Preventing accidental transmission of human transmissible spongiform encephalopathies

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The mechanism by which humans became infected with the BSE agent is discussed, and the matter of potential person-to-person transmission of TSEs through contagion or medical procedures is considered. There is some discussion regarding the current evidence relating to whether or not the blood of humans infected with TSEs is infectious. Considerable emphasis is placed on the fact that TSE agents are known to be relatively resistant to decontamination by procedures that are effective with conventional micro-organisms, including (under worst-case conditions) the autoclaving procedures used to sterilise surgical instruments. Methods for providing additional re-assurance with regard to the safety of instruments are described. Safety in the pathology laboratory is discussed extensively because TSE agents are not inactivated by the usual processes used to fix tissues, and such laboratories will receive fixed tissues that are still highly infectious as far as TSE agents are concerned.

From the preceding chapters, the reader will have become aware of the unusual characteristics of the transmissible spongiform encephalopathies (TSEs), and the unconventional nature of their incompletely-characterised causal agents. One notable property of these agents is their remarkable degree of resistance to chemical and physical inactivation procedures that are effective with conventional micro-organisms.

Transmission of bovine spongiform encephalopathy to humans

The emergence of variant Creutzfeldt-Jakob disease (vCJD) in the UK during the mid-1990s as a new human disease has been convincingly shown to be associated with the infection of humans by the same agent that causes bovine spongiform encephalopathy (BSE) in cattle. Among the possible vectors for infection that have been investigated, food remains the most likely candidate. As will be discussed, BSE-like agents survive the temperatures associated with the cooking of food. In the late 1980s, legislation was introduced in the UK whereby potentially
infected bovine tissues were required to be removed from the food-chain. Prior to this, infected bovine tissues could have been used as/in (or have contaminated) foodstuff. It is also acknowledged that the degree of BSE contamination of food-products would have escalated progressively throughout the 1980s as the scale of the cattle epidemic increased. It was also recognised retrospectively that the regulations introduced in the late 1980s had not been rigorously enforced until some time thereafter. However, UK beef products are now extremely safe with regard to the BSE agent because: (i) there is now a rigorous application of the original regulations which is confirmed by the regularly-published findings of the inspectors that visit abattoirs, rendering plants, etc: (ii) a number of other regulations were introduced to fortify those already in place (for example, abattoir practices were changed so that cross-contamination between potentially BSE- and non-BSE-infected tissues should not occur); and (iii) regulations were introduced to prohibit the use of mechanically recovered meat (MRM) in food products. MRM had been considered to be a product that had a high risk of containing BSE infectivity in the past².

Person-to-person transmission of TSEs

Due to the known relative resistance of TSE agents to inactivation, there has been on-going concern regarding the potential transmission of sporadic Creutzfeldt-Jakob (sCJD) from affected to unaffected individuals through the inadequacy or failure of marginally-effective disinfection or sterilisation processes applied to medical devices and surgical instruments. In the past, sCJD has been transmitted accidentally from person-to-person through neurosurgical procedures as a consequence of using inappropriate techniques to sterilise instruments or devices that had been in contact with the brain tissue of sCJD-infected individuals¹. A survey also showed that individuals who had been subjected to neurosurgery were at a higher risk of developing sCJD in later years compared with the controls³. This might have resulted from the use of inadequate sterilising procedures for neurosurgical instruments in the past, but the study did not provide any information regarding the sterilisation procedures that had been used.

In addition to the cases that are known or suspected to be associated with the failure of sterilisation systems applied to instruments or medical devices, a number of cases of iatrogenic sCJD has arisen from two other sources. The treatment of humans with hormones derived from cadaveric human pituitary glands has resulted in a significant number of cases of sCJD among the recipients⁴. Validation studies carried out on the chromatographic system used most recently in the UK for hormone
production showed that this system was capable of inactivating infectivity, or removing it from the end-product. Also, a common feature of the UK cases was that they had received treatment between 1974 and 1976 with hormone prepared by procedures that preceded the chromatographic method. The use of pituitary-derived growth hormone was discontinued in the UK in 1985, by which time a genetically-engineered alternative was available; however, the pituitary-derived product was still used thereafter in other parts of the world.

Commercially prepared cadaveric dura mater from humans is used in surgical repair techniques and has also caused a significant number of iatrogenic cases of sCJD. The only validation study that has been published with regard to the TSE-related safety of commercially-produced dura mater showed that the level of TSE infectivity was reduced substantially, but not completely, by the manufacturing process. Initially, it was considered that any CJD infectivity that might be associated with dura mater was likely to be associated with either its natural contact with potentially-infected cerebrospinal fluid or through contamination with infected brain tissue during its collection. However, later studies involving dura mater from scrapie-infected mice demonstrated that this tissue does become infected, and that the infectivity levels are only around 100-fold less than those found in the brain-tissue of terminally-affected individuals.

The emergence of vCJD in the mid 1990s, and the subsequent occurrence of 127 cases in the UK by September 2002 has escalated the level of concern regarding potential person-to-person transmission through surgery. This is partly due to the fact that it is not possible to predict at present how many more cases will occur, but also because many more tissues (especially those of the lymphoreticular system [LRS]) become infected in vCJD- compared with sCJD-affected individuals. Preliminary evidence from the study of vCJD-infected individuals, and the much more wide-ranging studies of scrapie in sheep and laboratory animals, indicate that infectivity is likely to be present in LRS tissues for some considerable time before the central nervous system (CNS) becomes infected which is when clinical disease manifests itself. Surgeons are, therefore, likely to be carrying out procedures involving deliberate or incidental invasion of LRS tissues in individuals that have no signs of neurological disease but are incubating vCJD.

Although various examples of known and potential accidental transmission have been cited above, there is no evidence that human CJD-like diseases are normally contagious. There are no known examples of transmission from affected individuals to others simply by social contact within domestic or hospital environments. This would lead one to conclude that no, or very little, infectivity is released from infected individuals in urine, faeces, saliva and other secretions, or...
aerosols produced by coughing. Although there is a paucity of actual experimental data to support this contention, it is strongly supported by the circumstantial evidence, at least for sCJD. Even sexual contact between affected and unaffected individuals has not been found to enhance the risk of the unaffected partner or any resulting progeny becoming infected. However, this risk-factor does change significantly for the progeny, but not the sexual partners, of individuals that happen to be affected by the much less common forms of CJD-like diseases that appear to be directly associated with inherited mutations in the PrP gene. As has been discussed in previous chapters, these constitute around 15% of all cases of CJD-like diseases in humans. Based upon the above general observations the World Health Organization has concluded that the nursing at home of sCJD patients by family, friends or care-workers does not expose these individuals to any apparent risk.

While there is strong evidence that sCJD does not readily transmit from one individual to another simply by social contact, a somewhat more precautionary view might be appropriate with regard to vCJD because the disease is relatively new and clearly has a different pathogenic mechanism. Nevertheless, the existing epidemiological data relating to vCJD have not apparently shown any clustering of cases within families or other social groups living closely together. However, given the lengthy incubation periods associated with CJD-like diseases, one may have to wait for some time to conclude that person-to-person transmission of vCJD does not occur.

Although there is the theoretical possibility that medical personnel might acquire TSEs from patients through accidents such as needlestick injury, there are no known examples of this type of transmission. Until 1993, sCJD had been observed in 24 individuals who had been healthcare workers of various types, including a pathologist and two technicians who had worked in neurohistopathology laboratories. However, there was no evident association between their development of sCJD and any occupational exposure to the CJD agent. Furthermore, there was an interval of 40 years between the recognition of CJD as a distinct clinical condition and the suspicion that it might be infectious. Even though sCJD is a relatively rare disease, brain tissue from CJD-infected individuals must have been handled world-wide without significant precautions during this period by pathologists and laboratory personnel, but without any apparent increased incidence of the disease in such individuals. Nevertheless, the accidental transmission of sCJD to human recipients of CJD-contaminated human growth hormone by intramuscular injection demonstrates that occupationally acquired disease through trauma is a possibility. This is supported by the data from experiments in which mouse-passaged scrapie infectivity was relatively efficiently transmitted to mice through skin scarification.
However, the human growth hormone that was injected was derived from pituitary glands, and the scrapie-infected mouse-tissue used in the scarification experiments was brain. Both of these tissues are known to harbour relatively high levels of TSE infectivity, at least in individuals that display, or are close to displaying, clinical symptoms. Apart from severe trauma incidents in the emergency departments of hospitals, the only other exposure of medical personnel to CNS and LRS tissues is likely to be during surgery, autopsy or in pathology laboratories. This is in sharp contrast to the level of exposure experienced by medical personnel that are only exposed to needlestick or similar injuries during minor procedures such as the collection of blood from overt or subclinical cases of CJD. As will be discussed, it is still an open question as to whether the blood of humans infected with TSEs contains any infectivity. The current evidence is that, if it does, it must be at a relatively low level. For medical personnel that are likely to be exposed only to needlestick or comparable injury, the risk of acquiring CJD-like diseases from patients seems to be very low at present.

With regard to dental surgery, there is no evidence that sCJD has ever been transmitted from person-to-person as a consequence of dental treatment. The current opinion with regard to vCJD is that blood-associated risks associated with dentistry are likely to be absent or very low, and that minor surgical procedures should not introduce any increased element of risk. A slightly more cautious approach is appropriate with regard to the large nerves that might be traumatised during dental treatment until more is known about their capacity to become infected with the vCJD agent.

**Blood-associated risks for medical personnel and patients**

There are several reports in the literature that the blood from cases of sCJD has been found to be infectious when assayed in laboratory animals but these claims do not stand up to rigorous scrutiny. It has been observed\(^\text{13}\) that ‘one or more aspects of each report are puzzling...and invite a more cautious appraisal than might be accorded by the casual reader’. Numerous studies have shown no evidence of human-to-human transmission of sCJD through the transfusion of blood or plasma, or the administration of plasma-derived therapeutic products\(^\text{14,15}\), suggesting that the risk to medical personnel from needlestick injury must be very small. In carefully conducted studies, low levels of infectivity have been detected in the blood of mice challenged with mouse-passaged agents derived from either humans with Gerstmann-Straussler-Scheinker syndrome (a CJD-like disease) or cattle with BSE\(^\text{16,17}\). However, infectivity was only detectable in mouse blood during the terminal phase
of their disease when the animals displayed unequivocal signs of neurological disease. Although these experimental data are indicative, they provide no definitive evidence regarding the risk of human-to-human transmission of sCJD or vCJD via a blood-borne route. Recent unpublished, but widely-circulated, data from the Institute of Animal Health’s Neuropathogenesis Unit in Edinburgh, UK indicate that BSE has been transmitted from several experimentally-infected sheep to other sheep by blood transfusion when the blood was collected from the donor sheep during both the pre-clinical and clinical phases of the disease. This observation is considered to be of potential relevance to the question as to whether or not the blood of subclinical cases of vCJD might harbour infectivity. This relates to the fact that, like humans with vCJD, the LRS tissues of sheep with experimentally-induced BSE become infected before the onset of clinical disease. Also, the sheep experiments were not hampered by the ‘species barrier’ effect through which the efficiency of transmitting these types of diseases is more effective within a species rather than between species. Therefore, some caution has to be placed on the finding that blood from vCJD-infected individuals was not found to be infectious when injected intracerebrally into mice. This caution relates not only to the ‘species barrier’ effect but also to the relatively small volumes that could be injected into the brains of mice compared with the 400 ml volumes that were transfused to sheep. It would thus seem that the jury is still out with regard to the question as to whether or not the blood of subclinical or clinical cases of vCJD is infectious.

Inactivation methods

Over the past 50 years, an ever-increasing catalogue of decontamination measures that are ineffective against TSE agents has accumulated. It is anticipated that the agent that causes vCJD will share this property (because it is the BSE agent), but this has not yet been formally demonstrated. Even some inactivation procedures that were previously considered to be completely effective are now known to provide a substantial degree of, but not complete, inactivation. Such procedures include exposure to 1 M sodium hydroxide for an hour at room temperature, gravity-displacement autoclaving at 132°C for an hour, and porous-load autoclaving at 134–138°C for 18–60 min. Nevertheless, the recommended use of sodium hypochlorite solutions containing at least 20,000 ppm of available chlorine still appears to be an effective method although it is not a particularly user- or product-friendly procedure. Despite the doubts about the efficiency of achieving complete inactivation by either sodium hydroxide exposure or
autoclaving, a number of studies have indicated that complete inactivation can be achieved by combining these procedures consecutively or simultaneously, even at an autoclaving temperature of 121°C. In addition, an indication that these conditions provide a good degree of ‘overkill’ has been provided by studies in which the 301V strain of mouse-passaged BSE agent was completely inactivated after boiling in 1 M sodium hydroxide for only 1 min. The 301V agent is known to replicate to relatively high titres in mouse brain, and is the most thermostable mouse-passaged agent that has yet been identified.

As has already been discussed, one particular area of current concern is the potential for vCJD to be transmitted from patient-to-patient through surgical procedures. This concern arises from the probable wide-spread distribution of infectivity in the LRS of subclinical cases, and the known unreliability of steam sterilisation under worst-case conditions. Although it has been suggested that the washing processes to which instruments are subjected before they are steam-sterilised will annul any TSE-related problems, this view is not universally accepted. A more cautionary approach does seem to be appropriate, at least in the UK where a recent review of disinfection and sterilisation standards in hospitals revealed deficiencies in many of the hospitals that were inspected.

Bloody, mucoid or purulent materials are most easily removed from instruments and devices by washing them immediately after they have been used. If these materials are allowed to dry on the surfaces of instruments, their subsequent removal becomes a much more difficult task. This well-recognised fact led to the traditional practice whereby nursing staff in wards and theatres often wash soiled instruments locally, before sending them on for further washing, and then sterilisation, in specialised central surgical sterilisation departments (CSSDs). However, in recognition of the fact that such practices expose medical personnel to risks through possible trauma or the aerosols that are generated, there is an increasing trend towards discouraging the practice of local washing. While this will make it more difficult to achieve effective washing in CSSDs, the practice of local washing cannot be robustly defended since it does constitute some element of risk for those involved, and potentially for others in the same working area. It is, therefore, important to ensure that washer-disinfectors in CSSDs are fit for their purpose and are being operated under the optimal conditions set out in the appropriate guidelines. The effectiveness of the washer-disinfector process is critical to the whole washing/decontamination/sterilising process because, as has been previously stated, ‘if it isn’t clean you are not going to sterilise it’.

The safest method of dealing with instruments and devices is to make them single-use and dispose of them by incineration. Clearly, this option
is often not available, and other methods have to be used for disinfection and sterilisation. Nevertheless, it is currently recommended that all instruments used neurosurgically on patients with known or suspected TSEs should be disposed of by incineration. With regard to the more general aspect of processing instruments, there are additional measures that can be applied before steam-sterilisation to enhance the level of confidence that TSE infectivity will not survive. These have been listed and prioritised by the World Health Organization\textsuperscript{10} and are shown in Table 1. However, it is appreciated that these methods could not be readily incorporated into the systems used for the large-scale routine processing of instruments in CSSDs, and that their application would have to be confined to dealing with instruments that had been used in potentially risky situations.

### Table 1 Methods that can be applied to heat-resistant instruments for inactivating TSE agents arranged in order of effectiveness

<table>
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<tr>
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<th>Method Description</th>
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<tr>
<td>1</td>
<td>Gravity-displacement autoclaving at 121°C for 30 min in NaOH. Clean, rinse and subject to routine sterilisation</td>
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<tr>
<td>2</td>
<td>Immerse in NaOH or NaOCl for 1 h. Rinse. Immerse in water and expose to gravity-displacement autoclaving at 121°C for 1 h. Subject to routine sterilisation</td>
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<tr>
<td>3</td>
<td>Immerse in NaOH or NaOCl for 1 h. Rinse, and transfer to an open pan. Expose to porous-load autoclaving at 134°C for 1 h. Subject to routine sterilisation</td>
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<tr>
<td>4</td>
<td>Immerse in NaOH and boil for 10 min. Rinse and subject to routine sterilisation</td>
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<tr>
<td>5</td>
<td>Immerse in NaOCl (preferred) or NaOH for 1 h at ambient temperature. Rinse and subject to routine sterilisation</td>
</tr>
<tr>
<td>6</td>
<td>Porous-load autoclaving at 134°C for 18 min</td>
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NaOCl, sodium hypochlorite solution containing 20,000 ppm available chlorine; NaOH, 1 M sodium hydroxide solution.

**TSE-related safety with regard to fixed tissues**

Safety precautions to protect personnel from infectious agents are obviously appropriate in laboratories that receive unfixed tissues for rapid diagnosis or other purposes. In contrast, it is generally considered that formalin- or glutaraldehyde-fixed tissues received by pathology laboratories will be largely free from any infectious agents because of the well-known disinfection effect of aldehydes. However, with CJD-like diseases these ground-rules change because their causal agents survive fixation in formalin or glutaraldehyde. When hamster brain containing $10^{10.2}$ ID\textsubscript{50}/g of the 263K strain of scrapie agent was fixed in formol saline for 48 h, only 1.5 logs of infectivity were lost\textsuperscript{29}. Even after full histological processing, the titre loss was only 2.8 logs\textsuperscript{29}. Glutaraldehyde fixation is also known to permit survival of TSE infectivity\textsuperscript{30,31}. 
Consequently, the handling of fixed CJD-infected tissues by medical and paramedical personnel has been viewed as a potentially risky activity, and a number of procedures have been recommended to reduce this risk. One suggestion has been to fix such tissues in formol saline containing sodium hypochlorite\textsuperscript{32}. As has been discussed, high concentrations of sodium hypochlorite inactivate TSE agents, but there has been no validation of its effectiveness when combined with formalin. The addition of phenol to formol saline has also been suggested\textsuperscript{33–35}, but the basis of these proposals was flawed\textsuperscript{36} and phenolised formalin was shown subsequently to be not only ineffective but also produce poor fixation\textsuperscript{37,38}. Sections, stained with haematoxylin and eosin, prepared from scrapie-infected formol-fixed brain tissue that had been autoclaved at 134°C for 18 min retained sufficient integrity to permit quantitative scoring of spongiform changes in brain tissue\textsuperscript{39}, and it was suggested that autoclaving at 126°C for 30 min\textsuperscript{40} or 132°C for 6 min\textsuperscript{41} could be used to inactivate CJD infectivity in formol-fixed brain. However, mouse- or hamster-passaged scrapie agent in formol-fixed brain has been shown to survive porous-load autoclaving at 134°C for 18 min\textsuperscript{42} or gravity displacement autoclaving at 134°C for 30 min\textsuperscript{28} with titre losses of less than 2 logs. The only procedure that has been shown to result in significant losses of infectivity titre in formol-fixed tissues, without significant loss of microscopic morphology, is a 1-h exposure to concentrated formic acid\textsuperscript{37}. In that study, the level of infectivity in hamster brain infected with the 263K strain of scrapie agent was reduced from $10^{10.2}$ ID\textsubscript{50}/g to $10^{1.3}$ ID\textsubscript{50}/g. With mouse brain infected with CJD agent, the original titre of $10^{8.5}$ ID\textsubscript{50}/g was reduced to $10^{2.3}$ ID\textsubscript{50}/g. However, in another study, where mouse brain infected with the 301V strain of BSE agent was fixed using paraformaldehyde-lysine-periodate, a necessary prerequisite for the subsequent immunocytochemical investigation that is an important aspect of TSE investigation, the degree of inactivation by formic acid was calculated to be 2 logs less than that achieved with formol-fixed 263K-infected hamster brain, despite the equivalent levels of infectivity of the two agents\textsuperscript{43}. This suggests that either infected tissues fixed with paraformaldehyde-lysine-periodate are less amenable to the inactivating effect of formic acid than those fixed with formalin, or that there is a fundamental difference in the susceptibility of the 263K agent compared with 301V; alternatively, both factors may contribute to this observation. These studies demonstrate that, although substantial degrees of inactivation can be achieved, there is no known decontamination procedure that can guarantee the complete absence of infectivity in TSE-infected tissues that have been processed by histopathological procedures.

It is inappropriate to use autoclaving for the disposal of TSE-infected fixed tissues because fixation in alcohol or formalin has been shown to enhance considerably the resistance of infectivity to inactivation by autoclaving\textsuperscript{28,42,44}. 

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