Transmissible Encephalopathies in Animals

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ABSTRACT

Scrapie in sheep and goats is the best known of the transmissible encephalopathies of animals. The combination of maternal transmission of infection and long incubation periods effectively maintains the infection in flocks. A single sheep gene (*Sip*) controls both experimental and natural scrapie and the discovery of allelic markers could enable the use of sire selection in the control of the natural disease. Studies of experimental rodent scrapie show that neuroinvasion occurs by spread of infection from visceral lymphoreticular tissues along nerve fibers to mid-thoracic cord. The slowness of scrapie is due to restrictions on replication and cell-to-cell spread of infection affecting neuroinvasion and subsequent neuropathogenesis. Probably both stages in mice are controlled by *Sinc* gene, the murine equivalent of *Sip*. The glycoprotein PrP may be the normal product of *Sinc* gene. Posttranslationally modified PrP forms the disease specific "scrapie associated fibrils" and may also be a constituent of the infectious agent. Scrapie-like diseases have been reported in mink and several species of ruminants including cattle. All of them may be caused by the recycling of scrapie infected sheep material in animal feed. The human health implications are discussed.

INTRODUCTION

The title of this paper refers to scrapie and a group of related diseases of the central nervous system (CNS). These diseases are caused by a class of infectious agents which are notable for their physicochemical stability and their apparent failure to stimulate any kind of protective host response to infection. The biochemical nature of these agents is still a matter of controversy but many workers consider them to be outside the known range of viruses and viroids. For this reason the term "unconventional viruses" is often used. The terms "virinos" and "prions" have entered the literature as synonyms for the scrapie family of agents but this may be premature. Such terms are best used to identify the specific hypothetical concepts about the nature of these agents that prompted their introduction.

The most radical concept, embodied in the much published "prion" hypothesis (1), is that the scrapie agent is an infectious protein which somehow directs its own replication. The "virino" hypothesis (2,3) draws upon the large body of evidence for the existence of a scrapie-specific genome, which is likely to be nucleic acid. It suggests that the putative scrapie nucleic acid is very small, not translated and therefore dependent on a host-coded protein to form an infectious agent. Taxonomically, "virinos" would fit in between viruses and viroids.

Several naturally occurring diseases are caused by members of the scrapie family of agents. Typically these diseases are associated with very long incubation periods (often several years). Overt clinical signs have a chronic progressive course over weeks or months and invariably culminate in death. Pathological lesions occur only in the central nervous system (CNS). The characteristic lesion seen by light microscopy is a noninflammatory, vacuolar degeneration of grey matter areas of the brain and spinal cord (hence the generic term, "spongiform encephalopathies"). In addition brain extracts contain large numbers of characteristic amyloid fibrils seen by negative stain electron microscopy (4). These fibrils are known as scrapie-associated fibrils (SAF: sometimes called "prion rods") but they are an important diagnostic feature of the related diseases as well (5).

Scrapie-associated fibrils are easily purified from clinically affected brain (6). They consist mainly of a membrane glycoprotein (7) PrP, whose messenger RNA is present in many types of cell (8) but is found in relatively high concentrations in normal neurons (9). In the course of infection, this protein is modified posttranslationally (8,10), accumulates in brain (11) and acquires the ability to form SAF. Sometimes, modified PrP is deposited extracellularly to form large cerebral amyloid plaques visible by light microscopy (12,13). In appearance, these plaques resemble the amyloid plaques associated with Alzheimer's disease (AD) but the latter are formed from a protein that is quite different from modified PrP (14). There are other fundamental differences in the pathology of AD and the spongiform encephalopathies and it is emphasized that AD is not known to be transmissible.

Scrapie in sheep and goats is the best understood member of the transmissible spongiform encephalopathies (15). Two related diseases of humans are kuru and Creutzfeldt-Jakob disease (CJD) but neither appears to be epidemiologically related to scrapie (16). This is not true

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of transmissible mink encephalopathy (TME), a rare disease of ranch-reared mink which occurs when animals are sufficiently exposed orally or by scarification to scrapie infected feed (17). Transmissible mink encephalopathy can be experimentally transmitted from mink to mink (for example by injection of infected tissue) but there is no evidence that this occurs naturally. In other words, mink are "dead-end" hosts for exogenously acquired scrapie infection (18).

Transmissible mink encephalopathy provides an important precedent which is relevant to the appearance of scrapie-like diseases in five species of captive ruminants in the last ten years. These are chronic wasting disease (CWD) of mule deer (19) and Rocky Mountain elk (20) in the USA, single cases of spongiform encephalopathy in nyala and gemsbok in the UK (21), and a large scale epidemic of bovine spongiform encephalopathy (BSE) in the UK (22,23).

Of these diseases, BSE is the most important. It has the pathological hallmarks of the whole group (vacuolation, and SAF formed from modified PrP; 22,24). Transmissibility to mice has recently been demonstrated (25). Epidemiological evidence strongly suggests that BSE is caused by scrapie infection getting into the cattle population via meat and bone meal supplements to concentrated feed stuffs (23). Differences in the level of concentrate feeding can account for the 30-fold lower incidence of BSE in beef suckler herds compared to dairy herds. If, like mink, cattle are "dead-end" hosts for scrapie infection, then the ban (introduced in the UK in mid-1988) on the feeding of animal derived protein to ruminants should lead to the disappearance of BSE. However, the long incubation period, estimated to be from 2.5 to 8 years (23), means that no reduction in incidence can be expected until 1992 at the earliest. The current incidence of BSE is about 1 in 1000 of adult dairy cattle so a very large number of cases (tens of thousands) will have occurred by the early 1990's (23).

A more worrying scenario is that infection, having got into the cattle population, may be able to spread from cow to calf or cow to cow. In other words BSE may become an endemic infection of cattle as scrapie is of sheep. At the moment there is no evidence one way or the other but if it did occur, endemic BSE would be as difficult to control as scrapie.

The occurrence of BSE has reemphasized the importance of scrapie research from both fundamental and practical points of view. Considerable progress has been achieved in many areas of scrapie research. What follows is a brief, selected synthesis of recent findings which reveal something of the way these diseases work. Two recent books give a more comprehensive account of current knowledge of the subject (26,27).

NATURAL SCRAPIE

There are no conceptual difficulties with the epidemiology of scrapie infection in sheep and goats. Infection is most commonly transmitted from ewe to lamb, both up to time of parturition and afterwards when ewe and lamb run together (15). The age-incidence curve (with a peak in the fourth year) reflects the incubation period of maternally transmitted infection. There is also horizontal spread of infection between unrelated adults and this can account for some of the scrapie cases in old sheep (28). However the mechanisms of maternal and horizontal spread of infection are not fully understood. Transplacental infection and infection via milk are two possibilities. The placenta from an infected ewe is a good source for the spread of infection to unrelated animals (29) and the high physicochemical stability of the scrapie agent (30) is a major factor in the buildup of contamination on farm premises. There is no direct evidence that semen or ova can become infected and there is much interest in the possibility that embryo transfer may provide a basis for importing sheep genetic material with a reduced risk of importing scrapie (31).

Sheep can be experimentally infected by oral dosing (e.g. with fetal membranes; 29) or by scarification (32) and these are likely to be natural routes of infection. Pathogenesis studies of natural scrapie show that the earliest sites of infection include tonsil, ileum and proximal colon as well as spleen and lymph nodes from a variety of sites (33). Only later is there evidence of scrapie replication in the CNS. This pattern is consistent with infection via the alimentary tract. The early and persistent infection of extraneural tissues may play a role in initiating neuroinvasion (especially with less neuroinvasive scrapie strains: see 34). In addition the lymphoreticular system (LRS) is likely to be important epidemiologically in providing a reservoir from which infection can be spread maternally and horizontally. An infected ewe can transmit scrapie to several successive lamb crops before she develops the disease (15). This is an efficient way by which scrapie infection is maintained in a flock. In contrast, studies of experimental TME in mink indicate very low levels of infectivity in the LRS which could be a major reason why there is no natural spread of infection from mink to mink (18).

Although scrapie is caused by an infectious agent, host genetic factors have a considerable influence on the development of disease in sheep (but, interestingly, not in goats). Selective breeding studies have been carried out with Cheviot (35), Herdwick (36) and Swaledale (37) sheep injected with either Cheviot or Swaledale sources of scrapie. Together these studies suggest that all sheep carry a gene, *Sip*, which controls scrapie incubation period (38). This gene has two alleles, sA and pA. Most sheep carrying the sA allele will develop experimental scrapie (i.e. sA is dominant for susceptibility) but incubation periods are usually longer in the heterozygotes than in the homozygotes (34). Sheep homozygous for pA are far less susceptible. They rarely develop the disease after subcutaneous injection of scrapie but they can be susceptible to intracerebral injection, after extremely long incubation periods. This means that the response of individual *Sip* genotypes depends on the route of injection and probably the effective dose of scrapie (34). There is evidence that *Sip* gene also controls natural scrapie (39) but studies of Suffolk (40) and Ile-de-France (41) sheep suggest a recessive pattern in which the most susceptible genotype is *Sip* sAsA.

The susceptibility of the sApA heterozygotes to experimental disease contrasts with their relative insusceptibility to the natural disease. In theory,

this difference could be used to reduce the incidence of natural scrapie by using *Sip* pApA sires to eliminate the *Sip* sAsA genotype (34). Such a strategy would depend on the reasonable but unproven assumption that most wild-type strains of scrapie interact with the alleles of *Sip* gene in the same way. There is also the question of which stages of scrapie pathogenesis are controlled by *Sip*. At the moment this question remains unanswered. However, there is good evidence that *Sinc* gene in mice is the homologue of *Sip* in sheep (38). The major action of *Sinc* gene is on scrapie multiplication in the nervous system, not the LRS(42). If *Sip* works in the same way, it could control neuroinvasion and subsequent events in the nervous system on which the development of clinical disease depends. This means that even though *Sip* sApA sheep may not develop the disease they could be persistent carriers of infection in the LRS and contribute to the spread of infection within flocks (34). It is difficult to study this possibility without either a laboratory test for scrapie infection or convenient markers for the alleles of *Sip* gene. Recently, restriction fragment length polymorphisms have been found which can identify the alleles of *Sinc* gene in mice (43-45). Another study has sought similar allelic markers for *Sip* gene with encouraging results (46). Reliable markers could be invaluable to further studies of scrapie epidemiology and the development of new strategies for disease control.

PATHOGENESIS OF EXPERIMENTAL SCRAPIE

Studies of short incubation models of scrapie (60-250 days) have revealed much about the pathogenesis of the disease after infection by nonneural, peripheral routes such as subcutaneous (s.c.), intraperitoneal (i.p.) and intravenous (i.v) (47). Infection is rapidly and widely distributed from the site of injection via the blood supply. The 1000-fold range in the efficiency of infection by different routes (i.v. > i.p. > s.c.) appears to be related to the extent of this immediate and short-lived viremic phase (48). There is no evidence that blood-borne infection can directly establish scrapie replication in the CNS but replication is rapidly established in spleen, lymph nodes and some other tissues of the LRS (47).

Neuroinvasion is the key stage in the pathogenesis of disease. With all the nonneural parenteral routes, the first site of replication in the CNS is in the thoracic spinal cord. The main neuroinvasive site is located in the region between thoracic vertebrae 4 and 9. This and other findings strongly suggest that infection spreads along visceral sympathetic fibers which enter this region of the spinal cord as part of the splanchnic nerve complex (see 47). Direct proof that infection can spread from the peripheral nervous system to the CNS was obtained by injecting scrapie into the sciatic nerve (49).

Serial splenectomy studies showed that spleen plays a major role in neuroinvasion but it is only needed for a few weeks to initiate the process (50). However spleen is not the only tissue from which neuroinvasion can take place. In splenectomized mice, infection spreads to the thoracic spinal cord from other visceral sites of scrapie replication, almost certainly lymph nodes (50). Splenectomy has no effect on pathogenesis after intragastric infection. In this case, early scrapie replication takes place in Peyer's patches and the evidence favors spread of infection to the thoracic spinal cord via the enteric and sympathetic nervous systems (51). The same pathway probably applies to natural scrapie because, as mentioned earlier, those parts of the intestines which contain Peyer's patches are among the earliest tissues in which infection can be detected (33).

Once scrapie replication is established in the thoracic spinal cord, infection spreads slowly to the rest of the cord, enters the brain via the medulla and eventually reaches anterior regions of the brain (47). There is also centrifugal spread of infection to other parts of the peripheral nervous system. Two estimates put the rate of spread of infection within the CNS at about 1 mm/day (47). Good evidence that scrapie can spread within neurons comes from studies of intraocular infection; the earliest appearance of infectivity in brain is in the contralateral superior colliculus which is the major projection area of retinal ganglion cells (52). Experiments involving intraocular infection and removal of the eye at various times afterwards gave an estimate for the rate of intra-axonal spread of scrapie. This value was also about 1 mm/day (53).

The neuronal targeting of infectivity provides a simple basis for understanding the neuropathogenesis of scrapie. For example, the duration of the replication phase in brain (from first detection to the onset of clinical disease) varies considerably with the route of infection: intraocular > intracerebral injection of anterior brain > intraspinal infection (thoracic cord) and all the nonneural peripheral routes (47). These differences have two implications. First, much of the infectivity and consequential brain damage is irrelevant to the pathogenesis of disease which depends on infectivity reaching and replicating in a limited number of "clinical target areas" (CTA). Secondly, the spread of scrapie infection in neural tissue is restricted and the site of entry (or of injection) in the CNS determines the pathways by which infection can reach the CTA (47,54). Infectivity entering the brain from spinal cord (after i.p., s.c or i.v. injection) is targeted more directly to the CTA than i.c. injected scrapie when more complex pathways are involved, presumably involving several neuron-to-neuron steps. Scrapie replication in the CTA then drives the production of lesions which leads to the clinical disease.

The nature of the primary lesions is not known. An attractive hypothesis is that modification of normal PrP, and other presumably essential proteins, causes progressive dysfunction (and maybe death) in certain populations of neurons. Major unanswered questions concern the normal function of PrP and the nature of the posttranslational modifications that are caused by scrapie which result in the formation of SAF and extracellular amyloid cores (55).

CONTROL OF SCRAPIE PATHOGENESIS

One of the great paradoxes of scrapie and the related diseases is why they are so slow when no known host responses are induced to slow them down. Recent studies have revealed

two mechanisms underlying the long incubation periods of scrapie. Both mechanisms are related to restrictions on scrapie replication within cells and on the spread of infection between cells.

There seems to be a universal occurrence of plateau concentrations of scrapie infectivity in the LRS (47). Plateaux develop well in advance of clinical disease and persist for the remaining life-span of the host. Plateau titers comparable to those in the LRS also occur in the peripheral nervous system but always at a concentration at least tenfold lower than the maximum titers found in the CNS. With one major exception, infectivity plateaux occur in the spinal cord and brain. The exception is the 263K strain of scrapie in hamsters, the fastest of all scrapie models, in which disease develops before replication in brain becomes limited.

There is evidence that scrapie replication takes place in stable, long-lived cell populations: radiation resistant cells in the LRS (56,57) and neurons. Plateau titers can therefore be seen in terms of a finite number of intracellular "sites" for the infectious scrapie agent in a finite number of nonreplaceable cells. The concept of site limitation is strongly supported by competition experiments in which the prior injection of a "slow" scrapie strain can completely prevent a second, much "faster" scrapie strain from producing the disease. Competition can occur with either the intracerebral or the i.p. route of injection confirming site limitation in both LRS and CNS (58,59).

In addition to the limitations on the number of scrapie permissive cells, there are also restrictions on the cell-to-cell spread of infection. In the CNS, this is shown by the differences in the duration of the replication phase in brain according to the site of entry or of injection of scrapie (see above). In terms of the neuronal targeting of infectivity, the basis of this restriction could be differences in the physiological and neurochemical properties of connecting neurons which would create a hierarchy of scrapie permissive cells according to their accessibility (47,54). The gradual slowing down of replication in brain, as the plateau is approached, would occur as fewer and less accessible cells in the nonclinical target areas remained. High plateau titers would then mask the continuing spread of infection and replication in the clinical target areas which must be among the last permissive cells to be reached. The same principles apply to the cell-to-cell spread of scrapie infection in the LRS, as described below.

Comparisons of a range of short and long incubation models in rodents show that, with all of them, scrapie replication in the LRS begins quite soon after infection (47). The major differences occur at the two subsequent stages of pathogenesis.

The first is exemplified by the 87V strain of scrapie injected i.p. into *Sinc* p7p7 mice. Depending on the dose injected, either a proportion of mice develop scrapie after long and extremely variable incubation periods (450-700 days; 60) or neuroinvasion fails to occur and there is no clinical disease at all (61). The restriction on neuroinvasion presumably acts at the cellular interface between the LRS and the peripheral nervous system. It can be bypassed by injecting 87V scrapie intracerebrally when all the mice develop the disease after highly uniform incubation periods. The limited neuroinvasiveness of 87V scrapie provides an obvious model for the existence of infected carriers in sheep scrapie (see earlier).

Most other scrapie models give highly uniform incubation periods after i.p. infection. A comparative study of five of them (including two very slow models) showed no restriction on neuroinvasion which was initiated within a few weeks of infection (42). The differences in incubation period were due to differences in the overall rates of replication and spread of infection, from neuroinvasive sites in the peripheral nervous system to the clinical target areas in the CNS. These five scrapie models differed in either the strain of agent or the *Sinc* genotype of the mice. Therefore both factors interact to control scrapie neuropathogenesis (42).

In conclusion, the pathogenesis of scrapie is controlled at two stages; neuroinvasion and the subsequent replication and spread of infection in nervous tissue. *Sinc* gene in mice acts at the neural stage of pathogenesis (and may control neuroinvasion as well). It has already been suggested that *Sip* gene may control natural scrapie in the same way.

GENES AND GENOMES IN SCRAPIE

There is biological evidence that each allele of *Sinc* gene codes for a product (presumably protein) which forms a multimeric structure involved in the overall process of scrapie replication (62,63). The evidence stems from the restrictions on scrapie replication and spread of infection described above, and the variety of allelic interactions by which many scrapie strains are recognized (38,63). However the nature of the protein(s) coded by the alleles of *Sinc* gene has remained unknown until recently, when two major discoveries were made.

First, restriction fragment length polymorphisms (RFLP) associated with *PrP* gene were found which correlated with the s7 and p7 alleles of *Sinc* (also called *Prn-i* gene; 43,45). Crossbreeding studies established a close linkage between the *PrP* and *Sinc* genes. A similar linkage of *PrP* and *Sip* genes in sheep is implied by the discovery of RFLP's which correlate with the *Sip* alleles sA and pA (46). Secondly, *PrP* gene sequence studies revealed two amino differences between PrP of *Sinc* s7s7 mice and *Sinc* p7p7 mice (44). This second finding suggests that the *PrP* and *Sinc* genes may be one and the same; in other words that PrP is the *Sinc* gene product. If this is true, the high level of *PrP* expression in normal neurons (9) and the control of scrapie neuropathogenesis in mice by *Sinc* gene (42) invite speculation that PrP may form sites involved in (a) the spread of infection to neurons and from one neuron to another, or (b) with scrapie replication within neurons.

These developments emphasize the increasing importance of PrP in scrapie pathogenesis; PrP is likely to be the product of *Sinc* gene and modified PrP forms the disease specific fibrils, SAF. In addition there is a substantial copurification of scrapie infectivity with purified SAF from scrapie brain (6,11,64), and other evidence links modified PrP with

infectivity (65). This association suggests that modified PrP may be either the infectious protein of the "prion" hypothesis or else the protective host-coded protein required by the "virino" hypothesis.

The idea that PrP may be involved with both the etiological agent of scrapie and with the host genetic control of incubation period is causing confusion in some minds. It is important to recognize the fundamental difference between a disease of genetic origin and the genetic control of an infectious disease. There is overwhelming evidence that scrapie is caused by an infectious agent which exhibits the properties of strain variation and mutation (38,66,67). This means that the scrapie agent has a genome which contains scrapie-specific information. The fact that, in sheep and mice, a single host gene and the scrapie genome have an interdependent action in controlling incubation period must not obscure the independent existence of these two factors.

On the "virino" hypothesis the scrapie genome is probably a small, nontranslated nucleic acid. The idea that *Sinc* (and probably *Sip*) gene may provide the precursor of the protein that protects the scrapie nucleic acid offers a very simple basis for the control of incubation period by a host gene product interacting with the scrapie genome (2). It is therefore important to find this putative nucleic acid genome.

On the "prion" hypothesis, modified PrP becomes the genome of the agent. Somehow, it must direct the posttranslational modification of normal, noninfectious, PrP to produce more of the modified, infectious form. If, in addition, PrP is the *Sinc* gene product, then the effect of the alleles of *Sinc* gene will be determined by the host-coded amino acid sequence of normal PrP. Subsequently, normal PrP must be modified posttranslationally, in as many specifically different ways as there are scrapie strains (at least ten) capable of replicating in a given host genotype. These are the stringent conditions for the replication of a proteinaceous genome. It is difficult to see how they might be met until the differences between normal and modified PrP are known (11,55).

IMPLICATIONS ARISING FROM THE NEW SCRAPIE-LIKE DISEASES

At the time of writing, over 3500 cases of BSE have occurred in the UK. This enormous epidemic has stimulated a reassessment of some features of the scrapie family of diseases.

First, it is interesting that the five most recent diseases have all appeared in domesticated or captive ruminants. Indeed, with the exceptions of humans and mink, ruminants are the hosts for all the known spongiform encephalopathies. There are 172 species of four-chambered ruminants and 169 of these are in either the *Cervidae* (41 species) or *Bovidae* (128 species) families. So far, a scrapie-like disease has appeared in members of five out of the nine corresponding subfamilies (*Cervinae, Odocoileinae, Bovinae, Hippotraginae and Caprinae*). It rather looks as though rumination may be associated with a certain proclivity to infection by scrapie-like agents.

The second point is that the comparatively high efficiencies of infection by the parenteral routes used in studies of scrapie pathogenesis (47) may have overshadowed the importance of the oral/alimentary route in natural infection (51). The latter (along with scarification) is clearly involved in natural scrapie, especially in the horizontal spread of infection (29,33) Oral infection, combined with scarification, is strongly implicated in the transmission of human kuru during endocannibalism of dead relatives (68,69) and also in the transmission of scrapie to mink to give TME (17). In the latter case, scarification probably comes into play when there is fighting between littermates at feeding time. Finally, the transmission of scrapie to cattle (to give BSE) is clearly associated with the ingestion of contaminated feed (23). Nothing is known of the origin of CWD in mule deer and Rocky Mountain elk (19,20), or of the spongiform encephalopathies in a nyala and a gemsbok (21); but the advent of BSE makes a scrapie source of infection by the oral route seem very likely.

A third point arising from BSE is that exposure to scrapie infection is not just by the feeding of untreated sheep carcasses or offal, as was originally supposed with TME (17). Infected sheep material is incorporated into meat and bone meal supplements and some infectivity apparently survives the rendering process. For many years now, material from scrapie cases and from preclinically infected sheep may have posed a threat to livestock fed on concentrates. The recycling of contaminated sheep protein could have contributed to the occurrence of natural scrapie: there would be no species barrier to impede transmission to sheep and such occurrences would be hard to detect against a background of endemic scrapie. Contamination of concentrated feedstuffs may also have been the unsuspected cause of TME in the USA in outbreaks where mink had not been fed untreated sheep carcasses or offal (70). A similar explanation has been mentioned for the isolated occurrences of CWD in mule deer and Rocky Mountain elk. The fact that BSE has only been reported in the UK offers limited reassurance to other countries in which a threat may exist due to endemic scrapie or the importation of contaminated meat and bone meal.

At the same time the scale of the outbreak of BSE in the UK means that additional factors must have increased the exposure to scrapie infection, but with regional variations to account for the greater incidence of BSE in the southern part of the country. Moreover, computer modelling of the outbreak suggests a relatively sudden increase in exposure which started in 1981/82 (23). Possible factors include a greater inclusion of sheep heads for rendering and changes in the rendering process; for example, the use of lower temperatures and the greatly reduced use of organic solvents to extract fats. Theoretically, another possibility is the sudden emergence of a mutant scrapie strain which just happened to be more pathogenic for cattle. However the form of the epidemic would require the simultaneous emergence of this mutant strain in many flocks (or cattle herds) throughout the country: this does not seem likely (23).

In the wake of BSE, it now seems possible that all the scrapie-like diseases in animals originate from endemic scrapie infection in sheep and goats. If the level of infection is high enough, the direct feeding of sheep

tissue or rendered products can enable infection to cross the species barrier and cause disease in other hosts, especially ruminants. Therefore, the recycling of animal proteins in feedstuffs should either be curtailed or the conditions of rendering changed to take account of the high physicochemical stability of scrapie agent and inactivate it (71).

What can be said about the scrapie-like diseases of humans? Because of the precedent set by TME, the possibility that CJD is caused by exposure to scrapie has been intensively studied ever since the transmissibility of CJD was demonstrated in 1968 (72). A large number of investigations have failed to show any epidemiological link between scrapie and CJD (16). Sheep and goats are not a major reservoir of CJD infection and no other animal reservoir has been identified. In the author's opinion, CJD could be a relatively common, nonpathogenic infection of man which only rarely causes clinical disease (see 73). This hypothesis means that the epidemiology of the disease is not an accurate reflection of the epidemiology of the infection. Unfortunately, the hypothesis cannot be tested until there is an efficient (nonbiological) test for the infectious agent.

Kuru is a special case. It may have originated from a case of CJD (68), but the practice of ritual cannibalism of dead relatives selectively passaged neuropathogenic strains within the population (73). Because there was no maternal transmission of this infection, the cessation of cannibalism has resulted in the gradual disappearance of kuru (69). (It will be remembered that there is no maternal transmission of infection in mink and the hope is that the same will apply to cattle.) In conclusion, it may be that only two of the scrapie-like diseases are maintained in nature as endemic infections; scrapie in sheep and goats and CJD in humans.

A final question concerns the public health risks posed by BSE. On the one hand there is the evidence that scrapie can infect other species to produce diseases such as TME and BSE; on the other, the absence of an epidemiological link between scrapie and CJD suggests that even if scrapie could infect humans, usually it does not. For this reason alone, BSE is unlikely to be a major threat to humans.

However the risk cannot be regarded as nonexistent, for two reasons. First, the widespread use of bovine products in food, vaccines, medicines and surgical devices may add significantly to the total human exposure. The second reason has to do with crossing the species barrier when at least two processes can occur (67,74). One of these, the "donor species effect", involves unknown modifications to pathogenesis which can reduce the efficiency of infection at the first passage in the new host. The other involves strain selection from preexisting mixtures or of mutants derived from single scrapie strains. Indeed, experimental passage from one species to another (e.g. from mice to hamsters) is an effective way of isolating mutant strains. It is therefore possible that the transmission of scrapie from sheep to cattle may alter the population of scrapie strains to which humans are exposed. If the strains selected by cattle have a greater pathogenicity for humans than sheep strains, the risks to humans would be increased accordingly.

From a practical standpoint, it is virtually impossible to quantify these risks. Strain typing is a protracted exercise (38,66,67) and even if a selection of scrapie strains in cattle was demonstrated, there is no way of evaluating directly their increased or decreased pathogenicity for humans. Neither are there diagnostic tests of infection (other than bioassay) to measure the level of human exposure. And the long incubation periods of these diseases mean that it could be at least a decade before an actual risk revealed itself by an increased incidence of CJD. Because of these difficulties there is no alternative but to assume that BSE poses a real risk, however small, and take precautionary steps to reduce it to an absolute minimum.

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