Glyceride Structure of Fats[1,2]

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A SOMEWHAT anomalous situation exists in the work on the glyceride composition of fats at the present time. A good deal of evidence has been obtained which indicates that the amounts of the various glyceride types, S_3, S_2U, SU_2, and U_3, in natural fats are closely approximated by a random or restricted random distribution of the fatty acids on the glyceride molecules. This evidence has been obtained by fractional crystallization (1, 2, 3), oxidative cleavage of unsaturated acids followed by fractionation (3, 4), and by countercurrent distribution (5, 6, 7). Calculations of both of the above distributions are based on the assumption that all the possible positional isomers occur in the amounts predicted by chance.

On the other hand, equally good evidence has been obtained to show that only certain of these positional isomers occur in natural fats or at least that certain isomers predominate. This evidence has been obtained by x-ray diffraction studies and thermal analysis of individual glycerides obtained from certain fats (8, 9, 10) and by lipase hydrolysis of fats (11, 12, 13).

This apparent anomaly was pointed out by Vander Wal in a recent paper (14). However, as he suggested, these two sets of data are not necessarily conflicting since one deals only with the amounts of the glyceride types and the other with the configuration within a glyceride type. Mathematically at least it is quite possible to have the amounts of the glyceride types equivalent to the amount predicted by a random distribution but to have only one specific isomer for each glyceride type. A possible explanation of how such an arrangement could come about in the biosynthesis of fats is the basis of this paper.

It is difficult to imagine any scheme other than random attachment of the fatty acids at each stage in triglyceride synthesis which would result in random amounts of the glyceride types in the final product. Assuming then that such a random attachment takes place, this must be followed by an intramolecular rearrangement at some stage in the synthesis which gives rise to a predominance of a particular positional isomer. Such a rearrangement could take place after the addition of two or three fatty acids, and these additions could be in the order 1, 2, 3, or 1, 3, 2 on the glycerol molecule. Examination of these possibilities, in the light of the data available in the literature and some of our own data on the lipase hydrolysis of natural fats, showed that the assumption of a random attachment in the order 1, 2, 3 with an intramolecular rearrangement at the 1, 2, diglyceride stage gave a good correlation of the data.

In the majority of fats which have been tested, a preference has been shown for the saturated acids in the 1 and 3 positions and the unsaturated in the 2 position, *i.e.*, the S_2U fraction is predominately symmetrical and the SU_2 predominately unsymmetrical. For this class of fat, termed Type I, the proposed scheme of fatty acid distribution is shown in Figure 1. A random attachment in the 1 position,

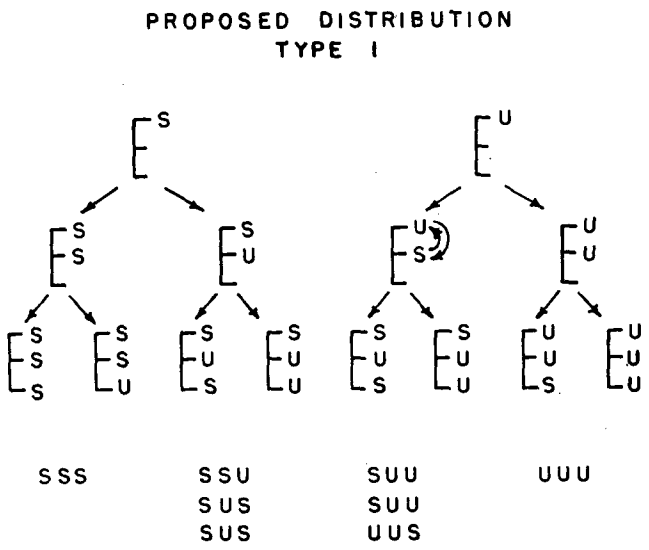

FIG. 1.

followed by a random attachment in the 2 position, gives rise to four possible diglycerides. There is no advantage to be gained in rearranging the disaturated or the diunsaturated glycerides. The preferred arrangement of the mixed glycerides for this type of fat is 1-saturated, 2-unsaturated so that the diglyceride having this configuration remains unchanged and the one having the opposite configuration rearranges. A further random addition in the third position gives eight possible triglycerides. These glycerides are grouped according to type at the bottom of the figure. As in a strict random distribution, there are one possibility for the formation of S_3 and U_3 and three possibilities for the formation of S_2U and SU_2. Unlike a strict random distribution however, the S_2U consists of two symmetrical isomers, SUS, to one unsymmetrical isomer, SSU, and the SU_2 is all the unsymmetrical form, SUU. In effect, we have a distribution that gives random amounts of the various glyceride types but with predominance of the saturated acids in the 1 and 3 positions.

The one known fat which does not conform to the Type I pattern is pork fat. In this fat, termed Type II, there is a predominance of the saturated acids in the 2 position and unsaturated acids in the 1 and 3 positions. The proposed distribution scheme for this type of fat is given in Figure 2. Random attachment in the 1 and 2 positions gives the four diglycerides as before. The preferred arrangement of the diglycerides in this case is 1-unsaturated, 2-saturated so that the mixed diglyceride having this configuration remains unchanged and the one with the opposite configuration rearranges. A further random

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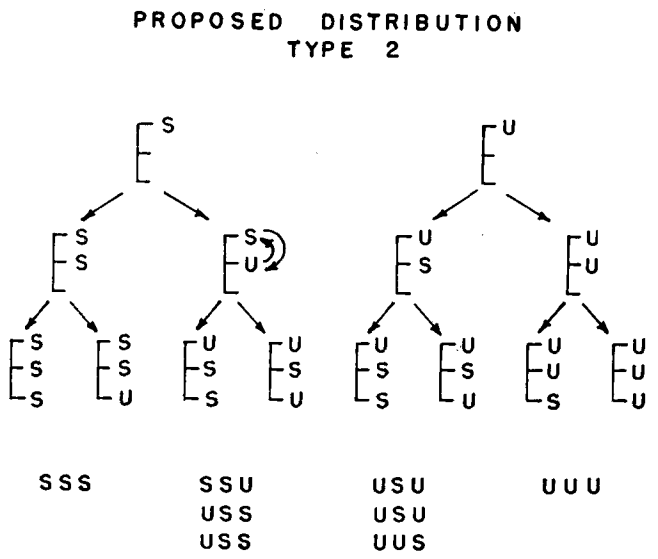

FIG. 2.

attachment in the 3 positions gives eight triglycerides as before, and these are grouped according to glyceride type at the bottom of the figure. Once again, there are one possibility for the formation of S_3 and U_3 and three possibilities for S_2U and SU_2. The S_2U in this case is all the unsymmetrical isomer, and the SU_2 has two symmetrical isomers to one unsymmetrical isomer.

It should be stressed that we are not proposing that either the glycerol or fatty acids exist free *in vivo*. It is likely that both are present as some complex. For example, the proposed scheme would fit in with *a*-glycerol phosphate, being a precursor of triglycerides.

In the case of restricted random distribution, the theory proposed by Kartha can be modified by restricting the amount of disaturated diglyceride rather than the amount of trisaturated glyceride. The amount of disaturated diglyceride is limited to that amount which on the random attachment of the third acid gives rise to the experimentally determined amount of trisaturated glyceride. This modified restricted random distribution can be used for both Type I and Type II fats. This modification gives good agreement with the experimental results of Luddy *et al.* (3), who found that certain fats do not fit either a random or restricted random distribution. The calculations involved in the modified restricted random distribution are illustrated below for the lard, analyzed by Luddy *et al.*

Composition of lard S–39.4%
U–60.6%

Determined amount of S_3 – 2.8%

Amount of disaturated diglyceride (S_2) to give this amount of S_3 is $\frac{2.8\%}{39.4\%} = 7.1\%$.

Amounts of diglycerides formed on random attachment are

SS $39.4\% \times 39.4\% = 15.5\%$
US $2 \times 39.4\% \times 60.6\% = 47.8\%$
UU $60.6\% \times 60.6\% = 36.7\%$

Amount of SS to be interchanged with UU to give 2 US is $15.5\% - 7.1\% = 8.4\%$.

Restricted amounts of diglycerides are

SS $15.5\% - 8.4\% = 7.1\%$
US $47.8\% + 2 \times 8.4\% = 64.6\%$
UU $36.7\% - 8.4\% = 28.3\%$

Amounts of triglycerides on the third random attachment are

SSS $7.1\% \times 39.4\% = 2.8\%$
SSU $7.1\% \times 60.6\% = 4.3\%$
USS $64.6\% \times 39.4\% = 25.4\%$
USU $64.6\% \times 60.6\% = 39.2\%$
UUS $28.3\% \times 39.4\% = 11.2\%$
UUU $28.3\% \times 60.6\% = 17.1\%$

The compositions of lard, chicken fat, palm oil, and cottonseed oil calculated in this way are given in Table I along with the experimental values of Luddy *et al.* The agreement is better than either a random or restricted distribution.

TABLE I

Calculated Compositions of Fats and Experimental Values of Luddy *et al.* (3)

	S_3	S_2U	SU_2	U_3
Lard				
Experimental	2.8	27.4	54.8	15.0
Proposed distribution	2.8	29.7	50.4	17.1
Random distribution	6.1	28.3	43.4	22.2
Restricted random distribution	2.8	33.4	43.0	20.8
Chicken fat				
Experimental	2.3	17.9	49.2	30.6
Proposed distribution	2.3	19.1	46.0	32.7
Random distribution	2.8	19.3	44.2	33.7
Restricted random distribution	2.3	20.0	44.2	33.5
Palm oil				
Experimental	9.4	48.1	39.3	3.2
Proposed distribution	9.4	48.6	38.4	3.6
Random distribution	16.2	40.6	33.8	9.4
Restricted random distribution	9.4	52.2	31.0	7.4
Cottonseed oil				
Experimental	0	14.5	51.0	34.5
Proposed distribution	0	14.3	51.6	34.1
Random distribution	1.9	15.7	43.0	39.4
Restricted random distribution	0	18.4	43.3	38.3

Support for the contention that there is an intramolecular rearrangement at the 1,2-diglyceride stage was found first in the work of Savory, Flanzy, and Desnuelle (11), who divided a number of fats into fractions consisting largely of S_2U and SU_2. These fractions were then hydrolyzed by pancreatic lipase, and the iodine values of the original fraction and of the liberated fatty acids were determined. As shown earlier by Savory and Desnuelle (15) and by Mattson and Beck (16), such hydrolysis is specific for the 1 and 3 positions, and the expected iodine value of the liberated fatty acids can be calculated for the proposed distributions. For example, in the S_2U fraction, the mole fraction of unsaturated acids in the original fraction is one-third, and in the 1 and 3 positions in Type I fats it is one-sixth (Figure 1). The iodine value of the liberated fatty acids would therefore be expected to be one-half that of the original fraction. The iodine values of the liberated fatty acids calculated in this way are given with the experimental values found by Desnuelle *et al.* in Table II for beef, mutton, and pork fats and for palm oil. The modified restricted random distribu-

TABLE II

Lipase Hydrolysis of Fat Fractions

Fat fraction	I.V. of total fatty acids	I.V. of liberated fatty acids	
		Found by Desnuelle *et al.* (11)	Calculated for proposed distribution
S_2U beef	30	17	15
SU_2 beef	57	45	43
S_2U mutton	37	21	19
SU_2 mutton	67	51	50
S_2U pork	39	57	58
SU_2 pork	70	82	91
S_2U palm	50	30	13
SU_2 palm	73	59	55

tion was used to determine the proportions of the various isomers for pork fat and palm oil. Agreement between experimental and calculated values was good in all cases except the SU_2 fraction from pork fat and the S_2U fraction from palm oil. The iodine values of both of these fractions varied considerably from those calculated from the composition of the original fats.

The same type of calculation applied to the fractions above can be applied to the lipase hydrolysis of whole fats. The amounts of each isomer can be calculated as the product of the mole fractions of the three acids times the number of possibilities for the formation of that isomer. The mole fraction of saturated acids liberated on lipase hydrolysis is then the sum of the amount of each isomer, multiplied by the mole fraction of saturated acids in the 1 and 3 positions of the isomer.

Mattson and Beck (12) have hydrolyzed lard with pancreatic lipase and analyzed the liberated fatty acids. For 36 mol percentage of saturated acids in the original lard they found 22.1 mol percentage of saturated acids in the liberated fatty acids. Calculations on the basis of the proposed theory, assuming a restricted distribution with 2.8 mol percentage S_3, predicts 21.9 mol percentage of saturates in the liberated fatty acids.

To determine if the theory gave equally good agreement with other fats, a number of animal and vegetable fats were hydrolyzed by pancreatic lipase in this laboratory, and the liberated fatty acids were analyzed. Details of the procedure are given in the experimental section. The results are summarized in Table III. Agreement was good in all cases.

TABLE III
Lipase Hydrolysis of Whole Fats

Fat	Mole % saturated acids		
	Whole fat	Liberated acids	
		Calculated	Found
Beef	58	70	68
Mutton	49	61	57
Pork	44	25 [a]	26
Chicken	31	42	38
Mouse	33	43	41
Rat	33	45	44
Hamster	23	32	31
Gopher	15	21	23
Guinea pig	30	40	36
Human	38	50	46
Olive oil	15	22	21
Safflower oil	10	14	14
Cacao butter	60	89 [b]	85

[a] Restricted distribution assuming 2.8% S_3.
[b] Restricted distribution assuming 2% S_3.

Very recently Mattson and Lutton (13) have published results on the lipase hydrolysis of whole fats, in which the resulting monoglycerides were recovered and analyzed. The compositions calculated for these monoglycerides for the proposed theory and the experimental values of Mattson and Lutton are given in Table IV. Some of the more saturated vegetable fats hydrolyzed by Mattson and Lutton are not included in the table as no data were available for the calculation of a restricted distribution. Although there is a general agreement between calculated and experimental values there is more variation than in Table III. At least part of the divergence in these two tables may be caused by differences in the methods used to recover and analyze the hydrolysis products.

TABLE IV
Lipase Hydrolysis of Whole Fat by Mattson and Lutton (13)

Fat	Mol % saturated acids		
	Whole fat	Monoglycerides	
		Calculated	Found
Soybean	13	2	0
Olive	15	2	1
Peanut	21	4	1
Cottonseed	30	9	11
Palm	49	19 [a]	13
Cacao butter	60	3 [b]	10
Rat	15	2	17
Pig	22	40	44
Dog	34	12	29
Human	33	11	19
Pig	36	62 [c]	71
Horse	39	15	21
Beef	54	29	29
Sheep	58	33	33

[a] Restricted distribution assuming 9.4% S_3.
[b] Restricted distribution assuming 2.0% S_3.
[c] Restricted distribution assuming 2.8% S_3, Type II distribution.

Experimental

Ten animal fats and three vegetable fats were hydrolyzed by pancreatic lipase. The animal fats were from freshly obtained adipose tissue cold-extracted with chloroform with the exception of the mouse fat, which was total body fat. The olive oil and cacao butter were commercial samples, and the safflower oil was hot-extracted from the seed with a low-boiling petroleum solvent. The lipase was a commercial sample that was given two additional cold-extractions with acetone in a high-speed homogenizer.

One gram of fat was emulsified with 50 ml. of water containing 30 mg. of elvanol.[3] Two hundred milligrams of lipase dispersed in 5 ml. of a 1% salt solution were added to the fat emulsion, and the hydrolysis was allowed to proceed for 10 min. at 45°C. The pH was maintained at 8 by the addition of 0.1 N NaOH from an automatic titrator. After 10 min. a 10-ml. sample was withdrawn and added to a solution of 10 ml. of 1 N HCl and 20 ml. of ethanol. The total lipids were extracted from this solution with Skellysolve F,[4] and the Skellysolve was removed by evaporation under reduced pressure. The resulting lipids were treated with an ether solution of diazo-methane, which converted the liberated fatty acids to their methyl esters. After evaporation of the ether and excess diazo-methane, a sample of the lipids was injected directly into a gas-phase chromatographic unit. Under the conditions of operation of the chromatographic unit only the methyl esters representing the liberated fatty acids came through the column. The composition of the liberated fatty acids was obtained from the areas under the peaks on the chromatographic chart. The chromatographic unit used was a Beckman GC 2 with a 6-ft., ¼-in.-in-diameter column containing 15 g. of packing, consisting of 4 parts Celite to 1 part of an adipic acid-diethyleneglycol polymer (17). A temperature of 200°C. and helium flow-rate of 80 ml. per minute were used. A chart for a typical run is shown in Figure 3.

From the size of the sample injected and the total area of the peaks the extent of hydrolysis could be estimated. Twenty to 30% of hydrolysis was obtained in all cases in 10 min. In the case of beef and pork fats, samples were taken at 5, 10, and 20

[3] A polyvinyl alcohol partially acetylated.
[4] Petroleum solvent with boiling range of 35–58°C.

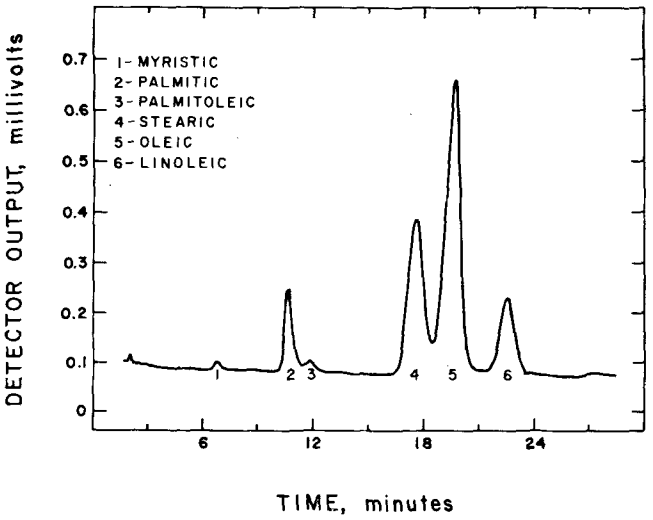

FIG. 3. Chromatograph chart for liberated fatty acids from pork fat.

min. hydrolysis times. There was no change in the composition of the liberated fatty acids over this time-interval. Hydrolysis of a sample of symmetrical oleodistearin prepared synthetically showed less than 1% oleic acid in the liberated fatty acids. The hydrolysis appeared therefore to be completely selective for the 1 and 3 positions under the conditions used.

The compositions of the original fats were also obtained by gas-phase chromatography. The fats were converted to their methyl esters by refluxing 100 mg. of fat in 10 ml. of methanol containing 1 mg. of sodium methoxide for one hour. A few drops of acetic acid were added to destroy the catalyst and the solvent evaporated to give the esters which were injected into the chromatographic unit.

Summary

A theory has been presented for the formation of fats which gives the amounts of the various glyceride types equivalent to a random or modified restricted random distribution and at the same time gives a predominance of specific positional isomers. The basis of the theory is a random attachment of the fatty acids at each stage of glyceride synthesis with an intramolecular rearrangement to a preferred form at the 1,2-diglyceride level of fat formation. The theory gives a good correlation of much of the data available on glyceride structure at the present time.

Like all previous theories, the present proposal is based on the analysis of the final products of fat synthesis without any definite knowledge of the mechanism of the synthesis. Much more work is required on the glyceride structure of fats and the mechanism of their biosynthesis before the distribution pattern of the fatty acids in natural fats can be definitely established.

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New Fat Products: Glyceride Esters of Adipic Acid[1]

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MANY practical applications as lubricants and coatings for the food industry have been visualized for highly viscous edible oils. A previous publication (6) has shown that the acylation of symmetrical diglycerides of edible fat-forming acids with dibasic acids like fumaric, succinic, and adipic produces compounds which possess a high viscosity and other unusual properties. Structurally these compounds are essentially two diglyceride molecules joined by simple ester linkages to a molecule of short-chain dibasic acid.

These esters might be expected to be edible and digestible since fumaric and succinic acids occur as metabolites in the Krebs cycle for the metabolism of fats. Recently Horn and co-workers (9) have provided evidence, based on the acute and chronic administration of adipic acid to laboratory animals, that adipic acid is physiologically comparable to citric and tartaric acids and can be added to food products with safety. While available information indicates that the glyceride esters of adipic acid as well as those of fumaric and succinic acids are edible, recommendations as to whether or not these compounds can be used as foods must await the outcome of tests now under way.

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