GM1 GANGLIOSIDOSIS IN FRIESIAN CALVES

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PLATES CXIV–CXX

The clinical features of a neuronal lipid storage disease in four Friesian calves from two herds have been described by Donnelly et al. (1972). The condition has since been recognised in four more calves from these herds and has been confirmed histochemically in a total of seven cases.

Clinical signs were noticed in the first month of life. Affected calves were slow to feed, dull, reluctant to move and ataxic. Progression of the disease resulted in coma and death at 6 to 9 mth. Histopathological findings were ballooning of neurons with lipid.

This paper describes the morphological and histochemical findings in seven of these calves. The results of lipid analyses of brain tissues from three affected calves and one control calf also are presented.

MATERIALS AND METHODS

The central nervous system (CNS) was examined in seven calves and liver, kidney, spleen, lymph node, adrenal, skeletal muscle, coeliaco-mesenteric ganglion, brachial plexus and retina were examined in four calves. Tissues for histological examination were fixed in calcium formol, embedded in paraffin and sectioned at 6 μm. Tissues for lipid histochemistry were fixed in calcium formol, quenched in liquid nitrogen and sectioned at 10–15 μm in a cryostat at –20°C.

Sections were stained with Harris’ haematoxylin and eosin, cresyl fast violet and phosphotungstic acid-haematoxylin (PTAH). Sections were impregnated with the silver methods of Holmes and Weil-Davenport. The following methods, as recommended by Adams (1965), were used for lipid histochemistry: acid haematein, luxol fast blue (LFB), Nile blue sulphate, oil red O (ORO), osmium tetroxide-α naphthylamine (OTAN)—alcian blue, performic acid—Schiff (PFAS), periodic acid-Schiff (PAS), acetylation/PAS, diastase/PAS, Sudan III, Sudan Black (SB) and toluidine blue.

The PAS, LFB and SB techniques were used before and after pyridine extraction.

A dimedone blocking technique was used with PAS (Bulmer, 1959).

Tissues for electron microscopy consisted of medulla removed at necropsy from two cases and fixed in formol calcium for 3 wk. These tissues were trimmed into pieces no larger than 1 sq mm and washed overnight at 4°C in veronal acetate buffer pH 7-4. The tissues were postfixed in veronal acetate buffered 1 per cent. osmium tetroxide at the same pH for 1 hr, dehydrated in graded alcohols and embedded in Araldite. Thick sections (1 μm) were stained with toluidine blue and Mallory’s borax methylene blue (Richardson, Jarett and Finke, 1960). Thin sections (60–90 nm) were mounted on uncoated copper grids, double stained with uranyl acetate and lead citrate and examined with a Hitachi HS 7 electron microscope.

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Lipid analyses were carried out by Mr H. Goodwin of the Department of Neurochemistry, Institute of Neurology, the National Hospital, Queen Square, on fixed brain material from three clinical cases and one control 6-month-old calf. Fixed liver and spleen from one of the clinical cases also were analysed.

RESULTS

Macroscopic observations

Most affected animals were in poor bodily condition. Coronal sections of the cerebrum showed bulging of the cortical grey matter above the level of the white matter with distinctive darkening of the grey matter-white matter junction (fig. 1). Slight coning of the posterior median lobe of the cerebellum with some excess cerebrospinal fluid were found in one case. Other organs were grossly normal in all cases.

Histological observations

Similar changes are present in the CNS in all cases with some slight variation in the intensity of the lesions. Neurons throughout the CNS are equally involved. They are ballooned and have a foamy or vacuolated appearance (figs. 2–4). Typically the nuclei of the neurons are displaced to an eccentric position with Nissl's granules aggregated around them (fig. 5). In the cerebellum, Purkinje cells have foamy cytoplasm or are contracted and pyknotic; the Golgi type II cells in the granular layer are swollen and foamy (figs. 3 and 4). Neuronal loss is not a feature.

Numerous spheroidal or ellipsoidal bodies are present at the level of the nuclei in the floor of the fourth ventricle, in the white matter of the cerebrum and cerebellum and occasionally in the granular layer of the cerebellum. These bodies have a granular appearance and a moderate to strong affinity for eosin. The silver impregnation technique indicates that they are axonal swellings (figs. 6 and 7).

Swollen glial cells are present in the white matter of the cerebrum and cerebellum. These cells are identified as reactive astrocytes with PTAH and the Weil-Davenport method (fig. 8). Many blood vessels in these areas have distended Virchow-Robin spaces containing light accumulations of round cells. These cells have the morphological characteristics of macrophages and some have fine cytoplasmic vacuolation. Increased numbers of microglia are found in grey matter areas particularly in the brain stem and cervical cord.

Neurons in the ganglion cell layer of the retina and in peripheral ganglia have swollen perikaryons with foamy cytoplasm.

Reticuloendothelial involvement is an inconstant and unspectacular feature. Sections of lymph-node from two cases and spleen from a third contain small numbers of swollen macrophages some of which exhibit fine cytoplasmic vacuolation. In the lymph-nodes these macrophages are found in the sinuses of the medullary zone; in the spleen they are present in the red pulp and at the periphery of germinal centres. No detectable lesions are found in other organs.
Histochemical observations

The histochemical reactions of the storage material are summarised in table I. Considerable variation in neuronal reaction is present with Baker’s acid haematein. Most neurons are negative or faintly positive but occasional cells show a very positive reaction (fig. 9). This feature is not seen with any of the other tests and is unrelated to the location of the positively reactive cells. The LFB, ORO and SB reactions are positive in all neurons (fig. 10). In frozen sections the PAS reaction is strongly positive in all neurons (fig. 11). There is a positive PAS reaction in some astrocytes and in some axonal spheroids.

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Acid haematein</td>
<td>+/−</td>
</tr>
<tr>
<td>Luxol fast blue (LFB)</td>
<td>+ + +</td>
</tr>
<tr>
<td>Pyridine extraction/LFB</td>
<td>−</td>
</tr>
<tr>
<td>Nile blue sulphate</td>
<td>Blue</td>
</tr>
<tr>
<td>Oil Red O (ORO)</td>
<td>+ + +</td>
</tr>
<tr>
<td>Osmium tetroxide-α naphthylamine</td>
<td>Blue</td>
</tr>
<tr>
<td>(OTAN)-alcin blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Performic acid-Schiff</td>
<td>+ + +</td>
</tr>
<tr>
<td>Periodic acid-Schiff (PAS)</td>
<td>+ + +</td>
</tr>
<tr>
<td>Acetylation/PAS</td>
<td>−</td>
</tr>
<tr>
<td>Diastase/PAS</td>
<td>+ +</td>
</tr>
<tr>
<td>Dimedone (30 min. at 60°C)/PAS</td>
<td>−</td>
</tr>
<tr>
<td>Pyridine extraction/PAS</td>
<td>−</td>
</tr>
<tr>
<td>Sudan III</td>
<td>+ + +</td>
</tr>
<tr>
<td>Sudan black (SB)</td>
<td>+ + +</td>
</tr>
<tr>
<td>Pyridine extraction/SB</td>
<td>−</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>Blue</td>
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</table>

In paraffin processed tissues the PAS reaction is greatly reduced or eliminated in many neurons but persists strongly in some. The PFAS reaction is negative in all neurons (fig. 12). The positive PAS reaction and the strong affinity for fat soluble dyes are eliminated by pyridine extraction for 18 hr at 60°C.

The neuronal storage material has a granular appearance with the LFB and PAS techniques (fig. 13). Similar but smaller LBF-positive granules are identifiable in some of the perivascular round cells. The granularity of the storage material is clearly visible in 1 μm sections of Araldite embedded tissues (fig. 14).

There is a reduction in the intensity of the staining reaction of the white matter of the cerebrum and cerebellum with LFB and OTAN resulting from a paucity of myelinated fibres. The number of oligodendrocytes is noticeably reduced. There are many axonal swellings in these areas. They contain fine or coarse granules which stain red-brown with OTAN and are limited by myelin sheaths. Small aggregates of deep red-brown granules found between the nerve fibres are probably located within astrocytes. Osmiophilic deposits are not present.
Ultrastructural observations

Numerous round to oval multilamellar bodies, 0.5 to 2.0 μm in diameter, are present in the cytoplasm of neurons and glial cells. The lamellae are arranged in a concentric fashion around a granular core (fig. 15).

Neurochemistry

Thin-layer chromatography on the brain samples from affected calves showed marked accumulation of the major monosialoganglioside (GM1); GM2 ganglioside was absent. A normal ganglioside pattern was found in the brain of the control calf. The results of the quantitative analyses of these samples are given in table II. No evidence of abnormal storage of GM1 ganglioside was detected in samples of liver and spleen from case No. 323.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Specimen</th>
<th>Total NANA* (mg NANA/100 g dry tissue)</th>
<th>Distribution of NANA (per cent)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GM1</td>
</tr>
<tr>
<td>64</td>
<td>Cortex</td>
<td>665</td>
<td>73-4</td>
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<tr>
<td></td>
<td>White matter</td>
<td>603</td>
<td>71-7</td>
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<tr>
<td>323</td>
<td>Cortex</td>
<td>586</td>
<td>82-4</td>
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<tr>
<td></td>
<td>White matter</td>
<td>490</td>
<td>82-9</td>
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<tr>
<td>212</td>
<td>Cortex</td>
<td>600</td>
<td>66-2</td>
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<td></td>
<td>White matter</td>
<td>373</td>
<td>64-5</td>
</tr>
<tr>
<td>Control</td>
<td>Cortex</td>
<td>227</td>
<td>31-8</td>
</tr>
<tr>
<td></td>
<td>White matter</td>
<td>64</td>
<td>38-6</td>
</tr>
</tbody>
</table>

* n-acetyl neuraminic acid.

DISCUSSION

The morphological, histochemical and analytical data presented in this study are consistent with a diagnosis of GM1 gangliosidosis. This type of storage disease has been described in man (Gonatas and Gonatas, 1965; O'Brien et al., 1965; Derry et al., 1968; O'Brien, 1969; Suzuki, Suzuki and Kamoshita, 1969) and cats (Baker et al., 1971; Blakemore, 1972). The disease has not been recorded previously in cattle.

Two types of GM1 gangliosidosis have been described in man. Type I is characterised by storage of the specific ganglioside in brain and viscera, storage of a mucopolysaccharide structurally similar to keratan sulphate in viscera, and skeletal abnormalities resembling those seen in Hurler's syndrome (O'Brien). Type II is characterised by storage of the specific ganglioside in CNS, without
GM1 gangliosidosis

Fig. 1.—Coronal section of cerebral hemisphere showing cortical swelling and darkening of the grey matter-white matter junction. × c. 1.5.

Fig. 2.—Swollen neurons with foamy cytoplasm in the medial vestibular nucleus. One neuron contains multiple vacuoles. Haematoxylin and eosin (HE). ×100.
FIG. 3.—Foamy Golgi type II cells (arrows) in the granular layer of the cerebellum. The nuclei of the Purkinje cells are displaced. HE. ×100.

FIG. 4.—Purkinje cells are sparse and pycnotic. HE. ×40.
GM1 GANGLIOSIDOSIS

Fig. 5.—Aggregation of Nissl's granules around displaced nuclei. Cresyl violet. × 200.

Fig. 6.—Axonal swellings in the cerebral white matter. Holmes' silver impregnation. × 200.
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Fig. 7.—Axonal torpedoes (A) in the granular layer of the cerebellum. Holmes' silver impregnation. ×400.

Fig. 8.—Reactive astrocytes in the cerebral white matter. Weil-Davenport. ×200.
**Fig. 9.**—Neurons exhibit variable phospholipid content. Myelin (right) stains intensely. Frozen section. Acid haematein. ×200.

**Fig. 10.**—The cytoplasm of neurons stains positively for lipid. Frozen section. Sudan black. ×200.
GM1 gangliosidosis

**Fig. 11.**—Strongly positive periodic acid-Schiff reaction in neurons in the coeliacomesenteric ganglion. Frozen section. Periodic acid-Schiff and haematoxylin (PASH). ×40.

**Fig. 12.**—Negative performic acid-Schiff reaction in neurons in the midbrain. Myelinated fibres stain positively. Frozen section. Performic acid-Schiff. ×100.

**Fig. 13.**—Granular appearance of the storage material in neurons in the coeliacomesenteric ganglion. PASH. ×400.
Fig. 14.—Granular inclusions in a neuron in the nucleus gracilis. Formol calcium and Os O₄. Mallory's borax methylene blue. × 950.

Fig. 15.—Membranous cytoplasmic bodies in a neuron in the nucleus gracilis. Electron micrograph. × 7000.
GM1 GANGLIOSIPOSIS

notable visceral or skeletal involvement (Derry et al.). As opposed to type I which develops earlier, clinical signs of type II GM1 gangliosidosis may not be apparent until the 12th mth and death, following a period of slow progressive deterioration, may not occur until 5 yr of age. The absence of skeletal deformities and visceral changes from the affected calves in the present study suggests that the disease in these calves more closely parallels the human type II form of GM1 gangliosidosis. These findings contrast with one chemically confirmed case in a cat where hepatic involvement was demonstrated (Blakemore). The disparity which exists between affected calves and infants in age of onset and rate of progression of the disease, possibly reflects the obvious differences in rates of growth and maturation which exist between these two species.

Bulging of the cut surface of the cerebral cortex may be specific for this condition in cattle. This change was consistently observed in the seven calves which we examined and a similar change does not appear to have been described in the brains of cattle affected with other types of neurological disturbance. The convolutional pattern of the cerebral gyri was described as normal or atrophic in infants with GM1 gangliosidosis (Landing et al., 1964; Gonatas and Gonatas; Derry et al.). Macroscopic cerebral change was not detected in affected cats (Baker et al.; Blakemore).

The microscopic findings conform to those described in man relative to the degree of neuronal swelling and gliosis (Norman et al., 1959; Landing et al.; Derry et al.) but differ somewhat with respect to demyelination and neuronal degeneration, features commonly seen in affected infants. There is no firm evidence of demyelination in calves although the staining reactions of the cerebral myelin are reduced. Neuronal necrosis is not prominent. Our studies also failed to reveal any change in the renal glomerular tufts where vacuolation of the epithelial cells has been described in both types of the human disease (Derry et al.; O'Brien). There is, in addition, very little evidence of reticuloendothelial involvement in the cases in this series. Vacuolated swollen reticuloendothelial cells in liver and spleen are seen in both types of human GM1 gangliosidosis (Derry et al.; O'Brien et al.).

Axonal swellings, similar to those described in these cases, have been recorded in a number of neuropathological conditions in cattle in which the primary change was in the perikaryon (Whittem and Walker, 1957; Barlow, Linklater and Young, 1968; Hartley, 1971; Jolly, 1971). The exact aetiology of these swellings has not been ascertained. Jolly however observed that, in pseudo-lipidosis of Angus calves, they contained structures resembling residual bodies. Our studies suggest that some of the swellings contain material which has histochemical similarities to the glycolipid in the perikaryons. In support of this, ultrastructural studies indicate that laminated bodies are present within nerve cell processes (Sheahan and Donnelly, unpublished observations).

The ultrastructural examinations reported in this study were performed as a diagnostic aid prior to chemical confirmation. The appearance of the intra-neuronal multilamellar bodies is similar to the membranous cytoplasmic bodies described in GM1 gangliosidosis in man (Gonatas and Gonatas) and cats (Blakemore). Structurally similar inclusions have been described also in
Tay-Sachs disease (GM2 gangliosidosis) in man (Terry and Weiss, 1963). The probability that these inclusions are lysosomal in origin has been accepted by most investigators.

The chemical analyses demonstrate a marked increase in total neuraminic acid (NANA) content in the CNS of affected calves. The relative distribution of NANA in the various ganglioside species is very similar to that described in human GM1 gangliosidosis (Suzuki et al.). The failure to detect an abnormal ganglioside pattern in samples of liver and spleen from one affected calf is consistent with the histological and histochemical studies which were also conducted on these tissues.

A remarkable reduction in the beta-galactosidase activity of tissue extracts from human cases of GM1 gangliosidosis has been demonstrated by Okada and O'Brien (1968). Preliminary studies indicate that a similar reduction in the activity of this enzyme exists in extracts of nervous tissue from affected calves (Donnelly, Sheahan and Kelly, 1973).

The suggestion that this disease in calves may be a result of an hereditable metabolic error (Donnelly et al., 1972) is substantiated by the present designation of the storage material and the occurrence of further cases in the two herds during a second breeding season. There is definite evidence of inbreeding in one of these herds and the paternal and maternal great-grand sires and great-great-grand sires of one affected calf are the same two bulls. These observations are comparable with the recognised familial occurrence and the probable transmission in an autosomal recessive manner of GM1 gangliosidosis in man (Okada and O'Brien; O'Brien).

This condition in Friesian calves may prove a useful model for studies of GM1 gangliosidosis.

SUMMARY

Structural, histochemical and chemical observations on a storage disease of Friesian calves have been described. The primary lesion was ballooning of neurons with glycolipid. The glycolipid was shown to be GM1 ganglioside. Firm evidence of visceral storage was absent. It was suggested that the disease might be analogous to type II GM1 gangliosidosis in man.

We are grateful to Professor A. N. Davison of the Department of Neurochemistry, Institute of Neurology, Queen Square, London, for facilitating us with lipid analyses and commenting on the results. We wish to thank Professor J. Hannan, Department of Clinical Veterinary Practices, Trinity College, Dublin, for bringing one affected herd to our notice. We acknowledge the technical assistance of Miss Carmel Brannick.

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