CCLXV. THE BODY FATS OF THE PIG.
III. THE INFLUENCE OF BODY TEMPERATURE ON THE COMPOSITION OF DEPOT FATS.

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Henriques and Hansen [1901] compared the setting-points and iodine values of fats from the leaf and from different layers of the back fat of a pig, which had been fed on barley. From the results (summarised below) they concluded that the determining factor in the relative hardness or softness of the fats was the temperature of the site in which the fat had been laid down in the animal:

	Solidifying point ° C.	Iodine value			Body temperatures ° C.
Outer back fat					
outermost	—	60·0	Back tissue	1 cm. deep	33·7
inner layer	26·4	57·1		2 „	34·8
Inner back fat					
outer layer	28·0	51·8		3 „	37·0
innermost	27·7	50·6		4 „	39·0
Perinephric fat	29·6	47·7	Rectum		39·9

They obtained further support for this hypothesis by maintaining three pigs from the same litter for two months at different (but in each case approximately constant) temperatures—one at 30–35°, one at 0° and one at 0° but covered with a sheepskin coat: the iodine values of the outermost layers of the back fats from these animals, at the end of the period, were respectively 69·4, 72·3 and 67·0.

In previous parts of this series [Bhattacharya and Hilditch, 1931; Banks and Hilditch, 1932], in the course of detailed analyses of fats from a number of pigs fed on various diets, the differences in the amounts of component acids which determine the general hardness or softness of the fats as a whole were defined. *Inter alia*, and in agreement with Henriques and Hansen, it was observed in all cases that the average composition of the outer layer of back fat (between the skin and the "streak," or thin layer of connective tissue) differed from that of the fat on the inner side of the streak. This difference consisted in the presence of somewhat less stearic and palmitic acids, and of correspondingly greater proportions of oleic acid, in the fat of the outer as compared with the inner layer; the proportions of linoleic and of myristic acid were almost the same in each layer.

The primary object of the experiments recorded in the present paper was to ascertain whether the respective layers of fat on either side of the "streak" are homogeneous, or whether there is a progressive alteration as the skin is approached; in other words, to extend the earlier work of Henriques and Hansen by obtaining more definite information concerning the component fatty acids in successive lateral sections of the back fat of a pig by means of the modern ester fractionation procedure. For this purpose, the central portion of the

whole of the back fatty tissue from a very fat sow was divided into five layers of approximately equal thickness (two from the "outer" portion between the skin and "streak," and three from the "inner" portion beneath the "streak").

The sow had been fed exclusively on a diet of maize meal, thirds and whey, and care was taken to select an animal which had not received any fish meal in order to exclude any possible entrance of marine oil acids from the latter into the body fat. The unusually high linoleic acid content and appreciable, if small, amounts of highly unsaturated acids of the C_{20} and C_{22} series observed by Banks and Hilditch [1932] in the body fats from a sow, whose food had contained 7 % of fish meal, had been attributed to the presence of the latter in the diet. In their proportions of linoleic and highly unsaturated C_{20} and C_{22} acids, however, the fats from the present animal were found to be remarkably similar to those of the previous case, and, as discussed later, it would now seem that, in these instances, the cause must be sought elsewhere than in the fish meal components of the feed.

The fat was extracted from the tissues by boiling them twice with acetone, after which the residual tissue was thoroughly pressed and finally washed three times with hot acetone. The last traces of solvent were removed from the fats by heating under vacuum at 100°.

Table I.

	Fat in tissue %	Extracted fat: Saponification equivalent	Extracted fat: Iodine value	Extracted fat: M.P. (open tube) ° C.	Mixed acids setting-point ° C.
Outer layer					
outside ("Outer I")	66	287·1	70·4	30·3	37·7
inside ("Outer II")	72	286·9	67·4	33·3	39·4
Inner layer					
outermost ("Inner I")	91	286·2	62·9	38·0	40·8
middle ("Inner II")	92	286·3	63·0	38·3	41·0
innermost ("Inner III")	88	286·5	62·8	38·3	40·9

The component acids present in each fat were determined by the same procedure (separation by means of the lead salts into "solid" and "liquid" acids, followed by fractional distillation under vacuum of the methyl esters of each of these groups of acids) as that followed in the previous study of sow body fat [Banks and Hilditch, 1932]. As in the latter case, the higher and the residual fractions from the methyl esters of the "liquid" acids possessed saponification equivalents and iodine values which indicated, in each instance, the presence in small amounts of highly unsaturated acids of the C_{20} and C_{22} series; their proportions were approximately calculated by the same method as that described in the paper cited.

Since it has recently been shown that palmitoleic, or a similar, acid occurs in small quantities in some body fats, for instance, in that of the rat [Banks *et al.* 1933], a portion (91·6 g.) of the methyl esters of the "liquid" acids from fat "Inner III" of the present series was distilled separately from that employed in the main analysis. About one-third of the whole (31·0 g.) was collected as a first fraction; this quantity was sufficient to include practically the whole of any unsaturated esters of acids lower in the series than oleic acid. This fraction was oxidised in acetone solution with potassium permanganate until practically all unsaturated material had disappeared and yielded 5·1 g. of almost saturated esters (iodine value 0·8, saponification equivalent 263·0). Since the original fraction oxidised had an iodine value of 89·8 and a saponification equivalent of

286·0, it follows that the unsaturated esters present therein must have had an iodine value of 107·3 and a mean equivalent of 292; the purest fraction of unsaturated C_{18} esters subsequently isolated at a later part of the original fractionation had iodine value 107·0, saponification equivalent 294·0. From this experiment it appears very unlikely that unsaturated acids of lower molecular weight than oleic acid occur in the body fat of the pig; if present, they cannot form much more than about 1 % of the component fatty acids.

Table II. *Summarised data for component fatty acids of the sow back fats.*

Acid	Solid acids S	Liquid acids L	Total	Fatty acids (excluding unsaponifiable matter) % (wt.)	% (mols.)
	Outer I (outer layer, outside). (203·0 g.*)				
	(33·7 %)	(66·3 %)			
Myristic	0·27	2·30	2·57	2·6	3·1
Palmitic	22·61	1·19	23·80	23·8	25·6
Stearic	10·12	—	10·12	10·1	9·8
Oleic	0·70	45·53	46·23	46·3	45·0
Linoleic	—	15·13	15·13	15·2	14·8
C_{20-22} unsaturated	—	1·96	1·96	2·0	1·7
Unsaponifiable	—	0·19	0·19	—	—
	Outer II (outer layer, inside). (201·7 g.)				
	(37·0 %)	(63·0 %)			
Myristic	0·14	2·66	2·80	2·8	3·4
Palmitic	22·84	0·58	23·42	23·5	25·1
Stearic	12·98	—	12·98	13·0	12·6
Oleic	1·03	41·87	42·90	43·0	41·8
Linoleic	—	15·59	15·59	15·6	15·3
C_{20-22} unsaturated	—	2·09	2·09	2·1	1·8
Unsaponifiable	0·01	0·21	0·22	—	—
	Inner I (inner layer, outermost). (203·7 g.)				
	(39·6 %)	(60·4 %)			
Myristic	0·39	2·53	2·92	2·9	3·5
Palmitic	24·23	0·57	24·80	24·9	26·6
Stearic	14·51	—	14·51	14·5	14·0
Oleic	0·47	42·19	42·66	42·7	41·4
Linoleic	—	13·85	13·85	13·9	13·6
C_{20-22} unsaturated	—	1·06	1·06	1·1	0·9
Unsaponifiable	—	0·20	0·20	—	—
	Inner II (inner layer, middle). (200·8 g.)				
	(39·7 %)	(60·3 %)			
Myristic	0·20	2·57	2·77	2·8	3·3
Palmitic	24·44	0·97	25·41	25·5	27·3
Stearic	14·49	—	14·49	14·5	14·0
Oleic	0·57	40·70	41·27	41·3	40·1
Linoleic	—	14·46	14·46	14·5	14·2
C_{20-22} unsaturated	—	1·44	1·44	1·4	1·2
Unsaponifiable	—	0·16	0·16	—	—
	Inner III (inner layer, innermost). (310·0 g.)				
	(39·3 %)	(60·7 %)			
Myristic	0·08	2·92	3·00	3·0	3·6
Palmitic	23·78	0·72	24·50	24·6	26·2
Stearic	14·53	—	14·53	14·5	14·0
Oleic	0·90	41·84	42·74	42·8	41·6
Linoleic	—	13·66	13·66	13·7	13·4
C_{20-22} unsaturated	—	1·40	1·40	1·4	1·2
Unsaponifiable	0·01	0·16	0·17	—	—

* Weight of mixed fatty acids employed in the analysis.

Apart from these points, the fractionation analyses followed our usual practice, and it is perhaps sufficient (owing to considerations of space) to summarise the results (Table II) in the form adopted in several recent communications from this laboratory.

The relationships between the various component acids of this series of fats, compared on the basis of molar percentages, will be seen more clearly from the summary in Table III.

Table III.

(i) Molar distribution of individual acids.

Fat	Myristic	Palmitic	Stearic	Oleic	Linoleic	C_{20-22} unsaturated
Outer I	3·1	25·6	9·8	45·0	14·8	1·7
„ II	3·4	25·1	12·6	41·8	15·3	1·8
Inner I	3·5	26·6	14·0	41·4	13·6	0·9
„ II	3·3	27·2	14·0	40·1	14·2	1·2
„ III	3·6	26·2	14·0	41·6	13·4	1·2

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

	Total saturated acids	Total C_{18} acids	Stearic acid	Molar ratio of saturated to unsaturated acids
Outer I	38·5	69·6	9·8	0·63
„ II	41·1	69·7	12·6	0·70
Inner I	44·1	69·0	14·0	0·79
„ II	44·5	68·3	14·0	0·80
„ III	43·8	69·0	14·0	0·78

Discussion.

Before comparing the differences between the successive layers of fat from the back of this animal, we would point out that all the five fats share in common an unusually high content of linoleic acid and also a proportion of highly unsaturated C_{20-22} acids which, though small, is definitely higher than the amount (up to 0·4 %) shown by Brown and Deck [1930] to be characteristic for normal lards. Further, so far as these particular acids are concerned, all the present fats display a great similarity to those previously studied from a sow whose diet had contained about 7 % of fish meal.

In the present instance the diet cannot well account for these peculiarities. It is unlikely that fat present in the maize meal is a contributory factor since, although this fat is of a fairly unsaturated type, it is no more so than that of barley or oats, which have been employed by other workers and which in their experience have not given rise to this type of body fat in pigs. Moreover, the sow fats now in question show the normal proportion of palmitic and myristic acids, whereas the work of Ellis *et al.* [1931] showed, as the result of feeding cottonseed oil (a fat similar in linoleic acid content to maize oil) to pigs, that the proportion of palmitic acid in the hog back fats fell markedly as that of linoleic acid rose (with increasing proportions of cottonseed oil in the diet). In any case, of course, the appreciable amounts now observed of unsaturated C_{20} and C_{22} acids cannot be associated with any part of the diet.

We are accordingly inclined to the view that the high linoleic acid and appreciable unsaturated C_{20-22} acid contents of the sow fats are due to some cause other than the diet. The most obvious difference in the two categories is, as a matter of fact, in the ages of the animals from which the fats were taken. In the studies of Ellis *et al.* [1926; 1930; 1931] on the body fats of hogs fed on rations low in fat, and also in those of Bhattacharya and Hilditch [1931], and in

other cases in which a low content of linoleic acid (from 1 % to not more than about 8 %) has been observed, it is noticeable that the fats were taken from young animals not more than about seven or eight months old. The two sows, whose fats have been examined and found to contain about 13–15 % of linoleic acid and 1–2 % of highly unsaturated acids of the C_{20} and C_{22} series, were probably several years old when slaughtered. It thus seems very possible that these general differences in body-fat composition may be connected with the age of the animal rather than with its diet.

Turning now to the component fatty acids of the different layers of back fat from the same animal (Table III), it must be said that the composition of each of the three "inner" layers is identical, or, at least, any variation is within the limits of experimental error of the ester-fractionation method. The part of the "outer" layer next to the "inner" layer (but divided from it by the "streak") is also very similar to the "inner" layers, but here the stearic acid content is definitely lower. The outermost layer of all differs most in composition from the rest, for in this case the stearic acid content is about 4 units % lower, and the oleic acid correspondingly higher, than in the "inner" layer fats. Both sections of the "outer" layer of fat also contain slightly less palmitic acid than the "inner" layers, but this difference is not so marked as in the case of the stearic acid figures.

In Part II of this series of papers [Banks and Hilditch, 1932] it was pointed out that, in the depot fats of pigs and cattle, the molar content of C_{18} acids is always in the neighbourhood of 70 %, rising to about 73 % with very unsaturated fats and falling to about 65 % in the more saturated fats, and that the actual degree of relative saturation is controlled, almost wholly, by the relative proportions of stearic and oleic acids (linoleic acid being more or less constant in proportion for different fats from different parts of the body of the same animal). This feature is well illustrated by the present group of fats, in which the softer (outer) fats contain about 69·7 % of total C_{18} acids in their component acids, whilst the more saturated (inner) fats contain 69·0 % or somewhat less. The main difference responsible for the difference in consistency lies in the varying proportions of stearic (9·8–14·0 %) and oleic (45·0–*ca.* 41·5 %) acids.

The progressive increase in mean saturation and the close identity in composition of all the "inner" layers of fat are perhaps best seen by reference to the final column of Table III (ii), in which the molar ratios of saturated to unsaturated acids as a whole in each fat are given.

On the whole, the present data confirm the conclusion of Henriques and Hansen that the increase in saturation (*i.e.* in stearic acid content) of the back fat follows the increase in body temperature. The figures given by these workers for the "inner layers" of fat (iodine values 51·8 and 50·6) also indicate close similarity in composition, although the respective body temperatures quoted were somewhat different, *viz.* 37·0° and 39·0°. In our experiments, the actual amount of fat of constant composition formed at least two-thirds of the whole back fat, and increasing unsaturation (oleic acid content) was only marked in the extreme outer layers. Since, however, the total thickness of the back fat of our animal appears to have been greater than that of the pig studied by the earlier workers, we consider that this may well account for the minor differences between the two series of observations, and that our detailed analyses confirm, so far as the fat nearest the skin is concerned, the apparent connection between the fat composition and the body temperature which Henriques and Hansen pointed out.

Cuthbertson and Tompsett [1933] have recently observed that the outer and

inner layers of the panniculus adiposus abdominalis in obese human subjects possess substantially the same iodine values and suggest therefrom that an explanation of variation in fat composition based on dietary considerations is more probable than one based on temperature differences. It would appear, however, that, in the case of human subjects, the effect of clothing would minimise any temperature differences. The results obtained by Cuthbertson and Tompsett are accordingly of the nature which would be expected, in view of Henriques and Hansen's experiment with a pig wrapped in a sheepskin. More detailed examination of the fat from human adipose tissues than the mere determination of iodine values would have been of extreme interest.

We venture to add a few words upon the broader question of the supposed general dependence of fat composition in plants and in animals on the temperature at which the fat is laid down—a matter upon which there seems to be some confusion of thought at the moment. Thus, Hammond [1933] has recently said that "Fat to be of use as a source of energy in the body must be just fluid at the natural body temperature, and as a consequence the fat of cold-blooded animals (fish) is of very low melting-point, while the fat of the sheep which has a high body temperature (104° F.) is of higher melting-point than that of the bullock with a lower body temperature (101° F.)." Whilst it is clear that fats present in an animal (or plant) must be almost completely, if not wholly, liquid at the natural temperature of the organism, it does not necessarily follow that warm-blooded animals or tropical plants always produce fats of higher melting-point and more saturated character than cold-blooded animals or plants which are indigenous to cool regions.

The instances of fats of fish, sheep and bullock given in the quotation, for example, should be considered in conjunction with those of such animals as the rabbit (body temperature 103–104° F.) or the hen (104–108° F.). Whilst sheep fat contains only about 40 % of unsaturated acids (mainly oleic), rabbit fat contains nearly 70 %, most of which is linoleic acid, with appreciable quantities of still less saturated acids. Again, hen fat contains about 70 % of unsaturated (oleic and linoleic) acids, in spite of the high body temperature of the bird; this fat is, indeed, almost completely liquid at room temperature. Further, the rat, with a body temperature of 100° F. (lower than that of the rabbit) contains about the same high proportion of unsaturated acids [Banks *et al.*, 1933], but these consist almost wholly of oleic acid: the more unsaturated linoleic acid, present in great quantity in rabbit fat, is almost absent from that of the rat.

It has also become usual to connect the liquid, very highly unsaturated fats of fish with their low body temperature; yet marine mammals such as the whale, dugong (102–104° F.) or porpoise (96–98·6° F.) have body temperatures of the same order as those of land animals, whereas their fats are very closely similar in composition to the fish oils, and include the same series of highly unsaturated acids.

Similarly, the occurrence of fats, solid at the normal temperature of temperate regions, in the seeds of the members of a fairly large number of tropical plant families has led to the superficial generalisation that plants of tropical origin yield fats of high melting-point and those of cooler regions fats of low melting-point. Low-melting vegetable fats are, in actual fact, common to both temperate and tropical plants, and, while it is obviously true that tropical families such as Palmae or Guttiferae yield solid seed fats with high contents of combined lauric, stearic or other saturated acids, it is equally true that many plants of exclusively tropical habitat produce seed fats of a liquid and highly unsaturated character. Familiar instances are those of *Hevea brasiliensis*

(rubber seed oil), *Perilla ocimoides* (perilla oil), *Aleurites Fordii* (China wood oil), *Couepia grandiflora* (oiticica oil), *Carthamus tinctorius* (safflower seed oil), *Gossypium* sp. (cotton seed oil), whilst many others might be given.

No wide generalisation can therefore safely be drawn between temperature and the composition of natural fats as a whole. Fats which are solid at the normal temperature of plants or animals are obviously incompatible with their conditions of life, but, of animals and plants which exist under relatively warm conditions, some utilise fats of a relatively saturated (solid) character, but others resemble the cold-blooded animals and temperate plants in having fats of a more unsaturated and liquid type. The chief correlation which at present seems possible is rather with their biological relationships and classification than with temperature.

SUMMARY.

The back fat of a sow was divided into five layers and the composition of the mixed fatty acids present in each layer was determined. The component acids of the three "inner" layers (on the underside of the "streak") were found to be practically identical, but the outermost layer contained slightly less palmitic acid and about 4 % less stearic acid, these being compensated by the presence of correspondingly more oleic acid. The inner part of the "outer" layer was intermediate in composition between the last-mentioned fat and the three "inner" layers, but resembled the latter more closely.

The detailed analytical figures afford general confirmation of the conclusion of Henriques and Hansen [1901], that there is a close relation between increase in saturation of the fat and increasing body-temperature. At the same time, the greater part of the back fat now examined (*i.e.* the portion beneath the "streak") was evidently completely homogeneous and made up of the same mixture of mixed glycerides containing constant proportions of the various component acids.

The animal, from which the present fat was obtained, had received a diet of maize meal, thirds and whey; but the depot fatty acids were characterised throughout by the presence of about 15 % of linoleic acid and 1–2 % of highly unsaturated acids of the C_{20} and C_{22} series. These values are probably not due to the diet of the animal but are characteristic of pigs of considerable age.

The general statement, not infrequently encountered, that warm-blooded animals and plants of tropical origin produce more solid, saturated fats than cold-blooded animals or plants from cool regions is shown to be only partially true.

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