Inherited Copper Toxicosis in Bedlington Terriers

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SUMMARY: Chronic hepatitis and increased hepatic copper concentrations, from 1,600 to 6,361 µg/g dry tissue were found in 4 related, Australian-bred Bedlington terriers. Two dogs were asymptomatic and 2 were clinically ill with signs referable to liver dysfunction. Two dogs were treated with d-penicillamine. After one year there was no improvement in the histopathological liver changes in either dog or significant lowering of hepatic copper level in one dog. Aust. vet. J. 60: 235-238.

Introduction

In 1975 chronic progressive hepatitis associated with increased amounts of hepatic copper was described in related Bedlington terriers in the United States (Hardy et al 1975) and later was shown to be due to an inherited metabolic defect in the breed (Johnson et al 1980).

There are many similarities between the disease and Wilson's disease (hepatolenticular degeneration) in man (Sternlieb 1980). Both are inherited as autosomal recessive traits, are characterised by increased hepatic copper concentration which precedes hepatic injury and are accompanied by decreased biliary and increased urinary excretion with normal gastrointestinal absorption of copper (Su et al 1982a). Important features of Wilson's disease not found in affected dogs are decreased levels of serum or plasma copper and caeruloplasmin and the various extrahepatic signs related to accumulation of copper in other sites which include Kayser-Fleischer rings (copper deposits in the peripheral cornea), neurologic deficits, psychiatric disorders, kidney dysfunction and arthritis. Increased amounts of copper have been found in the kidneys and brain of affected dogs (Su et al 1982b), but clinical signs referable to these sites have not been reported. Although there are differences between these 2 diseases, important similarities make affected Bedlington terriers useful animal models for the study of Wilson's disease (Owen and Ludwig 1982).

Until now, there have been no reports on this disease occurring in Bedlington terriers outside the United States of America.

Materials and Methods

Four Bedlington terriers§ that had been bred and raised in Victoria were referred to the University of Melbourne Veterinary Clinical Centre. They were fed mixed diets that included a variety of commercial dog foods, fresh meat and table scraps.

Wedge biopsies of liver were obtained from each dog via laparotomy and portions were submitted for histopathology and quantitative copper analysis. Copper measurements of plasma and liver were made by flame atomic absorption spectrometry. Containers used for tissue and blood were washed in 20% nitric acid prior to use. Plasma caeruloplasmin levels were determined by oxidation of p-phenylenediamine (Bingley and Dick 1969) at pH 6.4. Haematology, plasma biochemistry, sulfobromphthalein (BSP) retention times and urinalyses were performed by standard methods.

Case Histories

Case 1: A 6-year-old bitch was presented with a history of abdominal enlargement for 17 days and loose stools for one week. There were no other complaints. The dog previously had been in good health and was in oestrus 2 weeks before the onset of signs.

When examined, the dog was in good body condition. Temperature, pulse and respiratory rates were normal. There was marked distention of the abdomen with a fluid wave easily demonstrated. Abdominal palpation and radiography were restricted by the large amount of fluid present. Mammary enlargement and lactation were present, consistent with pseudocyesis.

The only abnormalities found in the haemogram, urinalysis and blood chemistry were slight leucocytosis, neutropenia with left shift, cosinopenia and elevated plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. The BSP retention time was slightly increased. Plasma copper was increased and caeruloplasmin was normal (Table 1).

Ascitic fluid had properties of a modified transudate. It was slightly turbid and blood-tinted with a specific gravity of 1.025, protein content of 42 g/L and cell count of 1,000/mm³, consisting mainly of erythrocytes with some neutrophils, mesothelial and plasma cells.

Clinical signs associated with the original presentation did not recur following the laparotomy and liver biopsy. Treatment was commenced with 125 mg d-penicillamine¶ orally 12-hourly. The dog remained asymptomatic for the following 13 months until represented with a history of lethargy, anorexia, polydipsia and vomiting of 3 days duration. Oestrus had occurred 6 weeks before. There was a mucopurulent vaginal discharge and enlargement of the uterus seen in abdominal radiographs.

Pyometra was diagnosed and an ovariohysterectomy performed. With the owner's permission another liver biopsy was obtained in order to assess treatment and progress of histopathological changes.

Case 2: An intact female littermate of Case 1 had been clinically normal, however the owner requested investigation in view of the inherited basis of the disease diagnosed.

The only clinical abnormality detected was a soft systolic murmur heard loudest over the mitral area. In a range of examinations of blood and urine similar to that described in Case 1, the only abnormal findings were elevations of plasma ALT and caeruloplasmin (Table 1).

After the liver biopsy was obtained, this dog was treated

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§ Three of these dogs have been recently described in a talk (Studdert, V. P. (1982) — Aust. vet. J. 59: 128).  
¶ Cuprimine v, Merck, Sharpe and Dohme (Australia) Pty Ltd, South Granville, New South Wales
in the same way as Case 1. It remained asymptomatic for 14 months until a mucopurulent vaginal discharge appeared 6 weeks after oestrus. At this time there were no abnormalities in the haemogram or blood chemistry; the plasma ALT activity was within the normal range. The owner requested an ovariohysterectomy and gave permission for another liver biopsy.

Case 3: A 5-year-old male was presented with a 6-week history of lethargy, reduced appetite and weight loss. Signs developed after the dog was anaesthetised with thiopentone for minor surgery and experienced a very prolonged recovery period. The dog was thin and resented abdominal palpation. No other abnormalities were noted. Blood studies showed a slight neutrophilia and elevated levels of plasma ALT, copper and caeruloplasmin (Table 1).

This dog was a third and fourth generation descendant of the sire of dogs 1, 2 and 4.

Case 4: A 3-year-old bitch, by the same sire as dogs 1 and 2, was clinically normal. When presenting the dog for an elective spay, the owner requested that a liver biopsy be obtained and examined for evidence of copper toxicosis. A related dog, not included in this report, had died with signs of liver failure. The only blood examination obtained was a plasma ALT estimation which was found to be normal (Table 1).

**Results**

In 3 dogs, only minimal changes were detected in the gross appearance of the liver. It was slightly pale with rounded edges and with some areas resembling nodular hyperplasia. Microscopically, Cases 1 and 2 showed disorganisation of hepatic cords with foci of fatty infiltration, most striking in periaccinar areas. Small periportal accumulations of neutrophils and lymphocytes were present. Hepatocytes were swollen and the cytoplasm contained large numbers of refractile, light brown granules. These were identified as accumulations of copper by positive reactions to rubeanic acid and rhodanine stains (Figure 1). In Case 2, there were also large accumulations of dark brown pigment in Kupffer cells and macrophages which were positive for iron with Prussian blue stain. There were no significant differences between the first and second biopsies obtained from Cases 1 and 2. Histopathology was not done in Case 3.

In Case 4 the liver was grossly normal. Microscopically it showed similar but less severe changes than the other cases, with no evidence of lipidosis or fibrosis.

Hepatic copper concentrations were elevated in all 4 dogs. Analysis of the second liver biopsy from Case 1 was not obtained due to technical difficulties (see Discussion). The copper concentration in the second biopsy from Case 2 was

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**TABLE 1**

**Selected findings in 4 Bedlington terrier dogs with inherited copper toxicosis**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Case No. 1</th>
<th>Case No. 2</th>
<th>Case No. 3</th>
<th>Case No. 4</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma alanine aminotransferase (IU/L)</td>
<td>254</td>
<td>135</td>
<td>127</td>
<td>49</td>
<td>5-80</td>
</tr>
<tr>
<td>BSP retention (30 min)</td>
<td>5.6%</td>
<td>4%</td>
<td>1%</td>
<td>ND</td>
<td>&lt; 5%</td>
</tr>
<tr>
<td>Hepatic copper concentration (µg/g dry tissue)</td>
<td>3,930</td>
<td>4,641</td>
<td>6,361</td>
<td>1,600</td>
<td>49-317*</td>
</tr>
<tr>
<td>Plasma copper (µmol/L)</td>
<td>18</td>
<td>ND</td>
<td>13</td>
<td>ND</td>
<td>6.0-10.1†</td>
</tr>
<tr>
<td>Plasma caeruloplasmin (U/mL)</td>
<td>18</td>
<td>28</td>
<td>39</td>
<td>ND</td>
<td>6.3-19.8†</td>
</tr>
</tbody>
</table>

* Range of 4 normal dogs  
† Su et al (1982b)  
‡ Range of 6 normal dogs  
ND Not done  

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**Discussion**

Most clinical features of inherited copper toxicosis in Bedlington terriers are related to the chronic, slowly progressive hepatitis that is the major pathological feature of the disease. Affected dogs are asymptomatic early in their lives during the initial period of copper accumulation. There is considerable variation in the time of onset and manifestations of disease, but most dogs, including those described in this report, conform to the patterns of clinical disease that have been described (Hardy and Stevens 1977): older dogs, usually with a long history of gradual deterioration or unthriftiness, develop overt signs of liver dysfunction including ascites and jaundice and die from hepatic failure; acute, sometimes rapidly fatal episodes of illness (described by breeders as “The 4-day Problem”) usually occur in younger dogs (2 to 8 years) — signs may include depression, anorexia, vomiting, ascites and jaundice and many reports suggest stress, such as whelping and shipping, precipitate these episodes; some dogs remain asymptomatic, detectable only by abnormalities in liver function tests, microscopic and histochemical examination of liver tissue and copper assay.

Haematological findings are usually unremarkable, but haemolytic anaemia, which is associated with copper toxicity in other animal species (Hill 1977) and in Wilson’s disease has been reported in affected Bedlington terriers. It may be attributed to hepatic necrosis or intrahepatic redistribution of copper with increased levels of circulating copper causing injury to red cell membranes and resulting in intravascular haemolysis. This may contribute to or account for the very marked jaundice seen in some cases. It has also been suggested that this process occurs at subclinical levels during the progress of the disease because increased amounts of hepatic iron, as seen in Case 2, are commonly found (Ludwig et al 1980).

Biochemical findings are often normal except those which reflect liver function. In advanced cases decreased total serum proteins, albumin and prothrombin and increased alkaline phosphatase and bilirubin values may be found, but in earlier stages these parameters are as variable and inconsistent as the clinical signs. Elevation of plasma ALT is the most common biochemical abnormality of diagnostic value at all stages of the disease (Hardy and Stevens 1977; Twedd et al 1979; Su et al 1982b), but it too may be normal or unconvincingly elevated, particularly in younger dogs. The
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frequency of plasma ALT elevation increases with age (Twedt et al 1979), corresponding to the progressive hepatocellular damage rather than earlier uncomplicated copper accumulation. This can precede clinical or other biochemical abnormalities, as in Case 2, but may fluctuate, as found in Cases 2 and 4, falling to within the normal range, even when there is histological evidence of continuing hepatic injury.

Plasma copper and caeruloplasmin oxidase levels may be elevated in affected Bedlington terriers (Su et al 1982b). However the distribution of values overlaps considerably the range found in normal dogs so neither is a reliable diagnostic indicator of this disease.

Although other forms of liver disease must also be considered, the occurrence of clinical signs and biochemical abnormalities consistent with liver dysfunction must suggest this copper-storage disease when found in a Bedlington terrier. Clinical investigation should include a liver biopsy earlier than might otherwise be considered. For example, in Cases 1 and 3, only supportive medical treatment was considered until liver biopsy findings were known. In Case 2 a slight elevation of the plasma ALT in a clinically normal dog might otherwise have been an inadequate basis for obtaining a liver biopsy.

Accumulation of copper in the liver of affected dogs begins in the first year of life and can be seen as prominent brown granules which are copper-laden lipoplyosomes (Ludwig et al 1980) scattered in the cytoplasm of hepatocytes. These granules give a positive reaction to copper-specific stains such as rubaneric acid or rhodanise (Twedt et al 1979; Ludwig et al 1980). Histological evidence of hepatic injury, however, does not appear until later, an important feature that distinguishes this as a primary copper-storage defect rather than copper accumulation secondary to hepatic disease as occurs in some human diseases, mainly those associated with chronic cholestasis (Sternlieb 1980) and reported in dogs with chronic active hepatitis (Thorngburg et al 1981; Johnson et al 1982).

Twedt et al (1979) found these granules in 85% of affected Bedlington terriers aged between 6 and 12 months. They found progressive liver disease, which began as focal hepatitis, mainly in those aged between one and 8 years, with a peak incidence from 2 to 4 years. This was followed by a chronic hepatitis, characterised by piecemeal necrosis, inflammatory infiltrates and bridging fibrosis, seen most often after 4 years of age, and in late stages cirrhosis.

Hepatic copper concentrations in normal dogs ranged from 49 to 317 µg/g. This corresponds to other reports in which control dogs (nonaffected Bedlington terriers and dogs of other breeds) had hepatic copper concentrations of about 200 µg/g (range 91 to 377 µg/g) dry tissue (Twedt et al 1979; Ludwig et al 1980). Normal values for the dog are much higher than the normal concentration found in man which according to intensive studies of copper metabolism has been shown to be about 30 µg/g dry tissue (Sternlieb 1980). In contrast, the 4 affected dogs in this report had 8 to 35 times the normal concentration, with the lowest amount found in the youngest dog, Case 4. Levels above 850 µg/g dry tissue have been reported (Su et al 1982b).

Although hepatic copper concentrations of affected dogs are markedly elevated, care is required in collection, preservation and transportation of tissue or blood in order to avoid or minimise contamination with copper. Instruments and containers for collection and storage of specimens should be copper-free. Treatment with ethylenediaminetetraacetic acid (EDTA) or nitric acid has been used for this purpose. Freezing or drying and preservation-free storage of tissue is recommended. Formalin, particularly technical grades, should be avoided as in our experience it has been heavily contaminated with copper, probably from storage containers, leading to invalid results in the second liver copper analysis from Case 1.

Copper chelating agents are used successfully in the treatment of Wilson's disease. The drug of choice is d-penicillamine which binds and promotes urinary excretion of copper (Walshe 1977; Sternlieb 1978). It is a slow-acting agent which mobilises and gradually reduces tissue copper deposits. Definite clinical improvement occurs only after 3 to 6 months, which renders it ineffective in the acute episodes of hepatic necrosis and haemorrhage which may sometimes prove lethal. Except for these unfortunate situations, penicillamine has proved effective at all stages of Wilson's disease, preventing liver damage when given at a presymptomatic stage and causing remarkable healing of liver damage in quite advanced cases.

Most affected dogs would not be presented for diagnosis and treatment until clinical signs appear, usually corresponding to advanced liver disease. In contrast, many cases of Wilson's disease are detected in the presymptomatic stages as a result of the investigation of siblings of diagnosed cases in much the same way as Cases 2 and 4 in this report were identified. The relatively high cost of penicillin and the slow response of liver copper to treatment have reduced its use.

250 mg penicillamine orally per day has been recommended as treatment for affected Bedlington terriers (Hardy and Stevens 1977) but there are few reports of its effectiveness. Marked reduction of hepatic copper, from 5,298 to 228 µg/g dry tissue occurred in one dog following more than 2 years of treatment (Ludwig et al 1980). Despite this, cirrhotic changes had developed in the dog. The slightly lower hepatic copper concentration found in the second biopsy from Case 2 suggests the 14 months of treatment were not effective. It may represent the decrease in copper concentration found in affected dogs past 6 years of age (Twedt et al 1979) or merely a variation in sampling.

It would appear that the controlled therapy used in Wilson's disease in which cupriuresis is monitored accurately and individually establish an effective dose, has not been used in dogs. We propose to apply such methods in future studies of these and other affected dogs.

In humans, supplementary treatment in the form of low-copper diets and intestinal copper-binding agents, such as sulphated potash, has been used but is of minor importance in the control of the disease as compared to the role of a chelating agent. In order to be effective, severe restriction of copper, which is not easily achieved with meat-based diets, is required. The recommended daily copper requirement for dogs is 7.3 mg/kg of food (dry matter) (NRC 1974) and commercial dog foods often exceed this, depending on their content of organ meats. It is prudent to avoid foods known to have a high copper content such as liver and other organ meats, lamb, pork, seafood and cereal products. Tap water may also have a significant copper content especially if delivered by copper pipes. The dogs in this report have been fed a balanced diet of chicken and vegetables, supplemented with selected vitamin preparations, since their disease was diagnosed. Many mineral supplements and general 'tonics', which might be used in the treatment of chronically ill undiagnosed cases, contain copper or liver extract which has a high copper content.

This genetic metabolic defect is transmitted in Bedlington terriers as an autosomal recessive trait (Johnson et al 1980). Affected dogs, that is those that accumulate copper, are homozygous for the defective gene, heterozygotes are carriers and normal dogs are homozygous for the normal gene. Carriers are asymptomatic and at the present time can only be identified with certainty by test matings.

The dog epidemic occurred in Bedlington terriers outside the United States where it is known to have been present at least since 1959 (Padula 1973). It is unlikely that it has appeared in Australia as an identical, independent mutation in the same breed, but rather an indication that in both countries, where the breed is based mainly on dogs imported from the United Kingdom, it arises from common breeding stock in which the defective gene is present. The dog mentioned as the sire of Cases 1, 2 and 4 is common to all dogs in this report, but he is not found in the pedigrees of both sire and dam of every case. The 4 affected dogs reported here, like most of the 200 to 300 Bedlington terriers in Australia, are descended from 6 dogs imported from the United Kingdom between 1963 and 1967. Although there has been correspondence calling for reports of the disease in that
country (Smythe 1977; Cherry 1982), none have appeared in the veterinary literature.

In addition to the 4 cases in this report, 13 other confirmed or suspected cases are known to the authors, suggesting the trait is widespread in the breed in Australia. In the United States, attempts are being made to eliminate the trait by identification of affected dogs and selective breeding programs. Breeders in Australia, who until recently considered their stock free of the condition, are being encouraged to do the same.

Acknowledgments

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References


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A morphometric analysis of the changes with age in the skin surface wax and the sebaceous gland area of Merino sheep

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SUMMARY: Skin samples were collected from the midside of Merino ewes at 6 weeks, one year, and 3 years of age. The thickness of the surface wax and the number and area of sebaceous glands per unit length of skin were measured with an image analyser. Three-year-old sheep had a significantly (P < .05) thicker skin surface wax layer, (14.45 µ ± 1.45), than they did as lambs, (7.85 µ ± 0.88). Both the number (2.91 ± 0.15 per mm) and area (24.27 µ²/µ ± 1.54) of sebaceous glands per unit length of skin of 3-year-old sheep were significantly less than in lambs, which measured 4.10 ± 0.15 per mm and 39.30 ± 4.14 µ²/µ respectively and one-year-old sheep, which measured 3.99 ± 0.21 per mm and 35.43 µ²/µ ± 3.65 respectively.

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Introduction

The surface wax layer of the sheep is considered to be an important barrier to infection. Roberts (1963) found that older sheep were more resistant to Dermatophilus congolensis infection, because the older sheep better maintained the integrity of the wax layer. Beltscher (1937) found older sheep more resistant to fleece rot and fly strike than younger sheep. A number of factors may operate to bring about this increased resistance to infection and to environmental stress with age. The fleece of an older sheep may be a more effective barrier to environmental stress or the wax layer and epidermis may have a greater tolerance to bacterial and climatic insult.

In this study the mean thickness of surface wax and the number and area of sebaceous glands per unit length of skin


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Materials and Methods

The sheep used were Polled Merino ewes of the non-Peppin medium wool strain reared in the lower north of South Australia. Ten lambs were randomly selected from 90 ewe lambs of a May/June lambing. A skin sample was collected 6 weeks after the period of peak lambing, and again from the same sheep at one year and 3 years 3 months of age - the third age being referred to hereafter as 3-year-old.

Midside skin samples taken by the method of Carter and Clarke (1957) were fixed in 10% formol saline. These samples were then frozen in liquid nitrogen and longitudinal sections 30µ thick were cut serially on a cryostat. Fixation, dehydration and paraffin embedding may result in distortion or loss of the outer layers of the stratum corneum (Lloyd et al 1979).

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