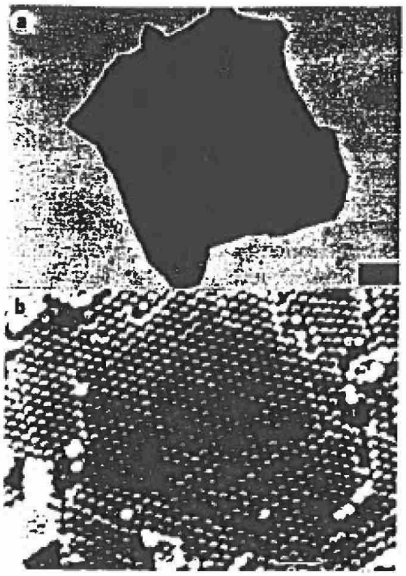

Figure 1 Micrographs of ordered latex templates. **a**, Optical microscopy in transmitted light. The different colours probably correspond to crystal domains of different symmetry or orientation. **b**, Scanning electron micrograph (SEM). Ordered latex layers display either hexagonal or cubic packing. Scale bars: 50 μm and 1 μm, respectively.

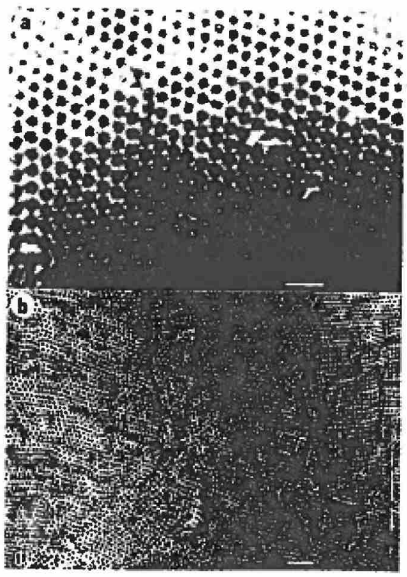

Figure 2 SEM of the microporous silica structures. Latex particles of **a**, 560 nm and **b**, 300 nm diameters were used as templates. Large ordered arrays of spherical cavities, representing a negative replica of the original colloidal crystal embedded in the silica, are seen. The silica flakes appear to be built up of many similar domains with different crystal orientations. Details of materials and methods are available on request from the authors. Scale bars, 1 μm.

media. The latex particles accumulated slowly on the membrane surface, building up closely packed, ordered layers roughly 10 μm thick. The deposited crystalline layer could be broken and detached from the membrane surface for analysis by light and scanning electron microscopy (Fig. 1).

To induce silica polymerization the microsphere surfaces had to be functionalized *in situ* by adsorption of the surfactant hexadecyltrimethyl ammonium bromide (HTAB). We soaked the crystalline latex layers with 0.02 M HTAB solution for 20 min, then removed the excess unadsorbed surfactant by washing briefly with deionized water. We mineralized the cavities in the arrays by passing 0.5 M silica solution through the latex-covered filter. The permeability of the layers decreased as the polymerization process continued, so that flow through the filter stopped in less than one minute. When the silica solution had gelled inside the colloidal crystal layer, we removed the excess solution and dried the latex/silica composite under vacuum. The latex templates inside the polymerized silica were removed by heating at 450 °C for 4 h, leaving silica flakes of very low density as the final product.

Scanning electron microscopy shows that the material is built up of three-dimensional ordered arrays of uniform pores. Examples of the discrete morphology of the material are shown in Fig. 2. By varying the size of the latex microspheres used, we were able to produce organized materials with pore sizes ranging from about 150 nm to 1 μm. A comparison between the repeat units of the silica replicas and the original latex crystals showed that the baked materials had shrunk by 20–35%, a value that is higher than, but comparable to, that in the M41S mesoporous silicas[4].

Our results show that it is possible to obtain highly structured silica materials in which the pore size, shape and ordering can be precisely controlled within a wide range that has previously been unattainable. The method is powerful and controllable, and could be adapted for large-scale production.

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The same prion strain causes vCJD and BSE

Epidemiological and clinicopathological studies, allied with pathological prion protein (PrP^{Sc}) analysis, strongly support the hypothesis that the human prion disease new variant Creutzfeldt–Jakob disease (vCJD) is causally related to bovine spongiform encephalopathy (BSE)[1,2], but considerable controversy remains. Distinct prion strains are distinguished by their biological properties on transmission to laboratory animals and by physical and chemical differences in PrP^{Sc} strains. We now find that the biological and molecular transmission characteristics of vCJD are consistent with it being the human counterpart of BSE.

We studied transgenic mice expressing only human PrP ($HuPrP^{+/+}$ *Prn-p*$^{o/o}$), which have been shown to lack a species barrier to human prions from one iatrogenic CJD case[3], comparing them with non-transgenic (FVB) mice. All of 16 further CJD cases, encompassing a wide range of clinicopathological phenotypes, all three PrP^{Sc} types reported in sporadic and acquired prion diseases[2] and all *PRNP* genotypes at polymorphic codon 129, a key determinant of genetic susceptibility to human prion diseases[4–6], were transmitted to these transgenic mice.

Almost all inoculated transgenic mice contracted disease with similar short incubation periods, consistent with a lack of species barrier to these isolates (Table 1). These transgenic mice express human PrP homozygous for valine at codon 129. However, there was no significant difference in mean incubation periods between inocula of the different codon 129 genotypes. PrP^{Sc} typing of these transmissions showed that the same prion types seen in sporadic and iatrogenic CJD (types 1–3) are produced, distinct from that seen in vCJD (type 4)[3]. Only occasional transmissions, at longer and variable incubation periods, were seen in FVB mice.

In contrast, efficient transmission of vCJD to FVB mice was observed (Table 1) although incubation periods were prolonged. Conversely, the attack rate of vCJD in the transgenic mice was reduced in comparison to typical CJD, and incubation periods were generally more variable and prolonged. Mean incubation periods to these six vCJD cases were similar in both types of mice. The clinical course in vCJD-inoculated transgenic mice was much longer than in transmissions of typical CJD. vCJD in humans is also associated with a long clinical duration[1]. Some mice, as well as showing typical neurological features, persistently walked backwards. This unusual clinical sign was not seen in transmissions of typical CJD, fatal familial insomnia or other

herited prion diseases[7].

BSE transmits efficiently to FVB mice[3], albeit with prolonged and variable incubation periods (Table 1) which fall to a consistent short incubation period of around 140 days on second passage (data not shown). Transmissions of BSE into the transgenic mice did not occur at incubation periods well beyond those of classical CJD[3], but we have now observed transmission with much longer incubation periods (Table 1). These transmissions resembled those of vCJD with a long clinical duration and backwards walking in some animals as well as the otherwise typical clinical features of mouse scrapie.

There were striking similarities in PrP deposition patterns between BSE- and vCJD-inoculated animals (detailed neuropathological studies will be published elsewhere). Such patterns are determined by host genotype as well as by agent strain[8]. We saw distinct patterns in the two types of mice, but, in each case, vCJD and BSE produced closely similar patterns. In vCJD- and BSE-inoculated non-transgenic mice, there were PrP plaques and diffuse PrP deposition. In vCJD- and BSE-inoculated HuPrP$^{+/+}$ *Prn-p*$^{o/o}$ transgenic mice we saw a predominantly pericellular pattern of PrP immunostaining (data not shown). PrP plaques are a rare feature of prion disease in mice. Occasional mock-inoculated transgenic mice showed weaker and less extensive pericellular PrP immunostaining, probably reflecting the high level of PrP^{C} overexpression in these mice. Western blotting for PrP^{Sc} was negative in all these controls.

We performed western blot analysis to determine the PrP^{Sc} types produced in these transmissions. We have previously shown that the PrP^{Sc} type seen in vCJD (type 4) has a ratio of glycoforms closely similar to that of BSE passaged in several other species[2]. vCJD-inoculated FVB mice produced mouse PrP^{Sc} with type 4-like glycoform ratios and fragment sizes indistinguishable from those in BSE-inoculated FVB mice (Fig. 1a,b).

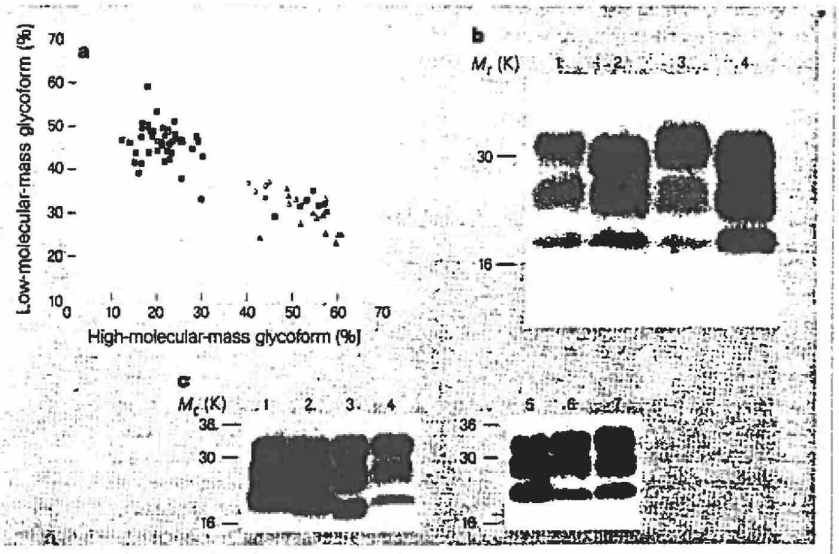

Figure 1 Transmission of prion diseases to mice. **a**, Scatter graph of proportions of protease-resistant PrP in the high-molecular-mass (di-glycosylated) and low-molecular-mass (mono-glycosylated) glycoforms in individual human cases and FVB mice with experimentally transmitted CJD, vCJD or BSE. Sporadic and iatrogenic CJD cases (PrP^{Sc} types 1-3), red squares; vCJD, yellow circles; transmissions of typical CJD to FVB mice, green squares; BSE to FVB mice, blue squares. Transmissions of vCJD to FVB mice, open triangles. **b,c**, Western blots of brain homogenates after pre-treatment with proteinase K using anti-PrP polyclonal antibody 95-108 (ref. 15) (**b**) or anti-PrP monoclonal antibody 3F4 (**c**). Methods were as in ref. 2 except that for PrP glycoform analysis a chemifluorescent substrate (ECF, Amersham) was used and ratios analysed on a Storm 840 Phosphoimager (Molecular Dynamics). **b**, Transmission of vCJD and BSE to non-transgenic (FVB) mice. Lane 1, human vCJD; 2, vCJD-inoculated FVB mouse (same case as lane 1); 3, BSE; 4, BSE-inoculated FVB mouse (same case as in lane 3). **c**, Transmission of vCJD to HuPrP$^{+/+}$ *Prn-p*$^{o/o}$ transgenic mice. Lane 1, human CJD, type-2 PrP^{Sc}; 2, transgenic mouse inoculated with CJD case from lane 1 showing type-2 pattern; 3, human vCJD case, type-4 PrP^{Sc}; 4, transgenic mouse inoculated with vCJD from lane 3 showing type-5 pattern; 5, human CJD case, type-2 PrP^{Sc}; 6 and 7, type-5 PrP^{Sc} pattern in vCJD-inoculated transgenic mice.

In transmission of vCJD to HuPrP$^{+/+}$ *Prn-p*$^{o/o}$ transgenic mice, where human PrP^{Sc} is generated, fragment sizes in inoculum and host can be directly compared. Again the PrP^{Sc} produced had type 4-like glycoform ratios. However, the fragment sizes differ from those in the inoculum and were indistinguishable from those in the type-2 PrP^{Sc} pattern[2] (Fig. 1c). We have designated this new pattern type 5.

A change of fragment size on passage in mice of a different codon 129 PrP genotype than the inoculum has been reported previously[2]. Type-1 PrP^{Sc}, seen in CJD cases of 129MM *PRNP* genotype, consistently converts to type-2 PrP^{Sc} on passage in these transgenic mice expressing 129VV human PrP. The glycoform ratios of the original inoculum are also maintained[2]. Abrupt changes in the biological properties ('mutation') of murine scrapie strains on passage in mice of different genotypes are well recognized[9]. We have not, however, been able to show PrP^{Sc} by western blotting in BSE-inoculated HuPrP$^{+/+}$ *Prn-p*$^{o/o}$ transgenic mice. This may reflect culling of many of these mice soon after clinical diagnosis rather than at a more advanced clinical stage. Though transmission of prion diseases without detectable PrP^{Sc} on primary passage has been reported[7,10], it will be important to confirm transmission by second passage studies.

The prion titres in these primary inocula are unknown but may be higher in the human cases, because cattle with BSE will have been culled before the terminal stages of disease. However, on clinical, pathological and molecular criteria, vCJD shows remarkable similarity in its transmission characteristics to BSE, and is quite distinct from all other forms of sporadic and acquired CJD. These data provide compelling evidence that

Table 1 Incubation periods for transmission of prion diseases to transgenic and wild-type mice

Type of inoculum	*PRNP* codon 129	No. of inocula	Transgenic: Affected/inoculated	Transgenic: Incubation period (days)	Wild type: Affected/inoculated	Wild type: Incubation period (days)
spCJD	MM	2*	66/67	210 ± 4	0/60	>450 or >600
spCJD	VV	1*	5/5	337 ± 11	1/5	471
spCJD	MV	2	15/16	218 ± 2	1/9	257
iCJD (GH)	MM	1	7/7	211 ± 5	1/5	318
iCJD (GH)	MV	1	4/4	195 ± 9	1/5	367
iCJD (GH)	VV	1	5/5	193 ± 4	0/5	>600
iCJD (DM)	MM	1	4/4	204 ± 6	2/4	569, 569
iCJD (G)	VV	1	8/8	187 ± 4	0/5	>600
vCJD	MM	6	25/56	228 ± 15	33/43	371 ± 17
BSE		5	10/28	602 ± 50	21/24	466 ± 26

Incubation periods (mean±s.e.m.) in HuPrP$^{+/+}$ *Prn-p*$^{o/o}$ transgenic mice and non-transgenic (FVB) mice. Methods and *PRNP* analysis were as ref. 2. All CJD cases were neuropathologically confirmed. BSE inocula were pooled brainstem and four individual confirmed cases. Most mice were examined by neuropathology and/or western blotting. Incubation periods are not given for mice that died without definite clinical diagnosis, despite neuropathological or western blot evidence. Numbers affected/inoculated exclude mice dying after inoculation or from intercurrent illness. Controls inoculated with PBS alone did not develop prion disease. spCJD, sporadic CJD; iCJD, iatrogenic CJD; GH, growth hormone; DM, dura mater; G, gonadotropin; MM, methionine homozygote; MV, methionine/valine heterozygote; VV, valine homozygote.

BSE and vCJD are caused by the same prion strain. Taken together with the temporal and spatial association of vCJD with BSE but not with scrapie or other animal prion diseases, and BSE transmission studies in macaques[11], this strongly suggests that vCJD is caused by BSE exposure. The theoretical possibility that both BSE and vCJD arise from exposure to a common unidentified source appears remote.

The production of a distinct molecular strain type on transmission of vCJD to mice expressing valine 129 human PrP suggests that BSE transmitted to humans of this genotype might produce a similar strain. Such cases may differ in their clinical and pathological phenotype to vCJD, but could be identified by PrP^{Sc} typing.

Although it has been argued that the species barrier resides in PrP primary structure differences between donor and host[12], our data emphasize that strain type can be as important. As prion propagation involves interactions between PrP^{Sc} and host PrP^{C}, and strains are associated with differences in PrP conformation and glycosylation[2,13], such PrP interactions may be most efficient if the interacting proteins are not only of the same sequence but have similar conformational preferences and glycosylation. Mismatch of codon 129 between inoculum and $HuPrP^{+/+}$ $Prn\text{-}p^{o/o}$ mice does not significantly affect CJD transmission, but this could differ for BSE. All vCJD cases have been 129MM genotype (ref. 14 and unpublished data). Although our 129VV mice are much less susceptible to BSE than to typical CJD, suggesting a substantial species barrier, 129MM human PrP mice could be more susceptible.

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'Male-stuffing' in wasp societies

Intracolony aggression within and between castes of social insects is common[1–3]. We have observed an unusual aggressive interaction between nestmates of the paper wasp *Polistes dominulus*. In response to foragers returning to the colony, females (workers) initiate aggressive encounters with males culminating with the male being forced head-first into an empty nest-cell ('male-stuffing'). 'Stuffed' males are unable to feed, so the behaviour seems to ensure that food is preferentially channelled to larvae, which are likely to be more closely related to the workers than are the adult males.

We observed two categories of stuffing. 'Initial stuffing' (Fig. 1) began with antenna-to-antenna contact and was followed by grappling, biting, and sting-threats. The aggressor then forced the recipient head-first into an empty cell. 'Repeated stuffing' was characterized by biting and pushing the abdomen of an individual whose head and thorax were already inside a cell.

We studied the behaviour by transcribing and analysing 26 hours of videotape. We saw stuffing behaviour only in colonies containing males ($n=5$ colonies) and not in those without ($n=6$ colonies; sexed by antennal morphology[2]; $\chi^2=21$, $P<0.001$). Stuffing was directed exclusively at males, despite their being greatly outnumbered by females (1:4.21) in colonies of both sexes (binomial test, $P<0.0001$). Of 66 stuffing events, 46 were directed at males from that colony (identified by marking them at eclosion); the remainder were of unknown origin. Queens ($n=5$) did not stuff males (0/66 events; binomial test, $P<0.1$). All stuffing was done by workers other than the returning forager.

Initial stuffing occurred soon after the return of a forager, whereas repeated stuffing occurred at random times (Fig. 2). Males that had been repeatedly stuffed remained in cells 6.35 times longer ($\bar{t}=384.29\pm43.01$ s; mean time ± s.e.m.) than the mean time between forager arrivals ($\bar{t}=60.53\pm2.25$ s; $n=833$). Thus, stuffing may function to preclude males from gaining access to resources gathered by the workers.

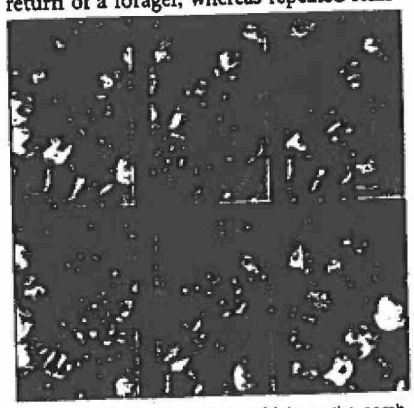

Figure 1 Initial male-stuffing. **a**, Male on the comb. **b**, Female (worker) approaches and antennates him, **c**, followed by biting and sting-threats. **d**, She stuffs him into an empty cell, **e**, and pushes on his abdomen. **f**, Male in the cell.

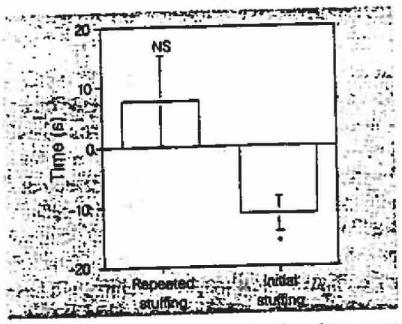

Figure 2 Difference between the time from most recent male arrival until stuffing and half the average interval between returns. A value of 0 is expected if male-stuffing is random with respect to arrivals. Initial stuffing ($n=32$) occurred shortly after a nestmate returned ($\bar{t}=18.86\pm2.89$ s; Wilcoxon signed-rank test $Z=-3.20$, $^*P<0.01$), but repeated stuffing ($n=34$) occurred randomly with respect to arrivals ($\bar{t}=39.88\pm7.51$ s; $Z=-0.18$, $P>0.8$; NS). Means ± s.e.m.

Limiting food consumption by males may maximize the inclusive fitness of workers, who should direct their help towards closely related kin[4,5]. Feeding future reproductive females provides a larger fitness pay-off than feeding adult males[6]. Workers from a colony containing one singly mated queen have a relatedness to sisters of 0.75. Workers are only related by 0.25 to brothers, 0.375 to nephews (worker-produced males) and are unrelated to immigrant males.

Assuming that female larvae are present, workers are more closely related to reproductive-destined larvae than to adult males. Even in circumstances where workers are, on average, equally related to male and female nestmates (such as brothers and half-sisters when the queen has mated more than once), feeding needy larvae may provide a larger inclusive fitness pay-off than feeding adult males, which can forage for themselves. Preferential channelling of resources to larvae, by stuffing males, may maximize the genetic self-interest of worker wasps.

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Human BSE

Jeffrey Almond and John Pattison

Two sets of studies, using different approaches, provide convincing evidence that the new variant of Creutzfeldt–Jakob disease is caused by the agent that is responsible for BSE in cattle. But they cannot tell us anything about the future number of cases of this variant disease.

On 20 March 1996 the UK government announced that a distinct variant of Creutzfeldt–Jakob disease (vCJD) had occurred in ten people in the United Kingdom over the previous 14 months[1]. Like CJD, the variant form results in brain damage and death; but it is pathologically and clinically distinct, not least in afflicting comparatively young people. The government also stated that the most likely cause of this apparently new disease was exposure to the agent that has caused the epidemic of bovine spongiform encephalopathy (BSE).

Since then, there have been more cases of vCJD and further evidence has accumulated that supports a link between the two diseases (Table 1). But to date there has been nothing as compelling as the results described by two papers in this issue — one, by Moira Bruce and colleagues[2], based in Edinburgh, appears on page 498; the other, by a group led by John Collinge (Hill *et al.*[3]), is on page 448. Both reinforce the conclusion that vCJD is distinct from other, sporadic forms of CJD (spCJD), and provide a convincing case that vCJD is caused by the same 'strain' of agent that has caused the BSE epidemic in Britain. In effect, vCJD is human BSE.

Both types of CJD, and other forms of transmissible spongiform encephalopathies (TSEs) such as scrapie, are thought to be caused by aberrant protein agents, which in turn cause the pathological modification of host-encoded prion proteins (PrPs). This is the 'protein only' hypothesis[4], although it still remains possible that some other kind of foreign macromolecule is involved (see box overleaf). The two new studies[2,3] are based on characterizing the strain of agent associated with vCJD and comparing it with strains from spCJD and from TSEs of other animals including BSE. The approaches differ slightly, however: although both groups use transmission potential as a defining character, Bruce *et al.*[2] concentrate on incubation period and pathology, whereas Hill *et al.*[3] focus on a particular feature — the so-called glycoform profiles — of the disease-specific PrP.

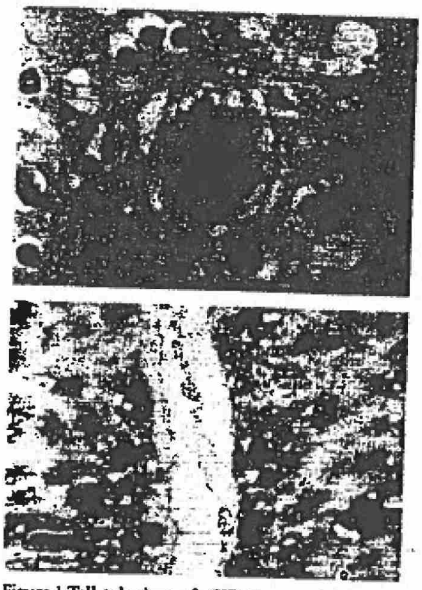

Figure 1 Tell-tale signs of vCJD. Top, cerebral cortex showing (centre) the characteristic florid plaque. Bottom, massive accumulations of PrP in the cerebellar cortex, revealed by immunocytochemistry. (Courtesy of J. W. Ironside, CJD Surveillance Unit, Edinburgh.)

Evidence for the existence of multiple strains of TSE agent comes mainly from the Edinburgh group[5] and is based on the observation that different strains will give reproducible incubation times and pathology in certain lines of inbred mice. To define strains of TSEs, they routinely use four or five mouse lines including homozygotes for prolonged scrapie incubation periods (p7p7), for short incubation periods (s7s7) and heterozygotes (p7s7). Strains of TSEs may differ from one another both in the incubation time in a particular mouse line and in the temporal sequence in which the different lines succumb to disease. Pathological damage is measured semi-quantitatively by scoring vacuolation in nine regions of the brain and is expressed graphically as a lesion profile or 'signature'. This signature is reproducible and characteristic of a given strain[6].

Previously, the Edinburgh group characterized eight cases of BSE from different periods in the epidemic, and from different areas: all displayed highly similar strain characteristics (their phenotype), which were moreover distinct from those observed over many years in TSEs isolated from sheep, mink and a mule deer. Importantly, strain phenotype can be maintained upon transmission between species (as assessed by re-assay in indicator mice). Thus, the BSE phenotype was maintained after experimental transmission to a pig, a goat and two sheep. Identification of the same phenotype in three cases of feline spongiform encephalopathy, an outbreak of which has occurred in the UK contemporaneously with BSE (albeit at a much lower level), and in cases in kudu and nyala in British zoos, has been taken as evidence that BSE has infected these species[6].

Although their current research is not yet complete, Bruce and colleagues[2] can now also conclude the following: that the strain of agent from vCJD is: (a) the same in each of the three cases they have studied; (b) different from that seen with other forms of CJD; and (c) indistinguishable from that of BSE. The mean incubation period of vCJD in one of their inbred lines of mice (RIII) is relatively short, which is characteristic of BSE whatever its source. Moreover, the signature produced in these mice is almost superimposable on that produced by BSE from cattle or from any other species accidentally or experimentally infected with BSE. The remaining lines of mice that have been inoculated with vCJD are still under observation. Experience predicts that the C57BL line of mice will be the next to develop signs of disease, and that is now happening (M. Bruce, personal communication). Given the data so far, it seems unlikely that the final results of these lengthy experiments will give a different picture — a picture which, incidentally, confirms the view that CJD occurring in farmers in recent years is in fact the sporadic form and is not related to BSE[1,7].

What of the work of Hill *et al.*[3]? Their research focuses on the fragment size and the ratio of di-, mono- and nonglycosylated forms of the disease-specific PrP protein after treatment with protease, a protein-digesting enzyme. This glycoform profile also seems largely to be maintained upon inter-species transmission, although

Table 1 Accumulated evidence of a link between BSE and vCJD

- **March 1996** Recognition of the emergence of vCJD as a predominantly UK disease (there has been one case in France), about ten years after the start of the BSE epidemic[1]. Given the rarity of the appearance of new TSEs generally, emergence of a new form in the UK at this time was always unlikely to be mere coincidence.
- **June 1996** Observation[18] that the characteristic vCJD pathology in humans (the production of florid plaques and prominent involvement of the cerebellum; see Fig. 1) could be reproduced almost exactly in rhesus macaques by inoculation with BSE.
- **October 1996** Publication of research showing that the glycoform profile of PrP from vCJD cases was distinct from classical CJD and identical to BSE[3].
- **October 1997** Publication of the studies by Bruce *et al.*[2] and Hill *et al.*[3], discussed here, which reinforce the conclusion that vCJD is indeed caused by the same agent that causes BSE, and make implausible any assertion that all of these observations are coincidental.

changes may occur in certain genotypes of recipient. Last year, using this parameter, the group obtained evidence that the vCJD agent was the same as that of BSE, although its possible similarity to other animal TSEs was not ruled out[4].

Their latest results[3] include a large number of transmissions to both transgenic mice expressing the human PrP gene (*Prn-p*) and their non-transgenic counterparts, and provide stronger evidence that the agent of vCJD is distinct from those of both spCJD and the iatrogenic form of CJD (a number of cases of which were caused by treatment of patients with growth hormone derived from human pituitary glands). By most criteria, vCJD and BSE are also highly similar: their glycoform profiles are indistinguishable, in both ratios and band sizes; mice suffering from the two diseases share unusual symptoms (some of the mice walk backwards); and although details of the pathology are yet to be published, the authors refer to "striking similarities" in PrP deposition patterns. In the line of inbred mice (FVB) used for this study, there are some differences in transmission potential of BSE and vCJD. This is particularly so in the human *Prn-p* transgenic animals, to which vCJD transmits more readily than BSE. These differences are, however, probably attributable to a 'species barrier' effect for BSE but not for vCJD.

Taken together, then, the two new sets of results complement each other and give a consistent message. But can we now draw firmer conclusions about the number of cases of vCJD that will occur in the UK? Unfortunately not. To date, there have been 21 confirmed instances in the UK (each one a tragedy in its own right, and our sympathy goes out to their families). The rate of new cases is not increasing, which provides some hope that the overall number will be relatively small, but it may take several years before we can be confident that this is not a period of comparative calm before a storm. Much depends on the average incubation time of vCJD: the longer the time, the higher the final figure is likely to be[9]. At present we cannot calculate the average incubation time of BSE in humans; nor is it possible to estimate the amount of infectivity (in terms of cattle or mouse infectious doses) required to infect a human.

One observation that bears on these issues is that all of the vCJD cases examined so far are homozygous for a common amino-acid polymorphism in the human PrP protein, namely methionine at position 129. This raises the possibility that people who are homozygous for valine at this position or who are heterozygotes (about 11% and 51% of the UK population, respectively) may be relatively resistant to infection, may be subject to longer incubation times[10] or may have different symptoms. The *Prn-p* transgenic mice used by Hill *et al.* have the valine 129 version of the gene and it will be interesting to compare transmission to similar mice with methionine at that position.

Finally, these latest results[2,3] also do not tell us anything more about the route by which the victims of vCJD were infected. There are various possibilities, including a common source for BSE and vCJD, and transmission from cattle to people through an intermediate species. But we still think that the most likely exposure was through eating beef products that included infected offal before it was banned from human food in late 1989. The report in the UK press of a case of vCJD in a vegetarian of 11 years' standing does not, we believe, invalidate this view; she may have inadvertently been exposed to contaminated beef products, or may have consumed infected beef before becoming a vegetarian.

A postscript. The UK government's decision, in March 1996, to point publicly to a probable link between BSE and vCJD was taken on the advice of the Spongiform Encephalopathy Advisory Committee (SEAC) — of which we were and are members, although here we are not writing in that capacity. At the time, the evidence connecting the two diseases was relatively slight and SEAC's advice was rightly questioned in both the scientific and popular press. Only rarely in such circumstances can science offer definitive evidence quickly, and decisions have to depend on the weighing of uncertainties. More such judgements may yet be required concerning BSE and human disease.

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'Protein only' prions?

The results of Bruce *et al.*[2] and Hill *et al.*[3] underline the need to gain a better understanding of the nature of the agents responsible for transmissible spongiform encephalopathies (TSEs). The most favoured view is that the agents are composed solely of an altered form of a host-encoded protein known as PrP and lack a foreign nucleic acid; this is the 'protein only' or prion hypothesis.

There are well [illegible] documented instances of TSE agents changing their characteristics, or phenotype, upon passage (repeated transmission through experimental animals). Indeed, Hill et al. report a change in fragment size upon transmission of BSE and vCJD to the [illegible] transgenic mice. [illegible] However, strain phenotypes can remain stable; thus the Edinburgh group of Bruce and colleagues have reported that different strains adapted to, and repeatedly passaged in, a single line of inbred mice retain their distinguishing features even though they must be composed of PrP protein with the same amino acid sequence. In addition, a single strain [illegible] illustrated by [illegible] retain its phenotype even when passaged through different hosts. In these circumstances [illegible] strain [illegible] maintained even though the agent [illegible] of PrPs of different amino acid sequence, raising the question of what determines strain phenotype. Clearly, in these cases, [illegible] primary structure of the PrP protein.

Supporters of the protein-only hypothesis argue that the small number of different strains that have been convincingly demonstrated so far can be explained by different conformations of the PrP protein, perhaps in combination with modifications such as glycosylation. Such differences of course need to 'breed true' upon passage.

On the other hand, opponents [illegible]

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DHNI0000041_123_0005