte Ratten		Ungewöhnte Ratten	
	Gesamtausscheidung g	% der Dosis ausgeschieden	Gesamtausscheidung g	% der Dosis ausgeschieden
5,6	0,13	2,4	0,54	9,6
5,6	0,44	7,8	0,63	11,2
5,6	0,84	15,0	0,78	14,0
5,6	0,90	16,1	0,87	15,6
5,6	1,12	20,0	1,19	21,2
5,6	1,17	20,8	1,49	26,6
5,6	1,27	22,7	2,06	36,7
5,6	1,31	23,4	3,36	60,0
5,6	1,59	28,5		
5,6	1,67	29,8		
11,2	0,76	6,8	0,42	3,8
11,2	0,79	7,0	0,60	5,4
11,2	0,87	7,7	0,65	5,8
11,2	1,76	15,7	1,14	10,2

Mit dem zur Adipinsäurebestimmung benützten gereinigten Ätherextrakt des Harns führten wir noch papierchromatographische Untersuchungen durch (über die Methodik siehe K. Lang und K. H. Bässler [8]). Außer der Adipinsäure und kleinen Mengen Oxalsäure und Bernsteinsäure, wie sie in jedem normalen Harn vorkommen, konnten wir keine Säure nachweisen. Es entstehen also keine Zwischenprodukte beim Abbau der Adipinsäure, die angegriffene Substanz wird vollständig im Stoffwechsel oxydiert. Dies deckt sich mit den Beobachtungen von Lang und Bässler [8] über den Abbau der Dicarbonsäuren durch das Cyclophorasesystem. Auch in diesen Untersuchungen zeigte es sich, daß die Affinität des Cyclophorasesystems zu der Adipinsäure nicht sehr groß ist, daß aber ein einmal angegriffenes Molekül der Säure sofort quantitativ zu CO_2 und HO_2 oxydiert wird.

Zusammenfassung.

In langfristigen Fütterungsversuchen (bis zu 33 Wochen) an über 200 Ratten wird gezeigt, daß Ratten Tagesdosen von weniger als 400 mg Adipinsäure ohne nachweisbare Schäden vertragen. Bei einer Dosis von

(die Hämoglobinwerte betrugen 69—74%) einen von der Norm abweichenden Befund. Die leichte Anämie aller Gruppen (bei der Kontrollgruppe ohne Adipinsäure war der Hämoglobinwert 70%) erklärt sich zwanglos aus der nicht optimalen Eiweißzufuhr.

Die *histologische Untersuchung*[1] ergab bei den Tieren, die weniger als 400 mg Adipinsäure pro Tag erhalten hatten, nichts Auffallendes. Die histologischen Befunde bei den mit größeren Adipinsäuredosen behandelten Tieren waren von der Höhe der Eiweißzufuhr unabhängig.

An der *Niere* ergaben sich keine spezifischen Befunde. Auffallend waren starke Regenerationsvorgänge in den Hauptstücken, kenntlich an einer unverhältnismäßig großen Anzahl von Mitosen. Dieser geringfügige Befund ist um so bemerkenswerter, als die Tiere längere Zeit (8—23 Wochen) regelmäßig beträchtliche Mengen Adipinsäure im Harn ausgeschieden hatten.

Auch an der *Leber* waren keine schwereren Veränderungen nachweisbar. Bei den Tieren, die 400 und 800 mg Adipinsäure erhalten hatten, ließ sich stellenweise eine Vergrößerung der Zellkerne sowie eine Vermehrung der Zahl der Zellen mit zwei und mehr Kernen beobachten. Eine Strukturveränderung der Kerne war nicht nachzuweisen. Mitunter wurde eine Vergrößerung des gesamten Zellvolumens festgestellt. Der auffallendste Befund in der Leber war eine Zunahme der Zahl der Kupfferschen Sternzellen, verbunden mit einer Vergrößerung dieser Zellen.

Der *Darm* bot das Bild eines chronisch entzündlichen Zustandes. Das Resorptionsepithel war höher als normal, die Zellen erschienen verlängert und plasmareicher; die Kerne waren sehr voluminös und basalwärts verlagert. Die Abgrenzung des Epithels gegen die Unterlage war unscharf und die Begrenzung gegen die tunica propria höckerig. Der Resorptionssaum des Epithels war stark verquollen. Die Zahl der Becherzellen war vermehrt. Es bestand ein starkes Regenerationsbestreben, kenntlich an den zahlreichen Mitosen in den Krypten. Das lymphatische Gewebe in der tunica propria war wesentlich vermehrt.

Die Unverträglichkeit höherer Adipinsäuredosen beruht also offensichtlich in der Hauptsache auf einer Schädigung der Darmschleimhaut, die zu schweren Durchfällen und einer verminderten Ausnutzung der Nahrung Anlaß gibt.

Die Tab. 5 zeigt das Ergebnis der Bilanzversuche. Die an Adipinsäure gewöhnten Ratten waren der Versuchsreihe III entnommen worden und hatten 20—25 Wochen vorher schon täglich die angegebenen Adipinsäuremengen erhalten. Wie man sieht, besteht kein deutlicher Unterschied zwischen den an Adipinsäure gewöhnten und den nicht gewöhnten

[1] Herrn Prof. Dr. Watzka vom Anatomischen Institut unserer Universität sind wir für die freundliche Auswertung der histologischen Präparate zum größten Dank verpflichtet.

gefütterten. Die Todesfälle verteilten sich wie folgt: 1 in der ersten, 3 in der zweiten, 5 in der dritten, 1 in der vierten Woche. Auch die Verteilung der Todesfälle auf die ersten Wochen zeigt, daß die Hauptschädigung in den Beginn der Versuchszeit fällt. Aus der Gewichtskurve und aus dem äußeren Verhalten der Ratten, die 400 mg Adipinsäure erhielten, ergab sich kein Hinweis auf eine Unverträglichkeit dieser Dosis.

Um etwaige toxische Wirkungen der Adipinsäure stärker in Erscheinung treten zu lassen, wurden in einer vierten Versuchsreihe Ratten bei einer Eiweißmangeldiät mit Adipinsäure behandelt. Das Futter bestand nur aus Weizen, ergänzt mit Lebertran. Dadurch war die Eiweißzufuhr auf 11% herabgesetzt. Zudem erhielten die Tiere damit ein biologisch nicht mehr so hochwertiges Eiweiß wie in den ersten drei Versuchsreihen.

Tabelle 4. *Gewichtszunahme und Mortalität von Ratten, die bei einer Eiweißmangeldiät (11% Protein) mit Adipinsäure gefüttert wurden.*
(Zahlen in Klammern = Anzahl der Tiere, aus denen die Mittelwerte errechnet wurden.)

Tägliche Adipinsäure-Dosis mg	Anfangsgewicht g	Gewicht nach 6 Wochen g	Gewicht nach 19 Wochen g	Zahl der verstorbenen Tiere
0	54	102 ± 16 (10)	200 ± 28 (5)	2
50	54	103 ± 9 (10)	179 ± 32 (7)	0
100	53	94 ± 14 (10)	172 ± 39 (5)	1
200	54	104 ± 13 (8)	182 ± 33 (5)	2
400	55	79 ± 14 (10)	144 ± 26 (5)	2

In der Tab. 4 sind die Befunde über das Wachstum der Tiere zusammengestellt. Durch interkurrente Todesfälle, für die eine plausible Todesursache nicht zu ermitteln war, und Tötung von Tieren zwecks anatomischer Untersuchung in der 7.—8. Woche nahm die Zahl der Versuchstiere in den späteren Wochen des Versuches stark ab. Der Versuch zeigt deutlich, daß die Toxizität der Adipinsäure bei einer unzureichenden Eiweißzufuhr größer ist als bei einer optimalen Ernährung. Während in den Versuchsreihen 2 und 3 sich kein Hinweis für eine Schädigung der Tiere, die bis zu einer Hemmung des Wachstums führt, ergeben hatte, war bei der verringerten Eiweißzufuhr das Wachstum der mit 400 mg Adipinsäure gefütterten Ratten signifikant gegenüber der Kontrollgruppe verzögert. Die Wachstumshemmung war auch noch nach 19 Wochen vorhanden. Die sonst beobachtete rasche Erholung der Tiere bei hoher Adipinsäurezufuhr war hier nicht eingetreten. Außer der Wachstumshemmung wiesen die Tiere keine äußerlich auffallenden Befunde auf, sie hatten keine Durchfälle, zeigten ein normales Haarkleid und waren nicht apathisch. Die Kontrolle des Blutbildes (Hämoglobingehalt), Zahl der Erythrocyten und Leukocyten, Differentialblutbild) ergab bei keiner Gruppe dieser Versuchsreihe außer einer geringgradigen Anämie

während dieser Zeit struppig und glanzlos. Im Verlauf der vierten und fünften Woche erholten sich diese Ratten. Sie wurden lebhafter, und ihr Fell wurde glatt und glänzend.

Tabelle 2. *Gewichtszunahme von Ratten, die 5 Wochen mit Adipinsäure gefüttert worden waren.*

Tägliche Adipinsäuredosis g	Anfangsgewicht g	Endgewicht g	Zahl der Versuchstiere
0	49 ± 7*	154 ± 20*	18
200	52 ± 7	156 ± 26	18
400	44 ± 5	139 ± 15	18
800	47 ± 7	100 ± 11	15

Da die Versuchsreihe II eindeutig ergeben hatte, daß die Zufuhr von 800 mg Adipinsäure im Tag zu Schädigungen der Gesundheit führt, wurde eine weitere Versuchsreihe unternommen, um bei einer langen Dauer des Versuchs (33 Wochen) zu klären, ob nicht schon 400 mg Adipinsäure im Tag toxisch wirken. Die wichtigsten Ergebnisse sind in der Tab. 3 zusammengefaßt.

Tabelle 3. *Gewichtszunahme und Mortalität von Ratten, die 33 Wochen hindurch mit Adipinsäure gefüttert wurden.*

Tägliche Adipinsäure-Dosis mg	Anfangsgewicht g	Gewicht nach 8 Wochen	Gewicht nach 33 Wochen	Zahl der Tiere	Zahl der gestorbenen Tiere
0	74	207 ± 35		11	4
400	74	183 ± 25	325 ± 25	9	4
800	73	154 ± 24	320 ± 31	4	10

In diese Versuchsgruppe waren einige gravide Weibchen aufgenommen worden. Es zeigte sich, daß auch die hohen Adipinsäuredosen ohne sichtbare Wirkung auf sie waren. Sie warfen Junge und waren auch in der Lage diese aufzuziehen. Diese Tiere sind in der Tab. 3 nicht mit berücksichtigt. Ebenso sind in dieser Tabelle nicht mit aufgenommen diejenigen Tiere, die zur histologischen Untersuchung getötet wurden. Die während der Versuchszeit verstorbenen Tiere sind bei den Gewichtsangaben gleichfalls nicht mit berücksichtigt. Auch in dieser Versuchsreihe ergab sich eine eindeutige toxische Wirkung der Dosis von 800 mg im Tag. Die Gewichtszunahme der mit dieser Dosis gefütterten Tiere war signifikant geringer als die der Kontrollgruppe. Außerdem zeigte diese Gruppe eine hohe Mortalität. Genau wie in der Versuchsreihe II erkrankten die mit 800 mg Adipinsäure gefütterten Ratten mit starken Durchfällen und wiesen ein struppiges und glanzloses Fell auf. Sie waren im Gegensatz zu den Tieren der anderen Gruppen apathisch. Aber auch in der Versuchsreihe III waren diese Veränderungen nur in den ersten drei Wochen zu beobachten. Die Tiere erholten sich zunehmend und erreichten nach 33 Wochen dasselbe Gewicht wie die mit 400 mg Adipinsäure

vollständig verzehrt wurde. Sie betrug anfänglich 10 g pro Tier und Tag und wurde mit steigendem Gewicht der Tiere entsprechend erhöht. Die Gewichtszunahme der Tiere wurde laufend verfolgt. Versuchsdauer 5 Wochen.

Versuchsreihe III. Männliche und weibliche Ratten mit einem Anfangsgewicht von 60—80 g erhielten im Tag 0 bzw. 400 und 800 mg Adipinsäure (mit NaOH) neutralisiert im Futter, das aus 80% Weizenschrot und 20% Vollmilchpulver bestand. Die Futtermenge betrug 10 g pro Tier und Tag und wurde mit dem Wachstum der Tiere entsprechend erhöht. Die Versuchsdauer betrug 33 Wochen. Nach 8 und 23 bzw. 25 Wochen wurden Tiere zur histologischen Untersuchung getötet.

Versuchsreihe IV. Männliche Ratten mit einem Anfangsgewicht von 40—60 g erhielten bei einem eiweißarmen Futter im Tag 0 bzw. 50, 100, 200 und 400 Adipinsäure (mit NaOH neutralisiert). Das Futter bestand nur aus Weizenschrot, ergänzt mit 0,2 cm³ Lebertran pro Ratte und Tag. Es enthielt 11% Protein (N × 6,25). Die Futtermenge wurde den Tieren, wie in den anderen Versuchsreihen beschrieben, zugemessen. Die Versuchsdauer betrug 19 Wochen. In der achten Woche wurden aus jeder Gruppe 3 Tiere zur histologischen Untersuchung getötet. In diesem Versuch wurde das Blutbild der Tiere verfolgt.

Bilanzversuche. An Adipinsäure gewöhnte und ungewöhnte Ratten wurden in Stoffwechselkäfigen gehalten und erhielten in 14 Tagen insgesamt 5,6 g (täglich je 400 mg) oder 11,2 g (täglich je 800 mg) Adipinsäure bei der Standarddiät zu fressen. Die an Adipinsäure gewöhnten Ratten waren Tiere, die 20 Wochen lang je 400 mg Adipinsäure pro Tag oder 25 Wochen je 800 mg Adipinsäure im Tag erhalten hatten. Der Harn jedes der Tiere wurde unter Toluol während der gesamten Versuchsdauer gesammelt. Die Adipinsäurebestimmung erfolgte dann nach der Methode von WEITZEL [7].

Das Ergebnis der Versuchsreihe I ist in der Tab. 1 zusammengestellt. Bei der in dieser Versuchsreihe angewendeten niederen Dosis ergab sich

Tabelle 1. *Gewichtszunahme von Ratten, die 4 Wochen hindurch mit Adipinsäure gefüttert worden waren.*

Tägliche Adipinsäure-Dosis mg	Gewichtszunahme g	Zahl der Versuchstiere
0	55 ± 13*	17
10	52 ± 14	18
20	62 ± 11	20
40	68 ± 16	17

* Mittlere Abweichung $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$.

weder in der Wachstumskurve noch im sonstigen Verhalten der Tiere etwas Auffallendes. Infolgedessen wurde eine zweite Versuchreihe mit einer wesentlich höheren Dosierung der Adipinsäure durchgeführt. Ihr Ergebnis ist in der Tab. 2 zusammengefaßt. Die Gewichtszunahmen der mit 200 und 400 mg Adipinsäure gefütterten Ratten zeigten gegenüber denjenigen der Kontrolltiere keinen signifikanten Unterschied. Dagegen war das Wachstum der Tiere, die 800 mg Adipinsäure im Tag erhalten hatten, signifikant, gegenüber dem der Kontrolltiere vermindert. Sie litten in den ersten 2—3 Wochen an schweren Durchfällen. Ihr Fell war

Lang-'53
Bib No. 34

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Über den Stoffwechsel und die Verträglichkeit der Adipinsäure.

Von

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Über den Stoffwechsel der Adipinsäure liegen schon eine Reihe von Mitteilungen [1—7] vor. Übereinstimmend haben alle Untersucher festgestellt, daß an Menschen oder Versuchstiere (Hunde, Kaninchen) verfütterte oder injizierte Adipinsäure nur zum Teil im Organismus verwertet wird. Ein mehr oder minder beträchtlicher Prozentsatz der verabreichten Dosis (etwa 5—50%) wird unverändert im Harn ausgeschieden. Die bisherigen Angaben beziehen sich lediglich auf kurzfristige Versuche, in denen die Adipinsäuredosis auf einen oder nur wenige Tage verteilt gegeben wurde. Im Zusammenhang mit dem Problem des Abbaues von Dicarbonsäuren im Organismus interessierte uns auch die Frage, wie der Tierkörper auf eine längere Zeit hindurch fortgesetzte Zufuhr der Substanz reagiert und ob sich eine Steigerung der Fähigkeit, die Substanz im Stoffwechsel zu verwerten, beobachten läßt.

Zur Klärung dieser Fragen verfütterten wir an Ratten täglich bis zu 800 mg Adipinsäure. Die Versuchsdauer betrug bis zu 33 Wochen. Da sich Schädigungen bei einem eiweißarmen Futter rascher und stärker zu entwickeln pflegen, wurde in einer besonderen Versuchsreihe die Wirkung der Adipinsäure bei einer Diät mit wenig und dazu nicht sehr hochwertigem Eiweiß überprüft. In den Fütterungsversuchen wurde das Blutbild der Tiere laufend kontrolliert. Außerdem wurden in Abständen Tiere getötet und anatomisch (makroskopisch und mikroskopisch) untersucht. In einer gesonderten Versuchsreihe wurden endlich noch genaue Bilanzuntersuchungen durchgeführt.

Methodik.

Versuchsreihe I. Weibliche Ratten von einem Anfangsgewicht von durchschnittlich 92 g erhielten bei einer Standarddiät täglich 0 bzw. 10, 20 oder 40 mg Adipinsäure im Futter. Die Standarddiät bestand aus 80% geschrotetem Weizen und 20% Vollmilchpulver. Die Tiere konnten von dem Futter ad libitum fressen. Die Gewichtszunahme der Tiere wurde laufend verfolgt. Versuchsdauer 4 Wochen.

Versuchsreihe II. Männliche Ratten mit einem Anfangsgewicht von 40—60 g erhielten im Tag 0 bzw. 200, 400 und 800 mg Adipinsäure (mit NaOH) neutralisiert. Das Futter bestand gleichfalls aus der Standarddiät (80% Weizenschrot und 20% Vollmilchpulver). Die Futtermenge wurde so bemessen, daß sie von den Tieren

diarrhea, alterations of the fur and the behavior of the animal, as well as significant retardations of growth. Aside from the findings in the intestine, the histologically detectable organ alterations are slight.

In the case of a protein-poor diet with 11% protein, 400 mg adipic acid per day are enough to inhibit growth.

Despite a considerable daily excretion of adipic acid in the urine, the kidneys reveal no marked anatomical alterations.

The degree of excretion of adipic acid is equal in the case of both rats accustomed to adipic acid and those unaccustomed to it. The organism does not attain the capacity to convert increased amounts of the substance.

The damages due to the feeding of larger doses of adipic acid affect above all the intestinal mucosa.

Literature

[1] BAER u. BLUM: Hofmeisters Beiträge 11, 101 (1908). — [2] MORI, Y.: J. of Biol. Chem. 35, 341 (1918). — [3] FLASCHENTRÄGER, B.: Hoppe Seylers Z. 159, 297 (1926). — [4] BERNHARD, K., u. M. ANDREAE: Hoppe Seylers Z. 245, 103 (1937). — [5] VERKADE, P. E., J. VAN DER LEE u. A. S. VAN ALPHEN: Hoppe Seylers Z. 250 47 (1937). — [6] VERKADE, P. E., J. VAN DER LEE u. A. S. VAN ALPHEN: Hoppe Seylers Z. 252, 163 (1938). — [7] WEITZEL, G.: Ber. Math. Phys. Klasse Sächs. Akad. Wiss. (Leipzig) 93, 9 (1941). — [8] LANG, K., u. K. H. BÄSSLER: Biochem. Z. 323, 456 (1953).

Table 5. Balance experiments on rats accustomed and unaccustomed to adipic acid.
The animals were fed 400 or 800 mg adipic acid daily for 14 days.
The total dose fed thus amounted to 5.6 or 11.2 g.

A.	B.		C.	
Verfütterte Gesamtdosis g	Gewöhnte Ratten 1. Gesamtausscheidung g	2. % der Dosis ausgeschieden	Ungewöhnte Ratten 1. Gesamtausscheidung g	2. % der Dosis ausgeschieden
5,6	0,13	2,4	0,54	9,6
5,6	0,44	7,8	0,63	11,2
5,6	0,84	15,0	0,78	14,0
5,6	0,90	16,1	0,87	15,6
5,6	1,12	20,0	1,19	21,2
5,6	1,17	20,8	1,49	26,6
5,6	1,27	22,7	2,06	36,7
5,6	1,31	23,4	3,36	60,0
5,6	1,59	28,5		
5,6	1,67	29,8		
11,2	0,76	6,8	0,42	3,8
11,2	0,79	7,0	0,60	5,4
11,2	0,87	7,7	0,65	5,8
11,2	1,76	15,7	1,14	10,2

Key:
A.= Total dose fed in g
B.= Accustomed rats
1. total excretion in g 2. % of the dose excreted
C.= unaccustomed rats
1 and 2 as above.

With the purified ether extract of the urine used for the determination of adipic acid, we performed paper chromatographic examinations. (For the methodology see K. LANG and K.H. BASSLER (8)) Aside from the adipic acid and small amounts of oxalic acid and succinic acid, as they occur in normal urine, we could detect no acid. Thus no intermediate products form in the decomposition of adipic acid; the substance attacked is oxidised completely in the metabolism. This agrees with the observations of Lang and Bassler (8) concerning the decomposition of the dicarbonic acids by the cyclophorasis system. In these examination as well it was shown that the affinity of the cyclophorasis system to adipic acid is not very great, but that a molecule of the acid once attacked is immediately oxidized quantitatively into CO_2 and HO_2.

Summary

In long-term feeding experiments (up to 33 weeks) on more than 200 rats it is shown that rats tolerate daily doses of less than 400 mg adipic acid without detectable harm. In the case of a dose of 400 mg, slight histological alterations result in the liver and kidneys, and marked ones in the intestinal mucosa. Disturbances of the growth or other exteriorly recognisable symptoms are not found in the case of this dosage. Daily doses of 800 mg cause

without adipic acid the hemoglobin value was 70%) can easily be explained by the insufficient protein intake.

The histological examination[1] revealed nothing remarkable in the case of the animals that received less than 400 mg adipic acid per day. The histological findings in the case of the animals treated with larger doses of adipic acid were independent of the size of the protein intake.

No specific findings were determined in the kidneys. Remarkable were marked regeneration processes in the major sections, recognizable from a very large number of mitoses. This slight finding is all the more remarkable because the animals excreted considerable amounts of adipic acid regularly in their urine for quite a long time (8-23 weeks).

No intensive alterations could be detected in the liver either. In the case of the animals that had received 400 and 800 mg adipic acid, an enlargement of the cell nuclei, as well as an increase in the number of cells with two and more nuclei could be observed in places. A structural alteration of the nuclei could not be determined. Occasionally an enlargement of the total cell volume was determined. The most remarkable finding in the liver was an increase in the number of Kupfer star cells, combined with an enlargement of these cells.

The intestine offered the picture of a chronically inflammatory condition. The absorption epithelium was higher than normal, the cells appeared elongated and richer in plasma; the nuclei were very voluminous and situated toward the base of the cell. The border of the epithelium toward the base was unclear and the border along the tunica propria was bumpy. The absorption border of the epithelium was markedly swollen. The number of goblet cells was increased. There was an intense effort at regeneration, evident from the numerous mitoses in the crypts. The lymphatic tissue in the tunica propria was significantly increased.

The intolerability of higher adipic acid doses thus obviously is based chiefly on damage of the intestinal mucosa, which causes heavy diarrhea and a reduced utilization of the food.

Table 5 shows the results of the balance experiments. The rats accustomed to adipic acid were taken from experimental series III and had already 20-25 weeks beforehand received daily the indicated amounts of adipic acid. As can be seen, there is no clear difference between the rats accustomed to adipic acid and those unaccustomed to it as concerns the excretion of the substance in the urine. The organism obviously does not achieve the capacity, after long-term administration of the substance, to convert the substance better in the metabolism, which is somewhat determined by an adaptive enzyme formation. Furthermore it can be seen that the degree of excretion -- at least in the areas examined -- is independent of the dose. Like other earlier researchers (7), we also find marked individual differences in the utilization of adipic acid.

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[1] We owe Prof. Dr. WATZKA of the Anatomical Institute of our University many thanks for his helpful analysis of the histological preparations.

But in experimental series II, these alterations were observed only in the first three weeks. The animals recovered gradually, and after 33 weeks reached the same weight as the animals fed with 400 mg adipic acid. The deaths were distributed as follows: 1 in the first, 3 in the second, 5 in the third, 1 in the fourth week. Even the distribution of deaths in the first weeks shows that the chief damage occurs in the beginning of the experimental period. No proof of an intolerability of the 400 mg dose can be found in either the weight curve or the apparent behavior of the rats that received this dose.

In order to allow certain toxic effects of adipic acid to manifest themselves more strongly, rats were treated with adipic acid in a protein-deficient diet in a fourth experimental series. The feed consisted only of crushed wheat, supplemented with cod-liver oil. In this way, the protein intake was decreased to 11%. In addition to this, the animals received a protein that was biologically not of as high value as that of the first three experimental series.

Table 4. Weight gain and mortality of rats that were fed adipic acid in a protein-deficient diet (11% protein)

Daily adipic acid dose mg	Initial weight g	Weight after 6 weeks g	Weight after 19 weeks g	No. of dead animals
0	54	102 ± 16 (10)	200 ± 28 (5)	2
50	54	103 ± 9 (10)	179 ± 32 (7)	0
100	53	94 ± 14 (10)	172 ± 39 (5)	1
200	54	104 ± 13 (8)	182 ± 33 (5)	2
400	55	79 ± 14 (10)	144 ± 26 (5)	2

In Table 4 are shown the findings concerning the growth of the animals. Due to intercurrent deaths, for which no plausible cause could be found, and killing of the animals for the purpose of anatomical examinations in the seventh and eighth weeks, the number of animals in the later weeks of the experiment decreased markedly. The experiment shows clearly that the toxicity of adipic acid is greater in the case of insufficient protein intake than in the case of optimal nutrition. While in experimental series 2 and 3 no proof of damage to the animals that leads to inhibition of growth was found, in the case of the reduced protein intake the growth of the rats fed with 400 mg adipic acid was inhibited significantly in contrast to the control group. Inhibition of growth was still manifest after 19 weeks. The otherwise rapid recovery of the animals observed in the case of high adipic acid intake did not occur here. Besides the inhibition of growth, the animals revealed no outer remarkable signs; they had no diarrhea, their fur was normal, and they were not apathetic. Checking of the blood picture (hemoglobin content, number of erythrocytes and leucocytes, differential blood picture) revealed no finding deviating from the norm, except for a slight anemia (the hemoglobin values amounted to 69-74%), for any of the groups of this experimental series. The mild anemia of all the groups (in the control group

adipic acid. Its results are shown in Table 2. The weight gains of the rats fed with 200 and 400 mg adipic acid are not significantly different from those of the control animals. On the other hand, the growth of the animals that received 800 mg adipic acid per day was significantly decreased in contrast to the control animals. During the first 2 to 3 weeks, they suffered from heavy diarrhea. During this period, their fur was shaggy and dull. In the course of the fourth and fifth week, these rats recovered. They became more lively and their fur became smooth and shiny.

Table 2. Weight gain of rats that were fed with adipic acid for 5 weeks

Daily adipic acid dose in g	Initial weight g	End weight g	Number of animals
0	49 ± 7	154 ± 20	18
200	52 ± 7	156 ± 26	18
400	44 ± 5	139 ± 15	18
800	47 ± 7	100 ± 11	15

Since experimental series II consistently showed that the addition of 800 mg adipic acid per day leads to disorders of health, another series of experiments was undertaken in order to clarify, over a longer experimentational period (33 weeks), whether 400 mg adipic acid per day already has a toxic effect. The most important results are shown in Table 3.

Table 3. Weight gain and mortality of rats that were fed with adipic acid for 33 weeks

Daily adipic acid dose in mg	Initial weight g	Weight after 8 weeks	Weight after 33 weeks	No. of animals	No. of dead animals
0	74	207 ± 35	-----	11	4
400	74	183 ± 25	325 ± 25	9	4
800	73	154 ± 24	320 ± 31	4	10

In this group, several pregnant females were included. It was shown that even the high adipic acid doses had no effect on them. They bore litters and were capable of suckling them as well. These animals are not considered in Table 3. Also not included in this table are those animals that were killed for the purpose of histological examinations. The animals that died during the experimental period are also not considered in the weight data. In this series of experiments as well, there was a uniform toxic effect of the dose of 800 mg per day. The weight gain of the animals fed with this dose was significantly less than that of the control group. Beyond this, this group demonstrated high mortality. Just as in experimental series II, the rats fed with 800 mg adipic acid fell ill with heavy diarrhea, and their fur became shaggy and dull. In contrast to the animals of the other groups, they were apathetic.

with NaOH daily. The feed was once again the standard diet (80% crushed wheat and 20% whole milk powder). The amount of feed was so measured, that it could be consumed completely by the animals. Initially, it amounted to 10 g per animal and per day, and was increased in proportion to the increasing weight of the animals. The weight gain of the animals was observed continuously. Duration of experiment: 5 weeks.

Experimental Series III. Male and female rats with an initial weight of 60-80 g received daily in their food, which consisted of 80% crushed wheat and 20% whole milk powder, 400 and 800 mg, respectively, of adipic acid neutralized with NaOH. The amount of feed amounted to 10 g per animal and per day and was increased in proportion to the growth of the animals. The duration of the experiment was 33 weeks. After 8 and 23 or 25 weeks, animals were killed for histological examinations.

Experimental Series IV. Male rats with an initial weight of 40-60 g received daily, in a protein-poor feed, 0 or 50, 100, 200 and 400 mg adipic acid (neutralized with NaOH). The feed consisted only of crushed wheat, supplemented with 0.2 cm^3 cod-liver oil per rat and per day. It contained 11% protein (N x 6.25). The amount of feed was matched to the animals as described in the other series of experiments. The duration of the experiment was 19 weeks. In the eighth week, 3 animals from each group were killed for histological examinations. In this experiment, the blood picture of the animals was followed.

Balancing experiments. Rats accustomed and unaccustomed to adipic acid were kept in metabolism cages and received, in 14 days, a total of 5.6 g (400 mg daily) or 11.2 g (800 mg daily) of adipic acid in a standard diet. The rats accustomed to adipic acid were animals that had received 400 mg adipic acid each per day for 20 weeks, or 800 mg adipic acid each per day for 2[illegible] weeks. The urine of each animal was collected in toluene for the duration of the experiment. The determination of adipic acid was then performed according to the method of Weitzel (7).

The result of experimental series I is shown in Table 1.

Table 1. Weight gain of rats that were fed with adipic acid for 4 weeks.

Daily adipic acid dose mg	Weight gain g	Number of experimental animals
0	55 ± 13*	17
10	52 ± 14	18
20	62 ± 11	20
40	68 ± 16	17

* Average deviation

In the case of the lower dose used in this experimental series, nothing remarkable resulted either in the growth curve or in other behavior of the animals. As a consequence, a second series of experiments was conducted with an essentially higher dosage of

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CONCERNING THE METABOLISM AND TOLERABILITY OF ADIPIC ACID

by

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A series of reports (1-7) is already available concerning the metabolism of adipic acid. All the researchers determined that adipic acid administered to humans or animals (dogs, rabbits) either in food or by injection, is only partly utilised in the organism. A more or less considerable percentage of the administered dose (about 5-50%) is excreted unaltered form in the urine. Data provided thus far relates only to short-term experiments, in which the adipic acid dose was distributed over one or a few days. In connection with the problem of the decomposition of dicarbonic acids in the organism, we were interested in the question of how the animal organism reacts to an administration of the substance continued over a long period of time, and of whether there can be observed an increase in the ability to utilize the substance in the metabolism.

In order to answer this question, we fed rats up to 800 mg adipic acid daily. The duration of the experiments was up to 33 weeks. Since damages generally develop more quickly and more intensively when a diet is poor in protein, the effect of the adipic acid was verified in a special series of experiments using a diet with very little, and not very high value, protein. In the feeding experiments, the blood picture of the animals was continuously checked. Beyond this, animals were killed at intervals and examined anatomically (macroscopically and microscopically). In a separate series of experiments, precise balancing examinations were performed.

Methodology

Experimental Series I. Female rats of an average initial weight of 92 g received 0 to 10.20 or 40 mg adipic acid daily in their food, which was a standard diet. The standard diet consisted of 80% crushed wheat and 20% whole milk powder. The animals were free to eat as they wished. The weight gain of the animals was observed continuously. Duration of experiment: 4 weeks.

Experimental Series II. Male rats with an initial weight of 40-60 g received 200, 400 and 800 mg adipic acid neutralized with

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Intermediary Metabolism of Adipic Acid

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Adipic acid has been used in recent years to supply acidity to food products. A comparison of its flavor contribution with that of citric and tartaric acids has shown equal performance in regard to its acid character.

Information concerning the intermediary metabolism of adipic acid by animals is limited and quite contradictory. A review of the literature indicates that adipic acid is metabolized by man to some extent (Stetten and Boxer, 1944; Stöhr, 1938). Adipic acid was not toxic to rabbits (Enders, 1941), rats (Enders, 1941), and humans (Bernhard and Andreae, 1937; Hanson, 1943; Weitzel, 1942). After feeding a diet containing adipic acid, the urine usually contained increased amounts of adipic or other organic acids. Adipic acid is frequently present in small amounts in the urine of normal individuals (Hanson, 1943). Mori (1918) reported an increase in oxalic acid excretion when adipic acid was injected subcutaneously in rabbits. On the other hand, Kabelitz (1943) reported that oral feeding of adipic acid to man did not influence the excretion of oxalic acid. Stöhr (1938) found that adipic acid, when fed to rats, failed to increase the liver glycogen. Catabolism of adipic acid by β-oxidation is repeatedly suggested, but has not been established conclusively.

In order to determine the metabolic changes that adipic acid undergoes, it became necessary to establish procedures that would quantitatively estimate the adipic acid and its metabolic products in tissues and urine. Since such procedures had not yet been worked out, investigations were undertaken toward this end.

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of acids containing even numbers of carbons in contrast to those having odd numbers of carbons. It would appear, therefore, that adipic, pimelic, suberic, and azelaic acids are either not oxidized in the rabbit, or are decomposed by methods other than direct β-oxidation.

CONCLUSIONS

1. The sodium salts of adipic, pimelic, suberic, and azelaic acids are mildly nephropathic agents when administered subcutaneously to rabbits, but are much less toxic than is sodium glutarate under similar conditions.

2. Since pimelic (C_7-) and azelaic (C_9-) acids are not more toxic than are adipic (C_6-) and suberic (C_8-) acids, it appears improbable that direct β-oxidation of dicarboxylic acids occurs, otherwise the above acids with odd numbers of carbons would yield glutaric acid, and lead to very severe kidney injury.

REFERENCES

(1) Day, J. N. E., Ross, G. A. R., and Stevenson, A.: Jour. Chem. Soc., 1920, cxvii, 660.
(2) Einhorn, A., and Lumsden, J. S.: Ann. Chem., 1895, cclxxxvi, 257.
(3) Geraghty, J. T., and Rowntree, L. G.: Jour. Amer. Med. Assoc., 1911, lvii, 811.
(4) Gierke, K.: Ann. Chem., 1864, cxxx, 207.
(5) Markownikoff, M.: Ber. chem. Ges., 1893, xxvi, 3089.
(6) Rose, W. C.: Jour. Pharm. and Exper. Therap., 1924, xxiv, 123.
(7) Rose, W. C.: Jour. Pharm. and Exper. Therap., 1924, xxiv, 147.

TABLE 3

Suberic acid

Rabbit 37, male, 2324 grams

DATE	ACID ADMINISTERED	PHTHALEIN RECOVERED	BLOOD			NOTES, ETC.
			Non-protein nitrogen	Creatinine	NaCl	
	grams	*per cent*	*mgm.*	*mgm.*	*per cent*	
July 4						No food; water ad lib.
July 5		71*	44.5	1.8	0.55	10:00 a.m., 8 cc. blood. 11:10 am., renal test
July 6			45.4	1.8	0.54	10:00 a.m., 8 cc. blood
July 6	2.0					12:00 noon, acid 15 injected subcutaneously as sodium salt in 15 cc. of water
July 7		59†	60.0	2.0	0.53	10:00 a.m., 8 cc. blood. 11:20 a.m., renal test
July 8			49.4	1.9	0.52	10:00 a.m., 8 cc. blood
July 9			45.0	1.9	0.51	10:00 a.m., 8 cc. blood. Experiment discontinued

*First period, 4 cc. urine containing 45 per cent dye; second period, 3 cc. urine containing 26 per cent dye. Total 71 per cent.

†First period, 3 cc. urine containing 39 per cent dye; second period, 4 cc. urine containing 20 per cent dye. Total 59 per cent.

TABLE 4

Azelaic acid

Rabbit 30, male, 2156 grams

DATE	ACID ADMINISTERED	PHTHALEIN RECOVERED	BLOOD			NOTES, ETC.
			Non-protein nitrogen	Creatinine	NaCl	
	grams	*per cent*	*mgm.*	*mgm.*	*per cent*	
July 27						No food; water ad lib.
July 28		70*	41.9	1.7	0.51	10:00 a.m., 7 cc. blood. 11:10 a.m., renal test
July 29			41.3	1.7	0.52	10:00 a.m., 6 cc. blood
July 29	2.0					11:30 a.m., acid 16 injected subcutaneously as sodium salt in 15 cc. of water
July 30		68†	51.1	2.1	0.50	10:00 a.m., 6 cc. blood. 11:10 a.m., renal test
July 31			60.8	2.1	0.51	10:00 a.m., 9 cc. blood. Experiment discontinued

*First period, 16 cc. urine containing 44 per cent dye; second period, 8 cc. urine containing 26 per cent dye. Total 70 per cent.

†First period, 9 cc. urine containing 52 per cent dye; second period, 6 cc. urine containing 16 per cent dye. Total 68 per cent.

observed. Evidently the six- to nine-carbon homologues of glutaric acid, when administered subcutaneously as their sodium salts, produce temporary interference with renal excretion, and in this sense are mildly nephropathic agents. On the other hand, they do not compare with glutaric acid in intensity of toxic action.

TABLE 2

Pimelic Acid

Rabbit 44, male, 1470 grams

DATE	ACID ADMINISTERED	PHTHALEIN RECOVERED	BLOOD			NOTES, ETC.
			Non-protein nitrogen	Creatinine	NaCl	
	grams	*per cent*	*mgm.*	*mgm.*	*per cent*	
October 20						No food; water ad lib.
October 21		81*	41.0	1.3	0.50	8:45 a.m., 8 cc. blood. 10:25 a.m., renal test
October 22			42.8	1.3	0.52	9:20 a.m., 6 cc. blood
October 22	2.0					12:35 p.m., acid 14 injected subcutaneously as sodium salt in aqueous solution
October 22		45†				8:45 p.m., renal test
October 23			68.6	1.5	0.52	8:25 a.m., 6 cc. blood
October 24		65‡	60.3	1.5	0.52	9:25 a.m., 7 cc. blood. 7:50 p.m. renal test
October 25			53.6	1.3	0.52	9:20 a.m., 6 cc. blood. Experiment discontinued

*First period, 9 cc. urine containing 60 per cent dye; second period, 8 cc. urine containing 21 per cent dye. Total 81 per cent.

†First period, 12 cc. urine containing 33 per cent dye; second period, 6 cc. urine containing 12 per cent dye. Total 45 per cent.

‡First period, 10 cc. urine containing 55 per cent dye; second period, 10 cc. urine containing 10 per cent dye. Total 65 per cent.

The data serve to emphasize the fact previously affirmed (7), namely, that glutaric acid manifests an unique behavior in the animal organism. Except for oxalic acid, it is decidedly the most toxic member of the homologous series.

No evidence was obtained of a difference in nephropathicity

TABLE 1

Adipic acid

Rabbit 16, male, 2160 grams

DATE	ACID ADMINISTERED	PHTHALEIN RECOVERED	BLOOD					NOTES, ETC.
			Non-protein nitrogen	Urea N	Creatinine	Sugar	NaCl	
	grams	*per cent*	*mg.m.*	*mgm*	*mgm*	*per cent*	*per cent*	
December 5								No food; water ad lib.
December 6		75*	45.5	22.2	1.4	0.114	0.47	10:00 a.m., 6 cc. blood. 10:20 a.m., renal test
December 6	2.0							7:00 p.m., acid 8 injected subcutaneously as sodium salt in 30 cc. of water
December 7		79†	58.3	33.3	1.7	0.127	0.45	9:30 a.m., 6 cc. blood. 10:00 a.m., renal test
December 7	2.0							6:30 p.m., acid 8 injected subcutaneously as sodium salt in 20 cc. of water
December 8		79‡	49.0	29.6	1.7			10:30 a.m., 7 cc. blood. 10:40 a.m., renal test
December 9	4.0							10:00 a.m., acid 8 injected subcutaneously as sodium salt in 30 cc. of water
December 9		20§						2:30 p.m., renal test
December 10		81¶	42.6	23.3	1.6	0.137	0.48	10:00 a.m., 7 cc. blood. 2:00 p.m., renal test. Experiment discontinued

*First period, 3 cc. urine containing 44 per cent dye; second period, 3 cc. urine containing 31 per cent dye. Total 75 per cent.

†First period, 6 cc. urine containing 70 per cent dye; second period, 2 cc. urine containing 9 per cent dye. Total 79 per cent.

‡First period, 3 cc. urine containing 57 per cent dye; second period, 3 cc. urine containing 22 per cent dye. Total 79 per cent.

§First period, 12 cc. urine containing 11 per cent dye; second period, 10 cc. urine containing 9 per cent dye. Total 20 per cent.

¶First period, 8 cc. urine containing 73 per cent dye; second period, 17 cc. urine containing 8 per cent dye. Total 81 per cent.

procedure and analysis were identical with those previously described (6).

The acids employed are briefly described below.

Adipic acid 8. A snow-white preparation made by recrystallizing a product obtained from the Eastman Kodak Company. Acid was free from oxalic acid, and melted sharply at 150° to 151°C. The melting points reported in the literature range from 149° to 153.5°C.

Pimelic acid 14. Prepared by the reduction of salicylic acid according to the procedure of Einhorn and Lumsden (2). Excess salicylic acid was removed by oxidation with alkaline permanganate. The pimelic acid so obtained was decolorized, and repeatedly recrystallized from water, benzene, and ether. Melting-point, 104° to 105°C.; theoretical, 105° to 106°C.

Suberic acid 15. Prepared by the oxidation of castor oil with nitric acid according to the directions of Markownikoff (5). Separation of the suberic acid from the crude mixture of suberic and azelaic acids was accomplished by the method of Day, Kon, and Stevenson (1). The acid was recrystallized seven times from hot water. The final product was snow-white, free from oxalic acid, and melted at 140.5° to 141.5°C. Theoretical melting point, 140° to 141°C.

Azelaic acid 16. The residue from the preparation of suberic acid, was used for the separation of azelaic acid, through its calcium salt, according to the procedure of Grote (4). The acid was recrystallize three times from hot water, without change in melting point. The latter was 105.2 to 106°C.; theoretical 106°C.

In order to economize space, we are presenting the results of only one experiment with each acid. The data detailed in tables 1 to 4 inclusive, are typical of other experiments made with the same acids. As will be observed, slight increases in non-protein nitrogen and creatinine, occurred in each case, following the acid administration, but the alterations in blood composition are generally quite small, and of short duration. In some animals (tables 1 and 2) the injections led to decreases in the output of phenolsulphonephthalein. As a rule, the blood pictures indicate complete recovery of renal function within forty-eight hours, although in a few experiments (the details of which are omitted), with larger doses of the acids, more prolonged effects were

cogen. Our experimental results thus supplement the data of Flaschenträger et al.[6], which have pointed out the great resistance of the higher dicarboxylic acids in the animal organism, as well as the findings of Edson[7], who found an absence of any antiketogenic effect of these dicarboxylic acids, which might be interpreted in the sense of a carbohydrate formation, in liver section experiments. In fact, in the latter's experiments, malonic acid was shown to be strongly ketogenic. Thus, higher dicarboxylic acids hardly enter the picture, within the framework of the intermediate metabolism, as being sources of a sugar formation.

Experiments: We used young male white or dappled rats, that had eaten nothing for 24 hours before the feeding. Weight of the animals after not eating: 110-130 g. Amount of dicarboxylic acids fed them (given as Na salt): 0.2-0.3 g. Absorption duration: 4-8 hours. Glycogen determination accomplished according to earlier indications.[5] (From the Medical-Chemical Institute of the University of Innsbruck).

Table. Behavior of the liver glycogen after feeding with dicarboxylic acids. (10 control animals: 0.074 _ 0.016 g %)

A.		B.	C.	D.
Zahl der Tiere		Dicarbonsäure	verfütterte Menge g	Leberglykogen pro 100 g Leber g%
8	a	Malonsäure . . .	0,2	0,040±0,012
6	b	Bernsteinsäure[6] .	0,15	0,909±0,060
6	c	Glutarsäure . . .	0,25	0,027±0,009
8	d	Adipinsäure . . .	0,25	0,066±0,007
6	e	Pimelinsäuren . .	0,25	0,038±0,011
6	f	Korksäure. . . .	0,3	0,043±0,014
4	g	Azelainsäure. . .	0,3	0,030±0,002
4	h	Sebacinsäure. . .	0,3	0,048±0,006

Key:
A. number of animals
B. dicarboxylic acid
C. amount fed in g
D. liver glycogen per 100 g liver, in g%
a. malonic acid b. succinic acid c. glutaric acid d. adipic acid
e. pimelic acid f. suberic acid g. azelaic acid h. sebacic acid

Literature

Literatur: [1] VERKADE u. Mitarbeiter, Hoppe-Seylers Z. 215, 225; 225, 230; 227, 213; 230, 207; 234, 21; 237, 186; 247, 111; 250, 47 (1933—1937) — Biochemic. J. 28, 31 (1934) — Vgl. auch FLASCHENTRÄGER u. BERNHARD, Helvet. chim. Acta 18, 962 (1935). — BERNHARD u. ANDREAE, s. 6. — [2] Literatur bei 5. — [3] SZENT-GYÖRGYI u. Mitarbeiter, Hoppe-Seylers Z. 236, 1 (1935). — [4] RINGER, FRANKEL u. JONAS, Chem. Zbl. 2, 704 (1913). — [5] STÖHR, Hoppe-Seylers Z. 217, 153 (1933). — [6] FLASCHENTRÄGER, Hoppe-Seylers Z. 159, 297 (1926) — FLASCHENTRÄGER u. BERNHARD, Hoppe-Seylers Z. 238, 221 (1936) — BERNHARD u. ANDREAE, Hoppe-Seylers Z. 245, 103 (1936). — [7] EDSON, Biochemic. J. 30, 1855 (1936).

Klinische Wochenschrift 17:47, 1663-4 (1938)

ON THE QUESTION OF THE FORMATION OF GLYCOGEN FROM DICARBOXYLIC ACIDS

by

Richard Stöhr

Verkade et al.[1] have proved that besides the usual oxidation in β-position, free fatty acids in the organism can also be oxidized in last position (ω-oxidation), whereby dicarboxylic acids form, the further decomposition of which takes place through one or both-sided ω-oxidation, to dicarboxylic acids with lower carbon count. When it was proved that under certain conditions dibasic acids of various chain lengths can be formed, these acids became of great interest as intermediate stages in the intermediate metabolism.

The formation of dicarboxylic acids through ω-oxidation of higher fatty acids is of particular interest to metabolic chemistry, insofar as it offers the organism the possibility of reaching directly the uncommonly important succinic acid stage (dicarboxylic acid with 4 carbon atoms); the formation of this acid was previously assumed to take place through dehydration of acetic acid or pyruvic acid (through α-, α'-diketoadipic acid)[2]. The significance of succinic acid for the metabolism is twofold: 1. Succinic acid is the first member of the succinic acid--fumaric acid--malic acid--oxalacetic acid system, in which end oxidation essentially is supposed to take place, and to which is ascribed, on the basis of the research of Szent-Gyorgi et al,[3] a dominant position in the dehydration processes in the muscle; 2. all members of the succinic acid--oxalacetic acid series are excellent glycogen formers[2], so that -- given a certain metabolic situation -- there is a possibility for a conversion into carbohydrate for all compounds whose decomposition leads into this system.

In this connection, the question was examined of whether an increase in liver glycogen can be determined in a feeding experiment on starving rats after administration of high dicarboxylic acids with <u>even</u> carbon count. Since according to Ringer, Frankel and Jonas[4] malonic acid is also supposed to operate glucoplastically, (experiments with phlorizine-diabetic animals), this acid, as well as higher dicarboxylic acids with <u>uneven</u> carbon count were also considered in our experiments, although the acids do not appear as intermediate products of the normal metabolism. Our feeding experiments thus encompassed dicarboxylic acids with 3-10 carbon atoms. They revealed that with the exception of succinic acid, whose easy conversion into glycogen we had already proved earlier with starving rats,[5] none of the dicarboxylic acids examined causes an increase in liver gly-

MORI, Y. (1918). The decomposition of muconic and adipic acids in the animal body. *J. Biol. Chem.* **35**, 341-351.

OCHOA, S. (1944). α-Ketoglutaric dehydrogenase of animal tissues. *J. Biol. Chem.* **155**, 87-100.

STETTEN, D., JR., and BOXER, G. E. (1944). Studies in carbohydrate metabolism I. The rate of turnover of liver and carcass glycogen, studied with the aid of deuterium. *J. Biol. Chem.* **155**, 231-236.

STÖHR, R. (1938). Zur Frage der Glykogenbildung aus Dicarbonsäuren. *Klin. Wochschr.* **17**, 1663-1664.

WEITZEL, G. (1942). Stoffwechselversuche mit Adipinsäure. *Ber. Verhandl. sächs. Akad. Wiss. Leipzig, Math.-phys. Kl.* **93**, 9-18.

WEITZEL, G. (1947). Die Bersteinsäureausscheidung bei Stoffwechselbelastung mit höhren *n*-Dicarbonsäuren. *Z. physiol. Chem. Hoppe-Seyler's* **282**, 185-191.

The presence of radioactive acetyl-γ-phenyl-α-aminobutyric acid after feeding γ-phenyl-α-aminobutyric acid and C^{14}-labeled adipic acid provides very strong evidence that acetate is a metabolite of adipic acid.

Radioactive glycogen was isolated following feeding of glucose and radioactive adipic acid.

Some of the metabolic products found in the urine are most certainly not direct degradation products of adipic acid, e.g., urea, but contain radioactive carbon, derived via carbon dioxide from adipic acid. This has been indicated by feeding tests with radioactive carbon dioxide followed by the isolation of traces of some of the same metabolic products in the urine.

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REFERENCES

BERNHARD, K., and ANDREAE, M. (1937). Stoffwechselversuche mit Dicarbonsäuren. *Z. physiol. Chem. Hoppe-Seyler's* **245**, 103-106.

BLOCK, K. and RITTENBERG, D. (1944). Sources of acetic acid in the animal body. *J. Biol. Chem.* **155**, 243-254.

BUSCH, H., HURLBERT, R. B., and POTTER, V. R. (1952). Anion exchange chromatography of acids of the citric acid cycle. *J. Biol. Chem.* **196**, 717-727.

DENISON, F. W., JR., and PHARES, E. F. (1952). Rapid method for paper chromatography of organic acids. *Anal. Chem.* **24**, 1628-1629.

ENDERS, A. (1941). Verträglichkeit und Ausscheidungsverhältnisse von Dicarbonsäuren. *Arch. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's* **197**, 597-610.

ETTINGER, R. H., GOLDBAUM, L. R., and SMITH, L. H., JR. (1952). A simplified photometric method for the determination of citric acid in biological fluids. *J. Biol. Chem.* **199**, 531-536.

FRIEDEMANN, T. E., and HAUGEN, G. E. (1943). Pyruvic acid. II. The determination of keto acids in the blood and urine. *J. Biol. Chem.* **147**, 415-442.

GOOD, C. A., KRAMER, H., and SOMOGYI, M. (1933). The determination of glycogen. *J. Biol. Chem.* **100**, 485-491.

HANSON, H. (1943). Untersuchungen über Nachweis und Isolierung von im Harn ausgeschiedenen Dicarbonsäuren. *Z. ges. exptl. Med.* **113**, 226-244.

KABELITZ, G. (1943). Untersuchungen über den Einfluss der Diacidogenen Fettsäuren, C_6 bis C_{11}, ihrer Glyceride und einiger Nahrungsfette auf die Oxalsäureausscheidung beim Menschen. *Klin. Wochschr.* **22**, 439-441.

KORNBERG, H. L., DAVIES, R. E., and WOOD, D. R. (1952). The metabolism of C^{14}-labeled bicarbonate in the cat. *Biochem. J.* **51**, 351-357.

MARKUS, R. L. (1950). Colormetric determination of lactic acid in body fluids utilizing cation exchange for deproteinization. *Arch. Biochem.* **29**, 159-165.

MARVEL, C. S., and RAND, R. D., JR. (1950). Separation of organic acids. *J. Am. Chem. Soc.* **72**, 2642-2646.

Six radioactive metabolites including adipic acid were found in the urine. Urea and glutamic, lactic, adipic, β-ketoadipic, and citric acids were identified as metabolites of adipic acid.

Experiments using radioactive C^{14} sodium bicarbonate have shown that carbon dioxide from the breath enters into some of the reactions with adipic acid. It also forms small quantities of acids in the urine. Carbon dioxide from adipic acid is probably responsible for the urea and citric acid found in the chromatograms. Kornberg *et al.* (1952) have shown by radioactive studies that intravenously injected sodium bicarbonate can be used to synthesize urea in the cat. Carbon dioxide ($C^{14}O_2$) alone did not produce radioactive citric acid although citric acid was present in approximately the same concentration as found in all the previous experiments.

$C^{14}O_2$ and nonradioactive adipic acid formed radioactive citric acid and it is suggested that the radioactivity was produced by an interaction of carbon dioxide and a metabolite of adipic acid. Negligible quantities of the other metabolites isolated from the adipic acid urine were produced from radioactive carbon dioxide.

The presence of β-ketoadipic acid in the urine strongly suggests that adipic acid undergoes a β-oxidation. β-Ketoadipic acid can in turn be metabolized to succinic acid and an active 2-carbon intermediate. We have shown that under appropriate experimental conditions, succinate and acetate can be demonstrated to be metabolites of adipic acid. Weitzel (1947) has shown that humans fed diets containing high levels of adipic acid show significant increases in the urinary succinic acid. The fact that no succinic acid was found in the radioactive experiments with animals not treated with malonic acid may mean that succinic acid was utilized more efficiently by the fasted animal. It is possible that the lactic acid found in the urine is an end product of succinic acid formed from adipic acid.

SUMMARY

Adipic acid is absorbed and metabolized by normal metabolic processes by the rat. When radioactive adipic acid was fed to fasted rats, metabolic products identified as urea, glutamic acid, lactic acid, β-ketoadipic acid, and citric acid, as well as adipic acid, were found in the urine.

The presence of β-ketoadipic acid provides some evidence that adipic acid is metabolized by β-oxidation in much the same fashion as fatty acids. Further evidence is provided by the appearance of succinate in the urine of rats fed radioactive adipic acid (1-C^{14}) and injected with malonic acid.

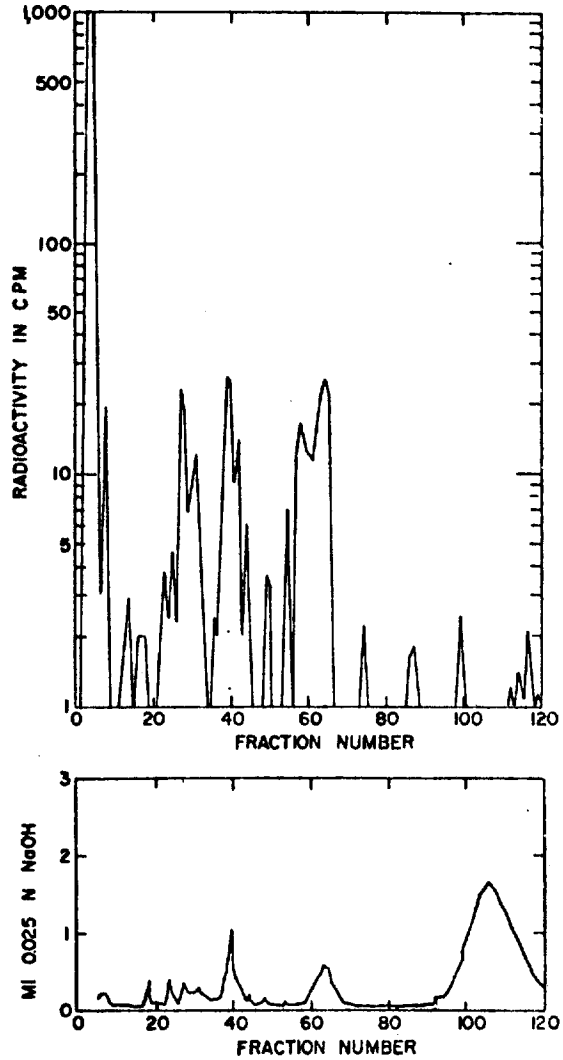

FIG. 9. Titration curve and distribution of radioactivity from urine of rat fed radioactive C^{14}-radioactive sodium bicarbonate.

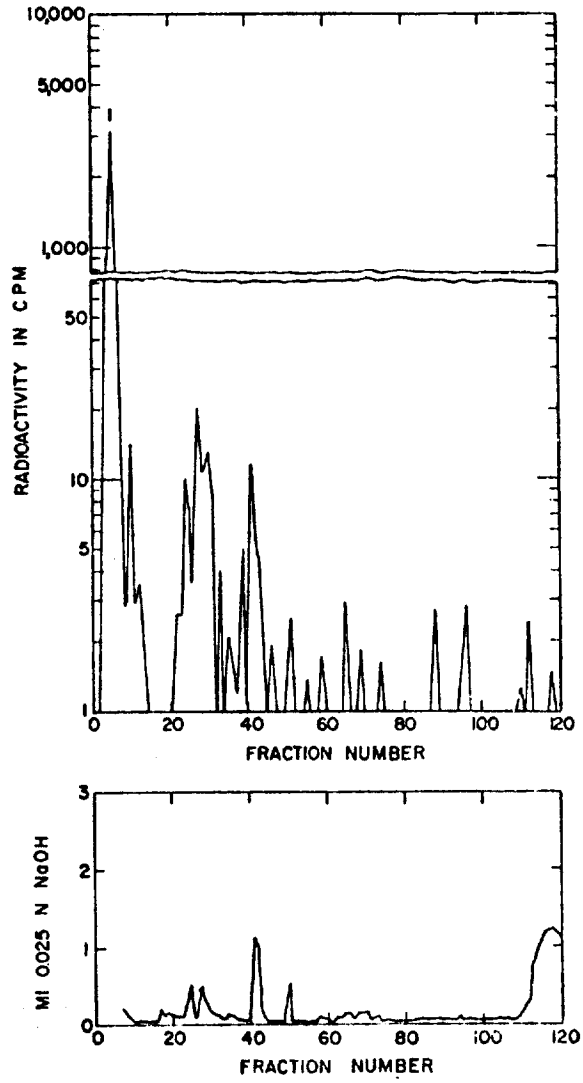

FIG. 8. Titration curve and distribution of radioactivity from urine of rat fed C^{14}-radioactive sodium bicarbonate and adipic acid.

labeled in the 2-carbon position. The carbon dioxide curves of the breath have a characteristic shape and in each case reach a maximum within 2 hours and then rapidly decline.

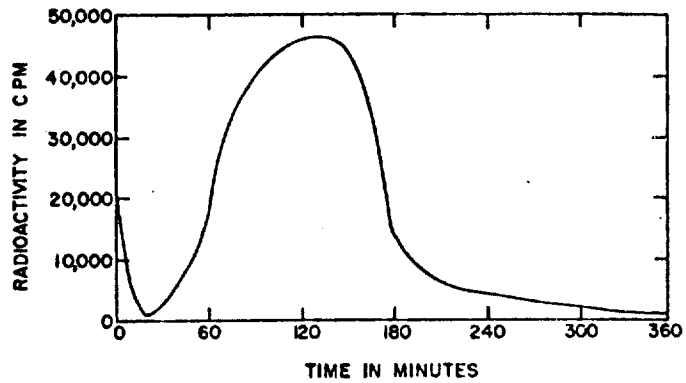

FIG. 6. Expired $C^{14}O_2$ in breath of rat fed radioactive C^{14} sodium bicarbonate and adipic acid.

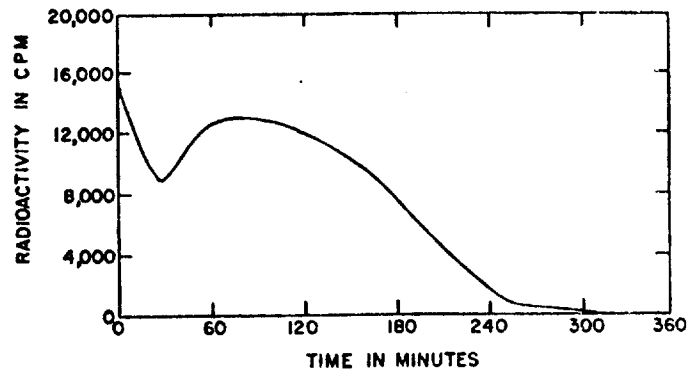

FIG. 7. Expired $C^{14}O_2$ in breath of rat fed radioactive C^{14} sodium bicarbonate.

The tissues from the sacrificed rats showed very little residual radioactivity. Of all the tissues examined, the highest activity appeared in the liver and kidney. Although negligible amounts of glycogen were isolated from the livers, the glycogen did not appear to be radioactive. However, when glycogen formation in the liver was encouraged by the oral administration of glucose with radioactive adipic acid, a high concentration of glycogen was isolated which was radioactive.

DISCUSSION

The experiments with radioactive adipic acid have shown that adipic acid is metabolized by the rat. At the concentrations fed, adipic acid is almost completely absorbed. Up to 70% of the radioactivity accumulates in the breath during the 24-hour experimental period. Adipic acid labeled with C^{14} in the 1-carbon position is metabolized faster than that

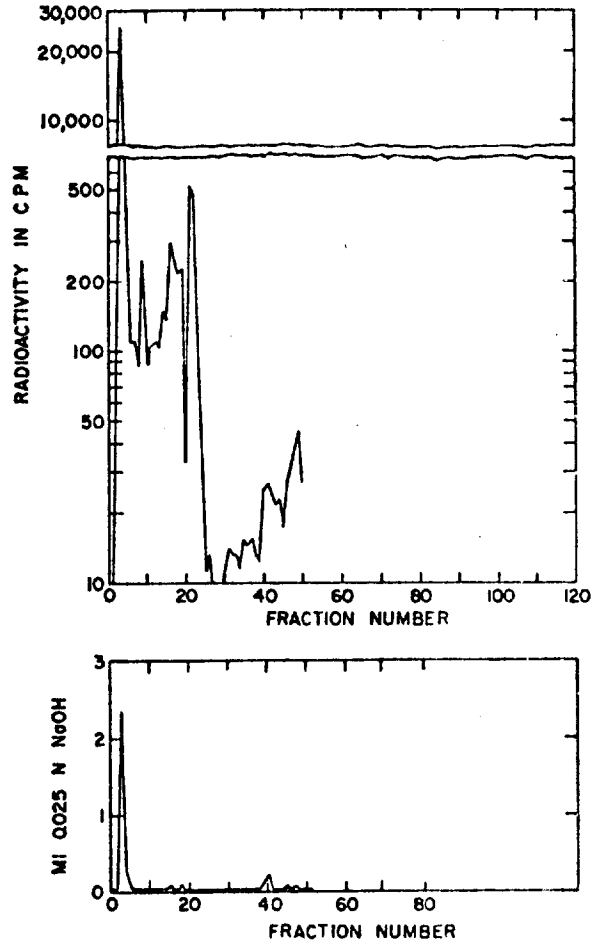

FIG. 5. Titration curve and distribution of radioactivity from urine of rat treated with malonic acid and fed 1-C^{14} adipic acid.

Formation of Succinic Acid from Radioactive Adipic Acid

If, as the presence of β-ketoadipic acid suggests, adipic acid undergoes β-oxidation during its metabolism, it should under appropriate conditions, be possible to demonstrate the formation of succinic acid and acetic acids *in vivo* from adipic acid.

Fasted, male, albino rats were fed by stomach tube approximately 50 mg adipic acid labeled with C^{14} in the carboxyl carbon in 3 ml water, then injected intraperitoneally with 2 ml of 0.5 *M* sodium malonate (Ochoa, 1944). Under these conditions, malonate inhibits the oxidation of succinate, but not acetate. Urine was collected for 24 hours, acidified with nitric acid to pH 1, and extracted with ethyl ether for 24 hours. After extraction, the ether was removed and the residue taken up in a mixture of 3 ml *n*-butanol and 7 ml of chloroform. A succinic acid marker was added, and the extract was chromatographed on a silicic acid column. Figure 5 shows that radioactive succinic acid as well as radioactive adipic acid was obtained from the urine of these treated rats.

Formation of Acetic Acid from Radioactive Adipic Acid

In order to accumulate acetate in the urine, male albino rats were fed 100 mg γ-phenyl-α-aminobutyric acid (Block and Rittenberg, 1944) and 25 mg carboxyl-labeled adipic acid with C^{14} per 100 g body weight in 5 g dog chow. Urine was collected for 48 hours, then extracted as described by Block and Rittenberg (1944).

The resulting acetyl-γ-phenyl-α-aminobutyric acid was repeatedly recrystallized from hot water until a constant melting point of 177° C was obtained. Radioactivity determinations showed that the coefficient of utilization as described by Block and Rittenberg (1944) was 13.3, indicating a high degree of utilization of part of the adipic acid for forming acetyl groups.

Radioactive Sodium Bicarbonate

In order to determine the role of radioactive carbon dioxide in the formation of radioactive metabolites from adipic acid in the tissues and urine, experiments were performed using radioactive C^{14} sodium bicarbonate alone and in the presence of nonradioactive adipic acid. The distribution of radioactivity in the breath and urine of rats as a result of two such experiments are shown in Figs. 6–9.

Peak 2. This peak corresponded to the elution peak of glutamic acid. No other peak was found in this region. The material in this peak gave a positive ninhydrin test.

Peak 3. This peak has not yet been identified.

Peak 4. This elution peak corresponded to lactic and β-ketoadipic acids.

Paper chromatography showed an R_f of the unknown corresponding to lactic acid in three developing solvents. The spot was radioactive. The chemical test of Markus (1950) gave a positive test for lactic acid.

β-Ketoadipic acid was identified by the ultraviolet spectrum of its phenylhydrazone. The unknown and pure β-ketoadipic phenylhydrazones gave identical absorption bands at 292 mμ. Furthermore, the phenylhydrazone was radioactive and represented 50% of the radioactivity.

Peak 5. The acids corresponding to this peak were adipic, succinic, and pyruvic acids. Paper chromatographic separation showed the radioactivity to be associated with adipic acid in three solvents. The Friedemann and Haugen (1943) test for pyruvate was negative.

Peak 5 material was fractionated further on a silicic acid column, which separates adipic, pyruvic, and succinic acids. Again, the radioactivity was associated only with adipic acid. Under the conditions of this experiment, succinic and pyruvic acids were not metabolites.

Peak 6. The identity of this peak has not been established. One possibility appeared to be malic acid, which was established by fractionation of known acids.

Peak 7. This peak corresponded in position to citric acid. The radioactivity associated with the fractionated citric acid was too low for paper chromatographic identification so that it was possible to use microchemical confirmation only. The microtechnique of Ettinger *et al.* (1952) showed high concentrations of citric acid in this peak.

Formation of Glycogen from Radioactive Adipic Acid

In order to determine whether adipic is converted to glycogen, fasted male albino rats were fed by stomach tube, 100 mg radioactive adipic acid labeled in the 1-C^{14} position and 400 mg glucose in water solution. At the end of 2 hours, the rats were sacrificed and the livers were analyzed for glycogen. The glycogen isolated under these conditions was radioactive.

the radioactivity did not correspond to an acid, but rather to a neutral or basic substance, such as creatine or urea. The peak gave a positive ninhydrin test. Mixed melting point determinations gave a melting point of 130° C. Melting point of pure urea was 132° C.

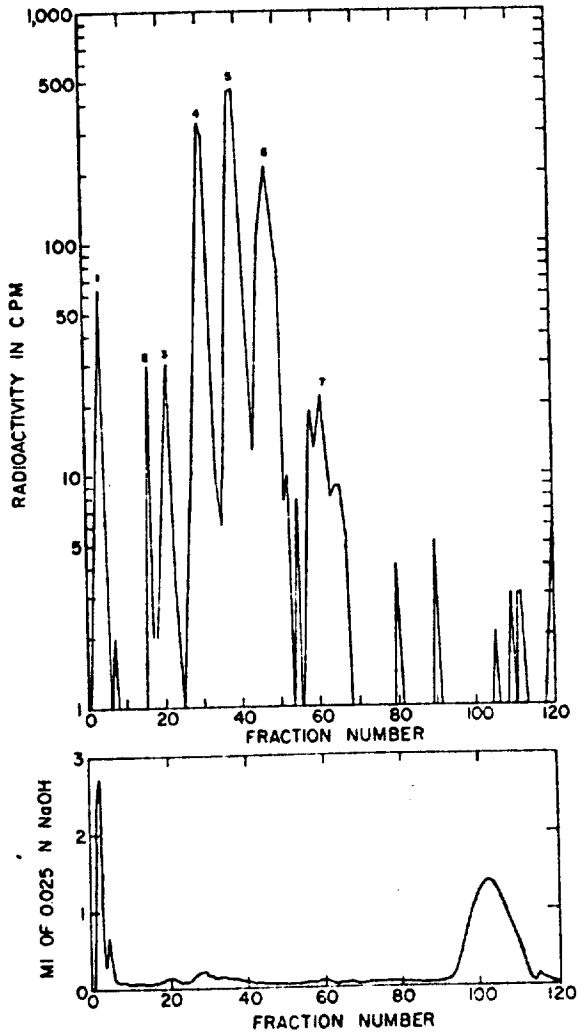

FIG. 4. Distribution of radioactivity and titration curve from urine of rat fed 2-C^{14} adipic acid.

known metabolite. (b) The R_f value by paper chromatography of the unknown must correspond to the known metabolite in at least two solvent systems. (c) The unknown must give a positive chemical test for the suspected known metabolite. (d) The reaction product of the chemical test must be radioactive.

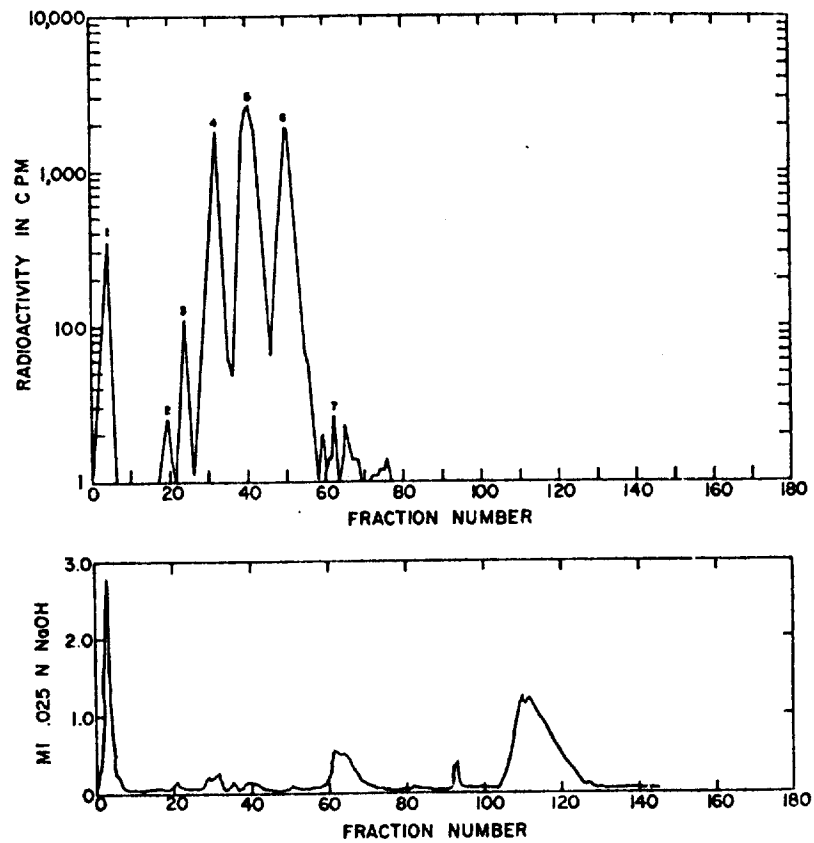

FIG. 3. Distribution of radioactivity and titration curve from urine of rat fed 1-C^{14} adipic acid.

The following tentative identifications have been made (see Figs. 3 and 4):

Peak 1. Paper chromatography showed the presence of urea. The radioactivity of the original fraction was adsorbed by IR-45 resin, so that

than that obtained with the 1-C^{14}-labeled acid. This would indicate that the carboxyl group is oxidized at a much faster rate than is the second carbon.

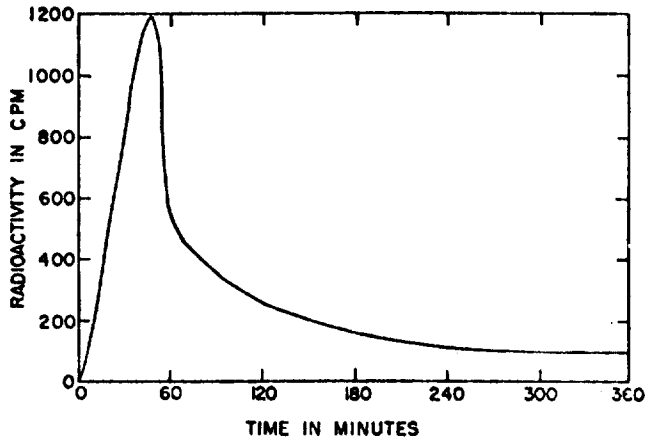

FIG. 1. Expired $C^{14}O_2$ in breath of rat fed 1-C^{14} adipic acid.

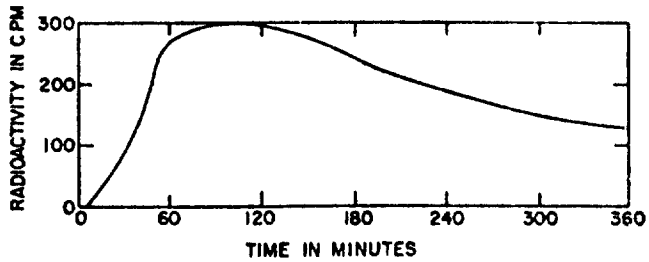

FIG. 2. Expired $C^{14}O_2$ in breath of rat fed 2-C^{14} adipic acid.

Figures 3 and 4 show the titration curves of base equivalent contained in each fraction and distribution of radioactivity in the urine of rats fed adipic acid labeled with C^{14} in the 1 and 2 positions, respectively. The curves appear to be identical in the number and distribution of radioactive peaks. In each case, seven radioactive peaks appear.

Identification of Peaks

Identification of a radioactive peak from the Dowex-1-formate column was considered to be dependent upon the following criteria: (a) The elution peak of the unknown must correspond to the elution peak of a

Identification of Isolated Compounds

Amino acids were identified on chromatograms after spraying with ninhydrin in alcohol and heating at 100° C; organic acids, by spraying with 0.2% alcoholic bromocresol green. Identification of a radioactive spot in the case of an amino acid, for which the color tests are very sensitive, was considered quite satisfactory if the radioactive area coincided exactly with the colored area when rechromatographed with a known substance. The indicator spray tests for organic acids were not considered sensitive enough to establish identity of the major spots.

Separation and identification of the acids, hydroxyacids, and ketoacids by the various solvent systems were not conclusive since these compounds tend to have similar R_f values ($R_f = 0.7$–0.9). The acids isolated from the Dowex-1-formate column were rechromatographed on silica gel columns (Marvel and Rand, 1950). Coincidence of the radioactive curves with the base equivalent curves of added known markers was taken as further evidence that the two acids are identical.

Lactic acid was determined by the chemical test of Markus (1950). The ketoacids were determined spectrophotometrically as the 2,4-dinitrophenylhydrazones. Citric acid was determined by the method of Ettinger *et al.* (1952); and pyruvic acid was tested by the procedure of Friedemann and Haugen (1943). Glycogen was isolated by the method of Good *et al.* (1933) as modified by Stetten and Boxer (1944). Urea was identified by mixed melting point techniques and chromatographic procedures.

RESULTS

Rats were fed C^{14}-labeled adipic acid by stomach tube and subsequently fasted overnight. From the analyses of the fractions recovered from the urine, it is apparent that during the fasting period, the radioactive carbon of the adipic acid was distributed into a number of fractions. The distribution of radioactivity in the various fractions and metabolites is shown in Figs. 1–5.

Figures 1 and 2 show the amount of radioactive carbon dioxide in the breath of rats fed 1-C^{14} and 2-C^{14} adipic acid, respectively, expressed as counts per minute. A comparison of the figures suggests that radioactive carbon dioxide resulting from the metabolism of adipic acid labeled in 1-C^{14} position reaches a maximum sooner than does the carbon dioxide resulting from the 2-C^{14}-labeled adipic acid. The maximum amount of carbon dioxide from the 2-C^{14}-labeled acid is considerably less

METHODS

Two samples of adipic acid labeled with C^{14} were used in these studies. One was labeled in the carboxyl group, and the other at the second carbon position.

Male albino rats (Carworth Farms), 150–250 g in weight, and raised on Gaines dog chow, were fasted for approximately 24 hours. They were then fed, by stomach tube, a solution containing approximately 50 mg radioactive adipic acid in 2–4 ml of water. The rats were immediately placed in individual metabolism chambers for the collection of respiratory carbon dioxide.

Urine collected during the experimental period under toluene was filtered through glass wool and lyophilized. The freeze-dried urine, which was a light yellow solid, was used in all fractionation experiments.

Fractionation of Urine Mixture

For the separation of the organic acids, columns containing the ion-exchange resin, Dowex-1 (200–400 mesh, formate form) were prepared as described by Busch *et al.* (1952); columns containing silica gel were prepared as described by Marvel and Rand (1950).

The freeze-dried urine was completely dissolved in a small amount of water and added to the Dowex-1 columns. The adsorbed acids were then eluted with formic acid. Fractions of the eluate were collected in test tubes with an automatic fraction collector. The tubes were placed in a vacuum desiccator over a mixture of calcium chloride and sodium hydroxide (2:1) and dried by infrared heat. As each tube dried, it was removed from the desiccator in order to minimize losses due to volatility. Water, free of carbon dioxide, was added to each tube and the contents titrated with 0.02 *N* sodium hydroxide using phenolphthalein indicator. From the results, a titration curve showing the base equivalent contained in each fraction was obtained. After titrating the eluates, the solutions were transferred to beakers and evaporated to a small volume in a warm stream of air. The concentrated solutions were quantitatively transferred to small glass dishes, dried, and scanned for radioactivity, using a Geiger-Müller counter. One-dimensional chromatograms on Whatman No. 1 filter paper were made of the eluates according to the method of Denison and Phares (1952). The positions of radioactive compounds on the chromatograms were determined by counting at intervals along the developed area with a thin-window Geiger-Müller counting tube.

produkte des normalen Stoffwechsels nicht auftreten. Unsere Fütterungsversuche erstreckten sich somit auf Dicarbonsäuren mit 3—10 Kohlenstoffatomen. Sie ergaben, daß mit Ausnahme der Bernsteinsäure, deren leichten Übergang in Glykogen wir schon früher bei hungernden Ratten nachgewiesen haben[5], keine der untersuchten Dicarbonsäuren eine Vermehrung des Leberglykogens bewirkt. Unsere Versuchsergebnisse ergänzen damit die Angaben von FLASCHENTRÄGER und Mitarbeitern[6] welche auf die große Resistenz der höheren Dicarbonsäuren im tierischen Organismus hingewiesen haben,

Tabelle. Verhalten des Leberglykogens nach Verfütterung von Dicarbonsäuren. (10 Kontrolltiere: 0,074±0,016 g%.)

Zahl der Tiere	Dicarbonsäure	verfütterte Menge g	Leberglykogen pro 100 g Leber g%
8	Malonsäure . . .	0,2	0,040±0,012
6	Bernsteinsäure[5] .	0,15	0,909±0,000
6	Glutarsäure . . .	0,25	0,027±0,009
8	Adipinsäure . . .	0,25	0,006±0,007
6	Pimelinsäuren . .	0,25	0,038±0,011
	Korksäure	0,3	0,043±0,011
4	Azelainsäure . . .	0,3	0,030±0,002
4	Sebacinsäure . . .	0,3	0,048±0,006

sowie die Befunde von EDSON[7], der im Leberschnittensaft eine antiketogene Wirkung dieser Dicarbonsäuren, die im Sinne einer Kohlehydratbildung gedeutet werden könnte, vermißt hat. Malonsäure erwies sich in den Versuchen des letzteren sogar stark ketogen. Höhere Dicarbonsäuren dürften somit im Rahmen des Intermediärstoffwechsels als Quelle für eine Zuckerbildung kaum in Frage kommen.

Versuche: Es wurden junge, männliche, weiße bzw. schwarze Ratten verwendet, welche vor der Fütterung 24 Stunden [illegible] hatten. Gewicht der Tiere nach dem Hungern: 110 [illegible]. Verfütterte Menge der Dicarbonsäure (als Na-Salz gegeben) [illegible] g. Resorptionsdauer: 4—8 Stunden. Glykogenbestimmung nach früheren Angaben[5]. *(Aus dem Medizinisch-Chemischen Institut der Universität Innsbruck.)*

Literatur: [1] VERKADE u. Mitarbeiter, Hoppe-Seylers Z. 215, 225; 225, 230; 227, 213; 230, 207; 234, 21; 237, 186; 247, 111; 250, 47 (1933—1937) — Biochemie. J. 28, 31 (1934) — Vgl. auch FLASCHENTRÄGER u. BERNHARD, Helvet. chim. Acta 18, 662 (1935) — BERNHARD u. ANDREAE, s. 6. — [2] Literatur bei 5. — [3] SZENT-GYÖRGYI u. Mitarbeiter, Hoppe-Seylers Z. 236, 1 (1935). — [4] RINGER, FRANKEL u. JONAS, Chem. Zbl. 2, 704 (1913). — [5] STOLLE, Hoppe-Seylers Z. 217, 153 (1933). — [6] FLASCHENTRÄGER, Hoppe-Seylers Z. 159, 207 (1926) — FLASCHENTRÄGER u. BERNHARD, Hoppe-Seylers Z. 238, 221 (1936) — BERNHARD u. ANDREAE, Hoppe-Seylers Z. 245, 103 (1936). — [7] EDSON, Biochemie. J. 30, 1[illegible]5 (1936).

Stoehr-'38
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ZUR FRAGE DER GLYKOGENBILDUNG AUS DICARBONSÄUREN.

Von

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VERKADE und Mitarbeiter[1] haben den Nachweis erbracht, daß freie Fettsäuren im Organismus neben der gewöhnlichen Oxydation in β-Stellung auch endständig oxydiert werden können (ω-Oxydation), wobei Dicarbonsäuren entstehen, deren weiterer Abbau durch ein- oder beiderseitige β-Oxydation zu Dicarbonsäuren mit niedrigerer Kohlenstoffanzahl erfolgt. Mit dem Nachweis, daß unter bestimmten Voraussetzungen zweibasische Säuren von verschiedener Kettenlänge gebildet werden können, haben diese Säuren als Zwischenstufen des Intermediärstoffwechsels allgemeines Interesse bekommen.

Die Bildung von Dicarbonsäuren durch ω-Oxydation höherer Fettsäuren gewinnt für die Stoffwechselchemie insofern besondere Bedeutung, als sie dem Organismus die Möglichkeit gibt, die für ihn ungemein wichtige Bernsteinsäurestufe (Dicarbonsäure mit 4 Kohlenstoffatomen) auf diesem Wege direkt zu erreichen, deren Entstehung bisher vorwiegend durch Dehydrierung der Essigsäure oder der Brenztraubensäure (über α-, α'-Diketoadipinsäure) angenommen worden ist[2]. Die Bedeutung der Bernsteinsäure für den Stoffwechsel ist eine zweifache: 1. die Bernsteinsäure ist das Anfangsglied des Systems Bernsteinsäure–Fumarsäure–Äpfelsäure–Oxalessigsäure, auf dem sich die Endoxydation im wesentlichen abspielen soll und dem auf Grund der Untersuchungen von SZENT-GYÖRGYI und Mitarbeitern[3] eine vorherrschende Stellung bei den Dehydrierungsvorgängen im Muskel zugeschrieben wird, 2. alle Glieder der Bernsteinsäure–Oxalessigsäure-Reihe sind ausgezeichnete Glykogenbildner[2], so daß — unter bestimmte Stoffwechsellage vorausgesetzt — bei allen Verbindungen, deren Abbau in diesem System einmündet, die Möglichkeit für einen Übergang in Kohlehydrat besteht.

In diesem Zusammenhang wurde nun die Frage geprüft, ob im Fütterungsversuch an hungernden Ratten nach Belastung mit höheren Dicarbonsäuren von *gerader* Kohlenstoffanzahl eine Zunahme des Leberglykogens nachzuweisen ist. Da nach RINGER, FRANKEL und JONAS[4] auch die Malonsäure glucoplastisch wirken soll (Versuche an phlorizinvergifteten Tieren), wurde sowohl diese Säure als auch die höheren Dicarbonsäuren mit *ungerader* Kohlenstoffanzahl in unsere Versuche einbezogen, obgleich die Säuren als Zwischen-