VII. DEPOSITION OF FAT IN THE LIVER AND CARCASS OF THE RAT ON DIETS HIGH IN FAT AND LOW IN LIPOTROPIC FACTORS

By HAROLD JOHN CHANNON, GEORGE NEIL JENKINS and JAMES ANDREW BUCHAN SMITH

From the Department of Biochemistry, The University of Liverpool

(*Received 30 November 1936*)

In continuation of previous studies of factors influencing the production of dietary fatty livers in rats, Channon & Wilkinson [1936] investigated the degree to which certain fats of widely varying chemical composition and physical properties accumulated in the livers when fed as 40% of a diet which was low in choline (choline intake about 1·5 mg. per rat per day) and which contained alcohol- and ether-extracted caseinogen 5, glucose 45, marmite 5, salt mixture 5 parts with 1 drop of cod liver oil per animal every 3 days. This work was undertaken in the first place in order to obtain information concerning the nature of the fatty acids which appeared in the liver under these different conditions and because knowledge of this kind should provide evidence as to the effect of the chemical nature of the food fat in fatty liver production. The results as far as the fatty liver problem is concerned are recorded by Channon & Wilkinson in the communication already cited.

The work described in the present paper deals in detail with the composition of the carcass fats of the rats in each of the six groups to which the different fats were fed, and it was carried out with three principal objects in view: (1) to determine whether the intensity of the fat infiltration in the liver is governed by the amount of depot fat deposited on the different diets, (2) to compare the composition of the carcass fat with that of the liver in the various groups and (3) to investigate in detail how the carcass fat of the rat is affected by including in the diets fats of very varied composition.

Experimental

The details of the feeding of the animals, with their food intakes and body weights, have already been published [Channon & Wilkinson, 1936]. Each group of 10 animals was given either beef dripping, palm oil, olive oil, cod liver oil, butter fat or coconut oil as 40% of its diet. After 14 days the rats were guillotined and their livers and alimentary canals removed. The carcasses from each group were pooled and covered with 5% aqueous NaOH in well-stoppered vessels and stored in the ice-chest. The body "fat" was then obtained by digestion of the carcasses with alkali at 100° until tissue disintegration was complete, followed by exhaustive extraction of the acidified liquor with benzene. The benzene was then evaporated to dryness and the fat freed from some non-fatty material by keeping in petroleum overnight. The evaporated petroleum filtrate was resaponified with alcoholic KOH and the fatty acids and unsaponifiable matter were separated in the usual way. Total weights, mol. wt. and I.V. of the fatty acids were then determined and are recorded in Table I, where are

Table I. *The amounts of fatty acids obtained from the carcasses in each group compared with the lipoid contents of the livers*

Fat in diet	Wt. of carcass (10 rats) g.	Wt. of fatty acids g.	Fatty acids in carcass %	Total lipoids in 100 g. liver g.	Lipoids in liver of 100 g. rat g.	Mean mol. wt. of carcass acids	I.V. of carcass acids
Beef dripping	1537	225	14·6	27·0	1·22	275	67·6
Butter fat	1534	171	11·1	30·7	1·46	277	65·7
Cod liver oil	1371	153	11·4	7·2	0·26	285·5	85·5
Olive oil	1489	220	14·8	15·6	0·53	280	77·7
Coconut oil	1460	191	13·1	20·5	0·80	270	59·2
Palm oil	1477	151	10·3	26·35	1·17	283	68·8
Control	1142	113	9·9	—	—	274	70·0

presented for comparison the yields previously obtained from the livers. The unsaponifiable matter amounted to 0·2–0·3 % of the carcass weight in each group.

Analysis of the acids

The absence of acids lower than decanoic. As butter fat and coconut oil both contain significant amounts of lower acids, it was necessary to find whether any of the latter were present in the carcasses of the groups to which these fats were fed. Consequently in these two cases after each saponification referred to above the solution was acidified with H_2SO_4, saturated with K_2SO_4 to salt out any lower acids which might be present and thoroughly extracted with petroleum. An aliquot of the extract containing some 5 g. of fatty acids was again saponified with alcoholic KOH, acidified with H_2SO_4 and the Reichert-Meissl value determined. In the coconut oil and butter groups the values were found to be 1·3 and 0·8 respectively, i.e. of the usual order for fats containing no measurable amounts of acids lower than decanoic.

The preparation of the methyl esters for distillation. The mixed fatty acids from each group were separated into liquid and solid fractions by the Twitchell process as modified by Hilditch & Priestman [1931]. Known amounts of the two fractions were then esterified (methyl alcohol-HCl) and the resulting esters tested to ensure that no free acid remained.

The absence of liquid saturated acids. As the presence of liquid saturated acids in cat's kidney has been reported by Turner [1931], it was thought essential to discover whether any measurable amount of such acids existed in the carcasses of the rats used in the present work. For this purpose a sample of the liquid acid fraction from the beef fat group was hydrogenated. 1·38 g. (I.V. 89·9) yielded on hydrogenation 1·3 g. (I.V. 1·9). As this product melted at 62°, it could not have contained any detectable amount of liquid saturated acid.

The presence of oleic and linoleic acids. A small portion of the liquid acids from each group was oxidized by the method of Lapworth & Mottram [1925]. In each case di- and tetra-hydroxy-derivatives were isolated and identified, thus establishing the presence of oleic and linoleic acids in each fat. In the case of the cod liver oil group, for example, 2·18 g. of the liquid acid fraction yielded 0·82 g. dihydroxystearic acid (mol. wt. 315, M.P. of crude product 126°) and 0·11 g. of the tetrahydroxy-derivative, the fraction of the latter least soluble in ethyl acetate melting at 172°. These yields correspond to the presence of 26 % oleic and 3 % linoleic acid in the original mixture. It is well established, however, that in cases such as this the yields of hydroxy-acids are always much below theory. Consequently the figures for oleic and linoleic acids found by oxidation

were very much lower than the corresponding values obtained by fractional distillation. In the present case the latter were 38 and 11 (maximum) respectively. The oxidation thus confirmed the distillation results in proving that both oleic and linoleic acids were present and that the former greatly exceeded the latter in amount. Oxidation of the liquid acids from the other five groups gave similar results.

The distillation of the methyl esters. The methyl esters of both the liquid and solid acids from all six groups were now subjected to fractional distillation *in vacuo*. Each fraction was saponified and traces of unsaponifiable matter were removed and the I.V. and mol. wt. of the acids determined. For the saturated mixtures the method of calculating the composition was based on the assumption that each fraction consisted of only two even-numbered saturated homologues, its small I.V. (if any) being due to oleic acid. As examples, the details of the entire process, together with the calculated compositions of the mixed solid acids from the palm and coconut oil groups, are recorded in Tables II and III.

Table II. *Fractionation of saturated esters from palm oil group*

Fraction	B.P. at 4 mm. up to °C.	Wt. of esters g.	Total saturated esters %	I.V. of acids	Mol. wt. of acids	Composition (%) C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	Oleic
1	151	2·78	6·64	0·2	255	0·2	6·4	—	—	—	—
2	157	4·83	11·53	0·5	253·5	1·2	10·2	—	—	—	0·1
3	158	11·90	28·41	1·2	258	—	26·0	2·0	—	—	0·4
4	159	3·48	8·31	1·3	261	—	6·7	1·5	—	—	0·1
5	160	6·03	14·40	1·7	262	—	11·1	3·0	—	—	0·3
6	162	5·43	12·96	4·0	273·5	—	4·9	7·5	—	—	0·6
7	164	4·53	10·81	5·9	277	—	2·5	7·6	—	—	0·7
Residue	—	2·91	6·95	17·2	315	—	—	—	3·4	2·2	1·3
Total	—	41·89	100·01	—	—	1·4	67·9	21·6	3·4	2·2	3·5
% of total mixed acids	—	—	—	—	—	0·4	19·9	6·3	1·0	0·7	1·0

Table III. *Fractionation of saturated esters from coconut oil group*

Fraction	B.P. at 4 mm. up to °C.	Wt. of esters g.	Total saturated esters %	I.V. of acids	Mol. wt. of acids	Composition (%) C_{14}	C_{16}	C_{18}	C_{20}	Oleic
1	122	2·93	6·59	0·2	236	4·7	1·9	—	—	Trace
2	130	3·04	6·84	0·1	229	6·6	0·2	—	—	Trace
3	140	4·00	9·00	0·5	240	5·1	3·9	—	—	Trace
4	148	7·37	16·59	0·8	248	4·7	11·7	—	—	0·15
5	150	7·88	23·29	1·1	261	—	14·6	3·1	—	0·2
6	152	10·34	8·87	2·8	263	—	17·8	4·8	—	0·7
7	163	3·94	11·11	9·5	267	—	5·4	2·6	—	0·9
Residue	—	4·94	—	17·5	294	—	—	4·8	4·1	2·2
Total	—	44·44	—	—	—	21·1	55·5	15·3	4·1	4·15
% of total mixed acids	—	—	—	—	—	6·8	18·0	5·0	1·3	1·4

For the liquid acids the method of arriving at the composition was not so simple. In the first place where some of the earlier fractions resulting from the distillation appeared to contain some solid acid, the Twitchell separation was again carried out. In Table IV, for example, it will be seen that fractions 1–4 were treated in this way. The nature of the solid acids so obtained was then deduced by making assumptions already used for the large solid fractions (Tables II and III). In the second place, by use of its I.V., the mol. wt. of each liquid

fraction was calculated as though it had been fully saturated. Then from the figures so obtained and the assumption that, if hydrogenated, each fraction would contain only two even-numbered homologues, it was possible to determine how much unsaturated C_{16}, C_{18}, C_{20} or C_{22} acid was originally present.

By this means the values obtained for the total amount of C_{18} unsaturated acids present in the original mixture may be considered reliable within the usual limits of the fractional distillation process, but owing to the fact that several C_{20} compounds with different degrees of unsaturation may have been present, it was impossible to arrive by calculation at the true proportions of the two C_{18} acids, oleic and linoleic. It was therefore decided to calculate the maximum amount of linoleic which could possibly have been present by assuming that all the unsaturated C_{20} acid possessed the minimum of one double bond. From the bromination experiment recorded later, it is quite clear that the C_{20} acids possessed on the average more than one double bond and so the amounts of linoleic acid actually present must have been less than those quoted in Tables IV and V, but as the maximum figures are themselves so low, any further alteration in this direction makes little difference, if any, to the final interpretation of the results.

For reasons which will now be given, the presence of palmitoleic acid was assumed in certain cases. In Table IV it is seen that fraction 1 gave rise to liquid acids having mean mol. wt. 247 and I.V. 78·1. As no liquid saturated acids were present and as the properties of the mixture could not be explained by the presence of either oleic or tetradecenoic acid, the observed figures appeared to be due to a mixture of palmitoleic and myristic acids, an assumption which satisfies both I.V. and mol. wt.

To confirm the presence of a lower unsaturated acid in the earlier fractions such as this, oxidation experiments were carried out in some instances. A typical example was that of the first fraction from the distillation of the unsaturated acids from the beef fat group. 3·02 g. (I.V. 85·0; mol. wt. 263) on oxidation [Lapworth & Mottram, 1925] yielded 1·6 g. of dihydroxy-derivatives having mean mol. wt. 303 and M.P. 123–125°. As $C_{18}H_{36}O_4$ and $C_{16}H_{32}O_4$ have mol. wt. 316 and 288 respectively, it is justifiable to assume that the original mixture consisted mainly of oleic and palmitoleic acids, together with a small amount of saturated acid.

Further difficulties arose as to how the composition of some of the small fractions of the liquid acids should be calculated, but these were largely overcome by methods which can be regarded as substantially reliable. One example will serve to illustrate this. Reference to Table V shows that when the fourth fraction was subjected to the Twitchell separation, the less saturated portion had mean mol. wt. 244 and I.V. 63·0. As the mixture had passed through the Twitchell process, any measurable amount of saturated acid present would be lower in the series than palmitic which always passes readily and almost completely into the more saturated fraction. To discover the nature of the saturated and other acids present, 2·085 g. of the mixture were oxidized [Lapworth & Mottram, 1925] giving 0·9 g. dihydroxy-derivatives. As these had mean mol. wt. 304 and M.P. 124°, oleic and palmitoleic acids must have been present in the original fraction. A little tetrahydroxystearic acid was also obtained at the same time, but it corresponded to an amount of linoleic acid too small to affect the calculation of the final composition for the mixture as a whole. 1·1 g. of the petroleum-soluble material from the oxidation just described were now carefully oxidized by the Bertram process as modified by Hilditch & Priestman [1931], giving 0·74 g. saturated acid (mol. wt. 229·5). From these findings it may safely

Table IV. *Fractionation of unsaturated esters from palm oil group*

Fraction	B.P. at 4 mm. up to °C.	Wt. of esters g.	Twitchell separation description	Total unsaturated esters %	I.V. of acids	Mol. wt. of acids	Composition (%)							
							Saturated				Unsaturated			
							C_{12}	C_{14}	C_{16}	C_{18}	Palmitoleic	Oleic	Linoleic	C_{20}
1	165	3·87	Solid	1·7	6·8	227	0·2	1·4	—	—	0·1	—	—	—
			Liquid	4·4	78·1	247	—	1·1	—	—	3·3	—	—	—
2	170	3·07	Solid	0·7	15·0	255	—	—	0·6	—	—	0·1	—	—
			Liquid	4·1	97·5	276	—	—	—	—	0·9	3·0	0·2	—
3	171	10·62	Solid	1·2	8·4	267	—	—	0·7	0·4	—	0·1	—	—
			Liquid	15·4	100·6	277	—	—	—	—	2·8	11·1	1·5	—
4	177	11·65	Solid	0·7	15·5	280·5	—	—	0·1	0·5	—	0·1	—	—
			Liquid	17·6	100·5	285	—	—	—	—	—	13·6	2·1	1·9
5	178	14·57	Liquid	22·9	105·5	285	—	—	—	—	—	16·3	4·1	2·5
6	179	7·50	Liquid	11·8	108·2	289	—	—	—	—	—	6·2	2·6	3·0
7	181	2·34	Liquid	3·7	121·5	285	—	—	—	—	—	1·9	1·3	0·5
Residue	—	10·07	Liquid	15·8	137·0	304	—	—	—	—	—	1·5	1·3	13·0
Total	—	63·69	—	100·0	—	—	0·2	2·5	1·4	0·9	7·1	53·9	13·1	20·9
% of total mixed acids	—	—	—	—	—	—	0·1	1·7	0·9	0·6	4·7	36·1	8·8	14·0

Table V. *Fractionation of unsaturated esters from coconut oil group*

Fraction	B.P. at 4 mm. up to °C.	Wt. of esters g.	Twitchell separation description	Total unsaturated esters %	I.V. of acids	Mol. wt. of acids	Composition (%)							
							Saturated				Unsaturated			
							C_{10}	C_{12}	C_{14}	C_{16}	C_{16}	Oleic	Linoleic	C_{20}
1	108	3·48	Solid	5·1	5·8	196·5	1·2	3·6	—	—	0·3	—	—	—
2	114	4·53	Solid	6·6	8·7	204	0·2	5·8	—	—	0·6	—	—	—
3	120	1·92	Solid	2·8	15·7	210	—	2·3	0·1	—	0·4	—	—	—
4	154	4·58	Solid	1·3	2·3	228·5	—	—	1·3	Trace	Trace	—	—	—
			Liquid	5·4	63·0	244	—	0·1	1·9	—	1·7	1·7	—	—
5	165	7·30	Solid	1·7	43·8	255	—	—	0·8	0·1	—	0·8	—	—
			Liquid	9·0	96·6	278	—	—	0·6	—	—	7·2	1·2	—
6	169	11·54	Liquid	16·9	99·3	275	—	—	2·1	—	—	11·0	3·8	—
7	170	20·70	Liquid	30·3	105·8	276	—	—	3·2	—	—	18·8	8·3	—
8	175	6·14	Liquid	9·0	109·4	284	—	—	—	—	—	6·5	1·9	0·6
Residue	—	8·18	Liquid	12·0	132·0	304	—	—	—	—	—	1·6	0·5	9·9
Total	—	68·37	—	100·1	—	—	1·4	11·8	10·0	0·1	3·0	47·6	15·7	10·5
% of total mixed acids	—	—	—	—	—	—	0·9	7·8	6·6	0·1	2·0	31·3	10·3	6·9

be concluded that the particular fraction under discussion consisted mainly of myristic, palmitoleic and oleic acids.

This experiment was typical of several carried out in different cases to confirm the methods adopted for calculating the composition of the fractions concerned. The type of difficulty which it was hoped to solve by these means occurred mainly in the smaller initial fractions from the distillation processes, and in most instances, therefore, possible minor errors in the assumptions made could not have materially affected the deduced final composition.

The presence of higher unsaturated acids. The results of the various analyses which are summarized in Table VI show that the carcass fats from each group contained 7–14% of higher unsaturated acids. To confirm the presence of such

Table VI. *The fat of the diet compared with that of the carcasses*

(*a*) = dietary fat. (*b*) = carcass fat.

	Beef dripping		Palm oil		Olive oil		Cod liver oil		Butter		Coconut oil	
Group	[1](*a*)	(*b*)	[2](*a*)	(*b*)	[3](*a*)	(*b*)	[4](*a*)	(*b*)	[5](*a*)	(*b*)	[6](*a*)	(*b*)
Up to C_8	—	—	—	—	—	—	—	—	5·8	—	8·4	—
Decanoic	—	—	—	—	—	—	—	—	2·2	—	7·2	0·9
Lauric	—	—	—	0·1	—	—	—	—	4·0	0·1	48·0	7·8
Myristic	4·5	1·1	4·1	2·1	1·1	0·1	4·8	1·6	10·4	3·5	17·5	13·4
Palmitic	30·6	22·7	40·1	20·8	9·7	17·1	6·4	15·6	26·1	25·9	9·0	18·1
Stearic	19·1	6·2	4·4	6·9	1·0	4·0	0·2	5·4	6·5	4·2	2·1	5·0
"Arachidic"	0·1	1·6	—	1·0	—	0·7	—	2·5	—	1·5	—	1·3
Behenic	—	—	—	0·7	—	0·5	—	—	—	—	—	—
Total saturated acids	54·3	31·6	48·6	31·6	11·8	22·4	11·4	25·1	55·0	35·2	92·2	46·5
Palmitoleic	—	2·5	—	4·7	—	4·2	16·2	9·5	—	5·0	—	3·1
Oleic	42·7	52·5	41·5	37·1	79·8	49·4	30·6	38·4	41·9	40·1	5·7	31·5
Linoleic	3·0	2·6	9·9	8·8	7·5	7·3		10·6	4·1	6·0	2·6	10·4
C_{20} unsaturated acid	—	10·5	—	14·0	—	13·8	30·2	11·8	—	10·3	—	6·9
C_{22} unsaturated acid	—	—	—	—	—	—	10·5	2·7	—	—	—	—
Total unsaturated acids	45·7	67·9	51·4	64·6	86·3	74·7	88·0	73·0	46·0	61·4	8·3	51·9

[1] Banks & Hilditch [1931]. [2] Hilditch & Jones [1931]. [3] Hilditch & Jones [1932].
[4] Guha *et al.* [1930]. [5] Hilditch & Sleightholme [1930]. [6] Collin & Hilditch [1928].

acids in the rat carcass, 21·81 g. of mixed fatty acids from the carcasses of an entirely separate group of animals chosen at random were brominated in ether at 0°. The ether-insoluble bromo-derivatives weighed 1·645 g. and contained 67·1% Br. This corresponds to the presence in the original mixture of 2·5% of higher acids having mean I.V. 324, and as the yield in such an experiment is always very much below theory, the amount actually present must have been considerably greater than the bromination experiment would suggest. The presence of these higher acids in appreciable amounts is therefore confirmed.

Since the methods used for detailed analyses of the fat from the beef dripping, cod liver oil, olive oil and butter fat groups were very similar to these recorded in Tables II–V, the final results only in these cases have been recorded. In Table VI therefore the composition of the carcass fat from each group is compared with that at present ascribed to the corresponding dietary fat.

The effect of coconut oil in the diet on the fatty acids of the liver. Channon & Wilkinson [1936] analysed the phosphatide and glyceride fatty acids of the livers from each group by separating them into solid and liquid fractions by the modified Twitchell process followed by the determination of mean I.V. and

mol. wt. As still more detailed information was required, it was decided to extend the investigation in the case of one of the six fats to include a complete analysis of the liver acids by fractional distillation so that the effect of diet on liver fatty acids might be studied more closely and might be compared in detail with the corresponding effect on the carcass fat. It was also hoped that the presence of any lower unsaturated acids might be revealed.

For this purpose 72 rats were given the same 40% fat diet for 21 days, the fat used being coconut oil. The animals were then guillotined, their livers dissected out and pooled, the liver fatty acids being obtained as usual in this laboratory. 378 g. of liver yielded 30·2 g. of fatty acids or 7·95% of the liver weight—a figure which is less than half that (18·6%) obtained previously [Channon & Wilkinson, 1936]. Although the livers were actually fatty, the reason for the much lower figure obtained in the present case is somewhat obscure.

28·96 g. of the acids were separated by the Twitchell process and the two fractions esterified and distilled. Owing to the very much smaller amount of the acids which was available in the present case for fractionation, it was somewhat more difficult to deduce the exact composition of some of the fractions than it was in the case of the carcass fats, but by methods resembling those already described, the approximate composition was arrived at. The carcass fatty acids were then also analysed, the results for both being compared below.

	Saturated						Unsaturated			
	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{16}	Oleic	Linoleic	"C_{20}"
Liver acids	—	7·2	8·7	23·5	10·0	0·3	3·5	16·9	3·1	25·2
Carcass acids	1·2	17·2	13·8	13·4	3·7	0·9	7·4	30·3	3·2	6·9

When the composition of the carcass fat in the previous coconut oil group (Table VI) is compared with that deduced in this latter experiment, it is seen that the influence of the diet has been even greater in the second case than it was before in that the percentage of lauric acid is more than doubled. This is probably due in part at any rate to the fact that the diet in the latter experiment was fed for 21 days instead of 14 days.

Discussion

Comparison of the total fatty acids present in the livers and carcasses. The percentage contents of fatty acids in both livers and carcasses are recorded in Table I. The lowest percentage of liver fat, 7·2%, occurred in the cod liver oil group in which the percentage of carcass fat was 11·4%. In contrast in the palm oil group, in which the carcass fat was 10·3%, the liver contained 26·3%—more than three times as much as that of the cod liver oil group. Similarly the highest liver fat, 30·7%, was found in the group receiving butter, in which the carcass fat was almost the lowest of the series (11·1%). These results show therefore that there was no apparent relationship between the total body fat of the animal and the percentage of fat in its liver under the conditions of these experiments in which pooled tissues were used, thus confirming the earlier work of Channon & Wilkinson [1934].

As already stated, Channon & Wilkinson [1936] subjected the phosphatide and glyceride fatty acids of the livers from each group to the Twitchell process and recorded the mol. wt. and I.V. for the fractions so obtained. Corresponding data have now been collected for the carcass fatty acids and so comparisons

Table VII. *Comparisons between the carcass fatty acids and those of the liver phosphatides and glycerides in the various groups*

				Beef dripping	Butter	Cod liver oil	Olive oil	Coconut oil	Palm oil
Carcass fatty acids	Saturated	*a*	% in original mixture	31·8	35·9	23·6	22·7	32·5	30·4
		b	Mol. wt.	269	260	265	266	252	261
	Unsaturated	*c*	Mol. wt.	289	283·5	302	291	277	292
		d	I.V.	90	100	106	98	89	100
Liver glyceride fatty acids	Saturated	*e*	% in original mixture	33·7	37·8	23·9	21·9	42·3	31·9
		f	Mol. wt.	267	—	310	267	257	269
	Unsaturated	*g*	Mol. wt.	300	296	313	289	266	298
		h	I.V.	106	116	176	109	101	121
Liver phosphatide fatty acids	Saturated	*i*	% in original mixture	39·5	41·7	42·5	42·2	42·7	40·4
		j	Mol. wt.	289	299	343	319	264	273
	Unsaturated	*k*	Mol. wt.	369	368	350	380	334	350
		l	I.V.	176	185	192	179	189	205
Comparisons made by observing certain differences		*e–a*		+1·9	+1·9	+0·3	−0·8	+9·8*	+1·5
		f–b		−2	—	+45	+1	+5	+8
		g–c		+11	+12·5	+11	−2	−11	+6
		h–d		16	16	70	11	12	21
		j–b		20	39	78	53	12	12
		k–c		80	84·5	48	89	57	58
		l–d		86	85	86	81	100	105

* The percentages of saturated acids recorded in Table VII are higher than those of Table VI. The latter are the more accurate, since they represent the results of the distillations; the former represent the results of the Twitchell separation only and are presented for comparison with the figures similarly obtained on the livers (see p. 49).

between them may be made. The carcass fatty acids must, of course, have contained phosphatide acids as well as those from the glycerides of the "depots", but the former would constitute only a small portion of the whole and their tendency would be to cause slightly higher mol. wt. and I.V. than the "depot" acids would have possessed by themselves. The various values obtained are compared in Table VII. Owing to the fact that the Twitchell separation in the case of the coconut oil group was exceedingly unreliable, owing to the relatively large amounts of lower acids present, it is impossible to include this group in the immediate discussion which follows. The values are, however, put on record with the others in Table VII and it should be noted that the unreliability of the Twitchell process in this particular case did not in any way detract from the accuracy of the final analysis as obtained by fractional distillation (Table VI).

Reference to Table VII (*e–a*) shows that very similar proportions of saturated acids occurred in the liver glycerides and in the carcass fat, and that the mean mol. wt. of these acids (*f–b*) were close, except in the case of the cod liver oil group. It therefore appears that the saturated acids occurring in the glycerides of the dietary fatty livers are in general similar both in proportion and in nature to those of the "depot" fat. At the same time it is fully realized by the authors how difficult it is to base deductions as to the relative nature of fatty acid mixtures on mean mol. wt. alone, but taking the results as a whole it is quite clear that this general tendency existed.

As far as the unsaturated glyceride acids are concerned a constant difference (*g–c*) was found in three cases, but in the others the variations were irregular. It therefore seems that the composition of the unsaturated acids of the liver glycerides differed markedly from that of the carcass unsaturated acids and to an extent which varied with the diet.

When the liver phosphatide fatty acids are compared with those of the carcass, no relationship is found to exist between either the percentages or the mol. wt. of the saturated fractions. Both varied irregularly. In the unsaturated acids on the other hand there were four cases where the I.V. were greater than those of the carcass acids (*l–d*) by an almost uniform amount and in three of these four cases moderately uniform differences also occurred in the mol. wt. (*k–c*). It is extremely difficult to decide the significance of these results.

In one case, that of coconut oil, the total liver fatty acids have been analysed and compared with those of the dietary fat (p. 47). As would be expected, there were still relatively large amounts (25%) of higher unsaturated acids in the livers, but there were also present 7% of lauric and 9% of myristic acid, due directly or indirectly to the diet. From the comparisons which have already been made above it is most probable that these two lower acids were in the glyceride fraction of the liver fat.

The proportion of saturated and unsaturated acids in the dietary and carcass fats. A comparison between the amounts of saturated acids of the body fats and published figures for the dietary fats can be made from Table VI and shows a definite parallelism. From this point of view the dietary fats fall into three groups: (i) coconut oil with saturated acids amounting to 92% of the whole, (ii) butter, beef fat and palm oil in which the saturated acids vary from 48 to 55% and (iii) olive oil and cod liver oil in which the corresponding figures are 12 and 11% respectively. The figures for the carcass fats for these three groups are (i) 46·5%, (ii) 35·2, 31·6, 31·6% and (iii) 22·4, 25·1%. It is interesting to note that when Gregory & Drummond [1932] fed rats for 10–13 weeks on diets containing no fat, 12% olein, 50% olive oil and also 12·5% stearin mixed with 12·5% laurin, the percentages of saturated acids in the body fats were 32, 22,

11 and 40·5%. Thus, although the times of feeding and the percentage of fat in the diets were very different in the present case, the general tendency is markedly the same, in that olive oil caused the lowest percentage and stearin mixed with laurin the highest.

The effect of diet on the individual fatty acids of the carcass fats. From Table VI two generalizations may be made. Firstly, the lowest value recorded for every acid, both saturated and unsaturated, in all the groups corresponds with the dietary fat also having the lowest content of each particular acid. Secondly, the extreme range of values for stearic (4·2–6·9%) and for "arachidic" acid (0·7–2·5%) are such that the variations are insufficiently great to be of significance in view of the limits of accuracy in the methods of analysis.

In the beef fat group the high palmitic acid content (22·7) reflects the fact that beef fat contains 30·6% of this acid, while the amount of oleic acid (52·5%) is high at the expense of both palmitoleic and linoleic acids, corresponding with the presence in beef fat of more than 40% oleic, less than 3% linoleic and the probable absence of palmitoleic acid.

In the butter group no acid lower than lauric has been stored, while myristic and palmitic acids were present to the extent of 3·5 and 26% respectively, the increase in these two members being mainly at the expense of oleic acid. These changes again are related to the composition of butter fat.

In the olive oil group all the saturated acids appear to have been decreased in amount, while the most marked change in the unsaturated acids is the high oleic content (49·4%), changes which correspond in general with the composition of olive oil. The figure of 49·4% for the oleic acid content in this case is certainly a little lower than that for the beef fat group, but the amount is scarcely significant, and if the total C_{18} unsaturated acids be considered in both cases rather than the oleic acid itself, more reliable figures are obtained for reasons already given (p. 44), and it is then seen that the resulting value of 56·7% for the olive oil group is slightly greater than that of 55·1% for the beef fat group.

Where cod liver oil was fed, the palmitic acid fell to 15·6%, while in the unsaturated acids there was an increase in palmitoleic acid from the average of about 4 to 9·5%. The C_{20} unsaturated acid is also slightly increased both in amount and in I.V., while only in this group is there any measurable quantity of C_{22} unsaturated acids (2·7%). These results show the marked influence of cod liver oil feeding.

In some ways the coconut oil group shows the effect of the dietary fat more strikingly than any other. Both decanoic (0·9%) and lauric acids (7·8%) were present, but acids lower than these did not appear to be stored. In addition the myristic acid is some 6–7 times greater than the average, while palmitic acid tends to be decreased. All these changes are to be related to the high proportion of decanoic, lauric and myristic acids with the relatively small amount of palmitic acid in coconut oil. Further, the low level of the two C_{18} unsaturated acids taken together is in accord with the composition of the dietary fat.

In the palm oil group, the figure for palmitic acid (20·8%), though higher than in most cases, is still less than that for the beef fat and butter groups, although palm oil contains more palmitic acid than either of the other two latter fats.

General conclusions

Three main conclusions may be drawn: (1) with regard to all the acids present, the composition of the body fat may be altered by appropriate feeding and certain acids not normally present may be introduced, (2) acids with less

than 10 carbon atoms are not stored and (3) the ease with which changes may be brought about differs amongst the various acids.

The first conclusion is in agreement with all previous workers in the field, though few have carried out detailed analyses. The second also confirms previous investigations. For example, Davis [1930], Cox [1933] and Eckstein [1929, 1, 2] found no storage of lower acids after feeding either the acids themselves or their derivatives.

The conclusion to which Barbour [1933] was led, that there is a level beyond which the saturated acids will not increase, whatever their proportion in the diet, is not confirmed in the present research. The level (25–27 %) found by Barbour has been exceeded in all but two of the cases published here, where the figures range from 22·4 to 46·5 %. At the same time it is clear that the different fatty acids present as the major components cannot be varied to an unlimited extent by the diet (e.g. the palmitic percentage is always between about three and four times that of stearic and between one- and two-thirds that of oleic).

It is of interest to compare the present findings with those of the only two groups of workers who have made similar studies of the fat of the rat. The true depot fats of rats receiving different diets have been analysed by Banks *et al.* [1933], while Klenk *et al.* [1935] have examined the fat of the skin, muscles and adipose tissue of rats fed on barley groats. Bearing the various experimental conditions in mind, these two sets of results correspond well with the present series with regard to the values for stearic, palmitic and total C_{18} unsaturated acids. They differ chiefly in the fact that in the present results the higher unsaturated acids in each case range between the relatively high values of 6·9 % in the coconut oil group and 14·5 % in the cod liver oil group and in doing so greatly exceed the corresponding amounts, if any, found by the other workers. In order that there should be no doubt as to the presence of these higher acids in the total carcass fat of normal rats, a sample of the acids from a separate group was brominated as described on p. 46, when it was proved that at least 2·5 % of the mixture consisted of acids of the arachidonic type. Since bromination is known always to indicate only a portion of the higher unsaturated acids actually present in such a mixture, there is no reason to doubt that these figures of 6·9–14·5 % are of the correct order. The difference between these particular values and the analyses of Banks *et al.* and Klenk *et al.* may be explained in part by the fact that the total carcass fat was used throughout in the present work and it would therefore contain a certain proportion of phosphatide, originally present as the "constant element" acids of Terroine [1936]. The same explanation will also apply to the fact that the other workers found no C_{20} saturated acid, while the average value found here was 1·7 %. It must also be pointed out, however, that in the work of Banks *et al.* the last fraction in the various fractional distillations of the solid acids constituted a higher percentage of the whole than in the present series, so that the tendency in their case would be for small amounts of higher acids, if present, to remain undetected. Similarly the figures quoted in Table VI for linoleic acid tend to be higher than those of Hilditch, Klenk and their colleagues, but oxidation experiments (p. 42) have confirmed the fact that the values recorded here for this acid, though maximum, are not far removed from the truth.

The results of Banks *et al.* in their group C, in which rats received 2–15 % cod liver oil in the diet for 10 weeks, may be compared with the cod liver oil group here which received 40 % for 2 weeks. The most noticeable points in the latter group are a pronounced fall in the palmitic and oleic acid contents. This change appears also to a lesser extent in their group C. There is also in both

cases little change in the proportion of linoleic acid, which might have been expected to increase in the depot fat when increased so much in the diet. One other point for mention is that in group C there appeared for the first time 8·5% of unsaturated C_{20} acids, while in the present work 40% of cod liver oil caused only a trifling increase in the amount of these compounds with the appearance of a small proportion of C_{22} unsaturated acids.

In their analysis of rat depot fats, Hilditch and his colleagues were unable to decide definitely whether palmitoleic acid was actually present or not, and therefore calculated their final figures for the composition of the mixed fatty acids in two ways, one in which palmitoleic acid was assumed to be present and the other in which it was not. Where they assume its presence, with one exception, they find higher values for palmitoleic acid than are quoted here. In the present work the existence of palmitoleic acid has been definitely confirmed and the calculated values for its content are believed to be accurate within the usual limits. One reason for making this claim is that a modification of the fractional distillation process previously applied by Irving & Smith [1935] in their analysis of pig liver fatty acids has also been used to great advantage in the present investigation. After the Twitchell process has been carried out on the total mixed fatty acids, there always remain in the liquid acids a few units % of saturated compounds due to the very slight solubility of lead stearate and rather greater solubilities of lead palmitate and lead myristate in alcohol. Consequently this small amount of saturated acid will ultimately interfere with the estimation of palmitoleic and oleic acids, unless the following modification is applied. When the methyl esters of the unsaturated acids have been distilled, the acids from the first few fractions should be separated by the Twitchell process into solid and liquid fractions of which the mol. wt. and I.V. are then determined. The process of arriving at the true constitution of these first few fractions is then greatly simplified and a very much more accurate estimation can be made of the palmitoleic acid content than was hitherto possible, although the increased accuracy is still only of the same order as that of the analysis as a whole. Reference to Table IV will illustrate this improvement in the method.

From the figures obtained in the liver experiment of Channon & Wilkinson [1936], it seemed possible that when coconut oil was fed to animals the livers might contain unsaturated acids of the C_{14} type. But when the fatty acids from the livers of animals receiving this diet were analysed, as recorded on p. 47, it was found that there could not have been present more than 2% of such acids and in fact it is very probable that none such existed. This does not exclude the possibility of desaturation either in the higher or lower acids, but it does show that the products of desaturation in the case of the latter are not stored in the liver in measurable amounts.

Summary

1. Six groups of rats were fed for 14 days on diets in which the common basal constituents were fat-free and of low choline content. Each diet contained in addition 40% of one of the following fats: beef dripping, butter, olive oil, cod liver oil, coconut oil and palm oil. The fatty acids of the pooled carcasses were subjected to a detailed analysis and the findings are compared with those of Channon & Wilkinson [1936] on the liver acids of the same animals. Further, a group of 72 animals was fed on the coconut oil diet for 21 days and the fatty acids of the livers compared with those of the carcasses.

2. Intense fatty livers resulted, varying from 30·7% in the case of the butter fat to 7·2% of the fresh liver weight for the cod liver oil, but no relationship was found to exist between the amount of fat in the livers and that in the carcasses.

3. The saturated acids of the liver glycerides were found to resemble closely those of the carcass both in the proportion of the total fatty acids and in their mean molecular weights, while the unsaturated acids were less closely related.

4. The liver phosphatide acids showed no relationship with the carcass fatty acids.

5. The effects of the various dietary fats on the amounts of the individual constituent acids of the carcass fats were very marked and are discussed in detail.

6. If desaturation of lower acids takes place, the products are not stored to any measurable extent either in the liver or in the carcass.

The expenses of this research were defrayed by a grant from the Medical Research Council, to whom we wish to express our thanks.

REFERENCES

Banks & Hilditch (1931). *Biochem. J.* **25**, 1168.
—— —— & Jones (1933). *Biochem. J.* **27**, 1375.
Barbour (1933). *J. biol. Chem.* **101**, 63.
Channon & Wilkinson (1934). *Biochem. J.* **28**, 2026.
—— —— (1936). *Biochem. J.* **30**, 1033.
Collin & Hilditch (1928). *J. Soc. chem. Ind., Lond.*, **47**, 261 T.
Cox (1933). *J. biol. Chem.* **103**, 777.
Davis (1930). *J. biol. Chem.* **88**, 67.
Eckstein (1929, 1). *J. biol. Chem.* **81**, 613.
—— (1929, 2). *J. biol. Chem.* **84**, 353.
Gregory & Drummond (1932). *Z. Vitaminforsch.* **1**, 257.
Guha, Hilditch & Lovern (1930). *Biochem. J.* **24**, 266.
Hilditch & Jones (1931). *J. Soc. chem. Ind., Lond.*, **50**, 171 T.
—— —— (1932). *J. Soc. chem. Ind., Lond.*, **51**, 805.
—— & Priestman (1931). *Analyst*, **56**, 354.
—— & Sleightholme (1930). *Biochem. J.* **24**, 1098.
Irving & Smith (1935). *Biochem. J.* **29**, 1358.
Klenk, Ditt & Diebold (1935). *Hoppe-Seyl. Z.* **232**, 54.
Lapworth & Mottram (1925). *J. chem. Soc.* **127**, 1628.
Terroine (1936). *Ann. Rev. Biochem.* **5**, 227.
Turner (1931). *Biochem. J.* **25**, 49.