The Component Glycerides of an Indian Sheep Body Fat

T. P. HILDITCH and R. K. SHRIVASTAVA, Department of Industrial Chemistry, University of Liverpool, England

THE constituent glycerides of a number of ox, sheep, and pig body fats have been studied in some detail in our laboratory on several occasions (1, 2, 3, 4). These fats were submitted to preliminary separation from solutions in acetone at various concentrations and temperatures down to 0°, fully-saturated glycerides and component acids being determined in the groups of glycerides thus separated. The technical importance of members of this important group of animal fats and the interest displayed at the present time in their glyceride structure offer good reason for further adding to the experimental studies of this group, which is characterized by the presence of important proportions of stearic as well as of palmitic and oleic glycerides and which may therefore be distinguished by the general term "stearic-rich animal body fats." Moreover, as exemplified in a recent communication by Riemenschneider *et al.* (5) and by other workers both in the United States and in our laboratory at Liverpool, the technique of partial segregation of mixed glycerides by crystallization from appropriate solvents has been much facilitated, subsequent to our earlier studies on animal body fats, by the use of temperatures down to —70°C.

We have recently had in our possession a specimen of fresh body fat from an Indian sheep. This has enabled us to make a further investigation of animal depot fat component glycerides, employing the more rigorous separations now obtainable by the use of lower temperatures (down to —30°) in crystallizations of the fat from acetone. We have thus been able to obtain component glyceride data of increased precision which, at the same time, offer an opportunity for a further review of the characteristic features of the glycerides of this interesting group of fats.

Experimental

The specimen of Indian sheep body fat examined was kindly placed at our disposal by our colleague K. T. Achaya, to whom it had been sent in May, 1947, from Bangalore. The sheep had been reared on the pasture diet (grass and leaves) normal in the hill country and mild climate of Bangalore. The fat was extracted in a fresh condition and sent to this country in a sealed tin. When opened, it was almost white in color with no sign of rancidity and had the following analytical characteristics: saponification equivalent 284.8, iodine value 39.4, with 0.3% of free fatty acid (as oleic) and 0.2% of unsaponifiable matter.

Component acids of the whole fat. Following our usual procedure, a determination was first made of the total component acids of the sheep body fat. The mixed acids (197.1 g.) from about 210 g. of the fat were first submitted to a lead salt separation from alcohol (6). The acids recovered from the lead salts which remained in solution in the alcohol were then crystallized from 10% solution in acetone at —50° for five hours. Each of the three groups of acids thus obtained was converted into methyl esters, and the latter resolved by distillation at 0.2-mm. pressure through an electrically-heated and packed column (7) into a number of fractions in the usual manner. The final composition of the Indian sheep body fatty acids, as thus determined, is shown in Table I.

The sheep body fat thus had a typical composition for its class, with palmitic acid approaching 30% (mol.), a high content (26.6% mol.) of stearic acid and 31.8% (mol.) of oleic acid as major components, together with minor proportions (of the usual order of magnitude) of myristic, arachidic, tetra- and hexadecenoic, octadecadienoic and unsaturated acids of the C_{20} (and perhaps C_{22}) series.

TABLE I

Component Acids of Indian Sheep Body Fat

Fraction		g.	%	Iodine value
A	Acids from insoluble lead salts	119.1	60.4	10.0
B	Acids from soluble lead salts, insoluble in acetone at −50°	42.6	21.6	76.2
C	Acids from soluble lead salts, soluble in acetone at −50°	35.4	18.0	97.3

Component acids (Increments % wt.)	A (60.4%)	B (21.6%)	C (18.0%)	Total	Excluding unsaponifiable	
					% (wt.)	% (mol.)
Myristic	1.3	0.4	1.2	2.9	2.9	3.4
Palmitic	23.2	3.0	1.6	27.8	27.8	29.5
Stearic	27.7	.....	.....	27.7	27.7	26.6
Arachidic	1.5	.....	.....	1.5	1.5	1.3
Tetradecenoic	.....	.....	0.3	0.3	0.4	0.4
Hexadecenoic	0.6	0.7	1.4	2.7	2.7	3.1
Oleic	6.1	17.1	9.7	32.9	33.0	31.8
Octadecadienoic	.....	.....	3.4	3.4	3.4	3.3
Unsatd. C_{20-22}	.....	0.3	0.3	0.6	0.6	0.6
Unsaponifiable	.....	0.1	0.1	0.2	.....	.....

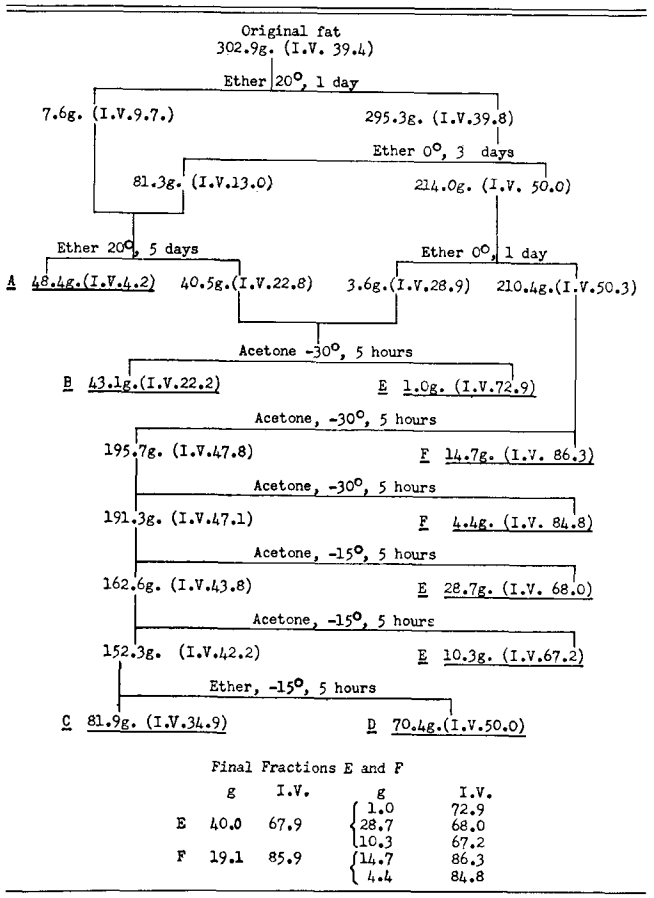
Component glycerides of the Indian sheep body fat. The fat (302.9 g.) was crystallized initially from ether at 0° or at room temperature, in order to separate the relatively large amounts of trisaturated and monounsaturated disaturated glycerides as far as possible from the more unsaturated classes of glycerides. The material left in solution was later crystallized from acetone at —30° and —15°. In this instance it was not found necessary or desirable to employ a temperature below —30°. In order to illustrate the details of the crystallizations, a crystallization chart showing all the operations (Table II) is given. All crystallizations were from 10% solutions in the solvents. The weights recorded in Table II have been corrected to the original weight of total fat to allow for minor mechanical losses and the small quantities used for determinations of iodine value.

The fractions A-F finally used for further analytical study are shown on the chart; in the cases of fractions E and F, two or three small fractions of closely similar iodine value were combined to give the final fractions.

The component acids in each of the fractions A-F were determined by our usual current procedure [preliminary crystallization of each group of mixed acids from ether at —30° (B, C, D) or —50° (E, F) followed by methylation of each group and fractional distillation of the methyl esters; the acids in fraction A were converted to esters and distilled without preliminary separation]. The final data from these

TABLE II

Crystallization of Indian Sheep Body Fat Glycerides
(All crystallizations from 10% solutions in ether or acetone; "corrected" weights)



Final Fractions E and F

	g	I.V.	g	I.V.
E	40.0	67.9	1.0	72.9
			28.7	68.0
			10.3	67.2
F	19.1	85.9	14.7	86.3
			4.4	84.8

analyses and the proportions and characteristics of the glycerides in the six fractions of the sheep fat are given in Table III.

The *fully-saturated glycerides* in the whole fat, and in fractions B and C, were determined by isolation after oxidation of the fats with potassium permanganate in acetone solution (8), and the component acids in each of the trisaturated glyceride fractions so obtained were determined by ester-fractionation

TABLE III

Indian Sheep Body Fat Glyceride Fractions

	A	B	C	D	E	F
Weight (g.)	48.4	43.1	81.9	70.4	40.0	19.1
Iodine value	4.2	22.2	34.9	50.0	67.9	85.9
Saponification equiv.	285.3	280.8	286.2	289.3	287.1	291.3
Glycerides						
% (wt.)	16.0	14.2	27.1	23.2	13.2	6.3
% (mol.)	16.0	14.5	27.1	23.0	13.2	6.2
Component acids (% wt.)						
Myristic	2.7	6.7	3.0	1.8	2.7	3.5
Palmitic	35.9	39.1	27.4	24.9	21.2	13.1
Stearic	53.7	26.7	29.5	21.0	8.8	.....
Arachidic	2.2	2.0	2.2	0.6	1.9	.....
Tetradecenoic	.....	0.4	0.3	0.6	1.2	1.5
Hexadecenoic	0.5	1.0	1.5	4.5	4.8	7.2
Oleic	4.9	21.1	32.6	42.6	48.7	63.7
Octadecadienoic	.....	1.2	2.5	3.6	8.8	7.4
Unsaturated C_{20-22}	.....	1.8	1.0	0.4	1.9	3.6
Component acids (% mol.)						
Myristic	3.2	7.8	3.6	2.1	3.3	4.2
Palmitic	38.0	40.9	29.3	26.8	22.6	14.0
Stearic	51.7	25.2	28.3	20.3	8.5	.....
Arachidic	1.9	1.7	1.9	0.6	1.6	.....
Tetradecenoic	.....	0.5	0.4	0.7	1.5	1.8
Hexadecenoic	0.5	1.1	1.6	3.9	5.1	7.8
Oleic	4.7	20.1	31.5	41.7	47.2	61.8
Octadecadienoic	.....	1.1	2.5	3.6	8.6	7.3
Unsaturated C_{20-22}	.....	1.6	0.9	0.3	1.6	3.1

(Table IV). The low iodine value (4.2) of fraction A justified the assumption that it consisted of trisaturated glycerides accompanied by a small proportion (15%) of monounsaturated disaturated glycerides. Insufficient of fraction D was available for isolation of trisaturated glycerides, and these were deduced by difference between the fully-saturated glyceride content of the whole fat and the sum of the increments of trisaturated glycerides in fractions A, B, and C [the component acids of this small increment (3.1% of the whole fat) were arbitrarily taken as 66.7% palmitic and 33.3% stearic].

TABLE IV

Trisaturated Glycerides in Indian Sheep Body Fat and Its Glyceride Fractions B and C

	Whole fat	Fraction B	Fraction C
Trisaturated glycerides % (wt.)	27.6	34.7	23.6
Trisaturated glycerides % (mol.)	28.0	35.2	23.2
Saponification equivalent	282.1	271.7	277.5
Component Acids (% wt.)			
Myristic	Not	16.4	8.4
Palmitic	deter-	50.3	47.3
Stearic	mined	33.3	44.3
Component Acids (% mol.)			
Myristic	Not	18.6	9.8
Palmitic	deter-	51.0	48.9
Stearic	mined	30.4	41.3

Discussion

Interpretation of component glyceride data (Tables III and IV). The data summarized in Tables III and IV can be utilized to define with some precision the various classes of mixed glycerides present in each of the fractions A-F of the sheep body fat. In fractions A, B, C, and D the proportion of trisaturated glycerides is known and accordingly, if triunsaturated glycerides are not also present, the proportion of monounsaturated disaturated and diunsaturated monosaturated glycerides is also determined. Triunsaturated glycerides are certainly not present in fractions A, B, and C, and probably also not to any appreciable extent in fraction D. The amount of triunsaturated glycerides likely to be present in the most soluble fractions, E and F, demands some further consideration.

In the first place, it will be seen that monounsaturated disaturated glycerides respectively form about 15%, 57%, 43%, and 23% of fractions A, B, C, and D; the three least soluble fractions, A, B, and C, contain over 75% of the total amount of this group of glycerides in the whole sheep body fat. Similarly, diunsaturated monosaturated glycerides predominate mainly in fractions D and E. In the most soluble fractions, E and F, there will certainly be no measurable proportion of trisaturated glycerides, and consequently their content of triunsaturated glycerides can be calculated to lie between the following limits: fraction F, between 73% and 45% (corresponding to between 4.6% and 2.9% on the whole fat), and fraction E, between 46% and *nil* % (corresponding to between 6.1% and *nil* % on the whole fat)—the higher alternatives representing mixtures of monounsaturated disaturated and triunsaturated glycerides with no diunsaturated-monosaturated glycerides present. It is clear from solubility considerations that the last named group will in fact be present in quantity in fractions E and F, and therefore the true

values for triunsaturated glycerides must be much closer to the minimum than to the maximum limits given. From another angle of approach, since fraction D contains only about 23% of monounsaturated glycerides, the proportion of the latter in fraction E must be still less; hypothetical contents of 20% and of 10% of monounsaturated glycerides in E would involve the presence in this fraction respectively of only 1.6% or 0.5% of triunsaturated glycerides in terms of the whole fat.

Consequently, although the quantitative evidence for the triunsaturated glyceride content of these fractions cannot be regarded as conclusive, it is felt that the considerations discussed above justify the course which has been followed and indicate that the amount of triunsaturated glycerides present can be little, if any, greater than that shown in the tables which follow.

It is interesting to note that Riemenschneider *et al.* (5), in the course of similar crystallizations of specimens of tallow and lard, reached the conclusion that their tallow contained 2.2% of triunsaturated glycerides while the lard contained a much larger amount —17.6% (mol.). The latter figure is, however, perhaps not surprising since the lard fatty acids had the unusually high content of 12.8% of linoleic acid (in addition to oleic 49.6, linolenic 0.8, and arachidonic 0.4%). With an oleic acid content of 49.6% the lard must have contained a fairly high proportion of triglycerides in which two oleic acid groups were present; the 14% of polyethenoid acids would contribute only one polyethenoid acidic group to any one triglyceride molecule so that if the remaining acyl groups were oleic, the result would be a triunsaturated glyceride. It is clear therefore that the observed 17.6% of triunsaturated glycerides is readily accountable in this manner.

In point of fact we have consistently observed in a number of fats, vegetable and animal, that the observed proportions of triunsaturated glycerides are well within the limits demanded by the possible association of one acyl group of a minor component unsaturated acid with two acyl groups of a major component unsaturated (usually oleic) acid. It will be seen that this is exemplified in fraction F of the present work, in which the 2.9% of triunsaturated glyceride increment can be more than accounted for in the form of mixed glycerides containing two oleic (1.93) groups and one minor unsaturated acid (0.97) group, the increments of oleic and of minor unsaturated acids in F being respectively 3.9 and 1.2%.

On the basis of the above discussion the glycerides in each of the fractions A-F of the sheep body fat can be divided into the categories trisaturated, monounsaturated disaturated, diunsaturated monosaturated, and triunsaturated (Table V). Further (as also illustrated in Table V) the distribution of the major component acids oleic, palmitic, and stearic in each of the glyceride fractions can also be estimated, on the assumption that each fraction contains only binary mixtures of the possible types present (e.g., glycerides containing either two, one, or no oleic group, etc.). These calculations do not indicate the presence of any tripalmitin, tristearin, or triolein although the first two of these have occasionally been reported in very small amounts in tallows (if present in the present instance, they would have been included with other mixed trisaturated glycerides in fraction A which was not further resolved in sufficient detail to reveal their presence). The analytical evidence points to the probability that the *(minor component) unsaturated acids other than oleic* only occur once in any triglyceride molecule in which they are present.

Up to this point the component glyceride data (as given in Table V) are derived solely *a)* from the observed contents of trisaturated glycerides, *b)* from considerations of relative solubility already discussed in regard to the triunsaturated glycerides likely to be present in the more soluble glyceride fractions, and *c)* (in regard to specific major component acids) from the assumption that in any given fraction binary mixtures (e.g., glycerides with no and with one oleic group, or glycerides with one and with two oleic groups, etc.) only are present. No particular theory of glyceride structure—"even" or "random"—is involved in the conclusions reached and expressed numerically in Table V. Further consideration of the data in Table V permits, however, a more definite glyceride structure to be assigned to several of the fractions, A-F. In some parts of these fractions, nevertheless, the available data are compatible with more than one interpretation in detail, and in such instances the further assumption may be

TABLE V

Categories of Component Glycerides in the Indian Sheep Body Fat

	A	B	C	D	E	F	Whole fat
Glycerides (% mol.)	16.0	14.5	27.1	23.0	13.2	6.2	100.0
Component acids (increments % mol.)							
Palmitic (and myristic)	6.6	7.1	8.9	6.7	3.4	1.1	33.8
Stearic (and arachidic)	8.6	3.9	8.2	4.8	1.3	.....	26.8
Hexa- (and tetra-) decenoic	0.1	0.2	0.5	1.0	0.9	0.6	3.3
Oleic	0.7	2.9	8.6	9.6	6.2	3.9	31.9
Polyethenoid C_{18} and C_{20-22}	.....	0.4	0.9	0.9	1.4	0.6	4.2
Component glyceride categories (increments % mol.)	(*a*) Distribution of trisaturated, disaturated monounsaturated, monosaturated monosaturated diunsaturated, and triunsaturated glycerides.						
Trisaturated	13.5	5.1	6.3	3.1	.....	.....	28.0
Disaturated monounsaturated	2.5	8.3	11.6	5.3	0.9	.....	28.6
Monosaturated diunsaturated	.....	1.1	9.2	14.6	12.3	3.3	40.5
Triunsaturated	.....	.....	.....	.....	.....	2.9	2.9
	(*b*) Distribution of (i) oleic, (ii) palmitic, and (iii) stearic groups.						
(i) Dioleo-glycerides	.....	.....	4.8	8.9	5.5	5.3	24.5
Mono-oleo-glycerides	2.5	8.7	16.0	11.0	7.7	0.9	46.6
(ii) Dipalmito-glycerides	3.8	5.7	4.8	3.1	.....	.....	17.4
Monopalmito-glycerides	12.2	8.3	17.2	13.8	10.3	3.3	65.1
(iii) Distearo-glycerides	9.7	.....	1.5	.....	.....	.....	11.2
Monostearo-glycerides	6.3	11.7	21.6	14.4	3.9	.....	57.9

TABLE VI

Possible Component Glycerides of the Indian Sheep Body Fat

	A	B	C	D	E	F	Whole fat % (mol.)
	(Increments % mol.)						
Trisaturated glycerides (28.0%)							
Tripalmitin	.....	0.5	.....	.....	.....	.....	0.5
Dipalmitostearins	3.8	4.6	4.8	3.1	.....	.....	16.3
Palmitodistearins	9.7	.....	1.5	.....	.....	.....	11.2
Monounsaturated disaturated (28.6%)							
Oleodipalmitins	.....	1.1	.....	.....	.....	.....	1.1
Oleopalmitostearins	2.5	7.1	11.7	5.2	1.0	.....	27.5
Diunsaturated monosaturated (40.5%)							
Palmito-oleo-unsaturated*	.....	0.5	1.9	3.4	5.1	.....	10.9
Palmito-di-unsaturated*	.....	0.7	.....	.....	.....	.....	0.7
Palmitodiolein	.....	.....	2.1	5.2	4.2	3.3	14.8
Stearo-oleo-unsaturated*	.....	.....	2.4	2.4	1.6	.....	6.4
Stearodioleins	.....	.....	2.7	3.7	1.3	.....	7.7
Triunsaturated glycerides (2.9%)							
Mono-oleo-di-unsaturated*	.....	.....	.....	.....	.....	0.9	0.9
Di-oleo-mono-unsaturated*	.....	.....	.....	.....	.....	2.0	2.0

*"Unsaturated" here indicates minor unsaturated components other than oleic.

made that, in any one fraction, the various component acids are likely to follow the general principles of "even" or "widest" distribution which we believe to be the fundamental characteristics of the glyceride structure of natural fats. Using the latter considerations only when an unambiguous solution cannot otherwise be reached, it is found that a possible composition of the glycerides in the whole fat—and one which we believe to be the most probable within the limits of analytical error of the whole procedure— is that illustrated in Table VI.

Conclusions

The component glycerides of an Indian sheep body fat have been investigated, employing the most recently developed experimental techniques, notably crystallization from solvents at low temperature.

The chief components of the fatty acids were palmitic (27.8), stearic (27.7), and oleic (33.0), accompanied by small proportions or traces of myristic (2.9), arachidic (1.5), tetradecenoic (0.4), hexadecenoic (2.7), octadecadienoic (3.4), and unsaturated C_{20-22} acids (0.6) (percentages by weight).

The chief constituent glycerides of the fat may be summarized as follows:

Fully saturated glycerides form 28% of the whole fat and consist largely of dipalmitostearins (16%) and palmitodistearins (11%); very small proportions of tripalmitin and possibly tristearin may also be present.

The monounsaturated glycerides (28.6%) consist largely of oleopalmito-stearins; small proportions of oleodipalmitin and perhaps also of hexadeceno-dipalmitins or -palmitostearins are also probably present.

Diunsaturated glycerides (40.5%) are the most prominent category of mixed glycerides in the sheep fat, with about 26% of palmitodiunsaturated and 14% of stearodiunsaturated glycerides. Probably nearly two-thirds of these are respectively palmitodiolein and stearodiolein, but in the remainder one of the minor component unsaturated acids takes the place of one (or in rare instances both) of the oleo-groups.

Finally, there are present about 3% of *triunsaturated glycerides,* which are made up of two (or one) oleic groups in association with one (or two) acyl groups of one or the other of the minor component unsaturated acids.

The Indian sheep body fat appears to differ somewhat from the English sheep body fats examined previously (4) in containing (in relation to its total saturated acid content) somewhat more trisaturated glycerides, definitely more of the diunsaturated glyceride group, and considerably less of the monounsaturated glyceride group.

REFERENCES

1. Hilditch, T. P., and Paul, S., Biochemical J., *32*, 1775 (1938).
2. Hilditch, T. P., and Pedelty, W. H., *ibid.*, *34*, 971 (1940).
3. Hilditch, T. P., and Murti, K. S., *ibid.*, *34*, 1301 (1940).
4. Hilditch, T. P., and Zaky, Y. A. H., *ibid.*, *35*, 940 (1941).
5. Riemenschneider, R. W., Luddy, F. E., Swain, M. L., and Ault, W. C., Oil and Soap, *23*, 276 (1946).
6. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 2nd Edition, p. 468, London: Chapman & Hall; New York: Wiley; 1947.
7. Longenecker, H. E., J. Soc. Chem. Ind., *56*, 199T (1937).
8. Hilditch, T. P., *op. cit.*, p. 513.