THE ROYAL SOCIETY

Transmissible spongiform encephalopathies

Statement by the Royal Society and the Academy of Medical Sciences

The Academy of Medical Sciences

Summary

- Further basic research is crucial to our understanding of Transmissible Spongiform Encephalopathies (TSEs) and the multi-factorial nature of their occurrence and natural transmission.
- It is important to encourage some high quality young researchers in this area through the establishment of prestigious 5-10 year Fellowships.
- The ban on recycling animal protein, for example using Meat and Bone Meal for ruminant food, should remain for the foreseeable future, and it is essential to explore alternative ways of disposing of carcasses.
- It is important to eradicate TSEs from all food animals; in the case of sheep and goats this should be assisted through the breeding of resistant animals and the selective culling of infected animals.
- Highly sensitive and inexpensive tests need to be developed for the routine testing of slaughtered animals and preclinical tests for use on live animals and humans.
- Further work is required on sterilising of surgical instruments and on the safety of blood transfusions.
- We also believe that urgent consideration needs to be given to the possibility of cross infection in those abattoirs that handle both food animal slaughter and the culling of over thirty month animals.
- There are prospects for the future development of therapies, but these will require public financial support.

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1 Introduction

Over the past decade, the Royal Society has taken a particular interest in the science associated with transmissible spongiform encephalopathy (TSE) diseases, which all appear to involve abnormal isoforms of prion related protein. In September 1993, the Society organised a discussion meeting on prion diseases[1], and in 1996 and 1997, it issued three statements on the developing science concerning Bovine Spongiform Encephalopathy (BSE) and TSEs in general[2 3 4]. Since that time, major advances have been made in our understanding of the basic science of prions and TSEs, and in the development of diagnostic tests, but many questions remain unanswered. There is an urgent need for more sensitive tests to diagnose TSEs in animals and vCJD in humans before clinical signs appear and for therapeutic agents to arrest the progress of infection. Furthermore, the likely extent of the vCJD epidemic is still uncertain, and BSE is no longer confined to the United Kingdom.

Following the report of the Phillips' BSE Inquiry[5], the Councils of the Royal Society and of the Academy of Medical Sciences decided to establish a joint Working Group to advise them on the current situation, and on whether a further statement was appropriate at this stage. The membership of the Group is listed at the end of this statement.

Since 1997 there has been a number of important reviews of the science of TSE including an extensive overview in Volume 2 of the Phillips Report[6], which largely details the science known up to 1996, but includes some important advances in knowledge since then. Two reviews, by Collinge[7] and by Aguzzi et al[8], appeared during the Group's work, and a number of other groups have been reviewing various aspects of the topic; the Food Standards Agency has published a report on BSE controls[9], and two parallel groups chaired by Professor Gabriel Horn and Professor Ian McConnell are re-considering the likely origins of BSE for MAFF and reviewing past and proposed MAFF Research Programmes respectively. We have not sought to duplicate the in-depth work of these other review groups, and the detailed coverage of the review articles is not repeated in this statement, which concentrates on some specific issues:

- gaps in our knowledge
- management of research
- diagnostic tests
- BSE in sheep and eradication of TSEs from all food animals
- disposal of infective material
- therapeutic agents

2 Transmissible Spongiform Encephalopathies

The first TSE to be described was scrapie in sheep in the first half of the 1700s, although it was only in the 1930s that it was shown to be transmissible within species. It was not until the 1960s that it was shown that scrapie belonged to the same family as rare fatal neurological diseases in humans – kuru, Creutzfeldt-Jacob Disease (CJD) and Gerstmann-Straussler-Scheinker disease[10]. A new cattle TSE, BSE, was first identified in 1986 and since then nearly 180,000 cases have been confirmed. This was eclipsed by the over 5 million cattle culled as a result of various schemes, most notably the ban on cattle over thirty months old entering the food chain (the OTM rule). As of 8 May 2001, since 1996 there have been 86 human deaths in the UK from a distinct new variant of CJD (vCJD), with a further 13 probable cases still awaiting confirmation. The infective agent for vCJD appears to be distinct from that of classical CJD diseases and is biologically indistinguishable from BSE.

All TSEs appear to involve the formation of an abnormal isoform of a naturally occurring cellular membrane protein (prion related protein PrP^{C}), usually referred to as PrP^{Sc}, which forms insoluble aggregates and which is accompanied by the destruction of brain cells and the proliferation of structural (glial) cells. The structure of the normal PrP^{C} glycoprotein is largely in an α-helical form, soluble and easily degraded, while PrP^{Sc} is largely in a β-sheet form, insoluble and resistant to degradation.

It has been known for some time that the susceptibility of sheep to scrapie was dependent on the precise sequence of amino acids encoded by the prion gene, with some genotypes being particularly resistant. It would also appear that genes other than the prion gene itself can influence susceptibility to scrapie. Genetic factors are also important for CJD. All vCJD victims identified so far are of a particular genotype, in which both copies of the prion gene encode the amino acid, methionine, at position 129 of the prion protein. However, all of the initial kuru victims were also homozygous for methionine or valine at this codon, while later cases were heterozygotes encoding for methionine/valine and thereby presumably conferring longer incubation times. Shorter incubation periods in animals homozygous for the prion protein gene are a common feature of all natural and experimental TSE infections.

One characteristic of TSEs is that transmission between animals of the same species is easily achievable experimentally. For intracerebral injection 100% of animals succumb over a wide dose range. Incubation periods are remarkably constant, even though they may be very long. As the dose is reduced, incubation periods lengthen until a point is reached where further dilution results in not all animals succumbing.

Transmission between species is much less efficient, and in some cases the recipients do not succumb even with high doses of infective agent. Where transmission is achieved, only a fraction of those exposed succumb and usually with greater variation in incubation period.

However, once transmitted to the new species, the agent becomes highly infectious for the new species, as in same-species infection. This "species barrier" is related to the molecular structure of the prion protein, mice expressing bovine PrP^C, for example, showing no species barrier to BSE. On the other hand, there must be other factors, as vCJD transmits more readily to non-transgenic mice than it does to transgenic mice expressing human PrP^C homozygous for valine at codon 129. A possible additional factor is the level of expression of PrP^C.

The fact that an animal does not display overt clinical signs of a TSE - induced disease does not necessarily mean that it is not sub-clinically infected. For example Collinge and co-workers[11] have shown that a strain of hamster prions thought to be non-pathogenic for conventional mice, nevertheless replicated to high levels in such mice without causing overt clinical disease. The prions produced were pathogenic when subsequently transferred to both mice and hamsters.

3 The main gaps in our knowledge and the crucial need to continue background basic research

Despite the significant progress made over the past few years, fundamental aspects of prion diseases still require resolution. Most importantly, the nature of the infective agent, whether it is purely the PrP^{Sc} isoform or PrP^{Sc} occurring in combination with some other molecule, is still not resolved. There is clear evidence that DNA or RNA cannot be involved at a level of more than 100 bases. Nevertheless, the mechanism remains unresolved as to how strain characteristics are retained during replication in hosts of different PrP genotypes if the infective agent is attributable purely to the prion protein, especially when the infection is retained through successive hosts. Work on human prions[12 13] has suggested that the prion protein may itself encode the strain information by differences in PrP^{Sc} conformation and glycosylation. This has been given support by recent studies on the aggregation of yeast prion-like protein in vitro[14].

Other major gaps in our knowledge include:

- the origins of BSE;
- the detailed nature of the PrP^C prion protein and its contribution to normal cell function;
- the mechanism of PrP^C to PrP^{Sc} conversion;
- the tertiary structure of PrP^{Sc};
- the mechanism of brain cell degeneration and the possible role of the transmembrane form of PrP^C;
- the relationship between different TSE diseases;
- the distinguishing features and replication of strain characteristics;
- the role of the lymphoreticular system in overcoming the species barrier between donor and recipient; and
- the genetics of host susceptibility.

We are still not sure about the origin of BSE. The Phillips report suggested that epidemiological evidence pointed to a start for the BSE epidemic in cattle in Great Britain around the early 1970s. This is uncertain because of the stochastic nature of the start of any new epidemic in an entirely susceptible population of hosts. The precise date could have been anywhere between the mid 1960s to the late 1970s. The Phillips report sets out the arguments for meat and bone meal (MBM) having been the main vector of BSE infectivity, including the reduction in incidence of BSE once controls on MBM were in place. However, it questions the original view that changes to the rendering process had allowed the infective agent from scrapie to be transferred to cattle in MBM rations. The Phillips report argues that this led to complacency over risks to human health, because the transmission of scrapie to humans had never been identified. Current levels of resolution indicate that BSE is a single novel strain, whereas the long established scrapie has many different strains, and this and the epidemiological evidence led the Phillips report to suggest that the source of BSE could have been a single chance mutation in a cow, sheep or other animal sent for rendering in the early 1970s, with subsequent waves of infected material being recycled in Southern England through waste from sub-clinically and clinically infected cattle in MBM. The high feed consumption of MBM in the UK could have increased the opportunity for transmission of the infectivity.

Apart from genetic effects on susceptibility, there may also be environmental factors. One hypothetical mechanism, mentioned in the Phillips report, involves an organophosphate insecticide coupled with excess manganese, which may increase susceptibility to BSE agents[15]. A recent report showing the effect of manganese on PrP[16] throws some light on this suggestion. The incorporation of manganese in place of copper makes the protein proteinase resistant and abolishes its anti-oxidant function; a new finding that will attract further examination. Neither abnormal folding nor proteinase resistance are, in themselves, definitive indicators of infectivity and, as the latter investigators note, the findings are a long way from explaining sporadic prion disease. The group chaired by Professor Gabriel Horn is carrying out further examination of the possible origins of BSE, and we await their report with interest.

Of particular interest is the relationship between the various TSEs. It has been shown through infection of mice that BSE and vCJD produce identical clinical signs, brain lesions and Western blot analysis profiles, which are different from those of sporadic CJD (spCJD) [12 17 18]. This relationship between BSE and vCJD infection has been confirmed in studies with macaque monkeys[19], which showed that BSE passaged through non-human primates remains highly infectious, with possible implications for human blood transfusion and surgical procedures. Furthermore, both younger and older animals succumbed

to BSE with similar pathology, although plaque deposition was greater and florid plaques more numerous in the younger macaques (This may point to vCJD cases being misdiagnosed in the elderly and hence not reported to the CJD Surveillance Unit). An unexpected finding from this work with macaques was that one of two strains of scrapie used in the controls produced incubation periods and lesion profiles in mice that were similar to those caused when the same strain of mice is infected by one form of sporadic CJD. Since spCJD occurs at a constant rate in all human populations, even in countries where scrapie is not present, a strain of scrapie is unlikely to be a major cause of spCJD. Further work in this area is clearly important to judge the significance of these findings.

We wish to stress the importance of epidemiological analysis; both in its own right, and also for the contribution it can make to improve understanding of key biological questions. To date epidemiological analysis has, for example, ascertained the primary route of transmission that caused the outbreak in Great Britain (the recycling of contaminated meat and bone meal feeds)[20], identified a potential role of vertical transmission[21 22 23 24], defined the optimum control policies to eliminate BSE by cattle culling and tracing[25] and provided estimates of the volume of infected material that entered the human food chain in the early stages of the BSE epidemic (via back calculation methods)[26]. For TSEs in general much remains to be investigated. The key areas are as follows.

- The importance of vertical transmission is uncertain for all TSEs. Epidemiological evidence points to its presence in BSE in cattle and in scrapie in sheep, but the precise mechanisms are uncertain. Some unanswered questions are, whether it occurs before birth, or whether other mechanisms, for example via colostrum, are involved.
- Is the likelihood of infection dependent on the age of the exposed host? Studies in 1996 suggested age-dependent susceptibility or exposure[25]. The relative importance of susceptibility and exposure remains unknown, but the problem is amenable to experimental study. This issue is also of importance to understanding the epidemiology of vCJD in humans.
- How do scrapie and other TSEs remain endemic in their major host species (eg. for chronic wasting disease (CWD) in mule deer in North America)? Vertical transmission alone cannot sustain endemic infection. Is direct horizontal transmission, for example via contaminated pasture, possible? This question is of obvious importance to any attempt to eradicate scrapie or to what may happen in the tail of the BSE epidemic in Great Britain.
- How transmission of BSE or scrapie and the incubation period distribution is dependent on factors such as the genetic background of the host or the dose of infecting material?
- How were the currently emerging epidemics of BSE in many different European countries initiated?

Little is known at present about all these important issues. Many of these key areas are being addressed by experimental pathologists, clinical research scientists, microbiologists, epidemiologists and field veterinarians.

We have considered the OTM cull, which so far has resulted in the slaughter of nearly 4.9 million animals. It is estimated that this and other restrictions have resulted in reducing the estimated number of diseased cattle entering the human food chain within one year of the development of clinical signs to less than one in the current year[27]. The declining number of animals with BSE will lead to greater confidence in future, but we agree with the Spongiform Encephalopathy Advisory Committee (SEAC) that changes to the rule should not be considered before January 2002. We do not believe that the present EU approved tests for infectivity at slaughter are an adequate substitute for the OTM rule. Such tests have unknown sensitivity and specificity in animals at different stages of the incubation period post infection. They can detect late stage infection when clinical symptoms of disease are nearly apparent – but little is known of their performance in early stage infection. This is an important research priority.

Other genes apart from different genotypes associated with the prion gene have been shown to have an effect on incubation times. For example, Prusiner and co-workers[28] have reported that loci on chromosomes 9 and 11 in mice affect incubation times. Another study[29] involving larger numbers of mice has shown relevant loci on chromosomes 2, 11 and 12. A recent paper[30] has shown that other genes have increased expression of PrP^C in TSE cases. These studies all have significant implications for the detailed pathogenesis of TSEs and the genetics of susceptibility and should prove fruitful areas for further investigation.

At present, it is not possible to reduce the wide uncertainty in the estimate of the total number of people who could be infected with vCJD beyond the currently published span of a few hundred to over 100,000 as an upper bound. Unfortunately, better estimates are unlikely to be available in the near future, and it should be noted that these estimates do not take into account the possibility that the early victims may have been individuals with a multi loci genotype conferring particularly short incubation periods.

The report on the investigation into a cluster of five vCJD cases in Queniborough, a small village in Leicestershire[31], found that the most likely cause of these cases was the consumption of meat bought from two local butchers where the slaughtering and meat preparation techniques led to contamination of meat with brain tissue. The situation at Queniborough is unlikely to have been very different from that in other similar sized communities, and suggests that some cases of vCJD are related to unusually severe exposure rather than greater susceptibility. It is possible that the cluster was related to a small number of

infected animals in the later stage of incubation entering the human food chain in the early 1980s. A study of the geographical distribution of all vCJD cases up to 10 November 2000[32] has shown some regional differences in incidence, but as yet there are no conclusive reasons for these variations.

The Queniborough cases reinforce the need for quantitative assessment of UK dietary exposure to BSE in Mechanically Recovered Meat (MRM) and brain contamination of, for example, head meat. The latter was used in processed meat products, some of which were eaten preferentially by young people, and to a greater extent by males than females[33].

There are many important specific questions about food animals that remain to be answered definitively, such as whether BSE infection exists in the national and EU sheep flocks. Further work is also required to quantify further any vertical and horizontal transmission of BSE in cattle.

With the development of more sensitive tests for infectivity, there is a need for investigations of the extent of infectivity during the development of TSE disease to be repeated. Many of the published data showing infectivity in different tissues were obtained using relatively insensitive tests, and it is important to confirm the apparent absence of the agent in several peripheral tissues.

There are four further points to which we would wish to draw attention in this section. First, in view of the expense of much of the work in this area, and the long lead-time to obtain results, it is most important for experiments and surveys to be designed carefully and for peer review of the proposed studies to include relevant statistical expertise.

Second, there are centres of expertise in TSE research throughout Europe and in the United States. The EU programme has brought some of these together, but there is still scope for further collaboration, especially with laboratories in Switzerland and the US.

Third, the Phillips report placed emphasis on the need for more research trained veterinary staff. Too few students in veterinary schools undertake training in scientific research (e.g. register for a PhD, or undertake intercalated BSc studies in specialist scientific disciplines). The reasons for this are complex and involve important issues such as: the initial selection of veterinary students motivated for a career in science; the lack of tenure-track career pathways for veterinary graduates in clinical research; and the need to locate centres of excellence in animal disease research within university veterinary schools. The problems are particularly acute in such fields as veterinary pathology, microbiology and epidemiology. Many of the issues pertaining to the maintenance and development of the academic base of the veterinary profession in veterinary schools and research institutes were identified by the Selborne Enquiry on Veterinary Research[34]. They include the dominance of non-veterinary graduates in relevant Research Council institutes. It is unclear how the veterinary profession, the university veterinary schools and the public funders of veterinary education and research (HE Funding and Research Councils) are addressing these urgent issues.

Finally, while there are still important studies that need to be undertaken to answer immediate practical problems, we would reiterate the final sentence of the Royal Society's 1997 statement, which stressed "the importance of also sustaining long-term research programmes that are investigative, innovative and curiosity driven". It is from such studies that the more original approaches to many of the issues raised below may be discovered.

4 Resources for research, and the need to continue to nurture the best post-doctoral researchers in the field

It is crucially important to create, maintain and provide access to research reagents and tissue banks with full documentation on provenance and, where appropriate, on infectivity measured against an agreed standard. These should include cattle tissue at varying incubation times following oral infection, as well as human CJD victims' tissues. The TSE Resource Centre at the Institute for Animal Health (IAH) is able to supply samples of laboratory TSE strains of known dose. As far as human tissue is concerned, we hope that the recent reports of post mortem tissue being retained without permission will not result in relatives being reluctant to give permission for retention of tissue samples especially from vCJD victims. Research reagents produced by publicly funded research should be made available to the TSE Resource Centre for the benefit of all TSE researchers.

Similarly all publicly funded human and animal surveillance data should be freely available to appropriate researchers.

Animal work in these areas requires category 3 containment facilities. These are available at the Veterinary Laboratories Agency (VLA), Weybridge and IAH (Compton and (more limited) Edinburgh sites). It is, however, vital that qualified TSE researchers elsewhere in the science base have access to appropriate containment facilities, but it would be prohibitively expensive to duplicate these extensively. Greater collaboration arrangements, including international exchanges, should be encouraged to make full use of existing facilities. This is particularly important to enable both research institute and university scientists to undertake basic research in parallel with the more directed studies designed to answer more immediate problems, but the full costs of maintaining TSE infected animals in containment for long periods of time has to be recognised.

Finally, TSE research, like many other biomedical activities, is a long-term process. It is difficult for postdoctoral researchers in these areas to establish themselves on the short timescale funding arrangements available to them. The Joint Funders Group of BBSRC, Department of Health, MAFF and MRC should give consideration to creating some prestigious longer-term (5 to 10 year) fellowships and longer-term grants targeted at fundamental research underpinning high priority biomedical problems such as TSE.

5. Development of highly sensitive tests

One of the main barriers to further advances, both in our understanding of the basic science and in the other more immediate issues discussed below, is the lack of sufficiently sensitive tests for TSE agent infectivity that can be carried out swiftly, rather than wait for bioassays. It is also most important for the sensitivity of new infectivity tests to be measured against the current most sensitive standards. For example, quantitative assessment needs to be standardised against the within species transmission where there is no species barrier. For BSE the most sensitive test is intracerebral injection into calves.

Improved tests to identify infected animals or to detect the infective agent are required for at least three purposes:

- to detect signs of infection (scrapie or BSE) in the brain or other tissues of food animals at slaughter prior to the development of clinical signs;
- to determine the prevalence of infection in food animals at an early stage of incubation by body fluids such as blood or urine, or mouth scrapes. If cheap and specific enough these could be used on large numbers of live animals to test for scrapie in sheep populations;
- to detect vCJD infection at a very early pre-symptomatic stage, especially to screen those at particular risk by means of a blood test. Such tests were also required to reduce the risk of transmission of infection through clinical procedures.

Priorities therefore include increasing the sensitivity of *in vitro* tests by one or two orders of magnitude, and the development of tests on blood or urine for example. Another priority is to have fast simple tests to distinguish between strains of infectious agents, eg scrapie and BSE in sheep.

The finding that plasminogen binds selectively to PrP^{Sc} [35] may be a promising approach to increasing the sensitivity of tests in the presence of PrP^{C}. The finding that the EDRF gene is under-expressed in erythroid cells in TSE infected animals[36] may prove to be a useful surrogate marker for use as a blood test.

The number of humans infected with prions, but currently asymptomatic, is unknown. There are concerns that secondary transmission of vCJD will result from use of prion-contaminated blood products and surgical instruments. vCJD has a pathogenesis distinct from other forms of human prion disease, with disease-related prion protein (PrP^{Sc}) readily detectable in lymphoreticular tissues[37]. Quantitation of such risks, and targeting of risk reduction strategies, is limited by lack of knowledge about relative prion concentrations in these and other peripheral tissues and the unknown prevalence of pre-clinical vCJD. A significant transmission barrier limits the sensitivity of bioassay. A high sensitivity immuno-blotting method for detection of PrP^{Sc} in vCJD tissues has therefore been developed[38], capable of detecting PrP^{Sc} in tissue homogenates when present at levels 10^4-10^5 fold lower than those found in brain.

This study showed that levels of PrP^{Sc} are not uniform in the lymphoreticular system, with consistently higher levels in tonsils. Other peripheral tissues studied were negative for PrP^{Sc} at this level of assay sensitivity with the exception of low levels in rectum, adrenal gland and thymus from a single vCJD patient. PrP^{Sc} was also detected in retina and optic nerve. However, vCJD appendix and blood (Buffy coat fraction) were negative for PrP^{Sc} at this level of assay sensitivity. From this initial work, it would appear that PrP^{Sc} is largely confined to the central nervous and lymphoreticular system in vCJD, with a range of other surgically important tissues having levels of PrP^{Sc} less than 10^4 – 10^5 of those in brain. It suggests that:

- rectal and other gastrointestinal tissues should be further investigated to assess risk of iatrogenic transmission via biopsy instruments;
- ophthalmic surgical instruments used in procedures involving the posterior segment of the eye, may represent a potential risk for iatrogenic transmission of vCJD;
- tonsil is the tissue of choice for diagnostic biopsy and for population screening of surgical tissues to assess prevalence of pre-clinical vCJD infection within the UK and other populations.

We commend the initiative of the Joint Funders Group (**http://www.mrc.ac.uk/**) in organising a recent workshop to bring together academic groups and companies developing or considering developing diagnostic tests for TSEs, and announcing a round of grants to support the development of such tests. A recent survey indicated that there were 22 firms involved in various ways in the development of tests, some of them the spin-off from academic studies.

6. The possibility of BSE in sheep and the eradication of TSEs in all food animals

The last statement from the Royal Society in 1997[4] discussed the issue of whether there was BSE in the

national sheep flock. This would be serious because it would be impossible to remove all the potentially infected lymphoid tissue at slaughter; this is not a problem in cattle, where lymphoid tissues are not infectious.

It is likely that many sheep were exposed in the early to mid-1980s to infected MBM. However, unless there was significant vertical and/or horizontal transmission of BSE the infection will have died out by now, because of the shorter life span of sheep. The possibility of vertical and horizontal transmission of BSE in sheep is currently being explored. Although not easy, studies of infectivity in urine and faeces are important in this regard.

A recent paper reports that an analysis of the results of an anonymous postal questionnaire to farmers on the incidence of scrapie[39] indicates there was no significant increase in "scrapie-like" cases in sheep during the 1980s, which would argue against a significant BSE epidemic having occurred in sheep.

The only satisfactory long-term solution is the eradication of TSE diseases from food animals. A choice has to be made between breeding from TSE resistant animals, selective culling of infective animals when suitable tests are available, and improving husbandry, particularly at birth, or a combination of these options. There are sheep genotypes that are particularly susceptible or resistant to scrapie[40 41 42]. A recent paper[43] has studied the difference in genotype distribution in two scrapie free and two infected flocks. All flocks had a mixture of both resistant and susceptible genotypes, and the changes in balance of the genotypes with age in infected flocks, compared with little variation between age groups in the scrapie-free flocks, is strong evidence that many scrapie cases go unrecognised even on farms that are reporting cases to MAFF.

We support the National Scapie Plan, and believe that it should be designed to remove TSEs from sheep and goat flocks. However, it will be most important to establish that the selected genotypes are resistant to replicating the agent and not just resistant to clinical disease, so that they have incubation periods longer than the animal's normal lifespan. In such circumstances, the animals could still be infectious and could also act as carriers. Nevertheless, it would be a mistake to hold up the start of this important programme of disease control, until more details of pathogenesis are resolved.

7 Destruction of infective material

We have considered the effect of infective agent stability on the disposal of large numbers of possibly infective animal carcasses and slaughterhouse waste, with respect to iatrogenic risk from CJD victims (of all forms). In the latter case, risks include cross infection from blood and blood products, and from dental and clinical instruments following tonsillectomies and neurosurgery procedures.

The reported persistence of infectivity in samples heated to 600^{O}C is surprising[44] and it is important that this finding should be corroborated. If it is true that the infective agent can retain some of its infectivity at such high temperatures this has significant implications for the sterilisation of medical equipment and the incineration of contaminated materials, and cross contamination in abattoirs. Clearly further research is needed to ensure that infective agents are completely destroyed.

We are particularly concerned to discover that there are abattoirs where both food animals are slaughtered and OTM cattle are culled. Under EU rules these cannot be undertaken on the same day, but it is important to ensure that chance of cross-infection from equipment or premises is eliminated.

The disposal of animal carcasses culled during the current foot and mouth disease outbreak has raised fears about the potential danger of disseminating BSE. On 31 March 2001, on the advice of SEAC, the Minister for Agriculture announced that all cattle over 5 years (born before the effective date of the effective "real" feed ban) must be burned or rendered, whereas cattle under 5 years, born after the effective date of the feed ban, could be buried with no substantive risk to the environment and to drinking water. The mass burning of carcasses in outdoor pyres raises concerns about the spread of the infective agent by aerosols, for example, and also the production of dioxins.

In the case of the disposal of animal tissue, in the UK there are over 430,000 tonnes of MBM in store[45], with possibly a further 200,000 tonnes of tallow, and further accumulations building up elsewhere within the EU. Quite apart from the considerable cost of storage of material, there is always a danger of leaks into the environment through, for example, infestation of rodents or invertebrates, which might then cause a health hazard in their own right. We have had a very preliminary look at the possibility of disposing of MBM through microbiological digestion on an industrial scale and through anaerobic pyrolysis at 850^{O}C to provide gases for electricity generation. Both are worth further investigation, and at least on the latter there appears to be potential for a return of a few £s per tonne compared with the significant cost of incineration of the waste.

Iatrogenic infection of sporadic CJD is well documented through the injection of human growth hormone prepared from cadaveric material inadvertently including CJD victims. This has led to fears that blood and blood products may be similarly affected, and persons who have resided in the UK for more than a specified period are now barred from donating blood in many countries. The whole blood of sheep, experimentally infected with BSE, has been shown to transmit infection after transfusion to one other sheep[46], but the infectivity of individual

components has not yet been identified, and much further work is required. As a precaution UK blood is leucodepleted, removing what are thought to be the most likely carriers of any infectivity. The costs of such preparations, however, need to be carefully weighed against the benefits.

The persistence of infectivity on the surface of metals has been investigated using stainless steel wires exposed to infective material. Five minutes exposure to infected mouse brain saturates the wire with infectivity. The wires can then infect mice after insertion into their brains for a little as 30 minutes. The wires bind less than 20ng of protein when eluted with 2M sodium hydroxide. No cases of CJD have been attributed to surgical instruments subjected to autoclaving. Nevertheless, this research should enable the conditions for sterilisation of surgical instruments to be defined, but further work is required using vCJD and spCJD, and for the work to be repeated in primates.

8. The prospects for therapy

By the time patients incubating CJD show clinical signs, the neurological damage may be too severe for recovery, although even in such cases some improvements may be possible. Blanket treatment even of high-risk patients could only involve drugs with at present unattainable safety levels. It is thus crucial that suitable, preferably non-invasive, diagnostic tests be developed that can identify early pre-clinical cases. It is particularly important to screen high-risk groups, such as familial cases, and haemophiliacs who have received blood products.

Work is hampered by not knowing what causes neurological damage, for example whether it is PrP^{Sc} or a toxic intermediate. Nevertheless, several teams are exploring a range of avenues for prophylactic and therapeutic drugs attacking various key stages in the progress of the infection, and this work should be encouraged.

The development of drugs that block the formation of PrP^{Sc} is potentially a promising area, for example:

a. localised reduction of PrP^{C} synthesis;
b. stabilising the PrP^{C} molecule to make the unwanted conformational change less energetically favourable;
c. destabilising PrP^{Sc} to make it less resistant to protease and enhance the decomposition of PrP^{Sc}.

Schenk et al[47] have shown that for transgenic mice that carry one of the mutations that cause Alzheimer's disease in man, immunization with β- amyloid peptide inhibits the formation of amyloid plaques and the associated encephalopathy. Later work[48 49], again using mouse models, has shown that such immunisation also apparently reduces learning dysfunction, which provides some encouragement for the proposition that dementia may be treated by disrupting the deposition of such plaques.

Another development involves antibodies against PrP to prevent and even reverse infection of sensitive tissue cultures by mouse prions.

It may also be possible to explore possible ways of attacking the infection at an earlier stage of its pathogenesis. Studies of scrapie in mice have demonstrated that mature follicular dendritic cells (FDC) expressing the host prion protein PrP^{C} are essential for agent replication in lymphorecticular tissue and subsequent spread to the nervous system[50 51]. Further work showed that inactivating mature FDCs delays the neuroinvasion of scrapie[52 53]. Investigations taking this forward have been reported[54 55] which show the importance of complement component (C3) and their receptors. The first of these used cobra venom to temporarily deplete mice of C3 and delay the onset of scrapie following peripheral injection of scrapie prions.

With the, hopefully, relatively small number of vCJD, sporadic and iatrogenic CJD cases, the economics of the development of therapeutic agents means that the work will have to be supported largely from public funds.

This statement has been endorsed by the Councils of the Royal Society and the Academy of Medical Sciences. It has been drawn up by a Working Group chaired by Professor Brian Heap FRS, Vice President and Foreign Secretary of the Society, and Master of St Edmund's College, University of Cambridge and consisting of:

Professor Roy Anderson FMedSci FRS (Imperial College)
Professor Peter Biggs FMedSci FRS
Dr Sheila Bird (MRC Biostatistics Unit, Cambridge)
Professor Colin Blakemore FMedSci FRS (University of Oxford)
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Glossary

For a more extensive glossary see that in Volume 2 of the Phillips Report at:

www.bseinquiry.gov.uk/report/volume2/glossary.htm

α-helix
One of two common forms of protein folding, where the amino acids turn regularly around themselves forming a right handed helix

β-form
The other major form of protein folding where the amino acids fold back on themselves to form sheets.

Anaerobic pyrolysis
Decomposition of material by heating in the absence of oxygen

Buffy coat
The layer of white blood cells above the red cells when blood is centrifuged.

Colostrum
Milk secreted by a mammal for first few days, which contains high levels of protein, vitimins and antibodies, etc.

Erythroid
Red blood cells

Genotype
The genetic constitution of a cell or an organism

Glycoprotein
A protein that has carbohydrate groups attached to the amino acid chain

Glycosylation
The addition of carbohydrates to proteins

Heterozygous
Having different forms of the same gene

Homozygous
Having identical forms of a whole gene or that part of the gene being referred to.

Iatrogenic infection
Infection caused by a clinical intervention

Isoform
Different three dimensional forms of the same molecule

Leucodepleted
Blood with white cells removed

Plasmin
An enzyme produced from plasminogen that removes blood clot protein from the blood stream

Plasminogen
The precursor of plasmin in the blood

Proteinase
Any enzyme that splits the bonds between amino acids in a protein

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