THE FATTY ACIDS OF BRAIN LIPOIDS.

Part i.

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(From the Physiological Laboratory of the University of Sydney.)

Contents.

The development of lipoid chemistry is reviewed by Bang* and by Glikin.† It is intended here to bring together the work done with regard to the fatty acids of lipoids.

Diakonow in 1868(1) showed that various fractions of phosphatides could be obtained, differing in the nature of their fatty radicle. He separated substances containing oleic and stearic acid; subsequently Strecker(2) added palmitic acid. Thudichum(3) showed that lecithin always contains oleic acid and another acid. Acids more unsaturated than oleic acid have been found by Henriques and Hansen(4), and Cousin(5) in lecithin of eggs and brain-substance; and by Erlandsen(6) in heart and voluntary muscle.

Thudichum noted the existence of unknown fatty acids in paramyelin, kephalinic acid in kephalin, and an oxyacid in amido- and sphingomyelin. Koch(7) obtained from kephalin dihydroxy-

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* Ergebnisse der Physiologie, Bd. vii. 1907.

† Handbuch der Biochemie (Oppenheimer), 1907.

stearic acid without oxidation. As regards the fatty acids of protagon, reliable data are wanting.

Although, however, much labour has been expended on lipoids, most of it has drifted in the wrong direction of examining by qualitative means substances ill-characterised and obtained by chance solvents. No problem in biochemistry could be more definite than that of determining the proximate constituents of various organs, and these must surely be known before we are concerned with the more complex structures into which they may be built. The most obvious step in advancing our knowledge of the lipoids is to examine the fatty acids. Hartley* has rightly entered the field in his examination of the fats of viscera. This author has shown that his results apply to a great extent to the lipoids. In the case of the brain, however, what is said of the fatty acids applies chiefly to those combined as lipoids, the quantity of free fat being negligible.

On the following page is given in tabulated form a review of our knowledge of the fatty acid radicles of lipoids up to the present time.

The object of this work is to examine the fatty acids of brain lipoids. Part i. deals with the total fatty acids as obtained by direct saponification of the brain.

LITERATURE.

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(2) STRECKER—Annalen der Chem. Pharm. Bd.cxlviii., s.77, 186.
(3) THUDICHUM—Chem. Konst. der Gehirns der Menschen u. Tieren, Tübingen, 1909.
(4) HENRIQUES & HANSEN—Skandr. Arch. für Physiol. Bd.xiv. p 3, 1903.
(5) COUSIN—Comptes Rendus Soc. Biol. tome 55, 1903.
(6) ERLANDSEN—H. S. Zt. Physiol. Chem. Bd. 54, ss.71, 83, 104, 1907.
(7) KOCH—Zt. Physiol. Chem. Bd. 36.
(8) ZUELZER—ib. Bd. 27, s.259, 1899.
(9) BASKOFF—ib. Bd. 55, s.395, 1908.
(10) KOSSEL U. FREYTAG—ib. Bd. 17, s.431, 1893.

* Hartley, Journal of Physiology, Vol. xxviii. p.353(1909).

Lipoid.	Class.	Origin.	Reference.	Nature of Fatty Acid.	Remarks.
Lecithin	Monamino-monophosphatides.	Egg yolk.	Henriques & Hansen(4)	Oleic and linoleic besides stearic and palmitic.	These were not pure lecithins.
,, ...	,,	Ox brain.	Cousin(5)	Linolenic besides others.	
			Henriques & Hansen.	Same as egg lecithin.	
,, ...	,,	Ox heart-muscle.	Erlandsen(6).	Fatty acids poor in hydrogen must contain linoleic series or higher.	Deduced from ultimate analysis.
,, ...	,,	Ox brain.	Zuelzer(8).	Palmitic acid.	Not very conclusive.
Kephalin	,,	Ox brain.	Koch(7).	Dioxystearic acid. Unsaturated acids.	Data not very complete.
Versalthin	Monamino-monophosphatide.	Ox pancreas.	Fränkel & Pari.	Myristic acid and an unknown unsaturated acid.	
Cuorin	Monamino-diphosphatide.	Ox heart-muscle.	Erlandsen.	Fatty acid with low percentage of hydrogen.	From ultimate analysis
Unnamed	Diamino-monophosphatide.	do.	Erlandsen.	Fatty acid $C_{22}H_{39}O_4$	This composition is calculated.
Jecorin.....	....	Horse-liver.	Baskoff(9).		
Protagon.....	....	Brain.	Thudichum(3).	Neurostearic acid $C_{18}H_{36}O_2$	
,, ...	.....	,,	Kossel & Freytag (10).	Stearic acid $C_{18}H_{36}O_2$	
Amido & sphingomyelin.	.....	Human brain.	Thudichum.	Oxyacid $C_{21}H_{36}O_4 - C_{22}H_{38}O_4$	
Total lipoids in tissues.	....	Liver (pig).	Hartley.	Linoleic acid. Liquid fatty acid of high mol. wt.	It was not determined whether this was present as lipoid or simply as fat.

Part i. Fatty Acids obtained by direct saponification of the whole brain.

i. *Total fatty acids in human brain.*
ii. *The nature of the fatty acids in the human brain.*

Of the methods which have been proposed for the estimation of the fatty acids in tissues, the one which is based on the soundest principle seems first to have been proposed by Liebermann.* This method has been adopted here with certain modifications† in estimating the total fatty acids in the brain, and also in obtaining larger quantity of the acids for examination. The modifications are necessary for tissues which, like the brain, contain much unsaponifiable matter.

i. Total Fatty Acids in Human Brain.

The fresh brain, freed as far as possible from superficial connective tissue and blood, was pounded in a mortar, and passed through a wire-sieve. The whole was then thoroughly mixed, and samples immediately weighed into small flasks, fifty grms. in each sample, and covered with 150 c.c. of alcohol. These samples were used subsequently as required. For saponification, samples were taken with 25grms. KOH, and heated for six hours in reflux condenser. The alcohol was partially removed, and the concentrated solution evaporated in a porcelain dish with the addition of sand and sodium bicarbonate. The hard, dried residue was finely powdered, and thoroughly extracted with anhydrous ether till the extracting fluid, on evaporation, left no significant residue of cholesterol. The cholesterol-free soap-powder was then decomposed by HCl, and the fatty acids extracted with ether, washed free of mineral acid, and dried in current of anhydrous CO_2.

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*Liebermann, Pflüg. Arch. 72, 360.

† This method has also been modified by Kumagawa and Suto (Biochemische Zeitschrift, Bd. ix., s.212), but the slight modifications which they have suggested do not entitle them to the claim to be authors of the method. Many subsequent authors also seem to be unaware that Liebermann was the author of this method.

The following figures give the total fatty acids and cholesterol found in moist brain-substance in three such experiments. The results are also given, calculated upon the quantity of solid matter in the brain, as found by drying to constant weight at 100°C. The fallacy in drying lipoid-containing substance in the air at 100°C. will be obvious, but the results are given thus, pending a better meaning of the term " total solids."

	Fresh Brain.		Calculated to Solids.	
	Fatty acid.	Cholesterol.	Fatty acid.	Cholesterol.
No. 1	5·28	2·19	24·07	9·98
2	4·82	2·28	21·98	10·40
3	4·95	2·18	22·57	9·94
Mean...	5·02	2·22	22·87	10·10

The brain-pulp gave, as average of four estimations, 78·07 % moisture, or 21·93 % total solid matter.

ii. The Nature of the Fatty Acids of the Brain.

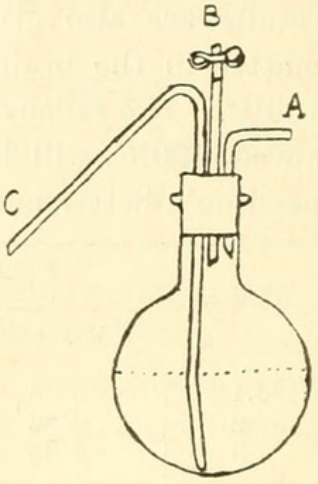
Method of obtaining the fatty acids.

The brain-substance was saponified in small lots (three), with potash, the whole operation being conducted in an atmosphere of coal-gas; the saponified mixture was then poured into a large flask and treated with excess of H_2SO_4(20 %), or HCl(1 in 4), freed from oxygen (by cooling in a stream of coal-gas), ether was added, and the whole transferred to a winchester quart and placed in a shaking machine for one hour; the coloured ethereal layer was separated and concentrated; the concentrated ethereal solution was then resaponified by the method of Kossel and Obermüller.* This elegant method is well adapted to work of this kind. The precipitated soaps were allowed to stand all night, then centrifuged, and washed repeatedly in the centrifuge with ether (five washings) till the ethereal solution was colourless.

The united sodium-soaps were preserved in dessicators in an atmosphere of CO_2.

* Kossel & Obermüller, H. S. Zeit. phys. Ch., Bd. xiv. 599(1890).

In the preliminary examination of the fatty acids the soaps were decomposed in the apparatus pictured below.



The object of this apparatus is to liberate and wash the fatty acid in an atmosphere of CO_2. Tube A is connected to a Kipp apparatus, and B is kept open.

When the separated fatty acid has risen to the surface, tube B is closed; the force of CO_2 then drives the watery solution out at C, which continues to siphon till tube B is opened. The apparatus is heated in a water-bath. The washed fatty acid is then transferred to a weighing bottle, and dried at 100°C. in a current of anhydrous CO_2.

Nature of fatty acids from human brain.

In a preliminary examination, the lipoids had not been completely decomposed, only a portion of the total fatty acids being split off. For complete splitting up of the lipoids by means of 20 % alcoholic KOH, six hours at a boiling temperature is required. An interesting observation was made, however, in that the fatty acids split off most easily from the lipoid bodies have a smaller iodine-absorption figure than those more difficult to split off.

The mean iodine-value of the total fatty acids from brain was found to be 81·3 % in twelve to fifteen hours, while a sample obtained by incomplete saponification gave a mean absorption of 51·3 % iodine(Hübl).

The fatty acids split off at an early stage by incomplete saponification are, when first obtained, pale yellow in colour but gradually darken on keeping, and rapidly at 100°C.; but when the saponification is continued for six hours, the fatty acids obtained are brown in colour.

These results point to the conclusion that the lipoids which most resist saponification contain fatty acids of an unsaturated nature.

Data for iodine-absorption :

No.1. 0·4784 grm. fatty acid absorbed 29·7 cc. N/10 I_2—78·85 % I_2.
No.2. 0·4447 grm. fatty acid absorbed 29·2 cc. N/10 I_2—83·3 % I_2.

Slightly impure oleic acid under same conditions :

No.1. 0·5 grm. absorbed 36·75 cc. N/10 Iodine—93·2 % I_2.
No.2. 0·5 grm. absorbed 36·20 cc. N/10 Iodine—91·9 % I_2.

Separation of saturated and unsaturated fatty acids.

The separation is based on tho solubility of the lead-soaps of the higher unsaturated fatty acids, and the insolubility of those of the saturated fatty acids in ether. The method of Dekonigh and Muter, with the modification of Drechsel, was used. Air was carefully excluded prior to determination of iodine-absorptions.

The unsaturated fatty acids of human brain.

The mean iodine-absorption of the liquid fatty acids was found to be 110·6 (in twelve hours, Hübl). Since, under exactly similar conditions, that for oleic acid was much less than this, it follows that the liquid fatty acids of the human brain are more unsaturated than oleic acid.

An approximate calculation of the quantity of linoleic acid which this would represent, shows that the liquid acids contain

Oleic acid..... 87·8 %.
(Equivalent of) linoleic acid ..... 22·2 %.

The saturated fatty acids of human brain.

The fatty acids which were obtained from the ether insoluble soaps, by decomposition with hot HCl, were washed acid-free with boiling water and dried. The ether-solution was decolourised with animal-charcoal, and gave, on evaporation, perfectly white fatty acids which set, on cooling, to an amorphous mass. The fatty acid had a faint odour of beeswax.

The melting point of the solid fatty acids was 51·4°C.

The mean molecular weight calculated from analysis of lead-soap was 318·7. This interesting result was immediately checked

by determination of the saponifying equivalent with KOH. The figures, though low compared with lead-estimation, are consistently high, and give a mean molecular weight of 308·3. It is probably difficult to obtain the lead-soap pure.

From these results there can be no doubt that the solid moiety contains fatty acids of molecular weight much higher than those of previously mentioned fatty acids. (The molecular weight of stearic acid is 284). The low melting point of the mixture is also significant, and does not correspond to any simple mixture of palmitic and stearic acids.

Data for mean molecular weight.

0·2872 grm. lead-soap gave 0·1033 grm. $PbSO_4$: mean mol. wt. = 318·7.

Saponification with alcoholic KOH

(1) 3·9812 grm. neutralised 26·0 cc. N/2 KOH, mean mol. wt. = 306·8.
(2) 0·9850 ,, ,, 31·8 cc. N/10 KOH ,, ,, ,, 309·7.
(3) 1·4786 ,, ,, 48·3 cc. N/10 KOH ,, ,, ,, 304·1.
(4) 1·4786 ,, ,, 48·6 cc. N/10 KOH ,, ,, ,, 306·4.

Separation of the saturated fatty acids.

This is based on fractional precipitation with magnesium-acetate.

The separation was carried out with 4·5 grams fatty acid, but the results prove definitely the existence of a fatty acid of high molecular weight in the brain as already mentioned.

Four small fractions were separated with the following results:

	Weight of fraction.	Mean mol. wt.	Melting point.
No.1....	1·17 g.	344·2	54·0 C.
No.2....	1·33	291·6	55·5
No.3....	0·68	288·0	
No.4....	0·40	338·0	52·1

No.4 was obtained by making filtrate from No.3 strongly ammoniacal, and is probably the same as No.1.

Fractions 2 and 3 had many characteristics of a mixture of palmitic and stearic acids, but the colour and low melting point prove them to be more complex.

Data for molecular weight determinations.

No.1.	1·1668 grm.	neutralised	33·9 cc. N/10 KOH.	Mean mol. wt. 344·2.
No.2.	1·3297 ,,	,,	45·6 cc. N/10 KOH.	,, ,, ,, 291·6.
No.3.	0·5154 ,,	,,	17·85 cc N/10 KOH.	,, ,, ,, 288 7.
No.4.	0·3595 ,,	,,	10·45 cc. N/10 KOH.	,, ,, ,, 338·3.

General conclusions.

(1) The fatty acids of the human brain are more complex than has previously been supposed.

(2) The liquid portion contains fatty acid more unsaturated than oleic acid, equivalent to not less than 22 % linoleic acid.

(3) The solid portion contains, besides stearic and palmitic acids, also a fatty acid of high molecular weight but low melting point. It is not crystalline, and probably belongs to a different group from stearic acid.

In conclusion, I beg to express my thanks to Professor Anderson Stuart, in whose laboratory this work was done; and to Dr. H. G. Chapman, at whose suggestion it has been undertaken.