A histochemical study of various forms of cerebral lipidoses

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SYNOPSIS A histochemical study was made of 13 cases of lipidoses of the central nervous system. Niemann-Pick's disease can be differentiated from other cerebral lipidoses by the finding that all affected cells store histochemically stainable phospholipids. Evidence has been adduced indicating that the material stored in infantile amaurotic idiocy is a complex containing ganglioside, cholesterol and possibly other constituents. The non-infantile forms of amaurotic idiocy were found to differ in essential points from the classical infantile form.

Most cases of cerebral lipidoses belong to the group of cerebromacular degenerations which includes the various types of amaurotic idiocy and Niemann-Pick disease. Classification and understanding of these disease processes have been hampered by lack of adequate criteria for distinguishing between the various diseases. Clinical and pathological data led to classification of cases of amaurotic idiocy in accordance with the age at onset, age at death, and the length of time between onset and death. The same criteria led some authors who studied cases of Niemann-Pick's disease with involvement of the nervous system to describe them as mixed cases of amaurotic idiocy with Niemann-Pick's disease.

Chemical studies have helped to determine the nature of the lipid moiety which was most severely deranged and to use this as a diagnostic criterion; but as most chemical studies were performed on relatively large pieces of tissue, two difficulties arose: 1, In most cases of lipidoses more than one lipid was abnormal in amount; 2, in some cases the 'dilution' of affected cells by normal or secondarily changed, e.g., demyelinated, tissue obscured the findings and the diagnosis.

The histochemical approach, and especially if coupled with a quantitative chemical study, may obviate some of these difficulties. Although far from perfect from the chemical standpoint, histochemistry can show positively the sites of the abnormal metabolites.

The following is a histochemical study of the central nervous system in 13 cases of lipidoses. It indicated that lipid complexes rather than pure

lipids are deposited in the cells and allowed certain conclusions to be drawn in relation to the classification of these diseases.

MATERIAL AND METHODS

This study is based on formalin-fixed material. The length of fixation varied from over 30 years to a few days. The material was in part derived from this laboratory. Professor F. Feyrter of Vienna (previously from Vienna, Graz, and Goettingen) also sent samples of tissues from various cases, some of which had been previously reported in the literature. Dr. R. M. Norman of Bristol sent samples of tissues from three other cases. Pieces from a brain of another case were given by Dr. S. A. Reif and U. Sandbank from the Assaf Harofe Government Hospital in Israel.

Seven of the cases were of the infantile type of amaurotic idiocy and three were of Niemann-Pick's disease. Three further cases were of other than infantile forms of amaurotic idiocy. One was a case of amaurotic idiocy with visceral involvement described by Norman, Ulrich, Tingey, and Goodbody (1959). The second was of late infantile amaurotic idiocy described by Tingey, Norman, Ulrich, and Beasley (1958). The third was a case of atypical amaurotic idiocy described by Wolman (1961).

All histochemical staining procedures were done on frozen sections. The staining procedures employed and the findings in the various cases are summarized in the Table.

DISCUSSION

The findings of this study as summarized in the Table allow three conclusions to be drawn and these will be discussed separately.

TABLE
HISTOCHEMICAL REACTIONS OF THE MATERIAL STORED IN NEURONES

	<i>Seven Cases of Infantile Amauro- tic Idiocy</i>	<i>Case Described by Norman et al. (1959)</i>	<i>Case Described by Wolman (1961)</i>	<i>Case of Late Infantile Amauro- tic Idiocy Described by Tingey et al. (1958)</i>	<i>Three Cases of Niemann-Pick's Disease</i>
Chemical and pathological data	Death in infancy; no storage in internal organs	Death in infancy; storage of lipid in parenchymatous organs	Death at age of 2½ years; no infor- mation available about storage in internal organs	Death at age of 4½ years; storage of lipid in internal organs	Death in infancy; storage of lipids in parenchy- matous organs
<i>Lipids</i>					
Sudan IV	±	±	±	+++	0
Sudan black	+ - + +	+	+	++++	+
Serra's procedure for bound lipids	++++	++ +	++ +	++++	++ +
Baker's procedure for phospholipids	0 - + +	±	±	0 - + + +	++ +
Luxol	±	+	+	+++	0
Schultz	++ +	+	++ +	0	+ - + +
Performic acid-Schiff	0	+	+	+++	±
Solubility in chloroform methanol (2 : 1)	0 - + +	±	±	0	±
<i>Carbohydrates and acidic groups</i>					
P.A.S.	+ - + + +	++	++ +	++++	± - +
Colloidal iron	+	0	0	0	±
Einschlussfärbung					
Orthochromatic	+ - + +	++	0 ±	++	0
Metachromatic	+ - + +	0	0	0	++
Orcinol	0 - + +	0	0	++	0 - + +
<i>Proteins</i>					
Millon	0	±	±	++ +	0 - ±
Ninhydrin-Schiff	0	±	±	++ +	0 - ±
Danielli's tetrazo method	0	0	0	++	0 - ±

DIFFERENTIATION OF NIEMANN-PICK'S DISEASE FROM AMAUROTIC IDIOCY In many cases of amaurotic idiocy phospholipids demonstrable by the Baker procedure, or earlier by the Smith-Dietrich procedure, are found in the affected neurones. Their amount is variable from one cell to another and some cells do not contain demonstrable amounts of phospholipids. The variability in the amounts of phospholipids in the different cells in single cases of amaurotic idiocy is believed to represent an important diagnostic criterion, as storing cells in Niemann-Pick's disease always contain high concentrations of phospholipids. The validity of this criterion is also borne out by the generally accepted concepts about the nature of these diseases. Niemann-Pick's disease is a sphingomyelin storage disease and each one of the cells affected by the process stores the 'specific' lipid. In amaurotic idiocy phospholipids represent 'non-specific' additionally stored lipids.

'NON-SPECIFIC' STORED COMPONENTS AND THEIR SIGNIFICANCE The Table shows that the material stored in the infantile cases of amaurotic idiocy always contains cholesterol in appreciable amounts. This finding, previously noted by other observers, *e.g.*, Diezel (1957), indicates that there is no more

reason to regard infantile amaurotic idiocy as a storage disease of gangliosides than to regard it as a cholesterolosis. The fact that chemical analysis showed the ganglioside storage and did not show the presence of increased amounts of cholesterol is probably related to the high content of cholesterol in the normal brain, so that increased amounts, in order to be apparent, have to be high enough to compensate for the dilution effect of surrounding tissue. Furthermore, amaurotic idiocy is often associated with secondary demyelinating changes in the white matter and the resulting loss of cholesterol may have often overcompensated for the increased cholesterol content in the neurones.

The constant presence of cholesterol in the cells of infantile amaurotic idiocy and the finding that the lipids are better stained by Serra's (1958) procedure for bound lipids (in which electron bonds between lipids and other constituents are split by urea) than by the simple Sudan black technique indicate that the material deposited in the affected cells is not a mixture of lipids but rather a complex which consists of gangliosides, cholesterol, and probably other constituents. The findings of Terry and Korey (1960) of membranous cytoplasmic granules in these cells also suggest that an organized complex containing

FIG. 1

FIG. 1. Frozen section of cerebellum of a case of infantile amaurotic idiocy stained with Sudan black B. The material stored in the cells did not stain intensely. $\times 105$.

FIG. 2

FIG. 2. Frozen section of the cerebellum of the same case stained by Serra's procedure. The treatment with urea markedly increased the sudanophilia of the stored material. $\times 105$.

lipids and other constituents is deposited in infantile amaurotic idiocy. It is suggested therefore that this disease is not a pure ganglioside storage disease, but a disease in which a complex containing ganglioside and cholesterol (? lipoprotein) is stored in the diseased cells. It is further suggested that the increase in 'non-specific' lipids in the various lipidoses, stressed by Uzman (1958), may in some instances represent the presence of lipid-complexes rather than deposition of pure lipids.

RELATIONSHIP OF OTHER FORMS OF AMAUROTIC IDIOCY TO INFANTILE FORM Gangliosides are strongly acidic lipids which stain metachromatically and can bind colloidal iron. The absence of metachromatic staining in the non-infantile cases of amaurotic idiocy studied might indicate either that little or no gangliosides are present in the stored cells, or that the gangliosides are firmly bound to other components. As orthochromatic staining was observed it suggests that acidic groups are present but

apparently not in a well-ordered fashion, which allows a polymerization effect of the dye molecules, and it is therefore probable that the non-infantile cases of amaurotic idiocy do not store gangliosides. This conclusion is borne out by chemical analysis (Tingey *et al.*, 1958).

The cells of the non-infantile cases were found to contain stainable amounts of protein, whereas the amount of protein in the stored material in infantile cases was too small for histochemical detection. Another difference between the material stored in the older cases and that stored in the infantile form was the presence in the older cases of stainable amounts of unsaturated lipids.

These findings indicate that whereas typical infantile amaurotic idiocy is probably a single nosological entity, the atypical forms and the non-infantile cases represent a series of processes which basically differ from the infantile form. As the non-infantile forms differ from the infantile forms (Tay Sachs) in the clinical course, in the age at onset,

the racial distribution, and in the nature of the stored substances, they should be regarded as different diseases.

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