CLXXXVI. THE COMPONENT FATTY ACIDS OF RAT BODY FATS.

BY ADAM BANKS, THOMAS PERCY HILDITCH
AND EDWIN CHARLES JONES.

From the Department of Industrial Chemistry, University of Liverpool.

(Received August 19th, 1933.)

DETAILED quantitative information upon the composition of the fatty acids combined in the depot fats of animals is abundant in the cases of the ox, sheep and pig, but is otherwise still scanty, except in a few isolated instances, such as the loin fat of the reindeer [Baughman *et al.*, 1929] and the body fat of the horse [Heiduschka and Steinruck, 1921; Grossfeld, 1931]. These two fats appear to fall into the same category as the tallows and lards, in that their component acids contain about 30 % (weight) of palmitic acid and substantial, but variable, proportions of stearic acid, the amount of the latter varying more or less inversely with that of the oleic acid present [*cf.* Banks and Hilditch, 1932]. Of bird depot fats, those of the goose [Bömer and Merten, 1922; Grossfeld, 1931] and the hen [Grossfeld, 1931] have been examined by resolution into "solid" and "liquid" acids by lead salt separation (Twitchell), or by oxidation (Bertram). The bird fats seem to belong to a different type from the animal fats mentioned, since the analyses quoted indicate the presence of only small amounts of stearic acid (usually not more than 5 %), and the proportion of palmitic acid is also lower (20–25 %). These differences, which are confirmed and extended, in the case of hen fats, by studies by the ester-fractionation method now proceeding in this laboratory, point to the desirability of collecting detailed quantitative data for the depot fats from a much wider variety of animals than that dealt with up to the present.

As a contribution to this end, we are able to communicate the results of analyses, by the ester-fractionation process, of four rat body fats and of the perinephric fat from wild rabbits (the latter analysis having been carried out by Mr J. R. Vickery in 1928, but not hitherto published). Burr and Burr [1930] pointed out that rats appear not to lay down linoleic acid in their reserve fats and suggested that they are unable to synthesise linoleic acid; according to these authors, the absence of fats containing linoleic acid from the diet causes the animals to develop the condition known as "scaly tail." Gregory and Drummond [1932] studied the depot and liver fats of rats fed on various diets by examining the analytical characteristics of the mixed "liquid" or mainly unsaturated acids obtained from the Twitchell lead salt separation. They confirmed Burr and Burr's observations that, with fat-free diets, the body fats were substantially devoid of linoleic acid, but found that this acid was present in the liver fats, from which it would follow that rats are able to synthesise this acid but do not deposit it in the reserve fat.

This peculiarity, the extensive use made of rats in nutritional experiments, and the desire already mentioned to extend the range of information on animal depot fat component acids, made us welcome an offer by Prof. Drummond to

place at our disposal, for study by the ester-fractionation method, specimens of depot fats collected from rats which had been fed (a) on mainly fat-free diets and (b) on diets supplemented by cod-liver oil. We are further indebted to Prof. Drummond for the information which follows regarding the origin of these four rat fats.

Each sample represented the whole of the adipose tissue fat dissected out from the subcutaneous, perinephric and mesenteric areas of a number of animals. Each rat yielded in all 5–6 g. of fat, and the total amount of available material in each specimen varied from 40 to 70 g. The latter quantity would have been sufficient for an accurate determination of a simple mixture of palmitic, stearic, oleic and linoleic acids; it will be seen, however, that the analyses were complicated by the apparent presence of small proportions of an unsaturated fatty acid of lower molecular weight than oleic acid (probably palmitoleic acid). Owing to this, it would be necessary to work with much larger quantities of material than were available in order to obtain data of the desired order of accuracy. The figures given for palmitic, stearic and linoleic acids, however, are probably very close to the truth, whilst that for oleic acid must be regarded as uncertain to within perhaps 3 or 4 units %. Fortunately, the general and characteristic features of the component acids are quite evident in spite of the minor uncertainties thus unavoidably present in some of the numerical data.

Three samples of rat fats were originally supplied.

Fat A was derived from rats fed on "fat-free" diets, but most of the animals had received daily doses of about 15 mg. of ethyl laurate as a carrier of vitamin A or carotene over periods varying from 3 to 7 weeks.

Fat B was from rats fed on different diets, none of which contained unsaturated fats. Most of these animals were on very low fat diets, but in some cases 10 % of the diet consisted of hydrogenated cottonseed oil; nearly all had supplements conveyed in ethyl laurate (as in A).

Fat C was from rats which had received diets containing from 2 to 15 % (in most cases 5 %) of cod-liver oil over periods of 10 weeks.

In view of the small amount of C available (*ca.* 40 g.), and of the interesting results disclosed, Prof. Drummond kindly undertook a special experiment in which 12 rats were fed for 12 weeks on a ration of "Glaxo casein" 20 %, rice starch 73 %, salt mixture 5 % and cod-liver oil 2 %. The resulting fat (D, 70 g.), however, did not reproduce the characteristics of fat C, possibly owing to the low proportion of cod-liver oil used.

Each fat was saponified, and the mixed fatty acids were divided into "solid" (mainly saturated) and "liquid" (mainly unsaturated) acids by the usual lead salt separation from alcohol (in presence of 0.5 % acetic acid). Each group of acids was then converted into methyl esters and fractionated under low pressure. The fractionation data are quoted in full (Table III) in order to make clear the basis of the deductions as to the composition of the mixed acids.

The general characteristics of the fats, all of which were almost entirely colourless and not completely solid at room temperature, may first be recorded (Table I).

Table I.

Fat	Diet	Saponification equivalent	Iodine value	Acid value	Setting-point of mixed fatty acids
A	"Fat-free"	281.0	60.5	34.8	37.2°
B	Mainly "fat-free"	283.3	57.3	17.0	38.2
C	2–15 % cod-liver oil	284.2	74.6	18.9	36.2
D	2 % cod-liver oil	280.3	57.4	20.0	39.6

The proportions of "solid" and "liquid" acids obtained from the mixed fatty acids of each fat in the preliminary lead-salt separation are given in Table II.

Table II.

Fat	"Solid" acids		"Liquid" acids	
	g.	%	g.	%
A	14.3	30.7	32.3	69.3
B	18.9	33.9	36.8	66.1
C	9.7	28.4	24.5	71.6
D	21.2	35.3	38.8	64.7

The details of the ester-fractionations are given in Table III. The last fraction in each case represents the residue left in the distillation bulb. In the distillations of the "liquid" esters the residues, after saponification, are freed from unsaponifiable matter, and the equivalents and iodine values of the recovered acids (free from unsaponifiable) are re-determined in order to estimate

Table III. *Fractional distillation of methyl esters of the "solid" and "liquid" acids.*

No.	"Solids"			"Liquids"		
	g.	Sap. Eq.	I.V.	g.	Sap. Eq.	I.V.
From fat A ("fat-free" diets).						
1	3.04	264.4	3.2	5.67	268.9	75.9
2	4.11	269.8	4.0	4.49	279.2	85.1
3	3.54	273.0	5.9	4.90	285.7	86.8
4	2.97	282.0	12.0	5.52	290.2	87.8
5	—	—	—	4.77	294.0	88.5
6	—	—	—	3.40	306.3	88.7
	13.66	—	—	28.75	(296.6)	(88.6) (Unsap.-free)
From fat B (mainly "fat-free" diets).						
1	3.33	264.8	2.0	4.41	273.0	75.5
2	3.15	268.5	2.5	4.55	282.4	82.1
3	2.79	269.1	3.4	4.38	286.7	84.2
4	3.86	271.8	4.2	5.39	292.3	84.3
5	2.64	281.5	11.3	4.82	294.1	85.6
6	—	—	—	5.02	296.3	85.8
7	—	—	—	4.04	305.0	89.3
	15.77	—	—	32.61	(296.7)	(88.0) (Unsap.-free)
From fat C (2-15 % cod-liver oil in diets).						
1	2.60	266.9	1.7	3.67	276.5	77.6
2	1.84	269.1	3.4	3.80	286.3	88.4
3	2.35	270.9	4.7	3.90	290.3	88.2
4	2.74	281.0	11.3	3.84	296.0	95.7
5	—	—	—	5.06	300.5	104.6
6	—	—	—	3.95	320.4	155.8
	9.53	—	—	24.22	(314.0)	(150.8) (Unsap.-free)
From fat D (2 % cod-liver oil in diets).						
1	2.94	266.2	0.9	12.12	277.4	80.8
2	3.70	267.7	1.9	4.62	288.0	87.1
3	3.60	269.6	2.1	4.52	289.6	87.4
4	4.83	271.9	3.6	4.18	295.6	88.1
5	4.31	280.3	9.9	4.66	294.7	88.8
6	—	—	—	6.12	303.8	93.9
	19.38	—	—	36.22	(295.5)	(91.4) (Unsap.-free)

the amount of unsaponifiable material present. The values so obtained (stated in terms of the corresponding methyl esters) are added in brackets in Table III. (All the unsaponifiable matter passed, in solution in alcohol, into the "liquid" acids.)

When the composition of the lower-boiling fractions of the "liquid" methyl esters from fats A, B and C was estimated on the assumption that no unsaturated esters were present of lower molecular weight than the C_{18} ester-mixtures (of respective iodine values 88.5, 85.8 and 95.7), it was found that the calculated mean equivalents of the saturated material in these fractions were much lower than that of methyl myristate. The lowest-boiling fraction of "liquid" esters from fats which have undergone oxidative rancidity often exhibits this feature, although not to the same extent as in the present instances; moreover, the rat fats now described had not become oxidised appreciably. An alternative explanation lay in the presence of an unsaturated acid of lower molecular weight than oleic acid and, although the amount of material at our disposal was insufficient to permit of any positive identification of such acid, some indirect evidence was obtained by modifying the fractional distillation of the "liquid" esters from fat D.

In this instance, a considerably larger first (lowest-boiling) fraction than usual was collected (12.12 g., or about one-third of the whole, and containing nearly all the esters of lower molecular weight than methyl oleate-linoleate). The amount (8.7 g.) not required for direct analysis was dissolved in acetone and oxidised with potassium permanganate in order to convert all unsaturated esters into acidic products; the residual neutral product was then re-submitted to the same oxidation process. After the double oxidation, the remaining neutral ester (0.97 g.) still possessed a small iodine value (5.1) and the fatty acids combined therein (after boiling with water to remove traces of nonoic, azelaic, *etc.*, acids) were solid and had a mean molecular weight somewhat higher than that of myristic acid. After correcting for the small residual iodine value of the product obtained from the oxidations, the corresponding weight of saturated ester present in the original fraction (12.12 g.) was 1.3 g. Assuming this to be methyl myristate (although actually small proportions of methyl palmitate were probably also present), the mean molecular weight of the unsaturated esters also present was about 282; calculated in terms of a mixture of methyl palmitoleate and oleate-linoleate (of mean iodine value 88.8), this corresponds with the possible presence of 4.8 g. methyl palmitoleate and 6.0 g. methyl oleate-linoleate in this fraction.

On this basis, the component fatty acids of fat D were made up approximately of myristic (4 %), palmitic (30 %), stearic (2.5 %), palmitoleic (?) (8.5 %), oleic (53 %) and linoleic (2 %) acids.

Since, therefore, the presence of small amounts of an unsaturated acid of lower molecular weight than oleic acid was definitely indicated, we have calculated the component fatty acids of all four fats on the basis that the "liquid" ester fractions are made up of methyl myristate, palmitoleate and oleate (or oleate-linoleate). The results are given in Table IV.

We are aware that the impossibility of definitely identifying an unsaturated acid of lower molecular weight than oleic acid in the present series renders the foregoing calculation somewhat unsatisfactory, and we therefore add, for fats A, B and C (Table V), the results of the alternative calculation, in which the absence of unsaturated acids with less than 18 carbon atoms in the molecule is assumed. This, however, involves the presence of small amounts of saturated acids (lauric and even capric) of lower molecular weight than myristic acid;

and we have not been able to detect the presence of any acids of lower molecular weight than myristic acid.

Table IV. *Component fatty acids of rat body fats A, B, C, D.*
(Assuming the presence of palmitoleic acid.)

Diet ...	A	B	C	D
	"Fat-free"	Mainly "fat-free"	2-15 % cod-liver oil	2 % cod-liver oil
	%	%	%	%
Myristic acid	5	4.5	5	4
Palmitic acid	24	28	23	30
Stearic acid	3	2	2.5	2.5
Palmitoleic (?) acid	8	7	5.5	8.5
Oleic acid	58	58.5	51.5	53
Linoleic acid	2	—	4	2
C ₂₀₋₂₂ unsaturated acids*	—	—	8.5	—

* In fat C the fractionation data clearly indicated the presence of highly-unsaturated acids of the C₂₀ and C₂₂ series in the highest-boiling and residual fractions of the "liquid" esters; their amount was approximately calculated by the same means as that employed in similar cases which have been encountered in pig body fat analyses [Banks and Hilditch, 1932].

Table V. *Component fatty acids of rat body fats A, B, C.*
(Assuming absence of any unsaturated acids of lower carbon content than C₁₈.)

Diet ...	A	B	C
	"Fat-free"	Mainly "fat-free"	2-15 % cod-liver oil
	%	%	%
Myristic and lower saturated acids	4.5	4	5
Palmitic acid	24	28	23
Stearic acid	3	2	2.5
Oleic acid	66.5	66	54.5
Linoleic acid	2	—	6.5
C ₂₀₋₂₂ unsaturated acids	—	—	8.5

DISCUSSION.

It will be understood, from what has already been said, that the values given for the amounts of oleic acid present depend upon whether a palmitoleic acid, or even some unsaturated acid of still lower molecular weight, is also present; the figures for oleic acid in Tables IV and V together represent, however, the extreme possible limits. Apart from this uncertainty, a number of points of interest emerge from the analyses.

Body fats (A and B) of rats fed on mainly fat-free diets. The results amply confirm the observations of the previous investigators, namely, that rats receiving no linoleic acid in their fatty diet deposit reserve glycerides from which that acid is almost or completely absent.

A feature which interests us especially, however, is the relative amounts of palmitic acid and of stearic acid present in these fats as compared with the corresponding proportions in the body fats of pigs, oxen and sheep. The proportion of palmitic acid is, indeed, of much the same order in all cases: expressed in molar percentages, the united amounts of palmitic and myristic acids (the latter being 4-5 % in each case) in the four rat fats lie between 30 and 35 %, a figure which is almost identical with that observed throughout a large series of lards and tallows [*cf.* Banks and Hilditch, 1931; 1932]. The proportions of stearic acid are, on the contrary, strikingly different in the body fats of the rat and of pigs, sheep and oxen. In the latter group stearic is definitely a major

component acid, occurring in widely varying amounts. In the rat body fats it is equally clearly a minor component and occurs in proportions relatively small compared with those of palmitic or oleic acid.

In other words, the component acids of the rat body fats consist essentially of 25–30 % of palmitic acid, the remainder (apart from small proportions (4–5 %) of myristic and 2–3 % of stearic acid) being made up entirely of unsaturated acids, in which oleic acid predominates, and from which linoleic acid is practically absent. In consequence, about 65–70 % of the rat fat component acids belong to the unsaturated (oleic) series, whereas in lards and tallow the unsaturated acids vary from less than 40 %, and rarely exceed 55 %, of the mixed fatty acids.

Fully-saturated glycerides were only present in very small proportions in either fat, specimen A containing about 2.5 % and specimen B about 3.5 %. The fatty acids present in the fully-saturated components had mean molecular weights of 260 (A) and 258 (B) (palmitic acid, mol. wt., 256), so that these consisted almost entirely of tripalmitin. This is, of course, another difference from the depot fats of pigs, sheep and oxen, since the latter, in addition to containing greater amounts of stearic acid, also contain fully-saturated components in proportions increasing with those of the stearic acid present, and consisting for the most part of mixed palmitostearins [Banks and Hilditch, 1931; 1932].

Body fats (C and D) of rats fed on diets which included cod-liver oil. The specimen C, which was derived from rats whose diet had contained from 2 to 15 % of cod-liver oil, was differentiated from the rest by the presence of a certain amount (about 8 %) of the highly-unsaturated acids of the C_{20} and C_{22} series which are present in cod-liver oil. Apart from this, the remaining acids appeared to be present in much the same relative proportions as when the diet of the animals was mainly fat-free, but the presence of linoleic acid had become definitely noticeable (4 %). Nevertheless, the linoleic acid content is surprisingly small when compared with that of the deposited unsaturated C_{20-22} acids, since each type of acid is present in much the same proportions in cod-liver oil [Guha *et al.*, 1930].

This feature is also peculiar in view of the observation of Gregory and Drummond [1932] that the body fat of rats fed on diets including olive oil contained unsaturated acids of which linoleic acid formed from 7 to 10 % (*i.e.* practically the same proportion as in olive oil itself). With the olive oil diets used by these workers, there was, moreover, a considerable decline in the saturated acid content of the body fats, namely, from 32 % (fat-free diet) to 22 % (12 % olive oil in diet) and 11 % (50 % olive oil in diet). The fact that, in fat C, the total content of saturated acids (30.5 %) was only slightly less than that in the fats from animals on mainly "fat-free" diets (32–35 %), suggests either that the added cod-liver oil was not freely utilised, or, more probably, that the average amount of cod-liver oil in the diets (about 5 %, *cf.* p. 1376) was insufficient to produce any marked alteration in the body fat. In the case of the pig, for example, addition of cottonseed oil to the diet does not cause definite modification of the body fat until the oil forms more than 4 % of the diet [Ellis *et al.*, 1931].

The body fat D (from rats which had received a diet including only 2 % of cod-liver oil) was of practically the same composition as fats A and B, and showed no increase in linoleic acid and no perceptible amount of the higher unsaturated acids. The only indication of the presence of the latter was a slightly increased iodine value (93.9) in the residual fraction of "liquid" esters

(Table III), but this was not accompanied by any perceptible increase in the equivalent (295.5) of these esters (after correcting for unsaponifiable matter). In this experiment, therefore, the characteristic cod-liver oil acids did not appear in the depot fat.

The perinephric fat of the (wild) rabbit. We are able to add some details as to the adipose tissue fat of another rodent, from an ester-fractionation analysis (made in this laboratory some years ago by Mr J. R. Vickery) of the component fatty acids of the perinephric fat from wild rabbits. This was a yellow-coloured, very unsaturated fat, almost liquid at the ordinary temperature, with a saponification equivalent of 285.2 and an iodine value of 124.0. The component acids were made up of myristic (4.5 %), palmitic (23 %) and stearic (4 %) acids, the remainder (68.5 %) consisting of unsaturated acids (apparently almost wholly of the C_{18} series) with a mean iodine value of 189.3. The latter acids yielded solid hexabromo-addition products equivalent to a content of 9 % of linolenic acid in the total fatty acids; it is therefore quite possible that the total amount of linolenic acid in the mixed acids approached 15–20 %, since the yield of "insoluble hexabromides" usually corresponds with not more than about 50 % of the linolenic acid present.

This rabbit perinephric fat closely resembles the rat fats in its proportion of palmitic, stearic and myristic acids and in its general content of about 30 % saturated and 70 % unsaturated acids. It is also similar in its fully-saturated glyceride content, namely, about 6–7 %, almost wholly tripalmitin (the latter triglyceride was also obtained from rabbit fat by fractional crystallisation by Klimont [1912]). The nature of the unsaturated acids in rat fats and in rabbit fats is, however, wholly dissimilar: whereas, in that of the rat, linoleic acid is practically absent, the rabbit fat described contained large amounts of this component (35–50 % of the mixed acids) and also appreciable quantities of linolenic acid.

SUMMARY.

The body fat of the rat belongs to a group in which the component acids are made up of palmitic (25–30 %), myristic (4–5 %) and small amounts of stearic (2–3 %), the remainder (about 65–70 %) consisting chiefly of oleic acid, apparently with small quantities of a palmitoleic acid; linoleic acid is, however, almost entirely absent.

When the diet of the animals includes cod-liver oil, some of the highly-unsaturated acids of the C_{20} and C_{22} series may occur in the depot fats, but otherwise little modification occurs, and the proportion of linoleic acid is only slightly increased.

The perinephric fat of a wild rabbit contained similar amounts of myristic (4.5 %), palmitic (23 %) and stearic (4 %) acids, but the unsaturated acids (68.5 %) differed widely from those of the rat fats, linoleic acid being the main component, while considerable amounts of linolenic acid were also present.

Fully-saturated components (almost wholly tripalmitin) were present only in small proportions in the body fats of either the rat (2–3 %) or the rabbit (6–7 %).

There seem to be at least two well-defined groups of depot fats characteristic of land (*i.e.* non-aquatic) animals.

(a) Fats with component fatty acids containing about 30–35 % saturated (usually 25–30 % palmitic) acids and for the rest unsaturated acids of varying type (oleic, linoleic, *etc.*). In these stearic acid is a minor component (not exceeding about 5 %), and fully-saturated components are only present in small

proportions and consist mainly of tripalmitin. The rodent body fats discussed in this paper, and probably also bird depot fats, fall in this category.

(b) Fats with component fatty acids made up of about 25–30 % palmitic and small amounts of myristic acids, the remaining 65–70 % consisting of stearic, oleic and linoleic acids. In this group (to which belong the depot fats of the pig, ox, sheep, horse, reindeer) the proportion of stearic acid is variable but usually important. The greater the amount of stearic acid (with correspondingly less oleic acid), the greater is the proportion of fully-saturated glycerides in the fat; in this group, these consist for the most part of mixed palmitostearins.

The persistence with which the palmitic acid content remains in the neighbourhood of 25–30 %, and that of myristic acid at about 4–5 %, throughout both groups is noteworthy.

We desire to thank the Department of Scientific and Industrial Research for a grant in aid of this work.

REFERENCES.

- Banks and Hilditch (1931). *Biochem. J.* **25**, 1168.
— — (1932). *Biochem. J.* **26**, 298.
Baughman, Jamieson and McKinney (1929). *Oil and Fat Ind.* **6**, (8), 11.
Bömer and Merten (1922). *Z. Unters. Nahr. Genussm.* **43**, 101.
Burr and Burr (1930). *J. Biol. Chem.* **86**, 587.
Ellis, Rothwell and Pool (1931). *J. Biol. Chem.* **92**, 385.
Gregory and Drummond (1932). *Z. Vitaminforschung*, **1**, 257.
Grossfeld (1931). *Z. Unters. Lebensm.* **62**, 553.
Guha, Hilditch and Lovern (1930). *Biochem. J.* **24**, 266.
Heiduschka and Steinruck (1921). *J. prakt. Chem.* **102**, 241.
Klimont (1912). *Monatsh. Chem.* **33**, 441.