LXX. THE COMPONENT GLYCERIDES OF HEN BODY FATS.

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(Received January 18th, 1935.)

THE methods which were recently employed in the study of the glyceride structure of a pig dépôt fat [Hilditch and Stainsby, 1935] have been applied to hen body fats, the component fatty acids of which were discussed by Hilditch *et al.* [1934]. For this purpose the material (abdominal and gizzard fats) which had been left over from the last-mentioned work was utilised, since the full analysis of the total component fatty acids of a fat is essential for the study of its component glycerides. The quantity of fat thus remaining at our disposal was not very large, but by using both abdominal and gizzard fats (the component acids of which (*cf.* Table I) were almost identical) it proved possible:

(i) to ascertain the proportion and nature of fully-saturated glycerides present in the original fat;

(ii) to carry out fractional crystallisation of a specimen of completely hydrogenated hen body fat;

(iii) to make a complete analysis of a specimen of the fat after it had been hydrogenated to a point (iodine value 14.6) at which (by analogy with pig dépôt fat) it was expected that all dipalmito-glycerides would have passed into the fully-saturated portion; and to carry out fractional crystallisation of the fully-saturated glycerides present in this partially hydrogenated fat.

The results obtained show that, although the fatty acids present in hen body fats are, in many respects, not dissimilar from those of an animal such as the pig, the composition of the mixed glycerides is quite different. The glycerides are much more heterogeneous in character: tri- C_{18} -glycerides and dipalmito-mono- C_{18} -glycerides are present in much greater quantities than in pig dépôt fat, with a corresponding diminution in the amount of monopalmito-di- C_{18} -glycerides.

The glyceride structure of bird fats is referred to by Amberger and Bromig [1921] and Bömer [1922], both of whom submitted goose body fat to fractional crystallisation. Amberger and Bromig detected dipalmitostearin, oleodipalmitin, palmitodiolein and possibly triolein, whilst Bömer suggested that the fat probably contained about 45 % triolein, 30 % palmitodiolein, 5 % dioleostearin, 3-4 % of dipalmitostearin and a very small amount of palmitodistearin.

EXPERIMENTAL.

The abdominal and gizzard fats employed were from the batch of 24 Light Sussex hens, which Mr Halnan and Dr Cruickshank of the Cambridge School of Agriculture had kindly placed at our disposal. The diet of the fowls and the general characteristics of the fats were described in the earlier paper [Hilditch *et al.*, 1934, p. 787], while the component acids of the fats are, for reference, again quoted below (Table I). The chief features of the component acids include the

palmitic acid content (27 %—a characteristic proportion for a land animal fat), low stearic and high linoleic acid contents and the presence of 8 % of the unsaturated palmitoleic ($\Delta^{9,10}$ -hexadecenoic) acid.

Table I. *Component fatty acids of depôt fats from 24 Light Sussex hens (aged 7 months).*

Acid	Weight percentages		Molar percentages	
	Abdominal	Gizzard	Abdominal	Gizzard
Myristic	0.1	0.1	0.1	0.1
Palmitic	25.6	25.3	27.3	26.9
Stearic	7.0	7.1	6.7	6.8
Palmitoleic	7.0	7.6	7.6	8.2
Oleic	38.4	36.6	37.1	35.4
Linoleic	21.3	22.8	20.7	22.2
C ₂₀₋₂₂ unsaturated	0.6	0.5	0.5	0.4

Fully-saturated glycerides of the hen depôt fat.

These formed only a small proportion (2.5 %) of the whole fat. In this respect the fat is comparable with rat, rabbit or the more unsaturated varieties of pig depôt fats, which also contain only small quantities of stearic acid, in addition to the more or less constant amount (25–30 %) of palmitic acid.

The gizzard fat (189 g.) was oxidised in acetone solution with powdered potassium permanganate, the unchanged neutral products of oxidation were re-submitted to similar oxidation until they possessed a negligible iodine value. Fully-saturated glycerides (6.8 g.) were thus isolated in a somewhat impure condition (iodine value 0.8, acid value 19.0 and including 0.32 g. of unsaponifiable matter).¹ After applying a correction for the unsaponifiable matter and acidic compounds (calculated as azelaodipalmitin) present in the crude fully-saturated glycerides, the content of the latter in the fat was calculated to be 2.5 %. Their mixed fatty acids (M.P. 59°) had a mean molecular weight of 260.3, corresponding with a mixture of about 85 % palmitic and 15 % stearic acids; the glycerides, after several crystallisations from ether, melted at 60°, and evidently consisted mainly of tripalmitin with some palmitostearins.

Estimation of tri-C₁₈-glycerides in the hen depôt fat.

(i) *Fractional crystallisation of the completely hydrogenated abdominal fat.* After hydrogenation to as low an iodine value as possible (1.5), the fat (77.7 g.) was submitted to systematic fractional crystallisation from ether. Six crystallisations in all were performed, the volumes of ether employed varying from 10 to 30 ml./g. of glycerides crystallised. Table II shows the weights and analytical characteristics of the fractions finally obtained, given in increasing order of solubility.

Assuming that fraction A (M.P. 68°) is a mixture of tristearin (sap. equiv. 296.7) and palmitodistearin (sap. equiv. 287.3), the fat (77.7 g.) contained 21.4 g. of tristearin, corresponding to a molar content of 27 % of tri-C₁₈-glycerides in the whole fat. If all the glyceride unsaturation in fractions E, F, G is credited to oleodistearin, a further 3 % of tri-C₁₈-glycerides must be added; for reasons which will appear later however it is quite possible that, in this particular hydrogenated fat, any residual unsaturation is due as much to oleodipalmitin as to oleodistearin.

¹ Part of the unsaponifiable matter (0.14 g.) was a yellow crystalline material, M.P. 120°, which was insoluble in ether.

Table II. *Fractional crystallisation of completely hydrogenated (abdominal) hen fat.*

(77.7 g., I.V. 1.5.)					
No.	g.	Glycerides		Corresponding acids (freed from unsap.)	
		Sap. equiv.	I.V.	Sap. equiv.	I.V.
A	42.1	292.0	—	—	—
B	2.7	287.2	—	—	—
C	6.3	284.3	—	—	—
D	4.4	282.3	—	—	—
E	6.3	279.8	2.6	267.3	2.5
F	9.2	279.4	1.0	266.7	1.1
G	6.7	287.7	9.5	268.9	8.7

If fractions B to G are similarly dealt with, on the basis of the observed equivalents, as binary mixtures of palmitodistearin and dipalmitostearin, or dipalmitostearin and tripalmitin, the original fat appears to have contained about 40 % of mono- C_{16} -di- C_{18} -glycerides, 29 % of di- C_{16} -mono- C_{18} -glycerides and 1 % of tripalmitin. In presence of more than small amounts of dipalmitostearin however the quantitative separation of the latter from monopalmitodistearin and tripalmitin becomes uncertain, and it is probably better to derive the composition of these groups from the total amounts of C_{16} and C_{18} acids present in the original fat, after deducting (a) the C_{18} acids determined as tristearin (in fraction A) and (b) the palmitic acid present as tripalmitin in the fully-saturated glycerides of the original fat.

On the latter basis, the results of the fractional crystallisation of the completely hydrogenated fat indicate the following approximate molar composition of the original glycerides: tri- C_{18} , 28–29 %; mono- C_{16} -di- C_{18} , 41 %; di- C_{16} -mono- C_{18} , 28–29 %, and tripalmitin, 2 %.

(ii) *Examination of a partially hydrogenated abdominal fat (I.V. 14.6).* Lack of material compelled us to confine our investigation to a single partially hydrogenated fat, instead of studying a series of these fats as in our previous work on the pig depôt fats [1934]. The abdominal fat (100 g.) was hydrogenated until its iodine value was reduced to 14.6. The product (84.4 g.), after oxidation with potassium permanganate in acetone, yielded 50.7 g. of crude fully-saturated glycerides (I.V. 0.6). Subsequent purification of the latter (50.0 g.) gave the following products: (a) almost pure fully-saturated glycerides, 47.2 g. (acid value 0.2, sap. equiv., 285.6); (b) less pure fully-saturated glycerides, 1.9 g. (acid value 8.2); and (c) acidic products of oxidation, 0.9 g. (acid value 130.0). The content of fully-saturated glycerides was therefore 58.8 % (wt.) or 58.5 % (mol.).

Fractionation of the methyl esters (27.2 g.) from the purified glycerides (a) showed that the component acids present were:

	% (wt.)	% (mol.)
Palmitic	32.8	35.1
Stearic	67.2	64.9

The general structure of the partially hydrogenated fat was thus as shown in Table III. Here however it is not possible, in the present instance, to differentiate between the saturated and unsaturated acids of the respective C_{16} - and C_{18} -series, because the shortage of material also prevented a separate analysis of the component acids of the whole fat. The latter can readily be derived by calculation from the iodine value of the fat and the corresponding figures for the original fat (Table I), but, the individual amounts of palmitoleic and of oleic

acids remaining unhydrogenated being undetermined, it is only possible to give the total molar contents of the C_{16} and C_{18} acids in each group. Since we are only concerned, for the moment, with the relative proportions of C_{16} and C_{18} acids in the mixed saturated-unsaturated glycerides, this information is sufficient for the purpose.

Table III. *Component acids (mols.) in the fully-saturated and non-fully-saturated parts of the hydrogenated hen fat of I.V. 14.6.*

	Mols. ...	...	Whole fat	Fully-saturated	Mixed saturated-unsaturated glycerides (by difference)
			100	58.5	41.5
Myristic			0.1	—	0.1
C_{16} (Palmitic + palmitoleic)			34.9	20.5	14.4
C_{18} (Stearic + oleic)			64.5	38.0	26.5
C_{20-22}			0.5	—	0.5

If, as in other fats which have been studied, the remaining mixed saturated-unsaturated components were at this stage only tri- C_{18} -glycerides with di- C_{18} -glycerides containing only one C_{16} (or other) radical, the 15 equivalents of non- C_{18} acids present in the mixed part would require, in order to form mono- C_{16} -di- C_{18} -glycerides, more C_{18} acids (30 equivalents) than are actually present. In other words, di- C_{16} -glycerides must still be present in quantity in the mixed saturated-unsaturated part of the hydrogenated fat at present under discussion.

This support of the evidence given by the fractional crystallisation ((i) above) of the completely hydrogenated fat received additional confirmation when the purified fully-saturated glycerides (a) (14.27 g.) from the partially hydrogenated fat were systematically crystallised from ether into the fractions summarised in Table IV.

Table IV. *Fractional crystallisation of purified fully-saturated glycerides (a) from the hydrogenated hen fat of I.V. 14.6.*

No.	g.	Glycerides		Corresponding acids (freed from unsap.)	
		Sap. equiv.	I.V.	Sap. equiv.	I.V.
A	4.82	291.9	—	—	—
B	3.75	286.2	—	—	—
C	3.30	283.6	0.7	271.0	0.7
D	2.40	282.5	2.1	269.8	2.2

Estimated as in the former case (*cf.* Table II, *et seq.*) these figures show that the molar composition of these fully-saturated glycerides was approximately 16 % tristearin, 65 % palmitodistearin, 14 % dipalmitostearin and 3 % tripalmitin (with 2 % of residual unsaturated glycerides, which might be either oleodipalmitin or oleodistearin). Since these fully-saturated components amounted to 58.5 % of the hydrogenated fat of iodine value 14.6, the proportions of the various glycerides present in the latter fat as a whole were respectively about 10 % tristearin, 38 % palmitodistearin and 8.5 % dipalmitostearin (with 2 % tripalmitin).

DISCUSSION.

Although, as we have already seen, the presence of palmitoleic acid, in addition to oleic and linoleic acids, in combination in the hen depôt fats complicates the interpretation of the experimental data, the latter afford a fair amount of information with regard to the component glycerides present and also give rise to certain suggestive inferences.

The very minor amounts of fully-saturated components in the original fat are, as already mentioned, quite in keeping with what has been observed in other animal fats of similar total palmitic and stearic acid contents. The non-fully-saturated glycerides however are sharply differentiated from other animal depôt fats which we have studied, in that they contain far more tri- C_{18} (unsaturated) glycerides and especially di- C_{16} -mono- C_{18} glycerides, with proportionately less monopalmito-di- C_{18} -glycerides than in other cases.

In considering the foregoing data, we would next direct attention to the following points:

(i) *The monopalmitodistearin produced by hydrogenation of hen fat.* If we compare the proportions of the various fully-saturated derivatives present in the completely and the partially hydrogenated hen fats, it is clear that palmitodistearin has been formed from palmitodi-“oleins” far more rapidly and completely than the dipalmitostearin results from its precursors:

Table V.

Mols. present (per 100 mols. of fat) of:	Completely hydrogenated fat	Partially hydrogenated fat
Tristearin	28-29	10
Monopalmitodistearin	41	38
Dipalmitomonostearin	28-29	8.5
Tripalmitin	2	2

The relative ease with which the monopalmito-derivatives pass into palmitodistearin is exactly parallel with the behaviour observed in pig depôt and other fats, and it is reasonable to assume that the same glycerides are concerned in each case, *i.e.* hydrogenation of β -monopalmitodi-“oleins” to β -palmitodistearin. If this view be accepted, it also follows that palmitoleic acid is not present to any appreciable extent in the form of monopalmitoleo-di- C_{18} -glycerides.

(ii) *Inferences regarding the dipalmitostearins produced by hydrogenation of the hen fat.* During hydrogenation, the monopalmitodistearin is not only produced much more rapidly than dipalmitostearin and tristearin, but the (slower) rates of production of each of the two latter glycerides are closely similar (*cf.* Table V): this naturally suggests a single cause for the relatively slow conversion into fully-saturated glycerides in the cases of both dipalmitostearin and tristearin. It was mentioned in our previous communication [1935] that, pending the results of configurational studies directed towards establishing the mechanism of such preferential hydrogenation, we incline to the view, as a working hypothesis, that the observed differences may be connected with relative difficulty of hydrogenation of an oleic radical in the β -, as compared with that of one in an α -position. In the present case, on this hypothesis, it follows that the unsaturated precursors of dipalmitostearin (as well as of tristearin) will contain an unsaturated acyl radical attached to the β -carbon atom of the glycerol molecule.

If this be so, any palmitic radicals originally present as such in these di- C_{16} -glycerides must have been attached to α -glyceryl carbon atoms. There remains

however a further consequence of these arguments: if the mono- C_{18} -glycerides (*v. supra*) are wholly or for the most part β -monopalmitodi-"oleins", the 8 % of palmitoleic acid present in the original fat must be assigned to the di- C_{18} -glycerides now under consideration. The latter would then, for the most part, be formulated as either α -palmito- β -oleo- α' -palmitolein or α -palmito- β -palmitoleo- α' -olein. It is of some interest that the palmitoleic acid content of the whole fat (8 % mol.) would suffice to give 24 % (mol.) of these glycerides, as compared with the observed total content (p. 601) of 28-29 % of di- C_{18} -glycerides.

It is also interesting to note that, in glycerides of this type, palmitoleic acid would be acting as an individual and independently of any other fatty acid. In other words, the glyceride structure formulated seems to preclude the view that palmitic and palmitoleic acids might be regarded as interconvertible, by means of desaturation or saturation.

(iii) *Distribution of the stearic acid (7 mols. %) present in the original fat.* Apart from the insignificant amount of stearic acid combined as palmitostearins in the 2.5 % of fully-saturated glycerides, all of this acid must be in the form of mixed saturated-unsaturated glycerides. It may be present (a) as α -stearodi-"olein", (b) as β -palmitostearo-"olein", or (c) possibly as α -palmito- β -palmitoleo- α' -stearin. If it were present exclusively in any one of these forms, the quantity of the respective mixed glyceride would be about 20 % (mol.) of the whole fat.

There is naturally no evidence from our experimental data as to the precise mode of combination of this stearic acid. It might be confined to one isomeride; perhaps more probably, it may be distributed amongst the different types. In the summary of component glycerides (Table VI) the three possibilities can therefore merely be indicated without offering any precise solution.

Table VI. *Approximate general composition of the glycerides of hen depôt fat.*

General type		% (mol.)
Fully-saturated	Mainly tripalmitin with small amounts of palmitostearins	2
Tri- C_{18} -glycerides	Tri-unsaturated (oleo-linoleins), but may include up to 20 % (mol.) of α -stearodi-"olein"	28-29
(β -) Monopalmitodi- C_{18} -glycerides	β -Monopalmitodi-"oleins", but may include up to 20 % (mol.) of β -monopalmitostearo-"olein"	41
Di- C_{18} -mono- C_{18} -glycerides	Probably α -palmito-"oleo"-palmitoleins, but might include up to 20 % (mol.) of α -palmito- β -palmitoleo- α' -stearin	28-29

The terms "olein" or "oleo"- indicate that linoleic as well as oleic radicals are concerned in these cases.

SUMMARY.

1. The chief component glycerides of the hen body fat studied are about 40 % (mol.) of monopalmitodi-"oleins", with nearly 30 % (mol.) each of mainly unsaturated tri- C_{18} -glycerides and of di- C_{18} -mono- C_{18} -glycerides. Fully-saturated glycerides (mainly tripalmitin) only form about 2 % of the fat. The proportion of stearic acid (*ca.* 7 %) combined in the fat may be present in the form of monostearo-glycerides in either of the three main groups; the combined amount of such monostearo-unsaturated glycerides will approximate to 20 % (mol.) of the fat. The individual distribution in the mixed glycerides of oleic and linoleic acids (present respectively to the extent of about 36 % and 21 % of the total com-

ponent acids) cannot be given, since the glyceride structure has been deduced mainly from study of the hen fat after partial or complete hydrogenation.

2. The 40 % of monopalmito-glycerides are almost certainly present in the form of β -palmitodi-“oleins” (or, to some extent, β -palmito-stearo-“oleins”). Consideration of the structure of the hydrogenated fats leads to the conclusions that the di- C_{16} -glycerides consist largely of α -palmito-derivatives, and that the 8 % of palmitoleic acid in hen fat is also probably mainly present in this group. The di- C_{16} -glycerides, on this basis, may be α -palmito- β -palmitoleo- α' -“olein”, α -palmito- β -“oleo”- α' -palmitolein, α -palmito- β -palmitoleo- α' -stearin, or a mixture of these.

3. The hen body fats are differentiated from the corresponding fats of pigs, sheep, cows and some other animals by their unusually large proportions of tri- C_{18} -(unsaturated)-glycerides and, especially, of the mixed semi-unsaturated di- C_{16} -mono- C_{18} -glycerides. The result is a more heterogeneous mixture of mixed triglycerides than in fats of the lard type. This is reflected in the physical consistency of the fats. A pig fat of similar mean unsaturation and not very different palmitic, stearic, oleic and linoleic acid contents forms a thin, semi-solid, almost homogeneous paste at atmospheric temperature; but the hen body fats separate into two well-marked phases—a clear liquid fat with a lower layer of solid glycerides (so-called “stearin”).

Grateful acknowledgment is made of a grant from the Department of Scientific and Industrial Research in aid of this investigation.

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