XIV. THE FATTY ACIDS OF THE HUMAN BRAIN.

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In the following communication evidence is brought forward that the solid fatty acids derived from the lipoids of the human brain do not consist chiefly of palmitic and stearic acids as has been claimed by certain investigators. The observation of Thudichum [1901] that the stearic acid of the human brain melts not at 69° but at 52° has been confirmed, and by fractional precipitation an acid isomeric with stearic acid has been isolated.

I have been unable to find any unoxidised fatty acids containing more than eighteen carbon atoms in the molecule. It has also been shown that at least 25 % of the solid fatty acids of the human brain are hydroxy-acids of higher molecular weight than stearic acid. From the close resemblance in properties which the cerebral hydroxy-acids show to the fatty acids found by Darmstaedter and Lifschütz [1896] in lanoline, it may be of some interest to suggest that we have here another indication of the relationship of the nervous system to other tissues of epiblastic origin.

The cause of the high molecular weight of the brain fatty acids.

About two years ago I pointed out [1910] that the mean molecular weight of the solid fatty acids of the human brain, as determined by titration, was considerably higher than that of stearic acid, this fact being confirmed by analysis of the lead soaps. I was not at that time acquainted with Thierfelder's paper on cerebronic acid [1905]. This acid occurs with other hydroxy-acids of high molecular weight in the precipitate of potash soaps difficultly soluble in alcohol, which is obtained when the saponified mixture from the fatty acids is allowed to cool. Moreover although the potassium salts of these hydroxy-acids are sparingly soluble in alcohol, they remain dissolved to a considerable extent in the presence of the other soaps and

subsequently separate amongst the first fractions precipitated as barium or magnesium soaps. The existence of hydroxy-acids in the brain other than cerebronic acid, with the possible exception of hydroxystearic acid [Koch, 1902] has not previously been noted. It is to the presence of these hydroxy-acids, however, that the observed high molecular weights are to be attributed. In the case of certain fractions it was observed that the apparent molecular weight varied enormously when the acid was regained by decomposition of the lead, barium or even the sodium soap by hydrochloric acid, the acid so regained possessing a higher molecular weight than before. The same phenomenon was observed in another way. When certain fractions of solid fatty acids of high molecular weight were converted into lead soaps, and these lead soaps subsequently exhausted with hot benzene or toluene (a process which Fränkel [1909] used to purify the lead soaps of the brain fatty acids), a decomposition sets in with the formation of a white precipitate rich in lead, and a brown coloured solution, which contains not lead soap, nor free fatty acid, but a material practically devoid of acid properties. It seemed clear that an anhydride or lactone formation had taken place in both the cases mentioned. That this view is correct is confirmed by the following fact. If the fatty acids are regained from the potash soaps difficultly soluble in alcohol, taking no precaution to avoid the dehydrating effects of mineral acids, it is found that the acid mixture so obtained is sparingly soluble in alcohol even at the temperature of ebullition, and after repeated exhaustion with hot spirit a portion is left which when dissolved in a mixture of chloroform and alcohol is found by titration to be only faintly acid; but if the same material is saponified the soap will be found completely soluble in hot alcohol and the material regained by decomposition with acetic acid is a true acid substance neutralising a considerable proportion of alcoholic potash. It is to the presence of anhydride or lactone-like derivatives of the hydroxy-acids that the observed variations in mean molecular weights as determined by titrating with potash, are due.

The lactone formation under the conditions described occurs most markedly, not with cerebronic acid, but with the hydroxy-acid melting at 73.5°.

Preparation of the fatty acids from the brain substance.

The brain after removal of connective tissue and washing was heated with 20 % potash solution. After cooling, the solution was acidified with hydrochloric acid and the fatty layer filtered off. This was then saponified with 10 % alcoholic potash for 15–24 hours. The solution was evaporated to

dryness with previous admixture of sodium bicarbonate, and the powdered residue extracted with anhydrous ether till the cholesterol was completely removed. The details of this process are described elsewhere [Grey, 1912]. The fatty acids were now converted into lead soaps and treated with ether, which was done with the aid of the centrifuge. The lead soaps insoluble in ether yielded acids with an iodine value of 27·5 %. From 150 g. of total fatty acids were obtained 67 g. of acids forming lead soaps insoluble in ether. The mean molecular weight of the solid acids was 320 as determined by titration. The iodine absorption is due chiefly to admixture with a solid unsaturated acid described later. The fatty acids from the lead soaps insoluble in ether were now neutralised with potash. The precipitate which separated on cooling is represented by fraction No. 1 in the accompanying table, and consists chiefly of hydroxy-acids. The filtrate was subjected to fractional precipitation by magnesium acetate, and the fractions 2-5 obtained. The substance remaining in solution (fraction 6) consists chiefly of the unsaturated acid melting at 42° to the presence of which the iodine-absorbing power of the solid fatty acids is due, earlier fractions being practically devoid of iodine value. The large loss of material was due to the volatilisation of the acids by steam.

TABLE I. *Showing the separation of 58 g. of solid fatty acid from the human brain.*

No. of fraction	How obtained	Weight	Melting point	Mean mol. weight by potash titre
No. 1	From potash soap insoluble in cold alcohol	14·6	84 -84·5°	420
No. 2	From first precipitate of magnesium soap	6·8	68·5-69°	399
No. 3	2nd ditto	6·5	55·5-56°	324
No. 4	3rd ditto	5·4	57 -57·5°	303
No. 5	4th ditto	8·0	47·2-47·6°	251
No. 6	Residue in solution ...	6·0	40 -41°	--

Loss 10·7

The hydroxy-acids of the human brain.

These were obtained from fraction No. 1 (*vide* Table I) and represent the acids forming potash soaps sparingly soluble in alcohol. They also separate from fractions 2 and 3 during the further purification of these. The potash soap was brought into solution by boiling alcohol and partially precipitated by magnesium acetate. The hydroxy-acid mixture so obtained was further fractionated by cooling the hot alcoholic solution of the acids, by the repetition of which process a fraction was obtained almost

insoluble in alcohol even when hot. This acid when dry was a white powder, and melted at 100–101°. The acid is not cerebronic acid. The main portion of the hydroxy-acids consisted of two other substances which were separated by recrystallisation from acetone and glacial acetic acid. The portion most soluble in acetone melted at 73·5°. The same substance was also obtained from the earlier fractions of magnesium soaps obtained from the potassium soaps soluble in alcohol.

In the course of these investigations the possibility of the formation of hydroxy-acids was considered, but since the chief hydroxy-acids found were not dihydroxy-acids, but monohydroxy-acids, they could not have been artificial products. It is possible however that small quantities of dihydroxy-acids may have resulted from the oxidation of unsaturated acids present. Experiments done with coal gas yielded practically the same iodine value as when no special precautions were taken. Moreover the isolation of acids of great degree of unsaturation and in particular of one containing six double bonds, indicates that oxidation during manipulation does not play any considerable part.

Nature of the hydroxy-acids.

Three distinct hydroxy-acids have been separated, but small quantities of others seem also to be present. The three acids isolated melted at 73·5°, 91° and 100–101° respectively. The melting point of this last corresponds to that of cerebronic acid, the quantity obtained was small, but judging by the result of a single combustion analysis the substance is certainly not cerebronic acid. On the other hand the acid melting at 91° corresponds closely in composition with cerebronic acid as determined by many analyses. The melting point of this substance remained constant after many recrystallisations from acetone and glacial acetic acid. The analyses of these acids are set forth in the accompanying table.

TABLE II.

No.	Melting point	Weight taken	CO ₂	H ₂ O	C %	H %	Empirical formula
1	100–101°	0·1296	0·3395	0·1376	71·44	11·80	C ₁₇ H ₃₄ O ₃ or C ₂₂ H ₄₄ O ₄
2 (a)	91·0°	0·1077	0·2960	0·1193	74·956	12·308	C ₂₅ H ₅₀ O ₃
(b)	"	0·1034	0·2851	—	75·174	—	
(c)	"	0·1967	—	0·2270	—	12·530	
(d)	"	0·1327	0·3660	0·1500	75·221	12·544	
(e)	"	0·2267	0·6267	0·2567	75·420	12·590	
				Mean	75·193	12·493	
3	73·5°	0·1433	0·3804	0·1730	72·398	12·073	C ₂₀ H ₄₀ O ₃
	"	0·1323	0·3526	—	72·614	—	
				Mean	72·506	12·073	

The composition of Thierfelder's cerebronic acid ($C_{26}H_{40}O_3$) is $C = 75.38\%$: $H = 12.56$ with which substance No. 2 will be seen to be in good agreement.

Substance No. 1 corresponds to the formula $C_{17}H_{34}O_3$ or $C_{22}H_{44}O_4$. The latter formula would be preferable since according to its melting point and other physical properties it can hardly be a lower member of the cerebronic acid series. In this case the acid would be a dihydroxy-acid and might have resulted by the oxidation of a pre-existing unsaturated acid of the formula $C_{17}H_{32}O_2$. Such an unsaturated acid has not yet been described as occurring in brain lipoids.

The acid melting at 73.5° corresponds approximately to the formula $C_{20}H_{40}O_3$ but it cannot be regarded as having been yet satisfactorily examined. It is characterised by the readiness with which it may be transformed into a non-acid lactone-like substance, as already explained.

The unsaturated fatty acids of the human brain.

Since the discovery by Henriques and Hansen [1903] of fatty acids in the brain more unsaturated than oleic acid, no separation of the unsaturated acids seems to have been attempted. The following preliminary separation is therefore described.

About two years ago the author observed that when bromine was added to the ether solution of the unsaturated fatty acids, the temperature being kept at 5° , a white precipitate separated which was found to contain 78–79% bromine, and therefore corresponded to a fatty acid more unsaturated than any previously described as occurring in lipoids. The amount of such highly unsaturated acid was small, nevertheless the result will be seen to be proof of the existence of unsaturated acids of the nature of clupanodonic acid in the human brain. The bromine separation has also shown the presence of the linoleic and linolenic acid series.

Separation of the unsaturated fatty acids.

An amount of 24.1 g. of unsaturated acids was dissolved in ether and cooled to 5° , and bromine added until a definite excess was present; the solution was kept in an ice chest all night, and the precipitate (0.52 g.) separated by decantation, and washed in centrifuge tubes with ether until all bromine compounds soluble in ether were removed. An aliquot portion of the ether solution of bromides was evaporated as a check on the amount in solution. From the weight of bromides obtained it is easy to determine the percentage of bromine absorbed by the acids, which was found to be 77.5%.

The ether was now removed from the main quantity of the soluble bromides, the latter washed free from acetic acid by hot water, and after drying shaken with ligroin of boiling point below 45°. A separation immediately followed, the larger portion being soluble while another portion was apparently insoluble in ligroin, but dissolved readily in chloroform. The quantities found together with the percentage of bromine in the fractions is set forth in the following table.

TABLE III. *The separation of 24.1 g. of unsaturated acids.*

Fraction of fatty acids	Weight of bromide found	Fatty acid which this represents	Bromine per cent.	Percentage of the acid in total un- saturated acids
Clupanodonic acid	0.52 g.	0.13	77.7	0.48
Acids of linoleic and linolenic series	5.64	2.37	58.0	9.83
Oleic acid series	34.23	21.43	37.4	88.92

Properties of the individual fractions of the unsaturated fatty acids.

(1) *Bromine compound insoluble in ether.* To the acid yielding this bromine compound I have given the name *clupanodonic acid* since it is still more unsaturated than clupanodonic acid. Although the quantity used in the analysis was small and the results must be regarded as only indicating the existence of such highly unsaturated acids, nevertheless the substance is quite definite and I have since prepared a gram of the bromide by precipitation of another quantity of unsaturated acids.

The *bromide* is a white powder, which turns a faint straw colour when heated to 120°. With further heating in a small tube it was found to blacken at about 200° and to decompose with further application of heat giving a sublimate and odour of stearic acid. Ignited on platinum foil the substance blackened and melted, burning without leaving any appreciable residue. The bromine compound is practically insoluble in ether, chloroform, and benzene, and only slightly soluble in cold alcohol. In hot alcohol it is more soluble and exhibits the properties of an acid. Titrated with alcoholic potash the molecular weight was found to be over 1000. This number was slightly low on account of the access of carbon dioxide owing to the slowness of the titration, and the titration was moreover carried out on a very small amount of material.

The bromine percentage was determined in two ways.

Data from bromine determinations.

(a) The substance was heated with nitric acid and silver nitrate.

0.2160 g. of bromo-acid gave 0.3946 AgBr. Br = 77.7 %.

(b) The substance was mixed with calcium oxide and ignited as in the method of Piria and Schiff.

0.0347 g. gave 0.0650 AgBr. Br = 79.2 %.

Calculated for dodecabromostearic acid Br = 77.9 %.

(2) *Brominated acids soluble in ether but insoluble in ligroin.* This fraction should contain any acids of the linoleic and linolenic series, and the percentage of bromine found is in agreement with this and points to the mixture consisting of both tetra- and hexa-bromine compounds. It would be premature however in view of the complexity of these lipid fatty acids, to calculate from the bromine figure the proportions of the bromine compounds of either series of which this mixture may consist. During the process of treatment with ligroin it was readily seen that at least two substances were present in the portion insoluble in that fluid for while most of the insoluble material was of a dark colour and of a plastic adhesive nature, a small portion was separated by repeated shaking and decantation which was of a light colour and amorphous. This amorphous material became of a glassy transparent nature when dried at 100° but did not melt at that temperature, whereas the main mass of the bromine compound which was insoluble in ligroin melted below 100°.

0.1556 g. of bromine compound heated with lime gave 0.2094 g. AgBr.

Br = 57.4 %.

Calculated for linoleic tetrabromide Br = 53.33 %.

„ „ linolenic hexabromide Br = 63.33 %.

(3) *Bromine compounds soluble in ether and also in ligroin.* This fraction is generally regarded as consisting of the dibromide of ordinary oleic acid. But in the present case it was found that the liquid fatty acid was admixed with a solid substance corresponding in composition to oleic acid and likewise forming a dibromide soluble in ether and ligroin. The fatty acid has not yet been examined in detail but it is evident that it occurs to a considerable extent mixed with the unsaturated liquid fatty acids of the brain and is difficult to separate from them. The acid is likewise obtained from the solid fatty acid mixture regained from the lead soaps difficultly soluble in ether, and is found left over in this separation, after fractional precipitation of the saturated acids as magnesium soaps (*vide* fraction 6, Table I).

The total bromides soluble in both ether and ligroin gave on analysis a percentage of bromine corresponding to oleic dibromide.

(a) 0.4507 g. gave 0.3880 AgBr. Br = 36.6 %.

(b) 0.4507 g. gave 0.4050 AgBr. Br = 38.2 %.

Calculated for oleic dibromide Br = 36.2 %.

Solid unsaturated fatty acid. This acid can only be briefly referred to. Further examination will be needed definitely to establish its nature. The acid was obtained from fraction No. 6 of the original magnesium soap separation, and also by exhaustion of the ether-insoluble lead soaps by hot benzene. It was purified by repeated crystallisation from acetone at a temperature of 5°. The melting point remained practically constant at 42°. The combustion figures show the substance to be of the nature of oleic acid.

(a) 0.1185 g. gave 0.1286 H₂O.

(b) 0.1323 g. gave 0.1467 H₂O; 0.3703 CO₂.

	<i>a</i>	<i>b</i>	Mean	Calculated for	
				C ₁₈ H ₃₄ O ₂	C ₁₈ H ₃₆ O ₂
C	—	76.34	76.34	76.60	75.66
H	12.10	12.32	12.21	12.06	12.61

The saturated fatty acids of the human brain.

The further separation of these acids obtained from fractions 2–5 of the original magnesium soap precipitation (*vide* Table I) was carried out in the usual way. The fractions were subdivided by precipitation with barium acetate and subsequently converted into silver salts which were submitted to combustion analysis. The fatty acids were regained from the silver salts and the melting points determined. In many cases the acids themselves were submitted to combustion analysis. As already pointed out, no acid of more than eighteen carbon atoms was found. The acids isolated and identified were ordinary stearic, palmitic and myristic acid, and in addition, in quantity quite equal to that of ordinary stearic acid, an isomer of stearic acid melting at 51–52°. This therefore confirms the discovery of Thudichum [1901] that the stearic acid occurring in greatest amount in the brain is not ordinary stearic acid melting at 69° but an isomer melting at 52° to which he gave the name neurostearic acid.

GENERAL CONCLUSIONS.

1. At least 25 % of the solid fatty acids of the human brain are hydroxy-acids, of which three have been isolated. Two of these at least are monohydroxy-acids and cannot therefore have been produced artificially by oxidation of unsaturated fatty acids.

2. The unsaturated acids contain besides oleic, linoleic and linolenic acids an acid still more unsaturated to which the name clupanodonic acid has been

given. It combines with twelve atoms of bromine. Such a compound has not previously been described as occurring in fats or oils.

There is present also a solid unsaturated acid melting at 42°, which is probably an isomer of oleic acid.

3. The saturated fatty acids contain normal stearic and palmitic acids and an isomer of stearic acid melting at 51–52°. The presence of myristic acid has also been confirmed.

4. In the resemblance between the hydroxy-acids of the brain, and those of lanoline, there seems to be additional evidence of the relationship of the nervous tissue to other tissues of epiblastic origin.

In conclusion I would express my thanks especially to Dr H. G. Chapman, at whose suggestion this work was begun, and to Professor T. P. Anderson Stuart, in whose laboratory the investigation was carried out.

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