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Clinical Communication

Polyglucosan body disease in a mixed-breed dog

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Abstract

AIM: To describe the histopathology of a previously unrecorded canine disease and deduce the cause of the lesions.

METHODS: Formalin-fixed tissues were processed into paraffin wax and epoxy resin for light and electron microscopy of variously stained sections of liver, brain, heart muscle and kidney.

RESULTS: Periodic acid Schiff (PAS) -positive bodies in liver and myocardium were typical of a polyglucosan body disease. Neurons contained coarse granular material that stained similarly to the polyglucosan bodies.

CONCLUSION: The nature, distribution and histochemistry of lesions observed are consistent with a putative diagnosis of Glycogen storage disease type IV, an inherited metabolic defect associated with a deficiency of glycogen-branching enzyme not previously reported in dogs.

KEY WORDS: *Polyglucosan body, glycogen storage, type IV, dog.*

Introduction

In contrast to human patients who may undergo extensive exploratory metabolic tests, inherited metabolic defects in animals are more commonly first diagnosed following necropsy and histopathological examination. Although appropriate frozen tissues may not be kept, much can be deduced about the biochemical defect from histopathological, histochemical and electron microscopic examinations of fixed tissues and subsequent comparison with similar lesions in humans, the same, or other animal species. Allowance needs to be made for inter-species or age variations in expression of the disease process. The present case concerns a polyglucosan body disease; it is an example of the use of deductive reasoning that suggests diagnosis of an enzyme deficiency consistent with a putative diagnosis of Glycogen storage disease type IV, previously unrecorded in dogs.

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Clinical History

Ill health in a 6–7 month-old female mixed-breed dog, described as a Labrador-cross by the owners, that weighed 9.8 kg was investigated over a 1-month period. She had a history of postprandial vomiting for the previous 3 weeks and the owner had noted an unusual gait. On first examination she had a normal temperature, a stilted hindlimb gait and loose greenish faeces. She was treated with bismuth salicylate and sodium bentonite (Peptosyl, Vetpharm (NZ) Ltd, Auckland, NZ), dipyrone and hyoscine (Buscopan, Boehringer Ingelheim (NZ) Ltd, Auckland, NZ), and a hypoallogenic diet (Hills I/D, Hill's Pet Nutrition, Topeka KS, USA). When presented again 2.5 weeks later, the bitch was dry retching and demonstrated a gag reflex when attempting to eat. Her forelimbs were weak, she had a stiff, stilted hindlimb gait and was reluctant to move. Her back was arched and the neck was stiff on ventral flexion and dorsal extension. The temperature was 39.5°C and a slight left shift was evident on haematological examination. There were no significant changes evident on radiological examination of the cervical spine and fore limbs. Dexamethasone was administered and the owners were requested to revisit with the dog next day. Three days later the dog was again presented having deteriorated considerably. She was unable to rise and showed postprandial regurgitation. A diagnosis of *Neospora caninum* infection was considered and treatment with Clindamycin (Antirobe, Pharmacia/Upjohn (NZ) Ltd) was initiated. Four days later her temperature was still elevated and because there were signs of pneumonia, trimethoprim sulphur was added to the treatment regimen. There was continued deterioration including the onset of seizures and weight loss for a further 5 days, at which time the bitch was euthanased with intravenous pentobarbitone.

Materials and Methods

At necropsy brain, spinal cord, heart muscle, liver and kidney were fixed in 10% formol saline. They were subsequently processed into paraffin wax. Microtome sections (3 μm) were stained with haematoxylin and eosin (H&E), PAS (with and without diastase pretreatment), luxol fast blue (LFB), sudan black, Best's carmine, toluidine blue and Lugol's iodine methods. Unstained sections were used for fluorescence microscopy. Formalin-fixed brain liver and heart were also post-fixed in 1%

H&E	Haematoxylin and eosin
PAS	Periodic acid Schiff
LFB	Luxol fast blue

osmium tetroxide and processed into epoxy resin. 'Thick' sections were stained with toluidine blue; 'thin' sections were stained with uranyl acetate and lead citrate.

Results

Gross pathology

Apart from light body condition, no gross lesions were noted.

Histopathology

On H&E staining, numerous smooth globular slightly basophilic bodies up to 15μm in diameter were evident within hepatocyte cytoplasm. These did not stain with sudan black, LFB or exhibit autofluorescence. They stained with Best's carmine and brilliantly with PAS even after pretreatment with diastase (Figure 1). In addition to these bodies, there were sometimes several other smaller PAS-positive coarse granules that had also withstood diastase pretreatment, as well as some smaller granules which did not. With Lugol's iodine, the larger bodies stained black, but smaller ones, including some which appeared to be aggregating into larger bodies, stained purple/brown. Moderate metachromasia was demonstrated using toluidine blue staining. Similar large and small bodies that had the same staining characteristics occurred within cardiac muscle cells (Figure 2) and Purkinje fibres. Autofluorescence was not present. No such bodies were noted in the kidney.

In the brain and spinal cord, neurons contained coarse granular material in the perikaryon that was slightly basophilic in H&E stained sections. This material was PAS-positive (Figure 3), even after diastase treatment, stained purple/brown with Lugol's iodine and was positive to Best's carmine stain for glycogen. It did not stain with sudan black, LFB or show autofluorescence when unstained sections were viewed with a fluorescence microscope.

Electron microscopy

In 'thick' sections, used to orient the tissues for cutting 'thin' sections, the coarse granules and globules described above stained light blue with toluidine blue. Ultrastructurally, the large globules in liver and heart muscle were moderately electron dense, had an irregular outline and were not surrounded by a bilayer membrane (Figure 4). Observation of variously sized globules indicated that the larger bodies probably developed by aggregation and fusion of the smaller ones in combination with the coarse granular material noted using light microscopy. At high magnification, the ultrastructure of these bodies was granular but granules were

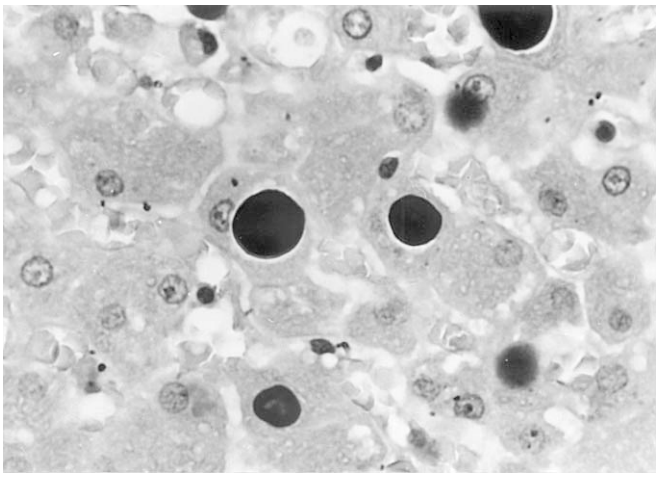

Figure 1. Polyglucosan bodies in hepatocytes. Paraffin PAS, 540x magnification.

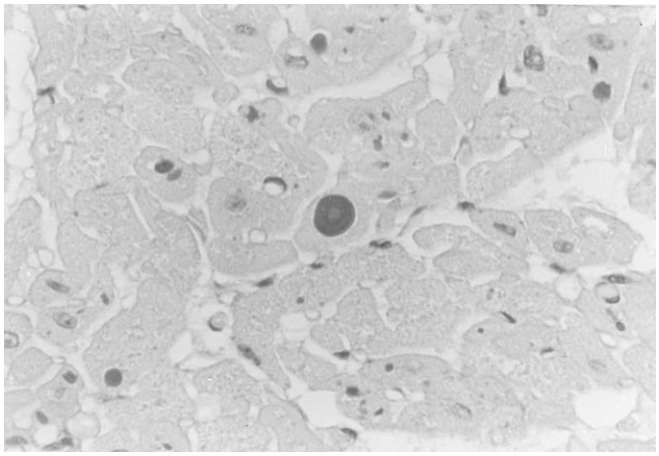

Figure 2. Polyglucosan and smaller bodies in cardiac muscle cells. Paraffin PAS, 500x magnification.

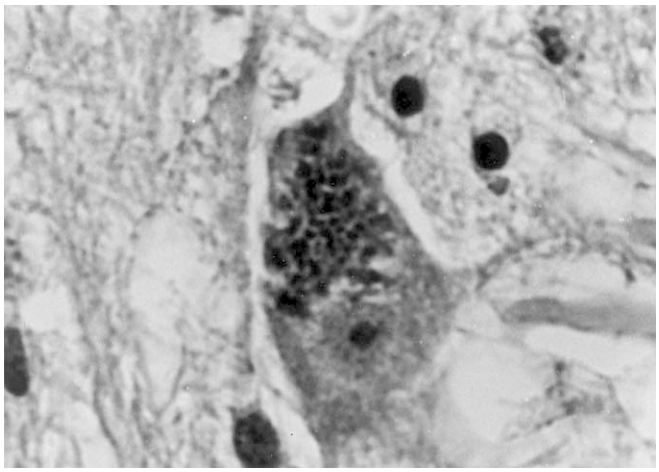

Figure 3. Neuron in the hind brain with coarse PAS-positive granules in the cytoplasm. Paraffin PAS, 650x magnification.

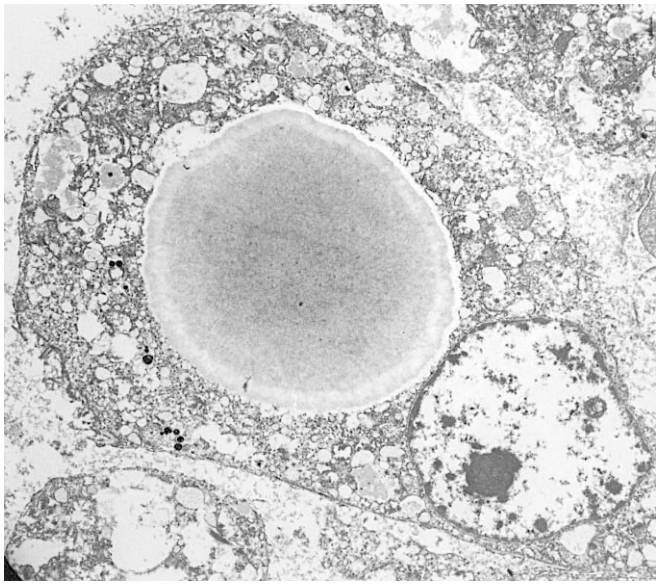

Figure 4. A single large polyglucosan body in an hepatocyte. Electron micrograph, 3400x magnification.

arranged in fingerprint-type profiles (Figure 5).

Electron microscopy of neurons confirmed that the intracytoplasmic inclusions were of a coarse granular nature (Figure 6). They appeared to have grown by fusion of smaller bodies to form irregular rounded aggregations that were not surrounded by a bilayer membrane. In contrast to these, other similar bodies were clearly seen within membrane bound organelles interpreted to be lysosomes (Figure 7).

Discussion

The lesions in liver and cardiac muscle are those of a polyglucosan body disease. Polyglucosan bodies are largely composed of polymerised glucose and are found universally in the brain and elsewhere in the nervous system of aged humans and less commonly in domestic animals, as corpora amylacea (Cavanagh 1999). In the central nervous system, they occur in most regions but are concentrated in some areas within the glial feltwork beneath the ependymal lining and pia mater, and within axons. Diseases characterised by polyglucosan bodies include Lafora body disease, Bielschowsky body disease, and type-IV glycogen storage disease, including an adult form also known as adult polyglucosan body disease (Cavanagh 1999). Lafora body disease, which is inherited as an autosomal recessive trait in humans, is also well described in dogs (Summers et al 1995; Cavanagh 1999), including a Basset hound in New Zealand (Jian et al 1970). However, Lafora-type polyglucosan bodies have a dense staining central core with a striking outer radiating pattern of less densely staining material and, although they occur in various tissues, are mainly found within the perikaryon of neurons (Cavanagh 1999).

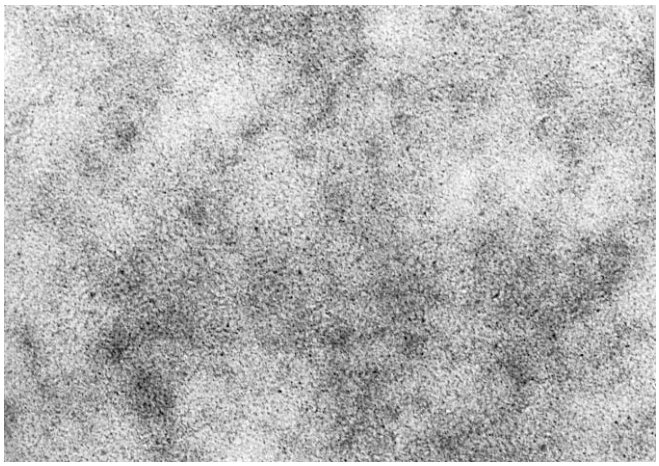

Figure 5. At the ultrastructural level, polyglucosan bodies in the liver had a granular appearance arranged in finger print type profiles. Electron micrograph, 103600x magnification.

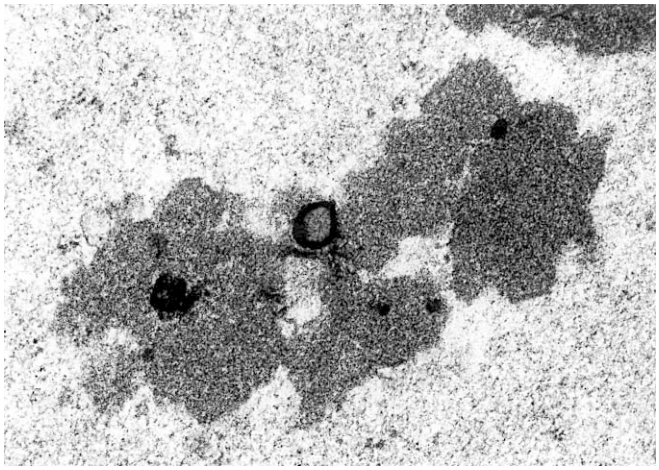

Figure 6. Ultrastructurally, accumulated material in neurons was coarsely granular and appeared to be formed by coalescence of smaller bodies. The conglomerates usually did not appear surrounded by a bilayer membrane. Electron micrograph, 31800x magnification.

Glycogen storage disease type IV is a rare disease associated with varying degrees of deficiency of the glycogen-branching enzyme that normally brings about branching of chains of glucosyl units so that they can be packaged into the relatively soluble α-particle, the main cellular reserve of glucose. In the deficient state, the resultant polyglucan that resembles amylopectin, has fewer α-1,6-linked branch points, longer α-1,4-linked glucose segments and is relatively insoluble (Chen and Burchell 1995). Ultrastructurally, in addition to the conventional α and β glycogen particles, fibrillar aggregations also occur.

The clinical signs and lesions of Glycogen storage disease type-IV vary depending on the mutation responsible, hence age of presentation and the species affected. In humans, infantile, juvenile and adult onset diseases have been described (Schochet et al 1970, 1971; Chen and Burchell 1995; Bao et al 1996; McKonkie-Rosell et al 1996; Lossos et al 1998; Cavanagh 1999). In infants there is failure to thrive, with gastrointestinal disturbances and hepatic failure due to cirrhosis of the liver. Signs of neurological involvement are uncommon in younger patients. In older patients, liver cirrhosis does not occur, but there may be progressive cardiac and somatic myopathy and progressive disorders of central and peripheral nervous systems.

The clinical signs, nature and histochemistry of the inclusions and their distribution in the bitch described above, are consistent with a putative diagnosis of Glycogen storage disease type-IV. Although skeletal muscle was not kept, the clinical history of muscle involvement and the nature of this disease in other species would imply that skeletal and probably smooth muscle of the upper gastrointestinal tract were also involved. Nevertheless, there are differences worth noting. In human forms of the disease, polyglucosan bodies, although occurring in the CNS, have a cytological location similar to that of corpora amylacea and, with

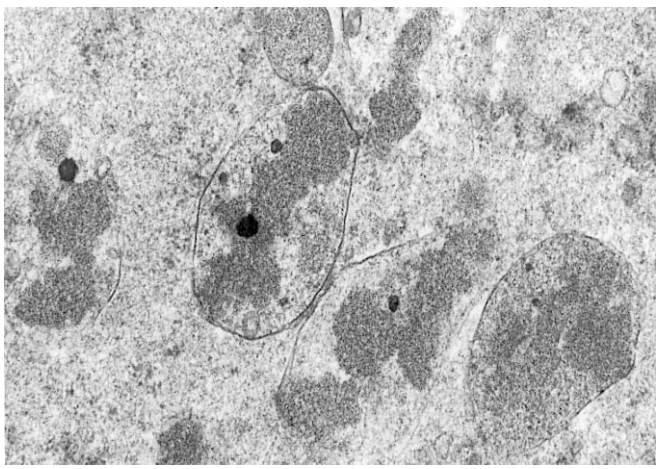

Figure 7. In some neurons the granular accumulated material was surrounded by a bilayer membrane. Electron micrograph, 31800x magnification.

the exception of 1 pair of affected newborn twins (Gallen et al 1986), are not described in the perikaryon of neurons. However, basophilic rounded inclusions occurred in neurons of Norwegian forest cats with glycogenosis type IV (Fyfe et al 1992). These appeared similar, although not identical, to those in the present canine case. Ultrastructurally, the neuronal inclusions in this were more coarsely granular than those in the liver and they appeared both free within the cytoplasm (Figure 5) and also within membranous organelles (Figure 6) that were interpreted to be secondary lysosomes. As this disease does not otherwise resemble Glycogen storage disease type II, a lysosomal storage disease due to a deficiency of α-glucosidase, it is postulated that the aggregations of abnormal polyglucan may have been taken into the lysosomal system by autophagy. In the liver, the ultrastructure of polyglucosan bodies was less fibrillar than the material in other reports, but this could be due to inadequate fixation for electron microscopy. Large polyglucosan bodies did not occur in the livers of affected Norwegian forest cats. This was attributed to cats normally having high hepatic gluconeogenic activity, storing little hepatic glycogen and mobilising little hepatic glycogen when fasted (Fyfe et al 1992).

Amylopectinosis, shown to be due to a deficiency of glycogen-branching enzyme, has also been diagnosed in 3 Quarter horses, a fetus, a neonate and a 1-month-old foal. There were prominent large polyglucosan bodies in cardiac and skeletal muscle (Render et al 1999). These were not noted in liver or brain but smaller granules with similar staining characteristics did occur in these tissues, being particularly abundant in motor neurons. Their small size may reflect the very young age of these animals. Two further cases of this disease in Quarter horse foals have been recorded (Valberg et al 1998).

The present report is the first putative diagnosis of Glycogen storage disease type IV in dogs. Confirmation and full characterisation of lesions must await further cases and demonstration of a deficiency of glycogen-branching enzyme. The history is relatively uninformative as to the primary breed carrying this mutation. The dog was described as a Labrador-cross by the owners, but the exact breeding was unknown. Given the obvious rarity of the putative mutant gene, close consanguinity would be expected in the immediate parentage so the mutation could equally have come from another breed.

Whereas the special enzymological tests to confirm a deficiency of glycogen-branching enzyme may not be readily available to practitioners, diagnosis may be aided by histopathological examination of muscle, liver and even skin biopsies. At necropsy of a suspected case, frozen tissues, as well as a full range of fixed tissues including skin, should be kept for further biochemical and histopathological investigation.

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