CCLXXVI. THE LIPOIDS OF SPLEEN AND LIVER IN VARIOUS TYPES OF LIPOIDOSIS¹.

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THE study of lipid distribution in derangements of lipid metabolism may be expected to throw new light on the physiology of lipid formation, transport and storage, just as the knowledge of carbohydrate metabolism is based to a great extent on the study of diabetes. At least three pathological entities of generalised "lipoid storage" disease or lipoidoses have been distinguished from clinical and pathological evidence and confirmed by chemical investigations: (1) Gaucher's splenomegaly, characterised by large deposits of the cerebroside kersin in spleen and liver; (2) Niemann-Pick's disease, in which the phosphatide and cholesterol contents of the viscera, the bone-marrow and the brain are increased at the expense of neutral fat; (3) Schüller-Christian's disease with replacement of bone tissue by cholesterol deposits. The outstanding chemical characteristics of these diseases have been described in case reports in the medical literature. However, since the methods used and the condition in which the organs are obtained from the *post mortem* room leave much to be desired from the point of uniformity, we took advantage of the simultaneous availability of fresh organs from several cases of these relatively rare conditions and studied the lipid distribution in spleen and liver of three cases of Gaucher's disease, two cases of Niemann-Pick's disease, and three control cases. As the methods of extraction have been discussed in another connection [Sobotka *et al.*, 1930; 1932] they will be only briefly described in the experimental part.

The synopsis in Table I shows that three cases of Gaucher's disease give concordant results, namely, shifts from the phosphatide fraction to the neutral fat fraction, and possibly an increase of cholesterol in comparison with the control analyses. The main characteristic consists in the increase of the balance in the acetone-insoluble lipid mixture after allowance is made for cholesterol and phosphatides. This increase is due to the presence of considerable amounts of the pathological cerebroside kersin [Lieb, 1924]. The cerebroside was isolated following Rosenheim's procedure [1914; 1916]. Spheruliths of kersin, completely free from phrenosin, were identified by Rosenheim's test with the selenite plate under the polarising microscope and 5 g. pure kersin were prepared from 52 g. of pooled acetone-insoluble material of spleen and liver (cases C and T).

The analysis of case H of Niemann-Pick's disease revealed remarkable increases of phosphatides and cholesterol esters over normal and the almost complete absence of neutral fat. The latter finding was duplicated in another case of Niemann-Pick's disease the analysis of which we owe to Dr J. Kahn, who will communicate his findings in detail elsewhere [1933]. A new feature in this

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Table I. *Synopsis of lipid analyses.*

Case	Weight moist g.	Lipoids g.	Tot. lip. Tot. sol.	Percentages of total lipoids					Free chol. Tot. chol.	Fatty acids
				Free chol.	Chol.-ester	Phosphatides	Neutral fat + E.A.	Balance (Cerebr.)		
Spleens										
Gaucher T	2615	280	50.6	11.6	0.5	17.7	33.6	36.6	97	44.8
Gaucher C	2100	132	31.7	6.5	7.5	33.5	26.4	26.1	60	51.6
Gaucher B	305	19.4	30.5	4.0	10.6	21.9	35.6	27.9	39	54.2
Gaucher average	—	—	—	7.4	6.2	24.3	31.9	30.2	—	50.9
Niemann-Pick H	180	14.7	45.8	9.6	6.9	62.6	1.2	19.7	70	47.9
Niemann-Pick (Kahn)	205	25.9	58.5	25.8	11.7	21.8	2.3	33.4	80	22.5
Controls (3)	—	—	13.6	10.1	1.5	41.5	25.9	21.0	91	54.7
Livers										
Gaucher T	3400	252	32.1	7.5	11.5	26.6	22.7	31.7	67	45.5
Gaucher C	4100	304	34.3	9.6	11.5	15.5	33.1	30.3	58	47.6
Gaucher B	405	45.6	46.6	9.7	6.4	31.1	27.1	25.7	72	50.7
Gaucher average	—	—	—	8.9	9.9	24.4	27.6	29.2	—	47.9
Niemann-Pick H	500	60.5	33.4	4.9	11.3	67.9	0.3	15.6	47	46.1
Niemann-Pick (Kahn)	180	20.6	41.7	20.4	6.9	28.5	7.8	36.4	84	30.5
Controls (2)	—	—	23.3	4.9	2.3	39.1	24.3	29.4	79	51.7
Brains										
Gaucher B	805	50.1	38.4	8.6	42.6	23.8	17.3	7.7	25	51.8
Niemann-Pick H	630	48.8	45.6	3.4	25.5	53.5	0.0	17.6	18	48.7

case was the great increase in cholesterol. One portion of the acetone extract consisted practically of pure cholesterol which crystallised in large characteristic colourless platelets from the pigmented fluid. Cholesterol ester was almost absent. Parallel with this increase and with the decrease in the amount of neutral fat, is the reduction in the quantity of phosphatides, especially pronounced in the spleen. The increase in the balance of the acetone-insoluble fraction (*minus* cholesterol and phosphatides) is at least partly due to the presence of kersin of which 1.8 g. could be crystallised and identified; the presence of phrenosin was definitely excluded. Products of partial hydrolysis of phosphatides as phosphatidic acids [Chibnall and Channon, 1927] and of cerebrosides such as psychosin [Thudichum, 1884] and acyl-sphingosines might contribute to this fraction.

The common fatty acids, stearic, palmitic and oleic acids, are presumably distributed between cholesterol ester, neutral fat and monoaminophosphatides (lecithins and kephalins), while the higher acids, especially lignoceric acid, are found in diaminophosphatides (sphingomyelin) and in cerebrosides. Thus, we may estimate the amount of the ordinary fatty acids from the percentages contained in cholesterol ester (43.3 % oleic acid), phosphatides (70.3 % for stearo-oleolecithin) and triglycerides (95.6 % for stearopalmito-oleoglyceride). Any free fatty acid, originally present or formed by decomposition, would be included in the figure for neutral fat according to the procedure of extraction. As will be seen from the last column of Table I, the total amount of fatty acid varies around 50 % for all cases investigated with the exception of Kahn's case.

The lipoids of animal tissues are intimately interlocked with each other through the presence of common groups as exemplified in the following scheme. The interconversion of lipoids can thus be interpreted as "exchange esterification" brought about by the hydrolytic and synthetic activity of esterases [Sobotka, 1930]. In the lipid disorders under investigation, the enzymic regulation of these equilibria seems to be disturbed. Substances, useful under normal conditions as transport forms, are not further metabolised, but are apt to clog the organs, especially those of the reticuloendothelial system. This is the case in Gaucher's disease with kersin, while in Niemann-Pick's disease (case H), lecithin and cholesterol ester accumulate at the expense of neutral fat as indi-

cated in the scheme above by the arrows. This process went even further in Kahn's case with subsequent depletion of the common fatty acids.

Liver esterase. A study of the esterase in the liver specimens strongly supports the assumption of enzymic deficiency. The esterase contents of twelve livers were determined by the saponification of methyl butyrate. Four livers from cases of Gaucher's disease and one liver from a case of Schüler-Christian's disease contained diminished amounts of esterase when compared with three normal livers (accidental violent death). Liver esterase was also diminished in a case of fatty degeneration (alcoholism) and in an atrophied specimen, and it was almost absent in tuberculosis. The liver of Kahn's case of Niemann-Pick seemed to contain no esterase at all while the liver of our case H was not included, as this enzymic study was begun in the later course of the work.

EXPERIMENTAL.

The fresh organs were chopped and extracted with cold acetone and subsequently in a Soxhlet apparatus with hot acetone, ether and finally with alcohol. White matter precipitated from the hot acetone extract upon cooling. Ether and alcohol extracts from the same organ were combined for analysis. All fractions were analysed for total and free cholesterol, phosphorus and Kjeldahl nitrogen. The results for the cold acetone extract and for the supernatant of the hot acetone extract were combined. Allowance was made for the fatty acid bound with cholesterol, and the balance—after deduction of the weights of cholesterol, cholesterol esters and phosphatides calculated as lecithin—was assumed to consist of neutral fat and free fatty acid. The analytical results for the white matter from the hot acetone extract were added to those for the ether *plus* alcohol extract. The amounts not accounted for as cholesterol or phosphatides comprise the cerebrosides and other less soluble phosphorus-free lipoids. From these fractions kerašin was isolated, if present, according to Rosenheim's procedure. The white matter was extracted with tetralin [Page, 1930]; the tetralin extract was poured into excess acetone, and the precipitate was extracted with light petroleum, leaving all phosphatides behind. The material soluble in light petroleum was recrystallised from a mixture of equal volumes of chloroform and alcohol and from 90 % acetone. The white substance obtained had $[\alpha]_D = -9.2^\circ$; it contained 1.96 % N (calculated for kerašin, 1.73) and yielded on hydrolysis 23.0 % (calculated, 22.2) sugar by Bertrand's method, calculated for galactose. It contained less than 0.05 % phosphorus and crystallised from pyridine in spheruliths which proved to be pure kerašin in the selenite plate test.

The measure of esterase is the esterase unit (E.U.), equal to that amount of enzyme which hydrolyses 0.2 g. of methyl butyrate in 20 cc. of a buffer solution to an extent of 25 % in 60 minutes at 30° . 8 cc. of a $M/3$ buffer mixture of $(\text{NH}_4)_2\text{HPO}_4$ and NH_3 in the proportion 9.5 to 0.5 were used for 20 cc. reaction

mixture making the initial p_H 8.2. The amount of hydrolysis was determined by titration with $N/5$ NaOH with thymolphthalein as indicator.

One part chopped fresh liver was allowed to stand for 7–14 days with 3 parts glycerol. The strained glycerol extract was used in a dilution equivalent to 100 mg. fresh liver or 25 mg. dry liver tissue for 20 cc. of reaction mixture. The enzymic strength of a material was defined by Willstätter and Memmen [1924] by its esterase value (E.v.) which equals the amount of E.U. in 10 mg. Thus, the amount of E.U. calculated in the above tests from the amount of butyric acid liberated, multiplied by 10/25, gives the E.v. of a specimen. Aqueous ammoniacal extracts of acetone-dried liver tissue are always weaker than the glycerol extracts from fresh liver.

The E.v. of three normal livers were 1.76 (0.54 for acetone-dried sample), 1.60 and 1.42, those of the Gaucher cases 0.69 (T), 0.27 (C), 0.23 (B), and 0.80 (Bayonne; acetone-dried sample, 0.16). In the last case, Gaucher's disease was an incidental *post mortem* finding in an accidental death. The liver from a child with Schüler-Christian's disease had an E.v. of 0.27, that from a case of Niemann-Pick's disease (Brooklyn) of which only an acetone-dried specimen was available, had no activity whatsoever. Other diseased livers, examined for comparison, included fatty degeneration with E.v. 0.57, a liver atrophy with E.v. 0.55, and a tuberculous liver with the low E.v. of 0.13.

SUMMARY.

The lipoids of liver and spleen in three cases of Gaucher's disease were analysed and compared with the findings in two cases of Niemann-Pick's disease and with normals. The classification of these diseases according to the chemical nature of the lipoids deposited is criticised. The various shifts in lipid distribution are explained by deficiency of lipolytic enzymes. Deficiency of liver esterase is demonstrated experimentally in these and similar cases.

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