Lesions akin to transmissible spongiform encephalopathy in the brains of rats inoculated with immature cerebellum

Their significance in the aetiology of these diseases*

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Summary. Fourteen BD IX rats were inoculated intracerebrally with a homogenate prepared from the immature cerebellar cortex of 10-day-old rats, when synaptogenesis is at its peak in this species. Eight controls were inoculated with mature cerebellar cortex. Transient ultrastructural changes were observed between 2 and 23 weeks’ incubation in those animals which had received an inoculum of immature cerebellum. These changes pointed to a re-activation of embryonic or neo-natal growth mechanisms and were identical to those occurring in kuru-inoculated spider monkeys. With longer incubation histopathological lesions such as intracytoplasmic vacuolation, chromatolysis and neuronophagia appeared in neurons of the brain stem reticular formation. Such features are common in all the spongiform encephalopathies. All controls were negative. It is suggested that the transmissible agent in these diseases might be the factor which influences the various stages of normal neuronal maturation. A hypothesis is developed which would reconcile the “infectious” character of these diseases with a genetic factor and explain the “unconventional” behaviour of the agent as well as the mode of its transmission.

Key words: Transmissible spongiform encephalopathy – Scrapie – Experimental kuru – Neuronal vacuolation – Re-activation of embryonic and neo-natal growth mechanisms

Ever since it was established that scrapie of sheep could be transmitted by intracerebral inoculation from an affected to a healthy animal [12], an extensive search for other transmissible, degenerative diseases of the central nervous system has been in progress. In due course it was proven that, besides scrapie, encephalopathy of mink [10], kuru [15] and Creutzfeldt-Jakob disease [16], a group of four conditions collectively designated as “transmissible spongiform encephalopathies”, and now widely believed to be caused by the same agent, could be transmitted in a similar fashion. This agent is remarkable, since it is resistant to any number of insults which would inactivate conventional viruses, causes no immune reaction in the host and no inflammatory change in the tissues and can not be visualized by electron microscopy. It is variously referred to as a “slow virus” [32], an “unconventional virus” [14], a “virino” [13] or a “prion” [29]. The search for it has been intense, but despite decades of work by several research teams, relatively little has been achieved to further our understanding of the aetiology of these diseases or the character of the agent which causes them (for a full bibliography see [1, 17]). It, therefore, became obvious that a completely new approach to this question was indicated and a pilot experiment to this effect will be reported here.

Material and methods

This study is based on the examination of the brains of 22 adult BD IX rats inoculated intracerebrally into the left frontal region with 15 to 20 µl of a 50% suspension of rat cerebellar cortex in phosphate-buffered physiological saline pH 7.4. The animals were divided into two groups; group I comprising 16 young adult rats, group II 6 middle-aged rats (see Table 1). Both groups were subdivided into sections A and B, animals in section A receiving an inoculum prepared from the cerebellar cortex of 10-day-old rats (see discussion), whilst those in section B (inoculated controls) received an equivalent inoculum prepared from the cerebellar cortex of adult rats. (Figure 1 illustrates the stage in the development of the cerebellum in a 10-day-old rat.) Litters from five different dams were used for inoculation; i.e. two siblings from dam 4; nine from dam 7; three from dam 11; four from dam 16; and three from dam 17. The dam of male 991 in...
group II B was not known. A third group of animals (un-inoculated controls) comprised five old, normal rats aged between 72 and 130 weeks.

In the 14 rats of groups IA and IIA the inoculum (designated im1, 2 or 3) was prepared under sterile conditions from the cerebellar cortex of five 10-day-old rats (litter mates and siblings of 11/4-6 in group IA) and used either fresh or after being stored in liquid nitrogen for later use. In the eight rats of groups IB and IIB (inoculated controls) the inoculum (designated ml or m2) was prepared from the cerebellar cortex of two adult rats (m1 = 48-day-old male, litter mate of 4/2 and 3 in group IIA; m2 = 9-month-old male) and used fresh. All further details are listed in Table 1.

None of the rats developed any obvious clinical signs and all (except one) were anaesthetized at various stages after inoculation and perfused via the ascending aorta with a modified one-half strength Karnowsky [22] fixative. (Rat 16/9 died of a fresh haemorrhage in the region of the red nucleus and was fixed by immersion in 10% formal saline.)

After removal from the skull, the fixed brain was divided sagittally, the left half being used for light microscopy, the right half for electron microscopy. Of the left half, four coronal blocks comprising the whole of the cerebral hemisphere and one sagittal block containing half the brain stem and cerebellum, were embedded in paraffin wax, cut at 7 μm and stained with haematoxylin-eosin or cresyl violet (Nissl method). Sample sections were treated with the periodic acid Schiff reagent or immunoprecipitated with silver nitrate (Marsland-Glees method). In 11 cases (including three inoculated controls), surviving from 30 to 108 weeks, and in five old, un-inoculated controls, the brain stem and cerebellum were cut in serial sections, mounting and staining three consecutive sections in every ten with cresyl violet.

From the right half, blocks for electron microscopy were taken from the following regions: dorsal frontal, medial and lateral cortex, striatum and cerebellar vermis. (With hindsight blocks from the brain stem should have been included. Unfortunately, however, this was omitted at the time of processing the material.) The blocks were post-fixed for 2 h in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, washed in buffer, dehydrated through graded ethanol, block-stained in saturated uranyl acetate in ethyl alcohol for 2 h, washed for 30 min in changes of ethyl alcohol and propylene oxide and embedded in araldite (a technique used routinely in this department). Thin sections, 80 nm, were cut on an LKB III ultramicrotome and stained at room temperature for 2 min with uranyl acetate (saturated solution at room temperature in equal parts of 50% ethanol and 100% methanol), followed by 10 min in Reynolds's lead citrate; they were examined under an AEI 6B and Hitachi H600 electron microscope. Thick sections 1 μm, stained with 1% toluidine blue, were available for orientation.

The counts of synapses in the cerebellum between spines of Purkinje cell dendrites and parallel fibres were carried out under the electron microscope on coded grids at a magnification of ×15,000 in two matched pairs (7/1-7/7 and 7/2-7/8); these were all siblings from the same litter surviving 22 and 30 weeks, respectively. An area of 7600 μm² from samples at various depths of the molecular layer was covered in every case. In addition counts were done in two old, un-inoculated controls (aged 72 and 130 weeks, respectively).

**Results**

**Histopathological findings**

The brains appeared macroscopically normal, the inoculation site could not be located with the naked eye. Microscopically, however, it was seen in five cases, where it appeared as a clean fibrous scar with remnants of haemosiderin scattered along the needle track. There was no evidence of inflammation. Although every effort had been made to place the inoculum as superficially as possible, in one case the needle had entered the lateral ventricle and this had led to a loosening of the overlying cerebral white matter and corpus callosum. In every case the leptomeninges appeared normal with no evidence of meningitis. In two animals a pituitary adenoma was found after removal of the brain from the skull.

The cerebral and cerebellar hemispheres appeared essentially normal in all cases. Status spongiosus of the grey matter did not occur anywhere in the brain or at any stage of incubation. There were no plaques or other amyloid deposits in any region of the brain. A search for growth cones on the dendrites of Purkinje cells in the cerebellum remained negative. Pathological changes were entirely confined to the brain stem of those animals inoculated with immature cerebellum, and here they affected almost exclusively the neurons of the reticular formation. Many of these neurons showed central chromatolysis with eccentrically placed nuclei, swelling of the cytoplasm and dissolution of the Nissl substance (Fig. 2). In some cells a small vacuole was located within the chromatolytic cytoplasm. Others contained larger intracytoplasmic vacuoles with preservation of the Nissl substance (Fig. 3 A-C). The vacuoles would either lie singly or in small clusters (Fig. 3 D, E), in some instances they...