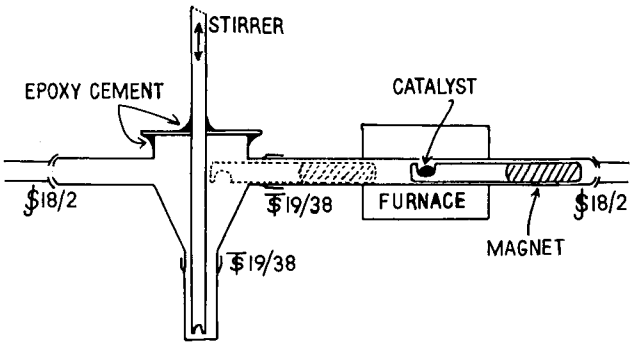

FIG. 2.

Stirrer A is made by collapsing a Pyrex tube, blowing three or four equally-spaced peripheral holes 2 or 3 mm below the cavity top, and then cutting the tube about 2 mm below the holes. The degree of agitation produced depends on hole diam and on the viscosity of the reaction mixture as well as on amplitude: jet formation and turbulence occur in water with holes of ca. 1 mm diam, but 2-mm holes are required with methyl linoleate. A stirrer of 0.6 mm I.D. produces satisfactory agitation of 0.2–0.5 ml liquid in a 1-cm I.D. vessel. Smaller quantities can be stirred by reducing the size of both stirrer and vessel.

The essentials of the reaction vessel assembly show in Figure 2. A gas-tight seal is made with epoxy cement between stirrer shaft and a 1/8-in. thick Neoprene disc and between the latter and the reaction vessel "head." Catalyst is placed in a depression in a movable tube within a side-arm of the head. The reactant in the vessels is degassed, saturated with hydrogen, and the catalyst is reduced. The catalyst tube is then moved from the furnace zone by use of an external magnet. The side-arm, and consequently the rod within it, is then rotated, so that the catalyst drops into the reaction vessel. The apparatus, in conjunction with conventional burette and gas-handling systems, is being used to study the selective hydrogenation of methyl linolenate.

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Nutritional Studies of Polyglycerol Esters[1]

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Abstract

Palatable polyglycerol esters of various mol wt were prepared with fatty acids from cottonseed and peanut oils. The wt gains of weanling male rats fed 1 g/day polyglycerol esters in 5 g of a basic diet were compared with those of matching rats fed 1 g, 0.5 g, and no lard in 5 g basic diet. After three weeks, all rats were given free access to diets containing 8% polyglycerol esters or lard. The polyglycerol esters were utilized as well as lard for wt gain. Intestinal absorption of the fatty acids from the polyglycerol esters was the same as with lard. Autopsies and histological examination of the tissues revealed no abnormalities attributable to the consumption of these materials. Appearance of the animals was normal throughout the experiment. The epididymal fat of the animals was similar regardless of the polyglycerol structure fed. No polyglycerols were detected in the body fat. From these results, it is concluded that the polyglycerol esters of both low and high mol wt were nutritionally similar to naturally occurring fats and that they were nontoxic.

Introduction

POLYGLYCEROL ESTERS have been of considerable interest to the food technologist for may years. Heretofore, only very limited application has been achieved owing to the inability of prior processing techniques to remove the unfavorable color, flavor, and odor of these compounds.

Polyglycerol esters of suitable color, flavor and odor have now been developed by utilizing improved processing techniques. Because their apparent and potential utilization in diversified food application has received wide attention, an investigation of the nutritional properties of these esters was undertaken.

Previously, Bodansky and co-workers (1) demonstrated that the lower mol wt polyglycerol esters were completely harmless when fed at high levels in the diet, even during continued long-term feeding of these compounds. The present study is designed to extend the scope of their study by including the higher mol wt polyglycerol esters.

Both the lower and higher mol wt polyglycerol esters were examined for their capacity to be utilized for energy during caloric restriction and for growth during ad libitum feeding; for the extent to which they are absorbed under restricted and ad libitum feeding; and for their effects on the fatty acid composition, wt and pathology of certain organs.

Experimental Procedures

Briefly, the polyglycerol esters were produced as follows: glycerol was polymerized with a suitable catalyst, utilizing newly developed processing techniques, to form the desired polyglycerol ranging from a diglycerol (2 glyceryl radicals) to a triacontaglycerol (30 glyceryl radicals). Subsequent esterification with the appropriate fat, oil or fatty acid yielded the desired polyglycerol ester.

The polyglycerol esters studied and the analytical values obtained for these esters show in Table I.

Weanling male rats of the Sherman Strain, born

[1] Presented at the AOCS Meeting, Atlanta, 1963.
[2] Deceased.

TABLE I

Analysis of Polyglycerol Esters

Test Materials	Identity	I.V.	Sap. No.	Hydroxyl No.	% Mono-glyceride	% Free glycerine
Drewmulse 125–14	Diglycerol hydrogenated cottonseed oil ester	41.3	130.1	327.8	21.7	3.2
Drewmulse 125–20	Triglycerol hydrogenated cottonseed oil ester	39.6	121.9	361.5	20.3	3.3
Drewmulse 137–75	Hexaglycerol peanut oil ester	58.8	123.8	297.5	12.5	1.8
Drewmulse 137–76	Hexaglycerol hydrogenated cottonseed oil ester	47.4	128.3	303.3	14.4	2.0
Drewmulse 137–71	Nonaglycerol peanut oil ester	58.6	120.0	299.1	10.9	1.7
Drewmulse 137–72	Nonaglycerol hydrogenated cottonseed oil ester	46.6	122.4	296.8	10.8	1.7
Drewmulse 137–68	Decaglycerol peanut oil ester	54.6	121.6	305.5	11.6	1.9
Drewmulse 137–69	Decaglycerol hydrogenated cottonseed oil ester	44.2	121.4	293.1	10.1	1.5
Drewmulse 137–79	Triacontaglycerol peanut oil ester	55.8	120.6	296.2	10.2	1.6

and reared on Rockland rat diet, when 28 days old (average wt 65 g) were distributed into matching groups consisting of eight rats/group. The animals were housed in individual cages which occupied four shelves of two animal racks. Cages were arranged so that corresponding animals in all groups occupied the shelves at the same level. In this manner, the effect of a possible temp gradient existing between lower and upper portions of the racks was minimized, thereby reducing the chances for experimental error.

All animals were given 5 g basic diet shown in Table II on a daily basis. At the end of this one-week preliminary feeding period, the animals were reweighed and exchanged with those occupying the same shelves, where necessary, thus ensuring equal groups on the basis of average wt gain during this period.

For the following three weeks, each animal was placed on a restricted food intake which consisted of 5 g basic diet/day. In addition, the reference groups were fed 0,0.5 and 1.0 g prime steam lard. The experimental groups were fed 1.0 g test material. The average body wt of the animals were recorded at weekly intervals. Prime steam lard values, estimated caloric equivalents, and calculated caloric values were determined for each test material. The capacity of these materials to serve as sources of energy during restricted caloric intake were evaluated according to the technique of Rice et al. (2).

Following the three-week period of restricted food intake, the animals were transferred to the diet given in Table III, which contained 8% test material or lard.

The group originally fed the basic diet was given the new diet with fat replaced by carbohydrate. The reference group which had been fed 0.5 g lard was discontinued at this time. The body wt for each group of eight male rats were recorded at weekly intervals throughout the study. At the end of the experiment, the animals were sacrificed. Autopsies were performed on all of the animals and sections of various tissues were taken for histological examination. The epididymal fat pads were weighed and saved for lipid analysis.

Fecal fat excretion, consisting of examination and determination of the total lipid extract, absorption and digestibility values of the test materials were determined during the three-week restricted and eight-week ad libitum feeding periods. A modification by Kaunitz et al. (3) of the method of Hoagland and Snider (4) was used for the determination of fecal fat. The total feces excreted in four days by two rats in each group having body wt nearest the average of their group were pooled and analyzed for their lipid content. Examination of fecal lipid extracts was achieved by TLC on silicic acid plates which were developed in a solvent system consisting of petroleum ether:ethyl ether:glacial acetic acid; 80:20:1. The plates were stained with iodine so that the degree of fractionation which occurred may be observed.

For depot fat analysis, the epididymal fat pads were macerated in clean sand and their lipids extracted with petroleum ether. The fatty acids of the extracted lipids were subjected to GLC analysis and their polyol portion analyzed for per cent glycerine. Unless otherwise indicated, fatty acid compositions of extracted lipids were determined by GLC.

For GLC analysis, the methyl ester of the extracted lipid was prepared by reacting 5 g of the lipid with 3 ml methanol containing 0.3% $NaOCH_3$ at 85C for ten min. The ester was extracted with petroleum ether and washed neutral to phenolphthalein and analyzed on a Barber-Colman GLC using a radium cell. A 0.1 μl sample of ester was injected into a six-ft column of Chromosorb W, 80–100 mesh, coated with Glutarate LAC 38 at 180C. The cell temp was 230C, applied cell potential was 750 v, flash heater temp was 290C, and sensitivity was 1×10^{-6}. Argon was used as the carrier gas at a flow rate of 150 ml/min.

Results

Caloric Values. Prime steam lard values, estimated caloric equivalents and calculated caloric values for each polyglycerol ester tested show in Table IV. Prime steam lard values were obtained by plotting the average cumulative wt gains of the reference groups at weekly intervals against the amount of lard fed. The average cumulative wt gains of the test groups were then interpolated on the appropriate reference curve and the amt of prime steam lard giving equivalent growth was determined for 1.0 g of each test material.

In general, utilization of the polyglycerol esters differed according to whether they were derived from a liquid oil or a partially hydrogenated oil. During the first week of restricted food intake, the estimated

TABLE II

Composition of Diet Fed During Preliminary and Restricted Feeding Periods

Ingredients	Content (%)
Casein	42.07
Sucrose	12.61
Cellulose	3.16
Salt mixture (Jones & Foster)	6.31
Cornstarch	29.22
Vitamin E mix[a]	1.36
Vitamin B mix[b]	1.27
Wesson oil	4.00

[a] dl Alpha tocopherol powder, Nutritional Biochemicals Inc.
[b] Rice et al. (2).

TABLE III

Composition of Diet Fed During Ad Libitum Feeding[a]

Ingredients	Content (%)
Casein	30.00
Dextrose	56.00
Salt mix	3.50
Calcium carbonate	0.50
Cellulose	2.00
Lard, test material, or additional dextrose	8.00

[a] Supplemented with adequate amt of all vitamins and with 2% linoleic acid.

TABLE IV

Caloric Values of Test Materials During Restricted Feeding Period

	1st Week		1st and 2nd Weeks		1st, 2nd and 3rd Weeks		Calculated caloric values
	Prime steam lard value	Caloric equivalent[a]	Prime steam lard value	Caloric equivalent	Prime steam lard value	Caloric equivalent	
125–14	0.86	7.74	0.76	6.84	0.73	6.57	7.5
125–20	0.80	7.20	0.76	6.84	0.66	5.94	7.4
137–75	0.94	8.46	0.74	6.66	0.72	6.48	7.8
137–76	0.64	5.76	0.61	5.49	0.64	5.76	6.7
137–71	0.74	6.66	0.67	6.03	0.66	5.94	7.4
137–72	0.67	6.03	0.66	5.94	0.67	6.03	....
137–68	0.80	7.20	0.76	6.84	0.66	5.94	7.6
137–69	0.74	6.66	0.66	5.94	0.68	6.12	6.8
137–79	0.59	5.31	0.57	5.13	0.59	5.31	6.9

[a] Lard is assumed to yield 9.0 cal/g.

caloric values of the polyglycerol peanut oil esters were rather close to the calculated values; however, during the next two weeks utilization declined by 10–20%. Similarly, the polyglycerol cottonseed oil esters were utilized somewhat less than those from the polyglycerol peanut oil esters, but there was little difference between them by the end of the third week.

Growth. The values for gain in body wt and the survival rates for all groups during the preliminary, restricted and ad libitum feeding periods show in Table V. There was a significant difference ($P < 0.1$) in the wt of the control group fed the fat free and 8% lard diets. All groups were heavier than those fed the basic diet. The differences were significant in those groups fed the tetraglycerol hydrogenated cottonseed oil ester, decaglycerol peanut oil ester and decaglycerol hydrogenated cottonseed oil ester. It should be noted that these groups attained nearly the wt of the group fed lard despite the fact that they weighed less at the beginning of the ad libitum feeding period. Furthermore, the test materials did not have the caloric value equivalent to that of lard.

Absorption and Digestibility. The wt of the lipids, mainly fatty acids, recovered from the feces, the wt of the fatty acids eaten during the collection period as calculated from the percentage content of fatty acids of the test materials, and the percentage of fat utilized by the rats fed during the restricted and ad libitum feeding period show in Table VI. These values indicate that the fatty acids of all the test materials were almost completely absorbed. For purposes of comparing the amt of test material eaten with the amount excreted, the fatty acid content of the former had to be used since only the fatty acids were recovered after hydrolysis of the fecal lipids and the glycerol moiety of the test materials was relatively large.

The fatty acid compositions of the extracted fecal lipids, in general, reflected those of the test materials. The fecal lipids from the rats fed the polyglycerol hydrogenated cottonseed oil esters were higher in palmitic, stearic and oleic acids, and lower in linoleic acid than those of the groups fed the polyglycerol peanut oil esters.

TLC of comparable amounts of test materials and the corresponding fecal lipids showed that considerable fractionation was achieved. Even without identifying the individual spots, one can conlude that the polyglycerol esters were more polar than the fecal lipids. The fecal lipids of the test groups resembled those of the control groups, but appeared to have

TABLE V

Average Body Wt (g) and Survival Rates for Groups of Eight Male Rats Fed the Test Materials During Preliminary, Restricted, and Ad Libitum Feeding Periods

	Preliminary feeding period		Restricted feeding period			Ad libitum feeding period						Survival rate
Age (days)	28	35	42	49	56	63	70	84	91	105	112	.....
Basic diet	65	75	77	79	87	122	145	178	200	244	255 ± 7.0[a]	8/8
0.5 g lard	65	75	89	96	102							
1.0 g lard	65	75	98	113	126	157	177	216	237	272	290 ± 7.7	8/8
125–14	65	75	96	105	112	143	169	193	220	249	275 ± 7.3	8/8
125–20	65	75	95	105	109	139	175	198	227	262	288 ± 9.6	8/8
137–75	65	75	97	104	111	146	166	211	230	250	268 ± 3.6	8/8
137–76	65	75	92	100	108	143	156	187	206	247	262 ± 6.9	8/8
137–71	65	75	94	102	109	136	161	204	218	245	259 ± 7.5	8/8
137–72	65	75	92	101	109	144	155	196	214	254	266 ± 11.6	6/8
137–68	65	74	94	104	108	143	161	195	218	263	280 ± 7.6	8/8
137–69	65	75	94	105	110	139	160	202	221	261	283 ± 6.9	8/8
137–79	65	75	91	99	106	136	172	198	224	259	273 ± 9.3	6/8

[a] Standard error.

TABLE VI

Fatty Acid Excretion in Stool, Fatty Acid Intake and Utilization During Restricted and Ad Libitum Feeding for Groups of Eight Male Rats[a]

	Excreted lipids (mg)		Fatty acid intake (g)		% Utilization	
	Restricted	Ad libitum	Restricted	Ad libitum	Restricted	Ad libitum
Basic diet	54	397	0	0	0	0
0.5 g lard diet	113	.....	4.0	.....	97	....
1.0 g lard diet	247	.....	8.0	.....	97	....
Lard diet	.....	570	.....	9.5	....	98
Drewmulse 125–14	123	360	5.12	6.65	98	99
Drewmulse 125–20	218	410	4.8	7.36	96	98
Drewmulse 137–75	72	413	4.96	6.43	99	99
Drewmulse 137–76	146	301	5.12	4.73	98	102
Drewmulse 137–71	141	555	4.8	6.06	98	97
Drewmulse 137–72	65	485	4.88	5.9	99	99
Drewmulse 137–68	75	466	4.88	6.59	99	99
Drewmulse 137–69	66	439	4.88	5.96	99	99
Drewmulse 137–79	96	572	4.8	6.6	99	97

[a] To obtain excreted lipids in mg/day/rat and fatty acid intake in g/day/rat, divide all values by 8.

TABLE VII

Fatty Acid Composition of Rat Epididymal Fat

	C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	% Glycerine
Basic diet	....	1.5	34.4	18.1	1.0	35.4	9.6	10.7
Lard diet	....	0.7	28.2	6.2	1.7	52.5	10.8	10.5
Drewmulse 125–14	....	0.4	31.5	13.5	0.5	41.5	12.5	10.8
Drewmulse 125–20	....	0.4	33.4	11.7	0.3	42.2	12.0	10.2
Drewmulse 137–75	....	1.3	20.6	13.2	1.1	40.9	22.9	10.8
Drewmulse 137–76	....	1.6	29.6	12.0	2.0	40.2	14.7	11.1
Drewmulse 137–71	0.3	1.6	30.2	10.6	1.0	42.7	13.7	11.4
Drewmulse 137–72	0.2	1.1	29.0	9.5	2.7	46.1	11.4	10.6
Drewmulse 137–68	....	0.5	31.0	9.2	0.6	41.1	17.2	10.6
Drewmulse 137–69	....	1.1	29.2	11.2	1.7	43.5	13.5	11.0
Drewmulse 137–79	....	0.8	33.2	7.9	1.6	43.1	13.5	10.8

some spots which had been contributed by the test materials. No spots were discernible which did not appear to correspond with spots on the control fecal plates or on the test material plates.

Autopsies and Histopathology. Throughout the experimental period all animals had a normal appearance. However, during the course of the ad libitum feeding period diarrhea was observed in those groups fed the test materials. Autopsies performed at the end of the experiment and the histological examination of the liver, kidney and ileum revealed no abnormalities attributed to the consumption of any of the test materials.

Character of Depot Fat. The GLC analysis of the fatty acids of the extracted lipids from the epididymal fat pads and the per cent glycerine analyzed from the polyol portion of the extracted lipids show in Table VII. Results are in the range of glycerine and not that of the various polyglycerols. The glycerine content of the extracted lipid suggested that only triglycerides were present or that at least no appreciable amounts of polyglycerols were deposited.

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The Metabolism of *Trans, Trans*-Octadecadienoic Acid. Incorporation of *Trans, Trans*-Octadecadienoic Acid into the C_{20} Polyunsaturated Acids of the Rat[1]

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Abstract

Trans,trans-9,12-octadecadienoic acid-1-C^{14} was fed to adult rats. After four hr the animals were killed and the fatty acids isolated from their organ lipids. The 20-carbon fatty acids were isolated and degraded stepwise.

Radioactivity of the degradation products indicated that the fed acid was incorporated into the isolated C_{20} acids, mainly eicosatetraenoic acid probably with two *trans*-double bonds, while radioactivity throughout the chain gave evidence for a synthesis of eicosatrienoic and eicosatetraenoic acid from acetate derived from the fed material.

In a separate experiment, unlabeled *trans,trans*-9,12-octadecadienoic acid was fed to weaning rats for 14 days. Isolation of their fatty acids also gave evidence for the incorporation of the fed acid into eicosatrienoic and eicosatetraenoic acids containing *trans* double bonds.

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Introduction

PRIOR TO THE advent of isotopic tracer methods, the alkaline isomerization technique provided some insight into the transformations of the polyunsaturated acids. For example, an increase in a tetraenoic acid following the feeding of linoleate was taken as presumptive evidence that linoleate was transformed into arachidonate in the animal body (1). This idea was later confirmed by experiments involving the feeding of linoleic-1-C^{14} acid to rats, followed by the isolation and stepwise degradation of arachidonic acid formed in their organs (2).

Evidence obtained by the alkaline isomerization method has also indicated that some of the *trans* isomers of polyunsaturated acids may undergo similar transformation in the animal body. Thus, Holman (3) reported that feeding of *trans,trans*-octadecadienoate to fat-deficient rats resulted in some increase in both tetraene and hexaene content of the body fat, although deficiency symptoms were not alleviated.

The present experiments represent an attempt to interpret these results. While these studies were in progress, Blank and Privett (4,5) reported that dur-