The Specific Distribution of Fatty Acids in the Glycerides of Animal and Vegetable Fats

F. H. Mattson and E. S. Lutton

From the Procter and Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio

(Received for publication, June 19, 1958)

The structure of naturally occurring triglycerides has been studied over a number of years with little agreement about the distribution of the fatty acids, except as to the prevalence of mixed glycerides. Several types of arrangement, for example, even, random, partial random, and restricted random, have been proposed and defended. Hilditch (1) and Bhattacharyya (2) have recently reviewed the bases of these various theories.

One reason that a number of theories developed has been the lack of suitable methods for the isolation and characterization of glycerides. It is possible, with fairly quantitative yields, to separate the triglycerides of a natural fat into glyceride classes, *i.e.* trisaturated, disaturated, monosaturated, and triunsaturated. However, only with a limited number of fats has it been possible to separate major proportions of single glycerides which can be identified by thermal and x-ray diffraction techniques. For instance, it has been shown that 2-oleoyl distearin comprises approximately 60 per cent of kokum butter (3, 4), that 2-oleoyl dipalmitin is the principal single triglyceride of stillingia tallow (5), and that 2-oleoyl palmitoyl stearin is present to the extent of 50 per cent in cocoa butter (6–8). Thus, the conclusively identified disaturated glycerides of vegetable oils are exclusively 2-oleoyl disaturated. On the other hand, lard has a very high proportion of 2-palmitoyl stearoyl "olein," clearly in contrast with beef and mutton tallow (9, 10). Thus, the evidence, although scattered, is indisputable for the occurrence in several different organisms of specific glycerides in proportions far in excess of expectations for random distribution. Nevertheless, reports continue to appear containing data which are interpreted as supporting an element of randomness in glyceride organization (11, 12).

In 1956 Mattson and Beck (13) demonstrated the specificity of pancreatic lipase for the cleavage of ester linkages at the 1 and 3 positions of triglycerides and suggested that this offered a new tool for study of triglyceride structure, a tool with certain obvious advantages over previous means. This method of enzymatic hydrolysis has been applied by Savary, Flanzy, and Desnuelle (7) to fractions of eight natural glycerides. On the basis of iodine value and, in some instances, identification of the fatty acids, they concluded that the fatty acids were not distributed at random. In the present study the enzymatic technique, applied to twelve vegetable fats and seven animal fats, has yielded strong evidence for a high degree of specificity in fatty acid distribution in the glycerides of natural fats.

* These results were reported in part at the Meeting of the American Society of Biological Chemists in April, 1956.

EXPERIMENTAL

The method used in this study of triglyceride structure is based upon the specificity of pancreatic lipase for the cleavage of ester linkages at the 1 and 3 positions of triglycerides. Since this enzyme splits few, less than 10 per cent, of the fatty acids from the 2 position of glycerol, the monoglyceride formed from a triglyceride will contain almost exclusively those fatty acids which were esterified at the 2 position of the triglyceride. Similarly, the fatty acids of the original triglyceride not accounted for in the monoglyceride fraction must have been esterified with the 1- and 3-hydroxyl groups of the glycerol.

The procedures used in this study were: (*a*) digestion of the fat with pancreatic lipase, (*b*) isolation of the monoglycerides formed as a result of digestion, and (*c*) determination of the fatty acid composition of the isolated monoglyceride and of the original triglyceride. From the proportions of fatty acids in the monoglyceride and in the original triglyceride, the distribution of fatty acids in the triglyceride was calculated.

The fats used in this study were obtained from reliable commercial or private sources, so that the identity of the fats is certain. The rat and dog fat was obtained from adult animals that had been fed a fat-free diet for 1 month before they were killed. The special lard sample, supplied to us by Dr. E. H. Ahrens of the Rockefeller Institute for Medical Research, was obtained from a pig that had been reared to a body weight of 250 pounds on a diet containing 25 per cent of safflower seed oil.

The conditions of digestion and the isolation procedure have been described previously (14). Fatty acid composition was determined by a spectrophotometric method described elsewhere (15).

RESULTS AND DISCUSSION

The fatty acid compositions of the original fats and of the monoglycerides formed during digestion are given for the vegetable fats in Table I and for the animal fats in Table III. The fatty acids of the triglycerides not accounted for in the monoglyceride fraction were esterified in the 1 and 3 positions. The percentage of each fatty acid type which was on the 2 position in the original triglyceride was calculated from the values in Tables I and III (Table II). Because of the small amount of tetraenoic acid present in these fats the distribution of this fatty acid was not calculated, since such values could have considerable error in them. Values were not calculated for the dienoic and trienoic acids of many of the fats for the same reason. Where comparable, the values in Tables I and III are in substantial agreement with those of Savary *et al.* (7).

TABLE I

Fatty acid composition of triglycerides of series of vegetable fats and of monoglycerides formed from them by hydrolysis with pancreatic lipase

Vegetable fats	Iodine value	Fatty acid composition			
		Saturated	Monoenoic	Dienoic	Trienoic
		%	%	%	%
Soybean oil					
Original triglyceride	140.8	12.8	26.8	52.7	7.8
Monoglycerides formed	149.5	0.0	42.4	52.3	6.1
Olive oil					
Original triglyceride	87.2	14.8	74.2	10.4	0.6
Monoglycerides formed	101.0	1.3	85.8	12.4	0.5
Peanut oil					
Original triglyceride	98.2	20.9	49.7	29.2	0.2
Monoglycerides formed	128.2	1.4	55.7	42.8	0.1
Cottonseed oil					
Original triglyceride	111.0	30.0	18.5	50.7	1.0
Monoglycerides formed	145.8	10.7	18.3	70.0	1.0
Aceituno fat					
Original triglyceride	56.0	40.5	56.9	2.3	0.3
Monoglycerides formed	88.0	4.3	92.8	2.8	0.1
Palm oil					
Original triglyceride	57.3	48.6	39.8	11.1	0.5
Monoglycerides formed	93.1	13.4	70.1	16.2	0.3
Shea butter					
Original triglyceride	49.5	52.2	41.3	5.9	0.7
Monoglycerides formed	75.7	28.2	59.7	11.8	0.2
Dunkwa allanblackia					
Original triglyceride	41.3	55.5	43.6	0.3	0.5
Monoglycerides formed	90.9	1.8	96.5	0.6	1.1
Kokum butter					
Original triglyceride	37.5	59.3	39.8	0.8	0.1
Monoglycerides formed	83.4	3.7	94.2	2.0	0.1
Cocoa butter					
Original triglyceride	39.7	59.9	36.4	3.5	0.2
Monoglycerides formed	96.7	9.8	88.3	1.6	0.3
Borneo tallow					
Original triglyceride	29.5	68.4	30.6	0.8	0.2
Monoglycerides formed	78.8	14.8	83.0	2.0	0.2
Stillingia tallow					
Original triglyceride	26.7	70.3	28.8	0.7	0.2
Monoglycerides formed	72.2	21.9	75.9	2.2	0.0

The fatty acids of the vegetable fats are not randomly distributed (Table II). If the distribution were random, all the values presented in Table II would be 33 per cent. This is particularly apparent in the case of the saturated fatty acids. The series of vegetable fats is made up of members containing from 13 to 70 per cent of saturated fatty acids. Of this series, shea butter had the highest proportion of its saturated acids in the 2 position, but this was only 18 per cent of the total saturated fatty acids present. Although 70 per cent of the stillingia tallow fatty acids are saturated, only 10 per cent of them are found on the 2 position; in kokum butter only 2 per cent of the saturated acids, which are 59 per cent of the whole, are found on the 2 position.

Since there was no preliminary fractionation of the original triglycerides into classes according to degree of saturation, the departure from randomness of fatty acid distribution may be even more marked than is indicated here. For example, palm oil usually contains about 5 per cent of trisaturated glycerides (9). This would account for about one-half of the saturated fatty acids found in the 2 position. Thus the diunsaturated and monounsaturated glycerides would have few molecules in which a saturated fatty acid was in the 2 position; by far the main proportion would be in the 1 and 3 positions.

From this and earlier evidence it is concluded that the disaturated triglycerides of vegetable fats are preponderantly of the 1,3 disaturated configuration which on a random basis has only one-half the probability of the 1,2 (and 2,3) configuration; the monosaturated triglycerides are largely 1 saturated.

The data, in general, do not indicate a specific distribution of the individual unsaturated acids with respect to each other, although there are some differences apart from experimental error. If there is a certain randomness in the distribution of the individual unsaturated acids, it could well account for the

TABLE II

*Proportion of each type of fatty acid that is esterified with 2 position of triglycerides of several animal and vegetable fats**

Fats	Fatty acid			
	Saturated	Monoenoic	Dienoic	Trienoic
	%	%	%	%
Vegetable				
Soybean oil	0	53	33	26
Dunkwa allanblackia	1	74	67	
Peanut oil	2	37	48	
Kokum butter	2	79		
Olive oil	3	38	40	
Aceituno fat	4	54	41	
Cocoa butter	5	81	15	
Borneo tallow	7	90		
Palm oil	9	59	49	
Stillingia tallow	10	88		
Cottonseed oil	12	33	46	33
Shea butter	18	48	67	
Animal				
Beef	18	49	88	
Horse	18	31	69	65
Sheep	19	51	68	
Human	19	38	61	
Dog (fat-free diet)	28	31	58	44
Rat (fat-free diet)	39	22	78	
Pig	65	16	17	
Pig (safflower seed oil diet)	67	15	27	

* Per cent of fatty acid type esterified with the 2 position = $\frac{\%\text{ of fatty acid type in monoglyceride formed}}{\%\text{ of fatty acid type in original triglyceride} \times 3} \times 100$.

TABLE III

Fatty acid composition of triglycerides of series of animal fats and of monoglycerides formed from them by hydrolysis with pancreatic lipase

Source of fat	Iodine value	Fatty acid composition				
		Saturated	Monoenoic	Dienoic	Trienoic	Tetraenoic
		%	%	%	%	%
Rat*						
Original triglyceride	96.6	14.8	67.0	15.8	0.8	1.6
Monoglycerides formed	111.1	17.2	44.5	37.1	0.5	0.7
Pig*						
Original triglyceride	127.1	22.3	17.7	59.4	0.0	1.1
Monoglycerides formed	95.4	44.0	8.1	47.1	0.0	0.3
Dog*						
Original triglyceride	74.0	33.9	53.0	10.7	1.5	0.9
Monoglycerides formed	86.6	28.8	49.8	18.5	2.0	0.8
Human						
Original triglyceride	68.0	32.9	59.3	7.0	0.2	0.4
Monoglycerides formed	86.1	19.0	67.4	12.9	0.2	0.5
Pig						
Original triglyceride	70.9	36.0	50.3	12.6	0.5	0.4
Monoglycerides formed	32.7	70.7	22.5	6.4	0.3	0.1
Horse						
Original triglyceride	85.7	39.1	39.4	9.7	11.0	0.8
Monoglycerides formed	130.7	20.7	37.0	20.2	21.6	0.5
Beef						
Original triglyceride	43.4	53.5	43.5	2.2	0.6	0.2
Monoglycerides formed	68.3	29.2	63.8	5.4	1.3	0.3
Sheep						
Original triglyceride	43.0	57.6	38.3	3.2	0.7	0.2
Monoglycerides formed	69.2	33.2	58.7	6.6	1.1	0.4

* The rat and dog fat were obtained from animals maintained on a fat-free diet for 1 month before they were killed; the lard was from a pig reared on a diet of 25 per cent safflower seed oil.

degree of randomness reported for linseed, soybean, and safflower seed oils (11, 12, 16).

The animal fats as a group present no pattern. The proportion of any given fatty acid on any position differs widely among the various fats. The only common feature appears to be that the fatty acids are not randomly distributed.

The fat of the pig is of particular interest, because it is the only one in which the saturated fatty acids are predominantly in the 2 position. This is true regardless of whether or not the lard is high in oleic acid or linoleic acid. This confirms the earlier work of Hilditch (9) and Quimby *et al.* (10).

Beef, horse, sheep, and human fat tend to resemble vegetable fat with a degree of selective positioning of saturated acids at the 1 and 3 positions. Of the species studied, only the rat and dog have their saturated fatty acids distributed in an approximately random fashion. There is a rather strong tendency toward location of linoleic acid at the 2 position of the triglycerides in all species except the pig. A greater degree of nonrandomness than in the case of vegetable fats is the picture among animal fats for relative oleic-linoleic acid positioning. It is clear then that any approximate correspondence of an animal's content of trisaturated glycerides with the probable values (17) is a coincidence rather than a demonstration of random fatty acid distribution.

In neither the case of animal nor vegetable fats is there support for the proposal of Kartha (18) that natural glyceride arrangement is random except for a sufficient reduction of trisaturated glycerides to maintain adequate mobility of the fatty stores.

A degree of selectivity in natural glyceride configuration is in keeping with the findings of Hanahan's (19) studies of the fatty acid distribution in beef, dog, guinea pig, and rat liver, and hen's egg lecithin. However, the saturated fatty acid is located on the 2 position of the lecithin of these species, whereas this positioning is seen only in the triglycerides of the pig.

SUMMARY

By means of pancreatic lipase, which specifically removes the fatty acids esterified with the primary hydroxyl groups of glycerol, the location of the fatty acids in the glycerides of a

number of naturally occurring vegetable and animal fats has been studied. It is concluded that:

1. Naturally occurring triglycerides exhibit a high degree of specificity of fatty acid distribution, so that random distribution does not occur in either vegetable or animal fats.

2. In the vegetable fats the saturated fatty acids are predominantly esterified with the primary hydroxyl groups of glycerol.

3. No general pattern of distribution prevails among the animal fats, although nonrandom distribution is evident. This is particularly apparent with respect to oleic-linoleic acid distribution.

4. Lard is unique in that of all the fats studied, it is the only one in which the saturated fatty acids are predominantly in the 2 position.

REFERENCES

1. Hilditch, T. P., *Ann. Rev. Biochem.*, **22,** 125 (1953).
2. Bhattacharyya, S., *Indian Soap J.*, **22,** 67 (1956).
3. Hilditch, T. P., and Murti, K. S., *J. Soc. Chem. Ind. London*, **60,** 16T (1941).
4. Lutton, E. S., *J. Am. Chem. Soc.*, **68,** 676 (1946).
5. Lutton, E. S., and Jackson, F. L., *J. Am. Chem. Soc.*, **72,** 3254 (1950).
6. Chapman, D., Crossley, A., and Davies, A. C., *J. Chem. Soc.*, 1502 (1957).
7. Savary, P., Flanzy, J., and Desnuelle, P., *Biochim. et Biophys. Acta*, **24,** 414 (1957).
8. Lutton, E. S., *J. Am. Oil Chemists' Soc.*, **34,** 521 (1957).
9. Hilditch, T. P., *The chemical constitution of natural fats*, 3rd edition, John Wiley and Sons, Inc., New York, 1956.
10. Quimby, O. T., Wille, R. L., and Lutton, E. S., *J. Am . Oil Chemists' Soc.*, **30,** 186 (1953).
11. Dutton, H. J., and Cannon, J. A., *J. Am. Oil Chemists' Soc.*, **33,** 46 (1956).
12. Scholfield, C. R., and Hicks, M. A., *J. Am. Oil Chemists' Soc.*, **34,** 77 (1957).
13. Mattson, F. H., and Beck, L. W., *J. Biol. Chem.*, **219,** 735 (1956).
14. Mattson, F. H., and Beck, L. W., *J. Biol. Chem.*, **214,** 115 (1955).
15. Official and Tentative Methods of the American Oil Chemists' Society, Chicago (1954).
16. Scholfield, C. R., and Dutton, H. J., Paper presented at the 49th Annual Meeting of the American Oil Chemists' Society, Memphis, Tennessee, April, 1958.
17. Norris, F. A., and Mattil, K. F., *J. Am. Oil. Chemists' Soc.*, **24,** 274 (1947).
18. Kartha, A. R. S., *J. Am. Oil Chemists' Soc.*, **30,** 326 (1953).
19. Hanahan, D. J., *J. Biol. Chem.*, **211,** 313, 321 (1954).