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## Observations on the histology and possible pathogenesis of lesions in the central nervous system of sheep with swayback

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Swayback or enzootic ataxia is an ataxic disorder of newborn and young lambs which is associated with low levels of copper in the tissues. Only one case of a similar syndrome has been reported in an adult (McDonald, 1942) and the concept of an insult during a vulnerable period in the development of the central nervous system (CNS) as postulated by Davison & Dobbing (1966) is clearly relevant.

Lesions may be found in three divisions of the CNS: the cerebral hemispheres, large neurones in the brain stem and spinal cord and in the white matter of the spinal cord (Innes & Shearer, 1940; Barlow, Purves, Butler & Macintyre, 1960; Howell, Davison & Oxberry, 1964).

Several changes have been reported in the cerebral hemispheres, including swelling, gelatinous softening and, more commonly, cavitation of the cerebral white matter. Such changes were found in 60% of the lambs examined by Innes & Shearer (1940), in 40% of those seen by Barlow *et al.* (1960), in 20% of lambs seen by us in Liverpool, but they appear to be rare in Australian lambs.

The gross lesion is similar to the cystic change found in human infantile encephalopathies (Courville, 1959; Spais, Palsson & van Bogaert, 1961), a lesion which may be caused by anoxia (Courville, 1959; Clarke & Anderson, 1961). It is also similar to the lesion found in infants who have survived the mother's attempted suicide by coal gas inhalation during late pregnancy (Schwedenberg, 1959). Howell & Davison (1959) found that the activity of the copper-dependent enzyme cytochrome oxidase was significantly lower in the CNS of swayback lambs than in normal controls. McDonald (1942) suggested that cerebral cavitation is found only

when the copper content of the ewe and of the foetus *in utero* is very low. Thus cytochrome oxidase activity in the developing brain of such lambs may be markedly impaired, and we have suggested (Howell *et al.* 1964) that the cerebral lesions may be a direct result of this marked impairment.

Chromatolysis and necrosis of large neurones in the brain stem and spinal cord are seen in all swayback lambs (Barlow *et al.* 1960; Barlow, Field & Ganson, 1964; Howell *et al.* 1964), and Barlow (1963) has demonstrated histochemically that the most severe reduction in cytochrome oxidase activity occurs in the groups of nerve cells that show the morphological lesions. Fell, Mills & Boyne (1965) demonstrated that cytochrome oxidase activity was lowered in neurones of the brain stem and spinal cord of clinically normal but copper-depleted lambs. The changes in these neurones may be a direct result of lowered cytochrome oxidase activity or, as Barlow (1963) has suggested, mitochondrial 'ageing' may also play a part.

Together with lesions in neurones, changes in the white matter of the spinal cord are constantly present in swayback. The lesions are found in the lateral columns below the point of entry of the dorsal nerve root and in the ventral columns adjacent to the median fissure.

Innes & Shearer (1940) and Innes & Saunders (1962) were of the opinion that this lesion was a secondary degeneration of motor pathways. Barlow *et al.* (1960) considered the lesion to be an inhibition in development which involved nerve cells, myelin sheaths and glia, and Howell *et al.* (1964) concluded that the lesion was the result of abnormal myelination. The findings of Barlow *et al.* (1960) and of Howell *et al.* (1964) suggested that lesions in spinal cord white matter, and in large neurones, and the changes in the cerebral hemispheres may be separate manifestations of swayback, whereas Innes & Shearer (1940) and Innes & Saunders (1962) maintained that the changes in spinal cord white matter were the direct result of damage to higher centres.

In the early 1950's in California many pregnant ewes were vaccinated with a modified live blue tongue virus. Many of them gave birth to ataxic lambs. Gross abnormalities were seen in the cerebral hemispheres of some of the lambs, and Innes & Saunders (1962) considered that this lesion showed 'no essential difference from classic swayback'. However, the affected lambs had normal spinal cords (Cordy & Shultz, 1961; Young & Cordy, 1964). Only a proportion of swayback lambs have lesions in the cerebrum yet all have lesions in the spinal cord.

It is possible, however, that the tracts arise in the brain stem and in particular from the red nucleus (Innes & Shearer, 1940). I have transected the brain stem of five lambs *in utero* and they were removed some 5–6 weeks later, just before the expected time of birth. The spinal cords of these lambs were normal and completely comparable to those of their unoperated twins which acted as controls (Howell, to be published).

In 1964 Howell *et al.* examined five lambs with swayback and four controls, all less than 1 month old. In the swayback lambs there was an obvious loss of myelin sheaths in the specified areas of the spinal cord. These areas contained Marchi positive pigment but degenerate myelin could not be demonstrated using the

OTAN method, which is free from Marchi artifact (Adams, 1959). Biochemical analysis of thoracic cord yielded only trace amounts of cholesterol esters and equal quantities were present in controls and swayback lambs. An analysis of myelin lipids showed that there was a smaller content in the swayback lambs whereas non-myelin lipids were not affected. From these results Howell *et al.* (1964) concluded that the lesion was one of faulty myelination rather than demyelination and that this was due to impaired lipid biosynthesis which could be directly attributed to copper and cytochrome oxidase deficiencies (Howell & Davison, 1959). These results have recently been confirmed and preliminary results indicate that some of the myelin that is laid down is immature (Howell, Davison & Oxberry, in preparation).

Such faulty myelination might have produced focal myelin aplasia or retarded myelination, and any abnormal myelin might subsequently degenerate. We therefore decided to keep swayback lambs alive for as long as possible in order to study any progressive changes in the lesions of the spinal cord white matter.

Two 2-month-old and two out of three $3\frac{1}{2}$-month-old sheep had lesions similar to the five 1-month-old swayback lambs examined previously (Howell *et al.* 1964). There were fewer myelinated fibres, more stroma, and in OTAN stained sections black-staining products of myelin degeneration were absent. In one of the three, $3\frac{1}{2}$-month-old animals and in a $6\frac{1}{2}$-month-old animal black degenerating myelin was present in OTAN stained sections. This material was also present in four of five 8-month-old swayback animals, but in one of these the material was present only at the site below the point of entry of the dorsal nerve root. Confirmatory evidence of myelin degeneration was obtained by the iron haematoxylin and Sudan II method (Lillie, 1965) and in some of the older animals gliosis was present (Howell, Davison & Oxberry, in preparation).

Our results indicate that the basic lesion in the white matter of the spinal cord is due to abnormal myelination which can be related to faulty lipid synthesis. The lesion persists and if the lamb lives some of the abnormal myelin degenerates.

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## Recent studies on galactosaemia, phenylketonuria and homocystinuria

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Galactosaemia, phenylketonuria and homocystinuria are inborn errors of metabolism as the term was defined by Garrod (1908). In all three lack of an enzyme causes a metabolic block and accumulation of the substrate of the missing enzyme, and all three are inherited as Mendelian recessive characters. Unlike Garrod's original examples, galactosaemia, phenylketonuria and homocystinuria are often accompanied by damage to the central nervous system and the majority of those affected are mentally retarded (Woolf, 1962*a*, 1963; Gerritsen & Waisman, 1966).

### *Galactosaemia*

*Basic biochemistry.* Galactose is converted into glucose, mainly in the liver but to some extent in many other tissues. The first step is phosphorylation to galactose-1-phosphate, a reaction catalysed by galactokinase. Galactose-1-phosphate reacts in the presence of galactose-1-phosphate uridylyltransferase, with uridyl diphosphate glucose, to yield uridyl diphosphate galactose, and this is converted into uridyl diphosphate glucose by the enzyme epimerase. In galactosaemia the second enzyme, galactose-1-phosphate uridylyltransferase, is absent or inactive. In consequence galactose-1-phosphate accumulates intracellularly and, by feed-back inhibition of galactokinase, causes the accumulation of galactose in all the body fluids.

Galactose-1-phosphate acts as a competitive inhibitor of phosphoglucomutase, blocking, to an extent depending on the relative concentrations, the main route for utilization of glucose-1-phosphate and glycogen.

*Clinical features.* In general, infants with galactosaemia fail to gain weight properly, their livers are enlarged, and often they are jaundiced. They may die in liver failure during the first few weeks or months of life. Survivors develop cataracts and show some renal tubular dysfunction. The cataracts may be the result of accumulation of dulcitol in the lens. Almost all surviving untreated galactosaemics are mentally retarded, with intelligence quotients (IQ) 30–70. Apart from this retardation, there is very little evidence of damage to the central nervous system, perhaps because only the less severely affected survive beyond infancy. If treatment is started in early infancy, about two-thirds of the patients have normal intelligence; the longer the time between birth and start of treatment, the lower the final IQ.