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MINIREVIEW

Scrapie, an Unconventional Virus: The Current Views (42490)

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History. Scrapie, a slow progressive fatal infection affecting the central nervous system (CNS), occurs naturally in sheep and goats (1) and was first described in the scientific literature of the last century by Besnoit and Morel (2). Due to a number of common features five other degenerative neurological diseases of the CNS have been grouped together with scrapie. Two of the others are animal diseases, transmissible mink encephalopathy (TME) (3) and wasting disease of mule deer and elk (4, 5), and the other three are human diseases, kuru, Creutzfeldt–Jakob disease (CJD), (6) and Gerstmann–Sträussler syndrome (7, 8).

In 1899 Besnoit (9) attempted to transmit scrapie by inoculating a ewe with brain tissue from a sheep with scrapie. Later Cuillé and Chelle (10) transmitted scrapie from one sheep to another, demonstrating the filterable nature and viruslike properties of the agent. The infectious nature of the scrapie agent was also shown in England during the 1930s by a major vaccine accident in which formalized tissue of louping-ill virus vaccine caused a large number of scrapie cases in 18,000 sheep in a flock formerly unaffected by scrapie.

The agent appears to replicate at a very slow rate with apparently no eclipse phase and requires many months to reach high titers (11). The characteristic features of the disease are both structural and functional. Pathological changes are restricted to the CNS without involvement of any other organs, and the disease is fatal. The late Björn Sigurdsson (12), because of the long incubation period, listed Rida as an example of slow infections and proposed the concept of slow or latent virus infection. Chandler successfully transmitted the disease in the early 1960s to mice (13) and then serially in both mice and rats. This facilitated laboratory investigations and confirmed the transmissible nature of scrapie. Later a more rapid form of the disease was developed in hamsters by passage from mice (3, 14).

Kuru was the first human neurological disease shown to be caused by a slow virus by its successful transmission to chimpanzees by the intracerebral inoculation of a suspension of human brain from a patient (15, 16). The disease has since been transmitted to other nonhuman primates (17). In man, transmission has been clearly demonstrated to be due to cannibalism of contaminated tissue (18).

For more than three decades the unusual properties of the scrapie agent have attracted considerable research attention. Numerous attempts have been made to define its chemical composition and classify it with other infectious agents such as viruses and the viroids of plants. However, there is still very little detailed knowledge of the nature of the scrapie agent. The unusual biological properties of the agent such as its resistance to nucleases, to irradiation with ultraviolet light and to divalent cation hydrolysis; its physicochemical stability and its apparent lack of specific antigenicity (19–22) have made it of interest in itself and also a useful laboratory model for the spongiform encephalopathy disease processes.

Over the last 20 years attempts have been made to purify and characterize the scrapie agent, but it has yet to be purified to a level free from contaminants (23–26). Early studies suggested that the scrapie agent was distributed throughout all subcellular brain fractions (24, 26). Various extraction procedures failed to release the agent from membrane fractions, and it was suggested that the agent was intimately associated with membranes of the infected cell (27).

Repeated failure to find typical virus particles consisting of nucleic acid and protein in scrapie-infected brains and the unusual resistance of infectivity to procedures that normally inactivate nucleic acids, have forced some

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radical rethinking about the nature of the scrapie agent and other slow viruses. Several hypothetical structures have been proposed, involving every major class of macromolecules as an important component. Examples include a small DNA virus (28), replicating abnormal polysaccharide within cellular membranes (27), a replicating polysaccharide on its own (29), naked nucleic acid (30, 31), or nucleic acid surrounded and stabilized by a polysaccharide coat (32, 33). It has also been suggested that the agent is exclusively composed of protein (18, 34–36). So far the structure has proved both unusual and difficult to define. Nevertheless it has been suggested that the scrapie agent behaves as a conventional neurotropic virus (37). Further, it has been known for some years that the agent is an independent pathogen exerting control over a number of characters (38). Dickinson and Fraser (39) and Fraser (40) have demonstrated strain variations of the scrapie agent with different incubation periods and distribution of the lesions.

From these investigations four major hypotheses on the nature of the scrapie agent have emerged. They are (i) the viroid hypothesis that the agent is composed of a nucleic acid of low molecular weight which is not encapsulated (30); (ii) the virino hypothesis, proposed by Dickinson and Outram (41), in which the agent has nucleic acid protected by host protein (the nucleic acid does not code for the protein, but might have regulatory functions within the cell analogous to those proposed for viroids); (iii) the prion hypothesis, proposed by Prusiner (35), in which the infectious agent is composed of protein molecule(s) and has no nucleic acid; (iv) the hypothesis in which the agent is filamentous in nature and is related to the "scrapie associated fibrils" (SAF) identified by negative staining by electron microscopy (42–44). These hypotheses will be discussed in turn with the significance of tubulovesicular particles that have been observed in thin sections of brains in scrapie and other spongiform encephalopathies (45–48).

Hypotheses. *The viroid hypothesis.* Although there have been a number of claims that nucleic acid is associated with the scrapie agent, attempts to demonstrate infectious nucleic acid by various methods have failed (49–51). Recent kinetic irradiation–inactivation reanalysis studies have suggested that the scrapie agent would have nucleic acid genome equivalent in size to a small virus (0.75×10^6 Da single-strand virus and 1.6×10^6 Da double-strand virus) (44) but a nucleic acid of this size has not been convincingly demonstrated. Thus it is unlikely that the agent is a piece of naked nucleic acid. Known plant viroids, when compared to the scrapie agent, differ in their sensitivity to various physical and chemical inactivation procedures. Further, viroid nucleic acid does not code for a protein(s) while protein appears to be an integral part of the scrapie agent. For these reasons the scrapie agent is unlikely to be a viroid.

The virino hypothesis. This proposes that the scrapie agent has a viruslike structure composed of a low molecular weight (by viral standard) scrapie-specific nucleic acid protected by a protein which is host-derived (38, 41). The nucleic acid acts by inducing the disease and replication of the infective nucleic acid is accomplished exclusively by host enzymes. The virino hypothesis explains many of the unconventional properties of the agent such as the difficulty in purifying the agent, its resistance to uv radiation and absence of an immunological response in (susceptible) infected animals. Recently German *et al.* (52), used two-dimensional fingerprinting analysis of the fractions prepared from healthy or scrapie hamster brain tissue(s) by density gradients to demonstrate that the RNAs from infected hamsters contained oligonucleotides that were not present in the RNAs isolated from healthy hamsters. They (52) have since produced fresh evidence that the scrapie agent may contain an essential nucleic acid that is protected by cellular components from inactivation by uv radiation.

The prion hypothesis. Prusiner investigated the proteins of scrapie infection (53). He used detergent and enzyme treatment, polyethylene glycol and $(NH4)_2\ SO_4$ precipitation followed by density gradient ultracentrifugation and claimed a 10^2- to 10^3-fold purification of the scrapie agent-associated protein. Further sodium dodecyl sulphate (SDS) gel electrophoresis increased this to a 10^3- to 10^4-fold purification (54). Analysis of these partially purified fractions by polyacrylamide gel electrophoresis (PAGE) revealed a diffuse protein band with a size of 27–30K designated PrP27-30 or PrP, which was not seen in control preparations (53, 54). Evidence from other sources has confirmed that polypeptides of the same

27–30K range are found in partially purified scrapie preparations from 263K strain of hamster and ME7 strain of mice brains (55–57). Antibodies have been raised in rabbits to the SAF and 27–30K polypeptide from both hamster and mice preparations (55, 58). Both antisera react in Western blots with the 27–30K protein as well as with a number of other low molecular weight polypeptides. However, these antisera failed to detect antigen in scrapie-infected hamster or mice brains, suggesting that *in vivo* the antigen is not available for reaction with the antibody (59). Both the PrP and infectivity showed similar resistance to digestion by trypsin and staphylococcal V-8 protease (60).

Prusiner (35, 61, 62) has reiterated his belief in the involvement of protein in the infectious process and introduced the term "prion" for the scrapie agent to distinguish it from both viruses and viroids (30, 31). He (35, 62) considers that proteinaceous infectious particles cause scrapie and that the agent may consist solely of protein with no nucleic acid whatever (62). Evidence for the prion concept has come largely from the fact that infectivity in hamster brain homogenates is resistant to a variety of treatments expected to destroy nucleic acid, but is abolished by several protein-specific reagents (62, 63). This demonstrates that apart from any other classes of macromolecules, protein may be an essential part of the scrapie agent. This property is not unique to the scrapie agent because all conventional viruses normally depend on a protein coat for their integrity and infectivity. Also all other microorganisms (except viroids) such as bacteria and mycoplasmata contain proteins.

Prusiner (62) made the assumption that scrapie and CJD are caused by prions and because of the very small size of the individual prions concluded that the agent is too small to contain nucleic acid, and this has been further supported by his failure to detect any scrapie-specific nucleic acid in highly infectious preparations. Some additional evidence that nucleic acid is absent is produced by the relative resistance to heating to 65°C in the presence of zinc ions, its photochemical inactivation after treatment with the psoralens, and resistance to treatment with 0.5 *M* hydroxylamine (64). Most animal and plant viruses, as well as bacteriophages, are inactivated by hydroxylamine (65, 66) except for the paramyxoviruses which, like scrapie agent, are also resistant. If the prion really lacks DNA and RNA, how does the agent replicate and survive? There are two possibilities. Either the PrP must bind to a specific host DNA sequence and act as a derepressor or the prion must violate part of the central dogma of molecular biology which is that genetic information is transferred from nucleic acid to proteins (35, 38, 62, 67). There are a number of objections to this hypothesis, such as the existence of several different genetically stable strains of scrapie (39, 40, 68), host variation, and the adaptation of the agent from one host species to another by repeated passage as shown by a reduction in the length of incubation. This implies that there must be a precise copying process to ensure stability of strains of the agent (39, 68–71). The absence of a nucleic acid suggests the involvement of a previously unknown system. Either the genetic information is encoded within the infectious protein or there is a range of proteins (72). Hadlow (73) observed that the scrapie agent when passaged in mink retains its ability to infect goats, but loses its ability to infect mice. This adaptation of the scrapie agent to different species and strains of animals would suggest that the scrapie agent behaves like other viruses with a genome which modifies and acquires a protein shell from the host cell during replication, which influences the host adaptability to a new host. The evidence supporting the concept that the scrapie agent is composed solely of protein is far from conclusive (38, 74) and Prusiner himself considers that the scrapie agent might contain some nucleic acid as well as protein (35, 62). Neither Prusiner nor other workers have as yet demonstrated whether the protein(s) essential for infectivity is an integral part of the agent or just associated with it. It is reasonable to conclude that the infectivity of the scrapie agent requires a protein(s) and also important to remember that purified proteins do not retain scrapie infectivity (61, 75).

The purification and structural studies (61) of the major scrapie prion protein PrP27–30K have revealed that PrP27–30K contains at least 17 naturally occurring amino acids. The data demonstrated a single N-terminal amino acid sequence for PrP27–30K with no known homology with other known proteins (61). The polypeptides in this analysis were purified by

a procedure which included extensive proteinase K treatment, sodium dodecyl sulfate disruption at 100°C, and HPLC size-exclusion chromatography. Amino terminal sequence data suggested that the prion polypeptide is a major component of the infectious preparation, but the results of polyacrylamide gel electrophoresis, antisera, silver staining, and radioiodination studies have demonstrated the presence of four cross-reacting proteins of low molecular weight as well as one protein of higher molecular weight in extensively purified preparations (55, 61). All these additional data suggest that other components are also present. Successful sequencing of regions within the prion protein will provide an approach to seeking the nucleic acid that encodes for it and this may help to resolve some of the controversies over the nature of the scrapie agent. Very recently, two independent studies showed that the 27–30K "prion" protein is in fact the product of a normal cellular gene which is expressed to the same extent in brains of infected and uninfected animals (76, 77) and at lower levels in other organs of healthy animals (77). Oesch *et al.* (77) also reported several other important findings. Western blot analysis was used to identify the prion protein-related polypeptide in the brain homogenates from infected and uninfected animals with and without proteinase K treatment. They observed in homogenates which were not treated with proteinase K a cross-reacting polypeptide of 33–35K that migrated more slowly than the prion protein. On this evidence the authors suggested that PrP27–30K was probably derived from PrP33–35K and that antigenically related protein was partially resistant to proteinase K digestion. These findings explained that although both normal and scrapie-infected brain homogenates contained PrP33–35K protein, the same purification procedures as used for isolation of the prion from scrapie infected brains when applied to normal hamster brains did not yield PrP27–30K (53, 54). Thus it would appear that the prion-related protein was the product of a normal cellular gene. Recently Wietgrefe *et al.* (78) constructed a complementary DNA library from messenger RNAs (mRNA) extracted from scrapie-infected mice. They screened the library differentially and identified a gene whose expression was increased in scrapie. They (78) further demonstrated that the complementary DNA corresponding to this identified gene hybridized preferentially and focally to cells in the brains of scrapie-infected animals and also in the senile plaques in brains of patients in Alzheimer's disease. These findings provide evidence that the scrapie agent induces increased expression of at least one mRNA that may be used as a possible marker for identification. From the published results it is now clear that the PrP27–30K prion protein is a product of a normal cellular gene that is expressed to a similar extent in infected and uninfected animals (76–78). Similar gene expression has been observed in some other slow and persistent virus infections (79). It has been also demonstrated that visna, HIV, and the PrP27–30 possess similar protein sequences (80). The similarity of the gene for scrapie-related PrP27–30 and the *pol* proteins of human immunodeficiency virus HIV and visna virus suggest that the host protein(s) could mediate some of the neurological degeneration associated with these infectious agents. Therefore it appears that the precursor of PrP is a normal protein, synthesized in cells of both mouse and hamster brain. While the impressive studies by the research groups of Chesebro and Oesch (76, 77) have provided strong evidence that the PrP27–30K polypeptide is encoded by a normal cellular gene, there is considerable experimental evidence that suggests that the PrP27–30K is inseparable from scrapie infectivity (54–57). It is possible that the prion protein in normal and infected cells could be identical, but acquires different properties from binding to another molecule, for example, nucleic acid, to form a capsid or an envelope for nucleic acid or nucleoprotein. The term "prion hypothesis" has yet to be substantiated (44, 59); therefore, the term has a restricted meaning in that the agent is composed only of protein.

Scrapie-associated fibril hypothesis. Abnormal fibrils designated "scrapie-associated fibrils" (SAF) were first demonstrated by Merz *et al.* (42) using negative contrast staining techniques by electron microscopy from scrapie-infected brains, and subsequently have been observed in other spongiform encephalopathies (81, 82). SAF are recognizable structures and have been considered as excellent candidates for the scrapie, kuru, or CJD agents (6, 42–44, 59, 81, 82), a form of the infectious agent (83), or that SAF might rep-

resent component(s) of the agent (82). SAF have been found only in scrapie and related diseases caused by unconventional viruses (42, 81, 82) and not in brain tissue of normal or in a variety of other human and animal diseases exhibiting similar histopathological and

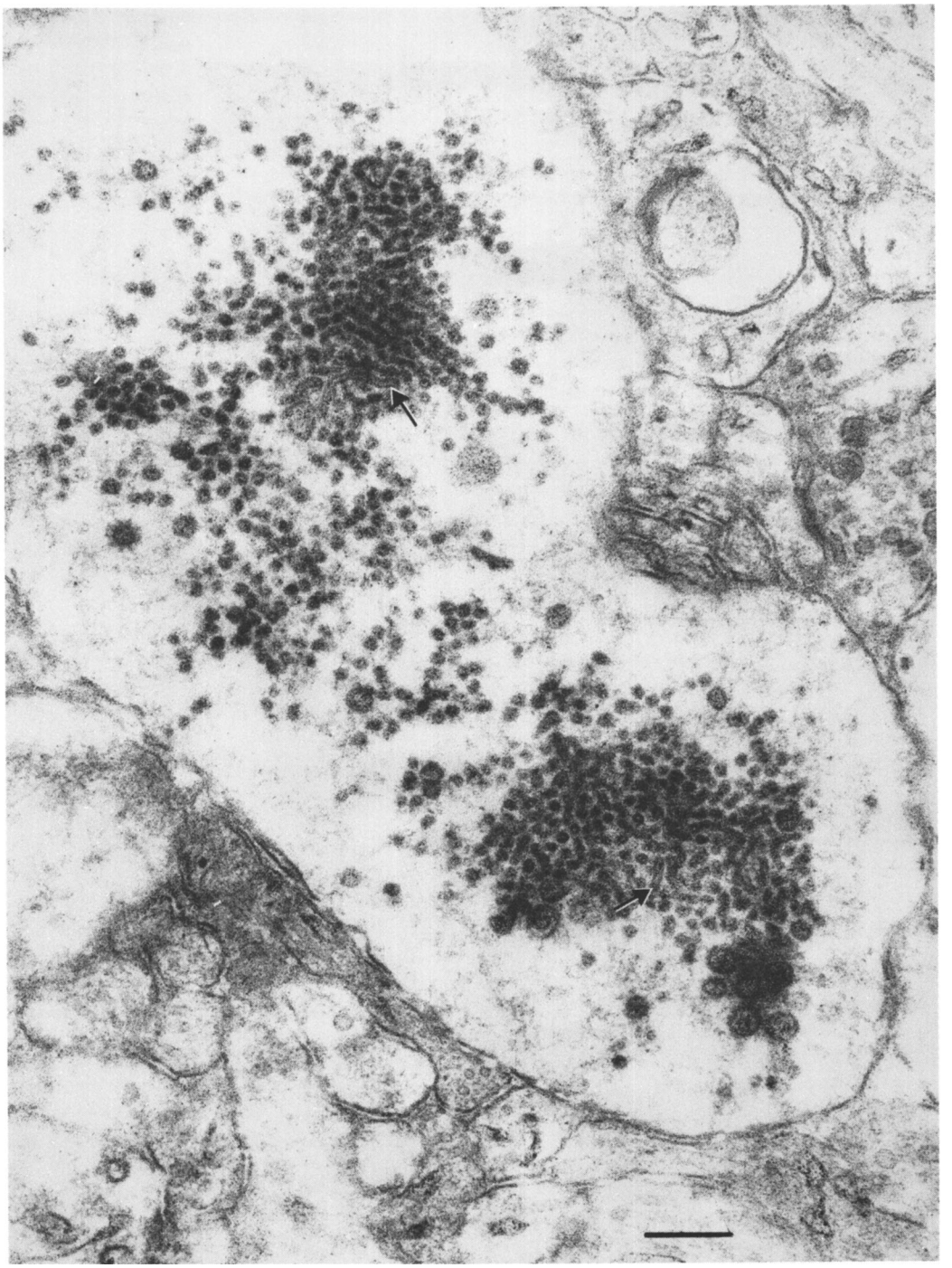
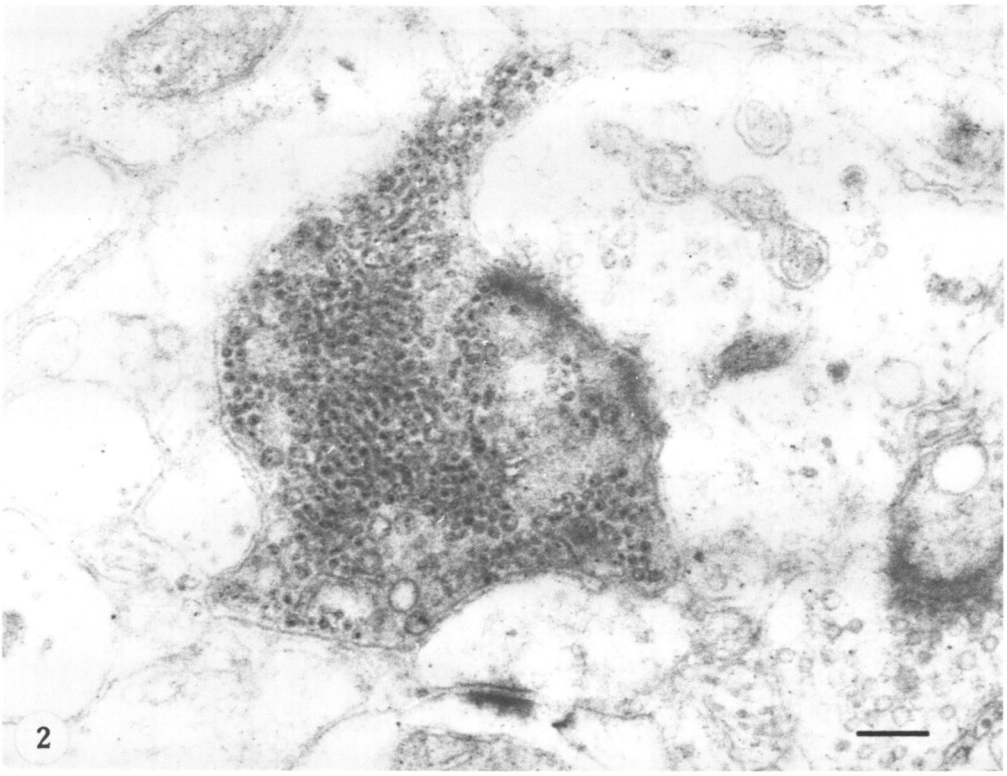


FIG. 1. Section from mouse scrapie brain cortex 3 months IC postinoculation. Note transversely and longitudinally cut scrapie-associated particles (arrows). Bar = 250 nm.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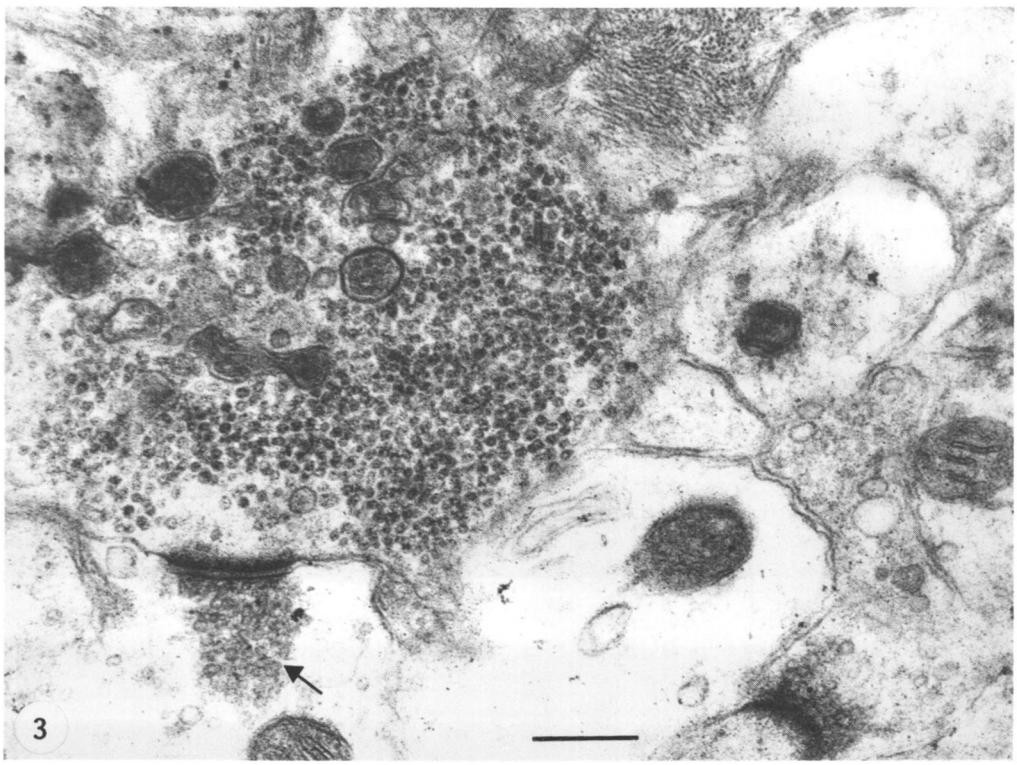
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ultrastructural features. The occurrence and number of SAF have been shown to parallel the increase in infectivity (81, 82, 84). Diringer *et al.* (43, 56) have shown a high degree of copurification between SAF and infectivity, which is also high in relation to protein concentration. They also showed that SAF were the only abnormal structures seen by electron microscopy of infectious extracts (43). SAF proteins have also been isolated from scrapie-infected hamster brains, and analyzed serologically and biochemically have also been shown to consist of a polypeptide of 27–30K molecular weight similar to the PrP26–30K proteins demonstrated by the Prusiner group (53, 54). Although SAF may contain nucleic acid, none has yet been demonstrated. The physicochemical properties and infectivity have been determined only on crude or partially purified extracts of SAF fractions used for immunizing which may have contained up to 10% impurities (85). Further there are limitations in the assays due to the long incubation period and to the fact that even in one experiment titration gives variable estimates of infectivity (86). It is difficult to determine the degree of purity of fractions containing SAF and it remains to be seen if the increase in the apparent infectivity of fibrillary material is due to coincidental copurification of the actual agent. Prusiner *et al.* (87) reported rod-shaped structures in prion preparations which the authors called aggregates of the PrP27–30K protein (PrP) or "prion rods." From the biochemical and immunological evidence so far presented it appears that the PrP27–30K, SAF, and "prion rods" are the same structures (43, 56–58, 87–89). The main differences between the prion (35) and the SAF (42, 59) hypotheses appear to hinge on the exact chemical composition of the structures since both have the same 26–30K polypeptides which cross-react antigenically (53, 54–57). Prion rod/SAF share some morphological and Congo red-staining properties with amyloid (42, 84) and paired helical filaments (90), both of which are a major feature of brain pathology in Alzheimer's disease and are also observed in some strains of scrapie, kuru, CJD, and the Gerstmann–Sträussler syndrome. It has recently been shown in material from two different strains of scrapie-infected hamsters that separation of the SAF antigen into various bands can result from different degrees of glycosylation of a single peptide chain (75). Further sequence data obtained by the authors (75) has shown that SAF/PrP is an amyloidlike peptide of low molecular weight, and this confirmed earlier biochemical observations (61). Like amyloid (91), prion rods have been shown to have a fibrillar structure by electron microscopy and to give green birefringence after Congo red staining. Because the plaques could be shown to react with anti-prion rod/SAF antibodies (55, 87) and could be stained with Congo red, Prusiner (89) speculated that in Alzheimer's disease, scrapie, and the other scrapielike diseases, the amyloid formed may be due to paracrystalline arrays of "prion" polymers and that these amyloid plaques may be analogous to some inclusion bodies. Immunoperoxidase staining with antisera to SAF or the PrP27–30K protein showed small regions of immunoreactivity in subependymal areas in scrapie-infected hamsters, and these were also stained with Congo red (55, 87). This indicates that prion/SAF have some common staining properties, but there is no homology between PrP27–30K and amyloid protein. If the prion rods/SAF is an amyloid precursor the "prion" proteins are unlikely to be the infectious agent, since they are part of normal tissue. There is no evidence that Alzheimer's disease, where amyloid is plentiful, has ever been transmitted to an animal (92).

Amyloid plaques occur late in the disease process in the brains of scrapie-infected mice (93–95). The SAF proteins have also been shown to develop only after a long delay in the disease process, when there is disease-specific incorporation of [^{3}H]leucine and [^{3}H]uridine labels (96). Amyloidlike SAF

FIG. 2. Section from hamster brain cortex of 90 days postinoculation. Note scrapie-associated particles in postsynaptic terminal. Bar = 200 nm.

FIG. 3. Section from human CJD brain cortex showing a postsynaptic terminal containing scrapie-associated particles. The scrapie-associated particles are readily distinguishable from synaptic vesicles (arrows). Bar = 250 nm.

therefore seem to be formed from a preexisting brain protein which normally turns over only very slowly (96). These recent kinetic data also indicate that the SAF protein is produced prior to scrapie agent replication (97) rather than being a constituent of the scrapie agent itself (97). This is consistent with the well-established hypothesis that amyloidoses are diseases resulting from abnormal protein degeneration (98, 99). It is important to prove conclusively whether either the SAF or the prion protein is a necessary part of the scrapie agent. However, at the moment it appears more likely that scrapie infectivity copurifies with SAF because of the high carbohydrate content of the SAF proteins (75). An ultrastructural difference between Alzheimer's plaques and those of scrapie are that although both have the clas-

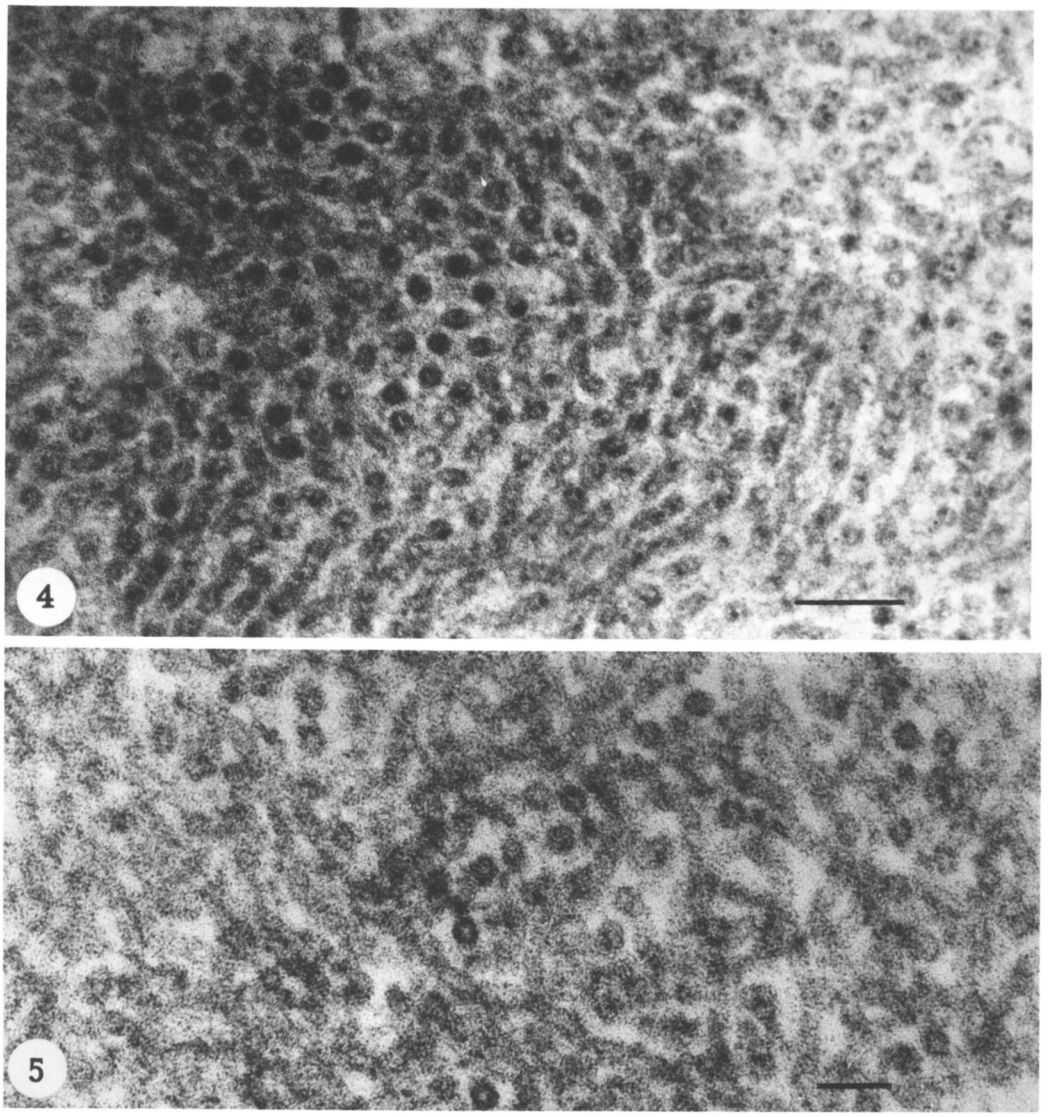

FIG. 4. High magnification electron micrograph of longitudinally cut scrapie-associated particles. Bar = 100 nm.

FIG. 5. Electron micrograph of a vero cell inoculated with measles. Note tubulofilamentous nature of the nucleoprotein similar to scrapie-associated in Fig. 4. Bar = 50 nm.

sical type of amyloid core, many of the degenerating neurites (axons and dendrites) in the former contain paired helical filaments which are absent in scrapie plaques (100). Conversely, many of the neurites in scrapie contain tubulovesicular particles but not in Alzheimer's disease (46, 93).

Various attempts to stimulate an immune response in susceptible hosts using immunizing schedules with or without adjuvants have failed (101–104) and several studies have shown that scrapie prion PrP27–30K SAF/prion is not highly immunogenic. However, rabbit antisera have been produced against both SAF and the PrP27–30K purified from infected hamster brains (55, 58). The antibody to the prion protein cross-reacts with both the prion rod and the SAF from scrapie, kuru, and CJD and with amyloid deposits in the brains of scrapie-infected hamsters (6).

Tubulovesicular particles. Among the variety of viruslike particles observed by electron microscopy of material from natural scrapie in sheep and from experimental scrapie of mice and rats (45–47, 105–109) one type of tubulovesicular particle has been found most frequently (45–47, 107, 109, 110). Many of the particles are circular and are occasionally seen as short tubulofilaments (Figs. 1–4). The overall diameters of both forms are 22–26 nm and the true nature of these scrapie-associated particles is not understood. Prusiner *et al.* (35, 87) and Carp *et al.* (59) do not consider the scrapie-associated tubulovesicular particles to be the infectious agent or even a consistent component of the pathology of spongiform encephalopathies because they were thought to be absent from brains of hamsters with experimental scrapie (105, 110) in which the highest concentration of agent has been reported to occur (3). However, Narang *et al.* (111) have recently found identical scrapie-associated particles in thin sections of the brains of hamsters infected with the 263K strain of scrapie. Failure to demonstrate the tubulovesicular particles in hamsters in previously reported studies (105, 110) may have been due to difficulties in sampling or because the studies had been carried out early in incubation period rather than at the peak of the disease.

After a lengthy search in previously defined regions using ruthenium red as a tracer, it has been possible to demonstrate the scrapie-associated particles in scrapie-infected mice inoculated by four different routes. Similar tubulovesicular particles have been observed in the brains of sheep naturally infected (47), in mice and hamsters inoculated with different strains of scrapie agent, and also in chimpanzees with Creutzfeldt–Jakob disease (112). A careful search in sections of brain from normal and sham-inoculated animals has failed to reveal similar particles (45–47). Recently, examination of tissue from human biopsies taken from the cerebral cortex of naturally occurring Creutzfeldt–Jakob disease has demonstrated particles similar to the 22- to 26-nm diameter scrapie-associated particles (48).

These scrapie-associated particles can be readily distinguished from normal neurotubules and synaptic vesicles (45, 46, 109). Apart from their size and different configuration ultrastructurally the scrapie-associated particles resemble the nucleoprotein capsids of measles virus (Fig. 5), suggesting that the tubulovesicular particles in scrapie might also be helical. However, in measles alignment of viral nucleoprotein occurs under the membrane of infected cells to form budding virus particles (113). In scrapie no such alignment of the scrapie-associated particles was observed and it might be that the scrapie-associated particles represent the complete infectious unit. Like measles virus the scrapie agent is not inactivated by hydroxylamine (65, 66). It appears that the scrapie-associated particles develop as spheres which then gradually extend in two directions to produce tubulofilamentous forms with swollen ends (personal observations). When the specimens are tilted in the electron microscope the particles appear to be linear aggregates of spherical particles (105). In previous tilt studies up to ±45° (109) it was apparent that not all particles were spherical, but that some were tubular.

Direct comparison of the resistance to inactivation of scrapie with viruses of similar size places the scrapie agent with the smaller viruses as opposed to with those of subviral size as suggested by a number of authors (see Refs. (18, 25, 35) for reviews). Both the extreme resistance of the scrapie agent to sterilization by ionizing radiation (114) and the gel filtration studies (35) have been taken to prove that the agent is quite small, but a thorough reexami-

nation of the existing evidence suggests that the infectious scrapie agent is not as small as some have claimed (115). Recent kinetic studies by Rohwer (44) demonstrated that the scrapie infectious agent is viruslike in size and susceptibility to inactivation, and that resistance is limited to a small subpopulation of the total infectivity. The calculations of the target size have given minimum molecular weights ranging from 64 to 150K (44, 114) and it has been suggested that multiple copies of the agent might exist within a single infectious particle due to aggregation (87, 116, 117). Alternatively the agent repairs itself as does the polyoma virus which is also resistant to ionizing radiation (114, 118, 119). Multiple copies of the agent might exist due to helical nature of the capsid arrangement as in paramyxoviruses. This would also explain the size variations seen in the scrapie agent. There is no convincing experimental evidence that the minimal infectious unit is less than 20 nm in diameter. Further evidence that the scrapie agent is comparable in size to viruses has come from the fact that, like known viruses, the infective particles do not penetrate into dilute agarose–acrylamide electrophoretic gels (120).

The scrapie-associated tubulovesicular particles observed appear to be of the right order of estimated size for the scrapie agent (44, 59). Further evidence comes from the fact that the aggregates of scrapie-associated particles are found in synaptic terminals and that enriched fractions of synaptic terminals have the highest titers (121).

The tubulovesicular particles are scrapie specific and appear to be at the very least a consistent part of the ultrastructural pathology of spongiform encephalopathies. The tubulovesicular particles may be the infectious agent of these diseases. Further work is being directed toward determining the possible relationship of the scrapie-associated particles to scrapie, Creutzfeldt–Jakob disease, and kuru.

In conclusion, it appears that purified "prion," PrP27–30K, SAF, and SAF-protein have been used as synonymous expression and are essentially noninfectious. Although considerable evidence is argued in favor of PrP27–30K being a component of the scrapie agent, one must consider the possibility that the scrapie agent could reasonably be a conventional virus with a small genome. The infectivity in the prion/SAF fractions is likely to be due to the copurification of the scrapie agent. The designation "prion" used for the infectious pathogens causing spongiform encephalopathies has been proposed without prejudgment of its infectious nature and physical composition. The nature of the tubulovesicular particles and their association with the disease still remains to be established.

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