TABLE VII

Distribution of Triglycerides in Chicken Fat

Method	S	S2 [a]	S_3	S_2U	SU_2	U_3	SUS	SSU	USU	UUS
Experimental Values (8)	31.30	.....	3.00	19.00	50.00	28.00	10.00	9.00	12.00	38.00
Complete Random	31.30	.....	3.07	20.19	44.32	32.42	6.73	13.46	14.77	29.55
Restricted Random	31.30	.....	3.00	20.27	44.36	32.37	6.76	13.51	14.79	29.58
Gunstone, Theory 1	31.30	.....	0.00	22.04	49.81	28.14	22.04	0.00	0.00	49.81
1-,3-Random,-2-Random (Original method)	31.30	26.12	3.00	20.19	44.52	32.29	8.49	11.70	11.42	33.11
1-,3-Random,-2-Random ("X-Cubic" method)	31.30	26.12	3.00	20.19	44.52	32.29	8.49	11.70	11.42	33.11

[a] %S in the 2 position of the glycerol moiety.

TABLE VIII

Distribution of Triglycerides in Linseed Oil

Method	S	S2 [a]	S_3	S_2U	SU_2	U_3	SUS	SSU	USU	UUS
Experimental Values (8)	7.80	0.00	0.00	0.00	26.00	74.00	0.00	0.00	4.00	22.00
Complete Random	7.80	.....	0.05	1.68	19.89	78.38	0.56	1.12	6.63	13.26
Restricted Random	7.80	.....	0.00	1.70	20.00	78.30	0.57	1.13	6.67	13.33
Gunstone, Theory 1	7.80	.....	0.00	1.37	20.66	77.97	1.37	0.00	0.00	20.66
1-,3-Random,-2-Random (Original method)	7.80	0.00	0.00	1.37	20.66	77.97	1.37	0.00	0.00	20.66
1-,3-Random,-2-Random ("X-Cubic" method)	7.80	0.00	0.00	1.37	20.66	77.97	1.37	0.00	0.00	20.66

[a] %S in the 2 position of the glycerol moiety.

A series of calculations were made on several representative fats containing varying amounts of saturated fatty acids. The computer program was applied to the calculation of the glyceride distribution of 70 different fats representing 116 analyses which have been published by other investigators. The results of two such comparisons (chicken fat and linseed oil) are illustrated in Tables VII and VIII. The comparisons obtained indicated that the 1,3-random-2-random hypothesis of VanderWal (4) and Coleman (6) best approximated the values obtained experimentally by other investigators. The suggestions put forth by Gunstone (7) also yielded good approximations of the actual results when glyceride types were considered, but diverged when values for isomeric forms were compared. Although complete data are not available for most fats reported in the literature (in most cases only the percentages of glyceride types are available), this agreement was found to be generally true for those fats where complete data were available as well as for those where only the percentages of glyceride types has been reported.

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Structure of the Intestinal Mucosa and Lymph Glycerides of Rats after Absorption of Fats Containing Elaidic Acid[1]

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Abstract

Elaidic acid was given to rats either as free acid or triglyceride (trielaidin or mixed glycerides transesterified with elaidic acid). The intestinal mucosa and lymph triglycerides were isolated and their structure determined by pancreatic lipase. The elaidic acid level was determined by GLC using capillary columns.

Results showed a marked tendency for elaidic acid to be located at the external positions of the triglyceride molecule beginning with the lymph. The results are discussed in relation to the absorption process and triglyceride synthesis.

[1] Presented at the AOCS meeting in Houston, Texas, 1965.

Introduction

IN PREVIOUS WORK made in cooperation with Raulin (6, 7), we have demonstrated that when rats and pigs are fed rations containing elaidinized peanut oil, the *trans* fatty acids are found predominantly in the external positions of the depot triglycerides (TG). This positional specificity occurs in spite of the fact that the distribution of saturated and unsaturated acids between the internal and external positions is different in these two species.

It seemed worthwhile, therefore, to study the location of elaidic acid in the TG molecule throughout the digestive process. This paper describes experiments in which the mode of incorporation into lymph triglycerides of different forms of dietary elaidic acid was determined. The relative degree of incorporation

TABLE I

Concentration of Elaidic Acid in the TG and MG Resulting from Its Lipolysis

Diet fat	Sample	Elaidic acid		Proportion of elaidic acid in β-position
		TG	MG	
		mole %	mole %	
EE[a]	Total lymph (24h)	29.2	10.7	12
EE	Total lymph (0–6h)	17.8	14.2	26
	(6–24h)	9.6	8.0	27
FE[b]	Chylomicrons (24h)	44.7	23.0	17
EE (after butter)	Chylomicrons (24h)	71.0	48.0	23
FE " "	" "	33.5	28.0	28
FE " "	Total lymph (24h)	34.8	17.4	16
FE " "	" " (0–6h)	28.0	13.0	20
FE " "	" " (6–24h)	37.0	18.0	16
FE plus lard	Total lymph (24h)	25.8	10.0	11

[a] EE = esterified elaidic acid. FE = free elaidic acid. MG = monoglyceride. TG = triglyceride.
[b] In this experiment the fatty acid of the diet are composed of 70% elaidic acid and 30% of palmitic acid as in EE. Values in parentheses indicate length of the lymph collection period after administration of the diet.

of elaidic and oleic acids into lymph and intestinal lumen and mucosa triglycerides was also ascertained.

Procedures

Materials

The free elaidic acid (FE) used in the feeding experiments tested 99% pure by capillary gas-liquid chromatography (GLC).

The esterified elaidic acid (EE) consisted of mixed triglycerides produced by the $SnCl_2$-catalyzed esterification of a 70/30 molar mixture of elaidic and palmitic acids with glycerol.

Methods

The lipids of both the intestinal mucosa and the lymph were extracted in the cold with methylal/methanol (4/1, v/v). The lipids of the intestinal lumen were extracted using the method of Bloor (1). The lipid classes in each sample were separated by a combination of silicic acid chromatography and thin-layer chromatography (TLC). The free fatty acid fractions were esterified with diazomethane. The other lipid classes were first saponified, acidified and the extracted fatty acids methylated with diazomethane. Triglyceride structure was determined using a pancreatic lipase hydrolysis technique described elsewhere (2).

Gas Chromatography

In the first series of experiments, a modified Barber-Colman model 10 gas chromatograph was used with 150 ft long x 0.010 in. I.D. capillary columns. The columns were coated with Apiezon L in the manner described by Litchfield et al. (3). Operating conditions were: column temperature, 200C; argon flow through column, 0.7 ml/min; injection port temperature, 280C. The column resolution factor (4) between elaidic and oleic acids was equal to 2.0 and methyl stearate exhibited a resolution of 40,000 theoretical plates (HETP = 0.86 mm).

In the second series of experiments, both packed columns on a Barber-Colman gas chromatograph and capillary columns on a Perkin-Elmer model 226 gas chromatograph were used. The packed columns were 6 ft long x 4 mm I.D. and were filled with 80/100 mesh Chromosorb W impregnated with DEGS polyester (25%, w/w). Operating conditions were: column temperature, 185C; injection port temperature, 245C; argon ionization cell temperature, 220C; cell voltage, 1250 v; gas pressure at top of column, 30 psig; and scavenger gas flow, 60 ml/min. Operating conditions for the capillary columns (same as described above) on the Perkin-Elmer gas chromatograph were: column temperature, 200C; column gas flow, 0.6 ml/min nitrogen; sample vaporization temperature, 250C; flame ionization detector temperature, 220C; air flow, 300 ml/min; hydrogen flow, 29 ml/min. Under these conditions, the column resolution factor (4) between elaidic and oleic acids was 1.30, and methyl stearate exhibited a resolution of 22,000 theoretical plates (HETP = 2.25 mm).

To determine the quantities of *cis* and *trans* octadecenoates present, the total fatty acid composition of a mixture was first determined by GLC on a packed DEGS column. This gave the mole percent of total (*cis* and *trans*) 18:1 acids present. The proportions of *cis* and *trans* octadecenoates was then calculated from their respective areas in the capillary gas chromatogram. Morever, since the radioactivity of the 18:1 acids had been measured, it was possible to calculate the proportions of tritiated oleic acid to total oleic acid by taking solutions of known composition and radioactivity as standards.

Recovery of labeled fatty acids was accomplished with a Packard fraction collector placed at the outlet of a DEGS packed column. A glass wool filled cartridge was used for collecting. Radioactivity of the collection fractions was measured with a Packard Tri-Carb model 527 liquid scintillation spectrometer.

TABLE II

Fatty Acid Composition of the Dietary Fat and of the "Free Fatty Acid" fraction in the Intestinal Lumen; Fatty Acid Composition and Structure of Intestinal Mucosa and Lymph Triglycerides as Determined by Pancreatic Lipase Hydrolysis[a]

	16:0	16:1	18:0	18:1	18:1-H[3]	18:1	18:2
				mole%			
Diet				(*cis*)	(*cis*)	(*trans*)	
Free fatty acids	33.5	—	5.0	—	29.0	29.5	2.4
Intestinal Lumen							
Free faty acids	49.5	—	6.4	8.5	7.9	22.0	4.4
Intestinal Mucosa							
TG[b]	34.4	3.8	8.8	29.9	6.3	5.6	11.1
MG[b] resulting from lypolysis	32.6	4.0	3.4	41.2	2.5	3.0	13.2
Proportion in β-position	31.6	35.0	12.8	45.9	13.5	17.7	39.5
Lymph							
TG	25.6	5.4	9.9	15.7	16.1	11.0	9.1
MG resulting from lypolysis	33.5	3.0	2.9	22.9	9.8	8.0	16.8
Proportion in β-position	43.5	18.5	9.8	48.6	20.2	24.2	61.5

[a] All fatty acids have been taken into consideration for the calculation of the figures in the table. However, only the quantitatively more important acids are shown.
[b] TG = triglycerides, MG = monoglycerides.

Experimental

I

Adult rats of the Wistar strain, weighing 220–250 g each, were maintained on the "Medi-Labo" commercial diet containing 4% fat (25% linoleic acid) prior to beginning the experiments. The rats were first fasted for 15 hr and then fed a synthetic fat-free diet to which 20% of the desired lipid was added. About 90 min after the meal, their major or main lymphatic ducts were cannulated. The lymph was collected at 0C for periods varying for 0 to 24 hours after the meal. In certain cases, chylomicrons were isolated by centrifugation. It was difficult to obtain enough lymph after a meal consisting of only elaidic acid, either free or esterified in the form of mixed palmitoyl-elaidoyl triglycerides. To obtain a satisfactory yield it was necessary either to add lard to the diet (1 part of lard for every 2 parts of elaidic acid) or to cannulate the rats after the administration of a ration made of butter or lard, wait about 20 hr until the lymph cleared and then feed the 20% elaidic acid ration. The TG from the intestinal lumen, intestinal mucosa, and lymph isolated and their fatty acid and triglyceride structures analyzed.

II

A second set of experiments with tritium-labeled oleic acid was also run. In these experiments the composition of the ration was 4 parts of the synthetic fat-free diet plus one part of free fatty acids. The molar composition of these fatty acids is given in Table II. The animals were operated on after being given a meal containing sunflower oil. They were allowed to drink ad libitum. About 30 hr later, when the lymph had become clear again, they were fed the experimental meal. The lymph was then collected and its TG analyzed for fatty acid composition and triglyceride structure.

In the experiments concerning the intestinal lumen and mucosal lipids, the rats were sacrificed 2 hr after the meal. The first two-thirds of the small intestine was excised and washed with 30 ml of a 0.9% solution of NaCl. The mucosa was scraped off. The TG were isolated and analyzed as before.

Results and Discussion

First Series of Experiments

The fatty acid composition of the lymph triglycerides is not given because it changed with the presence of different fats in the diets. Therefore, only the level of elaidic acid in the original triglycerides and in the monoglycerides (MG) resulting from their lipolysis are presented in Table I. The table also gives the proportion of elaidic acid in the β-position in the triglyceride molecule calculated, as indicated by Mattson and Volpenhein (8) by the relation

$$\frac{\text{mole percent of elaidic acid in MG} \times 100}{\text{mole percent of elaidic acid in TG} \times 3} = \text{proportion of elaidic acid in } \beta\text{-position}$$

It is evident that the level of elaidic acid in the TG fraction varies markedly from one experiment to another. The only explanation for that may be the presence of different fats in the diets and also their different absorption by different animals. However, the elaidic acid is located in all cases preferentially in the external positions of the triglyceride molecule. This was previously demonstrated in adipose tissue (6,7) and can now be extended to the lymph triglycerides.

These results are quite surprising, especially when considered in relation to the digestive process, since ordinarily the internal chains of lymph triglycerides are identical or almost identical to those of the alimentary triglycerides. In the case of the EE diet, one should have found elaidic acid in the β-position in a proportion of at least 33%.

In view of the variations detected, it is difficult to make a rigorous comparison between the amount of elaidic acid in the β-position and the form—free or esterified—administered. However, since the experimental level of elaidic acid never reached a value of 33%, and since preliminary in vitro experiments demonstrated that pancreatic lipase does not have any particular specificity for *trans*-18:1 acid (2), it is probable that etiher total hydrolysis of triglycerides may have taken place in the lumen, or the monoelaidin eventually formed during luminar hydrolysis might not have been well absorbed.

Second Series of Experiments

Before discussing the results concerning the triglyceride structure, the fatty acid composition of the intestinal contents, mucosa and lymph will be briefly considered. It can be seen in Table II that the free fatty acids of the intestinal contents, which represented about 70% of the total lipids, have a composition different from that of the ration. Since our experimental procedure allows the distinction between the tritiated dietary oleic acid and the unlabeled exogenous oleic acid, it can be seen that, starting from the intestinal lumen, the dietary oleic acid is diluted by an equivalent amount of endogenous oleic acid and is found at a level three times lower than elaidic acid. The same degree of dilution can also be detected in the lymph. The dilution is more marked in the mucosa. This is understandable if the type of lipids normally present in the mucosa are taken into consideration.

Comparison of the levels of elaidic acid and of tritiated oleic acid in the intestinal contents and lymph seems to indicate, in accordance with a recent report by Coots (3), that elaidic acid is less rapidly absorbed than oleic acid.

Concerning the triglyceride structure of the lymph and mucosa lipids, it can be seen that, as in the previous experiments, the levels of elaidic acids in β-position were clearly less than 33%.

If the radioactivity in oleic acid is used as an indication of the position occupied by this acid, something very striking is apparent. Indeed, the results point out that the proportion of exogenous oleic acid in the β-position is quite different from that of endogenous oleic acid in the same position: the endogenous oleic acid is located preferentially in the β-position, whereas the exogenous acid is distributed mostly between the α- and α'-positions, as is elaidic acid. Had this distinction not been established by the use of tritiated oleic acid and by the isolation of individual fatty acids after GLC, a total content of oleic acid in the β-position of about 34% would have been found. This figure is similar to that found by Savary et al. (10) after the administration of a mixture of free fatty acids.

These experiments also provide an explanation for the fact, reported by Mattson and Volpenhein (9), that labeled oleic acid, given in the free form, is found more abundantly in the external positions of lymph glycerides.

Finally, the behavior of elaidic acid can be compared to that of oleic and stearic acid. Elaidic acid, as stearic acid, seems to be absorbed with difficulty when given in large doses in the absence of other lipids. Also, similar to stearic, elaidic acid occupies preferentially the external positions of the triglyceride molecule. However, since in accordance with the present results, exogenous oleic acid has similar behavior, it is difficult to ascertain whether this distribution is not the result of a competition with other fatty acids with a more marked affinity for the internal position.

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