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#### TRIGLYCERIDE FATS IN HUMAN NUTRITION

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#### The Chemical Constitution of Natural Fats

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The purpose of this communication is to give a brief summary of the composition of the natural fats which come within the scope of a discussion upon the subject of triglyceride fats in human nutrition. This will be done by considering, first, typical compositions of human and other mammalian fats (depot, liver and milk) and, subsequently, the compositions of the vegetable and marine animal fats which are most commonly utilized as sources of human dietary fat.

To describe these fats in terms of their constituent mixed glycerides would be too complex and lengthy; fortunately this is unnecessary for the present purpose. All natural fats are complicated mixtures of mixed glycerides, but each individual mixed triglyceride is, in nearly all instances, elaborated according to the operation of a simple principle, often referred to as 'even' or 'widest' distribution of the fatty acid groups between the glycerol molecules. This prevailing type of glyceride structure may be briefly described (Hilditch, 1947, p. 16) by saying that as soon as a given fatty acid forms about 35% of the total fatty acids in a fat it will occur at least once in every triglyceride molecule of the fat, and that no simple triglycerides containing three groups of the same fatty acid will occur until such acid forms nearly two-thirds, or more than two-thirds, of the total fatty acids. Each fatty acid, moreover, acts independently of the rest in competing for union with glycerol according to this general plan. This general rule, most clearly recognizable in the comparatively few natural fats which contain only two or three major component acids, has been found, with very few exceptions, to hold throughout the vegetable and animal kingdoms. In two classes of animal fats only does it fail accurately to describe the general glyceride structure of natural fats: animal depot fats which are unusually rich in mixed glycerides containing stearic groups, and milk fats in which the lower saturated acids from butyric to lauric are markedly prominent. In these classes it has been suggested (Hilditch, 1947, pp. 302, 304) that the specific glyceride types present are the result of biochemical modification of an original preformed mixture of glycerides (chiefly palmito-oleins) assembled on the usual 'evenly distributed' basis.

Since, therefore, the differences between natural fats are almost wholly conditioned by the different fatty acids which are thus distributed in the triglyceride molecules, a fairly clear picture of the constitution of the fats is to be obtained by considering merely the proportions of the various fatty acids present in the whole fat. Fairly abundant data on this aspect exist, a number of which will now be quoted in illustration of the various kinds of fats concerned in nutrition. To give references to each analysis quoted is impracticable for reasons of space, and unnecessary because the reader will find full literature references (where not specifically given in this paper) elsewhere (Hilditch, 1947).

## Component acids of animal fats

Depot fats. Table 1 shows typical figures for the component acids of the adipose tissue fats of the hen and goose, and of a number of the more common domestic animals. The most prominent feature of these is the tendency towards a constant proportion of about 25–30% of palmitic acid, and the C<sub>18</sub> acids together form another approximately constant amount of 60–65% of the total acids. These are usually mainly oleic acid, but diene acids closely similar to, but not identical with, linoleic acid may occur to a varying, but usually small, extent. In a number of animals, especially (but by no means only) in ruminants, stearic acid becomes prominent and may form from about 15 to 30% of the total acids, the content of oleic acid being correspondingly reduced. It is only in these 'stearic-rich' depot fats that a seeming departure from the usual 'even distribution' rule is noticed in the glycerides, suggesting that preformed oleoglycerides may have been reduced to stearoglycerides.

Table 1. Typical component acids of animal depot fats (Percentage by weight.)

	Saturated acids  C <sub>14</sub> Palmitic Stearic			Unsaturated acids					
Animal				C <sub>16</sub>	Oleic	Octade- cadienoic	C <sub>20-22</sub>		
Hen	1	26	4	7	43	18	r		
Goose	8*	20	6	3	42	7	2		
Rat	5	28	2	7	58				
Cat	4†	29	17	4	41	2			
Pig, outer back	1	28	12	3	48	6	2		
inner back	1	30	16	3	41	7	2		
Sheep, perinephric	3	25	28	I	37	5	1		
back	3	28	16	I	47	4	I		
Ox, perinephric	3	29	21	3	41	2	I		
Horse, perinephric	3	20	7	4	39	22‡	2		
Man	3	24	8	5	47	10	3		

<sup>\*</sup> Also 12% lauric acid.

Horse fats (Holmberg & Rosenqvist, 1949; Brooker & Shorland, 1950) are exceptional in possessing less palmitic acid than usual, with much more linoleic acid and some linolenic acid, the latter acids differing from the polyene C<sub>18</sub> acids of other animal fats in being the forms which occur in vegetable fats.

<sup>†</sup> Also 3 % lauric acid.

<sup>‡</sup> Also 3 % linolenic acid.

Human depot fat (Cramer & Brown, 1943) is similar to many other animal depot fats except that its palmitic content is slightly lower, and its content of diene C<sub>18</sub> acids somewhat higher; the stearic acid content is not very great.

Liver fats. The general composition of animal liver lipids may be illustrated (Table 2) from data for the pig, sheep and ox given by Hilditch & Shorland (1937).

Table 2. Typical component acids of animal liver fats

			(Percentag	e by weigh	t.)				
	Saturated acids			Unsaturated acids					
Animal	$C_{14}$	Palmitic	Stearic	$\mathbf{C}_{16}$	$C_{18}$	$C_{20-22}$			
Liver glycerides									
Pig	_	23	9	9	47 (-2·4H)	12 (-6·8H)			
Sheep		22	13	5	45 (-2.8 H)	15 (-7·4H)			
Ox	1	30	7	11	40 (−3·oH)	11 (-6·9H)			
			Liver pl	hosphatides	i				
Pig		12	17	5	40 (-2·2H)	26 (-6·5H)			
Sheep	_	13	23	9	28 (-3·1H)	28 (-7·5H)			
Ox	-	13	27	5	27 (-3·oH)	28 (-7·5H)			

Liver lipids include considerable amounts of phosphatides as well as glycerides; the composition of the two classes is distinct, as will be clear from the following summary:

	Type of fat					
Acid	Liver phosphatides	Liver glycerides	Depot glycerides			
Palmitic (fairly constant) Stearic (rather variable) Unsaturated acids:	Lower High	High Low	High High			
$C_{16} \\ C_{18} \\ C_{20-22}$	Medium Lower (c. – 3H) High (c.–7H)	Higher High $(c3H)$ Medium $(c7H)$	Very low High (c. –2H) Very low			

Those acids which are common to depot and to liver lipids occur in quite different proportions in the three groups, and liver lipids also contain much more unsaturated acids of the  $C_{16}$  and  $C_{20-22}$  series (the latter being quite highly unsaturated) than depot fats.

Milk fats. Typical compositions for a variety of animal milk fats are shown in Table 3.

In the milk fats it is well to compare the proportions of component acids on a molar as well as on a weight basis, owing to the wide range of their molecular size (e.g. butyric 88, oleic 282). Sow-milk fats (and, indeed, a number of other milk fats, those of the dog, rat, mouse, cat) are almost identical in composition with their depot fats, but at the other end of the scale the ruminant animals produce considerable proportions of butyric and other saturated acids of unusually low molecular weight in their milk fats. Some other milk fats (horse, camel) are intermediate in character and, though containing small proportions of caprylic, capric and lauric acids, include little or no caproic or butyric acids.

Unsaturated acids Saturated acids C16 Octadecadieand  $C_6$ C14 C18 Animal  $C_4$  $C_8$ C10  $C_{12}$ C16 lower Oleic noic Percentages by weight Pig Man Horse Sheep Goat Cow I Buffalo ΙI Percentages by mol. Pig Man Horse I Sheep Goat Cow Buffalo 

Table 3. Typical component acids of animal milk fats

Human milk fat seems to be a type of its own; it contains some myristic and lauric, and a little capric acid, but none of any lower molecular weight.

In all these milk fats (except that of the horse) the proportion of palmitic acid is of the same order (22-28%) and the lower saturated acids, when present, are compensated for by lower proportions of oleic and/or diene  $C_{18}$  acids. Any explanation of milk-fat production in the animal must, it is clear, take due account of these clearly marked differences in fatty acid composition, and also of the virtual constancy of the large palmitic acid content.

# Production of storage fat by animals

It is clear that animals can synthesize fat (e.g. from carbohydrate) and also can lay down fat (or at least certain types of fat) from assimilated dietary fats.

Synthesized storage fats. That animals convert carbohydrates into fat was of course clearly demonstrated by the classical work of Lawes & Gilbert (1860, 1877); recent quantitative studies of the component acids of fats synthesized in the rat, pig and sheep have been contributed respectively by Longenecker (1939a, b), Hilditch, Lea & Pedelty (1939), and Hilditch & Pedelty (1941). Typical data for pigs grown on a low-fat diet (the fatty contents of which were known) are shown in Table 4.

It will be seen that not only was the total weight of fat produced more than three times that of the whole of the dietary fat taken, but that certain dietary fatty acids were not effectively absorbed, and that clear evidence of fatty acid synthesis was confined to palmitic, stearic, palmitoleic and oleic acids. The same state of affairs was

Fatty acid In diet Deposited Difference Saturated Below C<sub>14</sub> 0.11 Trace - 0.11 Myristic + 0.05 0.24 0.29 1.28 Palmitic 4 8 1 8.27 + 6.99 Stearic 0.25 3.99 + 3.74 Unsaturated C<sub>16</sub> Oleic 0.89 + 0.67 0.22 3.24 13.87 + 10.63 Linoleic 1.82 3.30 1.48  $C_{20-22}$ 0.87 0.13 0.74 Total 9.51 29.53 +20.02

Table 4. Synthesized fat in deposited pig fat

Increase of palmitic: increase of stearic+oleic:: 1:2.06 (wt.) or 1:1.9 (mol.).

observed in the sheep fats and, moreover, in both pigs and sheep the ratio of increase (over and above that possibly due to dietary fat) of palmitic acid to that of combined oleic and stearic acids was very close to 1:2 (mol.), suggesting that palmitodioleins are the main glycerides initially synthesized from non-fatty sources in these animals.

Assimilated dietary fats. The evidence on the nature of fats assimilated by animals from dietary fats is too abundant to be dealt with in any detail here, and attention will be focused only on one or two points which seem especially pertinent.

- (1) In general, a fat can be assimilated provided that it becomes almost completely fluid at the body temperature of the animal (i.e. fats which melt completely by about 40° are almost completely digestible).
- (2) It is not yet certain how far an animal can utilize fatty acids other than those which it can itself synthesize (e.g. palmitic, stearic, oleic, hexadecenoic). It has not yet been adequately demonstrated whether other acids (e.g. erucic acid of rape oils, elaeostearic acid of tung oil) are also compatible with animal metabolism, although it is well recognized that such oils may be initially accepted without apparent metabolic disturbance.
- (3) Acids with less than twelve carbon atoms in the molecule are not stored in animal reserve fats even when present in the dietary fats administered. Thus butyro-glycerides are not found in depot fats even when the animal has been fed on butter. Lauric acid (as glycerides in coconut fat) appears in rat depot-fats to the extent of only about 60% of that in the ingested oil (Longenecker, 1939c).
- (4) Vegetable fatty oils containing characteristic acids different from those in animal fats, when ingested by animals, frequently cause the deposition of a certain amount of such specific acids as glycerides in the depot fats; but these are apparently not laid down in the relative proportions in which they were present in the ingested oils. In other words, the composition of the depot fat of an animal fed richly on a specific vegetable oil is in no way the mean of that of the latter and of its own synthesized fat. On the contrary, ingestion of a fatty oil in quantity frequently causes undue diminution of a component acid common to both the normal animal fat and to the ingested oil, and increase in other component acids beyond those normally present

in either of these fats. This is shown notably in the work of Ellis & Isbell (1926) on pigs fed with groundnuts or soya beans, and of Ellis, Rothwell & Pool (1931) on pigs fed with varying proportions of cottonseed oil in the diet. The latter data are reproduced in Table 5, from which it will be seen that with 12% of cottonseed oil in the diet, the palmitic acid content of the pig depot-fat fell to below that of either the oil or the control depot-fat, and that the stearic acid increased considerably beyond either that in the oil or the control fat; on the other hand, linoleic acid (the major component acid in cottonseed oil) appeared in the depot fat. Increase in stearoglycerides in the depot fats of other animals (e.g. cow, buffalo) fed on cottonseed oil or oilcake has also been observed.

Table 5. Influence of dietary fat (cottonseed oil) on pig back-fat

Component fatty acids

Cottonseed	(percentage by weight)							
oil added to basal diet		Saturated	Unsaturated					
(%)	C <sub>14</sub>	Palmitic	Stearic	Oleic	Linoleic			
Nil	2,	25	14	50	9			
4	1	25	21	40	13			
8	1	22	23	36	18			
12	I	14	26	32	27			

## Component acids of some vegetable fatty oils

The composition of vegetable fatty oils from seeds or fruit coats covers a very wide range, and has little in common with that of the animal fats already discussed, except in so far that palmitic and oleic acids are often major components in both groups. On the whole, vegetable fats are much more unsaturated than animal fats, and frequently contain large proportions of linoleic as well as oleic glycerides. Other unsaturated acids are found in specific cases, such as linolenic, erucic, elaeostearic, petroselinic, lauric, myristic, arachidic, chaulmoogric. The qualitative nature of seed-fat acids usually follows very closely the botanical family to which the plant belongs, and in some instances is specific for a given genus. In not a few instances (most notably in the seed fats of the family Palmae) there is also considerable quantitative resemblance in the proportions of the component fatty acids of seeds from related botanical species or genera.

By way of illustration, the component acids of twelve of the vegetable fats and fatty oils most used for edible purposes are listed in Table 6. It will be seen at once that in many of these the unsaturated acids form the greater part of the glycerides, although in others saturated acids predominate. Moreover, the saturated acids also vary widely in nature as well as proportion, although palmitic and stearic are on the whole the most common.

# Component acids of marine animal fatty oils

Here again we encounter a type of fatty acid mixture completely different from any discussed above: although the fatty acids in the liver lipids of land animals have some small degree of affinity with the typical body or liver fats of fishes or marine mammals.

Table 6. Composition of some vegetable fatty oils

Typical component acids (percentage by weight)

Fatty oil	Saturated	Oleic	Linoleic	Linolenic	Erucic			
Coconut	92	7	I					
Palm kernel	83	16	I	_				
Cacao butter	59	39	2	_				
Palm	46	44	10		_			
Cottonseed	26	29	45	_				
Olive	13	79	8	_				
Groundnut	18	56	26	_				
Soya bean	15	23	55	7				
Sunflower seed	12	31	57	<u> </u>				
Maize	12	46	42	_				
Linseed	10	20	17	53	_			
Rape	9	16	13	8	49*			

Also 5 % eicosenoic acid.

Individual saturated acids (percentage by weight)

	C <sub>8</sub>	C <sub>10</sub>	C12	C <sub>14</sub>	C <sub>16</sub>	$C_{18}$	C <sub>20</sub>	C22	C <sub>24</sub>
Coconut	9	7	48	18	. 9	1	_		_
Palm kernel	3	6	50	16	6	2			
Cacao butter		_			24	35			_
Palm	_		_	1	39	6	_	_	
Cottonseed	_		_	Ţ	22	2	1		_
Olive	_	_	_	1	10	2	_	_	
Groundnut		_		_	9	3	2	3	I
Soya bean	_	_	_		10	4	r	_	_
Sunflower seed	_		_		6	4	I	I	
Maize	_	_	_		8	3	r		Trace
Linseed			_	_	5	4	I	_	
Rape					3	2	I	2	1

The general type of fatty acids of marine animals is roughly as follows. Saturated: up to 20% of the whole, but often less (palmitic predominating). Unsaturated: palmitoleic  $(C_{16})$  is fairly prominent (10-18%);  $C_{18}$  acids are usually the major component (25-40%), mainly oleic, with some octadecatetraenoic;  $C_{20}$  and  $C_{22}$  acids are (with palmitoleic) the characteristic acids of marine animal oils and may form together nearly half of the total acids (e.g. herring oil), down to (in other instances, for example, whales or freshwater fish) only 15-20%. In all cases these higher acids consist mainly of unsaturated acids with four, five or six ethenoid bonds, accompanied by a little monoethenoid acid.

It will thus be seen that the component acids of the glycerides of the more primitive animal organisms are far more complex in character than those of the more developed land animals.

### Hydrogenated fats

Many fatty oils are partly hydrogenated in technical practice before being incorporated into edible fats. The composition of such hydrogenated fats of course depends upon that of the original fat and upon the degree to which hydrogenation has been pursued. In general, a fatty oil is only processed to a melting-point between, say, 30 and 45°, and at these stages there is still as a rule a preponderance of unsaturated

(usually mainly monoethenoid) glycerides. This is because the hydrogenation process is largely selective in operation, i.e. saturated glycerides are not produced until nearly all linoleic (or most other polyethenoid glycerides) have been converted to the monoethenoid condition. It should further be borne in mind that the product of reduction of, for example, a linoleo-group is not wholly an ordinary oleo-group, but that considerable amounts of elaidoglycerides and also lesser amounts of cis-trans equilibrium mixtures of other isomeric oleoglycerides are produced in quantity. Thus a hydrogenated vegetable fat (e.g. groundnut or cottonseed oil), and still more so hydrogenated whale oil or herring oil, will contain component acids not present either in the original oils or normally in any of the fats of the higher animals.

#### Conclusion

In conclusion two points may be emphasized:

- 1. The above figures should convince the biochemist that quantitative consideration of the proportions of the different fatty acids of natural fats is essential to research on fat metabolism.
- 2. Iodine values and mean molecular weights, so generally utilized in biochemical investigations, are useless as guides to the real composition of these widely varying and often complex mixtures of fatty acids.

Fortunately, where saturated, oleic and linoleic acids are the only components of a fat, these can now be approximately evaluated on 3-5 g. of fat or even less, by a spectrophotometric method (Hilditch, Morton & Riley, 1945) in conjunction with the iodine value; in more complex mixtures of fatty acids, such as those of whale oil (Hilditch & Maddison, 1948) or fish oils (Hilditch & Pathak, 1948), resolution into mixtures of mainly saturated, mainly monoethenoid, and polyethenoid acids can be effected by successive crystallization from acetone at  $-60^{\circ}$  and ether at  $-40^{\circ}$ , a process which may be applied to as little as 10 g. or so of mixed acids. For fully detailed work on these complex oils, however, fractionation of the methyl esters of each group of acids is necessary, and for this purpose at least 100-200 g. of mixed fatty acids are essential.

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