CLXV. THE FATTY ACIDS OF PIG LIVER.
III. A GENERAL ANALYSIS.

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In two earlier communications, Channon *et al.* [1934, 1, 2] described their investigations on the nature of the octadecenoic acids of pig liver and discussed in detail the bearing of their results on the question of the desaturation of fatty acids in that organ. In continuation of these experiments the original fatty acids which had been prepared from 100 kg. of pig liver were subjected to further analyses by different methods. They were treated on a scale sufficiently large to give the results something of that completeness which they require, if ever they are to contribute materially to the elucidation of problems concerning fat metabolism in the animal body.

In view of the recognised importance of the liver in fat metabolism, it is surprising to find how little serious work has been carried out so far to establish the precise nature of the fatty acids contained in the mammalian liver. The chief contributors to this subject have been Hartley [1909], who analysed the fatty acids from pig liver, discovering in them the highly unsaturated arachidonic acid, and Klenk and Schoenebeck [1932] who made a thorough examination of those present as glycerides and phosphatides in ox liver. Work in similar fields published from various laboratories in recent years, such as the discovery of a liquid saturated acid in cat's kidney by Turner [1931] and of other liquid saturated acids in tubercle bacilli by Anderson [1929] and Spielman [1934], as well as the evidence put forward by Bosworth and Brown [1933] and by Bosworth and Sisson [1934] for the presence in butter fat of arachidonic and lower unsaturated acids, indicates that a thorough investigation of the lipins from different parts of the animal body has already been too long delayed. There can be little doubt that results accumulating in the course of time from researches of this type will ultimately bring to light the essential details of certain biological processes of which for the present the explanation remains obscure. As a contribution to this end the present work was carried out.

Experimental.

The preparation of the fatty acids and their separation into saturated and unsaturated fractions by the Twitchell process, as modified by Hilditch and Priestman [1931] has already been described by Channon *et al.* [1934, 1]. The main points to be noted for the present communication are these. The original acids had an iodine value of 110 and a mean molecular weight of 299. By the lead soap-alcohol procedure the mixture was shown to contain 34 % of saturated acids (I.V. 2; mol. wt. 277), and 66 % of unsaturated acids (I.V. 153; mol. wt. 321).

Estimation of the saturated acids by the Bertram method.

This consisted of oxidising samples of both the original mixture and the unsaturated fraction with permanganate and precipitating the petroleum-soluble products twice as magnesium soaps [Bertram, 1927].

(*a*) *The original acids.* 5·035 g. gave 1·750 g. saturated acids, or 34·8 %.

(*b*) *The unsaturated acids.* 5·226 g. gave 0·197 g. saturated acids. This is equivalent to 3·8 % of the unsaturated fraction, or to 2·5 % of the original acids.

In summarising these results it can be said that the content of the saturated acids was found to be 34·8 % by the Bertram process alone, and 36·5 % (*i.e.* 34·0+2·5) by the two methods combined. That this higher figure of 36·5 % approximated more nearly to the truth was proved later by distillation methods.

Analysis of the saturated acids.

210 g. of methyl esters were prepared from the saturated acids and fractionally distilled at 2 mm. The fractions were saponified, traces of unsaponifiable matter removed and the melting-points and molecular weights of the acids determined. Assuming that no fraction ever contained more than two adjacent homologues, the amounts of the various fatty acids present were calculated from the results arranged in Table I.

Table I.

Fraction	B.P. at 2 mm. (°) up to	Weight of acids g.	M.P. (°)	Mol. weight	Weight of each fatty acid present: Myristic g.	Palmitic g.	Stearic g.	"Arachidic" g.
1	136	1·8	53·5–55·5	249·5	0·4	1·4	—	—
2	141	5·6	57·0–59·0	251	1·0	4·6	—	—
3	141	20·6	58·5–59·5	255·5	0·4	20·2	—	—
4	145	18·4	60·0–61·0	258	—	17·1	1·3	—
5	153	27·7	55·0–56·0	263	—	20·8	6·9	—
6	160	18·6	56·0–57·0	269·5	—	9·6	9·0	—
7	165	75·8	68	285	—	—	73·1	2·7
8	167	13·6	68·5–69·0	286	—	—	12·6	1·0
9	Residue	13·5	62	297	—	—	7·2	6·3
	Total	195·6*	—	—	1·8	73·7	110·1	10·0

* This figure indicates a loss of less than 3 % of the total saturated fraction, or 0·9 % of the original mixed acids, and was most probably distributed very evenly over the various fractions.

Although reference is made in the discussion to this method of calculating the composition, two points should be explained at this stage. The acids lower in the series than palmitic have been estimated as myristic because, owing to the solubility of the lead soaps of lower members in alcohol, it is extremely improbable that any acid with less than 14 carbon atoms could ever be found in the saturated fraction from the Twitchell separation. Again, for calculation purposes the acids higher than stearic have been grouped together as "arachidic", although it is fully realised that behenic and lignoceric acids were probably also present in this fraction.

	% of saturated acids	% of original mixed acids
Myristic	0·9	0·3
Palmitic	37·7	12·5
Stearic	56·3	18·6
"Arachidic"	5·1	1·7
Loss	—	0·9
Total	100·0	34·0

Analysis of the unsaturated acids.

Various methods have been applied to the problem of determining the constitution of the unsaturated fraction. These are now described in proper sequence below, the first consisting of fractional distillation, followed by hydrogenation of samples from each fraction as used by Klenk and Schoenebeck [1932].

Distillation followed by hydrogenation. 80·5 g. methyl esters prepared from 77·5 g. unsaturated acids were distilled as usual. After the weights and iodine values of each fraction had been recorded, fractions 1–4 were saponified, traces of unsaponifiable matter removed and the fatty acids isolated. Fraction 1 was not studied further, but the acids from 2, 3 and 4, being semi-solid at room temperature, were subjected to the Twitchell separation and the resulting liquid acids were hydrogenated. 1·0–1·5 g. of the esters from each of the remaining fractions were hydrogenated with palladium black and hydrogen at room temperature and the saturated fatty acids isolated, their molecular weights and melting-points being determined. By assuming once again that only two adjacent homologues were present in any one fraction, the amounts of the different acids could be calculated. The results are summarised in Table II.

Table II.

	Methyl esters				Twitchell separation				Hydrogenated acids						
Fraction	B.P. at 1 mm. (°) up to	Wt. g.	% of total unsaturated acids	I.V.	Description	% of total unsaturated acids	I.V.	Mol. wt.	Mol. wt.	M.P. (°)	% C_{14}	% C_{16}	% C_{18}	% C_{20}	% C_{22}
1	100	0·63	0·8	65·4	Only 0·2g. acids obtained		61	287	—	—	—	—	—	—	—
2 *a*	140	4·42	5·5	80·0	Solid	1·2	4	233	—	—	1·0	0·2	—	—	—
b					Liquid	4·3	104	275	273	55–57	—	1·7	2·6	—	—
3 *a*	152	7·78	9·7	97·3	Solid	1·6	8	257	—	—	—	1·5	0·1	—	—
b					Liquid	8·1	119	281	281	60–62	—	0·9	7·2	—	—
4 *a*	155	9·16	11·4	117·7	Solid	1·0	18	266	—	—	—	0·6	0·4	—	—
b					Liquid	10·4	122	284	288	62–64	—	—	8·9	1·5	—
5	156	10·02	12·5	129·9	,,	—	—	—	291·5	63–65	—	—	9·1	3·4	—
6	160	8·94	11·2	143·6	,,	—	—	—	290	63–65	—	—	8·8	2·4	—
7	161	11·27	14·1	155·2	,,	—	—	—	291	62–63	—	—	10·6	3·5	—
8	165	6·46	8·1	179·4	,,	—	—	—	300	62–63	—	—	3·5	4·6	—
9	170	5·89	7·4	222·0	,,	—	—	—	312	64–66	—	—	—	7·4	—
10	175	2·67	3·3	234·2	,,	—	—	—	315	65–67	—	—	—	2·9	0·4
11	Residue	12·80	16·0	183·8	,,	—	—	—	332	64–66	—	—	—	4·6	11·4
	Total	80·04	100·0	—	—	—	—	Saturated acids (total)			1·0	2·3	0·5	—	—
								Unsaturated acids (total)			—	2·6	50·7	30·3	11·8

For comparison with these results the following theoretical figures are quoted. For the methyl esters of palmitoleic, oleic and linoleic acids the iodine values are 95, 86 and 173; for methyl esters of C_{20} acids, having 1, 2, 3, 4 and 5 double bonds, they are 78, 158, 238, 319 and 402; for methyl esters of C_{22} acids with 2, 3, 4 and 5 double bonds the corresponding figures are 145, 219, 294 and 369. The molecular weights of the fully hydrogenated C_{16}, C_{18}, C_{20} and C_{22} acids are 256, 284, 312 and 340 respectively.

The conclusions which may be drawn from the results set out in Table II are as follows:

1. The result of the Bertram analysis already mentioned has been very well confirmed in that the unsaturated fraction from the Twitchell separation yielded

on fractional distillation 3·8 % of saturated acids (fractions 2*a*, 3*a* and 4*a*). These obviously consisted of palmitic acid, associated with smaller amounts of stearic acid and lower homologues.

2. From a consideration of the melting-points of the hydrogenated fractions, it is clear that no liquid saturated acid could have been present in the original mixture, a fact which cannot be definitely proved at present, except by hydrogenation experiments.

3. The unsaturated acids contained a small amount of lower homologues, which, estimated as palmitoleic acid, amounted to 2·6 % of the total liquid fraction (fraction 2*b* and 3*b*). This finding was confirmed later.

4. Although the assumption that the hydrogenated fractions each contained only two adjacent homologues has yet to be discussed, the principle as used in the present instance indicated that the mixed unsaturated acids were made up of C_{18} compounds to the extent of approximately 50 %, the major part of the remainder consisting of C_{20} and C_{22} acids; while from the iodine values of the higher fractions and the molecular weights of their hydrogenated derivatives, it seemed most probable that the C_{20} and C_{22} acids contained 2, 3 and 4 double bonds.

This last conclusion is of particular interest, for in their work on octadecenoic acids of pig liver, Channon *et al.* [1934, 1] oxidised portions of these liquid acids by methods which were known to give extremely good yields of hydroxy-derivatives, if applied to pure samples of oleic and linoleic acids. Yet their results suggested that the C_{18} acids did not amount to more than about 28 % of the unsaturated fraction, whereas the present experiment suggests the presence of 50 % of such acids. It has already been stated by Lapworth and Mottram [1925] that their method of oxidation gives considerably reduced yields of hydroxy-derivatives if more highly unsaturated acids are present. It was accordingly decided to distil a large amount of methyl esters prepared from the liquid acids and to oxidise samples of the various fractions obtained. By this means it was hoped to segregate the highly unsaturated acids into the higher fractions and so obtain more representative yields of the hydroxy-derivatives from the C_{18} acids in the lower-boiling fractions.

At the same time the opportunity was taken of investigating further the small amounts of saturated and palmitoleic acids already mentioned as being present in the liquid mixture from the Twitchell separation.

Distillation and subsequent oxidation. 208 g. methyl esters were distilled *in vacuo* into eight fractions. The acids from the first four of these were treated by the modified Twitchell process, and then samples of the liquid acids from all the fractions were oxidised by Lapworth and Mottram's method [1925], a second oxidation being carried out in each case on the petroleum-soluble products to ensure that reaction was complete. From each fraction, in addition to the usual di- and tetra-hydroxy-derivatives, there was obtained a certain amount of fatty acid which was soluble in light petroleum, and which, although virtually saturated, was yet only semi-solid at room temperature. Although this substance has not so far been investigated, the yield obtained from each fraction has been recorded, together with its molecular weight, in order to allow calculations to be made should future work explain its occurrence and origin. It is worthy of note that its molecular weight increased with the molecular weight of the acids from which it was derived. The possibility of this substance being unoxidised material is excluded by the fact that it no longer possessed any appreciable iodine value, while the results both of the Bertram oxidation and of the distillation-hydrogenation experiments definitely exclude the possibility that this peculiar product pre-

Table III.

		Distillation					Oxidation										
							Dihydroxystearic acid					Tetrahydroxystearic acid			Semi-solid petroleum-soluble acids		
Fraction	Description	B.P. at 2 mm. (°) up to	Wt. of acids g.	Mol. wt.	I.V.	% of total unsaturated acids	Wt. oxidised g.	Wt. g.	Mol. wt.	M.P. (°)	% of total unsaturated acids	Wt. g.	M.P. (°)	% of total unsaturated acids	Wt. g.	Mol. wt.	% of total unsaturated acids
1	Solid	140	0·8	213·5	4·9	0·5*	—	—	—	—	—	—	—	—	—	—	—
	Liquid		1·7	248·5	69·4	1·0*	1·508	0·31	300	118–122	0·2	None	—	—	0·39	246	0·3
2	Solid	150	1·6	237	4·2	0·9*	—	—	—	—	—	—	—	—	—	—	—
	Liquid		2·7	266	87·2	1·5*	1·134	0·60	303	118–122	0·8	None	—	—	0·33	267	0·4
3	Solid	160	1·3	252	4·5	0·7*	—	—	—	—	—	—	—	—	—	—	—
	Liquid		3·4	277	87·3	2·0*	2·014	0·76	310	120–123	0·8	0·07	165	0·1	0·60	289	0·6
4	Solid	170	3·7	254	13·7	1·9*	—	—	—	—	—	—	—	—	—	—	—
	Liquid		38·0	280	103·3	19·2*	2·064	0·97	315	127	9·0	0·14	165	1·3	0·35	290	3·3
5	Liquid	175	16·2	280	110·1	8·1	2·489	1·13	319	126–128	3·6	0·22	165	0·7	0·35	289	1·1
6	Liquid	195	40·0	290	132·2	20·0	2·370	0·72	319	127–128	6·0	0·25	160	2·1	0·31	310	2·6
7	Liquid	195	22·5	304	183·3	11·2	2·139	0·30	317	122–123	1·6	0·025	Lost	0·1	0·32	333	1·7
8	Liquid	Residue	32·0	344	200·0	16·0	11·97	None	—	—	—	None	—	—	2·74	336	3·7
Total	—	—	163·9	—	—	83·0	—	—	—	—	22·0	—	—	4·3	—	—	13·7

* These figures have been corrected for small amounts removed after distillation and before Twitchell treatment for determination of iodine values and molecular weights.

existed as such in the liquid acids. That strange by-products are formed by this mild oxidation of these complex mixtures was indicated in every fraction by the fact that a certain amount of unsaponifiable matter was also isolated from the oxidation products, whereas all such matter had been removed from the acids as a whole before their analysis was begun. The results of these distillation-oxidation experiments are set out in Table III.

Several interesting facts should be observed. In the first place only 83 % of the original acids was recovered. The loss of 17 % of the material was due to much decomposition which took place in the undistilled residue, yielding by-products insoluble in light petroleum and not recorded in the table. This fractionation of 208 g. methyl esters was a much longer process than that previously carried out on 80·5 g. (Table II), and this fact, combined with the slightly higher pressure and correspondingly higher boiling-points, accounted for the bulk of the loss. Nevertheless, it may be assumed that the decomposition and consequent loss in iodine value which it necessarily entailed took place entirely in the undistilled residue, in which fraction the most highly unsaturated and therefore the most unstable acids would occur. It may therefore be surmised that as far as oleic, linoleic and palmitoleic acids are concerned, the quantitative results remain unaffected.

With regard to the hydroxy-derivatives, their melting-points are recorded in Table III and are typical for the usual products obtained by this means before purification by fractional crystallisation, while the molecular weights quoted for the dihydroxystearic acid from fractions 5, 6 and 7 approximate closely to the theoretical value of 316. It is of importance to observe that oleic acid was shown by these results to be present in all the fractions except the last, and that linoleic acid was detected in all but numbers 1, 2 and 8. The bearing of this finding on the distillation method of analysis as applied to unsaturated fatty acid mixtures is discussed later.

Palmitoleic acid. The presence of palmitoleic acid in the unsaturated fraction, which was already suggested by the results in Table II, was confirmed in the present instance by the molecular weights and melting-points of the dihydroxy-derivatives from fractions 1, 2 and 3. When the first of these was analysed, the values obtained were C, 67·3; H, 11·0 %; mol. wt., 300. For an equi-molecular mixture of dihydroxypalmitic and dihydroxystearic acids, the corresponding figures should be 67·5, 11·35 % and 302. It is therefore probable from both experiments that the content of palmitoleic in the unsaturated acids was 2–3 %.

The saturated acids from the unsaturated fraction. The Bertram oxidation and the results of Tables II and III all suggest that the saturated acids still remaining in the liquid fraction amounted to approximately 3·8 %. In order to analyse these, each of the solid portions obtained in the last experiment was fractionally recrystallised from acetone and its nature investigated.

From fractions 3 and 4 the solid acids (mol. wt. 252 and 254), consisted mainly of palmitic acid. They both gave crops of crystals from acetone, melting between 57·5 and 61·5° and typical C and H figures of C, 74·5; H, 12·5 %. ($C_{16}H_{32}O_2$ requires C, 74·9; H, 12·6 %; mol. wt., 256.)

After recrystallisation, fraction 2, which had mol. wt. 237, melted at 54–56° and therefore most certainly contained palmitic and myristic acids. Fraction 1 (mol. wt. 213·5) gave three crops and a mother-liquor, the details of which are shown in Table IV.

(Required for $C_{10}H_{20}O_2$: C, 69·8; H, 11·6 %; for $C_{12}H_{24}O_2$: C, 72·0; H, 12·0 %; and for $C_{14}H_{28}O_2$: C, 73·7; H, 12·3 %.) It must be concluded from these experi-

ments that this small amount of solid acid, which is not separated in the saturated fraction in the Twitchell method, but is estimated by a Bertram oxidation on the unsaturated fraction, consisted mainly of palmitic with small quantities of myristic, lauric and possibly *n*-decanoic acids. Table II also suggested the presence of stearic acid in small amounts.

Table IV.

Crop	Weight (g.)	M.P. (°)	C %	H %	Probable constitution
1	0·11	55·5–57·5	74·2	12·65	Mainly palmitic acid
2	0·08	45 –46	—	—	Lauric mixed with myristic acid
3	0·18	46	72·4	12·1	
Mother-liquor	0·40	—	69·7	11·5	Possibly *n*-decanoic acid present

The detection of highly unsaturated acids by oxidation. As shown in Table III, 11·97 g. of the undistilled residue were oxidised by the usual method. After extraction with ether, the resulting aqueous solution (12 l.) was evaporated to 700 ml. and the residue allowed to cool. Much inorganic material crystallised out along with small amounts of organic acids and some tar. These were filtered off and the residue extracted three times with boiling absolute alcohol. 0·3 g. of a crude mixture was obtained and was fractionally crystallised from aqueous alcohol and from water after treatment with charcoal. The first crop (0·04 g.) had M.P. 200°, but the major part, even after exhaustive attempts at purification, melted constantly at 186° and gave on analysis C, 55·2; H, 9·35 %. The mineral salts were then extracted three times with boiling 70 % alcohol, and 0·6 g. of a very impure organic acid was isolated. By repeated recrystallisations from both water and aqueous alcohol after charcoal treatment, 0·2 g. of a pure product was prepared, M.P. 195°. (Found: C, 54·5; H, 9·1 %. Required for $C_{20}H_{40}O_{10}$: C, 54·5; H, 9·1 %; and for $C_{22}H_{44}O_{10}$: C, 56·4; H, 9·5 %.) It therefore seems most probable from these figures that the fraction extracted by absolute alcohol was a mixture of octahydroxyarachidic and octahydroxybehenic acids, while that isolated by 70 % alcohol was the former of these alone. Owing to the large amounts of mineral salts and the solubility of these hydroxy-compounds, the extraction cannot be regarded as in any way complete. The experiment does show however that, in addition to the usual arachidonic acid first discovered by Hartley [1909], there is probably also present in liver fat the corresponding C_{22} acid with four double bonds.

Bromination experiments. All the known unsaturated acids form soluble liquid bromo-derivatives, in addition to solid isomerides, and usually the former can neither be identified nor estimated. This explains why bromination has never been regarded as a satisfactory method of analysing fatty acid mixtures. New interest in such a process has recently been stimulated however by the work of Ault and Brown [1934] who have suggested a method, to which reference is made later, for estimating arachidonic acid by this means.

50·3 g. of the unsaturated acids were therefore brominated in ether at 0° in the usual way. The insoluble product was filtered off and washed with ether and then excess bromine removed from the filtrate and washings by sodium thiosulphate solution. On removing the ether and taking up the residue in light petroleum (B.P. 80–100°) and leaving at 0° for one night, a further small amount of insoluble bromo-derivative crystallised out. This was filtered off, the filtrate taken to dryness, dissolved in light petroleum (B.P. 40–60°) and kept at 0° for some days, when crystalline tetrabromostearic acid was deposited. The results are summarised in Table V, where it should be noted that the saturated acids

in the unsaturated fraction are taken as being 4 % (the nearest unit) and the true data for the petroleum-soluble bromides appropriately corrected for that amount.

Table V. *Bromination of 50·3 g. unsaturated acids.*

No.	Description	Wt. g.	M.P. (°)	Bromine %	Corresponding wt. of unsaturated acids g.	Original unsaturated acids %	Calculated I.V. of these acids
1	Insoluble in ether	13·2	240 indef.	68·3	4·18	8·3	342
2	Insoluble in high-boiling petroleum	1·75	„	68·0	0·56	1·1	337
3	Tetrabromostearic acid	2·0	112–113	53·3	0·93	1·8	181
4	Soluble in cold petroleum	77·0	—	45·0	42·4	84·3	130
5	Saturated acids	2·0	—	—	2·0 (sat.)	4·0	—
	Total accounted for				50·0	99·5	

The absence of linolenic acid. Normally when linolenic acid is present in a mixture, it yields a hexabromostearic acid soluble in benzene, insoluble in ether and melting at 180°. To search for this substance, 2·9 g. of the ether-insoluble products of bromination (Table V), were thoroughly extracted with benzene, but the two fractions so obtained melted at the same temperature (240° with decomposition). The absence of linolenic acid was further confirmed by the oxidation experiment already described in which octahydroxyarachidic acid was isolated (p. 1364), for in that case no trace of hexahydroxystearic acid could be found. It is therefore safe to conclude that, if this unsaturated acid were actually present, the amount must have been extremely small.

Apart from this result, the bromination experiment added little to the information already gained. It confirmed the presence of ordinary linoleic acid and of higher unsaturated acids of the C_{20} or C_{22} type, but unfortunately with such a complex mixture nothing of any value could be concluded from the soluble bromide fraction (Table V, No. 4), which constituted such a large proportion of the whole. The minimum amount of highly unsaturated C_{20} and C_{22} acids present can however be stated from fractions 1 and 2 to have been 9·4 % of the total unsaturated fraction.

Discussion.

The general analysis just described has confirmed and extended the pre-existing knowledge of the fatty acids contained in mammalian livers and has in several aspects tested the methods by which they may be investigated.

The relative values of the Twitchell and Bertram processes were well illustrated. There is no doubt that the lead soap separation as modified by Hilditch and Priestman [1931] gave an exceedingly good separation of the saturated and unsaturated acids, whilst the small amount of saturated compounds still remaining in the liquid portion was readily estimated by a Bertram oxidation, carried out on the liquid acids rather than on the original mixture. The value so found for these residual acids was confirmed twice by distillation experiments.

The method of calculating the amounts of different acids present by distillation of the esters, followed by estimations made on the assumption that each fraction never contains more than two homologues, has been much used by

Hilditch and his co-workers and has been recently discussed in detail by Hilditch [1934]. Provided that a sufficiently large number of fractions be taken, it is most improbable in the case of the saturated acids alone that any one of the fractions will contain more than relatively small traces of a third homologue. Even in a case, such as the one described in the present communication, where only nine fractions were taken, the final results calculated on this basis probably approximated to within a few units of the true value in the large figures recorded. At the same time it is difficult to assume a greater degree of accuracy than this, for even if it be allowed that the presence of a third homologue be negligible, other small but appreciable errors may arise, as for example in determining the mean molecular weight of each fraction, a figure on which the whole process of deducing the composition depends. Provided these and other small errors at this stage neutralise each other, as they may often do, the conclusions will approximate closely to the true values; but if on the other hand they happen to accumulate, the figures finally arrived at for the main constituents will only approach within a few units of the actual percentages which a more accurate method would yield. Unfortunately a more accurate method for this purpose has not yet been devised.

The liquid acids present a still more difficult problem. In the present case the initial mixture of unsaturated acids was too complex for proper analysis to be made by fractional distillation. The failure of the process for this purpose was shown by the fact that oleic and linoleic acids were proved by oxidation methods to be present in the majority of the fractions, even though the boiling points of these differed in the extreme by as much as 35°. Again distillation followed by hydrogenation made it certain that C_{20} and C_{22} acids, having two, three and four double bonds also existed in the same fractions. Consequently it was impossible to elucidate satisfactorily the composition of the unsaturated liver acids by simple calculation from such fractions, although such a procedure may well be applicable to the less complex fatty acids of the glycerides and phosphatides from plant sources.

Hydrogenation, therefore, as applied by Klenk and Schoenebeck [1932] appears to be the most satisfactory approach so far devised for the analysis of such mixtures, the actual hydrogenation either preceding or following the fractional distillation. Such a method eliminates the danger of overlooking the presence of liquid saturated acids and, taken in conjunction with the other data acquired, it goes far towards yielding a true estimate of the various constituents. For the acids under discussion it has shown better than any other method the relative amounts of C_{16}, C_{18}, C_{20} and C_{22} compounds and has suggested their relative degrees of unsaturation. The figures so obtained for the C_{20} and C_{22} acids, which must have had two or three double bonds, was surprisingly large, but they have confirmed very closely the corresponding values found by Klenk and Schoenebeck in their work on the acids from ox liver. Unfortunately it has been impossible to prepare any derivatives by which these compounds might be characterised. The same method gave the content of C_{18} acids as approximately 50 % of the liquid portion and, from the yields of di- and tetra-hydroxy-derivatives, whether obtained by oxidising the total acids as in Part I of this series [1934, 1] or each of the various fractions resulting on distillation, it may be assumed that the quantity of oleic acid was about five times greater than that of the linoleic acid. The amounts of these acids in the unsaturated fraction should therefore have been of the order of 42 and 8 % respectively.

In at least two ways the presence of a lower unsaturated acid, which was most probably palmitoleic, was proved.

On investigating the saturated acids still remaining in the liquid fraction and obtained from it on distillation, the presence of lauric, myristic and possibly *n*-decanoic acids was shown, in addition to palmitic and traces of stearic acids. According to the theory of β-oxidation of fatty acids, these lower even-numbered members might well be expected to appear in small amounts in the liquid fraction of the liver acids. Any acids having less than 10 carbon atoms would have been previously removed in the preparation of the total acids from the liver, owing to their solubility in water or their capacity to distil readily in steam.

With regard to the true amount of arachidonic acid present, Ault and Brown [1934] have prepared pure arachidonic acid in different ways and brominated the pure product. They found that no matter how the acid was made, 1 g. on the average yielded approximately 0·842 g. of polybromide, instead of the theoretical value of 3·1 g. They then assumed that on brominating a mixture of acids this proportion of ether-insoluble polybromide would remain the same and so they suggested the following formula:

$$\% \text{ of arachidonic acid} = \frac{\text{Weight of ether-insoluble bromide from 100 g. of mixed acids} \times 100}{84{\cdot}2}.$$

When this was applied in the present instance, the content of arachidonic acid became:

$$\frac{13{\cdot}2 \times 100 \times 100}{50{\cdot}3 \times 84{\cdot}2}\% = 31{\cdot}2\ \%.$$

Now if it may be assumed for the moment that fraction 4 in Table V consisted entirely of the bromo-derivatives of a C_{20} acid with one double bond, together with the corresponding derivatives of arachidonic acid, the maximum amount of the latter acid which could possibly have been present may be calculated to be 26·5 %. That it must actually have been considerably less than this figure is obvious, for from the distillation-hydrogenation experiment it is certain that the petroleum-soluble fraction (No. 4, Table V) must have contained derivatives of acids all with higher iodine values than that of a C_{20} acid with one double bond, and these would all considerably decrease this estimated maximum for the content of arachidonic acid.

The method of Ault and Brown therefore did not apply in the present case. This might have been due to either of two reasons. In the first place, it is most probable that some highly unsaturated C_{22} acids were present in small amounts in the original mixture, as well as C_{20} compounds other than the ordinary arachidonic acid. These would naturally make it unfair to test the validity of the procedure on such a sample of acids.

In the second place the high value obtained in the present instance by the application of this method might have been due to the fact that in the presence of other unsaturated acids the proportion of arachidonic acid yielding a solid ether-insoluble bromide is greatly enhanced. Such an explanation is possible in the light of the work of Rudy [1932], who found that on brominating acids of this type the relative proportions of the different forms produced varied considerably with such factors as the type of solvent and the speed of bromination. That they may also vary according to the type and proportions of the other acids present in the mixture seems very possible.

The following table shows a summary of the approximate composition of the pig liver fatty acids as far as it may be deduced from these experiments. From the discussion as a whole it is clear that the figures cannot be other than approximate.

	Saturated acids %	Original mixture %
n-Decanoic and lauric acids	1	0·4
Myristic acid	2	0·7
Palmitic acid	39	14·0
Stearic acid	53	18·8
"Arachidic" acid	5	1·7
Total	100	35·6

	Unsaturated acids %	Original mixture %
Palmitoleic acid	2·5	1·5
Oleic acid	44	28
Linoleic acid	8	5
Linolenic acid	Absent	—
C_{20} acid	31	20
C_{22} acid	12	7·5
Total	97·5	62·0

Total loss = 2·4 %.

The C_{20} and C_{22} acids probably existed in the mixture as compounds having from two to four double bonds.

SUMMARY.

The pig liver fatty acids have been analysed by bromination and distillation experiments, the latter being followed both by oxidation and by hydrogenation processes. The results are discussed in full and summarised immediately above.

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