CXX. THE FATTY ACIDS OF PIG LIVER.

I. THE OCTADECENOIC ACIDS AND THE DESATURATION THEORY.

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THE accumulation of fat which occurs in the liver under the action of many substances, which is seen in numerous clinical conditions and which may be produced also by dietary methods, is the outstanding proof of the fact that the liver plays a fundamental part in fat metabolism. The desaturation theory, associated with the names of Leathes and Hartley, according to which fatty acids of the depot fat are transported to the liver, where they are desaturated and passed on to the tissues in this more readily oxidisable form, was put forward in an attempt to interpret this phenomenon. This theory is based on two lines of evidence: the effect of the food fat on the nature of the liver fatty acids [Leathes and Meyer-Wedell, 1909] and the investigations of Hartley [1909] on the unsaturated acids of the liver and depot fat of the pig. Considerable evidence has been put forward recently which suggests that the basis on which the desaturation theory rests is far less secure than had appeared. The demonstration by Leathes and Meyer-Wedell [1909] that the iodine value of the liver acids of cats and rats, which had been fed on highly unsaturated fish oils, might exceed that of the acids of the oil administered, was interpreted as evidence for desaturation. The extensive work of Sinclair [1932, 1, 2] seems to lend considerable support to the suggestion made by Joannovics and Pick [1910] that the increase in iodine value of the liver fatty acids caused by feeding oils of high iodine value observed by Leathes and Meyer-Wedell was due not to desaturation but to preferential absorption of the more unsaturated acids from the portal circulation. Sinclair has shown that the iodine value of the fatty acids of the liver phosphatides is rapidly raised by the administration of very small amounts of a highly unsaturated fat such as cod-liver oil, whilst the lowering of this high value by substitution of the cod-liver oil by a relatively saturated fat takes a much longer time. These and many other results of Sinclair imply an ability on the part of the tissues and particularly of the liver preferentially to take up the more unsaturated acids from the fat present in the food and tenaciously to retain them. At the present time therefore the selective retention by the liver cells of the more unsaturated acids seems probable and, if this can be established, one of the lines of evidence on which the desaturation theory rests will be invalidated. The second and perhaps more conclusive work which points to liver desaturation of fatty acids was provided by Hartley [1909] who isolated from the liver acids of the pig a $\Delta^{12,13}$ oleic acid and a $\Delta^{9,10;12,13}$ linoleic acid, while the ordinary $\Delta^{9,10}$ oleic acid was absent. Neither of these two former acids was present in lard which, however, was found to contain

$\Delta^{9, 10}$ oleic acid and stearic acid. Hartley concluded that the liver cells possessed the power of introducing a double bond into the 12 : 13 position, so that on mobilisation to the liver stearic acid was converted into $\Delta^{12, 13}$ oleic acid and similarly $\Delta^{9, 10}$ oleic acid was converted into $\Delta^{9, 10; 12, 13}$ linoleic acid.

This attractive evidence seems strong support for the liver desaturation theory, especially as the $\Delta^{12, 13}$ oleic acid was unlikely to have arisen from the food fat, for such an acid has not been found in natural products. It was perhaps surprising, therefore, when Turner [1930] failed to obtain any evidence for the presence of this particular oleic acid in sheep liver, in which only $\Delta^{9, 10}$ oleic acid was found to be present. In spite of the conflicting evidence which the work of Sinclair seems to provide and the doubt suggested by the results of Burr and Burr [1929; 1930; Burr et al., 1932], which will be mentioned later, confirmation of the proof of the presence of this $\Delta^{12, 13}$ oleic acid in liver would yet provide strong evidence for the desaturation hypothesis. We have undertaken an investigation of the fatty acids of pig liver with this object in view.

<div align="center">EXPERIMENTAL.</div>

<div align="center">*Preparation of the liver fatty acids.*</div>

The preparation of the crude fatty acids was carried out industrially in the following manner. 100 kg. of pig liver obtained direct from the slaughter-house were minced and mixed with a solution of potassium hydroxide. The mixture was allowed to stand until dissolution had occurred, when it was extracted six times with a non-chlorinated industrial solvent mixture. The material left on evaporation of the solvent (unsaponifiable matter and unhydrolysed lipoid substances) was saponified with alcoholic potash and the unsaponifiable fraction removed. The alkaline liver liquors (320 l.) were acidified with hydrochloric acid and the fatty acids removed by solvent extraction. The soap solutions resulting from the saponification and extraction of the unsaponifiable fraction were acidified and treated similarly. The crude fatty acids as received by us weighed 4·37 kg. and contained a considerable amount of the higher-boiling fractions of the solvent used for extraction. Contaminating impurities were removed by dissolution of the crude material in light petroleum (40 l.) and filtration after standing overnight. Evaporation of the light petroleum was followed by removal of the bulk of the industrial solvent by distillation on a boiling water-bath at 1 mm. The partially purified material was then saponified in the usual way with alcoholic potassium hydroxide. Removal of the unsaponifiable fraction by extraction with ether removed also the remainder of the industrial solvent. The fatty acids after purification by treatment with light petroleum weighed 1·835 kg. (i.v. 110, mol. wt. 229). The yield, 1·84 % of the fresh liver weight, is probably about 60 % of the total acids originally present in the liver in different forms. The extraction of acidified liver tissue after hydrolysis is an extremely difficult operation, especially in bulk, and the yield obtained therefore is to be regarded as satisfactory. Such a yield makes it unlikely that the material is not representative of the whole.

<div align="center">*Removal of the saturated acids.*</div>

The oxidations described later were carried out after the removal of the saturated acids either from the bulk preparation described above or from various small laboratory preparations made by exhaustive alcohol extraction of minced liver with subsequent preparation of the fatty acids from the alcoholic extract. The Twitchell process, as modified by Hilditch and Priestman [1931], applied

to 5·033 g. of the bulk preparation (i.v. 110, mol. wt. 299) gave 1·675 g. of saturated acids (i.v. 2·5, yield 33·3 %). A similar result was obtained on the large scale. 670 g. of the same acids yielded 231 g. of saturated acids (i.v. 2, mol. wt. 277, yield 34 %) and 440 g. of unsaturated acids (i.v. 153, mol. wt. 321). The proportion of saturated acids in the other preparations was of the same order, the highest figure being 39 %.

The preparation of the dihydroxy-acids from the unsaturated fraction.

Since the main object of this work was to establish the structure of the acids containing one double bond, it was desirable to explore the available methods for oxidation in order to determine the conditions under which greatest yields of dihydroxy-acids could be obtained. By oxidation of pure oleic acid in 0·1 % solution with alkaline potassium permanganate at 0° for 5 minutes, Lapworth and Mottram [1925, 1] obtained 95 % of the theoretical yield of dihydroxy-stearic acid. These authors state that the yield is depressed if the oxidation is carried out in the presence of more than a certain maximum of less saturated acids. The liver unsaturated acids consist of a complex mixture of acids from oleic acid to acids of more than twenty carbon atoms and having four and five double bonds. When such a mixture is oxidised under the conditions in which the greatest yield is obtained from pure oleic acid, the yield of dihydroxy-derivatives may clearly be seriously diminished. In order to determine the optimum conditions a series of comparative experiments, in all of which oxidation by alkaline permanganate has been used, have been carried out on the unsaturated fraction itself, and also on the material remaining after various processes have been carried out in attempts to concentrate the acids containing one double bond. Before describing these experiments, mention is made of the fact that when a mixture of acids, which has been freed from unsaponifiable matter, is oxidised by alkaline permanganate, there are produced substances which may be extracted by ether from the alkaline solutions of the oxidation products. The nature of this unsaponifiable fraction generated during oxidation has not been investigated. It is a complex mixture derived from the more unsaturated acids. It has been removed at various stages of the oxidations and it is mentioned here to explain the appearance of unsaponifiable fractions in the results which follow.

The method of Lapworth and Mottram [1925, 1]. 19·07 g. of the unsaturated fraction (i.v. 193) from a small laboratory preparation of fatty acids were dissolved in 20 l. of ice-cold water containing 14·0 g. potassium hydroxide. 14·6 g. potassium permanganate dissolved in 700 ml. ice-cold water were now added quickly with stirring, and after 5 minutes the oxidation was stopped by the addition of concentrated hydrochloric acid and sodium bisulphite solution. After removal by filtration, the insoluble oxidation products were dried *in vacuo* at room temperature over phosphorus pentoxide and extracted with light petroleum (B.P. 80–100°) to remove unoxidised acids and unsaponifiable material. The undissolved residue consisting of di- and tetra-hydroxy derivatives was then separated into its two main components by boiling with ether, the tetra-derivative being practically insoluble in that solvent. The portion soluble in light petroleum (6·54 g.) had i.v. 59·3 and mol. wt. 444. From it 1·03 g. of unsaponifiable matter were removed, yielding a mixture of acids of mol. wt. 383 and i.v. 69. 4·38 g. of these acids soluble in light petroleum were now re-oxidised under exactly the same conditions. The oxidation products insoluble in water

were filtered off, dried and extracted with petroleum. The petroleum-soluble portion was oxidised a third time. The combined yields of the various products from the three oxidations were: dihydroxy-derivatives 2·06 g.; tetrahydroxy-derivatives 0·70 g.; a semi-solid mixture of saturated acids soluble in petroleum 2·393 g. Allowing for the removal of portions of the oxidation products for analytical purposes, the yield of dihydroxy-derivatives was 13·0 % of the unsaturated acids or 8·0 % of the original mixed acids from the liver.

The method of Hartley [*1909*] *used by Turner* [*1930*]. The essential difference in the method used by these workers, as compared with that of Lapworth and Mottram, was that they carried out their oxidations in solutions which were approximately 13 times more concentrated. Following their procedure, 9·28 g. of the unsaturated acids (I.V. 193) were dissolved in 92 ml. 4 % potassium hydroxide, 280 ml. water were added and, after cooling to 0°, the mixture was oxidised by the addition of 400 ml. of 1·5 % potassium permanganate solution also at 0°. The oxidation was terminated after 10 minutes by treatment as in the previous experiment. A second oxidation of the petroleum-soluble fraction of the oxidation products was carried out under similar conditions. The final yields were: dihydroxy-derivatives 0·20 g.; tetrahydroxy-derivatives 0·15 g. This amount of dihydroxystearic acid corresponds to 2·5 % and 1·5 % of the unsaturated and of the original mixed acids respectively. The corresponding figures for the Lapworth and Mottram process were 13·0 and 8·0 %. The latter process, carried out at great dilution, is clearly far more efficient for the quantitative isolation of hydroxy-derivatives of the singly unsaturated acids present in liver. Lapworth and Mottram's statement as to the effect of the presence of more highly unsaturated acids in depressing the yield of dihydroxy-acid from oleic acid in a mixture made it likely that this yield of 13 % might be considerably below the value obtainable if the more highly unsaturated acids were first removed. For this reason attempts were made to increase the yield by removing as much of the unsaturated acids as possible from the mixture. For this purpose, two methods were employed.

Removal of more unsaturated acids. When certain of the more unsaturated acids are brominated, isomeric soluble and insoluble bromides are produced. The latter may be removed and the soluble product debrominated. On rebromination a further amount of insoluble bromide is formed. This principle was employed in an attempt to free the acids containing one double bond from the more unsaturated acids, alternate bromination and debromination being employed until no further insoluble bromide could be obtained. 19·56 g. of the unsaturated acids (I.V. 193) brominated in ether at 0° yielded 7·48 g. of ether-insoluble bromo-acids and 35·58 g. of soluble products. The latter were dissolved in light petroleum (B.P. 40–60°) and further insoluble bromides, which were precipitated after standing for 24 hours at 0°, were removed. The petroleum-soluble fraction, 31·57 g., contained 47·1 % Br. 30·34 g. of this product on debromination by the method of Rollet [1909] yielded 14·05 g. of fatty acid of I.V. 132. On rebromination of 13·85 g. of these acids, a further 1·21 g. of ether-insoluble bromide was removed. The remainder (Br 43 %), which remained completely soluble in light petroleum after standing for 2 days at 0°, was debrominated as before. By repetition of this bromination-debromination process, there were finally obtained 10·62 g. of fatty acids of I.V. 98. 10·1 g. of the latter acids when oxidised by the method of Lapworth and Mottram yielded 1·98 g. of dihydroxy-products (M.P. 130° indefinite) and 0·429 g. of tetrahydroxy-products (M.P. 168° indefinite) the former yield corresponding to 10·9 % of the original unsaturated acids, as compared with a yield of 13 % by direct oxidation

of the unsaturated acids without preliminary application of the bromination process. Unsuccessful attempts were also made to apply the Twitchell process to the mixture of soluble brominated acids in an endeavour further to remove the polybromo-acids.

The second method used for removing the more unsaturated acids was the lithium soap process which has been frequently described in the literature in various forms (in particular Moore [1919]). 22·15 g. of unsaturated acids from the large preparation (I.V. 153) were dissolved in 90 ml. absolute alcohol and 1·8 g. lithium hydroxide dissolved in 22·5 ml. water were added. After standing at 0° overnight, about 3 g. of lithium soaps were removed. A further 1 g. of lithium hydroxide dissolved in 100 ml. 80 % alcohol was added and the mixture maintained at 0° for 24 hours. The combined alcohol-insoluble lithium soaps yielded 6·01 g. of acids (I.V. 96·1, mol. wt. 286, 27 % of the total) while 15·6 g. of the more unsaturated acids (I.V. 171, mol. wt. 327, 70·4 % of the total) were obtained by decomposition of the soluble soaps. 4·755 g. of the more saturated acids yielded on oxidation by the method of Lapworth and Mottram 2·68 g. of dihydroxy-compounds (2·40 g., M.P. 127°; 0·28 g., M.P. 112°) together with 0·21 g. tetrahydroxy-derivatives (M.P. 168°). 10·72 g. of the more unsaturated fraction yielded by similar oxidation 1·23 g. dihydroxy-compound (M.P. 126°) and 0·05 g. of tetrahydroxy-derivatives. For comparison with these results a sample of the original unsaturated acids (I.V. 153) was directly oxidised without being subjected to the lithium treatment. The results of this experiment are summarised and compared with others in Table II.

The effect of increasing the permanganate concentration on the yield of the oxidation products. In the oxidation experiments so far described, the proportions of the reagents used by Lapworth and Mottram were rigidly adhered to. Since the starting material used by these workers was oleic acid (I.V. 90·4), whereas the acids used in the present work were far more unsaturated, it was considered desirable to investigate the effect on the yield of the dihydroxy-fraction of increasing the permanganate concentration proportionally to the increase in the iodine value.

The results of all these variations of the conditions of oxidation on the yields of the products are set out in Tables I and II. Table I shows that the yield of dihydroxy-acids by the Lapworth and Mottram method is about five times as great as that obtained by Hartley's method, while the yield of tetrahydroxy-compounds is about two and a half times as great. The separation of the more unsaturated acids by bromination has given poorer yields of both products when compared with the direct oxidation by the former method. Increase in the permanganate concentration causes a fourfold increase in the yield of the tetrahydroxy-fraction (Exp. 6), but slightly depresses the yield of dihydroxy-products. Table II shows a slightly increased yield of the two products as a result of the lithium soap treatment (*cf.* 4 and 5). The increase in the amount of the dihydroxy-fraction, which was the main objective, was however insufficient to compensate for the extra labour involved in the lithium soap separation, and accordingly direct oxidation of the unsaturated acids was employed in the large scale preparation. Tables I and II show the variations which may occur in the proportions of singly unsaturated acids present in the mixed acids from different liver samples. In Exps. 2 and 5, in which the oxidations were carried out under identical conditions, but on different samples of acids, the yields of dihydroxy-products were 8·0 and 14·8 % respectively of the original mixed acids. Hartley [1909] obtained yields of dihydroxy-compounds varying from 2·6 to 4·5 % of the mixed pig liver acids.

Table I. *Yields of di- and tetra-hydroxy-acids obtained under various conditions of oxidation.*

The unsaturated acids used in these experiments formed 61·5 % of the total mixed acids, and had I.V. 193.
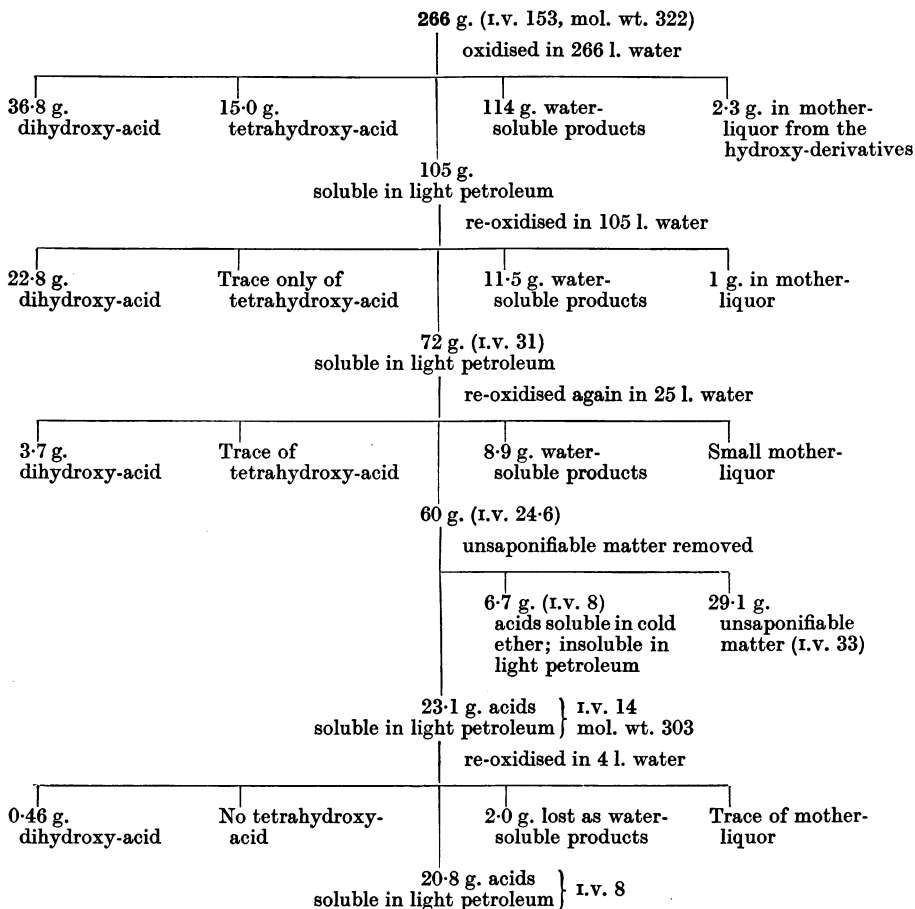
Method used	% yield from unsaturated acids		% yield from original acids (calc.)	
	Di-hydroxy	Tetra-hydroxy	Di-hydroxy	Tetra-hydroxy
1. Hartley's method of oxidation of unsaturated acids	2·5	1·6	1·5	1·0
2. Lapworth and Mottram's method on the unsaturated acids	13·0	4·2	8·0	2·6
3. The singly unsaturated acid in the mixed unsaturated acids first concentrated by bromination experiments and then oxidised by Lapworth and Mottram's process	11·0	2·4	7·0	1·5

Table II. *Yields of di- and tetra-hydroxy-acids obtained by the Lapworth and Mottram method.*

The unsaturated acids used in these experiments constituted 66·1 % of the original liver acids and had I.V. 153.

Particular acids oxidised	% yield from acids from lithium soaps		% yield from total unsaturated acids		% yield from original mixed acids (calc.)	
	Di-hydroxy	Tetra-hydroxy	Di-hydroxy	Tetra-hydroxy	Di-hydroxy	Tetra-hydroxy
4. Acids from insoluble lithium soaps	56·3	4·4	15·65	1·2	10·34	0·8
Acids from soluble lithium soaps	11·5	0·5	8·3	0·33	5·5	0·2
Total by this method	—	—	23·95	1·53	15·84	1·0
5. Direct oxidation of mixed unsaturated acids	—	—	22·4	1·0	14·8	0·7
6. The same as the preceding, except that the permanganate has been increased	—	—	21·0	4·1	13·9	2·7

The preparation of the dihydroxy-acids on a large scale. 266 g. unsaturated acids (I.V. 153, mol. wt. 322) were oxidised in 266 l. water at 4°, with the proportions of potassium hydroxide and permanganate recommended by Lapworth and Mottram. The second oxidations of the material soluble in light petroleum were carried out in exactly similar manner. In the third and fourth oxidations the volume of water was decreased because of the low iodine value of the petroleum-soluble fractions which indicated that not more than a part of them could be oleic acid. The successive stages in the procedure are best illustrated by the scheme (p. 846), which is followed by a brief summary of the final results.

266 g. (I.V. 153, mol. wt. 322)

oxidised in 266 l. water

| 36·8 g. dihydroxy-acid | 15·0 g. tetrahydroxy-acid | 114 g. water-soluble products | 2·3 g. in mother-liquor from the hydroxy-derivatives |

105 g. soluble in light petroleum

re-oxidised in 105 l. water

| 22·8 g. dihydroxy-acid | Trace only of tetrahydroxy-acid | 11·5 g. water-soluble products | 1 g. in mother-liquor |

72 g. (I.V. 31) soluble in light petroleum

re-oxidised again in 25 l. water

| 3·7 g. dihydroxy-acid | Trace of tetrahydroxy-acid | 8·9 g. water-soluble products | Small mother-liquor |

60 g. (I.V. 24·6)

unsaponifiable matter removed

| 6·7 g. (I.V. 8) acids soluble in cold ether; insoluble in light petroleum | 29·1 g. unsaponifiable matter (I.V. 33) |

23·1 g. acids ⎱ I.V. 14
soluble in light petroleum ⎰ mol. wt. 303

re-oxidised in 4 l. water

| 0·46 g. dihydroxy-acid | No tetrahydroxy-acid | 2·0 g. lost as water-soluble products | Trace of mother-liquor |

20·8 g. acids ⎱ I.V. 8
soluble in light petroleum ⎰

N.B. Water-soluble products are calculated by difference.

The results are summarised in Table III.

Table III.

Wt. of unsaturated acids used	266 g.,	equivalent to 402 g. of original mixed acids
,, crude dihydroxy-derivatives	63·8 g.,	,, 56·9 g. of "oleic" acid
,, crude tetrahydroxy-derivatives	15·0 g.,	,, 12·1 g. of "linoleic" acid
,, saturated acids	20·8 g.	
,, acids soluble in cold ether, insoluble in light petroleum*	6·7 g.	
,, unsaponifiable matter*	29·1 g.	
,, water-soluble products etc. (by difference)†		140·4 g.

* The nature of these oxidation products was not investigated further.

† These products would consist mainly of a mixture of the further cleavage products of small molecular weight derived from all the different unsaturated acids, together with the hydroxy-derivatives of the highly unsaturated acids (C_{20} and C_{22}).

Investigation of the dihydroxy-fraction.

In view of the possibility that the three yields of dihydroxy-acids obtained in the successive oxidations, 36·8, 22·8 and 3·7 g. respectively, might differ in nature on account of preferential oxidation of one or more acids, each was

separately fractionally crystallised from a mixture of alcohol:ether (1:1). The yields and properties of the resultant six fractions are recorded in Table IV.

Table IV. *Fractionation of the dihydroxy-acids.*

	Fraction	Weight g.	M.P. ° C.	C %	H %	Mol. wt. (titration)
From first oxidation	1 a	28·7	126 –126·5	67·75	11·3	314
36·8 g.	1 b	5·2	119·5–122 (indef.)	67·1	11·2	314
From first re-oxidation	2 a	15·3	129·5–130·5	68·4	11·45	312
22·8 g.	2 b	5·6	117·5–120·5	68·5	11·4	315
From second re-oxidation	3 a	2·1	128·5–129	68·5	11·5	317
3·7 g.	3 b	1·2	117·5–120·5	68·6	11·4	315
$C_{18}H_{36}O_4$ requires				68·35	11·5	316

The analytical figures for fractions 1 a and 1 b suggest the presence of some tetrahydroxy-acid caused by the slight solubility in the very large volumes of boiling ether which were used in the separation of the crude di- and tetra-hydroxy-acids from each other. These fractions were accordingly combined, extracted with the minimum volume of boiling ether and the soluble dihydroxy-product was then fractionally crystallised from alcohol-ether. Following the removal in this way of 5 g. of crude tetrahydroxy-acid from fraction 1, all the fractions were successively crystallised from this solvent, and by this means the material was finally divided into a series of fractions with the following compositions and properties:

Table V. *Composition and properties of the dihydroxy-acids.*

Fraction No.	Weight g.	M.P. ° C.	C %	H %
1	36·0	130·5–131	68·4	11·3
2	1·3	128	68·1	11·3
3	0·8	124–125	68·4	11·6
4	8·1	117–120	68·45	11·4
5	1·7	114	67·7	11·2
$C_{18}H_{36}O_4$ requires			68·35	11·5

Table V shows that at least 70 % of the dihydroxy-acids consist of a compound melting at 130·5–131°. The melting-points and combustion analyses of fractions 2–5 imply the existence of three compounds: one, M.P. 130·5–131° (fraction 1); another of low carbon content, M.P. 114° (fraction 5); and an intermediate compound which constitutes the main bulk of the material, apart from fraction 1, namely fraction 4, 8·1 g., M.P. 117–120°. Fraction 4 was repeatedly recrystallised from various solvents, but no effective further purification was obtained. This fraction appears to be a mixture of two dihydroxystearic acids, while fraction 5 is possibly the dihydroxy-derivative of an acid of lower carbon content.

The constitution of dihydroxystearic acid, M.P. 130·5–131°.

This was investigated by the method of Lapworth and Mottram. To 10 g. of the acid dissolved in 6 l. water containing 30 g. potassium hydroxide cooled to below 6° were added 4 l. 1 % aqueous potassium permanganate also cooled to below 6°. The mixture was well shaken and after remaining at room temperature for 4 hours the oxidation was stopped by the addition of sodium bisulphite solution and concentrated hydrochloric acid. A slight precipitate,

formed at this stage was filtered off. It weighed 0·12 g. and melted at 126° and was therefore probably unchanged dihydroxystearic acid. The amount of acid actually oxidised was therefore 9·88 g. The aqueous filtrate after neutralisation with sodium carbonate was reduced to a volume of 900 ml. and was then extracted thoroughly with ether.

Monobasic acid. The residue left after the removal of the ether was distilled in steam for 3 hours. The distillate was extracted with light petroleum (B.P. 40–60°). The solution, first dried over sodium sulphate, yielded on evaporation of the solvent 3·965 g. of liquid acids. 3·81 g. of these acids were distilled. 3·332 g. distilled at 234°. Further fractionation yielded 2·334 g. having B.P. 238° and mol. wt. (determined by the silver salt method) 144·2, 142·6. The zinc salt was prepared (found: C, 54·7; H, 8·5 %). The small amount of acid boiling above 240° was not further investigated. The theoretical figures for *n*-octanoic and *n*-nonanoic acids are as follows:

	Yield g.	B.P. ° C.	Mol. wt.	Of zinc salt C %	H %
n-Octanoic acid	4·50	236–237	144	54·6	8·6
n-Nonanoic acid	4·91	253–254	158	56·9	9·0

Dibasic acid. The residue from the distillation in steam (about 70 ml.) was filtered while hot from traces of tar and the dibasic acid allowed to crystallise; yield 4·29 g., M.P. 135–136° with previous softening. Suberic acid requires 5·44 g., M.P. 141°. On concentrating the aqueous filtrate, a further 0·28 g., M.P. 120° (indefinite) was obtained. These products were divided into four fractions by crystallisation from ether.

1. 2·522 g. M.P. 140·5–141°; mol. wt. 174·0; C, 55·3; H, 8·1 %.
2. 1·083 g. M.P. 140–141°.
3. 0·218 g. M.P. 135–137°.
4. 0·27 g. M.P. 100° indefinite.

Suberic acid, $C_8H_{14}O_4$, requires C, 55·15; H, 8·1 %; mol. wt. 174; M.P. 141°. The fourth fraction was converted to its magnesium salt, which was crystallised from water. The least soluble portion yielded 0·05 g. of acid of M.P. 102·5°. Azelaic acid melts at 106°. The oxidation products were therefore suberic and *n*-octanoic acids, together with small amounts of the acids having one more carbon atom, *i.e.* azelaic and *n*-nonanoic acids. In their oxidation of pure oleic acid, Lapworth and Mottram [1925, 2] obtained an 80 % yield of suberic acid and a 70 % yield of *n*-octanoic acid. They did not isolate azelaic or *n*-nonanoic acids from their oxidation products. They also estimated the amount of oxalic acid produced from the carbon atoms adjacent to the double bond. This was not sought in our experiments. These results show therefore that at least 70 % of the singly unsaturated acid present in this sample of pig liver is $\Delta^{9,10}$ octadecenoic acid.

Investigation of the dihydroxystearic acid, M.P. 117–120°.

4·048 g. of this product were oxidised and the products isolated in exactly the same way as in the previous experiment. None of the substance remained unoxidised.

The monobasic acid. This weighed 1·505 g.; mol. wt. 145. 1·252 g. on distillation gave three fractions, (*a*) 0·5 g., B.P. up to 220°; (*b*) 0·41 g., B.P. 221–235°; (*c*) 0·27 g., B.P. 236–240°. By redistillation of fractions (*a*) and (*b*) there were

obtained (i) 0·31 g., B.P. 220°; (ii) 0·28 g., B.P. 221–235°. The mol. wt. of fraction (i), determined by the silver salt method, was 136·4. This together with its boiling-point precludes the presence of n-hexanoic acid. The zinc salt was prepared from fraction (b) and gave on analysis C, 55·5; H, 8·8 %. The theoretical yields and the mol. wts. and boiling-points of the possible acids which might be present are:

Acid	Yield	Mol. wt.	B.P. °C.
n-Nonanoic	2·02	158	253–254
n-Octanoic	1·84	144	236–237
n-Heptanoic	1·66	130	224
n-Hexanoic	1·49	116	206
Crude product (found)	1·505	145	up to 240

With the small amount of material available, it was not possible to carry the investigation further. Study of the boiling-points and molecular weights of the various fractions, together with the initial weight of the crude oxidation product, make it appear certain that n-hexanoic acid could not have been present unless in traces. The mixture of acids probably consisted of n-heptanoic, n-octanoic and n-nonanoic acids.

The dibasic acid. The aqueous residue from the distillation in steam was filtered from 0·24 g. of a dark brown oil and from the filtrate there crystallised 1·73 g. dibasic acid, M.P. 126–129°, clearing finally at 132°. On concentrating the mother-liquor, a second crop of 0·28 g. separated, M.P. 120° (indefinite). Total crude dibasic acid, 2·0 g. The theoretical yields of sebacic, azelaic and suberic acids would have been 2·59 g., 2·41 g. and 2·23 g. respectively. It is to be noted that in both crops the product differed in melting-point from that obtained at the corresponding stage from the oxidation of the higher-melting dihydroxystearic acid (p. 848). On recrystallisation from ether, the first crop still melted at 120–125° and had mol. wt. 175. Further recrystallisation from ether had no apparent effect. Both fractions were therefore converted into their magnesium salts and these fractionally crystallised from water. The acids from the most and from the least soluble salts were obtained and recrystallised both from water and from ether. The most soluble magnesium salt gave a dibasic acid, which, after recrystallisation, weighed 0·32 g.; M.P. 139–141°; C, 55·15; H, 8·1 %; mol. wt. 175 (by titration). The acid from the least soluble magnesium salt weighed 0·28 g., crystalline plates, M.P. 133–134°; C, 59·60; H, 8·9 %; mol. wt. 206. The theoretical data for four possible dibasic acids are:

	M.P. °C.	C %	H %	Mol. wt.
$C_{11}H_{20}O_4$	124	61·1	9·3	216
$C_{10}H_{18}O_4$	134	59·4	8·9	202
$C_9H_{16}O_4$	106	57·4	8·5	188
$C_8H_{14}O_4$	141	55·15	8·1	174

The dibasic products of oxidation therefore contain suberic and sebacic acids; and it follows that in all probability azelaic acid is a third component, because, apart from any other consideration, suberic and sebacic acids present alone in a mixture would be very readily separated, whereas these two components were only obtained in the present instance with difficulty. The presence of other dibasic acids in very small traces is also possible. In Table VI are given the products which would arise from a number of acids oxidised under the conditions used by us. The presence of suberic and n-octanoic acids and also of part of the azelaic and n-nonanoic acids in the oxidation products in this experiment shows

Table VI.

Acid	Main products	By-products
9:10-Dihydroxystearic acid	Suberic and n-octanoic acids	Azelaic and n-nonanoic acids
10:11 ,,	Azelaic and n-heptanoic acids	Sebacic and n-octanoic acids
11:12 ,,	Sebacic and n-hexanoic acids	Nonamethylenedicarboxylic acid and n-heptanoic acid
12:13 ,,	Nonamethylenedicarboxylic acid and n-pentanoic acid	Decamethylenedicarboxylic acid and n-hexanoic acid

that the lower-melting dihydroxy fraction is a mixture and that the $\Delta^{9, 10}$ dihydroxy-derivative has not been entirely removed by fractional crystallisation. The yields and analytical data suggest that about one-half of the fraction may be the 9:10 compound. It therefore remains to account for the apparent formation of sebacic and n-heptanoic acids. Under the conditions of oxidation used, the presence of these products suggests that the hydroxy-groups were originally in the 10:11 positions. This view, however, can only be put forward with considerable caution. Until the oxidation products can be obtained in far greater quantity to enable their isolation in pure form, no definite statement can be made. These results do however indicate the presence of an acid which is neither $\Delta^{9, 10}$ nor $\Delta^{12, 13}$ octadecenoic acid, and which probably forms not more than 10 % of the total dihydroxy-fraction.

Oxidation of ordinary oleic acid. At this stage it was felt wise to prepare the dihydroxystearic acid from ordinary oleic acid, in order to see whether it could be fractionated into high- and low-melting products of a similar nature. Accordingly 14·27 g. oleic acid (which still contained a little saturated acid) were oxidised by Lapworth and Mottram's method and yielded 13·27 g. dihydroxystearic acid, M.P. 128°. Allowing for the 1·67 g. of saturated acids which were removed by light petroleum, the yield was 94·0 % of theory. On fractionating the hydroxy-derivative as in the large experiment on p. 847 there were obtained 10·35 g., M.P. 130°; 1·0 g., M.P. 128° and 0·47 g., M.P. 122°, the mother-liquor of 1·4 g. being a semi-liquid product. This fractionation was very similar in its results to that described for the hydroxy-compounds from liver, except that in this case there was no corresponding lower fraction, with a melting-point of 117–120°, which remained unchanged after several recrystallisations.

DISCUSSION.

These results show that at least 80 % of the octadecenoic acid of pig liver is ordinary oleic acid ($\Delta^{9, 10}$), while some 10 % may be the $\Delta^{10, 11}$ acid. No evidence was obtained of the presence of the $\Delta^{12, 13}$ acid obtained by Hartley [1909]. This latter result is the more striking because the $\Delta^{12, 13}$ acid was the only acid found in pig liver by that author. It was isolated by him as the dihydroxy-acid, M.P. 129°, which was further oxidised. From the steam-volatile oxidation products, calcium and barium salts were prepared and these contained the percentages of metal required by theory for n-hexanoic acid. It is to be noted however that the decamethylenedicarboxylic acid, which should have been produced under the conditions of oxidation used by him along with n-hexanoic acid, was not isolated. Hartley contrasts the melting-point of his dihydroxy-acid, M.P. 129·5°, with that obtained by Edmed [1898] by oxidation of oleic acid, which gave a dihydroxy-acid, M.P. 134°, as evidence for the dissimilarity of his compound from the $\Delta^{9, 10}$ acid. Robinson and Robinson [1925], however, who record M.P. 132° for 9:10-dihydroxystearic acid prepared from

pure oleic acid, comment that the melting-points (up to 136°) previously re-corded in the literature are too high.

Little can be said at present concerning the small fraction of the acid which was isolated in this work as a dihydroxy-acid, M.P. 117–120°. The material available in the work carried out by Hartley [1909] and Turner [1930] did not allow them fractionally to crystallise their dihydroxy-products, and no corre-sponding compound was obtained.

We feel that our results further weaken the available evidence for desatura-tion of fatty acids by the liver, for it will be recalled that Turner [1930] failed also to obtain evidence of the presence of any octadecenoic acid in sheep liver other than the $\Delta^{9\ 10}$ acid. Bearing in mind the extensive researches of Sinclair [1932, 1, 2] which show the remarkable avidity with which the liver removes unsaturated acids from the portal circulation and the tenacity with which it retains them when the unsaturated fat in the diet is substituted by a saturated one, the original results of Leathes and Meyer-Wedell can be interpreted equally well for the present as being due to preferential absorption of fatty acids or as being due to desaturation.

From another direction also considerable evidence is accumulating which seems to cast doubt on the validity of the desaturation hypothesis. The extensive literature which is accumulating to show that animals will not grow on a diet which is rigorously freed from fat, but that normal growth is restored when very small amounts of linoleic or linolenic acids are administered, has given rise to the conception of "essential" fatty acids which implies a very limited ability of the animal to synthesise certain unsaturated acids at least [Burr and Burr, 1929; 1930; Burr et al., 1932]. If this finding is substantiated, it is of particular importance to emphasise that one of the "curative" acids is linoleic acid, which on the desaturation hypothesis should be produced from depot oleic acid by introduction of a double bond into the 12:13 position.

SUMMARY.

1. With a view to obtaining further evidence on the theory of desaturation of fatty acids in liver, the octadecenoic acids of pig liver have been investigated by oxidation of the unsaturated acids and subsequent further oxidative degra-dation of the dihydroxy-acids.

2. Description is given of experiments carried out with a view to increasing the yields of dihydroxy-acids when the mixed unsaturated acids are oxidised with alkaline permanganate.

3. Fractional crystallisation of the latter acids shows the presence of at least two octadecenoic acids. One of these is shown to be $\Delta^{9,\ 10}$ octadecenoic acid (ordinary oleic acid) and to constitute at least 85 % of the octadecenoic acids present. The second dihydroxy-acid, M.P. 117–120°, yielded on further oxidation a mixture of monobasic and dibasic acids which could not be satisfactorily separated on account of the small amount of material available. The evidence so far obtained from study of the oxidation products suggested the presence of a $\Delta^{10,\ 11}$ acid.

4. The $\Delta^{12,\ 13}$ octadecenoic acid isolated by Hartley [1909] from pig liver was shown to be absent.

5. The bearing of these results on the liver desaturation theory is discussed.

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