Neurovisceral Glucocerebroside Storage (Gaucher’s Disease) in a Dog

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Abstract. An 8-month-old Sydney Silky dog that was ataxic and hyperkinetic was found to have a neurovisceral storage disease. Typical Gaucher cells were seen in the liver, lymph nodes and cerebellum, but not in the spleen. Ultrastructurally, the storage bodies in Gaucher cells contained tubular structures, and many neurones contained laminated cytosomes, accumulations of a ‘wispy’ material and rarely tubular material. Chemically, the liver and brain contained glucocerebroside.

Over the last decade there has been an increasing awareness of the presence of neurovisceral storage diseases in domestic animals. The inherited lysosomal storage diseases in animals have been reviewed recently by JOLLY and BLAKEMORE [8] who discuss their similarity with comparable entities in man. This report describes what is probably the first recorded case of a disease in the dog that is similar to Gaucher’s disease in man.

Clinical History

A pedigree 8-month-old male Sydney Silky dog had gradually progressive nervous signs for approximately 1 month. On presentation for euthanasia it had marked incoordination with a wide-based stiff gait, generalised trembling, hyperkinesis, and over-correction of movements. There was exaggeration of local limb reflexes, but placing reflexes were normal. When resting in sternal recumbency the dog appeared normal. It has not been possible to ascertain if any of its litter mates were similarly affected.
Materials and Methods

A sample of blood was taken for haematology, then at autopsy the following samples were taken and immersed in 10% formal saline: whole brain and cord, eyes, lumbar spinal nerves, lung, heart, spleen, liver, pancreas, adrenal, thyroid, kidney, bladder, salivary gland, tonsil, testicle, striated muscle, aorta, duodenum, ileum, and lymph nodes from head, thorax, and abdomen.

After fixation the brain was sliced transversely and samples taken at seven representative levels. Blocks were also taken from cervical, thoracic and lumbar spinal cord. These and portions of the other viscera were processed for histopathological examination by conventional methods, embedded in paraffin wax and stained with haematoxylin eosin (HE). Selected sections of brain were also stained by the luxol fast blue – periodic acid-Schiff – haematoxylin (LFB – PAS – H) and LFB – Holmes silver methods [6]. Sections of liver and lymph nodes were also stained by the PAS – H method.

Small portions of formalin-fixed brain from the dorsal thalamic region, dorsal hippocampus, and the cerebellar folia together with liver and lymph node were taken for electron microscopy. These were post-fixed in osmium tetroxide, dehydrated with ethanol and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate and examined at 50 kV in an Hitachi Hs8 electron microscope.

Samples of formalin-fixed liver and cerebral hemisphere were submitted to the Department of Neurochemistry at the National Hospital, London, for lipid analysis.

The tissue used for ultrastructural and biochemical studies had been fixed in formalin for 2 years prior to examination.

Results

Haematology

The only abnormal finding was a total white cell count of 16,900/mm³, of which 92% were mature neutrophils, 3% small lymphocytes, 3% monocytes, 1% small lymphocytes with vacuolated cytoplasm and 1% normoblasts. This indicates a mild leucocytosis with neutrophilia, lymphopenia and eosinopenia.

Morbid Anatomy

No gross abnormality was seen.

Light Microscopy

The liver had focally disseminated small groups of intrasinusoidal cells (fig. 1). Each group consisted of up to about 10 large cells, some of which may have coalesced. They had one or more vesicular nuclei or a shrunken one, all usually peripherally located. The cytoplasm was weakly eosinophilic and amorphous and occasionally contained a few small vacuoles. With PAS
the cytoplasm ranged from diffuse pale pink to dark red and occasionally contained a few distinct PAS-positive filaments. These cells had the characteristic morphology of Gaucher cells [10]. Several of these cellular aggregates were infiltrated by other cells with a small irregular hyperchromic nucleus and no obvious cytoplasm.

All the lymph nodes examined and the tonsil had marked hyperplasia of the cortical elements with prominent lymphatic nodules. In addition, all nodes had mild to severe diffuse infiltration by large cells similar to those in the liver. They were most profuse at the corticomedullary junction and extended into the medulla within the medullary cords. There was no invasion of the cortical or medullary sinusoids. None of these cells was seen in the spleen or tonsil.

Throughout the brain, but not in the cord, variable numbers of neurones were present that had a distended pale cytoplasm. This resulted from loss or peripheral displacement, or both, of Nissl granules and replacement by either a foamy, finely vacuolated cytoplasm or numerous weakly eosinophilic, PAS-negative granules. These changes were most obvious in neurones of the dorsal and lateral thalamic nuclei and the dorsal hippocampus (fig. 2). They were present in fewer numbers also in the cerebral cortical grey matter, inferior colliculus, oculomotor nucleus, cochlear nucleus, trigeminal motor nucleus, superior olivary nucleus, dentate nucleus, fastigial nucleus and the ventral pontine grey matter. There was no apparent involvement of Purkinje cells, the inferior olivary nucleus, hypoglossal nucleus, vagal nucleus or the retina.

Focally within the dorsal cortical grey matter there were a few to many very shrunken neurones with pyknotic nuclei whose cytoplasm sometimes showed definite foamy vacuolation. There were a few degenerate neurones with a distinct eosinophilic cytoplasm and a fragmenting nucleus. Neuronophagia did not occur.

In the cerebellum there was a widespread, mild to severe, atrophy of the granular cell layer. In the worst affected folia there was a virtual absence of granule cells with part replacement by large cells with a finely vacuolated cytoplasm and either one or two large vesicular nuclei or a peripheral shrunken nucleus (fig. 3). There were also degenerating Purkinje cells.

In all parts of the superior olivary nucleus there was an almost complete loss of neurones together with a moderate diffuse gliosis. There was also a probable loss of neurones from the cochlear nucleus.

At all levels of the brain there was a mild to moderate spongy vacuolation of white matter; in many instances the vacuoles were empty, but some contained a swollen axon or a lipid phagocyte. There was also breakdown of
Fig. 1. Liver, groups of Gaucher cells. HE.

Fig. 2. Hippocampus, swollen neurones with foamy cytoplasm. HE.

Fig. 3. Cerebellum, Gaucher cells present (arrow) in granular layer and loss of granule cells. HE.
myelin sheaths. The areas most severely affected were the central white matter of the cerebral hemispheres, corpus callosum, optic tracts, cerebral peduncles, trapezoid body, central cerebellar white matter and spinocerebellar and corticospinal tracts. Similar vacuolation was present around the nuclei with neuronal changes, particularly about the superior olives. A few axonal spheroids were present in the ventral pontine grey matter.

Throughout the central nervous system the parenchymal blood vessels were prominent with an apparent swollen, PAS-positive endothelium. There were also a few perivascular cells with copious foamy cytoplasm.

In the kidney there were numerous distinct, round, eosinophilic, intranuclear inclusion bodies up to 3 μm in diameter in the epithelium of the proximal convoluted and descending straight tubules, particularly at the corticomedullary junction. Epithelial cells of the urinary bladder and adrenal cortex had foamy vacuolation of the cytoplasm. No obvious abnormalities were seen in choroid plexus, retina, thyroid, pancreas, salivary gland, duodenum, ileum, lung, heart, aorta, skeletal muscle or testicle.

Ultrastructure

The Gaucher cells in the liver had differing appearances related to the amount of storage material present. When they contained appreciable amounts, their surface was made up of several layers of fine processes (fig. 4). The stored material was clearly membrane bound when it was compacted, but when it was more dispersed a limiting membrane was not always apparent. The stored material consisted of twisted, branching, tubular structures 40 to 60 nm in diameter and of indeterminate length (fig. 4). The storage areas in some instances were in continuity with the extracellular space. The Gaucher cells also contained a small number of laminated bodies similar to those seen in neurones. The preservation of the tissue was not sufficient to allow more detailed morphological observations.

The cells in the lymph node had a similar appearance and contained the same storage material as the Gaucher cells in the liver.

Neurones commonly contained two types of stored material, which was present in differing amounts depending on the location of the cell. The neurones from the dorsal thalamic area contained large numbers of membrane-bound laminated bodies (fig. 5); the periodicity of the electron-dense lamellae was 5–6 nm (fig. 6). The laminated contents of these bodies were often confluent with areas of 'wispy' material (fig. 5, 7), which may or may not have been membrane bound. The areas of 'wispy' material were often quite extensive and at higher magnification were composed of irregularly orientated
Fig. 4. Liver, part of a Gaucher cell showing folding of cell surface (s) and material stored in tubules (t) in cytosomes.

bilamellar material that sometimes had a periodicity of 5 nm. This material was also seen, together with laminated bodies, in the granule cells of the cerebellum. The neurones in the hippocampus contained fewer laminated bodies and less ‘wispy’ material than the neurones in the thalamus. Tubular material similar to that seen in the Gaucher cells of the liver was not found in neurones. One neurone, however, contained an area of tubular material with a diameter of 20–30 nm (fig. 8), and another contained a mixture of
Fig. 5. Thalamus, neuronal ‘wispy’ storage material (w) can be seen in continuity with laminated cytosomes (c).

Fig. 6. Thalamus, part of a laminated cytosome; the periodicity of the lamellae is approximately 5–6 nm.

Fig. 7. Thalamus, ‘wispy’ material in the cytoplasm of neurones.
'wispy' material and irregular tubules approximately 10 nm in diameter (fig. 9). Neither Purkinje nor glial cells contained stored material of any kind. Typical Gaucher cells were seen in the cerebellum in areas where there was degeneration of granule cells but not elsewhere in the brain.

**Chemical Findings**

The results of the cerebroside analysis on the brain and liver are given in table I. Large amounts of glucocerebroside were found in the liver and significant amounts in the brain. Normally cerebroside is present as galactocerebroside.

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<thead>
<tr>
<th>Table I. Cerebroside content of liver and brain</th>
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<tr>
<td>g/100 g dry tissue</td>
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<tr>
<td>Liver</td>
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<tr>
<td>total cerebroside</td>
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<tr>
<td>glucocerebroside</td>
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<td>Brain, white matter</td>
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<td>total cerebroside</td>
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<td>glucocerebroside</td>
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<td>Brain, cortex</td>
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**Discussion**

The abnormal cells seen in the liver, lymph nodes, and cerebellum of this dog are indistinguishable by light and electron microscopy from those described as Gaucher cells in man [7, 10, 11]. These observations, together with the detection of glucocerebroside in liver and brain, are sufficient evidence to diagnose a glucocerebroside storage disease that has obvious similarities to Gaucher's disease in man.

As far as we can ascertain this represents the first substantiated case of a disease in animals that is very similar to Gaucher's disease in man. Previously, the visceral form of this condition has been suspected, once in a pig [12] and once in a sheep [8].

Gaucher's disease in man has been shown to be associated with a deficiency of the enzyme glucocerebrosidase [4], which results in storage of glucocerebroside in cells of the reticuloendothelial systems. The disease has several clinical forms [10]: the infantile form shows a variety of nervous signs including strabismus, retroflexion of the head, spasticity, bulbar paralysis, and occasionally tremor; whereas in the juvenile and adult forms the main char-
Fig. 8. Thalamus, tubular material present in a neurone, 20–30 nm in diameter.

Fig. 9. Thalamus, a mixture of tubular and ‘wispy’ material in a neurone. The tubular elements (single arrow) are 15–20 nm in diameter, and the ‘wispy’ material has a periodicity of 7–8 nm (double arrow).
acteristics are splenomegaly and hepatomegaly, only very rarely are nervous signs present.

In our canine case three distinct forms of storage material were seen with the electron microscope in brain neurones; these were tubules, ‘wispy’ material, and laminated cytosomes. ADACHI et al. [1] found tubular material in neurones, but we saw this in one cell only. Much commoner was the extensive accumulation of ‘wispy’ material, both free and associated with the laminated bodies. The nature of this material, previously unrecorded in animal lipidoses, is unknown; however, it has a morphology similar to that of the material found in Buscaino bodies, which has been considered to resemble cerebrosides [2, 5]. The appearance of our laminated bodies is similar to the membranous cytosomes reported by ADACHI et al. [1] in a case of early infantile Gaucher’s disease in man. They are also similar to the cytosomes seen in conditions in which there is storage of ganglioside; in particular they resemble the zebra bodies of gargoylism [3]. SVENNERHOLM [14] reported alteration in the ganglioside ratio in cases of infantile Gaucher’s disease, and he concluded that the glucocerebrosidase that accumulates is derived from ganglioside. ADACHI et al. [1] observed apparent continuity between tubular material and laminated cytosomes, as we observed apparent continuity between the ‘wispy’ material and the laminated bodies. It may be possible, therefore, that the ‘wispy’ material represents either a specific storage product, an alteration in the morphology of the stored substance (caused by prolonged storage in formalin), or a mixture of ganglioside and cerebroside.

Our canine case differs clinically from the cases seen in children in that incoordination and tremor were the main signs and there was no splenomegalgy or hepatomegaly. There were considerable morphological neurovisceral similarities, however, between our case and some of the cases in children [10], except for apparent noninvolvement of the spleen. These clinical and pathological differences again indicate that although specific lysosomal storage diseases in animals are very similar to the related human disorders they are not always identical [13].

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References


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