CCXXXIX. THE FATTY ACIDS OF PIG LIVER.

II. FURTHER EVIDENCE ON THE OCTADECENOIC ACIDS AND THE DESATURATION THEORY.

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IN a recent paper in which we reported the results of investigations into the chemical nature of the octadecenoic acids of pig liver the conclusion was reached that at least 85 % of these were present as the $\Delta^{9:10}$ compound, while the remaining 15 % did not contain any $\Delta^{12:13}$ acid [Channon *et al.*, 1934]. This result was contrary to that obtained in a similar study by Hartley [1909], for the only octadecenoic acid found by him was the $\Delta^{12:13}$ derivative. The disagreement in these results is of importance, for that of Hartley has provided one of the main lines of evidence on which the theory of the desaturation of fatty acids in the liver is based.

Bearing in mind the large quantities of pig liver used in the preparation of the fatty acids both by Hartley and by ourselves and taking into consideration the number of animals concerned, it seems at least improbable that the divergence in our respective results can be attributed to a fundamental difference in the composition of the original acids. It is true that neither Hartley nor ourselves paid attention to the previous diet of the animals used, but present knowledge of the appearance of the fatty acids of the food in the liver does not suggest that any particular acid would appear in the liver to the complete exclusion of others of similar chemical nature, even if that acid were present in the food in considerable amount. Further, as far as we are aware, the presence of $\Delta^{12:13}$ octadecenoic acid has not been reported in natural products except by Hartley. Hence disregarding the possibility of chemical idiosyncrasy in the respective groups of animals, these considerations have led us to attempt to find an explanation of these contradictory results on the basis that our somewhat different methods of preparation of the hydroxy-acids and of their further oxidation might have caused different products to be formed. We are aware, of course, that Hartley had no difficulty in identifying the $\Delta^{9:10}$ compound as the only octadecenoic acid in lard, and that by similar methods he failed to find this acid in liver. Nevertheless it seemed important to investigate the matter further by carrying out the various stages exactly as Hartley had done in order that the results might be compared, and we were fortunate in having at our disposal sufficient of the original acids used by us in our previous investigations to make this possible.

We have accordingly investigated both the possibilities that the disagreement in the results might be due to some change taking place either in the further oxidation of the dihydroxystearic acid or in the preparation of that

product from the original acids. To test the former of these 5 g. of the higher-melting dihydroxystearic acid, prepared from the liver acids and proved by a recognised method to be a 9:10 derivative as described in our original paper, were oxidised exactly as described by Hartley, who used the method of Edmed [1898]. We had no difficulty in isolating *n*-octanoic and suberic acids, as the main products of oxidation. Further, when 112 g. of the original liver acids were treated throughout as described by Hartley, we were again able to isolate these same two products.

Hence our original finding that the main octadecenoic acid of pig liver is the $\Delta^{9:10}$ compound was confirmed by carrying out the oxidations according to Hartley's procedure. It has to be borne in mind however that Hartley's recognition of the presence of the $\Delta^{12:13}$ acid was based on partial evidence only. He prepared and then oxidised the dihydroxystearic acid and removed the volatile acid products by distillation in steam. Calcium and barium salts were prepared from the steam distillate, and these salts were found to contain the percentage of calcium and barium required for the corresponding salts of hexanoic acid. This monobasic acid was not further characterised. Nor was the dibasic acid, which must necessarily have been formed at the same time, isolated or identified in any way. Hartley suggested that his dibasic acid had either (a) been oxidised to oxalic acid which he found in considerable quantity as a product of oxidation, or (b) that it was so similar in properties to the original unchanged dihydroxystearic acid as to be inseparable from it in the amounts which he had available. From our experiments carried out at a temperature of 80° quite good yields of the crude dibasic acid were obtained, and the occurrence of oxalic acid has been explained by Lapworth and Mottram [1925, 2] who investigated the work of Edmed more fully. Edmed stated that under his conditions (identical with those used by Hartley), $\text{CH}_3 \cdot (\text{CH}_2)_7 \cdot \text{CHOH} \cdot \text{CHOH} \cdot (\text{CH}_2)_7 \cdot \text{COOH}$ (a 9:10 derivative) yielded $\text{CH}_3 \cdot (\text{CH}_2)_7 \cdot \text{COOH}$ (nonanoic acid) and $\text{HOOC} \cdot (\text{CH}_2)_7 \cdot \text{COOH}$ (azelaic acid). But when more fully investigated it was shown by Lapworth and Mottram that, although this type of oxidation does take place to some extent, another set of products is simultaneously formed by the cleavage taking place in such a way as to give $\text{CH}_3 \cdot (\text{CH}_2)_6 \cdot \text{COOH}$ (octanoic acid), $\text{C}_2\text{H}_2\text{O}_4$ (oxalic acid) and $\text{HOOC} \cdot (\text{CH}_2)_6 \cdot \text{COOH}$ (suberic acid). Lapworth and Mottram did not indicate what proportion of the dihydroxystearic acid yields octanoic, suberic and oxalic acids when Edmed's method is used, but our results, in which we obtained octanoic and suberic acids as the main products by both methods, indicated that the main oxidation is still of this latter type. If that be true in all cases, irrespective of the position of the double bond, then the finding of hexanoic acid as a main product of oxidation simultaneously with oxalic acid by Hartley would not indicate the presence of a $\Delta^{12:13}$ acid, but would suggest the presence of a $\Delta^{11:12}$ compound, thus: $\text{CH}_3 \cdot (\text{CH}_2)_5 \cdot \text{CH}(\text{OH}) \cdot \text{CH}(\text{OH}) \cdot (\text{CH}_2)_6 \cdot \text{COOH}$ (*i.e.* 11:12) would yield as the main products $\text{CH}_3 \cdot (\text{CH}_2)_4 \cdot \text{COOH}$ (hexanoic acid), $\text{C}_2\text{H}_2\text{O}_4$ (oxalic acid) and $\text{HOOC} \cdot (\text{CH}_2)_5 \cdot \text{COOH}$ (sebacic acid). In this connection it is interesting to observe that from the 15 % or so of lower-melting dihydroxystearic acid prepared by fractional crystallisation of the main bulk as described in our previous communication, we obtained a certain small amount of sebacic acid; the monobasic acid however, at all times difficult to characterise definitely when present, as in this case, in a mixture, appeared to be heptanoic rather than hexanoic acid. This fact, taken in conjunction with the first part of our present discussion, does not suggest that any explanation can be given along these lines for the finding by Hartley of hexanoic acid, particularly when it is realised that he was able to show by identical methods

and without difficulty that the octadecenoic acid of lard was the usual $\Delta^{9:10}$ compound.

Unfortunately further information cannot be obtained from the melting-point of the dihydroxy-acid itself for the effect of the position of the two hydroxy-groups upon the melting-point is often too slight to make this factor a reliable indication unless an adequate amount of material is available to enable rigorous purification to be accomplished.

We ourselves have had two very great advantages in carrying out this work. In the first place, we have been able to make use of the method initially suggested by Robinson and Robinson [1925] and elaborated by Lapworth and Mottram [1925, 1] for the preparation of the hydroxy-acids—a method which gave from seven to eight times the yield obtainable by the procedure available to Hartley in 1909. In the second place, we have had very much larger quantities of material on which to work. Perhaps these two facts alone might be sufficient to account for the divergence in the two sets of results.

Our experiments described below therefore throw no further light on the problem under discussion, except to confirm our previous work and to show that the disagreement between the results of Hartley and ourselves is not due to the different oxidation technique employed.

EXPERIMENTAL.

I. *Oxidation of dihydroxystearic acid prepared by the method of Lapworth and Mottram [1925, 1] and oxidised by the method of Hartley [1909].*

5 g. dihydroxy-derivative, M.P. 130.5–131°, obtained from pig liver as described in our previous communication, were dissolved in 166 ml. water containing 3.31 g. potassium hydroxide. The solution was heated to 80°, and 400 ml. water containing 5.3 g. potassium permanganate were added, the mixture being shaken and then allowed to cool. After removal of the manganese dioxide by filtration, the colourless filtrate was acidified with 10 % H_2SO_4 , when a voluminous white precipitate was thrown down. This weighed 3.8 g. and consisted mainly of unchanged dihydroxystearic acid. This material was reoxidised as before, and again all the permanganate was decolorised and some unchanged substance recovered. The latter was oxidised under the same conditions a third time, when no dihydroxystearic acid remained unchanged and the permanganate was definitely in excess.

The monobasic acid. As was done by Hartley, the filtrate from the unchanged dihydroxy-derivative after the first oxidation was distilled in steam. The distillate was exactly neutralised (36 ml. 0.1 *N* NaOH being required), and evaporated to a small bulk. On adding calcium chloride solution, a white precipitate was formed. This calcium salt was filtered off, washed and dried. It weighed 0.48 g. (Found by ignition: Ca, 12.30 %. $(\text{C}_8\text{H}_{11}\text{O}_2)_2\text{Ca}$ requires Ca, 14.8 %; $(\text{C}_8\text{H}_{15}\text{O}_2)_2\text{Ca}$ requires Ca, 12.27 %. Hartley [1909] found: Ca, 14.7 %.) As confirmation the calcium oxide from a second analysis of 0.1734 g. was washed into a small flask with hydrochloric acid and precipitated as oxalate, the latter being titrated with permanganate. 10.35 ml. *N*/10 permanganate solution were required. Whence Ca = 11.9 %.

It is therefore clear that the lowest monobasic acid formed by either method is in our case octanoic acid and that the hexanoic acid found by Hartley on similar evidence is absent. This conclusion is confirmed by studying the dibasic acid.

The dibasic acid. The residue left after distilling all the three oxidation-liquors in steam was concentrated to about 50 ml. when 1.28 g. dibasic acid,

M.P. 135°, crystallised out. A second crop was obtained, weighing 0.15 g., M.P. 102–118°. This probably consisted of crude azelaic and suberic acids mixed. The first crop was recrystallised, yielding 0.63 g. (C, 55.1; H, 8.0 %; M.P. 141.5°; mol. wt. 174.6. Suberic acid, $C_8H_{14}O_4$, requires C, 55.15; H, 8.05 %; M.P. 141°; mol. wt. 174.) A small third crop of very crude dibasic acid (0.2 g.) was also obtained. The total yield corresponds approximately to 60 % of the theory for suberic acid. The isolation of this particular dibasic acid alone suggests that the method adopted for the oxidation plays no part in accounting for the difference in the results obtained by Hartley and by ourselves.

II (a). *The preparation of dihydroxystearic acid by Hartley's method.*

By this procedure the Twitchell process used by us in the original work was not carried out. 112 g. of the original mixed liver acids (i.v. 110, mol. wt. 300) were mixed with 112 ml. potassium hydroxide solution (sp. gr. 1.145), and the mixture was dissolved in 4.6 l. of water cooled to 0°. 4.6 l. 1.5 % potassium permanganate also at 0° were added slowly with vigorous shaking. After 10 minutes a saturated solution of sodium bisulphite was added and the whole acidified with hydrochloric acid. The white precipitate so formed was filtered off, dried in a vacuum-desiccator and extracted as usual with light petroleum. The latter extracted 35.0 g. acids (i.v. 36). This corresponds well with Hartley's results at this stage.

The portion insoluble in light petroleum was washed free from a brown gummy substance by cold ether. From the residue consisting of di- and tetrahydroxystearic acids, the dihydroxy-acid was removed by extraction with warm ether. The total yield of the crude dihydroxy-derivative amounted to 2.2 g., M.P. 125° (indef.). After one recrystallisation from 70 % alcohol (charcoal) the M.P. rose to 127°. This yield of approximately 2 % corresponds to that of 15 % obtained by the more recent methods previously used by us on the same batch of liver acids.

Lest any of this product should be lost in further processes of purification it was oxidised immediately without further treatment.

II (b). *Oxidation of the dihydroxy-derivative so formed by Hartley's method.*

1.5 g. of this substance were oxidised according to the details given in section I above, except that only one oxidation was carried out [*cf.* Hartley, 1909, p. 364]. Only a portion of the 1.5 g. was therefore affected, which accounts in part for the lower yields of oxidation products. This enabled the use of bisulphite to be avoided, because all the permanganate was used up, and there was thus no chance of sulphite interfering at the end in the formation and analysis of the calcium salt.

The monobasic acid. The manganese dioxide and then the unchanged dihydroxystearic acid were filtered off, the filtrate distilled in steam, the distillate neutralised exactly and evaporated to a small bulk. After adding calcium chloride, the calcium salt was filtered off. It weighed 0.15 g. On ignition 0.1463 g. gave 0.0248 g. CaO. Whence Ca = 12.1 %. (Theory 12.27 %.) In confirmation the Ca in this CaO was estimated by the oxalate-permanganate method. 8.80 ml. *N*/10 permanganate were required; whence Ca = 12.0 %.

The dibasic acid. This was obtained in two crops: (1) 0.23 g., M.P. 127° (indef.) and (2) 0.14 g., M.P. 122–128°. These were combined and recrystallised, and some oily product removed.

First crop 0.07 g., M.P. 139°. (Found: C, 54.94; H, 8.06 %. Suberic acid ($C_8H_{14}O_4$) requires C, 55.15; H, 8.05 %.)

It is therefore again quite certain that the products of oxidation contain octanoic and suberic acids and that the different methods adopted are not the source of the disagreement in results.

SUMMARY.

In a previous paper [1934] it was shown that at least 85 % of the octadecenoic acids of pig liver is the $\Delta^{9:10}$ compound. This result is at variance with that of Hartley [1909] who found the $\Delta^{12:13}$ acid as the sole compound of this nature present. In an effort to explain these conflicting results a further sample of the original liver acids has been submitted to treatment identical with that used by Hartley. The monobasic and dibasic acids isolated as further oxidation products of the dihydroxy-derivatives were octanoic and suberic acids, confirming our original finding that the main octadecenoic acid is the $\Delta^{9:10}$ compound.

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