ON THE NATURE OF THE FAT CONTAINED IN THE LIVER, KIDNEY, AND HEART.

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THERE are many indications that in the active cellular organs of the body the fat exists not so much in the form of simple glycerides of the higher fatty acids as in that of lecithin and similar complex bodies. A more complete knowledge of such complex substances is desirable in order to understand the rôle played by fats in the life of the cell, and something may be learnt from the study of the fatty acids obtained from them by saponification. For this reason Liebermann's method, with slight modifications, has been used.

In the course of experiments which have been carried on for some time by Leathes[1] it has become more and more obvious that the higher fatty acids obtained from the liver or heart or kidneys, behave, in certain respects, differently from those which may be obtained by the same methods from adipose connective tissue. From the organs a larger amount of higher fatty acids soluble in ether but insoluble in petroleum ether is always obtained, and if the acids soluble in petroleum ether are exposed to the air, or still more if they are heated, a part is found to have become insoluble in this solvent. Furthermore, on applying the method proposed by Farnsteiner, for separating acids of the linoleic and linolenic series from acids more saturated, evidence was obtained for the presence in considerable amount of acids less saturated than oleic acid. This seemed to point to the presence, in the fats of the organs examined, of unsaturated acids different from those occurring in the fat stored in adipose connective tissue. It seemed possible that if these differences could be elucidated, some light might be thrown on the nature of the changes which fats undergo when they leave the connective tissues, and enter the stream of metabolic change in the active organs of the body.

With this end in view, I proceeded, at the suggestion of Dr Leathes, to whom I am indebted for much kindly help and advice, to

collect the fatty acids liberated from the liver and other organs of different animals, and to examine their properties and attempt the identification of the several acids contained in the mixture. In the present paper I shall confine myself to the results of the examination of certain properties of the acids.

The first property to be examined was the power of absorbing iodine, which, as is familiar, is a measure of the degree to which fatty acids are unsaturated. The iodine value, the weight, that is, of iodine absorbed by 100 parts of the fat, has been found in the case of the fats extracted by ether from the organs to be somewhat higher than that of the fat from the connective tissue. Thus Woltke[2] found that the iodine value of the fat from the liver of a normal dog was 83·1, from the heart muscle 84·9, and from the kidney 78·0, whereas the connective tissue fat had an iodine value of 62·8. Leick and Winckler[3] obtained the following values: heart muscle fat 83·5, connective tissue fat 59·1 in the case of normal dogs, and in the case of sheep, heart muscle fat 67·7 and connective tissue fat 43·9. Rosenfeld[4] gives the following figures for the iodine value of the heart muscle fat of four dogs: 53·47, 58·55, 60·4, 41·8. It will be observed that the iodine value of the organ fats is somewhat higher than that of the connective tissue fat. As the iodine value of olein is 85·6, it has been assumed that the higher iodine values obtained for fats derived from the organs indicate that they contain relatively more olein than the connective tissue fat. The experimental results given later in the paper show clearly that this difference between the fat of the various organs and that contained in the connective tissue is not to be explained simply by the presence of varying proportions of olein.

By the method described below, the iodine value for the fatty acids obtained from connective tissue fat, it is true, is of the same order as that found by previous investigators; but the values for the fatty acids from the organ fats are higher than the iodine value of oleic acid, and far higher than any before recorded[1]. The numbers varied from 115 to

[1] While this work was in progress a paper appeared (Erlandsen, *Zeits. f. physiol. Chem.* LI. p. 71, 1907) in which a substance cuorin, obtained from ox-heart muscle, is described. This substance is a monoamido-diphosphatide and yields on hydrolysis fatty acids, the mean iodine value of which is 130·1. The lecithin from the same source yields fatty acids having a mean iodine value of 110.

In this connection it is interesting to note that Henriques and Hansen examined the fatty acids obtained from the lecithin of the yolk of egg, and from the high iodine values they obtained, argued that a considerable portion of the acids in the lecithin must belong to the linoleic series. (*Skand. Archiv f. Physiologie*, XIV. p. 390.)

135. This indicates the presence of fatty acids of the linoleic series ($C_nH_{2n-4}O_2$). The unsaturated acids were separated from the saturated acids and the iodine value of the former determined. This was found to be greater than that of linoleic acid and indicates the presence of acids of the linolenic series ($C_nH_{2n-6}O_2$). An experiment on the bromination of the unsaturated acids not only confirmed this, but also afforded evidence of the presence of an acid or acids containing four unsaturated linkages.

The nature of these fatty acids is being more completely investigated.

Method of preparing the fatty acids. The method which has been most frequently used for the extraction of fat is that of Rosenfeld. This method consists in extracting the dried tissue with alcohol and chloroform and taking up the united extracts with ether. It is well known that this ether extract does not consist wholly of neutral fat. Glikin[5] has examined such ether extracts and has shown that some samples contain 16·12 % lecithin and 3·66 % "ptomaine." Leathes has shown that on saponification with alkalies, the ether extracts yield only 65 % of fatty acids, whereas pure animal fat from the connective tissues yields about 95 % of fatty acids. Since it would be difficult to obtain the fats in a pure form from these ether extracts, it was decided to prepare the fatty acids from the organs by direct saponification. The method adopted has the advantage of simplicity and rapidity, permits of the use of large quantities of materials, and gives a final product consisting of little else but the higher fatty acids.

The method as carried out on the liver will be described in full, but the same procedure has been followed with the heart muscle and the kidney, and the connective tissue fat which has been examined has been taken through every stage of the treatment in precisely the same way.

All visible adherent fat etc. was first carefully removed from the organ, the liver substance cut into small pieces and finally minced in a machine. In most cases the whole of the minced tissue was used for experiment. It was placed in a large flask, and a solution of caustic potash (500 gm. potash and 500 c.c. water) added. 250 c.c. of this solution were used per kilogram of fresh tissue. The mixture was heated on the water-bath and shaken at intervals. When all the tissue had dissolved, methylated spirit was added, and the heating continued, with further addition of spirit, until saponification was complete. The solution was then cooled, diluted somewhat with water and a 10 %

solution of sulphuric acid added until the presence of free mineral acid could be detected in the solution. The acidified solution was heated on the water-bath until the precipitated fatty acids had risen to the surface; the lower layer of clear fluid was then syphoned off. A large volume of water was added, the whole heated on the water-bath until the precipitated acids collected on the surface and the lower layer of wash liquor syphoned off as before. The washing was continued until the wash liquor was no longer acid, and almost colourless. The precipitate was transferred to a filter and left to drain overnight, after which it was dried *in vacuo* over sulphuric acid for 2 or 3 days. When dried as completely as possible the precipitate was extracted in a Soxhlet apparatus with petroleum ether (B.P. 45°—65°) for 6 or 8 hours. The petroleum ether extract was diluted with 2 litres, or more, of the same solvent and allowed to stand for 24 hours. At the end of this time a dark brown substance separated from the solution. This substance is apparently soluble in a concentrated solution of fatty acids in petroleum ether, but insoluble in a more dilute solution. It solidifies to a hard mass. The amount obtained varied in different experiments, and frequently contained nitrogen and sulphur. It has not been further investigated. It was removed from the solution by filtering, and the petroleum ether distilled off from the filtrate on the water-bath, the last traces being removed by heating at reduced pressure in a slow current of dry carbon dioxide. On cooling, the fatty acids solidified to a yellow or light brown mass, consisting of an oily and a crystalline part.

The amount of higher fatty acids obtained from the various organs was determined and is expressed in the following tables in units per cent. of fresh substance used. The iodine value of the mixed higher fatty acids was determined immediately after the petroleum ether, in which they were dissolved, had been distilled off. Wijs' method for determining the iodine value has been used throughout.

The following table contains the results obtained for the higher fatty acids from the various organs of the pig, the goat and the dog.

Source of fatty acids	Weight of fresh substance	Fatty acids %	Iodine value of fatty acids
Pig:			
Liver E	700 gms.	2·86	126·4
Liver F	950	2·58	134·1
Liver G	870	2·44	134·7
Kidney	180	2·37	116·2
Heart Muscle	230	1·75	134·4
Pericardium	—	—	66·1
Connective tissue	—	—	53·9

Source of fatty acids	Weight of fresh substance	Fatty acids %	Iodine value of fatty acids
Goat:			
Liver A	535	2·38	113·5
Liver B	370	3·42	119·6
Kidney (2 pairs)	118	—	120·6
Kidney (7 pairs)	420	2·11	116·3
Heart muscle (2 hearts)	117	1·69	128·2
,, ,, (4 ,,)	195	2·07	107·7
Pericardium	—	—	43·5
Connective tissue A	—	—	35·9
Connective tissue B	—	—	43·8
Dog:			
Liver A	360	2·76	114·6
Liver B	200	2·78	127·6
Kidney B	70	2·71	120·1
Heart muscle B	87	2·20	127·2
Pericardium B	—	—	63·3
Connective tissue B	—	—	60·5

A series of experiments on the higher fatty acids from the human liver and heart muscle has been carried out. The results are given in the following table: an abstract of the post-mortem report is also given:—

Source of fatty acids	Weight of organ	Weight of fresh substance	Fatty acids %	Iodine value of fatty acids
Liver, X.	1380 gms.	1240 gms.	3·98	117·8
Liver, A. P.	450	350	2·59	126·4
Liver, W. H.	1685	800	2·85	123·0
Liver, L. R.	275	245	2·35	128·0
Heart muscle, W. H.	—	102	2·80	120·0
,, ,, H. S.	—	162	2·36	127·9
Omentum	—	—	—	65·0

X. Cause of death: appendicitis and general peritonitis. No further details available.

A. P. Age 15 months. Cause of death: burns, meningitis. Liver pale and soft, but showed no other pathological changes.

W. H. Age 56. Cause of death: intestinal obstruction and lobar pneumonia. Body jaundiced. Peritoneum healthy. Liver pale, soft and greasy. Heart healthy.

L. R. Age 7. Very poorly nourished. Disease: tubercle of lungs, bronchial and mesenteric glands, spleen and liver. Liver showed only a few miliary nodules, principally on the serous surface, otherwise it appeared quite normal.

H. S. Age 54. Cause of death: chronic nephritis, renal calculus, uraemia. Body very fat. Peritoneum healthy. Epicardial fat of the heart very much increased and viscus enlarged. Left ventricle hypertrophied, and other chambers dilated. Moderate atheroma of aortic cusps and great vessels.

The results given in all the above tables differ in a striking manner from those previously obtained, examples of which are given (p. 18): the

iodine value of the higher fatty acids from the organs is much higher than the iodine value of the "ether extracts" obtained by other methods. As the iodine value of a neutral fat is somewhat lower than that of the corresponding fatty acid (*e.g.*, the iodine value of olein is 85·6, that of oleic acid 90·0) slightly higher values than those previously obtained were to be expected. But instead of an increase in iodine value of about 5 %, increases of more than 50 % have been obtained in many cases. Experiments have been carried out with the object of determining the cause of this large increase in iodine value. From one portion of a pig's liver, an ether extract was prepared according to the method of Rosenfeld, and this was compared with the higher fatty acids obtained by saponification of another portion of the same liver.

50 grams of fresh liver tissue were dried by heating first on the water-bath, then in an oven at 105°, and finally *in vacuo* at 100° for two days. The whole was finely powdered before the final drying *in vacuo*. The dried substance was boiled for 15 minutes with alcohol, and then extracted for 6 hours with chloroform, boiled again for 15 minutes with alcohol and again extracted for 6 hours with chloroform. The united alcohol and chloroform extracts were taken up in ether, the ether distilled off, the ether extract weighed, and the iodine value determined. The ether extract was then saponified, the fatty acids precipitated with sulphuric acid, washed, dried, and extracted with petroleum ether. The fatty acids were weighed and the iodine value determined.

600 grams of the tissue from the same liver were saponified in the usual way and the iodine value of the higher fatty acids determined. The results are given in the two following tables:

Extraction method.

Fresh tissue	Dry substance	Ether extract (on dry substance)	Iodine value of ether extract	Fatty acids from ether extract	Iodine value of fatty acids
50 gms.	14·27 gms.	18·24 %	78·4	69·4 %	113·3

Saponification method.

Fresh substance	Fatty acids %	Iodine value of fatty acids
600 gms.	3·35	120·0

It will be observed that the iodine value of the "ether extract" (78·4) is of the same order as that obtained under similar conditions by previous observers. If this ether extract consisted of pure neutral fat, on saponification it should yield 95 % fatty acids and the iodine value of these fatty acids should be about 83. Quite different results are, however, obtained by saponification of the ether extract. Only 69·4 % higher fatty acids are obtained, this being in agreement with Leathes[1] and Paton's[6] results, and the iodine value of these fatty acids is 113·3, this figure being slightly lower than that obtained for the fatty acids obtained by direct saponification. It is thus evident that the ether

extract contains substances other than neutral fat, and it is to the impurity of the ether extracts that the low figures previously obtained for the iodine value must in part be ascribed.

Changes undergone by the fatty acids on exposure to air. Specimens of fatty acids were left exposed to the air for varying intervals of time and the iodine value determined at the end of that time. The following results were obtained:—

Source of fatty acids	Iodine value of freshly prepared acids	Time exposed to the air	Iodine value after exposure
Pig Liver, B.	123·0	8 weeks	65·0
		14 ,,	60·1
Pig Liver, G.	134·7	2 ,,	116·0
		8 ,,	70·8
Human Liver, W. H.	123·0	1 ,,	113·3

The higher fatty acids from an ox liver, prepared by Leathes, were examined after being exposed to the air for two years. It was found at the end of this time that only about one half of the higher fatty acids dissolved in petroleum ether. The iodine value of this soluble portion was 48·3. The portion insoluble in petroleum ether was resinous in appearance. It dissolved almost completely in ordinary ether. The iodine value of this portion soluble in ordinary ether was 59·4.

The fatty acids from the organs undergo change on exposure to the air, shown by the decrease in iodine value. This change is similar to that undergone by the so-called "drying oils"; these oils or fats are glycerides of the doubly or trebly unsaturated linoleic and linolenic acids. This change occurs more rapidly at higher temperatures.

Further examination of the fatty acids from pig's liver. The results given above indicate that the fatty acids from the liver, the kidney, and the heart muscle, have iodine values of the same order. Whether the acids from the three organs are identical is a matter for further investigation. The following experiments were carried out with fatty acids from pig's liver, as this was the most convenient source from which fairly large quantities could be obtained.

I. *Further purification of the fatty acids.* Cholesterol and unsaponified matter may be present along with the fatty acids extracted by petroleum ether. In order to remove these, if present, the following experiment was performed.

20 grams of fatty acids from pig's liver were dissolved in a 30 % solution of caustic soda, an excess of alkali being used. The alkaline solution of the soaps was extracted for 56 hours with ether in a continuous extraction apparatus. The alkaline solution was then acidified with 10 % sulphuric acid, the precipitated acids extracted with petroleum ether,

and the solvent evaporated off on the water-bath. The product obtained was faintly yellow in colour. The iodine value was determined.

Iodine value of fatty acids before the above treatment = 130·0.
Iodine value of fatty acids after the above treatment = 125·3.

Thus, the high figures obtained for the iodine value of the fatty acids in the experiments previously described are not due to substances other than fatty acids.

II. *Determination of mean molecular weight.* The mean molecular weight of the fatty acids obtained as the final product in the above experiment was determined by titration with deci-normal sodium hydrate solution.

0·5238 gm. mixed fatty acids required 18·0 c.c. $\frac{N}{10}$ NaOH.

Mean molecular weight = 291.

0·5684 gm. mixed fatty acids required 19·7 c.c. $\frac{N}{10}$ NaOH.

Mean molecular weight = 288.

The molecular weight of stearic acid is 284.

III. *Separation of the saturated and unsaturated acids.* The separation is based upon the solubility of the lead salts of unsaturated fatty acids in ether.

The fatty acids were dissolved in caustic potash, and the excess of alkali neutralised with acetic acid. A 7 % solution of lead acetate was heated to boiling and gradually added, with constant shaking, to the hot solution of the soaps. On cooling, the clear supernatant fluid was poured off and the lead soaps washed three times with warm water, and then dried. The lead soaps were warmed gently with ether, the ethereal solution filtered off and shaken with 20 % HCl. The precipitated lead chloride and aqueous solution were separated, the ethereal solution washed with water, and the ether evaporated off, the last traces being removed in a stream of dry carbon dioxide. The iodine value of the unsaturated acids was determined.

Source of fatty acids	Iodine value of mixed fatty acids	Iodine value of unsaturated acids
Pig Liver, B.	123·0	189·3
Pig liver, H.	134·0	203·2
Ox liver, A.	111·4	173·8

Since any oleic acid present in the original fatty acids would be contained among the unsaturated acids thus obtained, and since the iodine value of oleic acid is 90 and even that of linoleic acid only 181, it is clear that if the mixed unsaturated acids have an iodine value higher

than 181, this must be due to the presence of acids more unsaturated than linoleic acid. Further, the more oleic acid is present in the mixture, the more there must be of these acids more unsaturated than linoleic acid.

IV. *Bromination of the unsaturated acids.* Unsaturated acids readily unite with bromine forming addition products. Dibromo- and tetrabromo-compounds (obtained from acids of the oleic and linoleic series respectively) are easily soluble in the common organic solvents. Hexabromo-compounds (obtained from acids of the linolenic series) are sparingly soluble in the same solvents, and an octo-bromo-addition product has been described ($C_{17}H_{26}O_2Br_8$) which is almost insoluble.

The high iodine values of the unsaturated acids from the liver indicate the presence of fatty acids containing three and possibly four unsaturated linkages, and should give on bromination insoluble addition products.

Two grams of unsaturated acids were dissolved in carbon tetrachloride and the solution immersed in ice. 1 c.c. of bromine was added, drop by drop. The whole was allowed to stand, cooled, in a stoppered flask for three hours. A white solid separated; this was filtered off, repeatedly washed with cold carbon tetrachloride, dried and weighed: weight, 0·76 gm. The substance became very dark-coloured at 240°, but did not melt. To this dried, insoluble addition product 70 c.c. of dry ether were added, shaken repeatedly and the part still insoluble in ether filtered off, dried, and the bromine estimated by Carius' method.

0·2023 gm. substance gave 0·3261 gm. AgBr	Br = 68·5 %
Calculated for $C_{18}H_{30}O_2Br_6$	Br = 63·3 %
Calculated for $C_{18}H_{28}O_2Br_8$	Br = 69·8 %

Thus the presence of fatty acids containing three unsaturated linkages is confirmed, and evidence obtained of the presence of an acid or acids still more unsaturated than these.

Conclusions.

1. The higher fatty acids from the liver include in addition to saturated acids and acids of the oleic series, considerable quantities of acids of the series $C_nH_{2n-4}O_2$, $C_nH_{2n-6}O_2$, and possibly $C_nH_{2n-8}O_2$.

2. The fatty acids from the kidney and the heart muscle have iodine values of the same order as those of the liver fatty acids, pointing to the presence in these organs, too, of similar acids, in similar amounts.

3. The higher fatty acids from the organs undergo change on exposure to the air. The iodine value diminishes and a great portion of the fatty acids becomes insoluble in petroleum ether.

4. The unsaturated fatty acids yield, on bromination, addition products insoluble in ether, carbon tetrachloride, alcohol, acetic acid etc., similar, therefore, in their properties to the brominated acids of the series $C_nH_{2n-8}O_2$.

REFERENCES.

(1) Leathes. Proc. Physiol. Soc. pp. i–iii. (This Journal, xxxi. pp. 1–3. 1904).
(2) Woltke. Maly's Jahresb. p. 77. 1901.
(3) Leick and Winckler. Schmiedeberg's Archiv, xlviii. p. 163. 1902.
(4) Rosenfeld. Schmiedeberg's Archiv, lv. p. 180. 1906.
(5) Glikin. Maly's Jahresb. p. 83. 1903.
(6) Paton. This Journal, xix. p. 167. 1896.