Sphingomyelin of Red Blood Cells in Lipidosis and in Dementia of Unknown Origin in Children

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Histological and chemical examinations of biopsy specimens from cerebral tissue of children suffering from undiagnosed progressive brain disease are performed increasingly. Important information is provided, which, though rarely of therapeutic value, does increase precision of diagnosis, prognosis, and genetic advice (Cumings, 1965a, b, c; Poser, 1962; Adams, 1965). This is especially true of the chemical investigations, and it is likely that chemical analyses will challenge the current classifications of progressive brain disease. Amaurotic idiocy has already proved to be more heterogeneous than was thought hitherto, while supposedly different diseases, such as Niemann-Pick's and gargoylism, have more in common than earlier classifications suggested.

However, brain biopsy is still a procedure not to be undertaken lightly, and it would be an advantage if comparable information could be gained by a less drastic method. We have been interested in the possibility of finding abnormalities in easily accessible tissue, such as red blood cells, which might correlate with abnormalities in the constitution of the brain. In this paper we give the results of a study of the sphingomyelin content of erythrocytes in children with various types of progressive brain disease.

Material and Methods

The patients included in this study are enumerated in the legend to the Fig. Nearly all were more than 2 years old. The group of children with juvenile amaurotic idiocy came from different hospitals; in only some of these cases was the diagnosis verified by histological and biochemical investigation of brain tissue. All the other patients were under our care, the diagnosis being verified in nearly all by means of biopsies.

The 10 patients with progressive brain disease (dementia and neurological abnormalities), in which

no diagnosis could be made, are briefly described in Table I. Incomplete investigation made it impossible to reach a diagnosis in Cases 7 and 8. The molar concentrations of phospholipids in the red blood cells have been determined as described by Hooghwinkel and Niekerk (1960), Hooghwinkel and Borri (1964), and Hooghwinkel, Borri, and Bruyn (1966). The amounts of the various phospholipids of red blood cells have been expressed as molar percentages of total phospholipids. Absolute values of phospholipids depend a good deal on size and shape of the

FIG.—Sphingomyelin of red blood cells, as a percentage of phospholipids, in six groups of cases.

I: normal values, 17 cases (○: < 8 years; ●: > 10 years), mean $\pm$ 2SD; II: juvenile amaurotic idiocy, 12 cases; III: mucopolysaccharidosis, 7 cases; IV: other diagnoses (○, Gaucher's disease, 3 cases; ×, Niemann-Pick's disease, 2 cases; □, sudanophilic leucodystrophy, 1 case; ■, metachromatic leucodystrophy, 1 case); V: dementia of unknown cause, 10 cases; VI: controls with severe cerebral atrophy after brain damage in early life.

Received August 26, 1968.

TABLE
Details of Patients with

Case No.	Sex	Early Development and Progression of Disease	Age at Time of Investigation (yr.)	Family History	Physical and Neurological Symptoms and Signs*	Ocular Symptoms
1	F	Normal till 1½ yr.; then gradual dementia	3	Negative	No recognition; severely mentally retarded; spastic tetraplegia, irregular myoclonic jerks, fine head tremor	Vision uncertain; otherwise normal
2	M	Slow till 5 yr.; then gradual but progressive dementia	10	Negative	Functioning at IQ 30 (2 yr. later completely demented); coarse features but neither hepatomegaly nor bone abnormalities; myoclonic jerks; spastic tetraplegia; PEG, diffusely enlarged ventricles	Bilateral cataract; fundus probably normal
3	F	Normal till 4 mth.; then rapid dementia	6	Brother died from similar illness, no diagnosis made	Completely demented; spastic legs; PEG, symmetrically enlarged ventricles	Bilateral cataract, probably blind
4	F	Normal till 6 mth.; then severe regression	2½	Negative	Severely demented, hyperkinesia, convulsions, abnormal postures; PEG, cortical atrophy rt. frontal area	None
5	F	Normal till 9 mth.; then rapid regression	Nearly 2	Negative	Severely demented, primitive reflexes, spastic tetraplegia; fever of unknown cause	None
6	M	Normal in first few mth.; then slow development	4	3 of 5 sibs mentally retarded, with retinal abnormalities and liver enlargement	Severely retarded but motor development reasonable; nystagmus, liver much enlarged; PEG, slight increase of ventricle size	Pigment increase
7	F	Normal till 3 mth.; then rapid dementia with epilepsy	1½	Negative	Completely demented, spastic tetraplegia, probably blind, frequent convulsions	None
8	M	Normal till 6 mth.; then rapid regression	1	Consanguineous parents; maternal brother died in infancy from convulsions and head enlargement	Retarded, loss of contact; vision reduced, stereotypic aimless movements; PEG, cerebral atrophy	Tapetoretinal degeneration
9	M	Normal till 7 yr.; failing at school; from 9 yr. increasingly atactic with <i>petit-mal</i> epilepsy; intellectual functions declined; IQ 100 at 7 yr.; 49 at 14 yr.	14	Insufficient data; ataxia in paternal family?	Ataxy, loss of co-ordination; enlarged liver and spleen; normal acid phosphatase	None
10	M	Normal till 5 yr.; then decline of mental functions without other serious signs	8	Negative	Physical and neurological examination normal; PEG, slightly enlarged ventricles; psychologist, dementing, organic picture, IQ 55 (88 2 yr. before)	Except for myopia no abnormalities

* PEG = pneumoencephalogram.

† TLC = thin-layer chromatography.

Sphingomyelin of Red Blood Cells in Lipidosis and in Dementia of Unknown Origin 199

I Dementia of Unknown Origin

EEG	Sphingomyelin (% of P-lipids in erythrocyte)	Biopsy			Diagnosis
		Tissue	Histology	Chemistry	
No normal background activity, periodic bursts of high-voltage delta waves	29·4	Brain	No abnormalities	Moderate decrease of myelin lipids in white matter, sphingomyelin moderately decreased in white matter, strongly decreased in grey matter	Dementia of unknown origin, with spastic paraplegia and myoclonic jerks
Almost continuously spike-and-wave activity	28·0	Brain	Typical picture of amaurotic idiocy; many neuronal cells and a number of glia cells swollen with PAS + ve material	Decrease of myelin lipids; decrease of sphingomyelin content of white matter; marked increase of gangliosides (G5 and G6) on chromatography	Dementia of unknown cause; probably agangliosidosis; much increased urinary excretion of acid mucopolysaccharides
Severe diffuse abnormalities most prominent in rt. hemisphere	29·7	Brain	Focal perivascular necrosis with gliosis around these areas	Slight decrease of myelin lipids and moderate decrease of sphingomyelin	Familial dementia of unknown origin
Bilateral spikes and waves, sometimes synchronous	30·1	Brain	No abnormalities	Decrease of sphingomyelin in grey and white matter; generalized decrease of myelin lipids	Dementia of unknown origin
No abnormalities, but at age 4 yr. cerebral activity much diminished	31·4	Rectal mucosa; suralis nerve brain	No abnormalities one Hortega glia cell infiltration Specimen lost	Not done	Dementia of unknown origin; normal sphingomyelin in red cells
Isolated theta and delta waves of spikey character in rt. temporal area	26·3	Liver		Almost complete absence of sphingomyelin with long fatty acid chains	Familial oculo-cerebral syndrome, with visceral involvement; investigations of patient and sibs incomplete
Severe generalized epileptic activity	29·9				Dementia of unknown origin; cerebral biopsy planned
Normal	26·8				Oculo-cerebral syndrome of unknown origin, low sphingomyelin in RBC, hence amaurotic idiocy unlikely
Slow background activity; no specific abnormalities	29·1	Lymph-node; bone-marrow; liver	Foam cells Foam cells Foam cells	Cholesterol much increased; sphingomyelin only slightly increased; normal lipid hexose content; TLC† did not show increase of glycolipid	Clinically suggestive of Niemann-Pick's disease, but chemical analysis of liver specimen against this
Diffusely abnormal; paroxysmal activity on photo-stimulation	29·7	Sural nerve	Normal		Dementia of unknown origin, too well to justify brain biopsy

TABLE II

Molar Distribution of Phospholipids, Expressed as Percentage of Total Phospholipid, in Red Blood Cells (mean and range)

	Sphingomyelin	Lysophosphatidylcholine plus Phosphatidylcholine	Phosphatidylethanolamine
Normals	32.2 (30.0 - 34.0)	40.6 (37.4 - 41.7)	27.2 (24.5 - 29.5)
Juvenile amaurotic idiocy	32.5 (30.2 - 35.5)	39.6 (36.2 - 43.1)	27.9 (26.0 - 29.7)
Mucopolysaccharidosis	27.8 (24.6 - 29.8)*	40.6 (38.6 - 43.7)	31.5 (29.3 - 34.4)
Niemann-Pick's disease	26.7 (26.6 - 26.8) }	46.5 (44.6 - 48.4)	26.3 (24.8 - 27.9)
Gaucher's disease	29.1 (27.6 - 30.4) } *	42.8 (41.6 - 43.3)	28.1 (28.0 - 29.1)
Metachromatic leucodystrophy	31.8	40.1	28.0
Sudanophilic leucodystrophy	29.6	39.0	31.4
Controls (see text)	31.9 (27.3 - 34.6)	39.2 (37.5 - 40.8)	28.9 (26.1 - 32.3)
Dementia of unknown cause	28.8 (26.3 - 31.4)*	43.0 (39.1 - 44.8)	28.1 (24.2 - 31.3)

* Significantly lower than normal, $p < 0.01$ (Wilcoxon).

red blood cells, and they vary with changes in the ratio of total cell surface to the total cell volume; this ratio itself may be influenced by the lipid composition of the membrane. Thus, it is more likely that a metabolic disturbance will manifest itself in a change in the percentage distribution of the various phospholipids.

Patients with dementia of unknown origin. The patients in this group either showed normal development before the illness or had shown slow development from birth. In all, however, there was a clear-cut intellectual deterioration, together usually with epileptic symptoms and other neurological symptoms. Before a biopsy was done, extensive investigations were undertaken, of blood, urine, and CSF (including electrophoresis and cytology). Urinary excretion of amino acids and mucopolysaccharides was always checked. In most cases the urine was investigated for metachromatic material and/or arylsulphatase-A. Pyridoxine deficiency and dependency were excluded. White blood cells and cells in CSF were examined for abnormal granules and vacuoles. EEG's were made repeatedly (Dr. K. Mechelse). Biopsy of rectal mucosa or sural nerve was made in a few cases. It was often difficult to make a histological diagnosis from these specimens, and, at least in our laboratories, impossible to analyse biochemically such small amounts of tissue.

If a progressive cerebral disease were present, and no diagnosis could be arrived at using the above-mentioned methods, brain biopsy was planned. With permission from the parents, a biopsy was taken from the prefrontal lobe by means of a small craniotomy. The specimens were investigated both histologically and biochemically. In two cases biopsy was refused by the parents. In one case the biopsy was postponed, because of intercurrent illness. If visceral involvement was suspected we preferred to take a biopsy from the liver. In Table I we summarize the relevant data of the 10 patients in this group of dementia of unknown origin.

Results

Table II summarizes the results of the phospholipid determinations, and the Figure shows the distribution of the molar percentages of sphingomyelin in the red blood cells. In general the sphingomyelin content of plasma showed the same trend as that of red blood cells, but less consistently. Sphingomyelin percentages were abnormally low in many cases. The patients suffering from amaurotic idiocy were exceptional, in that in all these cases sphingomyelin values were within normal limits. The control group, consisting of patients with severe cerebral atrophy after brain damage, usually due to a purulent meningitis many years earlier, also showed normal values. The one case of metachromatic leucodystrophy also had normal sphingomyelin in red cells; we do not know yet if this is typical for this disease. The group of dementias of unknown origin showed low sphingomyelin values in all cases but one.

Discussion

A low sphingomyelin content of red blood cells is perhaps explicable in those patients whose disease is associated with accumulation of sphingolipids in the central nervous system and/or other tissues. This is the case both in Hurler's disease (Ledeën *et al.*, 1965; Borri, Hooghwinkel, and Edgar, 1966) and in Tay-Sachs's disease (Svennerholm, 1962) where gangliosides are stored in the brain; in Gaucher's disease (Rosenberg and Chargaff, 1958; Janse and Hooghwinkel, 1967) where cerebroside are accumulating in liver and spleen; and in Niemann-Pick's disease where there is accumulation of sphingomyelin (Hooghwinkel and Borri, 1964). Lowered sphingomyelin concentration of red blood cells might also have been

expected in metachromatic leucodystrophy, where sulphatides are accumulating in the brain (Austin, 1965).

The only phospholipid based on sphingosine and not glycerol, and which is therefore to be regarded as a sphingolipid, is sphingomyelin which normally constitutes 32% of all phospholipids in the red blood cells. The normal values found in the control group were as expected: no metabolic disorder was present and therefore the large loss of cerebral tissue is not reflected in any change of lipid content of red blood cells. The normal values in juvenile amaurotic idiocy also accord with the fact that in this illness no disorder of sphingolipid metabolism has been demonstrated. Though histological studies have suggested a storage disorder, amaurotic idiocy has not been thought to be a typical lipidosis.

The other diseases listed in Table II and in the Figure are all examples of disorders of lipid storage, the sphingolipids accumulating in the central nervous system and/or the visceral organs. The red cells of these patients showed a decrease in sphingomyelin in nearly every case. The fact that the group of 10 patients in whom no diagnosis could be made showed a similar decrease of sphingomyelin content of red blood cells suggests that in these patients also there was some abnormality of lipid metabolism.

The change in sphingomyelin content of red blood cells may be indicative of a more generalized disturbance of sphingolipid metabolism. It is known that accumulation of various sphingolipids is caused by absence or deficiency of catabolic enzymes. In Gaucher's disease there is a deficiency of cerebrosidease (Brady, Kanfer, and Shapiro, 1965); in Niemann-Pick's disease a deficiency of sphingomyelinase (Brady *et al.*, 1966), and in metachromatic leucodystrophy a deficiency of arylsulphatase (Mehl and Jatzkewitz, 1965). It is suggested that in the metabolic pool a shortage develops of sphingosine which is the precursor both of cerebral sphingolipids and of the sphingomyelin in red blood cells. The relative deficiency of sphingomyelin may be compensated for by an increase in lecithin and cephalin. Evidently neither demyelination nor loss of cerebral tissue by themselves result in low sphingomyelin content of red blood cell membranes, judging by the normal values usually recorded in the control group.

We think from the investigation described above that the following conclusions may be drawn.

(1) Disturbances of sphingolipid metabolism can be shown by biochemical analysis of red blood cells.

(2) Juvenile amaurotic idiocy is most probably not caused by an accumulation of sphingolipids in the brain.

(3) Examination of red blood cell phospholipids should precede more drastic diagnostic investigations in childhood dementia.

(4) Cerebral symptoms together with a decreased relative concentration of sphingomyelin in red blood cells indicate a progressive brain disease associated with derangement of sphingolipid metabolism.

Summary

Sphingomyelin concentration of red blood cells, measured as a percentage of phospholipid, was studied in children suffering from progressive brain disease of different types. Normal values were found in 12 cases of juvenile amaurotic idiocy, and also in a control group of children with severe atrophy of the brain consequent on infection of CNS many years earlier. Low values were found almost uniformly in children with mucopolysaccharidosis (Hurler's disease), in Niemann-Pick's disease, and in Gaucher's disease. One patient with sudanophilic leucodystrophy also showed a slightly low value, and a normal value was obtained in one child with metachromatic leucodystrophy.

A further group of 10 children with progressive brain disease was studied, in whom no exact diagnosis could be made, despite extensive investigation which often included brain or liver biopsy. In 9 of these 10 patients, red blood cell sphingomyelin content was decreased, suggesting an abnormality of sphingolipid metabolism.

Estimation of red blood cell sphingomyelin content should precede more drastic investigations such as brain biopsy, as it may provide an important approach to the diagnosis of brain disease in children.

We are grateful to Professor W. Luyendijk and Dr. G. Th. van Beusekom, for performing the cerebral biopsies; to Dr. G. Th. A. M. Bots, Department of Pathology, Leiden, for examining the cerebral biopsy specimens; and to Dr. Steketee, director of the Dr. Willem v.d. Berghstichting at Noordwijk, for permission to study patients in his institution.

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