

marked alterations in the phospholipid partition, similar to those in the plasma, are produced, *i. e.*, an elevation in total phospholipid, cephalin and sphingomyelin to values higher than normal with the amount of lecithin decidedly decreased. A similar increase of ether-insoluble phospholipid (sphingomyelin) in plasma and cells during treatment of pernicious anemia has been reported by Kirk, although no consistent changes in other phospholipids were observed.⁵

In contrast to the changes in pernicious anemia are those accompanying the elevated total phospholipid in diabetic and essential lipemia.⁶ Not only the absolute amount of lecithin but also its proportion in the total was increased. Cephalin was higher, but not proportionately so, in the essential lipemia, whereas no cephalin whatever was found in the plasma of the diabetic lipemia. Sphingomyelin was increased slightly in the plasma of both. The phospholipid partition of the erythrocytes, however, was essentially normal.

Study of an individual with Schüller-Christian disease indicates that in this condition there is a tendency toward an increased amount of sphingomyelin, with a compensatory decrease of lecithin in both plasma and cells.

These preliminary data indicate the need for further investigation concerning the physiological rôles of the individual phospholipids, lecithin, cephalin, and sphingomyelin, in health and disease.

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Relation Between Cerebroside and Neutral Fat Contents of Blood Plasma, Erythrocytes and Stroma.

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That cerebroside may exist in small amounts in blood has been shown by the development and application of a titrimetric microprocedure for this group of lipids.^{1, 2, 3} This method, slightly modified, has been employed on lipid extracts of blood, stroma and brain. The data indicate that in erythrocytes, stroma, and brain, much of the

⁵ Kirk, E., *Am. J. Med. Sci.*, 1938, **196**, 648.

⁶ Bernstein, S. S., Williams, H. H., Hummel, F. C., Shepherd, M. L., and Erickson, B. N., *J. Pediat.*, 1939, **14**, 570.

¹ Kimmelstiel, P., *Biochem. Z.*, 1929, **212**, 359.

² Kirk, E., *J. Biol. Chem.*, 1938, **123**, 613.

³ Kirk, E., *J. Biol. Chem.*, 1938, **123**, 637.

lipid which formerly was designated as neutral fat* is cerebroside, whereas in plasma only negligible amounts of cerebroside are present. Furthermore, there are indications that the cerebrosides may comprise a significant fraction of the blood lipids in certain pathological conditions.

Method. Procedure for hydrolysis: The procedure of Kirk² was modified to facilitate hydrolysis of the cerebrosides and filtration of the hydrolysate. Aliquot samples of the lipid extract and blanks for initial and total reduction values² are measured into 10 cc glass-stoppered graduated cylinders. The solvents are evaporated carefully in a boiling water bath. To each of the samples for initial reduction are added 3 cc of water, and to each of those for total reduction 2 cc of 6 *N* hydrochloric acid. The cylinders are stoppered tightly and heated for 15 minutes in a boiling water bath to emulsify the lipids and hydrolyze the cerebrosides. After cooling, 1 drop of phenol red, 0.5 cc of 4% zinc sulfate solution ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), and 2 cc of 0.1 *N* sodium hydroxide are added to each sample for initial reduction, after which they are diluted to 10 cc and filtered through a medium weight filter paper. To each sample hydrolyzed with hydrochloric acid are added 1 drop of phenol red indicator and, in succession, the following: 40% sodium hydroxide (drop by drop) until the solution turns pink; 1 *N* hydrochloric acid until a yellow color is evident; and 0.1 *N* sodium hydroxide until a pink color develops. Finally, 0.5 cc of the 4% zinc sulfate solution and 2 cc of 0.1 *N* sodium hydroxide are added, the samples diluted to 10 cc and filtered through the medium weight paper.

Determination of reduction values: The sugar reduction values are determined with potassium ferricyanide according to the procedure of Hagedorn and Jensen.⁴ Aliquots of the filtrates are measured into 1 by 4 inch titer tubes. Exactly 2 cc of 0.02 *N* potassium ferricyanide solution[†] are added to every sample. The titer tubes are covered with agate balls (1 inch diameter), heated in a boiling water bath for 15 minutes and cooled. Just before titration, 3 cc of iodide-sulfate-chloride reagent,[‡] 2 cc of 3% acetic acid and a few

* Heretofore in determinations of the lipid partition of blood and tissues, all of the lipid unaccounted for as phospholipid or cholesterol and its esters has been included in the neutral fat fraction.

⁴ Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46; *ibid.*, 1923, **137**, 92.

[†] Potassium ferricyanide solution—1.65 g of potassium ferricyanide and 10.6 g of fused sodium carbonate in 1000 cc of water.⁴

[‡] Iodide-sulfate-chloride solution—10 g of zinc sulfate and 50 g of sodium chloride plus water to make 200 cc. Just before using add 0.5 g of potassium iodide per 20 cc of solution.⁴

drops of starch solution are added. Titration with standard 0.005 N sodium thiosulfate solution, to the disappearance of the blue color, is carried out with a microburette. The reduction values in terms of galactose and cerebrosides are calculated according to Kimmelstiel¹ and Kirk.²

Results and Discussion. Cerebroside analyses of plasma and erythrocytes are given in Table I. Small amounts of cerebrosides were detected in normal plasma and in plasma samples from polycythemia, Schüller-Christian disease, and diabetic lipemia; in the latter the neutral fat fraction was elevated ten-fold. A slight elevation of plasma cerebroside accompanied remission of pernicious anemia.

The cerebroside content of normal erythrocytes averaged 47 mg per 100 g of cells, thus comprising 9% of the total lipid (Table I). The red cells in severe pernicious anemia possessed an increased amount of cerebrosides which was further elevated during partial remission and returned to a more normal concentration with a complete remission. Similar observations have been noted by Kirk.^{3, 6}

TABLE I.
Cerebroside and Neutral Fat Content of Plasma and Erythrocytes.

	No. cases	Cerebrosides		Neutral fat*		Neutral fat minus cerebrosides	
		mg	%†	mg	%†	mg	%†
<i>Plasma (per 100 cc)</i>							
Normal	4	15	2	225‡	31‡	210	29
” §	13	42	8				
<i>Pernicious anemia</i>							
Severe	3	17	4	121	30	104	26
Partial remission	1	31	8	107	28	76	20
Remission	1	47	10	70	15	23	5
Diabetic lipemia	2	27	0.2	1854	39	1827	39
Polycythemia	4	9	1	69	20	60	19
Schüller-Christian	1	2	0.4	148	27	146	27
<i>Erythrocytes (per 100 g)</i>							
Normal	4	47	9	51‡	10‡	4	1
Normal§	13	51	12				
<i>Pernicious anemia</i>							
Severe	1	85	21	123	30	38	9
Partial remission	1	131	25	131	25	0	0
Remission	1	103	16	103	16	0	0
Diabetic lipemia	2	400	50	400	50	0	0
Polycythemia	4	56	8	110	14	54	6
Schüller-Christian	1	69	13	69	13	0	0

*Includes cerebrosides.

†% of total lipid.

‡Values from previous studies.⁵

§Values from Kirk.³

⁵ Williams, H. H., Erickson, B. N., Bernstein, S., Hummel, F. C., and Macy, I. G., *J. Biol. Chem.*, 1937, **118**, 599.

⁶ Kirk, E., *Am. J. Med. Sci.*, 1938, **196**, 648.

TABLE II.
Cerebroside and Neutral Fat Content of Stroma and Brain.

	No. cases	Cerebrosides		Neutral fat*		Neutral fat minus cerebrosides	
		mg	%†	mg	%†	mg	%†
<i>Stroma</i> (per 100 mg dry)							
Normal							
Human	5	1.7	14	1.3‡	12‡	0	0
Sheep	1	2.8	11	2.8	11	0	0
Horse	1	1.0	8	1.0	8	0	0
Polycythemia—human	7	1.9	9	3.0	15	1.1	6
Embryo—beef	6	1.6	8	2.1	10	0.5	2
Horse							
Tetanus antitoxin	4	1.4	8	3.2	19	1.8	11
Diphtheria antitoxin	3	1.1	7	1.4	9	0.3	2
<i>Brain</i> (per 100 mg, dry)							
Dog							
Normal§	2	4.0	10	12.0	28	8.0	18
Disulphide anemia§	2	7.9	19	11.6	28	3.7	9

*Includes cerebrosides.

†% of total lipid.

‡Values from previous studies.⁷

§Values from previous studies.⁸

In diabetic lipemia, a large part of the erythrocyte lipid consisted of cerebrosides (50% of the total lipid). This is in striking contrast to observations made on plasma, which contains only a small quantity. The erythrocytes in polycythemia and Schüller-Christian showed no abnormalities in cerebrosides.

Cerebrosides appear to be a structural component of the erythrocyte, for measurable amounts are present in the stroma (Table II). Similar to the lipid distribution of the intact erythrocyte, about 10% of the total stroma lipid—previously classified as neutral fat—now seems to consist entirely of cerebrosides. Likewise, the cerebroside fraction composed 10% of the total lipid of brain tissue, although this value does not account for the total amount (30%) of neutral fat.

⁷ Erickson, B. N., Williams, H. H., Bernstein, S. S., Avrin, I., Jones, R. L., and Macy, I. G., *J. Biol. Chem.*, 1938, **122**, 515.

⁸ Williams, H. H., Erickson, B. N., Beach, E. F., and Macy, I. G., *J. Lab. Clin. Med.*, in press.