PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **495th Meeting** *of the Society was held at the Universities of Glasgow and Strathclyde on Thursday, Friday and Saturday,* 29, 30 *and* 31 *May* 1969, *when the following papers were presented:*

NEUROCHEMICAL GROUP COLLOQUIUM ON 'SCRAPIE'

Structural Changes in Scrapie-Affected Brain

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Scrapie is essentially a disease of sheep and has been known in this country for more than 200 years. It derives its name from one of the outstanding clinical features of the disease: the rubbing or scratching, which leads to fall out of hair and excoriation of the skin. The disease was shown to be transmissible to sheep by injection by Cuillé & Chelle (1936), but a great advance was made when Chandler (1961, 1963) showed that it could be established in mice and rats, where the adapted agent has an incubation period of some 4–5 and 6–8 months respectively.

Essential pathological changes are limited to the central nervous system, though infective agent is widely distributed in the (histologically normal) tissues of the body, especially the spleen. Most recent work has been carried out on mice and rats.

For many years vacuolation of nerve cells, particularly the large motor type (such as occur in the reticular formation of the medulla or anterior horn of spinal-cord grey matter) was accepted as the hallmark of scrapie infection in the sheep, though small numbers of vacuolated cells were also to be found in old normal animals. In addition, vacuolation of background substance of the brain, gliosis and a fairly widespread and scattered nerve-cell degeneration was recognized as well as some sickness of myelinated nerve fibres that did not amount to demyelination. Astrogliosis has been emphasized as a cardinal feature of the disease in both sheep and goats.

In the mouse nervous system microvacuolation of ground substance comes on about 60 days after infection, and at about the same time or slightly before this there is enlargement of astroglia, which becomes much more easily stainable than it is under ordinary conditions.

In the opinion of several workers this activation of astroglia is the earliest structural change in scrapie infection.

Later the hypertrophy of astroglia becomes intense. It is not certain whether there is an actual increase in number of glial cells or whether there is simply enlargement and increased stainability of individual cells. The scrapie-affected glial cells are reminiscent of malignant transformation such as is seen in benign astrocytoma. It must be pointed out, however, that concentration of scrapie agent in spleen may exceed that in brain without any morphological change being visible in either the light- or the electron microscope.

Scrapie agent has some very remarkable properties, but in one respect it behaves like a classical neurotropic virus: the manner in which it makes its first appearance in the central nervous system after peripheral inoculation. By using astroglial activation as the first sign of change produced by scrapie agent the latter can be shown to settle first in the segments of neuraxis corresponding to the site of peripheral injection.

The vacuoles of scrapie-affected nerve cells are usually empty (though some authors have described granular contents). They appear to be derived from dilatation of endoplasmic reticulum. Microvacuolation of background substance in scrapie-affected mouse brain appears to be derived partly from expansion and fusion of astrocyte processes and partly from greatly dilated axis cylinders. The same great expansion of astroglial processes is seen in the rat. In most animals microglial cells are not affected in scrapie, but in the rat they are enlarged, and in the electron microscope are seen to contain many myelin-type inclusions. Also in the rat with scrapie, amyloid-like bodies have been found. Within cerebellar axis cylinders, orientated tubules have been described in considerable numbers. There is some evidence that they are more common in the old normal rat than in the young animal, though they make their appearance in the young rat with scrapie. They probably constitute a non-specific change that occurs in old age and is brought forward in scrapie infection.

Scrapie agent has proved very difficult indeed (if not impossible) to separate from cellular membranes. Careful electron-microscopic examinations have not revealed special changes in subcellular organelles, though mitochondrial disorganization, with production of a helical filiment surrounding a

central rod-like core, has been described. In view of the tendency of scrapie infectivity to be associated firmly (though not exclusively) with mitochondrial fractions these changes deserve further study.

Pattison (1965) has emphasized the remarkable symmetrical localization of the changes of scrapie. This is quite striking, but unilateral changes may be seen in the earliest stages (see above).

In summary, scrapie changes comprise:

(*a*) Neuronal vacuolation, more marked in some species than others and varying in degree with different strains of scrapie agent.

(*b*) Widespread non-specific nerve-cell degeneration.

(*c*) Myelin sickness, especially in long fibre tracts.

(*d*) Astroglial hypertrophy, regarded by several workers as the earliest and most clear-cut sign of scrapie change. The glial changes are reminiscent of those occurring in a low-grade astrocytoma and suggest the possibility that there has been widespread 'transformation' of glial cells in scrapie.

(*e*) Strict symmetry of histological changes in established disease.

(*f*) Changes that are usually associated with aging, e.g. amyloid bodies, ordered neuronal tubule formation.

(*g*) Changes much more marked in grey matter than in white.

Chandler, R. L. (1961). *Lancet*, i, 1378.
Chandler, R. L. & Fisher, J. (1963). *Lancet*, ii, 1165.
Cuillé, J. & Chelle, P. L. (1936). *C. R. Acad. Sci., Paris*, **203**, 1552.
Pattison, I. H. (1965). *Monogr. nat. Inst. nerv. Dis. & Blindn.*, no. 2, p. 249.

Biochemical Changes in Scrapie Affected Brain

By R. H. Kimberlin. (*Agricultural Research Council Institute for Research on Animal Diseases, Compton, near Newbury, Berks.*)

When mice are inoculated intracerebrally with scrapie-affected material there is an interval of at least 4 months before the appearance of clinical signs, and until recently very little was known of the biochemical changes underlying the development of the disease. Some early studies were carried out in sheep and goats involving mainly the analysis of brain and body fluids taken from affected animals, but these were almost entirely negative (Avery, Mills & Darcel, 1960; Darcel, Merriman, Beauregard, Avery & Kasting, 1963; Darcel & Avery, 1960; Darcel, Avery & Carson, 1961; Kasting & Darcel, 1963; Millson, West & Dew, 1960; Slater, 1965*a*,*b*). However, the transmission of scrapie to mice (Chandler, 1963) provided the research worker with a very convenient experimental form of the disease and nearly all present day knowledge of the problem refers to mouse scrapie. Scrapie is a disease that affects the central nervous system and it is not surprising that virtually all the biochemical abnormalities associated with the development of the disease are found in this tissue.

The degenerative nature of scrapie is reflected in the progressive decline in body weight that occurs soon after the onset of clinical signs. The change in body weight is accompanied by a similar decrease in brain weight. Analysis of the main constituents of brain shows that, although small changes in density and water content do occur, the decreased brain weight is due largely to a proportionate loss of lipid, protein and water (Mould, Dawson, Slater & Zlotnik, 1967; Kimberlin & Millson, 1967; Field, Caspary & Windsor, 1966). The total amount of nucleic acid is, however, unchanged, resulting in a small increase (10%) in the concentration of both DNA and RNA (Kimberlin & Millson, 1967). Histochemically the breakdown of lipid is reflected in the deposition of neutral fat in brain (Mackenzie & Wilson, 1966), and some slight changes have been reported in the fatty acid composition of myelin and whole brain from affected mice (Heitzman & Skipworth, 1969). An increased accumulation of periodate–Schiff-positive material has also been detected histochemically in scrapie-affected mice (Mackenzie, Wilson & Dennis, 1968*b*). Despite the breakdown of protein there is no gross change in the concentration of total free amino acids (Mould *et al.* 1967).

Extensive studies involving the use of radioactive tracers have revealed only minor changes in the metabolism of RNA and protein (Kimberlin, 1968; Millson & Hunter, 1968), and the respiratory activity of brain homogenates from scrapie-affected mice is decreased only in proportion to the loss of brain weight (Kimberlin & Millson, 1967). Similarly the activities of succinate dehydrogenase and cytochrome oxidase are unchanged in scrapie-affected mice, although it is noteworthy that both these enzymes have an increased activity in scrapie goats (Slater, 1965*b*).

It would therefore seem that even in the clinical stage of scrapie the biochemical abnormalities are not very striking. However, brain has a very heterogeneous cell population, and these studies only reflect the changes taking place in the tissue as a whole. The histopathological abnormalities of mouse scrapie include vacuolation of the ground substance, neuronal degeneration and hyperplasia of the astrocytes, and so it would seem that though some cells have a decreased metabolic activity others are more active. The enlargement of astrocytes is of interest since it may be that an altered metabolism in these cells is responsible for the