REVIEW ARTICLE

Nature of the Scrapie Agent: Current Status of Facts and Hypotheses

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Scrapie is a slow infection affecting the central nervous system (CNS) of sheep and goats (Dickinson, 1976). Experimentally the agent has been passaged in mice, hamsters and a number of other laboratory animal species. Kuru and Creutzfeldt-Jakob disease (CJD) are related diseases occurring in humans (Gajdusek, 1977). These diseases are caused by agents that have unconventional characteristics compared to those of known animal viruses (Gajdusek, 1977). The unusual physical, chemical and biological properties of the scrapie agent have led to an abundance of theories concerning its nature. Each of the major classes of macromolecules has been suggested as an important component of the scrapie agent. Hypotheses have suggested that scrapie agent is composed exclusively of protein (Griffith, 1967), exclusively of polysaccharide (Gibbons & Hunter, 1967), or exclusively of nucleic acid (Diener, 1972). Other hypotheses have suggested that it is a replicating membrane component (Gibbons & Hunter, 1967) or a filterable virus (Eklund et al., 1963). This is but a partial list of the theoretical constructs that have been proposed.

It has been established that, regardless of its unusual characteristics, the agent behaves as an independent pathogen exerting control over a number of its characteristics (Kimberlin, 1982a). Evidence for this includes the existence of strains of agent whose characteristics, such as incubation period and the pathological changes induced, are highly reproducible and are presumably under genetic control (Dickinson & Fraser, 1979; Fraser, 1979). The nature of the informational molecule is unknown, but a model with nucleic acid as the controlling element of heritable characteristics clearly has precedence in that it applies to all viruses and, in fact, to all living organisms. Adherents of the hypothesis of a nucleic acid-containing agent leave open the question of whether the nucleic acid codes for agent protein, as with standard viruses, or whether the agent is composed of regulatory nucleic acid and a host-derived protein component. The latter is the virino model for the nature of the agent (Dickinson & Outram, 1979; Kimberlin, 1982b). In contrast, there is a recent proposal which places scrapie in a realm that has no precedent in modern molecular biology. This is the prion hypothesis (Prusiner, 1982), in which the heritable specificity is induced by an infectious unit composed solely of protein.

In the following, we will review recent facts that bear upon the nature of the agent and relate them to the current hypothetical models of the agent.

THE FACTS

Scrapie infectivity is dependent on protein

A number of studies using a variety of chemical agents have indicated that protein is required for scrapie infectivity. Pronase (after acetone extraction or SDS treatment), proteinase K, SDS at high concentrations, phenol and urea inactivate a high proportion of scrapie infectivity (Millson et al., 1976; Millson & Manning, 1979; Cho, 1980, 1983; Lax et al., 1983). The most convincing evidence on this point was provided in a series of studies by Dr S. B. Prusiner and colleagues who used a range of concentrations and exposure times in their studies. The treatments included proteinase K (Prusiner et al., 1981a), diethylpyrocarbonate (McKinley et
al., 1981) and chaotropic ions (Prusiner et al., 1981 b). Thus, scrapie agent shares with all animal viruses a requirement for protein in order to achieve maximum infectivity levels (Dulbecco & Ginsberg, 1973, p. 1010).

**A 26K to 30K protein is associated with scrapie infectivity**

A protocol involving detergent and enzyme treatment, polyethylene glycol and (NH₄)₂SO₄ precipitations followed by density gradient ultracentrifugation was used by Prusiner et al. (1982) to purify scrapie agent from infected hamster brains. In this procedure, infectivity was purified 10²- to 10³-fold with respect to protein, retaining 1 to 10% of the initial infectivity. Later modification of this procedure led to a purification of 10³- to 10⁴-fold (Prusiner et al., 1983). Analysis of radioiodinated fractions of the partially purified scrapie infectivity by polyacrylamide gel electrophoresis (PAGE) revealed a diffuse protein band (27K to 30K) not seen in control preparations (Bolton et al., 1982). This protein band was resistant to proteinase K digestion (100 μg/ml, 25 °C, 30 min) under conditions in which most of the host proteins were degraded. Scanning laser densitometry was used to measure the concentration of this protein (designated PrP) in relation to the amount of infectious agent (McKinley et al., 1983 b). Although there is considerable scatter in the data, a correlation exists between the concentration of PrP and infectivity. Under conditions of prolonged treatment with proteinase K, PrP and infectivity exhibited similar degradation rates. Both PrP and infectivity tended to show similar resistance to trypsin and staphylococcal V-8 protease digestion. The kinetics of proteolytic digestion, on the whole, support an association between PrP and infectivity.

A protein with a molecular weight similar to PrP has been found in preparations of purified hamster scrapie (263K strain) using a different procedure (Diringer et al., 1983 a). This protocol also uses detergent and enzymic treatment [however, enzyme concentrations are at least tenfold lower than those used by Prusiner et al. (1983)] but in addition employs sonication and differential and rate zonal ultracentrifugation. Infectivity was purified 10⁴- to 10⁵-fold with respect to protein. Fractions of purified scrapie analysed by PAGE and silver staining revealed a diffuse protein band at 26K not seen in normal material. This protein band is similar to the PrP band (26K to 30K). The 26K to 30K protein is present in fractions with infectivity (Diringer et al., 1983 a). In recent studies using a modification of the procedure described by Diringer et al. (1983 b), various scrapie agent–mouse strain combinations were analysed (R. J. Kacsak et al., unpublished observations). A polypeptide of 26K to 30K, similar to the 263K protein, was found associated with all four mouse scrapie agents examined. However, in certain agent–strain combinations, polypeptides at lower molecular weights were also seen, usually at 24K to 25K and 21K to 22K (R. J. Kacsak et al., unpublished observations). Similar analyses of 263K hamster preparations yield only the 26K to 30K polypeptide, similar to that noted by other laboratories (Bolton et al., 1982; Diringer et al., 1983 a). Thus, evidence from three independent sources establishes that a polypeptide(s) in the 21K to 30K range is found in partially purified scrapie preparations.

Antibodies were raised in rabbits to the 27K to 30K polypeptide found in 263K-hamster preparations (Bendheim et al., 1984). These antibodies reacted in Western blots with the 27K to 30K polypeptide and with a number of polypeptides of lower molecular weights. The antibody also reacted with material in infected hamster brain. This material was clumped, visible by light microscopy and showed green–red birefringence after staining with Congo red. Although infectious agent is distributed throughout the infected hamster brain, this antiserum did not detect its presence, which suggests that most antigen is unavailable for reaction with antibody. Antibodies to the 26K protein have also been produced by Diringer et al. (1984), and R. J. Kacsak et al. (unpublished) have an antibody to the proteins derived from mice infected with the ME7 scrapie agent. These antibodies were also raised in rabbits. The latter antibody cross-reacts with the 26K protein and the anti-263K antibody cross-reacts with ME7 proteins.

In recent studies, amino acid analyses and a partial sequence of the 263K–hamster polypeptide have been reported (Prusiner et al., 1984). The amino acid analysis indicated high levels of glycine, glutamine and asparagine. The partial sequence encompassed 17 amino acids from the N-terminus. The analysis included a number of minor signals, which were interpreted
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as having been generated by the degradative effect of the high levels of proteinase K used in this procedure for partial purification of scrapie infectivity. In contrast, findings from H. Diringer’s group (personal communications) suggest that the N-terminus of the 263K polypeptide is blocked. Furthermore, the amino acid composition found by Diringer and colleagues is different in a number of respects; in particular, their results indicate much lower levels of methionine.

Scrapie is not a viroid

The requirement for protein clearly rules out the possibility that the scrapie agent belongs to the group of infectious agents termed viroids (Diener, 1972). Viroids are plant pathogens which are composed exclusively of nucleic acid; no protein is required for infectivity and phenol extraction readily yields infectious nucleic acid. Viroid nucleic acid does not code for protein, but it does act as template for progeny molecules, thereby maintaining heritable characteristics (Davies et al., 1974; Diener, 1981). The nucleic acid probably induces pathological change by disorienting cellular regulatory functions. Additional studies revealed that viroids and scrapie differ in their sensitivity to various physical and chemical inactivating procedures, further supporting the idea that scrapie is not a viroid (Diener et al., 1982).

Attempts to demonstrate infectious nucleic acid by phenol extraction of scrapie homogenates have failed (Marsh et al., 1974; Ward et al., 1974). This finding, however, does not distinguish scrapie from many conventional animal virus groups (Dulbecco & Ginsberg, 1973, pp. 1150–1151). Other attempts to demonstrate a novel, scrapie-specific, nucleic acid have failed to yield reproducible results (Hunter et al., 1976; Malone et al., 1979; Prusiner, 1982; Bountiff & Hunter, 1982). In the case of experimental CJD, nick translation techniques failed to provide any evidence for a wholly or partly double-stranded DNA (Manuelidis & Manuelidis, 1981). In preparations that have been partially purified with respect to infectivity (Marsh et al., 1984a), Dees et al. (1984) described low molecular weight RNA bands in scrapie preparations that were not seen in normal preparations. Whether these are associated with the infectious agent or are related to pathological processes remains to be determined. It should be noted that the usual strategy for analysis of a virus nucleic acid is first to isolate it from purified virus particles. Until recently, purified particles have not been available with scrapie; however, the structure described in the next section may provide such an opportunity.

A filamentous structure is consistently associated with scrapie

Until recently, electron microscopy had failed to reveal virus-like structures that were consistently seen in different agent–host combinations or that were observed reproducibly from laboratory to laboratory (Baringer et al., 1981; David-Ferreira et al., 1968; Narang, 1974). However, a finding published in 1981 could have critical importance in the study of the nature of the scrapie agent (Merz et al., 1981). Filamentous structures, called scrapie-associated fibrils (SAF), were found in negatively stained, detergent-treated membrane fractions from scrapie-infected mouse brain. Following staining with sodium phosphotungstate, SAF are seen as paired or quadrupled (4 to 6 nm) filaments that are helically wound around each other. The length of the fibrils varies between 50 and 400 nm depending upon the isolation conditions used, e.g. sonication leads to shorter fibrils (Diringer et al., 1983a). In limited studies, SAF have not been seen in thin sections of scrapie brain. The structures have been found consistently in preparations from four strains of mice and in hamsters infected with scrapie. Preparations from animals infected with any one of seven different scrapie strains have been shown to contain SAF (Merz et al., 1983a). They have not been seen in more than 100 preparations of normal brains, including age-matched mice and hamsters, very old mice and mice treated with cuprizone, a treatment which in part mimics the histopathological changes seen in some scrapie agent–host combinations. Other chemical treatments or virus infections that produce vacuolation and/or gliosis were also negative for SAF (Merz et al., 1984). These included triethyltin, chronic and acute infections with Semliki Forest virus and neurotropic retroviruses. All of the preparations were evaluated as coded samples. SAF can be distinguished morphologically from normal CNS cytoskeletal filaments (Merz et al., 1981, 1982, 1983a). Structurally they are not like any known
animal virus or virus product. SAF do resemble amyloid, a class of abnormal fibrillar structures seen in a variety of diseases, but they are not identical (Merz et al., 1981, 1982, 1983b). Ultrastructurally, SAF can be distinguished from (i) amyloid found in the CNS of certain scrapie agent–mouse strain combinations (Merz et al., 1982), (ii) the two major forms of amyloid (AL and AA) present in human cases of systemic amyloidosis and (iii) amyloid that has been made synthetically from normal proteins, e.g. insulin and substance P. SAF have also been seen in humans with CJD and in experimental CJD in mice, hamsters and guinea-pigs (Merz et al., 1983c; Manuelidis & Manuelidis, 1983). They have not been seen in preparations from humans with Alzheimer’s disease, Guam parkinsonism dementia or amyotrophic lateral sclerosis (Merz et al., 1982, 1984). In addition to brain preparations, SAF and infectivity have been observed in spleens obtained from scrapie- and CJD-infected animals (Merz et al., 1983c). The presence of SAF in spleen suggests they are closely associated with the agent and not just a product of the pathological changes occurring in scrapie- and CJD-infected brain.

In 1982, a report described ‘rods’ in partially purified scrapie preparations (Prusiner et al., 1982). In this study, the significance of the rods for scrapie was said to be unclear because a few structures of similar size and shape were found in control fractions. More recent reports from this group, in which the structures were referred to as rods (Prusiner et al., 1983) or ‘fibril-like’ (McKinley et al., 1983a), have indicated that they are seen only in scrapie preparations. Ultrastructurally, the rods or fibril-like material are indistinguishable from SAF, except in length, a difference which can readily be explained in terms of the vigorous initial tissue homogenization used (Prusiner et al., 1983). These authors suggest that the rods are aggregates of the agent which itself is said to be a polymer of two or three PrP molecules (Prusiner et al., 1983).

SAF were also observed in scrapie-infected hamster brains by a third group (Diringer et al., 1983b). In these studies, it was found that procedures designed to purify scrapie infectivity also purified SAF. Using rate-zonal centrifugation, SAF and infectivity banded over the same S value range (70S to 300S) (Diringer et al., 1983b). The work of Diringer’s group has been of central importance in suggesting a link between infectivity, SAF and the 26K to 30K protein. In their fractions with high specific scrapie infectivity, the 26K to 30K protein was the major species detected and SAF were the only structures visible by electron microscopy.

The sequence of events in this area has been detailed recently in several articles (Rohwer, 1984a; Kimberlin, 1984).

**Genetic control of scrapie agent–mouse strain interactions**

Over the past 20 years data have accumulated concerning the genetic control of scrapie agent–mouse strain interactions. In this work, a series of parameters have been shown to be controlled by both host and agent. These parameters include (i) incubation period (Dickinson & Meikle, 1971), (ii) the intensity of vacuolation in various selected regions of the brain (Fraser, 1979), (iii) the presence and/or frequency of amyloid plaques (Bruce et al., 1976), (iv) behavioural changes (Outram, 1972; McFarland et al., 1980), (v) host susceptibility (Kimberlin & Walker, 1978), (vi) weight gain during the preclinical phase of disease (Carp et al., 1984a). In the context of the ‘nature of the agent’ issue, the key point is the control exerted by scrapie agents.

For each of the parameters noted there are examples in which injection of the same strain of mouse with two agents yields markedly different results. A case in point is the incubation period marker. Inbred strains of mice were shown to differ in a gene termed *sinc*, an acronym for scrapie incubation (Dickinson & Meikle, 1971). Most mouse strains are s7s7 and these have a short incubation period for ME7 and a comparatively long incubation period for the 22A agent. In contrast, in p7p7 mouse strains, 22A has a shorter incubation period than ME7. The difference in incubation period between two agents in the same mouse strain can be considerable: in s7s7 strains the incubation period for ME7 is 130 to 150 days following intracerebral injection, whereas the incubation period for 22A is 380 days. There are other scrapie agents which have incubation periods greater than 500 days in s7s7 mouse strains. Even among scrapie agents which are similar with regard to their incubation period in s7s7 or p7p7 mice, there are marked differences. For example, the 139A and ME7 agents each have
### Table 1. Comparison of two scrapie agents, 139A and ME7, which have short incubation periods in s7s7 mice

<table>
<thead>
<tr>
<th>Biological comparison</th>
<th>ME7</th>
<th>139A</th>
</tr>
</thead>
<tbody>
<tr>
<td>White matter vacuolation</td>
<td>None</td>
<td>Extensive</td>
</tr>
<tr>
<td>Grey matter vacuolation</td>
<td>Varies in different locations, but extensive</td>
<td>Similar in different locations, low level</td>
</tr>
<tr>
<td>Amyloid plaques in VM mice</td>
<td>Modest number</td>
<td>None</td>
</tr>
<tr>
<td>Amyloid plaques in CBA mice</td>
<td>A few</td>
<td>None</td>
</tr>
<tr>
<td>Increase in weight, CBA mice, preclinical</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Increase in weight, SJL mice, preclinical</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Incubation period in CBA mice</td>
<td>150 days</td>
<td>115 days</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biochemical comparison</th>
<th>ME7</th>
<th>139A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of SAF purification using modified Diringer procedure</td>
<td>SAF easily isolated, high yield</td>
<td>SAF difficult to isolate, low yield</td>
</tr>
<tr>
<td>Effect of proteinase K on SAF proteins under non-denaturing conditions</td>
<td>Slight</td>
<td>Degraded &gt; 50%</td>
</tr>
<tr>
<td>Effect of proteinase K treatment on SAF proteins under denaturing conditions</td>
<td>Moderate</td>
<td>Completely degraded</td>
</tr>
</tbody>
</table>

Comparatively short incubation periods in s7s7 mice but show marked differences in a number of biological characteristics (Table 1) (Carp & Callahan, 1985). These include the presence or absence of white matter vacuolation, the extent and pattern of grey matter vacuolation, the capacity to induce plaques in VM and CBA mice, the capacity to cause weight increases in the preclinical phase of disease in SJL and CBA mice and incubation period in CBA mice. It is interesting that in addition to these biological differences these scrapie agents differ in the biochemical characteristics of their specific associated polypeptides and the ease with which SAF can be isolated from homogenates (Table 1). The specific polypeptide(s) associated with 139A preparations is much more susceptible to inactivation by proteinase K under both non-denaturing and denaturing conditions than is the ME7 polypeptide. In addition, attempts to partially purify SAF from ME7 homogenates by a modification of the Diringer procedure (Diringer et al., 1983b) are much more effective than from 139A homogenates. These differences are detailed in a recently submitted manuscript (R. J. Kacsak et al., unpublished).

A phenomenon consistent with a mutational event has been described in mice injected with scrapie agent 87A which forms plaques and has a long incubation period in s7s7 mice (350 days) and even longer in p7p7 mice (500 days) (Bruce & Dickinson, 1979). In these studies, an agent appeared randomly; termed 7D, it had characteristics similar to those of ME7, i.e. an incubation period of 150 days in s7s7 mice and 300 days in p7p7 mice with no plaque-forming capacity in s7s7 strains. The random appearance of this new agent in the population strongly suggests a mutational event.

### THE HYPOTHESES

The facts described above have been used in formulating three hypotheses regarding the nature of the agent. In one, the prion hypothesis, it is asserted that infectious agent is composed of a maximum of three protein molecules (Prusiner, 1982, 1984a). As such, the agent is thought to be devoid of nucleic acid. In this view, the filamentous structures observed in scrapie preparations are interpreted as a polymeric form of this infectious protein (Prusiner et al., 1983).

In the second hypothesis, the agent is said to contain a small nucleic acid which does not encode protein but serves as the template for its own reproduction (Dickinson & Outram, 1979). The protective protein associated with the agent is derived from the host. This is the virino hypothesis (Dickinson & Outram, 1979; Kimberlin, 1982b). In the third, the filamentous agent
Table 2. Current hypotheses concerning the nature of the scrapie agent

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>Nature of agent</th>
<th>Mode of replication</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prion</td>
<td>Protein only</td>
<td>Reverse translation, protein-directed protein synthesis or induction of host transcription</td>
<td>Prusiner (1982, 1984a, b)</td>
</tr>
<tr>
<td>Virino</td>
<td>Protein + nucleic acid; host-encoded protein with regulatory nucleic acid</td>
<td>Nucleic acid replicated by host enzymes with virino nucleic acid as the template</td>
<td>Dickinson &amp; Outram (1979); Kimberlin (1982a, b)</td>
</tr>
<tr>
<td>Virus</td>
<td>Protein + nucleic acid; protein encoded by virus-specific nucleic acid</td>
<td>As per standard animal virus</td>
<td>Eklund et al. (1963); Merz et al. (1983c); Carp et al. (1985)</td>
</tr>
</tbody>
</table>

hypothesis, it is postulated that the agent is the SAF and, as such, is the first filamentous animal virus (Merz et al., 1983c). This hypothesis places scrapie agent within the constraints of molecular virology since the structure can readily accommodate a nucleic acid as the required informational molecule. In this case, the nucleic acid codes for the protein of the agent. It should be emphasized that these three terms, prion, virino and fibrillar agent (SAF), describe hypothetical models of the agent. Accordingly, at present none of these terms can be used as a substitute for scrapie agent. These three hypotheses with appropriate references are summarized in Table 2 and discussed in detail below.

The prion hypothesis

The term prion was derived from the words proteinaceous infectious particle and has been used to denote the infectious agent of scrapie (Prusiner, 1982). Based upon its derivation, the term fails to provide criteria that distinguish the scrapie agent from most other infectious agents, e.g. bacteria, mycoplasmas and viruses, all of which, of course, contain protein. The only class of infectious agents eliminated is viroids (Diener et al., 1982). The definition of the term does not clarify the issue: “Prions are small proteinaceous infectious particles which are resistant to inactivation by most procedures that modify nucleic acids. The term prion underscores the requirement of a protein for infection; current knowledge does not allow exclusion of a small nucleic acid within the interior of its protein” (Prusiner, 1982). This definition still leaves us with three possibilities. First, there is a nucleic acid associated with protein and the nucleic acid codes for a scrapie-specific protein. Second, again there is nucleic acid and protein, but in this case the nucleic acid does not have the capacity to code for a protein; viroid nucleic acid lacks coding capacity. Third, the infectious agent is composed solely of protein. For the first possibility we already have a word, i.e. virus. The second possibility does require a new term and the word virino has already been suggested (Dickinson & Outram, 1979; Kimberlin, 1982b). The term prion can contribute to the current discourse on the nature of the agent only if its meaning is restricted to the ‘protein only’ possibility. Proponents of the term strongly imply that scrapie agent is likely to contain only protein. However, the presence of nucleic acid is usually mentioned as a possibility. The attempt to subsume within the single term, prion, both the ‘protein only’ and the ‘protein with nucleic acid’ concepts, has made it difficult to engage in precise dialogue about the term.

The evidence supporting the concept that scrapie is composed solely of protein is far from conclusive (Kimberlin, 1982b; Johnson, 1982). In the communication by Prusiner (1982) it was noted that “skepticism of the... model is certainly justified”. Inherent in the ‘protein only’ concept is the notion that the particle is too small to contain a significant number of nucleotides (Prusiner, 1982; Prusiner et al., 1983). The need to suppose that the infectious agent is too small is unfounded for two reasons. First, it has been reported that scrapie infectivity eluted from a Toyoda Soda TSK 4000 column with an apparent molecular weight of 50000 (Prusiner, 1982). However, the sample contained high concentrations of the detergent sulphobetaine 3-14 which has been shown to elute as a peak of micelles with an apparent ‘molecular weight’ of 50000
(Diringer & Kimberlin, 1983). Scrapie infectively bound to such a column could elute with the detergent micelles and thus give an erroneously low estimate of size. This finding does not invalidate the 50000 molecular weight estimate but it does raise doubts as to its correctness. In addition, Diringer & Kimberlin (1983) found that infectious agent in sulphobetaine 3-14 sedimented as a range of particles of molecular weights much higher than 50000. In recent studies, scrapie brain membrane preparations were detergent-extracted under non-denaturing conditions and the extracts were centrifuged to equilibrium density in either CsCl or Nycodenz (Marsh et al., 1984a, b). The peak of infectivity was found at a density of 1.28 g/ml in CsCl and 1.24 to 1.28 g/ml in Nycodenz. These results suggest that scrapie is a macromolecular complex with a density consistent with conventional viruses. Secondly, filtration data with a particle of unknown shape can only yield conclusive values for the volume of a structure if that structure is spherical. Fibrils with the same diameter could pass through if they were orientated perpendicularly to the filter. Although a spherical particle with a given diameter might not have enough room for the number of nucleotides required to act in either a regulatory or coding capacity, cylindrical structures of sufficient length with the same diameter would have the volume needed to contain functional amounts of nucleic acid. Based on the above considerations it is not necessary to postulate nucleic acid-free models of infectious agent.

Other data, based upon experiments using chemical and physical inactivating agents, suggest that scrapie infectivity is remarkably resistant to conditions that normally inactivate nucleic acid within conventional viruses (Prusiner, 1982). However, in each case the validity of the inference that scrapie agent is devoid of nucleic acid is brought into question by other considerations. These studies include the following.

(i) Scrapie infectivity is remarkably resistant to u.v. irradiation at wavelengths of 254 nm and 280 nm, whereas infectivity is susceptible at 237 nm (Alper et al., 1967, 1978; Alper & Haig, 1968). Although these results are different from most viruses, they are not unique to scrapie agent (Latarjet, 1979). In a recent report on scrapie sensitivity to ionizing radiation, inactivation rates were compared to those of viruses in which the molecular weight of nucleic acid had been determined, in many cases by sequence and restriction enzyme data (Rohwer, 1984b). These comparisons yielded nucleic acid molecular weight values of 1.6 × 10⁶ if scrapie contains DNA as its genetic component (similar to the smallest DNA animal viruses, the picodnavirus group) or 0.75 × 10⁶ if RNA is the genetic component (37% of the molecular weight of the picornavirus group). In another study, the u.v. sensitivity of membrane-associated scrapie infectivity was increased following treatment with chlorpromazine, a chemical which penetrates lipid bilayers and induces single-strand breaks in nucleic acids (C. Dees, personal communication). This finding suggests that the target for u.v. inactivation of scrapie infectivity is an essential nucleic acid.

(ii) Scrapie is resistant to nucleases (Millson et al., 1976), but so are conventional viruses because their nucleic acid is protected by a protein coat (Dulbecco & Ginsberg, 1973, p. 1144).

(iii) Photoinactivation with psoralens occurs by forming covalent linkages with nucleic acids. Several animal virus groups have been found to be inactivated by psoralens, but they do not affect scrapie (McKinley et al., 1983c). Picornaviruses are also unaffected by treatment with psoralens.

(iv) RNA polymers are hydrolysed to mononucleotides during exposure to 2 mM-Zn(NO₃)₂ at 65 °C for 24 h. The hydrolytic effect on DNA polymers is much less profound (Butzow & Eichhorn, 1975). Treatment with Zn has been shown to inactivate a viroid (Diener et al., 1982), but there is no effect on scrapie (Prusiner, 1982). The significance of this finding is reduced since there have been no reported attempts to use Zn to inactivate conventional viruses, nor were these controls attempted in the scrapie study.

(v) Hydroxylamine, which inactivates nucleic acids by interacting with the nucleotide bases, is another example of a compound purported to distinguish scrapie from known viruses on the basis of there being little or no nucleic acid in scrapie. However, the published experiment showing the effect of hydroxylamine on scrapie (McKinley et al., 1981) shows a rate of inactivation that is indistinguishable from that reported for T₄ phage (Tessman, 1968). Further, hydroxylamine does not inactivate intact tobacco mosaic virus (Schuster & Wittmann, 1963).
In summary, these inactivation experiments fail to establish a need to go outside the bounds of established molecular biology to invoke a 'protein only' scrapie agent (Kimberlin, 1982a, b; Johnson, 1982).

The virino hypothesis

In the virino construct, there is a low molecular weight nucleic acid and a host-derived protein. The nucleic acid is scrapie-specific but does not encode any protein. In this model, replication of agent nucleic acid is accomplished by host enzymes. The nucleic acid of the agent would probably induce disease by disrupting the regulatory functions of biochemically similar small nucleic acids of the host. This is probably also how viroid nucleic acid induces disease. In this model, the protective protein coat that probably establishes many of the characteristics of scrapie agent (e.g. nuclease resistance) is encoded by the host. The virino hypothesis would lead to a number of the unusual characteristics associated with scrapie infectivity: (i) the host derivation of the protein would explain the absence of an immunological response in susceptible infected animals, (ii) the host origin of the protein could also explain the difficulty of separating the infectious agent from host material (Hunter, 1972; Millson & Manning, 1979; Prusiner et al., 1981a), (iii) the small size of the nucleic acid would explain the resistance of scrapie infectivity to irradiation (Alper et al., 1978) and (iv) the low molecular weight of the nucleic acid could also contribute to difficulties in isolating and detecting nucleic acid in partially purified preparations.

The filamentous virus hypothesis

There is no example of an infectious filamentous structure among animal viruses but there are filamentous bacteriophages (Marvin & Hohn, 1969), and, indeed, approximately 40% of plant viruses are filamentous (Fraenkel-Conrat, 1979). These filamentous viruses have dimensions that are broadly similar to those of SAF and they clearly contain sufficient nucleic acid to encode their replication and their genetic specificity. Evidence suggesting that SAF may be a new example of such a filamentous infectious agent includes (i) SAF are found only in scrapie and related diseases caused by unconventional infectious agents (Merz et al., 1981, 1983c, 1984), (ii) SAF are not observed in normal preparations or preparations from a variety of pathological conditions that are not caused by scrapie-type agents, (iii) in general, the occurrence of SAF and their increase in number parallels increases in infectivity (Merz et al., 1983a, c), (iv) there is a high degree of co-purification of SAF with infectivity (Diringer et al., 1983a, b) and (v) SAF are not observed in normal preparations or preparations from a variety of pathological conditions that are not caused by scrapie-type agents, (iii) in general, the occurrence of SAF and their increase in number parallels increases in infectivity (Merz et al., 1983a, c), (iv) there is a high degree of co-purification of SAF with infectivity (Diringer et al., 1983a, b) and (v) in preparations of high specific scrapie infectivity (relative to protein) the only abnormal structure consistently found is SAF (Diringer et al., 1983a), also known as rods (Prusiner et al., 1983). Indeed, as a visible form of scrapie agent, SAF is presently the only candidate. The structure of SAF and/or the chemical nature of its protein might provide a basis for the insensitivity of scrapie infectivity to many of the chemical and physical inactivating processes that have been used.

The complete chemical composition of SAF remains a question. It is clear that the protein portion (of the 263K agent) is made at least in part of the 26K to 30K polypeptide (Diringer et al., 1983a; Prusiner et al., 1983). A search for nucleic acid is currently underway in a number of laboratories.

The relationship between SAF and amyloid

It has been suggested that the abnormal fibrillar structures that make up cerebral amyloid are composed of rods such as those seen in scrapie preparations (Prusiner et al., 1983). This suggestion was put forward on the basis of similar ultrastructural appearance and green–red birefringence after staining with Congo red (Glenner et al., 1974). The concept that the rods are composed of aggregates of infectious protein, prions, leads to the idea that amyloid is infectious. Since amyloid deposits are also seen in Alzheimer's disease, it was even postulated that amyloid in this and other conditions might be infectious (Prusiner et al., 1983; Prusiner, 1984a, b). There has been much speculation about the possible infectious aetiology of Alzheimer's disease (Carp et al., 1984b); however, thus far there is no evidence of a transmissible agent (Brown et al., 1982).
It must be stressed that substances besides amyloid, e.g. silk, produce green–red birefringence after Congo red staining (Glenner, 1980). Furthermore, it has been reported that SAF can be distinguished ultrastructurally from the amyloid seen in scrapie agent–mouse strain combinations that produce CNS amyloid plaques and from amyloid seen in human CNS diseases such as CJD and Alzheimer's disease (Merz et al., 1983b). There has also never been any evidence that amyloid is the infectious entity in those diseases associated with systemic amyloidosis, such as tuberculosis, rheumatoid arthritis and multiple myeloma. Lastly, although amyloid plaques are found in some scrapie agent–host combinations they are not seen in all combinations. In fact, the 263K agent in hamsters which has been used extensively in recent biochemical studies does not yield plaques nor do two other combinations used in many of the earlier biochemical studies (Hunter, 1972), the 139A agent in Compton White or in C57BL mice. Yet, preparations from all of these agent–host combinations yield SAF (Merz et al., 1981; Diringer et al., 1983a).

Concluding remarks

The enigma concerning the nature of the scrapie agent has both fascinated and frustrated researchers for many years. The findings outlined in this review combined with new data that will soon become available should be examined in the light of the three hypotheses presented. We will then be able to establish whether the scrapie agent is consistent with conventional molecular virology or represents a new form of infectious agent. In either case, understanding the nature of the scrapie agent will assist in our ability to study the epidemiology and pathogenesis of known slow infections and may provide means to detect similar agents in CNS diseases of unknown aetiology.

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REFERENCES


