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The Significance of Astroglial Hypertrophy in Scrapie, Kuru, Multiple Sclerosis and Old Age Together with a Note on the Possible Nature of the Scrapie Agent

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Zusammenfassung. Auf gewisse pathomorphologische Ähnlichkeiten zwischen Scrapie, Kuru, Multiple Sklerose und Altersdegeneration wird hingewiesen. Ein gemeinsamer Faktor in diesen Veränderungen wird vermutet, bei dem es sich um hypertrophische Vorgänge in Gliazellen, ähnlich einer gutartigen Neoplasie, handelt. Diese Veränderungen könnten mit einer Aggregation von Polysaccharidketten im Cytoplasma in Beziehung stehen. Derartige Polysaccharidaggregate könnten als Foci einer weiteren Aggregation wirken und einen Vorgang auslösen, durch welchen der Zellstoffwechsel tiefgreifend beeinträchtigt wird. Solche Aggregate von Polysaccharidketten könnten hinwiederum als sekundäre Foci wirken, so daß die Illusion eines sich selbst reduplizierenden Agens resultiert.

The century which has elapsed since Charcot (1868, 1872) gave an excellent clinical and pathological description of multiple sclerosis (MS) has not brought any real insight into the nature of the condition. The experimental encephalitis (later established as allergic in nature) produced by Rivers and his coworkers in the mid-thirties, though stimulating much research and clinical trial of immunosuppresive drugs in MS, has failed to bring a real understanding of the human disease. Meanwhile the introduction of the concept of "slow" or "latent" virus infection into our thinking by the pioneer work of the late Dr. Björn Sigurdsson (1954) and his successors in Iceland has reopened the question of the infective nature of multiple sclerosis. It has indeed been found possible to reinterpret clinical epidemiological observations in the light of an infective aetiology (Schapira et al., 1963). More recently the demonstration that at least one chronic "degenerative" condition of the nervous system (Kuru) can be transmitted to chimpanzees has strongly reinforced interest in attempts to transmit MS to experimental animals (Gajdusek et al., 1967). For the most part these have been negative but two positive results have recently been reported and in both cases scrapie developed in the recipient animals (Palsson et al., 1965; Field, 1966).

Scrapie has been known for more than 200 years as a disease of sheep widerspread in Europe and in many other parts of the world. Within recent years it has come to be looked upon as a paradigm of "slow" virus infections and great impetus to its study has been given

by its transference to small laboratory animals such as mice (CHANDLER, 1961) and rats (CHANDLER and FISHER, 1963).

In 1959 HADLOW drew attention to the pathological similarities between scrapie and kuru, a subacute or chronic degenerative disease of the nervous system apparently limited to the Fore linguistic group of people in the Eastern Highlands of New Guinea. This condition, first described by GAJDUSEK and ZIGAS (1957), was initially believed to be genetic, and there is no doubt that a strong genetic factor governs susceptibility to the disease. However it has now been possible to transmit the disease to chimpanzes (GAJDUSEK et al., 1967) though, so far, to none of a large number of other animal species tried. In scrapie, too, a genetic factor is of great importance (GORDON, 1959) and indeed PARRY (1962) was inclined to see the disease entirely in this light. However, there is no doubt that scrapie can be transmitted by inoculation of infected nervous (or other) tissues.

The link between Multiple Sclerosis (MS) and scrapie rests at present on two sets of experiments, one in Iceland (PALSSON et al., 1965) and one in Newcastle (FIELD, 1966). In both cases scrapie emerged in animals inoculated with multiple sclerosis material (sheep in Iceland; mice in Newcastle) and in both cases incubation period shortened and signs became more typical with passage — a phenomenon to be expected when the "species barrier" is crossed (PATTISON, 1965).

Although several electron microscope investigations of scrapie have been made (FIELD and RAINE, 1964, 1965; CHANDLER, 1966, 1967; FIELD et al., 1967) no viral particles of recognizable morphology have been found; nor have they been seen in MS material (FIELD and RAINE, 1964; PERIER and GREGOIRE, 1965). A careful search of chimpanzee kuru material (kindly supplied by Dr. D. C. GAJDUSEK) has likewise failed to reveal virus though unexpected "herring bone" like structures have been found in the granular layer of the cerebellar cortex and will be described elsewhere (FIELD and RAINE, 1967). In this animal too, amyloid deposits were seen in electron microscopic examination, many deposits being of very small size. Much larger amyloid deposits were found in the scrapie rat (though not in the mouse) by FIELD et al. (1967). These deposits occurred in young adult scrapie animals (8—9 months old) and were also seen, though in lesser amount, in normal old rats (18—20 months). An outstanding feature of the pathology of scrapie in mouse and rat (and also sheep and goat) is the marked hypertrophy of astroglial cells which appears to be the primary morphological event in the development of the experimental disease. Astroglial enlargement is also found in old rats though never to the same degree as occurs in scrapie. In the limited fields viewed in the electron microscope the appearances in old age may quite readily be mistaken for scrapie. Indeed the changes of scrapie appear to be an exaggeration of those which normally occur in old age. In kuru, too,

astrocytic enlargement has been described as a feature of the condition, though some workers have regarded it as non-specific when compared with "specific" degeneration of the cerebellar fibre systems (BECK and DANIEL, 1965). Other studies, however, emphasize the widespread changes in kuru (KLATZO et al., 1959; NEUMANN et al., 1964; KLATZO, 1965; KAKULAS et al., 1967). A curious feature of kuru is the occurrence of "plaques" (especially in the cerebellum) in the form of rounded, eosinophilic masses often showing a ray-like appearance at the periphery, and staining positively with the periodic acid Schiff test. Their central parts often show reticulin with Foot's stain. They are strongly argyrophilic with Bodian's stain but colour only palely with Congo red. In the electron-microscope kuru plaques are found to be made up of very large numbers of fibrils matted together but arranged rather radially at the periphery giving rise to the ray-like appearance seen in the light microscope. Banded reticulin is occasionally encountered and also considerable numbers of "herring-bone" like bodies presenting a honey-comb patterned appearance. These are also encountered elsewhere in the granular layer of the cerebellar cortex, for the most part in the glomeruli. Neither in the human nor in the small amount of chimpanzee material examined do these herring bone structures resemble the tubular arrays seen in normal old rats and with increased frequency in young adult scrapie rats (RAINE et al., 1967).

In multiple sclerosis the most striking pathological change is of course the dissolution of myelin sheaths (with relative sparing of axons, at least in the early stages) in circumscribed areas to form plaques. Astroglial overgrowth leads to the formation of a scar. Whilst degeneration of the myelin sheath is commonly regarded as the primary event — perhaps associated with degenerative changes in oligodendroglia — some older neurologists were impressed by the early changes seen in astroglial cells. Thus CHARCOT (1876) concluded that hypertrophy of glial cells was undoubtedly the first change in the development of multiple sclerosis plaques. He wrote "Nous pouvons essayer de rétablir dans l'ordre naturel de leur succession, les phénomènes qui composent l'altération dont il s'agit, et chercher ainsi à reconnaître le mode pathologique suivant lequel cette altération se constitue. Incontestablement la multiplication des noyaux et l'hyperplasie concomitante des fibrils reticulées de la neuroglie est le fait initial, l'antécédant nécessaire: l'atrophie dégénérative des éléments nerveux est secondaire, consécutive: elle a déjà commencé à se produire lorsque la neuroglie fait place au tissu fibrillaire, bien qu'elle marche alors d'un pas plus rapide."

The same conclusion was stated by ANTON and WOHLWILL (1912). They claimed that even ". . . in den frischesten Herden die Reaktion von seiten des Gliagewebes auffällig früh einsetzt und auffallend hochgradig ausfällt." They corroborated Marburg's observation that significant

(nennenswerte) myelin degeneration was not found without at least a swelling of glia cell nuclei. In a case of their own in which there were a large number of very fresh lesions ANTON and WOHLWILL found glial cell swelling around blood vessels before Spielmeyer preparations showed any myelin destruction.

Recently, JAKOB (1967) has also drawn attention to the hypertrophy of astroglial cells as an early event in the evolution of multiple sclerosis lesions. All who have studied cases of MS are impressed by the marked astroglial response which often seems disproportionately great for a simple repair, though there are certainly early lesions with an acutely necrotic appearance. Whilst at first sight multiple sclerosis and scrapie have very little in common, early astroglial activation is an important common feature, and it may be that the succeeding changes flow from this. In scrapie, the process is widespread in the nervous system, whilst in MS it is limited to areas in which lesions develop and their neighbourhood; i.e. it is a focal process. As in the case of scrapie no virus particles have been seen in electron-microscopic examinations of multiple sclerosis material. Glial cells from explanted fragments of scrapie affected brain grow with greater vigour than do those from normal material (FIELD and WINDSOR, 1965; GUSTAFSON and KANITZ, 1965) and the appearance of the astroglia in scrapie (especially in the mouse and rat) suggests they have undergone a benign neoplastic change. Enlarged convoluted glial nuclei are sometimes found in very recent MS lesions (e.g. in the very acute case reported by FIELD et al., 1962) where they may even bear close resemblance to the cells of a glioblastoma. Indeed the "grape-fruit" like bodies reported by FIELD et al. may also be found in such tumours. Can it be that the functional activity of the DNA of these glial cells has been altered in some way both in scrapie and in MS?

Some interesting recent observations may be briefly reviewed. PATTISON and JONES (1967) have suggested that scrapie agent is associated with a basic protein and has certain features in common with a histone. A possible mechanism for such linkage could resemble that between acid mucopolysaccharide and small protein peptide, where it is mediated by xylose and two molecules of galactose (DISCHE, 1966). Histones are known to modify the activity of DNA and it seems possible that scrapie agent might produce its action by altering the directive activity of nuclear DNA in astrocytes. In this way profound modifications of the astrocytes might be brought about leading to cellular hypertrophy and the appearances of benign neoplasia. Histones when exerting their inhibitory influence on DNA undergo preliminary acetylation (ALLFREY et al., 1964; POGO et al., 1966) and it has recently been found that nucleohistones extracted from the brains of animals incubating scrapie are also acetylated about 30% more than those from non-scrapie brains (CASPARY et al., 1967). This suggests that the nucleohistones of the brain in scrapie are in an

activated form at a time when morphological change is just beginning in astroglial cells. It seems highly improbable, however, that the scrapie agent is itself a histone.

Nevertheless it is tempting to suppose that in scrapie there may be some agent which over the space of about 10 or 12 weeks (the time required for earliest astroglial changes) interferes with activity of nuclear DNA so that the cells move in the direction of benign malignancy. Altered function of the nervous system can in fact be demonstrated by psychomotor testing of mice at about the time when the very earliest glial changes appear (SAVAGE and FIELD, 1965). Such chronic interference with glia might occur in MS with a considerably longer incubation period. POSKANZER et al. (1965) have shown that the clinical phenomena of MS can be interpreted in the light of a prolonged incubation period following exposure round about puberty. The emergence of scrapie when MS material has been inoculated into sheep (PALSSON et al., 1965) or mice (FIELD, 1966) awaits independent confirmation and it is possible that thymectomy with or without irradiation might facilitate transfer in small laboratory animals. An agent capable of "parasitizing" astroglial cells for prolonged periods leading to their hypertrophy followed by atrophy, could well be causative of the diffuse pathological changes characteristic of scrapie in animals, and, if operative focally, of the localised changes in MS. The importance of glial cells for the functioning of nerve cells and the maintenance of myelin sheaths is now recognized.

Recent work with kuru is of especial interest. So far as we know, kuru is limited to the Fore linguistic group of peoples of central New Guinea and occurs nowhere else in the world. It would appear to have a long incubation period and passage of the disease to experimental animals has been successful so far in chimpanzees only (GAJDUSEK et al., 1967). The genetic soil in kuru (both human and animal) — as in scrapie — is of the greatest importance for development of the disease. Again in kuru astroglial overgrowth is an important feature, both in the natural disease and in the experimental condition of the chimpanzee. Whether in kuru astroglial hypertrophy is the primary pathological disturbance remains to be determined.

In a study of the ultrastructural changes in scrapie rats RAINE and FIELD (1967) drew attention to some points of resemblance to those occurring in normal old animals. Thus, astroglia is often enlarged in the old animal, especially in the vicinity of blood vessels where the earliest scrapie changes are found. "Tubular arrays" of the type first seen by DUNCAN and WILLIAMS (1962) in certain cerebellar axis cylinders (probably the axons of Purkinje cells) are much more numerous in the normal old rat and the 8 months old scrapie rat than in the 8 months old normal animal (RAINE and FIELD, 1967). In scrapie rats amyloid deposits are commonly found. Many of the changes seen in kuru also resemble

those seen in the old brain. Laminated amyloid bodies are frequent in kuru as they are, of course, in the old brain.

The kuru plaques referred to above have been described also in scrapie (BECK and DANIEL, 1965) and their fine structure bears some resemblance to that of plaques in Alzheimer's disease (KIDD, 1964).

In kuru there are widespread degenerative changes in nerve cells and, as in old age (FERRARO, 1959), those of the olive are often markedly affected. PAS positive granular material accumulates within these nerve cells. Similar material is found in some non-kuru New Guinea brains especially in the older age range (personal observation).

There are thus several points of resemblance between scrapie, kuru and old age. Indeed scrapie and kuru show the changes of the latter in exaggerated form. In the case of multiple sclerosis the single important common factor is localized astroglial proliferation.

Despite the most intensive search no organism has been recognized in scrapie or in multiple sclerosis. The scrapie agent has many remarkable properties. It is generally agreed that it is unusually resistant to heat (though many of the experiments done to test its heat resistance are far from unobjectionable in their technical aspects) and to chemicals (such as formalin — which it will resist for as long as 28 months (PATTISON, 1965). Antibodies to scrapie agent have not so far been demonstrated. The agent is highly resistant to UV light and to ionizing radiation. Indeed ALPER et al. (1966) from their irradiation experiments have claimed that the "target size" of the agent is very small (perhaps as little as 3 mμ) and that it cannot contain nucleic acid. It has indeed been suggested that a new self-replicating particle may be in question, either a polysaccharide (FIELD, 1966) or one containing a small nucleic acid core stabilized by a non-antigenic and chemically resistant polysaccharide coat (ADAMS and CASPARY, 1967). Some direct evidence is now at hand that polysaccharide synthesis may be anhanced during the development of scrapie as compared with its level in normal animals (ADAMS et al., unpublished work, 1967). However, recent studies of the effects of ultraviolet and cobalt-60 radiation upon DNA tumour viruses and EMC virus (DEFENDI and JENSEN, 1967; NAFTALIN, 1967) have suggested that activity of virus may persist after heavy irradiation. Other interpretations may therefore be possible for the work of ALPER, whose experimental results have been fully confirmed in our own laboratory.

In a recent editorial article (Nature, (Lond.) 1967) some of the remarkably heterodox concepts which have recently entered scrapie research were considered especially in so far as they might stimulate further experiment. With this in mind some further discussion may be indulged.

The resemblances between the changes in old age, kuru, scrapie and (focally) in multiple sclerosis are sufficient to stimulate speculation on

what might be some common underlying factor(s). The stimulated appearance of astroglial cells in these conditions has been alluded to already and the possibility suggested that interference with their DNA might interfere with normal size control and fibrilogenetic mechanism. This release might be mediated by activated histone derepression of DNA but it is highly unlikely that the agent of scrapie or kuru is itself a histone. Mention has been given above to the suggestion that it might be a polysaccharide or polysaccharide-nucleic acid complex structure. This leads to difficulties in visualising a mechanism of replication, especially if the agent were simply a polysaccharide. However McMullen (1964) has suggested that "nucleoside diphosphate saccharides . . . are analogous to the activated amino-acid in protein template biogenesis and correspondingly enter into the same kind of . . . coping mechanisms involving polynucleotide structures . . ." Robbins et al. (1966) in reviewing polysaccharide biosynthesis point out that this interesting speculation has, so far, no experimental basis and go on to consider mechanisms involving participation of the cell wall membrane.

It may be, however, that replication in the sense that one molecule or chain divides into two, or acts directly as a template for a similar molecule, does not take place in the present instance. Thus it is conceivable that the aggregation of a small number of polysaccharide chains in the cytoplasm of an astroglial cell may occur and act as a "seeding focus" for normal constituents of the cell cytoplasm in the neighbourhood. This will lead to unbalance in the peripheral cytoplasm so that locally more polysaccharide will be made to redress the balance. In this way the cell will continue to elaborate more and more polysaccharide but the multiplication of molecules will not be dependent upon a template mechanism for polysaccharide like that currently held to obtain for nucleic acids. Moreover, a small fragment, comprising a few aggregated polysaccharide chains if transplanted to another cell will initiate the same "seeding out" process in its cytoplasm. In this way the illusion of a self replicating mechanism (in the DNA sense) might be created. Pirie (1966) likewise has drawn attention to the remarkable unwillingness of some substances to crystallize until the first focus appears and Oosawa et al. (1966) have given an example in relation to the polymerisation of actin and flagellin. If the ageing process in glial cells provided such a first focus — unusually early in "diseases" — then the process could go on with cell hypertrophy and apparent replication (i.e. increase in titre) of the active "seeding aggregations". These "seeding aggregates" might well be below the level of visibility in the electron microscope and of very small size and molecular weight as suggested by the latest estimates (Alper et al., 1966, 1967; Adams et al., unpublished work, 1967). Indeed if it is a question of aggregates of polysaccharide chains then no definite molecular weight may be assignable to the "aggregates" and it is an open question what

length of chain might serve as an active focus. This might account for the varying estimates of size put forward by different workers.

PIRIE (loc. cit.), whilst accepting that it is premature to speculate about the nature of normal cell components whether organelles, membranes or other structures, that may be caricatured by viruses, points out that it is nevertheless reasonable to look for possible candidates in the electron microscope, especially in the early stages of a disease. Could the remarkable accumulation of fibrils within astrocytes in scrapie be such "pseudo-viruses"? A detailed survey of their properties might be rewarding.

It is, of course, realized that many of the ideas put forward here are tentative, imprecise and leave some vital suggestions ill-defined. It is difficult to visualize, for example, a mechanism whereby agglomeration of polysaccharide chains might occur so that a "seeding focus" could become established within a glial cell. Important light might be shed on such possibilities from purely chemical studies. For example BENNET et al. (1967) have recently adduced evidence of self association of phenolics and bile acid derivatives, the effect apparently depending on the stereochemistry of the molecules involved, especially the position and number of attached hydroxyl groups. However, the value of any hypothesis derives largely from the experimental tests it can stimulate and the inculpation of polysaccharides in the mystery of scrapie has already led to much further work on these complex materials as well as new possibilities of "virus" structure.

Summary

Attention is drawn to certain resemblances between some of the pathological features of scrapie, kuru, multiple sclerosis and old age. It is suggested that there may be a common factor in these changes, that it is the hypertrophic change in glial cells, and that this resembles benign neoplasia. It is suggested that these changes are associated with aggregation of polysaccharide chains within the cytoplasm and that these foci "seed out" the further formation and aggregation of polysaccharide by the cell so that its metabolism is ultimately quite disturbed. Aggregates of polysaccharide chains may themselves act as further seeding foci so that an illusion of a self replicating agent may result.

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