Pathogenesis of Spongiform Encephalopathies Studies in Mice

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The view that scrapie-like diseases are caused by infectious protein (PrP) is inconsistent with the biological evidence for strain variation, arising mainly from work using mice (Dickinson & Meikle 1971; Bruce *et al.*, 1991). Inbred mice are analogous to culture media with which strains from scrapie and homologous diseases are discriminated and identified. Scrapie strains produce characteristic patterns of incubation period and neuropathology depending on host genotype, but which are scrapie strain encoded. **Crucial mistakes will arise if the wrong assumptions about the chemical identity of scrapie-like agents are unchallenged.** The constraints controlling the transmission of the various scrapie-like agents between species cannot be fully understood without knowledge of the chemical identity, the causal agent and the biochemistry controlling its replication.

Strains often co-exist as mixtures and this can lead to errors in interpretation such as when a change in agent properties is assumed to be host-instructed-(Carlson *et al.*, 1989) rather than host-permitted strain selection from the mixture. Using high dilution sub-passage in permissive hosts, cloned strains are obtained (Bruce & Dickinson 1979; Bruce & Fraser 1991). A single host genotype can propagate different cloned scrapie strains. This is inconsistent with the prion hypothesis which proposes that agent identity is host-instructed and encoded in the host's PrP gene and hence that one host genotype can only replicate one strain! For the same reason, the prion hypothesis is inconsistent both with the repeated isolation of the same BSE isolate from many different species and of a single scrapie strain in different mouse *Sinc* or PrP genotypes (Bruce *et al.*, 1991; 1994). One of the major observations which show that the scrapie agent possesses an independent genome arises from mutation which can occur when the agent is transmitted between or within a species of the same or different *Sinc* or PrP genotypes (Bruce & Dickinson 1987; Kimberlin *et al.*, 1989).

There are many examples of differences in lesion targeting and type which are determined by the strain of scrapie agent (Fraser 1993). For instance, in the single neuronal projection from the retina following intraocular infection in C57BL mice, the earliest spongy neuropathology in the superior colliculus occurs after 110 days with the ME7 scrapie strain but after 400 days with the 87A strain (Fraser 1982; Fraser & Dickinson 1985). These strain-dependent patterns of lesions targeting are inconsistent with the prion hypothesis because, as the PrP genotype of the target neurons of the C57BL mouse genotype must be identical, differences can only arise from the genetic information encoded by the different strains of the agent.

It has long been known that competition, and not synergism, occurs between strains such as when previous intracerebral or intraperitoneal infection with a "slow" strain ("slow" for a particular host genotype) competes with or blocks the replication of a "fast" strain presented by the same route (Dickinson *et al.*, 1972). This can lead to the complete exclusion of the fast strain (Dickinson *et al.*, 1975). This is evidence that strains can compete for some of the same replication pathway on the same neurone populations or on the same target cell in the lymphoreticular system (LRS).

When the agent is altered such as by prolonged boiling, 10% formalin treatment, or passage through a different host species, it is unable to replicate in the CNS even after intracerebral injection without an initial re-programming replication step in the LRS system (Dickinson & Fraser 1969; Brown *et al*, these Proceedings). Agents which altered using these and other physical or chemical exposures, such as treatment with ethanol or high doses of ionizing radiation or laser U/V light (Dickinson *et al.*, 1986; Fraser *et al.*, 1994) retain their phenotypic identity. These results show that strain characteristics are specified by the scrapie agent itself and not by a separate conventional virus, and that there is a molecular component of the agent which is altered

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by the treatment and independent of that encoding strain properties. This is consistent with an alternative explanation for agent variation, that the agent contains an informational molecule protected by a host-coded protein (Dickinson & Outram 1988).

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Abstract

PRP Genotype Variation in Cattle and Incidence of BSE

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The cattle disease bovine spongiform encephalopathy (BSE) is one of a family of similar diseases known as transmissible spongiform encephalopathies (TSEs) which affect various mammals. Polymorphisms and mutations of the PrP gene have been associated with the incidence of experimentally induced and natural TSEs in sheep, goats, laboratory mice and humans and so this study of the bovine PrP gene was undertaken to discover if there was a similar PrP genotype association with BSE in affected cattle. Although the sheep and human PrP genes are highly polymorphic, the bovine PrP gene is, in comparison, remarkably invariant. There are two polymorphisms of the coding region of the bovine PrP gene, in particular a difference in the number of an octapeptide repeated sequence (either 5 or 6 copies). Analysis of more than 350 cattle in Scotland revealed no frequency differences between the PrP genotypes of BSE and healthy cattle. There is therefore, no PrP maker which could be used to predict which cattle are at risk of developing BSE.

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