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 gargoilism, 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 ± 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.
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Early Development and Progression of Disease</th>
<th>Age at Time of Investigation (yr.)</th>
<th>Family History</th>
<th>Physical and Neurological Symptoms and Signs*</th>
<th>Ocular Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Normal till 1½ yr.; then gradual dementia</td>
<td>3</td>
<td>Negative</td>
<td>No recognition; severely mentally retarded, spastic tetraplegia, irregular myoclonic jerks, fine head tremor</td>
<td>Vision uncertain; otherwise normal</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>Slow till 5 yr.; then gradual but progressive dementia</td>
<td>10</td>
<td>Negative</td>
<td>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</td>
<td>Bilateral cataract; fundus probably normal</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>Normal till 4 mth.; then rapid dementia</td>
<td>6</td>
<td>Brother died from similar illness, no diagnosis made</td>
<td>Completely demented; spastic legs; PEG, symmetrically enlarged ventricles</td>
<td>Bilateral cataract, probably blind</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Normal till 6 mth.; then severe regression</td>
<td>2½</td>
<td>Negative</td>
<td>Severely demented, hyperkinesis, convulsions, abnormal postures; PEG, cortical atrophy rt. frontal area</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>Normal till 9 mth.; then rapid regression</td>
<td>Nearly 2</td>
<td>Negative</td>
<td>Severely demented, primitive reflexes, spastic tetraplegia; fever of unknown cause</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>Normal in first few mth.; then slow development</td>
<td>4</td>
<td>3 of 5 sibs mentally retarded, with retinal abnormalities and liver enlargement</td>
<td>Pigment increase</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Normal till 3 mth.; then rapid dementia with epilepsy</td>
<td>1½</td>
<td>Negative</td>
<td>Completely demented, spastic tetraplegia, probably blind, frequent convulsions</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>Normal till 6 mth.; then rapid regression</td>
<td>1</td>
<td>Consanguineous parents; maternal brother died in infancy from convulsions and head enlargement</td>
<td>Tapetoretinal degeneration</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>Normal till 7 yr.; failing at school; from 9 yr. increasingly atactic with petit-mal epilepsy; intellectual functions declined; IQ 100 at 7 yr.; 49 at 14 yr.</td>
<td>14</td>
<td>Insufficient data; ataxia in paternal family?</td>
<td>Ataxy, loss of co-ordination; enlarged liver and spleen; normal acid phosphatase</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>Normal till 5 yr.; then decline of mental functions without other serious signs</td>
<td>8</td>
<td>Negative</td>
<td>Physical and neurological examination normal; PEG, slightly enlarged ventricles; psychologist, dementing, organic picture, IQ 55 (88 2 yr. before)</td>
<td>Except for myopia no abnormalities</td>
</tr>
</tbody>
</table>

* PEG = pneumoencephalogram. † TLC = thin-layer chromatography.
### Sphingomyelin of Red Blood Cells in Lipidosis and in Dementia of Unknown Origin

#### Dementia of Unknown Origin

<table>
<thead>
<tr>
<th>EEG</th>
<th>Sphingomyelin (% of P-lipids in erythrocyte)</th>
<th>Tissue</th>
<th>Biopsy</th>
<th>Chemistry</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No normal background activity, periodic bursts of high-voltage delta waves</td>
<td>29·4</td>
<td>Brain</td>
<td>No abnormalities</td>
<td>Moderate decrease of myelin lipids in white matter, sphingomyelin moderately decreased in white matter, strongly decreased in grey matter</td>
<td>Dementia of unknown origin, with spastic paraplegia and myoclonic jerks</td>
</tr>
<tr>
<td>Almost continuously spike-and-wave activity</td>
<td>28·0</td>
<td>Brain</td>
<td>Typical picture of amaurotic idiocy; many neuronal cells and a number of glia cells swollen with PAS +ve material</td>
<td>Decrease of myelin lipids; decrease of sphingomyelin content of white matter; marked increase of gangliosides (G5 and G6) on chromatography</td>
<td>Dementia of unknown cause; probably agangliosidosis; much increased urinary excretion of acid mucopolysaccharides</td>
</tr>
<tr>
<td>Severe diffuse abnormalities most prominent in rt. hemisphere</td>
<td>29·7</td>
<td>Brain</td>
<td>Focal perivascular necrosis with gliosis around these areas</td>
<td>Slight decrease of myelin lipids and moderate decrease of sphingomyelin</td>
<td>Familial dementia of unknown origin</td>
</tr>
<tr>
<td>Bilateral spikes and waves, sometimes synchronous</td>
<td>30·1</td>
<td>Brain</td>
<td>No abnormalities</td>
<td>Decrease of sphingomyelin in grey and white matter; generalized decrease of myelin lipids</td>
<td>Dementia of unknown origin</td>
</tr>
<tr>
<td>No abnormalities, but at age 4 yr. cerebral activity much diminished</td>
<td>31·4</td>
<td>Rectal mucosa; suralis nerve brain</td>
<td>No abnormalities, one Hortega glia cell infiltration</td>
<td>Almost complete absence of sphingomyelin with long fatty acid chains</td>
<td>Dementia of unknown origin; normal sphingomyelin in red cells, Familial oculo-cerebral syndrome, with visceral involvement; investigations of patient and sibs incomplete</td>
</tr>
<tr>
<td>Isolated theta and delta waves of spikey character in rt. temporal area</td>
<td>26·3</td>
<td>Liver</td>
<td></td>
<td>Specimen lost</td>
<td>Dementia of unknown origin; cerebral biopsy planned</td>
</tr>
<tr>
<td>Severe generalized epileptic activity</td>
<td>29·9</td>
<td></td>
<td></td>
<td></td>
<td>Oculo-cerebral syndrome of unknown origin, low sphingomyelin in RBC, hence amaurotic idiocy unlikely</td>
</tr>
<tr>
<td>Normal</td>
<td>26·8</td>
<td></td>
<td></td>
<td></td>
<td>Clinically suggestive of Niemann-Pick's disease, but chemical analysis of liver specimen against this</td>
</tr>
<tr>
<td>Slow background activity; no specific abnormalities</td>
<td>29·1</td>
<td>Lymph-node; bone-marrow; liver</td>
<td>Foam cells</td>
<td>Cholesterol much increased; sphingomyelin only slightly increased; normal lipid hexose content; TLC+ did not show increase of glycolipid</td>
<td>Dementia of unknown origin, too well to justify brain biopsy</td>
</tr>
<tr>
<td>Diffusely abnormal; paroxysmal activity on photo-stimulation</td>
<td>29·7</td>
<td>Sural nerve</td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 intermittent 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 leukodystrophy 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 (Ledeen et al., 1965; Borri, Hooghkinkel, 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 Hooghkinkel, 1967) where cerebrosides are accumulating in liver and spleen; and in Niemann-Pick’s disease where there is accumulation of sphingomyelin (Hooghkinkel 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 katabolic 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 demylinization 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.

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