A CASE OF JUVENILE GAUCHER'S DISEASE WITH INTRANEURONAL LIPID STORAGE

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The juvenile form of Gaucher's disease with chronic involvement of the nervous system appears to be distinctly rare. Cases have been described by Evans (1916), Reiss and Kato (1932), Myers (1937), Bird (1948), and Brain (1954). Lipid storage was noted in the nerve cells in the only two cases completely examined histologically, namely those of Bird and Brain.

This report describes the pathological and biochemical observations made in a further case believed to belong to this rare variant of Gaucher's disease.

Case Report

The patient, a girl, was born on April 14, 1949, and died at the age of 8 years 9 months on January 16, 1958. She was the eldest of six siblings and her parents (non-Jewish) are alive and healthy. Delivery at term was uneventful and thereafter she apparently developed normally until the age of 5 years, when she started school. Her scholastic progress was unsatisfactory and she was transferred to a special school at the age of 6 years, where she was found to have a mental age of 3 years, to be restless, incontinent, destructive, and unsteady on her feet. Mental deterioration continued, necessitating her removal from school and she began to have major epileptic fits with clonic movements of the right side of the body in February 1957. Examination of the nervous system at that time was quite negative, and in particular the fundi oculi were normal. The erythrocyte sedimentation rate was 35 mm. in one hour. Electroencephalography showed bilateral, symmetrical, slow wave-and-spike discharges. Cerebrospinal fluid contained protein, 12 mg. per 100 ml., sugar 75 mg. per 100 ml., chlorides 736 mg. per 100 ml., and culture was sterile. A cerebral biopsy (April 23, 1957) revealed equivocal changes in some neurones which were thought to be consistent with, but not diagnostic of, cerebral lipidosis. During the subsequent months she became aphasic and developed athetoid movements in all limbs, general hyper-reflexia, bilateral extensor plantar responses and spasticity of the right arm. She was now confined to bed and despite anticonvulsant drugs, frequent brief epileptic attacks continued. At no time was either the liver or spleen palpable. She eventually died in an

emaciated state following a recurrent attack of bronchopneumonia.

Necropsy Findings

The body was that of a slender, poorly nourished girl, with normal-sized head, showing no skeletal abnormalities. The eyes were normal and the abdomen was not protuberant. The spleen was enlarged threefold but of normal shape, the capsule was healthy, and the splenic pulp showed no particular abnormalities. The lymph glands and bone marrow appeared normal. The liver was normal. The lungs showed a recent bilateral bronchopneumonia with scattered areas of collapse. The cardiovascular, genito-urinary, and endocrine systems were normal.

Histology

Spleen.—The pulp is diffusely infiltrated by numerous, closely packed, large cells with foamy vacuolated cytoplasm and a small, often eccentric, nucleus (Figs. 1 and 2). These cells have the appearance of lipid-storing histiocytes. The Malpighian corpuscles are rather small and probably reduced in number. The blood vessels, trabeculae, and capsule are normal.

Bone Marrow.—Bone marrow taken from a lumbar vertebra contains relatively normal haemopoietic tissue sparsely infiltrated with cells somewhat resembling those found in the splenic pulp.

Lung.—There is a recent acute bronchopneumonia. Foamy histiocytes are numerous not only in the pneumonic areas but also in other areas where the inflammatory changes are less marked. Some alveoli are distended solely by these cells which are similar to the "storage" cells in the spleen.

Liver.—There is a normal lobular architecture with no evidence of lipid storage in parenchyma. A few cells resembling foamy histiocytes are occasionally noted with difficulty in the sinusoids (Fig. 3).

The kidney, heart, and adrenal gland appear normal.

Examination of the Central Nervous System

Macroscopic.—The cerebrum, which weighed 1,020 g., was normal externally, except for the presence of a small scar on the convexity of the right frontal lobe which

FIG. 1

FIG. 2

FIG. 3

FIG. 1.—Splenic pulp infiltrated with groups of foamy histiocytes. Haematoxylin and eosin $\times 65$.

FIG. 2.—Spleen. Haematoxylin and eosin $\times 285$.

FIG. 3.—Liver. A few P.A.S.-positive histiocytes (darkly stained cells) are present in the sinusoids. P.A.S. $\times 285$.

FIG. 4.—Cerebral cortex. Note ballooned neurones. Thionin $\times 160$.

FIG. 5.—Pons. Group of enormously distended neurones. Haematoxylin and eosin $\times 160$.

indicated the biopsy site. Serial coronal sectioning showed merely slight uniform dilatation of the ventricles.

The brain-stem and cerebellum weighed 140 g. They were normal externally and on section, apart from slight dilatation of the aqueduct and fourth ventricle.

Microscopic.—Sections were examined from representative parts of the cerebral cortex, basal ganglia, brain-stem, and cerebellum. In all sections there is evidence of advanced neuronal lipid storage (Figs. 4 and 5).

Many neurones have disappeared. Of those which remain, some look relatively normal but others show varying degrees of ballooning of the cell body. Some cells are very markedly enlarged; from these the Nissl substance has disappeared, the cytoplasm is finely granular and the nucleus eccentric. Cells, without visible nuclei, and with their cytoplasm replaced by a large clear vacuole containing only a few granules, appear to be on the point of disintegrating.

This degeneration of nerve cells is associated with a gliosis proportional to the cell loss and in some areas with a proliferation of rod cells. It is well-marked in the cerebellum where there is gliosis of the molecular layer, and great reduction in the number of Purkinje cells, proliferation of Bergmann's layer of astrocytes, and

degeneration of the granular layer. Some of the remaining Purkinje cells are distended with lipid.

In the white matter throughout there is slight thinning of nerve fibres and myelin sheaths accompanied by a mild gliosis. Gliosis is most severe in the white matter of the cerebellar folia.

The leptomeninges show slight non-specific fibrosis.

The blood vessels are normal. A few fat granular cells are present in some of the perivascular spaces in most sections.

Staining Reactions of Stored Lipid

Brain (Frozen Sections).—The contents of the distended nerve cells failed to stain with Scharlach R, Sudan black B, and by Feyrter's method. With the periodic-acid-Schiff (P.A.S.) method, positive material was demonstrated in a few Purkinje cells and some of the cerebral neurones; however, the more abnormal the cell, the less well did its contents stain and grossly distended cells were P.A.S. negative. With the Gros-Bielschowsky method neurofibrils were displaced to the periphery of the ballooned cells and occasional torpedoes were demonstrated on the dendrites (Fig. 6).

Brain (Paraffin Sections).—With haematoxylin and eosin, fine eosinophilic or slightly basophilic granules

FIG. 6.—Medulla. Neurofibrils peripherally displaced by intra-neuronal lipid. Gross-Bielschowsky $\times 160$.

were found in the vacuolated cells. These granules varied in colour from blue to pale brown with Mallory's phosphotungstic acid haematoxylin. The cell contents were P.A.S. negative.

Spleen (Frozen Sections).—The contents of the majority of the foamy histiocytes stained positively with P.A.S. and Sudan black B, and a faint orange brown or not at all with Scharlach R.

Spleen (Paraffin Sections).—Most of the histiocytes contained fine P.A.S.-positive granules, although the reaction tended to be less intense than in the frozen sections. The Prussian blue reaction was practically

negative, only a small number of widely dispersed histiocytes containing haemosiderin granules.

Liver.—Paraffin sections only were available. Best's stain for glycogen was negative. With P.A.S. many liver cells contained a small quantity of very finely granular positive material. A few cells were distended with quite large quantities of positive material. These cells lay singly and occasionally in small groups in the sinusoids and appeared to be altered Kupffer cells and resembled the storage cells found in the spleen.

Biochemical Studies

Portions of formol-fixed brain, spleen, kidney, and liver were examined for their lipid contents.

Total phospholipid, sphingomyelin, total and esterified cholesterol, "galactose" cerebroside, and water were determined as in previous examinations (Cumings, 1953). Total hexosamine and neuraminic acid were estimated (Cumings, 1957) as well as true cerebroside (Radin, Lavin, and Brown, 1955).

The type of hexose in the cerebroside of the cerebral cortex and of the spleen was determined in the following manner. A methanol/chloroform extract of each organ was prepared according to the technique of Radin *et al.* (1955). The dried extract was hydrolysed and the lipid removed in the manner described by Montreuil, Boulanger, and Houcke (1953). The resulting hydrolysate was treated by an ion exchange resin (Woolf, 1953). The carbohydrates of the hydrolysate so treated, both before and after fermentation with yeast, were estimated by an anthrone method (Radin *et al.*, 1955). It was thus possible to estimate the galactose and glucose contents of the original cerebroside.

Another portion of the resin-treated hydrolysate was submitted to single-dimensional paper chromatography. The solvent system used was that of Montreuil *et al.* (1953) and after drying the paper it was treated by the method of Trevelyan, Procter, and Harrison (1950).

The presence of glucose was confirmed by a glucose spot reagent ("clinistix" glucose oxidase) and also by the formation of a glucosazone.

The results of lipid estimations in the brain and the organs are shown in Table I. It will be noticed that there are differences from the normal figures for a child of about the same age (see Cumings, Goodwin, Woodward,

TABLE I
LIPIDS IN THE ORGANS AND BRAIN

	Cerebral		Cerebellar		Spleen	Liver	Kidney	Normal Figures for Same Age	
	White Matter	Cortex	White Matter	Cortex				Cerebral White Matter	Cerebral Cortex
Total phospholipid	17.8	14.8	24.1	8.8	11.8	8.1	6.0	23.0	19.6
Sphingomyelin	10.0	3.2	8.8	3.5	6.7	0.6	1.2	5.6	4.3
Total cholesterol	10.3	6.9	17.2	8.3	8.0	1.9	1.6	15.7	5.5
Esterified cholesterol	0.4	0	0.2	0	0.3	0.1	0.1	0	0
"Galactose" cerebroside	20.7	17.5	31.7	15.9	7.6	6.0	8.5	17.9	4.1
True cerebroside	—	2.0	—	—	1.5	—	—	—	1.1
Total hexosamine	0.27	0.63	0.35	0.42	0.40	0.54	0.66	0.23	0.6
Neuraminic acid	—	0.34	—	0.20	—	—	—	0.07	0.35
Water (%)	73.7	85.6	81.2	86.0	72.5	71.8	78.6	71.3	83.7

Results in g./100 g. tissue (except water).

FIG. 7.—Chromatogram of hexoses in cerebroside of cerebral cortex (Cor) on the right. Glucose (Glu) and galactose (Gal) standards on the left.

FIG. 8.—Chromatogram of hexoses in cerebroside of the spleen, before yeast fermentation, on the left. Glucose (Glu) and galactose (Gal) standards on the right.

FIG. 9.—Chromatogram of hexoses in cerebroside of the spleen, after yeast fermentation, on the left. Glucose (Glu) and galactose (Gal) standards on the right.

and Curzon, 1958). The phospholipids in the cerebral white matter and the cortex are lost to some extent as is also the total cholesterol in the white matter, while the total hexamine and the neuraminic acid levels are normal. The cerebroside content of the cerebral white matter is decreased, but in the cortex it is increased both in the cerebellum as well as in the cerebrum. The spleen, kidney, and liver all show increased amounts of cerebroside as well as raised levels of total phospholipid. The normal figures for cerebroside in the spleen as given by Thannhauser (1953) are 0.1 to 0.6 g. per 100 g. dry tissue, so that the spleen of this patient contains a considerable excess of this lipid.

The results of the examination for the determination of the hexose in the cerebroside showed that in the cerebral cortex 63% of the hexose was glucose and 37% galactose, whereas in the cerebroside of the normal cortex all the hexose is galactose. In the spleen the figures were 82% glucose and 18% galactose. These findings were, in general, confirmed by the chromatographic procedures. The results obtained from the examination of the cerebroside in the cerebral cortex are shown in Fig. 7, where it can be clearly seen that both glucose and galactose are present. In Fig. 8 a similar result is demonstrated for the cerebroside of the spleen. By means of yeast the glucose portion of the cerebroside hexose in the spleen has been fermented and the chromatographic appearance is seen in Fig. 9.

Discussion

The histological findings clearly indicate that this case belongs to the group of so-called lipid storage disorders, the aetiology of none of which is known. The group includes the following conditions: Hand-Schuller-Christian disease, gargoylism, Niemann-Pick's disease, Gaucher's disease, and amaurotic family idiocy. A diagnosis may usually be reached by correlating the clinical and pathological data, but it is now well recognized that the chemical identification of the stored lipid in the viscera or the brain or in both is the only certain method by which a firm diagnosis can be reached. The histochemical methods available at present may not determine with absolute certainty the nature of the lipid for the principal reason that, with the exception of cholesterol, the lipids which accumulate in the affected cells are closely related to one another (Edgar, 1957). Nevertheless, histochemistry does form a useful adjunct to histological and biochemical studies.

Before discussing the chemical findings, it may be of interest to analyse briefly the clinical and pathological features of the present case in an attempt to fit it into one of the above categories.

Hand-Schuller-Christian disease can readily be excluded from the differential diagnosis for many reasons. It is perhaps sufficient to state that in this disease intraneuronal lipid storage does not occur;

the central nervous system, when affected, is involved usually in a secondary manner by the characteristic cholesterol granuloma extending from the skull or, more rarely, by scattered discrete granulomas within the brain (Feigin, 1956).

Ballooning of nerve cells, indicative of intraneuronal lipid storage, is found in gargoylism, in amaurotic family idiocy, and in Niemann-Pick's disease. There is some dubiety concerning the nature of the neuronal changes in Gaucher's disease in young children. The infantile form of the disease is rare, rapidly fatal, and the neurological symptoms are outstanding. The neuronal changes in most recorded cases appear to be of a non-specific degenerative nature (Diezel, 1957) but in a few cases slight evidence of neuronal lipidosis has been demonstrated (Norman, Ulrich, and Lloyd, 1956). Gaucher cells have been noted in the adventitia of some of the cerebral blood vessels (Norman, *et al.*, 1956; Barlow, 1957) but there was no intraneuronal lipid storage in Barlow's case. In the even rarer juvenile form of Gaucher's disease, neuronal lipidosis has been previously recorded in only two cases (Bird, 1948; Brain, 1954).

Gargoylism can readily be excluded because of the absence from the present case of skeletal changes and corneal opacities and the ease with which the substance stored in the spleen was stained after fixation (Dawson, 1954).

Visceral lipidosis, a constant feature of Niemann-Pick's and Gaucher's disease, has also been noted occasionally in amaurotic family idiocy. It is rare in the infantile variety (Tay-Sachs) though a case, only the second to be confirmed biochemically, has recently been reported (Norman, Ulrich, Tingey, and Goodbody, 1959) in which there was pronounced hepatomegaly and histological examination revealed foam cells in the liver, spleen, and many other organs, and also vacuoles in the parenchymatous cells of the liver and certain other glandular organs. Glasgow (1957) has reported upon a patient with amaurotic family idiocy, who is still alive, in whom typical foam cells were found in the sternal bone marrow. This patient also showed radiological evidence in the long bones of lesions which were suggestive of the deposits seen in Gaucher's disease. In the juvenile variety (Batten) visceral lipidosis has been noted rather more frequently (Schob, 1930; Sjövall, 1934).

Macular changes, usually present in Niemann-Pick's disease (cherry-red spot) and Batten's disease (pigmentary peppering and degeneration), were not noted in the present case. Moreover, patients with the former disease are chiefly Jewish and invariably die before the age of 2 years with prominent visceral involvement. Furthermore, the intraneuronal lipid

in Batten's disease stains bright orange with Scharlach R, is P.A.S. positive, and is insoluble in ordinary lipid solvents (Norman, 1958) in contrast to the present case in which negative results were obtained with Scharlach R and with P.A.S. after alcohol and chloroform extraction.

Thus an analysis of the clinical and pathological findings, by a process of exclusion, suggested that the case under consideration was one of juvenile Gaucher's disease and the chemical findings have substantiated this conclusion.

These various diseases have been shown by chemical analysis to have deposits of differing lipids: thus in Niemann-Pick's disease sphingomyelin in the organs and both sphingomyelin and gangliosides in the brain are accumulated. The brain in amaurotic family idiocy shows an increased content of ganglioside while in Gaucher's disease cerebroside is accumulated in the organs (Klenk, 1941; Thannhauser, 1953). The brain has rarely been examined and has been found to show no abnormality when histological evidence of abnormality was also lacking (Klenk, 1941; Thannhauser, 1953; Cumings, 1953).

The chemical examination for cerebroside in the brain in which there were recognizable histological abnormalities has not previously been made. Bird's case the total phospholipid and the sphingomyelin contents of the brain were normal but cerebroside were not estimated, even though they were found to be increased in the spleen.

A difference of opinion existed as to the nature of the hexose in the organ cerebroside, for Lieb (1924, 1927), Lieb and Mladenovic (1929), Epstein (1924), and Thannhauser and his colleagues (Ottenstein, Schmidt, and Thannhauser, 1948) all considered that it was galactose. However, later workers have shown that the cerebroside in the spleen contain glucose and not galactose (Klenk, 1941; Brante, 1951). As a result of the experiments recorded earlier it seems clear that the cerebroside deposited in both the brain and the spleen in this case contained glucose as the major hexose component.

These chemical findings would appear to confirm the clinical and histological findings in this case and make the diagnosis of Gaucher's disease certain.

Summary

The clinical, histological, and chemical findings in a case of juvenile Gaucher's disease are described in a young girl of 8 years. The cerebral and splenic cerebroside were increased and were shown to contain glucose in greater amount than galactose. Well-marked and abnormal deposition of lipid was found in the brain on histological examination.

The findings in this unusual case are discussed in relation to other reported cases of this condition.

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