and to increasing surface tension of the nonionic surfactant.

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Nutritional Effects of Dihydroxystearic Acid in Rats[1,2]

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Abstract

Two matching groups of weanling male albino rats were fed a purified diet containing 5% dihydroxystearic acid. All animals survived until they were killed; the first group was sacrificed at age 150 days and the second at 305 days. Fecal fat analyses showed that at least 80% of the ingested dihydroxystearic acid was not excreted. Growth was depressed during the first four weeks; this was followed by a period of rapid weight increase. The weight of the epididymal fat pads indicated depression of neutral fat deposition. Livers and adrenals were relatively heavy in the group killed earlier and essentially normal in those killed later. This and the eventual weight increase showed that the rats adapted themselves to the intake of the material. Gas liquid chromatography carried out on the methyl ethers of the epididymal fat did not reveal the presence of dihydroxystearic acid. The fatty acid composition of the lipids of serum, kidney, liver and epididymal fat was determined on the methyl esters of the lipid extracts.

Introduction

BIOLOGICAL WORK with oxidized fats has often been hindered by the inability of investigators to use pure materials. Furthermore, in many biological studies, the main attention has been given to polymerized fractions. In view of this fact, we decided to carry out a study on rats fed dihydroxystearic acid. This material is available in quantities sufficient for feeding studies and occurs in oxidized fats, probably in concentrations of a few tenths of one per cent (1).

Dihydroxystearic acid has been used previously in nutritional studies (2,3). Harris et al. found that feeding of this material was associated with an increase in the acetyl value of the carcass fat, suggesting deposition of the material in the tissues. However, the question of whether the material is actually deposited has not been answered satisfactorily.

Experimental

The 9,10-dihydroxystearic acid used in this experiment was prepared from oleic acid according to the method of Greenspan (4). The melting point of the material was 92–94C, which is close to values previously observed. The dihydroxystearic acid was fed together with saturated triglycerides of C_{10-18} acids with the following composition: C_{10}....5%; C_{12}....45%; C_{14}....25%; C_{16}....8%; C_{18}....16%. The latter triglyceride mixture was obtained from coconut oil by fractionation of the split fatty acids and their random reconstitution into triglycerides (5). This material is well absorbed and it was hoped that it would aid in the absorption of the high melting dihydroxystearic acid with little danger of oxidative interaction between the two.

Five per cent each of dihydroxystearic acid and saturated triglycerides were included in a diet containing 30% alcohol-extracted casein, 54% destrose, 2.5% USP XIII salt mixture, 0.5% calcium carbonate, 2% cellulose, 2% of a 75% linoleic acid concentration, and adequate amounts of all vitamins. The diet was fed to two matching groups of eight weanling male rats of the Columbia-Sherman strain. Their mothers had been given, during the later stages of pregnancy and during lactation, a low fat diet prepared by the replacement of the triglycerides, the dihydroxystearic acid, and the linoleic acid concentrate in the above diet by dextrose. One per cent of a safflower oil concentrate containing at least 98% linoleic acid was added.

One group of rats was killed with chloroform at age 150 days. Blood was drawn from the heart, the organs were weighed, sections were taken for histological studies, and the rest was frozen for lipid analyses. The serum liver, kidneys, and epididymal fat pads were extracted with 2:1 chloroform:methanol, for determinations of fatty acid composition of their lipids. Methyl esters were prepared for gas-liquid chromatography by transesterification. Chromatography was carried out on a Barber-Coleman instrument having a 6 ft × ⅛ in. i.d. glass column packed with ethylene glycol succinate on Chromosorb W. The column was operated at 200C with argon at 40 psi. A sample of the dihydroxystearic acid used in these studies and a sample of the lipid extract of the epididymal fat were converted to methyl ethers because this has been found to increase the volatility of hydroxy compounds (6). The ethers were chromatographed on a 4 ft × ⅛ in. i.d. glass packed column with neopentyl sebacate on Chromosorb W operated at 225C with argon at 40 psi. The standard dihydroxystearic ether emerged after 20 min. In order to determine the approximate amount detectable in the lipid extract, 0.2% of dihydroxystearic acid was added to the depot fat and the methyl-ethers of the mixture were chromatographed. A peak of ca. 2 mm in height appeared 20 min after the injection of the material corresponding to the peak obtained on the standard run with pure

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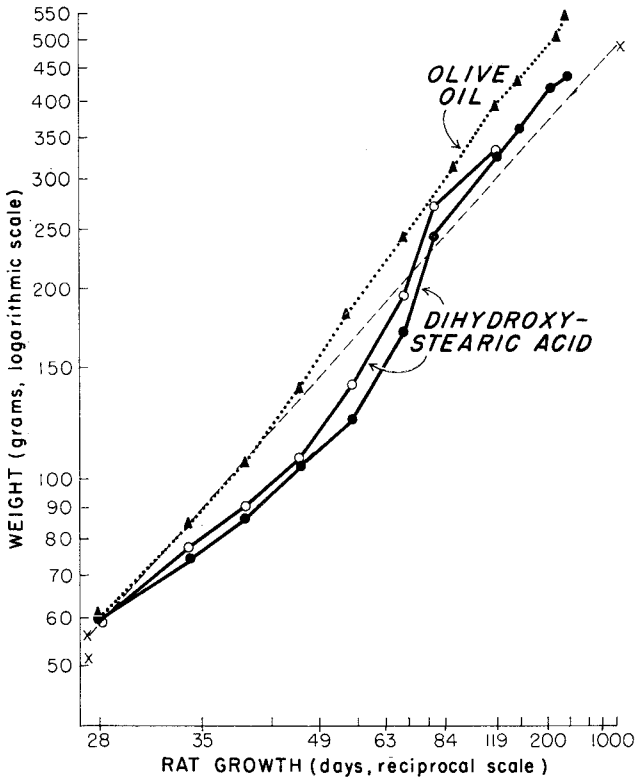

FIG. 1. Average weight gain curves of two groups of male rats fed dihydroxystearic acid. Group fed olive oil was drawn from same pool of weanling rats and placed on its diet at same time as experimental groups. Broken line represents usual weight gain curve of normally growing male rats fed commercial stock rations.

dihydroxystearic acid. Therefore, at least 0.2% of the dihydroxystearic acid in the fat mixture could have been detected.

The second group of animals was killed for the determination of organ weights when they were 305 day old.

In view of the high melting point of the dihydroxystearic acid and the resulting question of absorption, food intake and fecal fat excretion were determined on two rats over a four day period. The feces were dried, ground, boiled with 25% NaOH in methanol, acidified, and extracted with petroleum ether. During this period, the two rats together ate 101 g of diet containing 12.12 g of fat and linoleic acid, including 5.05 g of dihydroxystearic acid. They excreted 736 mg of lipid, which probably included some of the triglycerides. It is therefore certain that at least 80% of the ingested dihydroxystearic acid was not excreted in the feces.

The average weight gain curves of the groups fed dihydroxystearic acid are shown in Figure 1. Body weight is plotted on a log scale against the reciprocal of the age, a method which gives a straight line for normally growing animals (7). The broken line represents the usual weight gain of male rats fed a complete commercial stock ration. During the first four weeks on the diet, growth of the experimental animals was significantly depressed; a marked increase in weight occurred during the next three weeks; thereafter the weight gain continued at the expected rate. Whether or not the initially depressed growth was the result of a lower food intake is not certain. Eventually, the rats ate over 12 g of food per day (see above), which is normal for purified diets (8).

That the initial period of weight depression was due to the diet can be seen from the growth curve

TABLE I

Deviations [a] from Normal of Organ Weight—Body Weight Relationships of Male Rats Fed Dihydroxystearic Acid

Organs	Percentage of deviation at	
	150 days	306 days
Testicular fat pads	−12 ± 7.0 [b]	−25 ± 6.3
Liver	+13 ± 3.4	+1 ± 4.3
Kidneys	−5 ± 4.8	+2 ± 4.1
Adrenals	+22 ± 7.5	+3 ± 4.2
Heart	−4 ± 3.1	0 ± 3.8

[a] Difference between observed organ weight and "normal" organ weight for a rat of the same body weight as read from previously established organ weight—body weight curves for normal male rats. Deviations are expressed as percentages of the normal organ weights.
[b] Standard error.

of a group of male rats fed 20% of fresh olive oil instead of the dihydroxystearic acid and saturated triglycerides. This group, part of another experimental series, was drawn from the same pool of weanling rats as those under study here; they had the same initial average body weight and were placed on their diet at the same time. Their growth was essentially a straight line with a somewhat greater slope, which agrees with our previous experience with rats fed higher levels of fat than usually occur in commercial rations.

The organ weight data from the two groups fed dihydroxystearic acid are shown in Table I. The data are expressed in terms of percentages of deviation from the expected weights of organs in normal male rats of the same body weights. The expected weights were determined from organ weight—body weight curves obtained with data collected from over 400 male rats fed diets containing 10–30% of lard but otherwise similar to that used here (9).

The testicular fat pads of both experimental groups were lighter than those normally found in rats fed 10% of fat in our diet. Inasmuch as the saturated triglycerides used in this experiment have never been observed to interfere with neutral fat deposition, we would conclude that the dihydroxystearic acid was responsible for the smaller fat pads. Livers and adrenals were relatively heavy in the group killed at age 105 days but were normal in those killed later. Histological examination of the group killed earlier revealed no pathological changes.

The fatty acid composition of the lipids from testicular fat pads, serum, liver, and kidneys are shown in Table II. Chromatography of the methyl-esters of the epididymal fat did not indicate the presence of dihydroxystearic acid. The composition of the depot fat resembled most nearly that we have observed in rats fed a low-fat diet containing 2% of linoleic acid (5). However, the occurrence of appreciable amounts of C_{12} and C_{14} acid reflects the presence of these acids in the saturated triglycerides included in the diet (see composition given above). The serum lipids contained 11.2% of stearic acid, which is a high as that seen when rats are fed diets containing 20% of the saturated triglycerides.

TABLE II

Fatty Acid Composition of Lipids of Rats Fed Dihydroxystearic Acid

Acid	Fat pads	Serum	Liver	Kidneys
C_{12}	4.9	2.6	0.7	1.0
C_{14}	7.5	5.5	1.7	1.3
C_{14}:1	0.6	trace		
C_{14}:2				1.2
C_{16}	21.9	29.3	27.3	31.4
C_{16}:1	15.0	3.6	2.6	3.1
C_{16}:2				0.9
C_{18}	0.9	11.2	19.6	18.7
C_{18}:1	31.6	15.2	20.7	12.3
C_{18}:2	17.6	15.3	12.8	8.4
C_{20}:4	trace	18.0	14.7	21.7

Discussion

In feeding studies with dihydroxystearic acid triglycerides, Harris et al. found that rats fed this material grew better than their controls (2). This is in contrast to the present results with dihydroxystearic acid, but the fact that feeding of an acid gives different effects from feeding of its triglycerides has been observed repeatedly.

The weight increase after the initial weight depression, and the more normal weights of livers and adrenals in the animals killed at age 305 days, shows adaptation to the diet. We have observed this adaptation in animals fed diets containing oxidized fats (10). In the metabolism of dihydroxystearic acid, little of the ingested material was excreted but, on the other hand, none was deposited in the depot fat.

From these studies, it appears that the intake of dihydroxystearic acid does not have severely toxic effects. This would be in line with a previous observation by Larson et al., who found that the long term feeding of epoxidized soybean oil was well tolerated by rats (11). On the other hand, Nightingale et al. observed that the feeding of 25% of triglycerides containing ⅓ dihydroxystearic acid suppressed the vitamin K content of the rats' intestinal flora to such an extent that a severe disturbance of the blood clotting mechanism resulted (3).

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Gas Chromatography of *Cis-Trans* Fatty Acid Isomers on Nitrile Silicone Capillary Columns[1]

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Abstract

Three nitrile silicone polymers have been evaluated as liquid phases for gas chromatographic separation of the geometric isomers of methyl oleate, methyl linoleate, and methyl linolenate on capillary columns. A polymer of β-cyanoethylmethylsiloxane proved the most effective. This liquid phase separated oleate from elaidate, resolved the four geometric isomers of linoleate into three peaks, and divided the eight geometric isomers of linolenate into six peaks. Two other copolymers of dimethylsiloxane and β-cyanoethylmethylsiloxane gave poorer resolution of *cis-trans* isomers, but showed different elution patterns for the geometric isomers of linoleate and linolenate.

Introduction

OVER THE past few years, many workers have reported the ability of high-resolution gas liquid chromatography (GLC) columns to separate the *cis* and *trans* isomers of unsaturated fatty methyl esters. Apiezon or polyester liquid phases have usually been employed to make these separations. Previous accomplishments in this field were summarized in a recent review (1).

During recent investigations of the properties of several polar GLC liquid phases, it was noted that capillary columns coated with nitrile silicone polymers exhibited a remarkable ability to separate *cis* and *trans* isomers. We decided to investigate this effect further. This report describes the evaluation of three nitrile silicone polymers as capillary column liquid phases for separating the geometric isomers of methyl oleate, methyl linoleate, and methyl linolenate.

Procedures

Materials. The preparation and characterization of the pure fatty acid isomers and their mixtures used in this study have been previously described (1,2).

Gas Chromatography. A Barber-Colman Model 20 gas chromatograph equipped with a capillary column and an argon ionization detector was used for all gas chromatography analyses. Samples were injected into the flash vaporizer at 275–300C. By means of sample dilution with petroleum ether and a stream splitting arrangement, approximately 0.001 to 0.010 μl of methyl esters was placed on the capillary column. The detector cell was equipped with a radium ionization source and maintained at 220–240C. The ionization voltage applied to the cell electrodes was 1100 v. A scavenging flow of argon (55–65 ml/min) through the detector maintained an effective cell volume of a few μl.

Three nitrile silicones were evaluated as GLC liquid phases:

A. General Electric nitrile silicone XE-60, a copolymer of 50 mole % dimethylsiloxane and 50 mole % β-cyanoethylmethylsiloxane (3). This

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