XXXIV. THE BODY FATS OF THE PIG. II. SOME ASPECTS OF THE FORMATION OF ANIMAL DEPÔT FATS SUGGESTED BY THE COMPOSITION OF THEIR GLYCERIDES AND FATTY ACIDS.

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IN order to obtain evidence of the glyceride structure of pig depot fats parallel with that for tallows [Banks and Hilditch, 1931] it was necessary, owing to the relatively small proportions of fully-saturated glycerides, to employ much larger quantities of material than were available in the group of pig depot fats, the component fatty acids of which were recently described [Bhattacharya and Hilditch, 1931]. The whole of the leaf fat and a complete longitudinal section of the back fat, about 20 cm. wide, from a sow reared at the National Institute for Research in Dairying were therefore procured in order to make a detailed survey of the component glycerides in the perinephric fat and in the layers on either side of the central seam of connective tissue in the back fat. The diet of the animal for about 15 months prior to slaughter had consisted of a mixture of wheat middlings (6-12 parts), barley meal $(2\frac{1}{2}$ -5 parts) and fishmeal $(\frac{1}{6}-1)$ part) with about 1 part of either extracted soya bean meal, dried milk, or cheese.

The fatty tissue was extracted twice by boiling with acetone, after which the residual tissue was thoroughly pressed and finally washed three times on a filter with hot acetone: the last traces of acetone were subsequently removed by heating under vacuum at 100° .

The shoulder end of the back fat was noticeably softer than the main central portion and therefore about one-sixth of each end of both layers was extracted and examined for component fatty acids separately from the remainder. The fats isolated from the various parts of the animal are described in Table I.

Component fatty acids of the sow dep6t fats.

The method of determination was the same as that employed in the previous work on tallows and pig fats [Banks and Hilditch, 1931; Bhattacharya and Hilditch, 1931], and was described in detail in the former communication (p. 1170). A summary of the final analytical data for the component fatty acids of each of the above pig fats is given in Table III.

It will be observed that in each instance small proportions of highly unsaturated acids of the C_{20} and C_{22} series are included. The presence of these derivatives became evident during the fractional distillation of the methyl esters of each set of " liquid " (mainly unsaturated) acids (obtained in the preliminary lead salt separation), the iodine values and equivalents of the final fractions and residual esters being greater than the corresponding values for the C_{18} unsaturated esters present. To illustrate this, and also the manner in which the amount of C_{20-22} esters was estimated, we give in Table II the frac-

tionation data for the "liquid " esters of (i) the beef tallow " N" quoted in our earlier paper (p. 1170), (ii) the fat from the inner back layer of pigs fed on a diet containing ³ % of arachis oil [Bhattacharya and Hilditch, 1931], and (iii) the fat from the main central portion of the inner back layer of the sow dealt with in this paper.

The figures in brackets are the corresponding observed values for the residual esters after removal of unsaponifiable material, and show that esters of higher equivalent and iodine value occur in the final fractions of the "liquid " esters of the present series. The fatty acids from the latter were also treated in ether solution with bromine, when crystalline bromine addition compounds were precipitated: these were almost insoluble in boiling benzene, and when heated, blackened, but did not melt, at 225-230°. This is characteristic of the polybromides of the highly unsaturated marine animal oil acids of the C_{20} and C_{22} series. The proportion of these present was therefore estimated approximately from the mean iodine values of the C_{18} and C_{20-22} groups of esters present in each case. (The ester fraction of equivalent nearest to, but below, 296 was assumed to be composed only of C_{18} unsaturated esters; the mean iodine value of the C_{20-22} esters was deduced from the residual esters (freed from unsaponifiable matter) on the assumption that their mean equivalent [compare Guha, Hilditch and Lovern, 1930] was approximately 330.) The latter assumption, although of an approximate order, introduces little error, since the amount of C_{20-22} esters present is in all cases relatively very small; the calculated iodine values of the C_{20-22} esters present ranged from 170 to 240.

Table III. Summarised data for component fatty acids of the pig fats.

Table III (cont.).

* Weight of mixed fatty acids employed in the analysis.

The different component acids of each of the present group of fats exhibit numerical relationships of an exactly similar kind to those discussed in the former part of this series [Bhattacharya and Hilditch, 1931, Table III, p. 1954), as will be seen from Table IV.

The most notable feature is, perhaps, the tendency for the united molar percentage of stearic, oleic and linoleic acids to be in the neighbourhood of 70 %. Where the total molar content of saturated acids is highest (51 %, perinephric fat), that of the united C_{18} acids is lowest, and conversely: but whereas the most unsaturated (outer back, shoulder) fat contains little more than half as much saturated acids (30%) as the perinephric fat, its molar content of C_{18} acids has only increased to 73 %. The increase, between these two extreme cases, of about 8 $\%$ in an otherwise tolerably constant proportion of C_{18} acids is mainly compensated for by some diminution in the amount of palmitic acid, the myristic acid figures remaining throughout at about 5% .

It should next be observed that, as in the previous instances, the increase in stearic acid is mainly at the expense of oleic, and not linoleic, acid, the amount of the latter increasing only slightly (from 13 to 15 $\%$) with increasing unsaturation of the fats. This is additional evidence that the comparatively

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Table IV.

(i) Molar distribution of individual acids.

(ii) Molar percentages of the various groups of acids.

high stearic content of most animal depôt fats is attained by a process of saturation of oleic, rather than of linoleic, derivatives.

The amounts of linoleic acid in the mixed fatty acids are in all cases larger than in any of the fats discussed in Part I of this series, as will be seen from Table V.

> Table V. Molar content of linoleic acid in the total fatty acids of various pig fats.

* Possibly slightly low, owing to rancidity.

In consequence of these proportions, and of the fact that oleic acid diminishes as stearic acid increases, the ratio of linoleic to oleic acid increases in the group now being described from the outer back fats to the leaf fat $(i.e.$ as unsaturation decreases); this ratio was almost constant in all three fats from pigs whose diet had included arachis oil, and increased in the opposite sense (from the leaf fat to the outer back fat) in the remaining cases in which the diet contained relatively saturated, or no added, fat. We are thus led to believe that the relative amounts of these two unsaturated acids in any pig fat depend merely upon (i) the amount of stearic acid formation which has occurred, coupled with (ii) the amount of linoleic acid present in the mixed fatty acids as a whole; the latter depends to a large extent on the diet and increases, but within comparatively narrow limits, from the perinephric to the outer layers of the back fats.

The occurrence of 1-2 $\%$ of unsaturated acids of the C_{20} and C_{22} series in all the depot fats of a pig fed on a diet including fish-meal is noteworthy, since these proportions are definitely greater than those (up to 0.4%) which Brown and Deck [1930] have shown to be characteristic of normal lards; the details of typical ester fractionations given in Table II illustrate this clearly, and also show that in the pig fats dealt with in the first part of this series there is a slight tendency to increase in iodine value and saponification equivalent in the residual esters, which is consistent with the presence of traces of arachidonic acid as postulated by these investigators.

The diet of the animal which yielded the fats now under discussion contained on an average $7\frac{9}{6}$ of fish meal, and may thus have included about 0.7 % of fish oils; the latter will have contained about 20 % of the highly unsaturated C_{20} and C_{22} acids and an equal or perhaps somewhat greater amount of linoleic acid. Whilst we lack, unfortunately, precise data for the fat intake in the diet and the total amount of storage fat in the animal, it appears that the 1-2 $\%$ of unsaturated C_{20} and C_{22} acids in the latter might represent at least an appreciable proportion of the total quantity of these in the ingested fish meal; but, on the other hand, the recent observations of Brown [1931] go to show that lard from pigs fed on a diet which included 14 $\%$ of menhaden oil only contained 2.7 % of C_{20} and C_{22} acids, which were slightly less unsaturated than the corresponding acids of the oil itself.

It seems, then, that (as in the case of vegetable oils) the characteristic acids of ingested animal fat appear in pig depôt fats, but that the C_{20} and C_{22} acids are not stored so freely as oleic and linoleic acids; the latter, indeed, is laid down in unusually large amounts, although the amount present in the fish oil would not greatly have exceeded that of the C_{20} and C_{22} derivatives. This ready assimilation of linoleic acid into the present series of pig depot fats is in keeping with our previous observations on fats from pigs fed on a diet including arachis oil, and with those of Ellis and Isbell [1926], who showed that, in extreme cases, the linoleic acid contents of pig fats almost reproduced those of the vegetable oils (arachis or soya bean) which formed the main part of the diet.

These results, on the whole, corroborate the general trade experience that a fish meal diet leads to the production of extremely soft fat: the softness is due both to general increase in unsaturated components and, especially, to unusually large proportions of linoleic acid. The C_{20} and C_{22} acids are not present in sufficient quantity to add appreciably to the soft qualities of the fats, but on the other hand might well, with the onset of slight rancidity, be responsible for development of a fish-like taint in the flavour.

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Component glycerides of pig dep6t fats.

The fully-saturated glycerides of the central outer and inner back layers, and of the perinephric fat from the animal whose diet was described on p. 298 were isolated quantitatively by our usual procedure (oxidation of unsaturated components by means of potassium permanganate in acetone), as described in detail by Hilditch and Sleightbolme [1931]. In addition, two of the nine pig fats, the fatty acids of which were recently described by Bhattacharya and Hilditch [1931], were also submitted to this process (the quantity available of the remaining seven fats was insufficient for glyceride structure determination): the specimens utilised were perinephric fats from pigs (i) fed on a control diet and (ii) fed on the control diet $+3\frac{9}{6}$ of arachis oil.

The numerical details of the isolation and purification of fully-saturated glycerides are collected in Table VI: it will be observed that the small pro-

Table VI. Determination of fully-saturated glyceride $(F.S.G.)$ content.

portions of fully-saturated glycerides in the back fats necessitated the oxidation of very large amounts of the latter in order to obtain sufficient material for the subsequent determination of their component fatty acids by ester fractionation.

The purified fully-saturated glycerides "A" (Table VI) were hydrolysed and the resulting acids converted into mixed methyl esters, which were frac-

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tionally distilled in the usual manner in order to determine their composition. It may suffice to record the final results obtained (Table VII).

Table VII. Component fatty acids of fully-saturated glycerides of pig fats.

	Weight percentages			Molar percentages		
	Mvristic %	Palmitic $\%$	Stearic %	Myristic %	Palmitic $\%$	Stearic %
Outer back	0.3	52.2	47.5	0.3	$54 - 7$	45.0
Inner back	1.4	$55-1$	43.5	$1-6$	$57 - 5$	40.9
Perinephric	1.0	$55 - 6$	$43 - 4$	1.1	58.0	40.9
Perinephric (previous series, control diet)	1.6	59.3	$39-1$	1.9	$61-5$	$36 - 6$
Perinephric (previous series. control + 3% arachis)	2.2	46.0	$51-8$	2.5	$48 - 4$	49.1

The relationship between the molar percentage of fully-saturated glycerides and the total molar content of saturated acids in these pig fats is very similar to that which was observed in the cases of tallows [Banks and Hilditch, 1931] and of butter fats [Hilditch and Sleightholme, 1931]: indeed, when plotted graphically (Fig. 1) the whole series, ranging from the outer back pig fat (34 $\%$ saturated acids, 2 $\%$ F.S.G.) to a butter fat containing 72.5 $\%$ saturated acids and 41*5 % fully-saturated glycerides, lies on ^a fairly smooth curve.

The composition of the fully-saturated glycerides is not quite so regular as that of the similar portions of tallows and milk fats, the molar content of stearic acid lying between the extreme limits of 37 and 49 $\%$. In the three fats from different parts of the same animal, however, this figure varies only from 41 to 45 $\%$. On the whole the ratio of palmitic (with myristic) to stearic acid in the fully-saturated components in these pig depot fats is very similar to that in the corresponding glycerides of tallows. The content of myristic acid is distinctly lower than in tallow fully-saturated glycerides and, correspondingly, the melting-points (60.5°) of the fully-saturated components of the pig fats containing 41 mols. $\%$ of stearic acid were observed to be several degrees higher than those (54-54.5°) of tallow fully-saturated glycerides of similar stearic acid content.

Some features of the deposition of storage fats suggested by their structure.

The analyses reported, in this and preceding papers, of depot fats of pigs and cattle (and also, to a certain extent, those of cow milk fats) disclose a number of consistent similarities which will demand attention when the manner in which storage fats are laid down is considered.

The most arresting feature is the circumstance that, whatever the degree of saturation of a depôt fat, its molar content of C_{18} acids is in the neighbourhood of 70 $\%$ (rising to about 73 $\%$ with very unsaturated fats, and falling to about 65 $\%$ in the more saturated fats). The actual degree of saturation is controlled, almost wholly, by the relative amounts of stearic and oleic acids present in any given case.

If it were assumed, firstly [cf. previous papers, 1931, pp. 1180, 1960], that the primary phase of the glycerides which finally appear as depot fat is that of a comparatively unsaturated mixture produced by lipoclastic esterification of a mixture consisting, for example, of about 30 mols. of palmitic (including subordinate amounts of myristic) with about 70 mols. of oleic (with linoleic and probably subordinate amounts of stearic) acid, and secondly, that such initially-formed glycerides subsequently undergo hydrogenation *(i.e.* act as acceptors of hydrogen, in conjunction with some independent oxidation process proceeding in the same location), the observed approximate constancy of the C_{18} acid content in all normal depôt fats so far studied by ourselves and by other workers would follow as a matter of course. It is suggestive, moreover, to note that hydrogenation of the oleo-derivatives of preformed glycerides (but not necessarily of oleic acid prior to conversion into mixed glycerides) would inevitably produce a series of fats in which the relations between fullysaturated glyceride content and total saturation would be of the nature which we have found so uniformly characteristic for animal fats and, equally, so different from those of practically all the seed fats studied up to the present.

The observed tendency for the fully-saturated glycerides of depot fats to possess similar compositions (frequently in the region of 60 mols. of palmitic to 40 mols. of stearic acid) is also in consonance with the supposition that glyceride hydrogenation may be an essential factor in the sequence of changes leading to storage fat. Within the limits of saturation concerned in any of the animal fats we have examined, the composition of fully-saturated materials would be kept within a comparatively narrow range, because dipalmito-oleins (with only one unsaturated acid group) will attain, ceteris paribus, complete saturation more readily than palmitodioleins, and still more so than triolein.

The glyceride structure of cotton-seed oil after partial catalytic hydrogenation has recently been investigated by Hilditch and E. C. Jones [1932] and the results are perhaps worth comparing with those for animal fats, since cotton-seed oil resembles our hypothetical "initially synthesised" animal glyceride mixture in so far as it contains about ²⁵ % of saturated (nearly all palmitic) and ⁷⁵ % of unsaturated acids, with no fully-saturated glycerides. During hydrogenation in presence of nickel at 180° the first action is almost selective conversion of linoleic to oleic (and iso-oleic) glycerides, and little increase in stearic (and consequently negligible formation of fully-saturated) glycerides takes place; but, from the point at which linoleic derivatives have disappeared the process is chemically not dissimilar (except in temperature conditions) from that which we have suggested as operative in the animal. It is interesting to observe that the relationship between fully-saturated glyceride content and degree of saturation of the hydrogenated cotton-seed oils, which is illustrated in Fig. 1, follows throughout the similar values which have been traced in this and preceding papers for pig fats, tallows and butter fats; the coincidence, although not complete, is distinctly.more close than that with the curve for glycerides synthesised in the laboratory or with the mathematicallyderived graph, especially for fats of very small fully-saturated glyceride content. It should be added, moreover, that both in hydrogenated cotton-seed and hydrogenated olive oil there was marked selective hydrogenation of palmitoglycerides before tristearin was produced in noticeable amount. We propose to undertake similar hydrogenations of one of the more unsaturated pig fats and to examine the glyceride structure of resulting products, comparable in degree of saturation with some of the more saturated lards and tallows which we have investigated.

It is, we believe, sometimes overlooked that the occurrence of stearic acid as a major component (e.g. forming 10 $\%$ or more) of natural fats is practically confined to non-aquatic mammals; it rarely forms more than about $1\frac{9}{6}$ of marine animal oils or of vegetable fats, except in the case of the members of a very few. tropical families (Sapotaceae, Guttiferae, Sterculiaceae, Dipterocarpaceae). Its relative abundance in animals may be a consequence of its derivation from preformed oleic glycerides in order to produce the more saturated fats of higher melting-point.

Whilst the composition of storage fats thus leads us to favour the view that a hydrogenation of glycerides rather than of free fatty acids is an essential link in the process of their formation, it clearly leaves us unable to suggest with any confidence where this change may take place. In view of the evidence disclosed in Part ^I of this series [1931], including the results obtained by Ellis and co-workers [1930, 1931], we believe that the characteristic constancy at about 70 mols. $\%$ of total C_{18} acids in pig depôt fats is most closely maintained when the diet of the animal has only contained small proportions of fat, and accordingly this may point to fat synthesised in the animal primarily from carbohydrate as being the main seat of the suggested hydrogenation process; but this does not preclude the possibility that directly assimilated fat may also be affected. On the other hand, however, the evidence of Ellis and Zeller [1930] that linoleic acid in pig depot fat is derived exclusively from assimilated vegetable linoleic acid may indicate that assimilated fat from the diet does not undergo hydrogenation to any material extent.

The tendency observed, in the less saturated depot fats of a pig, for the component fatty acids to include somewhat more than 70 mols. $\%$ of C_{18} acids, and, conversely, for the C_{18} acid molar content to fall below this figure in the more saturated depot fats from the same animal, would be explicable if specific mixtures of glycerides were selectively withdrawn from the blood at different sites of deposition. This would presuppose the presence in the blood of a common stock of glycerides in which the chemical processes had been completed, a possibility which is perhaps less remote than the alternative of hydrogenation to varying degrees in the adipose tissues themselves.

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