Progressive Ataxia of Charolais Cattle Associated with Disordered Myelin

W. F. Blakemore and A. C. Palmer

Wellcome Laboratory for Comparative Neurology, School of Veterinary Medicine, Cambridge

R. M. Barlow

Animal Disease Research Association, Moredun Institute, Edinburgh

Received March 5, 1974; Accepted April 20, 1974

Summary. The pathology of a recently recognised progressive disorder of myelin in mature Charolais cattle is described. Clinical signs are first noticed at 8–24 months of age and progress from slight ataxia to recumbency over a period of 1–2 years. Lesions are microscopic and restricted to the white matter of the central nervous system. They are most marked in the internal capsule, cerebellar white matter and spinal cord and consist of multiple eosinophilic plaques. Ultrastructurally the lesions are associated with the nodal region of the myelin sheath. They are composed of disordered myelin, hypertrophied and hyperplastic oligodendrocytic tongues and many small processes which originate from these. There is evidence of myelin breakdown, but this does not elicit a phagocytic response. The aetiology of this condition is not known but the nature of the pathological process suggests a basic derangement of the myelin bearing cell.

Key words: Leucodystrophy — Myelin — Cattle — Oligodendrocyte — Node of Ranvier.

Introduction

A newly recognised nervous disorder of mature Charolais cattle was reported by Palmer et al. in 1972. The present paper describes the nature of the unique lesion present in these animals.

History and Clinical Signs

The observations made over the past 3 years are based on 8 cases followed to post-mortem examination. All but 1 were French bred, imported pedigree Charolais and came from 7 British herds¹. The last animal to be examined (8) was bred in England from imported parents. Three other pedigree cattle exported from France over the same period are known to have been destroyed after developing apparently similar clinical signs. Typical signs have been seen only in females, the youngest being just under 1 year old.

The clinical signs were principally those of weakness of a hind-leg which slowly progressed to ataxia. Involvement of all four limbs was apparent in some cases. Attendants had sometimes noticed slight incoordination, but the primary complaint was difficulty in rising which was accompanied by jerking movements of the hind-legs. The severity of clinical signs progressed slowly to recumbency.

Another curious but apparently characteristic feature was the type of micturition: urine was passed in an uneven squirting flow. When excited the animals

¹ See Addendum.
sometimes nodded the head jerkily, but otherwise there were no convincing indications of brain involvement such as visual deficit or change of temperament.

**Materials and Methods**

Details of the 8 animals that were examined post-mortem are given in Table 1. Cases 1 and 2 were obtained from an abattoir and a full examination was not possible; however, despite the animals being stunned with a captive bolt, sufficient intact brain was available for histopathological examination. Brain, spinal cord and sciatic nerve was taken from all cases (together with the anterior peroneal nerve in Case 4). Specimens from visceral organs were obtained from all cases except 1 and 2. Tissues were fixed in 10% formol saline, embedded in paraffin and sections were stained with haematoxylin and eosin (H. and E.) as routine. Frozen sections were prepared as necessary. Special histological staining methods included Luxol fast blue (L.F.B.), Methasol fast blue 2 G, Osmium tetroxide α naphthylamine (O.T.A.N.) for normal and degenerating myelin (Adams, 1958), Alcian blue for acidic mucopolysaccharides, Cajal's gold sublimate for astrocytes, Holmes and Glees' methods for axons, Mallory's phosphotungstic acid haematoxylin, toluidine blue, periodic acid-Schiff (P.A.S.) and combinations of L.F.B. and P.A.S. and L.F.B. with Holmes' axon stain. Frozen sections were also stained with Sudan black, Sudan III and IV, and with Nile blue sulphate. Motor end plates from the peroneus tertius muscle from Case 4 were stained by the choline esterase method of Coupland and Holmes, the terminal axons being stained by the Gros-Schulz silver method.

For electron microscopy tissue from cerebral and cerebellar white matter was examined from Cases 3, 7 and 8. Material from Cases 3 and 8 was obtained within 3 min and 15 min of death respectively and fixed by immersion in 4% glutaraldehyde. Material from Case 7 was obtained following bilateral carotid perfusion under deep Nembutal anaesthesia using 4%.

---

**Table 1. Age of onset, rate of progression and examinations carried out**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age when signs first detected</th>
<th>Age at death</th>
<th>Laboratory examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>?</td>
<td>32 months</td>
<td>Abattoir, Light microscopy</td>
</tr>
<tr>
<td>2</td>
<td>?</td>
<td>?</td>
<td>Abattoir, Light microscopy</td>
</tr>
<tr>
<td>3</td>
<td>24 months</td>
<td>36 months</td>
<td>Full P.M. examination, Light microscopy, Electron microscopy</td>
</tr>
<tr>
<td>4</td>
<td>12 months</td>
<td>23 months</td>
<td>Full P.M. examination, Light microscopy</td>
</tr>
<tr>
<td>5</td>
<td>15 months</td>
<td>24 months</td>
<td>Full P.M. examination, Light microscopy</td>
</tr>
<tr>
<td>6</td>
<td>8 months</td>
<td>17 months</td>
<td>Full P.M. examination, Light microscopy</td>
</tr>
<tr>
<td>7</td>
<td>15 months</td>
<td>23 months</td>
<td>Full P.M. examination, Light microscopy, Electron microscopy (perfused)</td>
</tr>
<tr>
<td>8</td>
<td>14 months</td>
<td>20 months</td>
<td>Full P.M. examination, Light microscopy, Electron microscopy</td>
</tr>
</tbody>
</table>
formaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer at pH 7.3. Tissue was post-fixed in 1% osmium tetroxide and dehydrated with graded ethanol. Following transitional stages in propylene oxide it was embedded in araldite. Thick 1–2 μm sections were stained with alkaline toluidine blue. Thin sections were cut with a diamond knife, collected on uncoated grids, stained with uranyl acetate and lead citrate and examined at 40 kV in an Hitachi HS8 microscope, or a Siemens Elmiskop 51.

Brain tissue from Cases 3 and 8 was also rapidly frozen for subsequent lipid and protein analysis. Brain tissue from Case 6 was taken with sterile precautions and rapidly frozen to –80°C for subsequent transmission experiments.

Results

No consistent gross pathological changes were present.

Neuropathology

Similar changes were present in all animals, differing only in degree. The lesions appeared as multiple eosinophilic plaques within the white matter of the brain and spinal cord (Figs. 1, 2, and 4). These were most frequent in the corpus medullare of the cerebellum extending into the white matter of the folia, the inferior, middle and superior cerebellar peduncles, and internal capsule, corpus callosum, optic tract, the lateral lemniscus, medial longitudinal fasciculus, pontine decussation, the ventral and lateral funiculi of the spinal cord. The plaques appeared granular and devoid of nuclei (Figs. 1, 2, and 4) and axons could be seen passing through them in silver stained thick sections (Fig. 3). In general, the tinctorial affinity of the plaques was similar to that of the surrounding myelin, i.e. they were pale pink with P.A.S., stained as hydrophilic lipids with the O.T.A.N. method, were L.F.B. positive and positive with Sudan Black and Sudan III and IV in frozen sections. The plaques also stained with Alcian blue at pH 5.7 but not at pH 2 or pH 3; they did not give a metachromatic reaction with toluidine blue. A slight purple staining after staining with Nile blue sulphate in frozen sections indicates that the plaques also contained material other than myelin.

In some areas the plaques appeared to be distributed around blood vessels (Fig. 1). In regions adjacent to plaques, ‘pre-plaques’ were often seen as a dilation and distortion of myelin sheaths. There was no evidence of lipid carrying phagocytes in or around the plaques. The neuropil around the plaques was often vacuolated (Fig. 2) and showed an apparent increase of glial nuclei. Some of these were reactive astrocytes whereas others appeared to be oligodendrocytes (Fig. 4); it was impossible to say whether there was a real increase of cells, or whether this impression was caused by displacement by the plaques of the cells from their usual site. Occasionally glial cells were present which had angular basophilic cytoplasm; these are believed to be abnormal oligodendrogia (Fig. 4).

In silver preparations axonal swelling and fragmentation was sometimes associated with plaques; infrequently at other sites (e.g. the spinal cord) axonal degeneration was present.

Despite the widespread lesions in the white matter, morphological alterations in grey matter were not pronounced. In two cases the cerebral cortex showed a fine vacuolar spongy transformation of the neuropil with dilation of perivascular spaces; there was also neuronal degeneration in the lateral vestibular nucleus and among the internuncial cells of the spinal cord.
Fig. 1. A collection of eosinophilic plaques in the white matter of the spinal cord. Isolated plaques (arrow) are present between normal myelinated fibres. H. and E. × 135

Fig. 2. Plaques in the white matter of the internal capsule. Note that the plaques are acellular, while collections of cells lie adjacent to them. H. and E. × 135
In Case 4 perivascular spaces were prominent in both grey and white matter. In Cases 1, 3, 4 and 8 reactive pericytes around vessels close to the lesions contained PAS-positive granules. In Case 4 there was some fibrinoid degeneration of small arteries in the spinal dura mater.

No significant changes were observed in peripheral nerve or motor end plates.

At the ultrastructural level the plaques seen by light microscopy were composed of axons surrounded by masses of small processes and disorganised myelin sheaths (Figs. 5–7). Large plaques contained more than one demyelinated axon (Fig. 6). These axons were usually normal but in some cases they were swollen and contained many lysosomes or unusual accumulations of endoplasmic reticulum; these abnormalities occurred either as the axon approached or was within the plaque. When the plaques were small (pre-plaques at the light microscope level) the axon was surrounded by small processes which in turn were surrounded by hypertrophied tongues of oligodendrocytic cytoplasm, the whole being contained within a thin myelin sheath (Fig. 7). Continuity between hypertrophied oligodendrocytic tongues and the small processes could be demonstrated (Fig. 8). These small processes were 0.05–0.3 µm in diameter and of indeterminate length. They demonstrated some preferential orientation as many lay with their long axes at right angles to the long axes of the surrounded axons (Fig. 7). When two processes touched, their membranes showed evidence of fusion and when they lay next to an axon, specialised contact points were sometimes seen, similar to those at normal nodes of Ranvier (Metuzals, 1965; Peters and Vaughn, 1970). Although the hypertrophied oligodendrocytic tongues contained many microtubules, which in some instances were clumped, these only occasionally extended into the small processes; other organelles infrequently present were small dense bodies and mitochondria (Figs. 7 and 8). The smallest processes were devoid of organelles.

At the edges of plaques small astrocytic processes intermingled with the small oligodendrocytic processes, but when the latter were contained within a myelin sheath this did not happen. The extracellular space around the plaques was expanded and material of medium electron density similar to normal 'gap substance' (Metuzals, 1965) was often present between the small processes (Fig. 6).

These changes were always associated with the nodal region (Fig. 5). Thus the length of the node of Ranvier was often greatly increased though in some cases the changes only affected the paranodal myelin at one side of the node (Fig. 5). At some nodes the paranodal myelin was not disorganised; instead the myelin sheath appeared thinner than normal for the size of the contained axon (Fig. 5) and in some instances the paranodal myelin at each side of the node was of different thickness. Long stretches of 'naked' axon also occurred, unassociated with evidence of hypertrophy or hyperplasia of oligodendrocyte tongues or small processes.

No evidence of myelin degeneration in the form of phagocytosis of myelin, or the presence of lipid droplet and 'lyre bodies' in microglial cells was present, but
Fig. 5. Two axons $a_1$ and $a_2$ with widened nodes of Ranvier. Axon $a_2$ shows no hypertrophy of oligodendrocyte tongues whereas this is marked at one side of the node of axon $a_1$. $o$ hypertrophied oligodendrocyte tongues, $p$ mass of small processes associated with axon $a_1$. $\times 5900$

Fig. 6. A plaque containing several axons ($a$) cut in transverse section, and masses of small processes ($p$). Gap substance is marked by a cross. $\times 15000$
myelin figures were frequently present both within plaques, in paranodal regions and adjacent to oligodendrocyte cell bodies situated near plaques (Figs. 9—10). These myelin figures did not contain axonal debris and in some cases (Fig. 9) they appeared to be within oligodendrocyte cytoplasm.
Fig. 7. An axon (a) cut in transverse section surrounded by a mass of small processes (p) which in turn are surrounded by hypertrophied oligodendrocyte tongues (o) and a thin myelin sheath (arrow). × 15500
Fig. 8. An hypertrophied oligodendrocyte tongue (o), containing numerous microtubules, extending from which is a fine process (arrow) similar to those present in Figs. 5—7. × 42000

Fig. 9. A naked axon (a) surrounded by myelin figures. × 9500
Fig. 10. Accumulation of myelin figures in the paranodal region (arrow) on one side of a node of Ranvier (n). × 13300

Fig. 11. An hypertrophied oligodendrocyte from near a plaque; the cytoplasm is extensive and contains many mitochondria. × 11500
Astrocytes in Cases 3 and 8 contained large numbers of filaments while those in Case 7 had fewer filaments but contained many large gliosomes (Hashimoto, 1969). The cell body region of most oligodendrocytes appeared normal, especially away from plaque-containing areas. However, in these regions, in Cases 3 and 8 in particular, cells with only some features of oligodendrocytes, but none of those associated with astrocytes or microglia, occurred. These cells had a lower cytoplasmic density and more mitochondria than normal oligodendrocytes (Fig. 11) and were judged to be abnormal oligodendrocytes. No degenerative changes were seen in oligodendrocytes and no other abnormalities were detected apart from thinning of some myelin sheaths which was common in some animals.

**General Pathology**

In the lumbar lymph nodes of both Case 4 and 5 the medullary sinuses had a superficial resemblance to cords of hepatic parenchyma, being packed with large macrophages amongst which foci of eosinophils were embedded. A similar change was present in the carcase lymph nodes of cases 4, 5, and 6. These changes seem to be sufficiently frequent to warrant mention though their significance is at present unknown.

**Discussion**

The ataxia observed in these heifers has an insidious onset and progressive course. It appears to be due to a specific disorder of central white matter which to our knowledge is distinct from other leucodystrophies of man or animals. The lesions are present at all levels of the CNS but are most advanced in the internal capsule, cerebellum and spinal cord. Peripheral nerves are not apparently affected. The basic abnormality is a demyelination of a very unusual type, associated not with degeneration, but rather with hypertrophy of oligodendroglia, in particular marked hypertrophy and hyperplasia of their cytoplasmic tongues from which extend a plethora of small processes. These proliferative changes occur in relation to the nodes of Ranvier and many of these are wider than normal. While the residual internodal myelin appears normal in appearance it is often thinner than would be expected for the size of the contained axon. Although neuronal and Wallerian-type degeneration have been seen these are not an essential part of the nodal lesion.

This disorder is thus a form of segmental dysmyelination which in the light of present knowledge appears to be without precedent. Normally one oligodendrocyte may myelinate many internodes (Peters and Proskauer, 1968; Mathews and Duncan, 1971) and it is reasonable to assume that axons myelinated by one cell will have nodal regions in close spatial relationship. In the present condition the plaques contain more than one axon and the paranodal region at both sides of the node is not always affected. Therefore, it is likely that the affected internodes which make up the plaque are derived from a single oligodendrocyte. Thus the defect which initiates the pathology must be a property of the oligodendrocyte rather than of the axon. Such a defect might be one solely of the oligodendrocyte or be related to the region of contact between the terminal loops of the myelin sheath and the axolemma. The nature of the disorder is at present not understood.

Preliminary neurochemical examinations of one case (3) (Davison, Banik, and Ramsey, personal communication) have not shown any abnormality in the lipid

---

2 See Addendum.
composition of white matter. The total protein content of isolated myelin (from cerebellar white matter) is lower than normal but acrylamide gel electrophoresis revealed no abnormal distribution of these proteins. The hypertrophy of oligodendrocyte tongues, with accumulations of myelin figures within them, seen in the present condition, is reminiscent of changes seen in 'quaking' mice (Watanabe and Bingle, 1972), a disorder in which the fatty acid composition of myelin lipids is thought to be abnormal (Baumann, 1970). These have not been examined in the present condition, but the similarity in the pathological changes suggests that some instability of myelin resulting from structural incompatibility may be the underlying lesion.

Some of the changes described may be of a secondary nature. Hypertrophy of oligodendrocyte tongues has been observed (W. F. Blakemore, unpublished) in association with the large axonal masses which develop in the spinal cord following 'dying back' of axons (Blakemore and Cavanagh, 1969). The abnormal oligodendrocytes observed near the plaques may develop as a result of the increased metabolism needed to support hypertrophied and hyperplastic cytoplasmic tongues. At the present time knowledge of the nature of the stimulus for hypertrophy appears central to the issues of aetiology and pathogenesis.

Acknowledgements. We wish to thank Mr. J. A. Fraser and Mr. A. L. Ogden for referring the cases to us and Professor A. Davison and Drs. R. Ramsey and N. L. Banik of the National Hospital for Nervous Diseases, Queen Square, London, for carrying out the chemical analysis. We wish to acknowledge the technical help of J. E. Payne and R. C. Patterson.

This work was carried out with the help of grants from the Wellcome Trust and the National Fund for Research into Crippling Diseases. W. F. Blakemore holds a Wellcome Research Fellowship.

References

Metuzals, J.: Ultrastructure of nodes of Ranvier and their surrounding structures in the central nervous system. Z. Zellforsch. 65, 719–759 (1965)

W. F. Blakemore, Ph.D.
Dept. of Veterinary Clinical Studies
School of Veterinary Medicine, Madingley Road
Cambridge CB3 OES, Great Britain