The cerebellum in mucopolysaccharidosis

A histological, histochemical, and ultrastructural study

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SYNOPSIS Studies in the morphology, histochemistry, and ultrastructure of the cerebellum, with special reference to the Purkinje cell dendrites, have been undertaken in eight cases of gargoylism. The results suggest that the demonstration of ovoid swellings of the Purkinje cell dendrites by the Cajal method for the cerebellum, together with certain histochemical findings, may enable a diagnosis of mucopolysaccharidosis to be made. Possible mechanisms for the formation of these swellings are briefly discussed.

Gargoylism is one form of generalized neuronal storage disorder. The term gargoyle was suggested by Ellis *et al* (1936) to describe the grotesque facial appearance associated with this abnormality. Since then other forms of the disorder have been described, presenting less distinctive clinical appearances but nevertheless manifesting defective mucopolysaccharide metabolism, and it is now recognized that typical biochemical changes may be present without any appreciable alteration in the facial appearance.

The rapid advances in biochemical knowledge which have been made in recent years have permitted further classification of the disorder, notably by Hoof and Hers (1968) into three variants and by McKusick (1969) into six variants, three of which are associated with severe mental subnormality. This emphasis on the biochemical changes has led to relative neglect of the morphological aspect, though Green (1948), Jervis (1950), Henderson (1952), Naidoo (1953), Dawson (1954), and Bishton *et al.* (1955) have described histological studies of the brains of gargoyles in some detail, and several other case reports are also on record (Wallace *et al.*, 1966, and Dekaban *et al.*, 1971). The ultrastructural changes seen in biopsy material from the central nervous system were first reported by Aleu *et al.* (1965), and further studies have been made by Wallace *et al.* (1966) and by Loeb *et al.* (1968).

We describe here the histology, histochemistry, and electron microscopy of the cerebellar cortex in eight cases of gargoylism.

METHODS

The pathological material consisted of eight brains, seven of which had been fixed in 10% formalin for periods varying from two months to 15 years. In only one case (in which necropsy was carried out eight hours after death) was unfixed material available for examination.

The following techniques were employed on frozen sections in an attempt to identify the stored material: Herxheimer's Sudan IV, Periodic acid Schiff, Alcian blue, Hale's colloidal iron, Luxol fast blue, Feyrter's tartaric acid thionin, and the Cajal silver impregnation method for the cerebellum. Extraction and digestion methods were also used. Cryostat sections of the unfixed material were stained by all the above methods save for the Cajal, which of course was performed on formalin fixed material.

The cerebellum from all cases except case 2 was examined by electron microscopy. The fresh material was fixed in cacodylate buffered glutaraldehyde. The formalin fixed material was diced into 1 mm cubes, well washed in cacodylate buffer to remove all traces of fixative, and post-fixed in osmium tetroxide. The tissue was then dehydrated in ascending grades of alcohol, passed through propylene oxide and embedded in Araldite. Ultra-thin sections were stained in uranyl acetate and lead citrate.

CLINICAL FINDINGS The clinical features of the

TABLE 1
CLINICAL FEATURES

No.	Age (yr), sex	Age at onset (yr)	Family history	Hepato-splenomegaly	Corneal opacity	Facial abnormality	Skeletal abnormality	Severe mental defect	Mucopoly in urine	Syndrome
1	6 F	Early 2	Nil	+	+	Typical	Lumbar kyphosis	+	+	Hurler
2	17 M	5	+	+	—	Typical	Dorsolumbar kyphosis	+	+	Hurler
3	8 F	3	+	—	—	Atypical	Some beaking of vertebrae	+	+	Sanfilippo
4	9 M	5	+	—	—	Atypical	No classical features	+	+	Sanfilippo
5	NK M	NK	NK	NK	NK	Typical	Dorsolumbar kyphosis	+	+	Hunter
6	6 NK	NK	NK	+	NK	Typical	Dorsolumbar kyphosis	+	+	Hurler
7	11 F	4	+	—	—	Atypical	No classical features	+	+	Sanfilippo
8	14 M	5	+	—	—	Atypical	No classical features	+	+	Sanfilippo

NK: not known.

TABLE 2
NECROPSY FINDINGS

No.	Weight of brain (g)	Leptomeninges	Dilatation of ventricles	Convolution pattern	Perivascular spaces in basal ganglia	Infiltration in other organs with mucopolysaccharide
1	1,260	Thickened	Slight	Some atrophy of gyri	Present	+
2	1,650	Normal	Marked	Normal	Present + cyst formation	+
3	640	Thickened and tough	Moderate	Shrinkage of gyri	Absent	+
4	780	Thick and fibrous	Mild	Normal	Absent	+
5	NK	Thickened	None	Normal	Present	+
6	NK	Thickened	Moderate	Some shrinkage of gyri	Present	+
7	780	Thickened, opaque, slimy	Moderate	Mild atrophy of gyri	Absent	+
8	1,080	Thickened, opaque, slimy	Moderate	Moderate atrophy of gyri	Present	+

TABLE 3
HISTOCHEMICAL RESULT

	Red Sudan dye	PAS	PAS after diastase	Alcian blue	Colloidal iron	Luxol fast blue
Spheroids	Weak	+++	+++	++	++	++
Purkinje cells	Weak	+++	+++	++	++	++

patients are summarized in Table 1. The patients' ages ranged from 6 to 17 years. All were grossly subnormal mentally and presented the typical clinical appearance, in that the four cases of Hurler-Hunter syndrome had the characteristic gargoyle facies, and the four cases of Sanfilippo type did not.

Urinary excretion of mucopolysaccharides was demonstrated in all, and the substance was positively identified as heparan sulphate in cases 7 and 8. Cases 3 and 4 were siblings, and cases 2, 7, and 8 each had one sibling suffering from the same disorder. Case 1 had no affected sibling, and in cases 5 and 6 no relevant family history was available.

NECROPSY FINDINGS These are summarized in Table 2. All the cases except one showed thickening

of the leptomeninges and mild to marked dilatation of the ventricles. Five cases showed gyral atrophy; three did not. There was very slight atrophy of the cerebellar folia in all the cases. Hepatosplenomegaly was present in all. All the organs examined histologically contained vacuolated cells in which mucopolysaccharide was demonstrated histochemically.

CEREBELLAR CORTEX *Histology and histochemistry* The findings were identical in all eight cases.

The Cajal method showed that the granular layer, the basket cells, the white matter, and the Purkinje cells were relatively well preserved; where any cell loss had occurred there was mild gliosis. The Purkinje cells were distended by argyrophilic granules, and there were large ovoid expansions on

FIG. 1 (left). Photomicrograph demonstrating the presence of ovoids on the Purkinje cell dendrites. Frozen sections. Cajal cerebellar stain, $\times 300$. FIG. 2 (right). Photomicrograph at higher magnification showing a limiting membrane of the ovoids and that they contain granules similar to Purkinje cell cytoplasm. Frozen section. Cajal cerebellar stain, $\times 600$.

FIG. 3. Strongly positive periodic acid-Schiff reaction in the ovoids. PAS, $\times 300$.

FIG. 4. Alcian blue staining of granular content of ovoids. Alcian blue, $\times 300$.

FIG. 5 (left). *Electron micrograph. Numerous round to oval multilamellar bodies in ovoids. These bodies are demonstrated transversely where numerous, and circular where less densely packed.* $\times 12,500$. FIG. 6 (right). *Electron micrograph. Higher power of 'zebra bodies' shown in Fig. 5.* $\times 45,000$.

many of the dendrites (Fig. 1). The ovoids appeared to have limiting membranes and were packed with granules similar to those seen in the cell bodies (Fig. 2). Very few axonal torpedoes were present (they were stained deep grey by this method).

The histochemical findings are summarized in Table 3 and illustrated in Figs 3 and 4. They confirm that the stained material in the ovoids is a glycolipid indistinguishable from that seen in the Purkinje cell bodies.

Ultrastructure Studies of the ultrastructure were undertaken in seven cases, a large number of sections known to include Purkinje cells and their dendritic

swellings being examined. Again the findings were similar in all seven cases.

Lipid inclusions of varying size were found in the cytoplasm of the cell bodies and in the ovoids. These inclusions consisted of alternating clear zones and dense bands of a single unit membrane (Fig. 5). They appeared transverse in places where they were tightly packed, and circular where they were more loosely arranged (Fig. 6). No 'granulo-membranous' bodies were seen in any of our cases.

DISCUSSION

There are several possible explanations for the development of the dendritic swellings which we

FIG. 7. *Electron micrograph. Demonstration of 'zebra bodies' in the Purkinje cell cytoplasm in a case of Tay-Sach's disease. $\times 4,000$.*

have seen in all these cases, and which have been described by other authors, including Jervis (1950) and Bishton *et al.* (1957). Three of these seem to merit consideration.

Such enlargements could obviously result from a degenerative process in the dendrites. There are, however, no other signs of degeneration in these structures, and the axonal torpedoes which usually accompany any such process in the Purkinje cells were very rarely seen in these cases, though the ovoids were very numerous. The Purkinje cells in other types of neuronal storage disease, though often damaged or destroyed, do not develop ovoids on their dendrites at any stage.

The possibility that such swellings could be an artefact, caused by the absorption of water during prolonged fixation in formalin, is eliminated by the finding of typical lesions in the one case where fresh tissue was examined. In fact, the material appears to be unchanged by prolonged formalin fixation (up to 15 years in one case).

We suggest, though we are, of course, unable to prove, that the ovoid bodies merely represent an overspill of the abnormal material with which the Purkinje cell bodies are distended.

Other swellings on the Purkinje cell dendrites are found in infantile neuroaxonal dystrophy, but these occur also on many other dendrites and on axons throughout the central nervous system (Cowen and Olmstead, 1963). Furthermore, these swellings vary greatly in size, ranging from 6 to 79 μm (*ibid*), whereas those which we describe vary only within the range 30–70 μm . There are probably histochemical distinctions also; in our cases the ovoids were stained only faintly by Sudan IV, whereas the swellings in neuroaxonal dystrophy are said to be strongly Sudan positive. We have not found, nor have we seen described, any such bodies in any other type of neuronal storage disease.

In all our cases examined by electron microscopy 'zebra bodies' were present both in the ovoids and in the Purkinje cell bodies. Such intracellular inclusions have been described by Aleu *et al.* (1965), Loeb *et al.* (1968), and Wallace *et al.* (1966) in gargoyles, but they have also been seen in gangliosidosis by Suzuki *et al.* (1968) and by us in a case of Tay-Sach's disease (Fig. 7); presumably, therefore, they are not in themselves specific of mucopolysaccharidosis.

It would appear from our findings that the presence of ovoid bodies having the histochemical features which we describe may be diagnostic of mucopolysaccharidosis, though, of course, we do not suggest that their absence necessarily excludes the diagnosis.

Much of the material used in this study was acquired by the late Dr R. M. Norman, from sources which we are unable to mention individually. We are indebted to Dr J. Apley, Dr N. Brown, Dr R. T. T. Harrison, and Dr C. A. Pennock for making the material and clinical details of cases 7 and 8 available to us, and we thank Mr S. Foster and Mrs P. Stirling for photographic and technical assistance.

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