HUMAN BRAIN LIPIDS AT VARIOUS AGES IN RELATION TO MYELINATION

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THE object of this work has been to examine the lipid constituents of human brains at different ages in order to follow the course of increasing myelination.

The principal myelin-forming constituents have been held to be cholesterol, cerebroside and sphingomyelin, as these substances show great increases in the white matter in adult life (Johnson, McNabb and Rossiter, 1949). The sphingolipids (i.e. cerebroside and sphingomyelin) are probably of more importance than the cholesterol, as the nerve sheath is almost entirely composed of them (Edgar, 1955).

Brante (1949) has estimated the lipids in the cortex and white matter at different ages. Johnson *et al.* (1949) give the lipid in the cortex and white matter of neonatal and adult brains.

The sphingomyelin, as estimated by Johnson *et al.* (1949), by difference between the total lipid P and the "KOH-splitable" P, will give unduly high results owing to the inclusion of the Cephalin B. This has been shown to be the case by Dawson (1954) who suggests that it is preferable to assess the sphingomyelin by difference between the total lipid choline and the lecithin choline. Results by both the methods are recorded here.

Cerebroside, as ordinarily estimated from the lipid galactose, includes the ganglioside.

Cephalin B and ganglioside do not contribute to the myelin sheath (Edgar, 1955), and it is important to take this fact into consideration.

Through the kindness of Dr. Brown of Southmead Hospital it has been possible to obtain brains of the following ages: Three brains a few hours after birth; and brains of 2, 4 and 9 days; 2 weeks; and 1, 2 and 9 months. Adult brains of 5, 19 and 65 years were obtained from other sources.

METHODS

The tissue was dried and extracted with 2 $CHCl_3$: 1 MeOH and again with $CHCl_3$. The extracts were taken to dryness, re-extracted with boiling $CHCl_3$, as advised by Brante (1949), filtered, and again taken to dryness. The lipid extract was dissolved in $CHCl_3$, made up to volume, and aliquots taken for the various estimations.

Cerebroside, by Brand and Sperry (1941). It was found best to take aliquots containing about 1·5 mg. cerebroside.

Cholesterol, by Sobel and Meyer (1945).

Phosphatide and *Sphingomyelin*+*Ceph. B.*, by Schmidt, Benotti, Hershmann and Thannhauser (1946).

Material for P estimation destructively digested with sulphuric and perchloric acid, neutralized with NaOH, and P estimated colorimetrically by the molybdic acid reagent.

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TABLE I

Lipids in the White Matter
(Nine Brains from Birth to 2 Months and Three Adult Brains. Results Given as Mean ± S.E.M.)

	Per cent. Dry Tissue					Per cent. Fresh Tissue				
	Birth to 2 Months	9 Months	5 Years	Adult	Adult/Infant Ratio	Birth to 2 Months	9 Months	5 Years	Adult	Adult/Infant Ratio
Cerebroside	4·7±0·9	8·1	12·0	12·2	2·60 : 1	0·52±0·13	1·67	3·58	3·52	6·80 : 1
Cholesterol	5·5±0·7	11·5	14·0	14·4	2·60 : 1	0·65±0·11	2·39	4·17	4·22	6·50 : 1
Phosphatide	22·9±1·7	20·7	26·3	24·5	1·07 : 1	2·55±0·29	4·30	7·73	6·87	2·70 : 1
Lecithin	10·6±0·9	8·2	8·7	8·0	0·75 : 1	1·19±0·20	1·72	2·59	2·24	1·90 : 1
Sphingomyelin+Cephalin B ..	1·8±0·7	2·2	6·2	6·1	3·40 : 1	0·20±0·08	0·46	1·80	1·72	8·60 : 1
Cephalin A	10·5±1·8	10·3	11·4	10·4	0·99 : 1	1·16±0·27	2·13	3·29	2·91	2·50 : 1

TABLE II

Lipids in the Cortex
(Three Brains from Birth to 2 Months and Three Adult Brains. Results Given as Mean ± S.E.M.)

	Per cent. Dry Tissue					Per cent. Fresh Tissue				
	Birth to 2 Months	9 Months	5 Years	Adult	Adult/Infant Ratio	Birth to 2 Months	9 Months	5 Years	Adult	Adult/Infant Ratio
Cerebroside	4·5±1·3	4·4	3·0	4·2	0·93 : 1	0·60±0·16	0·64	0·45	0·70	1·16 : 1
Cholesterol	5·2±0·6	5·8	5·2	5·7	1·10 : 1	0·72±0·24	0·84	0·77	0·94	1·30 : 1
Phosphatide	22·2±2·4	19·3	19·4	19·6	0·88 : 1	3·01±0·62	2·80	2·87	3·32	1·10 : 1
Lecithin	10·2±1·5	7·8	3·8	6·8	0·66 : 1	0·34±0·08	0·37	—	1·26	3·70 : 1
Sphingomyelin+Cephalin B ..	2·6±1·0	2·6	—	7·5	2·90 : 1	1·62±0·29	1·13	0·56	1·15	0·72 : 1
Cephalin A	9·4±1·5	8·9	—	7·8	0·83 : 1	1·05±0·34	1·30	—	0·91	0·87 : 1

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TABLE III

Sphingomyelin and Cephalin B in the Cortex and White Matter
(Per cent. Dry Tissue)

		White Matter							Cortex					
		Neo-Natal	1 Month	2 Months	9 Months	19 Years	65 Years	Adult/ Infant Ratio	Neo-Natal	1 Month	2 Months	9 Months	65 Years	Adult/ Infant Ratio
Sphingomyelin	0·5 0·1 Nil Nil Nil	0·1	0·8	1·8	1·7	2·0	2·4	22·0	0·2	0·6	0·7	1·0	2·7	13·4 : 1
Cephalin B	1·2 1·5 2·1 Nil 1·2	1·2	0·8	1·8	0·5	4·4	3·5	3·3	1·8	3·2	1·3	1·6	4·8	2·67 : 1

TABLE IV

Ganglioside in Relation to Cerebroside in the Cortex and White Matter
(Per cent. Dry Tissue)

	Cortex					White Matter				
	Neo-Natal	1 Month	2 Months	9 Months	65 Years	Neo-Natal	1 Month	2 Months	9 Months	65 Years
Cerebroside	3·5	6·0	4·0	4·4	4·4	5·6	4·6	4·5	8·1	12·6
Cerebroside: less neuraminic acid ganglioside	3·1	5·5	3·6	4·0	3·8	—	—	—	—	—
Cerebroside: less hexosamine ganglioside	2·4	4·1	2·9	3·4	3·0	3·6	3·8	3·6	6·7	11·6

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Total Choline, by Glick (1944); *Lecithin Choline*, by Brante (1949). Suspension in both cases made acid with HCl before filtration and precipitation of choline.

Sphingomyelin+Ceph. B.=Total lipid P less "KOH-splitable" P.

Sphingomyelin=Total Choline less Lecithin choline.

Hexosamine, by Elson and Morgan (1933) in hydrolysed lipid extract, with precautions advised by Rondle and Morgan (1955)—glucosamine HCl standard.

Neuraminic Acid, by Klenk (1941). Dried tissue separately extracted. In the absence of a neuraminic acid standard, the value of the normal adult cortex was assumed to be that given in literature.

Results

White Matter

Results for the period up to and including age 2 months have been averaged (Table I) because, with the exception of sphingomyelin, no marked changes were recorded up to this age. Further observations in this critical age group are desirable, since Brante (1949) has recorded increases in both cholesterol and cerebroside at age 2 months (one case).

The maximum increase in cholesterol and cerebroside occur between the ages of 2 and 9 months, and there is a further increase after 9 months to the adult stage.

The total phosphatide increases with age on a wet basis but not on a dry basis.

It will be seen from Table IV that the deduction from the cerebroside of the hexosamine ganglioside does not affect the increases of cerebroside with age after age 2 months. There is no neuraminic acid ganglioside in the white matter (Klenk, 1955).

The estimation of sphingomyelin (Table III) is not very accurate for small amounts, probably as stated by Brante (1949) "owing to differences in the determination of lecithin in relation to total choline". It is considered that the values for the neonatal period may be taken as negligible.

Cortex

The cholesterol and cerebroside show no increase with age (Table II). This also applies to the cerebroside after the neuraminic acid and hexosamine ganglioside have been deducted from it (Table IV).

The sphingomyelin does increase with age, and does not reach a nearly adult value at age 2 months, as it does in the white matter (Table III).

Ester Cholesterol (Table V)

There are very small quantities of this in the human brain. It occurs chiefly in the white matter and is at a maximum in my case at 9 months.

Table V

Ester Cholesterol (Per cent. Dry Basis)

	Neo-Natal	1 Month	2 Months	9 Months	19 Years	65 Years
White Matter	0·6	0·30	0·4	1·5	0·5	Nil
Cortex	Nil	Nil	0·1	Nil	0·3	0·3

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Discussion

It may be of interest to compare briefly these chemical findings in the developing brain with the degree of myelination as judged by the usual histological methods.

Comparison of the chemical results as a whole with the histological staining of myelin by the Weigert-Pal method or its modifications shows only an approximate correlation.

The white matter at 2 months stains more deeply than at the neonatal period, whereas there is no change in the content of cholesterol and cerebroside. The sphingomyelin however does show an increase.

There is only slight histological evidence of myelination in the cerebral white matter at the time of birth, whereas the content of cholesterol and cerebroside is not less than it is at age 2 months. The sphingomyelin content is however, negligible at birth.

The period at about 2 years when the white matter is very appreciably myelinated, does however correspond to a time when there are comparatively large amounts of all three myelin forming constituents. We have not yet obtained a brain of 2 years for analysis but it has been shown that maximum levels are obtained between 9 months and 5 years.

Summary

1. The lipid content of the cortex and white matter of brains at various ages has been assessed, with particular reference to the myelin forming constituents.

2. The neonatal brain contains considerable amounts of cholesterol and cerebroside, but negligible amount of sphingomyelin.

3(a). In the white matter no changes are recorded up to age 2 months, except in the sphingomyelin which increases. The cholesterol and cerebroside increase between 2 months and 9 months, and reach their maximum adult level somewhere between 9 months and 5 years. On a wet basis, the cholesterol, cerebroside and sphingomyelin reach about half their adult value at age 9 months.

3(b). In the cortex, the cholesterol and cerebroside reach adult value at age 2 months. The sphingomyelin reaches about a third of its adult value at 9 months.

4. The highest Adult/Infant ratios are recorded for sphingomyelin, and the lowest for lecithin.

5. The total phosphatide is the only Lipid which, when increased, does so only by virtue of the reduced water content.

6. There is some evidence that the sphingomyelin content (exclusive of Cephalin B) gives the best indication of the degree of myelination up to the age 2 months.

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