

Scrapie strain variation and mutation

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There are many strains of scrapie, distinguishable by their disease characteristics in genetically-defined mice. Numerous distinct strains have been isolated in the same mouse strain, indicating that scrapie agents have an informational molecule, independent of the host. Strain characteristics are stable on serial mouse passage under constant passaging conditions. However, changes in the species or mouse genotype used for passage may lead to changes in properties which are consistent with the selection of variants which replicate faster in the new host, rather than active modification of the agent by the host. The fact that this has been observed with biologically cloned strains is evidence for mutation in the scrapie agent. Transmissions to mice from natural scrapie and BSE suggest that strain variation exists in the field. These findings have important implications when considering the molecular nature of the scrapie agent and the details of agent-host interactions.

It has been known for many years that scrapie-like agents, like conventional microorganisms, exhibit strain variation. This was first observed over 30 years ago in two experimentally passaged scrapie isolates, which produced dramatically different clinical signs in goats, either a 'drowsy' or a 'scratching' syndrome.¹ Since then strain variation has been well documented for experimental scrapie in sheep,² mice^{3,4} and hamsters,^{5,6} CJD in mice^{7,8} and transmissible mink encephalopathy in hamsters.⁹

Our current understanding of strain diversity in scrapie is based largely on the long-term studies of Alan Dickinson and coworkers, working at the Neuropathogenesis Unit in Edinburgh, who have concentrated mainly on experimental mouse models.^{10,11} About 20 phenotypically distinct strains have been isolated in mice by serially passaging

scrapie or BSE from a wide range of sheep, goat and cattle sources. The methods used for strain discrimination have, of necessity, relied on simple measurements of disease characteristics, particularly the incubation period and the severity and distribution of pathological changes in the brain. Scrapie strains have also been found to differ in their clinical manifestations,¹² their ease of transmission to new species⁵ and their susceptibility to thermal inactivation.^{13,14}

There has been much speculation in recent years about the molecular nature of the scrapie agent. Its unusual physico-chemical properties (*see* Taylor, this issue), together with the failure to identify scrapie-specific nucleic acids in highly infectious brain extracts,¹⁵ have prompted the hypothesis that the agent is a protein devoid of nucleic acid.¹⁶ The candidate protein, PrP, is host coded, accumulates in abnormally protease-resistant forms in infected organs and copurifies with infectivity in extracts from these organs.¹⁷ The alternative view is that there are scrapie-specific nucleic acids which have not yet been detected because of the limitations of currently available techniques. According to the 'virino' hypothesis,¹⁸ these nucleic acids would be closely associated with and protected by host tissue components, which could be abnormal forms of PrP. Another view is that there is still the possibility that the scrapie agent is a virus, despite its unconventional properties.¹⁹

The existence of many distinct strains of scrapie is crucial to these arguments as it clearly demonstrates that the scrapie agent has an informational molecule which is independent of the host. We review here the characteristics of strains and their behaviour on passage in different host species and genotypes and go on to discuss the implications of these observations for molecular models of the scrapie agent.

INCUBATION PERIODS AND BRAIN PATHOLOGY

The most obvious way in which scrapie strains differ is in their incubation periods between initial infection and clinical disease in genetically defined hosts.³ If all experimental variables are kept constant, this measurement is remarkably repeatable; a single scrapie strain injected intracerebrally at high dose into a group of inbred mice will generally give standard errors of less than 2% of the mean incubation period. In mice the host *Sinc* (scrapie incubation) gene also exerts a major influence on the incubation period and this has been exploited to extend the scope of strain discrimination. Two alleles of *Sinc* have been identified, designated *s7* and *p7*.²⁰ It is almost certain that the *Sinc* gene encodes PrP and *Sinc*^{s7} and *Sinc*^{p7} mice consistently differ in the sequence of the protein, by two amino acids.²¹⁻²³

Each scrapie strain, injected under standard conditions, has a characteristic and highly reproducible pattern of incubation periods in the 3 *Sinc* genotypes of mice (the two homozygotes and the heterozygote F₁ cross) (Fig. 1).^{4,11} Scrapie strains differ, not only in their incubation periods within a single *Sinc* genotype, but also in their relative incubation periods in the two homozygotes; for example, the ME7 scrapie strain has a shorter incubation period in *Sinc*^{s7} mice than in *Sinc*^{p7} mice, but this ranking is reversed for 22A. Another striking difference between strains is in the dominance characteristics shown by the 2 *Sinc* alleles; with ME7 the incubation period in the F₁ heterozygote lies between those of the two parental genotypes, whereas with 22A the incubation period in the heterozygote lies beyond the parental range.

Scrapie strains also show dramatic and reproducible differences in the type, severity and distribution of pathological changes they produce in the brain (*see* also Fraser, this issue). The most prominent change seen in routine histological sections is a vacuolation of the neuropil, which is targeted to different parts of the brain, depending mainly on the strain of scrapie, but also to some extent on *Sinc* and other mouse genes. The distribution of vacuolar degeneration is the basis of a semi-quantitative method of strain discrimination in which the severity of pathology is scored from coded sections in nine grey matter and three white matter brain areas to construct a 'lesion profile'.²⁴ Each combination of scrapie strain and mouse genotype has a characteristic lesion profile^{4,10} which, unlike the incubation period, is not sensitive to the initial infecting dose. Lesion profiles can therefore be used to identify strains in samples with low levels of infectivity and to determine which strain kills the animal when a mixture of strains is injected.

The differences in pathology between scrapie strains can be demonstrated even more clearly in sections immunostained with PrP-specific antisera. There are extensive accumulations of PrP in the brain with all strains of scrapie, mostly in the form of diffuse or granular deposits in the neuropil in areas of vacuolar degeneration and amyloid plaques. As with vacuolation, there are clear and reproducible differences between scrapie strains in the distribution and severity of these changes.²⁵ As an illustration, Figure 2 shows PrP accumulation in different parts of the hippocampus with 4 strains of scrapie. The most selective strain, 87V, targets pathology precisely to a narrow sector, which correlates exactly with the distribution of the dendritic processes of pyramidal neurons within this sector. Precise targeting to particular identifiable groups of neurons is also seen elsewhere in the brain with 87V and other strains.

These results suggest that a fundamental difference between scrapie strains is their ability to recognize and replicate in different neuronal populations. However, as there is considerable overlap in target-

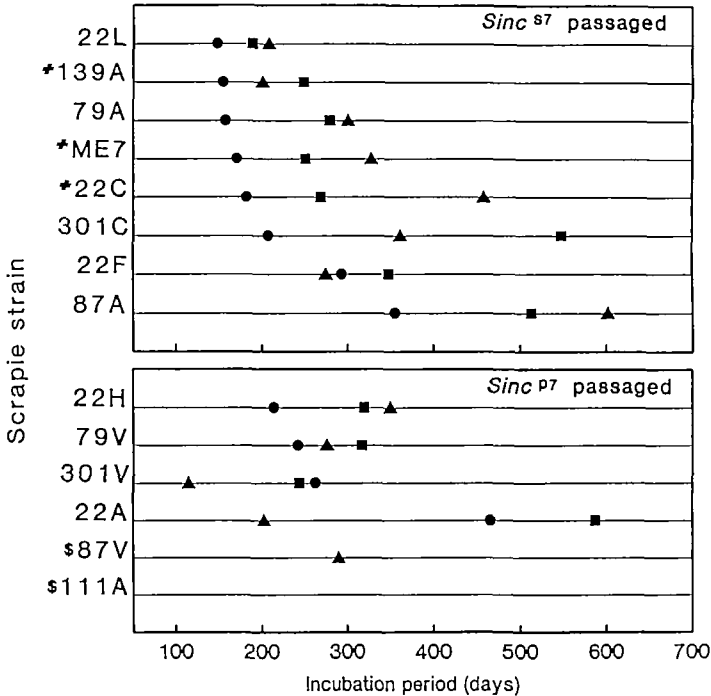


Fig. 1 Incubation periods following intracerebral injection of 1% brain homogenates for 14 scrapie/BSE strains in mice of the three *Sinc* genotypes: C57BL(*Sinc*⁸⁷)(●), VM(*Sinc*^{p7})(▲) and C57BLxVM(*Sinc*^{87p7})(■).
 *The properties of 139A, ME7 and 22C are unchanged when they are passaged in *Sinc*^{p7} mice.
 \$ The incubation periods for C57BL and C57BLxVM mice with 87V and for all three genotypes for 111A are longer than 700 days and some individuals do not develop clinical disease within their lifespan.

ing patterns seen with different strains, it is probable that a uniform group of neurons is capable of supporting the replication of more than one strain. Indeed, it has been demonstrated recently that two distinct scrapie strains can replicate in neuronal cultures containing a single cloned cell type.²⁶ Further evidence that different strains can use the same replication sites has come from 'blocking' experiments in which the intracerebral injection of a strain with a comparatively long incubation period has been shown to delay or prevent the replication of a shorter incubation period strain injected some time later.²⁷

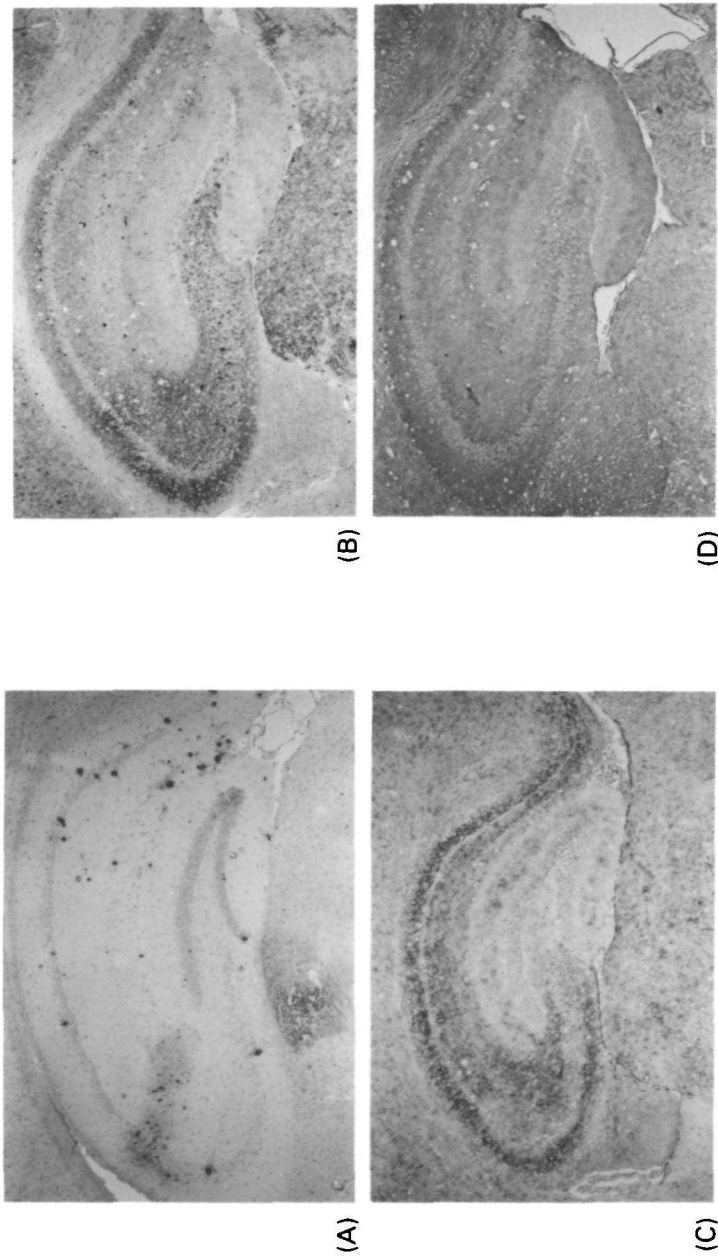


Fig. 2 Distribution of PrP accumulation, demonstrated by immunostaining, in the hippocampus of (A) *Sincp7* mouse with 87V, (B) *Sincp7* mouse with ME7, (C) *Sincp7* mouse with 22A and (D) *Sincp7* mouse with 79A.

ISOLATION OF STRAINS IN MICE

On transmission of a scrapie-like disease from its natural host to mice the incubation period is usually very long and there may be survivors. On subsequent mouse-to-mouse passage in a single genotype the incubation period shortens and stabilises after a few passages to give a strain with characteristic properties. These properties are stable indefinitely on further mouse-to-mouse passage, as long as the conditions of passage, particularly the mouse genotype, remain constant. In Edinburgh alone, 14 unequivocally distinct strains of scrapie and BSE have been isolated in mice (Fig. 1).¹¹ A further 5 isolates have unique disease characteristics, indicative of new strains, but are not yet fully stable.

Most primary sources have given rise to two different scrapie strains when passaged in *Sinc^{s7}* and *Sinc^{p7}* mice.^{10,11} Clearly, these differences are not simply imposed by the host as numerous strains have been isolated in the same mouse genotype and the same strain (e.g. ME7) has occasionally been isolated in both genotypes. On the other hand, this resolution of isolates into two distinct stable strains has always been consistent with the selection of strains which replicate more rapidly in the mouse genotype used for passage.¹⁰ For example, 79A and 79V were isolated from the goat 'drowsy' source, by passage in *Sinc^{s7}* and *Sinc^{p7}* mice respectively; 79A is quicker than 79V in *Sinc^{s7}* mice and 79V is quicker than 79A in *Sinc^{p7}* mice. Interestingly, for a single scrapie strain the incubation period is not necessarily shorter in the mouse genotype in which it has been isolated.

Once the incubation periods and pathological properties have stabilised, it cannot be assumed that the isolate contains only a single strain, as it is possible that minor variants are passaged together with the major strain in a stable mixture. For many experimental purposes this may not be significant, but it may become important if the isolate is passaged in a new host, as described below. In order to remove minor strains from an isolate it is necessary to biologically clone the strain, by several sequential passages using the minimum infecting dose.²⁸ This procedure has been shown to lead to a permanent change in the characteristics or behaviour of several isolates.

STABILITY OF MOUSE-PASSAGED STRAINS

The existence of many strains indicates clearly that scrapie-like agents carry some form of information which is independent of the host. As both the hypothetical 'protein-only' and 'virino' structures include a host component, an important question is whether and to what extent this strain-specific information is modified by the host in which the isolate is passaged. To investigate this, several mouse-passaged strains

have been serially passed in new mouse genotypes or species. Scrapie strains have been found to differ dramatically in the stability of their properties on passage in their new hosts (Fig. 3).

The characteristics of some scrapie strains, such as ME7, 22C and 139A, remain constant when the mouse genotype in which they are passed is changed from *Sinc*^{s7} to *Sinc*^{p7}. In contrast, some isolates change their properties when passed in the alternative *Sinc* genotype. Whenever such changes have occurred they have been consistent with the selection of strains with shorter incubation periods under the new passing conditions. In the case of an uncloned isolate this could simply be the separation of strains which have been propagated as a mixture since the original transmission to mice. However, there are examples of changes occurring in cloned strains, suggesting the generation and selection of new mutant strains. There is no evidence that the *Sinc* genotype of the mouse can actively modify the properties of a strain.

Much of the work on strain stability on passage in different *Sinc* genotypes has involved two strains derived from the SSBP/1 experimental sheep isolate, 22C and 22A; these studies are described in detail in a recent review.¹¹ Both 22C and 22A are stable when serially passed in the genotype in which they were isolated, *Sinc*^{s7} in the case of 22C and *Sinc*^{p7} in the case of 22A. However, when uncloned 22C is passed in *Sinc*^{p7} mice its properties change gradually over several passages to give a new strain, 22H, which is clearly different from 22A. This change in properties is not seen when cloned 22C is passed in *Sinc*^{p7} mice, showing that the change from 22C to 22H is not the result of modification by the new host. Rather, this is evidence that 22C and 22H coexisted in the early mouse passages of the isolate and that 22H was removed from the mixture by cloning. These results emphasise the need to use cloned strains in studies seeking host modifications of strain characteristics.

In contrast, 22A is unstable when the *Sinc* genotype in which it is passed is changed, even after it has been cloned. On serial passage in *Sinc*^{s7} mice the incubation period characteristics gradually change, eventually stabilising after several passages to give yet another new strain, 22F. The fact that this has been seen using cloned 22A suggests that 22F has been generated from 22A, either by a host-induced modification or by mutation, in the sense of a change in the scrapie-specific information of the agent. The same change has been seen in 16 separate *Sinc*^{s7} passage lines, although the number of passages required to regain stability has varied considerably. In some of these lines little or no change has been seen at the first passage in the new genotype, making it unlikely that the phenomenon is due to a modification of the agent by the host. These results are more consistent with the gradual selection of

a mutant strain, 22F, which has a shorter incubation period in the new passaging genotype than the parental strain, 22A.

Other evidence that scrapie strains can mutate has come from studies with 87A, which is unstable even when passaged in the *Sinc* genotype in which it was originally isolated.²⁹ The characteristics of 87A are stable when it is passaged at low dose in *Sinc*^{s7} mice but often change suddenly when it is passaged at higher dose in the same mouse genotype, to give a strain with much shorter incubation periods. This new strain is stable, even when passaged at high dose, and is always identical to ME7. This sudden change in properties is seen even after 87A has been cloned, suggesting that ME7 is a shorter incubation period mutant strain derived from 87A.

The studies described above show that the *Sinc* (or PrP) genotype of mouse used for passage has no consistent effect on the characteristics of scrapie strains, apart from selecting strains which replicate more quickly. Studies showing a change in properties when a 'Chandler'-derived isolate was passaged in a new *Sinc* genotype³⁰ cannot be taken as evidence of a host modification, as the authors claim, because an uncloned isolate was used; the 'Chandler' isolate has been shown elsewhere to contain a mixture of strains¹⁰ and the results reported by Carlson and coworkers are consistent with the resolution of this mixture on the basis of incubation periods in the two genotypes.

INTERSPECIES TRANSMISSION

As already noted, when scrapie is transmitted from one species to another the incubation period is usually long compared with later passages in the new species, a phenomenon referred to as the 'species barrier'. Even for the same change of passage species, the species barrier is highly variable between scrapie isolates. For example, the 263K strain transmits from hamsters to mice only with difficulty, producing very long incubation periods, whereas the 431K hamster-passaged strain has been transmitted to *Sinc*^{s7} mice with a minimal species barrier effect.⁵ There is also one natural scrapie-derived strain in mice, 111A, which has been passaged four times in *Sinc*^{p7} mice without the usual shortening of the incubation period (*see* Fig. 1).

In a series of experiments in which mouse-passaged scrapie strains were passaged in rats or hamsters and then re-passaged in mice (Fig. 4), Kimberlin and coworkers demonstrated that the species barrier has a number of contributing factors.^{6,31} They found that cloned 22A and cloned ME7 were completely unchanged after serial passage in hamsters and subsequent reisolation in mice. In contrast, the properties of cloned 139A and cloned 22C were permanently changed by passage in

hamsters, giving rise to new strains which were stable on serial passage in mice. Cloned 139A was unchanged by passage through rats. These results are evidence that the species barrier effect can be partly due to the selection of strains in the new host species, other than the major strains present in the original host.

Cloned mouse-passaged strains

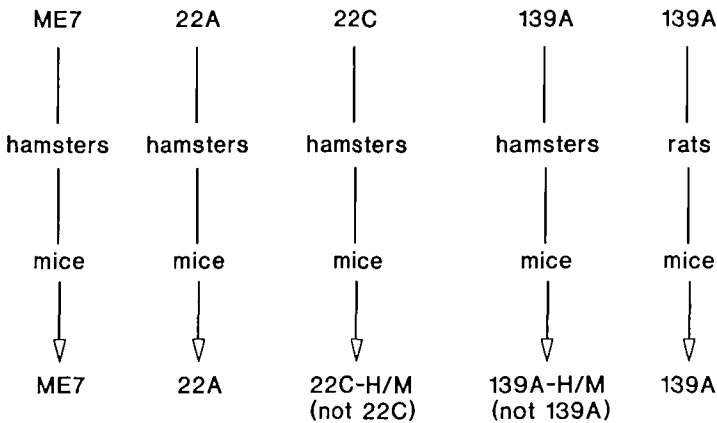


Fig. 4 Summary of experiments in which cloned mouse-passaged strains were serially passaged several times through Syrian hamsters or rats and then repassaged in mice.^{6,31}

The fact that a species barrier is seen even when the strain characteristics remain unchanged shows that other factors are involved, but only at the first passage in a new species. These are likely to depend on the association of infectivity with tissue components derived from the 'donor' species. The simplest effect is a reduction in the efficiency of initial infection, possibly as a result of more effective clearance and inactivation by the host of infectivity from the inoculum. There are also differences in pathogenesis between the first and subsequent passages in the new species. When there is no change in passing species, the incubation period following intraperitoneal infection is longer than that following direct intracerebral infection, usually by about 50%. However, in at least some interspecies passages, the intracerebral and intraperitoneal routes produce similar incubation periods (H Fraser and M Bruce, unpublished results; R H Kimberlin, personal communication).

A possible explanation is that the agent is unable to establish itself directly in the brain when it is associated with foreign tissue, but must be processed in peripheral organs, such as spleen, before it can replicate in the new host.

The assertion that there is the equivalent of a species barrier between mouse strains of different *Sinc* genotypes³⁰ is misleading. Transmission of scrapie strains between *Sinc* genotypes may sometimes result in the selection of new strains, as described above, but there is no great reduction in the efficiency of infection and there is the usual large difference in incubation periods between intracerebral and intraperitoneal routes.^{4,18}

STRAIN VARIATION IN NATURAL SCRAPIE-LIKE DISEASES

Because of the possibility of the selection of minor variants, it is not clear to what extent the mouse-passaged strains isolated from natural cases are representative of field strains. However, transmissions to mice can give some information about the extent of strain variation in the natural diseases.

In the case of transmissions of natural scrapie to mice the incubation periods and pathology in standard panels of mouse strains have varied widely between sources. However, on further passage in mice the majority, but not all, of the isolates from cases occurring in the UK have given varying combinations of the same 3 strains, 87A and ME7 in *Sinc*^{s7} mice and 87V in *Sinc*^{P7} mice (M Bruce, unpublished observations). Therefore these sources may not be as diverse as they appear on primary transmission to mice. A series of transmissions from Icelandic sheep have given at least 3 strains in mice, which are not yet fully characterised but clearly differ from those isolated from UK cases. In the USA, mouse passages have been set up from 5 natural scrapie sheep; again, the resultant strains have not yet been fully characterised, but differ between sources.³² It is likely that the variation in transmission results between sources depends on scrapie strain variation in the natural host, but it is also possible that 'donor' effects make some contribution at the first passage in mice, as described in the previous section.

Recent transmissions of BSE from cattle to mice³³ have been much more straightforward. So far, BSE has been transmitted from 6 unrelated cattle sources, collected at different times during the epidemic and from widely separated geographical locations within the UK. The results of these transmissions were remarkably similar to each other and differed from those in all previous and contemporary transmissions of scrapie (Fig. 5). All 6 BSE sources produced a characteristic pattern of incuba-

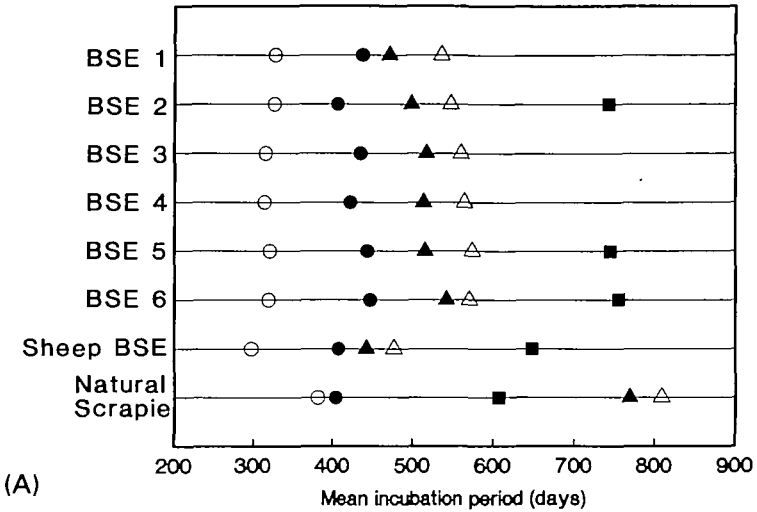
tion periods and pathology in a standard panel of inbred mouse strains and crosses. There were large and consistent differences in incubation period between mouse strains of different *Sinc* genotypes and also, surprisingly, between mouse strains of the same *Sinc* genotype (e.g. C57BL and RIII). This incubation period difference within *Sinc* genotypes was lost at the first mouse-to-mouse passage. Further passages in *Sinc*^{s7} and *Sinc*^{p7} mice have produced two strains, 301C and 301V, which differ from all strains derived from sheep or goat scrapie (Fig. 1).

The uniformity of transmission results with different sources of BSE suggests that each cow was infected with the same major strain of agent. The consistency of the pathology reported in cattle with BSE³⁴ also suggests that a single or a limited number of strains is involved. It is generally accepted that BSE originated from rendered scrapie-infected offal, included in high-protein feed supplements. The transmission studies described above suggest that the major strain of agent causing BSE in cattle is different from the strains causing scrapie in sheep. A possible explanation is that the high temperatures involved in rendering and subsequent passage through cattle have selected variant strains from sheep scrapie.

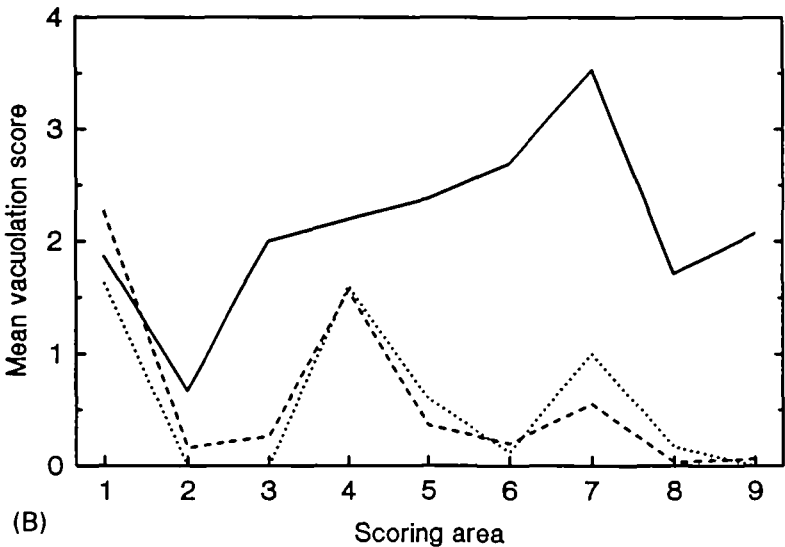
Recently, transmissions to mice have been achieved from other species with novel scrapie-like diseases, which were suspected to be related to the BSE epidemic; the sources were 3 cats, a greater kudu and a nyala. The results of all 5 transmissions were strikingly similar to results from cattle sources, indicating that these species were infected with BSE (H Fraser and G Pearson, personal communication). BSE from cattle has also been transmitted experimentally to sheep, goats and pigs and then from each of these species to mice. Again, the results of these mouse transmissions were similar to direct transmissions of BSE (unpublished observations). These results show, firstly, that the BSE agent is unchanged when passaged through a range of species and, secondly, that the donor species has little influence on the disease characteristics of BSE on transmission to mice.

PrP IN AGENT-HOST INTERACTIONS

There is little doubt that PrP plays a central role in pathogenesis, as it is almost certainly the *Sinc* gene product (*see* Scott, this issue). The biological effects of *Sinc* probably depend on one or both of the two amino acid differences observed between PrPs from *Sinc*^{s7} and *Sinc*^{p7} mice.²² As described above, the phenotypic properties of a mouse-passaged strain depend on specific and precise interactions between the scrapie informational molecule and the *Sinc* or PrP genotype of the host. It has been suggested that the incubation periods of mouse-passaged



(A)



(B)

Fig. 5 (A) Comparison of incubation period characteristics in a panel of mouse strains on primary passage to mice of 6 sources of cattle BSE, one source of experimental BSE in sheep and one contemporary source of natural sheep scrapie. The mouse strains were RIII(*Sinc^{s7}*) (○), C57BL(*Sinc^{s7}*) (●), VM(*Sinc^{p7}*) (▲), IM(*Sinc^{p7}*) (△) and C57BLxVM(*Sinc^{s7p7}*) (■). Data are not yet available for BSE 5 and 6 sources in C57BLxVM mice. (B) Lesion profiles at the primary passage for RIII mice infected with cattle BSE (—), sheep BSE (·····) and natural sheep scrapie (-----).

strains depend on the compatibility of PrPs between donor and recipient mice³⁰ but there is no experimental support for this. In fact, many of the observations cited in this review argue that the donor mouse genotype has no direct effect on strain characteristics. At the simplest level, it can be seen from Figure 1 that the incubation period for a single strain is not necessarily shorter in the genotype which matches that of the donor. Also, 3 out of 4 cloned strains tested have been unchanged by passaging in the alternative genotype and the fourth strain has changed only gradually, suggesting a host-permitted selection of a new strain, rather than a host modification.

Mismatching of PrPs in donor and recipient species may, on the other hand, be involved in some way in the species barrier. Transgenic mice carrying multiple copies of the hamster PrP gene have short incubation periods when intracerebrally infected with the Sc237 hamster-passaged scrapie strain (identical to the 263K strain used elsewhere), in contrast with the extremely long incubation periods seen in non-transgenic mice with this strain.³⁵ Also, there is evidence that the abnormal PrP in these animals is derived from the hamster rather than the mouse protein.³⁶ This shows that the Sc237 strain interacts preferentially with hamster PrP in the transgenic recipients, as would be expected from its very short incubation period in hamsters and its very long incubation period in non-transgenic mice. However, these results give no information about whether donor PrP type is critical in this interaction or about the effect of the transgene on efficiency of infection and details of pathogenesis.

Other investigations in transgenic mice into various aspects of the agent-host interaction and species barrier have so far been difficult to interpret. Problems in these studies have included the effects of transgene copy number and endogenous gene expression on incubation periods and the use of uncloned, poorly characterised scrapie isolates. The insertion of foreign or altered PrP genes into mice lacking their own PrP gene³⁷ will avoid some of these problems in future.

MOLECULAR MODELS OF SCRAPIE-LIKE AGENTS

The existence of many strains of scrapie is crucial to speculation about the nature of the scrapie agent as any valid model must include a molecule which carries strain-specific information. It is relatively easy to explain strains in terms of an agent-specific nucleic acid, in which case the genetic variation would be analogous to that seen in conventional microorganisms. It is more difficult to envisage how a protein alone could specify strain diversity. According to protein-only models the scrapie pathogen is PrP which has been modified in some specific

but as yet unknown way. This abnormal protein is suggested to induce the same modification in host PrP molecules, either by direct interaction or by causing a mistranslation of the gene.^{38,39,40}

The characteristics of strains do not depend, to any great extent, on the primary sequence of PrP in the animals in which they are passaged. Therefore, if scrapie-like agents do consist solely of PrP, strain-specific information is likely to reside in post-translational modifications of the protein. The relevant modifications could either be conformational or chemical, for example involving differences in glycosylation. There must be as many specific modifications as there are distinct strains and each must be able to 'replicate' itself accurately over many serial passages, apart from predictably generating other specific modifications. Also, multiple forms of modified PrP must be capable of retaining their separate identities when passaged as mixtures and such mixtures must be resolvable by biological cloning. A crucial question is whether a protein alone can fulfil all these criteria or whether it requires a separate informational molecule, such as a nucleic acid, as proposed in the 'virino' and virus hypotheses.

In order to reconcile the protein-only and 'virino' hypotheses, it has recently been speculated that the agent consists of a modified host protein which confers transmissibility and an inessential nucleic acid which determines strain characteristics.⁴¹ Such a model predicts that strain characteristics and infectivity can be separated by treatments which remove or destroy nucleic acids. At present there is no evidence that this separation is possible and, in fact, the phenotypic properties of several strains have survived exposure to high doses of ionizing or UV radiation (D Taylor and M Bruce, unpublished observations). A simpler explanation is still that the agent contains an essential small nucleic acid which is protected by its close association with host tissue components such as PrP (the 'virino' hypothesis).

Despite much speculation, there is very little direct evidence concerning the molecular basis of strain variation. No specific differences have been found in PrP from the same host species/genotype infected with different scrapie strains.⁴² On the other hand, the scrapie nucleic acid, if it exists, remains obstinately elusive.¹⁵ The issue will remain controversial until there is a direct identification of the informational molecule of the agent and the variations in it which lead to phenotypic diversity.

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