CV. THE FAT COMPOSITION OF MILK-FED RATS

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PREVIOUS analyses of rat body fats have indicated fatty acid compositions which vary considerably according to the diet. Banks *et al.* [1933], Klenk *et al.* [1935], Spadola & Ellis [1936] and Channon *et al.* [1937] have made detailed analyses of rat body fats. The earlier analyses indicated the presence of 25–30 mol. % palmitic acid, 4–5 mol. % myristic acid, small amounts of stearic acid (2–4 mol. %) and 65–70 mol. % unsaturated acids, chiefly oleic but including appreciable amounts of hexadecenoic acid (5–13 %) and a small amount of linoleic acid. These proportions were found in the more recent studies to be substantially altered by feeding cottonseed oil (Spadola & Ellis), palm oil, olive oil, cod liver oil and coconut oil (Channon *et al.*). Hydrogenated cottonseed oil, beef dripping and butter fat did not produce such considerable changes. In all the analyses reported, stearic acid appears as a minor component acid² (ca. 4 mol. % $\pm$ 2).

Dr S. K. Kon very generously placed at our disposal a quantity of body fat extracted from rats which were raised from weaning for eleven weeks on a (cow) milk diet alone. We report here a detailed analysis of this fat, the fatty acid composition of which was found to resemble in general both that indicated by the earlier analyses for rat body fat and that reported by Channon and co-workers for the carcass fat of rats fed for 2 weeks on a diet containing 40 % butter fat.

EXPERIMENTAL

The body fat used in this investigation was an ether extract of the rats used by Henry & Kon [1936] in an experiment on the effect of an exclusive milk diet (cf. original paper for feeding details, etc.). Immediately after killing, the intestines were removed and the carcasses were dried to constant weight at 60–65° and exhaustively extracted with ether in a Soxhlet apparatus. The ether extract represented approximately 14 % of the weight of the dried carcasses. It was stored in a completely filled bottle at –2° for several months before analysis could be undertaken. The “fat”, which was semi-solid at room temperature, had the following analytical values: sap. equiv. 284.3, iodine value (Wijs) 65.1.

Inasmuch as this total ether extract gave qualitative indication of the presence of phosphorus it was considered desirable to effect as completely as possible a separation of the phosphorus-containing substances, chiefly phosphatides. The whole “fat” was refluxed with four volumes of acetone, in which neutral fat is easily soluble but phosphatides are not. The solution was allowed to stand at 0° overnight. The supernatant liquid was decanted and the acetone

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² Our use of the terms major and minor component acids follows the suggestions given by Hilditch [1934]. The divisions are obviously not sharply defined but they represent large amounts as distinct from small amounts of the various acids and not large compared with larger amounts as interpreted by Anderson & Williams [1937].

treatment of the semi-crystalline dark-coloured residue was repeated. In a third similar extraction the material insoluble in cold acetone separated into two distinct layers, the upper one definitely crystalline yet admixed with a lower darker coloured viscous liquid closely resembling the ordinary phosphatides. This upper layer was decanted from the lower one and the two products were further separated by repeated crystallizations from acetone, the supernatant liquids each time being combined and added to the previous supernatant liquids. The phosphatide-like material did not dissolve in hot acetone. This enabled the separation from the crystalline precipitate, which formed only after cooling, to be accomplished more easily. The three products thus obtained from the preliminary separation were freed of acetone and weighed.

The crude glyceride from the combined supernatant liquids was dissolved in four volumes of hot acetone and further purified by the addition of 5 ml. of a concentrated methyl alcoholic solution of CaCl_2 . There was an immediate precipitation of a material insoluble in boiling acetone resembling that previously obtained. On standing overnight at 0° an even larger crystalline precipitate formed than in the previous treatment of the entire "fat". The same method as described above was used to separate the crystalline precipitate from the sticky substance insoluble in hot acetone.

Thus, the entire "fat" was resolved into glyceridic and non-glyceridic components. The largest part was the glyceridic portion completely soluble in both hot and cold acetone. The crystalline preparations from cold acetone have been reckoned as glycerides on account of their solubility in warm acetone and the absence of P and N. The remaining fractions were comparatively free from glycerides and were mainly mixtures of phosphatides, judging from the P and N contents. The weights, together with the various analytical values for the five preparations, are collected in Table I.

Table I. *General characteristics of the various fractions of rat body fat*

	g.	%	Sap. equiv.	I.V.	P %	N %
Glyceridic:						
A. Soluble in hot and cold acetone	488.3	70.9	285.9	71.9	Nil	Nil
B. Soluble in hot, insoluble in cold acetone	35.5	5.2	277.0	15.0	Nil	Nil
C. Precipitated from cold acetone after addition of CaCl_2	145.1	21.1	281.4	47.6	0.15	0.10
Non-glyceridic:						
D. Acetone-insoluble	12.7	1.8	287.2	65.3	2.54	1.23
E. Precipitated from hot acetone by CaCl_2	7.3	1.0	288.3	68.2	1.76	1.02

The three glyceridic fractions, together constituting 97.2% of the total ether extract, were further analysed after their conversion into methyl esters following the customary procedures of this laboratory [Banks *et al.* 1933; Dean & Hilditch, 1933, 1, 2; Hilditch & Longenecker, 1937]. An electrically heated fractionating column of high operating efficiency under equilibrium conditions [Longenecker, 1937] was used for all ester distillations. Details of the various analyses are given in Tables II and III.

The phosphatidic fractions were unfortunately too small in amount to justify us in attempting detailed analyses of their component fatty acids.

The fractionation and analytical data (cf. Table III) clearly indicated the presence of unsaturated esters of lower molecular weight than methyl oleate.

Table II. *Lead salt separation of the mixed fatty acids*

Glyceride	"Solid" acids				"Liquid" acids			
	g.	%	Corresponding methyl esters		g.	%	Corresponding methyl esters	
			Sap. equiv.	I.V.			Sap. equiv.	I.V.
A. Soluble in acetone*	77.9	27.4	274.8	10.8	206.9	72.6	293.2	86.5
B. Insoluble in cold acetone	20.2	77.0	277.5	5.6	6.0	23.0	280.8	53.8
C. Precipitated from cold acetone after addition of CaCl_2	46.2	39.9	275.0	6.6	69.6	60.1	289.8	78.7

* Steam distillation of the mixed acids prior to the lead salt separation yielded none of the lower molecular weight acids.

Table III. *Fractional distillation of methyl esters*

"Solid" acids				"Liquid" acids			
No.	g.	Sap. equiv.	I.V.	No.	g.	Sap. equiv.	I.V.
(a) Glyceride A, soluble in hot and cold acetone							
S 1	1.82	241.0	0.2	L 1	1.43	203.5	12.7
S 2	2.11	249.6	0.9	L 2	1.47	240.1	15.3
S 3	22.25	263.9	1.7	L 3	4.64	245.7	30.4
S 4	15.49	269.3	2.1	L 4	9.62	264.0	54.1
S 5	7.31	281.9	17.6	L 5	3.33	275.1	70.5
S 6	5.43	294.3	30.2	L 6	6.79	286.8	81.8
S 7	8.60	297.1	33.4	L 7	15.28	294.1	91.6
S 8	3.82	325.3	34.5	L 8	23.05	295.2	92.7
	66.83	(318.7*)		L 9	12.91	295.8	95.3
				L 10	9.86	296.9	95.8
				L 11	3.72	299.8	103.8
				L 12	6.58	302.4	108.7
				L 13	6.00	499.8	93.6
					104.68	(304.6*)	
(b) Glyceride B, soluble in hot, insoluble in cold acetone							
S 1	2.42	259.4	0.9	L 1	1.23	240.9	5.8
S 2	3.80	268.1	1.0	L 2	1.66	271.8	44.3
S 3	3.37	269.2	1.1	L 3	2.06	320.5	88.2
S 4	2.45	273.1	1.9		4.95	(305.2*)	(86.9*)
S 5	4.37	287.1	8.0				
S 6	2.46	316.0	23.5				
	18.87	(294.9*)	(12.2*)				
(c) Glyceride C, precipitated by CaCl_2 from cold acetone							
S 1	3.27	240.4	—	L 1	2.03	222.6	13.6
S 2	5.74	264.0	0.9	L 2	3.12	249.8	30.6
S 3	9.30	270.7	3.1	L 3	3.14	264.3	46.8
S 4	7.06	272.9	4.3	L 4	7.58	284.7	76.7
S 5	7.80	278.3	7.7	L 5	12.53	290.7	84.7
S 6	5.93	290.1	14.7	L 6	13.80	293.3	87.3
S 7	3.18	318.3	21.8	L 7	5.69	295.2	89.6
	42.28			L 8	2.41	297.4	90.9
				L 9	6.54	359.4	93.6
					56.84	(301.5*)	

* Esters freed from unsaponifiable matter.

Hexadecenoic acid has been recognized several times as a component of rat body fat [Banks *et al.* 1933; Klenk *et al.* 1935; Spadola & Ellis, 1936; Channon *et al.* 1937]; Spadola & Riemenschneider [1937] recently showed that its constitution is the same as that hitherto observed for the monoethenoid C_{16} acid in

fats from all other marine or land fauna, namely, $\Delta^{9:10}$ -hexadecenoic acid. The presence of an unsaturated C_{14} acid was indirectly established by the acetone permanganate oxidation [Armstrong & Hilditch, 1925] of the ester fraction L 3 (glyceride A, soluble in cold acetone). 3.20 g. of the original ester fraction (sap. equiv. 245.7, i.v. 30.4) yielded 2.32 g. of saturated acids having an equivalent weight of 244.9. Azelaic acid, m.p. 105° , showing no depression when mixed with the pure acid (m.p. 106°), was obtained from the oxidation products [cf. Hilditch & Longenecker, 1937]. From a consideration of these facts and from our knowledge of the performance of the fractionating column we feel justified in including the unsaturated C_{14} and C_{16} acids when calculating the individual compositions of the three neutral glyceride fractions (Table IV).

Table IV. *Component acids of neutral glycerides of rat fat*

(i) "A", soluble in hot and cold acetone. 300 g. hydrolysed for analysis

Component acids	"Solid" (27.4%)	"Liquid" (72.6%)	Total	% (wt.)	% (mol.)
Decanoic	—	0.40	0.40	0.4	0.6
Lauric	0.01	0.49	0.50	0.5	0.7
Myristic	3.25	3.40	6.65	6.8	8.0
Palmitic	15.30	4.70	20.00	20.3	21.3
Stearic	4.16	—	4.16	4.2	4.0
Arachidic	0.94	—	0.94	1.0	0.8
Tetradecenoic	—	1.47	1.47	1.5	1.8
Hexadecenoic	0.31	5.35	5.66	5.8	6.1
Oleic	3.40	48.58	51.98	52.9	50.4
Octadecadienoic*	—	5.95	5.95	6.0	5.8
Arachidonic	—	0.55	0.55	0.6	0.5
Unaponifiable	0.03	1.71	1.74	—	—

(ii) "B", soluble in hot, insoluble in cold acetone. 28.9 g. hydrolysed for analysis

Component acids	"Solid" (77.0%)	"Liquid" (23.0%)	Total	% (wt.)	% (mol.)
Lauric	—	0.32	0.32	0.3	0.4
Myristic	4.46	5.02	9.48	9.6	11.0
Palmitic	49.92	4.04	53.96	54.8	55.7
Stearic	18.41	—	18.41	18.7	17.1
Tetradecenoic	—	0.32	0.32	0.3	0.4
Hexadecenoic	0.32	2.83	3.15	3.2	3.3
Oleic	3.20	9.68	12.88	13.1	12.1
Unaponifiable	0.69	0.79	1.48	—	—

(iii) "C", precipitated by CaCl_2 from cold acetone. 127 g. hydrolysed for analysis

Component acids	"Solid" (39.9%)	"Liquid" (60.1%)	Total	% (wt.)	% (mol.)
Lauric	0.15	1.38	1.53	1.5	2.1
Myristic	3.98	2.37	6.35	6.4	7.5
Palmitic	25.02	4.71	29.73	30.1	31.3
Stearic	5.42	—	5.42	5.5	5.1
Arachidic	2.26	—	2.26	2.3	2.0
Tetradecenoic	—	1.09	1.09	1.1	1.3
Hexadecenoic	0.34	5.06	5.40	5.5	5.7
Oleic	2.73	42.03	44.76	45.3	42.8
Octadecadienoic*	—	2.19	2.19	2.2	2.1
Arachidonic	—	0.10	0.10	0.1	0.1
Unaponifiable	—	1.17	1.17	—	—

* Representing isomeric di-unsaturated C_{18} acids.

DISCUSSION

It is of interest to observe how the composition of the total neutral glyceride resembles or differs from that of the food. The composition of the former was calculated from the individual compositions of the three neutral glycerides A, B, and C and their respective percentages, 72.9, 5.4 and 21.7. These figures are compared with those for a representative analysis of cow milk fat [Hilditch & Longenecker, 1938] in Table V.

Table V. *Fatty acid composition of glycerides of rat fat and cow milk fat (% mol.)*

Acid	Rat fat	Milk fat
Butyric	—	8.1
<i>n</i> -Hexanoic	—	2.8
<i>n</i> -Octanoic	—	2.5
<i>n</i> -Decanoic	0.4	3.7
Lauric	1.0	4.4
Myristic	8.0	12.5
Palmitic	25.4	23.2
Stearic	5.0	7.6
Arachidic	1.0	1.0
Decenoic	—	0.4
Dodecenoic	—	0.5
Tetradecenoic	1.6	1.7
Hexadecenoic	5.9	3.7
Oleic	46.6	24.8
Octadecadienoic	4.7	2.9
Arachidonic	0.4	0.2

None of the lower molecular weight acids (up to C_{10}) of milk fat were stored and the C_{10} and C_{12} acids were not found in appreciable amounts. These acids represent a total molar percentage of 22.8 in milk fat but only 1.4 in the rat fat [cf. also Eckstein, 1929, 1, 2; 1930; Davis, 1930; Cox, 1930; Powell, 1930; Hughes & Wimmer, 1935; Channon *et al.* 1937; Schoenheimer, 1937].

Another noticeable difference between the two fats is the amount of oleic acid, which in milk fat is normally about 25% but in this rat fat was 46.4%. In the detailed investigation [Channon *et al.* 1937] of the effect of feeding 40% fat diets for 14 days on the composition of rat fat, butter fat was included and, having regard to the difference in the experimental feeding conditions, the results were very similar. The case of coconut oil feeding examined by these authors is also interesting in this connexion since they report the presence of 31.5% of oleic acid and 10.4% of linoleic acid in the carcasses of rats fed on this oil, although the latter contains the acids in much smaller amounts (5.7 and 2.6% respectively, Collin & Hilditch [1928]).

The data now reported closely resemble those previously obtained for rats on a low fat diet [Banks *et al.* 1933; Klenk *et al.* 1935; Spadola & Ellis, 1936] and are not significantly different from those of Channon *et al.* for rats on a diet containing 40% beef dripping or butter fat. They appear, therefore, to represent the "normal" composition of rat fat, namely, small amounts of myristic,¹ stearic, hexadecenoic (palmitoleic) and octadecadienoic acids, and approximate constancy of both palmitic acid (ca. 25 mol. %) and oleic acid (45–50 mol. %). Whether the unsaturated acids "normally" found are to be attributed to storage of those found in the food or to more complicated processes is a question difficult

¹ Myristic acid figures for rat fat are not usually so high as those reported in Table V. It is possible that the high amount present in butter fat has led to a deposition of this acid.

to answer. That the body is capable of absorbing and depositing fatty acids has been revealed in many cases by feeding unusual acids and later recovering them in the fat depots. As regards linoleic acid, Green & Hilditch [1935] have concluded that its origin in land animals is food fat. Anderson & Mendel [1928] calculated that it was possible (mathematically) for the small amount of fat in their diets to have accounted for the total depot fat produced. In our case the hexadecenoic, oleic and octadecadienoic acids may have been in effect concentrated from the supply present in the milk fat in a similar way to that described by Spadola & Ellis [1936] and Channon *et al.* [1937] as resulting from the feeding of relatively high amounts of fat containing larger proportions of unsaturated acids. Against this consideration, however, are the results of Channon and co-workers with olive oil (80% oleic acid and 8% linoleic acid) and palm oil (41% oleic acid and 10% linoleic acid). In both cases these workers found lower percentages of the respective acids in the carcass fat than in the food fat. To us it seems more logical that a more complicated mechanism tends to maintain a fairly constant composition of the body fat.

Barbour [1934] was led to the conclusion that, regardless of the nature of the dietary fat, the total saturated acids from rat body fat reached a threshold level at about 25–27%. It is unfortunate that he took into consideration the “solid” acids obtained from a Twitchell lead salt separation, as modified by Baughman & Jamieson [1930], instead of the true amounts of saturated acids. The well-known experience with this separation is, of course, that unsaturated acids are never completely removed from the “solid” acids (i.e. those obtained from the precipitated lead salts) and that the lower saturated acids are found in appreciable amounts in the “liquid” or soluble lead salt fraction. Whilst obviously Barbour’s results cannot be more exactly calculated, since a detailed examination of the component acids was not made, it appears that his results differ little from the more detailed analyses of Banks *et al.* [1933], Klenk *et al.* [1935], Spadola & Ellis [1936] and Channon *et al.* [1937]. These analyses indicated that the percentage of saturated acids might vary considerably (22.4–46.5%) but that there was an approach toward constant values at 32–35% (twelve cases). Higher values than these have been reported in only three cases, two by Spadola & Ellis as the result of low fat diets (37.4%) and cottonseed oil feeding (39.6%) and the other (46.5%) as the result of a high coconut oil diet [Channon *et al.* 1937]. In this latter case myristic and lauric acids accounted for 22.1% of the saturated acids, an unusually high percentage for these acids. From our own figures, the saturated acids represent 40.4% of the total. Here again, the lauric and myristic acid contents are somewhat higher than usual.

While the information available is by no means complete, the conclusion reached by Barbour seems essentially correct, i.e. that the rat does not store saturated acids beyond a certain “threshold” level. For the reasons mentioned above, we place this level at 32–35%, seven to ten units higher than that reported by Barbour.

The resolution of the total ether extract into predominantly neutral fat and predominantly phosphatide fractions again indicated the precautions that must be taken, owing to the separation of the di- and tri-saturated glycerides from cold acetone. Klenk & Ditt [1934] called attention to the material which crystallized out of the acetone solutions of glycerides from heart muscle. Hilditch & Shorland [1937] experienced similar difficulties in the resolution of liver fats as did Klenk & Schuwirth [1937] with rat fat. We were fortunate in having sufficient quantities of the neutral glycerides crystallized from acetone to make possible their quantitative fatty acid analyses. These analyses (cf. Table IV)

amplify the indications given by the saponification equivalents and iodine values of the respective fractions given in Table I. The material which crystallized out of cold acetone contained, as would be expected, high proportions of saturated acids. The amount of the crystalline precipitate (C) obtained on cooling the acetone solution, after addition of CaCl_2 to remove the last traces of phosphatides, was unexpectedly large. Both these crystalline precipitates (B and C) were glyceridic, and completely soluble in warm acetone, in contrast to the non-glyceridic portion, the phosphatides, which was not soluble in boiling acetone. When working with much smaller amounts of material, as is more often the case in a study of this kind, it is advisable to separate the phosphatides from the warm acetone solution of glycerides or to combine such crystalline precipitates with the glyceridic fractions. If these fractions cannot be wholly freed from P and N, they should preferably be analysed separately and corrections similar to those suggested by Hilditch & Shorland should be employed.

SUMMARY

The ether extract of rats fed from the time of weaning for 11 weeks on milk alone has been studied in detail. Crystalline glyceride mixtures obtained in the acetone separation of phosphatides were analysed separately from the main glyceride fraction (which was liquid at room temperature) by the ester fractionation procedure. A higher percentage of saturated acids, indicating the presence of di- and tri-saturated glycerides, was found in these crystalline glycerides than in the liquid glycerides. Precautions are indicated for the acetone resolution of glycerides and phosphatides.

The composition of the rat fat was only slightly altered from "normal" by the milk diet. Of the 22.8% (molar) of C_4 , C_6 , C_8 , C_{10} and C_{12} acids present in milk fat only 1.4 mol. % (C_{10} and C_{12} acids) were stored by the rat. The somewhat larger amount of myristic acid than is usually obtained from rat fats might be related to the quantity present in the milk fat. On a low fat diet, the rat appears to build a body fat of roughly constant composition. This usually contains small amounts (3–6%) of myristic, stearic, hexadecenoic and octadecadienoic acids with approximately constant amounts of both palmitic (25%) and oleic (45–50%) acids. The presence of tetradecenoic acid was indicated by this study, as was also that of decanoic, lauric, arachidic and arachidonic acids ($\pm 1\%$ each).

From a consideration of various data reported for rat fats, a threshold level of the saturated acids as suggested by Barbour [1934] seems likely to occur at 32–35 mol. % (somewhat higher than Barbour's original figures).

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