



# Inactivation of Transmissible Degenerative Encephalopathy Agents: A Review

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## SUMMARY

The unconventional agents that cause transmissible degenerative encephalopathies, such as bovine spongiform encephalopathy, scrapie, and Creutzfeldt-Jakob disease (CJD), are relatively resistant to inactivation by standard decontamination procedures. The only methods that appear to be completely effective under worst-case conditions are strong sodium hypochlorite solutions or hot solutions of sodium hydroxide. Other procedures that result in significant degrees of inactivation are described. The infectivity levels in histologically-fixed tissue can be reduced substantially by treatment with concentrated formic acid without adversely affecting the microscopic quality of the tissue. © 2000 Harcourt Publishers Ltd

KEYWORDS: BSE; scrapie; CJD; agent inactivation.

## INTRODUCTION

The transmissible degenerative encephalopathies (TDE) include bovine spongiform encephalopathy (BSE), scrapie in sheep and goats and Creutzfeldt-Jakob disease (CJD) in humans. A unique feature of TDE is that the host's normal PrP protein becomes modified as a consequence of infection (Carp *et al.*, 1985), and forms pathological deposits in affected tissues. The highest level of expression of PrP protein is in the central nervous system (CNS), where the pathological deposits are most evident. This is usually associated topographically with neuronal vacuolation (Bruce *et al.*, 1989), which is why these diseases are often described as spongiform encephalopathies. However, because spongiform vacuolation is not always present, these diseases are more accurately described as TDE (Taylor, 1991a).

The normal form of PrP is designated PrP<sup>sen</sup>, because it is sensitive to digestion by proteolytic

enzymes; the disease-specific form, designated PrP<sup>res</sup> because of its resistance to proteolytic digestion, forms pathological deposits. Although PrP<sup>sen</sup> and PrP<sup>res</sup> are chemically identical, there are differences in their tertiary structures. PrP<sup>res</sup> has a significantly higher ratio of  $\beta$ -sheets, and a lower ratio of  $\alpha$ -helical structures, compared with PrP<sup>sen</sup>. Although it is unknown whether TDE agents consist entirely of host-specific molecules such as PrP<sup>res</sup> or whether non-host informational molecules such as nucleic acids may be an essential adjunct (Bruce *et al.*, 1994; Chesebro, 1998; Farquhar *et al.*, 1998), it is generally agreed that PrP<sup>res</sup> is an important component. Regardless of their true chemical identity, TDE agents are relatively resistant to inactivation (Taylor, 1993, 1999a), as will be discussed in this review.

## STRAINS OF TDE AGENTS

Reference will be made to strains of TDE agents obtained by serial passage in laboratory animals. These are defined in Table I.

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Table I  
Nomenclature of strains of TDE agents

Strain	Source
ME7	mouse-passaged scrapie
22A	mouse-passaged scrapie
139A	mouse-passaged scrapie
263K	hamster-passaged scrapie
301V	mouse-passaged BSE
S. Co.	guinea pig-passaged CJD
Fukuoka-1	mouse-passaged CJD
Kitasoto-1	mouse-passaged CJD
K. Fu	hamster-passaged CJD
SY	hamster-passaged CJD

## PHYSICAL METHODS OF DECONTAMINATION

### *Irradiation*

Ionizing, ultraviolet and microwave irradiation have little effect on TDE agents (see, for example, Latarjet, 1979; Taylor & Diprose, 1996), and have no practical application in their inactivation.

### *Dry heat*

A small fraction of 263K infectivity survived exposure to dry heat at 360°C for 1 h (Brown *et al.*, 1990a), but the brain-homogenate had been lyophilized and was heated under anoxic conditions. Drying of scrapie-infected tissue is known to enhance its thermostability (Asher *et al.*, 1986, 1987). In contrast, when 7-mg samples of non-lyophilized, macerated, ME7-infected mouse-brain were exposed to dry heat, there was no detectable infectivity after an exposure to 200°C for 1 h, even though some infectivity survived exposure to 160°C for 24 h or 200°C for 20 min (Taylor *et al.*, 1996). However, 263K and 301V partially survived exposure at 200°C for 1 h (Steele *et al.*, 1999).

### *Autoclaving*

This section considers data derived from gravity-displacement (GD) and porous-load (PL) autoclaving studies. In GD autoclaves, air is expelled by allowing steam to migrate to the upper area of the chamber before progressively filling it. In contrast, air is removed from PL autoclaves by high vacuum. A major difference between GD and PL autoclaving is that in the latter steam rapidly floods the autoclave chamber during the sterilizing stage. This is relevant to some of the PL autoclaving data that will be discussed.

### *Gravity-displacement autoclaving*

GD autoclaving at 121°C for 90 min reduced the titre of 263K by 6 logs, but 3.4 logs survived (Prusiner *et al.*, 1984). This is in good agreement with the data obtained by Ernst and Race (1993) using the same agent and the same autoclaving regime, showing a 5.7 log reduction. Autoclaving the same agent at 134°C for 30 min reduced the titre by 5.3 logs, but 3.5 logs survived (Brown *et al.*, 1990a). Autoclaving at 126°C for 2 h inactivated 139A but not 22A (Kimberlin *et al.*, 1983), which was inactivated only after a 4 h exposure (Dickinson, 1976). S.Co. and 263K were reported to be inactivated at 132°C for 1 h (Brown *et al.*, 1986). Consequently, this procedure was adopted as a standard for CJD agent decontamination in the USA (Rosenberg *et al.*, 1986) and elsewhere. However, subsequent studies showed that this procedure does not guarantee complete inactivation (Ernst & Race, 1993; Pocchiari, 1993; Taylor, 1996). Autoclaving at 132°C for 4.5 h has been recommended (Prusiner *et al.*, 1984), and Ernst and Race (1993) have shown that 263K is inactivated by a cycle at 132°C for 90 min.

### *Porous-load autoclaving*

Fifty-milligram samples of macerated brain containing 22A or 139A were inactivated by exposure to PL autoclaving at 136°C for 4 min (Kimberlin *et al.*, 1983), and it was consequently recommended that a cycle of 134–138°C for 18 min, or six cycles of 3 min, should be used to inactivate CJD agent (DHSS, 1984). However, it was advised that instruments used in neurosurgery or ophthalmological surgery on known or suspected cases of CJD should be discarded rather than recycled after autoclaving. This advice was later extended to include other high-risk groups such as blood relatives of families with a known susceptibility to CJD, and individuals who had been recipients of (a) hormones derived from the pituitary glands of human cadavers, (b) dura mater graft material derived from human cadavers, or (c) human corneal grafts. It has been suggested that thorough cleaning could sufficiently reduce the amount of infectivity remaining on such instruments to enable them to be reliably sterilized by subsequent autoclaving (Ayliffe, 1993), but there has been concern about the associated possibilities for cross-contamination and occupational exposure (Taylor & Bell, 1993). The emergence of a new variant (nv) form of CJD, and its apparent association with the BSE agent (Bruce *et al.*, 1997) has heightened concern regarding the safety of

surgical instruments. This is because, unlike other forms of CJD, PrP<sup>res</sup> is readily detectable in the lymphoreticular tissues in cases of nvCJD, which suggests that instruments used in general surgery on suspect cases may also need to be discarded. The future scale of the new variant CJD epidemic is unknown, but dealing with surgical instruments generally could become a significant problem if it is large.

Conflicting data for PL autoclaving has resulted from using 340-mg samples of infected brain macerate (Taylor *et al.*, 1994). The larger samples were used because intact (unmacerated) tissue of approximately similar weights had been inactivated by PL autoclaving at 134–138°C for 18 min (Taylor & McConnell, 1988; Taylor, 1986, 1996), and might be closer to the maximum weight of infected tissue that might have to be dealt with by autoclaving in the course of human or veterinary healthcare (although no official advice has ever been issued in this respect). The experiments showed that, using 340-mg samples of macerate, BSE, ME7 and 263K were not inactivated completely by PL cycles of up to 1 h at 134–138°C; the residual titre was about two logs in the case of 263K. However, with such large samples, there was some smearing and drying of the brain-tissue onto the surfaces of the glass containers before autoclaving.

Scrapie agent is more resistant to inactivation by autoclaving when infected brain-tissue is dried onto glass or metal surfaces (Asher *et al.*, 1986, 1987). Consequently, the study involving 340-mg macerates is more relevant to everyday practice, and casts doubt on the appropriateness of the current UK recommendation to use a PL cycle at 134–138°C for 18 min to destroy such agents. In view of these uncertainties, experiments were carried out to assess the effectiveness of PL autoclaving cycles at 134, 136 and 138°C for times ranging between 9 and 60 min, using samples of infected brain macerates weighing either 50 or 375 mg. The agents used were: (a) 22A, which is known to be more thermostable than other strains of mouse-passaged scrapie agent (Dickinson & Taylor, 1978; Kimberlin *et al.*, 1983); (b) 263K, which had more recently been shown to survive PL autoclaving (Taylor *et al.*, 1994); and (c) 301V, which had not been tested previously. The results from these experiments (Taylor, 1999b) showed that 301V can survive exposure to 138°C for 1 h. However, 50-mg macerates of 22A-infected brain-tissue in which the infectivity levels were  $\geq 10^{7.2}$  ID<sub>50</sub> were inactivated by all of the 136°C

processes, which accords with earlier data (Kimberlin *et al.*, 1983); the same is true for the 50-mg macerates exposed for four different time-periods at 134°C. Paradoxically, one case was observed in mice injected with material from a 50-mg sample autoclaved at 138°C for 9 min, and cases were also observed in mice injected with material from 375-mg macerates autoclaved at 136 or 138°C (but not at 134°C). These data suggest that the thermostability of 22A strain was enhanced as the temperature of autoclaving was increased, and the difference between the 134 and 138°C samples was significant ( $P < 0.01$ ). With 263K, the starting titre was  $10^{8.3}$  ID<sub>50</sub>/g, and there was much the same degree of survival of the agent whether autoclaving was carried out at 134, 136, or 138°C, which would support the above hypothesis. For 301V, which had a starting titre of  $10^{2.6}$  ID<sub>50</sub>/g, the data are even more convincing, because 60% of the animals that were injected with material autoclaved at 134°C developed disease; the ratio for similar samples exposed at 138°C was 72%, and this is significant ( $P < 0.05$ ). These data indicate that simply increasing PL autoclaving temperatures and holding times would not necessarily be effective in achieving a reliable decontamination standard for TDE agents.

The lesser efficiency of inactivating partially smeared and dried macerated tissue, compared with intact undisrupted tissue, may relate to the very rapid heating that occurs in the film of dried material (compared with the bulk of the sample), and the consequent very rapid fixation of PrP<sup>res</sup> in the dried film. Protection by fixation has been shown to occur during the inactivation of poliovirus by formalin (Gard & Maaloe, 1959). Similarly, prior fixation in ethanol (Taylor, 1996) or formalin (Taylor & McConnell, 1988) has been shown to enhance considerably the thermostability of the scrapie agent. Although the molecular nature of TDE agents has not yet been characterized (Chesebro, 1998), it is likely that PrP<sup>res</sup> is a component, but it is uncertain what accounts for the characteristics of the subpopulation of scrapie agent that is intrinsically more thermostable than the main population. The stability of this subpopulation after autoclaving is shown in Fig. 1, which demonstrates that, after an initial large decline in titre, the residual infectivity resists further inactivation for 1 h. The dotted lines show that the predicted times of 5 or 23 min for complete inactivation, based upon the two extreme possibilities for the shape of the initial inactivation curve,

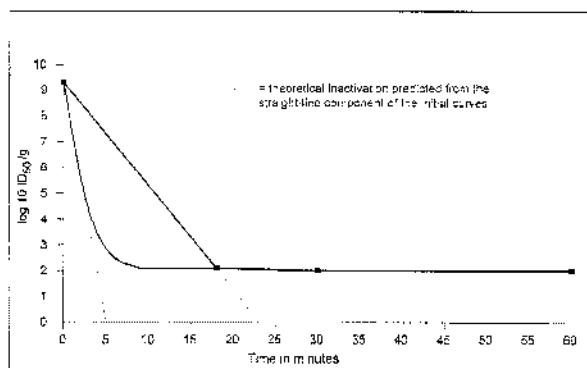


Fig. 1. The inactivation curve for the 263K strain of scrapie agent during autoclaving at 134°C showing the maximal and minimal effect between zero time and eighteen minutes

are highly inaccurate. The persistent stability of the thermostable subpopulation has also been demonstrated by the loss of only 1.7 logs of 263K after a second exposure to PL autoclaving that resulted in a loss of 3.3 logs during the first cycle (Taylor *et al.*, 1998a).

## CHEMICAL METHODS OF DECONTAMINATION

### *Acids and bases*

Mould *et al.* (1965) detected little inactivation of scrapie infectivity over the pH range 2–10, and there was little inactivation of S. Co. exposed to hydrochloric acid (pH 0.1) for 1 h (Brown *et al.*, 1986). However, exposing histologically-fixed tissues to concentrated formic acid, which solubilizes proteins, reduces TDE infectivity levels (by up to 8 logs) to around 100 ID<sub>50</sub>/g without significantly affecting the microscopic morphology of the tissue (Brown *et al.*, 1990b; Taylor, 1995; Taylor *et al.*, 1997b).

It has been reported that a 1-h exposure to 1 M sodium hydroxide (pH 14) inactivates S. Co. and 263K (Brown *et al.*, 1986). However, the sensitivity of these bioassays was reduced because it proved necessary to dilute the samples considerably to render them non-toxic for the recipient animals. Residual infectivity has been detected following treatment of 263K with 1 M sodium hydroxide (Diringer & Braig, 1989; Ernst & Race, 1993) even for periods of up to 24 h (Prusiner *et al.*, 1984). Kitasoto-1 and Fukuoka-1 have also been reported to survive exposure to 1 M (Tamai *et al.*, 1988) or 2

M sodium hydroxide (Tateishi *et al.*, 1988), respectively. More recent work with sodium hydroxide, involving ME7, 263K and BSE (Taylor *et al.*, 1994) has demonstrated that if the samples are carefully neutralized they can be injected without further dilution. Under these circumstances, infectivity can be shown to survive exposure to 2 M sodium hydroxide for up to 2 h. With 263K, although more than 5 logs of infectivity were lost following such treatments, around 4 logs survived.

Although autoclaving or exposure to sodium hydroxide are not completely effective *per se* for inactivating TDE agents, inactivation can be achieved by combining these procedures. Taguchi *et al.* (1991) and Ernst and Race (1993) described the successful inactivation of Kitasoto-1 and 263K by a sequential process involving exposure to 1 M sodium hydroxide, followed by GD autoclaving at 121°C for 30 or 60 min, respectively. Complete inactivation of 263K has also been reported after GD autoclaving at 121°C for 90 min in the presence of 1 M sodium hydroxide (Prusiner *et al.*, 1984). It has also been observed that if 22A is autoclaved at 121°C for 30 min in the presence of 2 M sodium hydroxide (without a prior holding period in sodium hydroxide), inactivation can be achieved (Taylor *et al.*, 1997a). More recently, it has been shown that 301 V, which is high titre and extremely thermostable, can be inactivated by boiling in 1 M sodium hydroxide for 1 min (Taylor *et al.*, 1999a).

### *Alkylating agents*

There is little effect on TDE agents after exposure to formalin (e.g. Fraser *et al.*, 1992), glutaraldehyde (Dickinson & Taylor, 1978), acetyleneimine (Stamp *et al.*, 1959),  $\beta$ -propiolactone (Haig & Clarke, 1968) or ethylene oxide (Brown *et al.*, 1982a; Dickinson & Taylor, 1978).

### *Detergents*

Mild detergents have little effect on TDE agents (Millson *et al.*, 1976), but sodium dodecyl sulphate (SDS) has some effect on CJD (Walker *et al.*, 1983) and scrapie infectivity (Millson *et al.*, 1976), which is enhanced by heat (Kimberlin *et al.*, 1983). Tateishi *et al.* (1991) have reported that boiling infected tissue homogenates for 3 min in 3% SDS is effective. However, survival of infectivity has been reported when 50-mg samples of macerated brain are boiled (or autoclaved at 121°C) in 5% SDS for 15 min (Taylor *et al.*, 1999b). The use of hot SDS must therefore be confined to dealing with

contaminated fluids. The only other detergent which reduces titre is sarkosyl, but at much higher concentrations.

#### *Halogens*

Sodium hypochlorite solutions containing up to 25 000 p.p.m. available chlorine have been reported to inactivate S.Co. and 263K (Brown *et al.*, 1986). In an extensive study with 22A and 139A, it was demonstrated that a solution containing 13 750 p.p.m. available chlorine is effective at 30 min, leading to the recommendation that 20 000 p.p.m. for 1 h should be used in practice (Kimberlin *et al.*, 1983). A study was conducted using two sources of BSE which were exposed for 30–120 min to solutions of sodium hypochlorite or sodium dichloroisocyanurate containing comparable levels of available chlorine (ranging from 8250 to 16 500 p.p.m.). No infectivity was detectable in any of the hypochlorite-treated samples, but infectivity was recoverable from samples which had been treated with the dichloroisocyanurate solutions (Taylor *et al.*, 1994). It was shown that, at the end of the various exposure periods, the dichloroisocyanurate solutions had released 3.5 times less chlorine than the hypochlorite solutions.

Only a modest reduction in scrapie infectivity was obtained using 2% iodine in sodium iodide for 4 h (Brown *et al.*, 1982b). Similar results were observed with scrapie and CJD agents using an iodophor containing 0.8% iodine (Asher *et al.*, 1981).

#### *Organic solvents*

There is little effect on TDE infectivity following treatment with acetone (Hunter & Millson, 1964), 5% chloroform (Wilson, 1955), ether (Gajdusek & Gibbs, 1968), ethanol (Dickinson & Taylor, 1978), 4% phenol (Wilson, 1955) or proprietary phenolic disinfectants (Brown *et al.*, 1983; Kimberlin *et al.*, 1983). Little inactivation of 22A and 301V was achieved when exposed to laboratory facsimiles of commercial solvent-extraction processes involving hexane, heptane, perchlorethylene or petroleum, as used traditionally by the rendering industry (Taylor *et al.*, 1998b).

#### *Oxidizing agents*

Exposure of scrapie agents to chlorine dioxide inactivated only a small proportion of infectivity (Brown *et al.*, 1982b). Treatment of scrapie agent with 3% hydrogen peroxide caused little inactivation (Brown *et al.*, 1982a). Scrapie-infected brain

homogenates were not inactivated by concentrations of up to 18% peracetic acid, but 2% inactivated intact brain tissue (Taylor, 1991b). These apparently anomalous results are considered to demonstrate the protective effect of aggregation which occurs in homogenized preparations.

#### *Salts*

The single report that sodium metaperiodate has a considerable effect on scrapie infectivity (Hunter *et al.*, 1969) is contradicted by several others (Adams *et al.*, 1972; Dickinson, 1976; Brown *et al.*, 1982b). Similarly, a claim that potassium permanganate inactivates all CJD and scrapie infectivity in homogenates of brain tissue (Asher *et al.*, 1981) is challenged by data from other experiments (Brown *et al.*, 1982a, 1986; Kimberlin *et al.*, 1983).

#### *Chaotropes*

The claim that urea is an effective scrapicide (Millson *et al.*, 1976) is not supported by other studies that have been reviewed (Brown *et al.*, 1986). High concentrations (> 4 M) of guanidine thiocyanate (GdnSCN) are known to be relatively effective with infected brain-homogenates. Lower concentrations, or the use of less chaotropic salts, such as guanidine hydrochloride (GdnHCl), are less effective. PrP<sup>res</sup> is denatured in 2–4 M GdnHCl, depending on the degree of purification, pH etc., and this correlates with some loss of infectivity. It has been reported that 0.4 M GdnSCN is an effective sterilant for some types of CJD-contaminated surgical instruments (Manuelidis, 1998). However, the study on which this claim was based did not involve the use of contaminated instruments, but used 10% homogenates of SY-infected brain-tissue (Manuelidis, 1997). Also, the sensitivity of the bioassays was reduced 200-fold by the need to dilute the treated brain homogenates to avoid toxicity in the assay animals, and the experiment was incomplete at the time of publication.

#### *Proteolytic enzymes*

Proteases such as trypsin in non-denaturing conditions have little effect (Hunter & Millson, 1967; Millson *et al.*, 1976), producing titre losses of <1 log which are not significant. However, broad spectrum proteases, such as pronase (Millson *et al.*, 1976) and proteinase K (Prusiner *et al.*, 1981), can reduce titre significantly after prolonged digestion times. Other proteases, including Quiagen™, may also be effective.

### Conclusions

Procedures that efficiently and/or rapidly fix proteins protect TDE agents from inactivation, and enhance their thermostability. Such treatments include alcohols and aldehydes and, paradoxically, rapid heating with steam. The development of user- and product-friendly inactivation procedures are likely to result from the study of methods, or combinations thereof, that disrupt protein structure rather than fixing it.

The most thermostable strain of TDE agent that has been isolated to date 301 V. Since there are no apparent differences in the sensitivity of different strains of TDE agents to chemicals, 301 V is the preferred generic model for inactivation studies at present.

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### REFERENCES

- ADAMS, D.H., FIELD, E.J. & JOYCE, G. (1972). Periodate—an inhibitor of the scrapie agents? *Research in Veterinary Science* **13**, 195–8.
- ASHER, D.M., GIBBS, C.J., DEWAN, A.R., KINGSBURY, D.T., SULMA, M.P. & GAJDUSEK, D.C. (1981). Effects of several disinfectants and gas sterilisation on the infectivity of scrapie and Creutzfeldt-Jakob disease. *Abstracts of the Twelfth World Congress of Neurology*, Kyoto, 20–25 September, p. 225.
- ASHER, D.M., POMEROY, K.L., MURPHY, L., ROHWER, R.G., GIBBS, C.J. & GAJDUSEK, D.C. (1986). Practical inactivation of scrapie agent on surfaces. *Abstracts of the IXth International Congress of Infectious and Parasitic Diseases*, Munich, 20–26 July.
- ASHER, D.M., POMEROY, K.L., MURPHY, L., GIBBS, C.J. & GAJDUSEK, D.C. (1987). Attempts to disinfect surfaces contaminated with etiological agents of the spongiform encephalopathies. *Abstracts of the VIIIth International Congress of Virology*, Edmonton, 9–14 August, p. 147.
- AYLFFE, G.A.J. (1993). Surgical instruments and disease transmission. *Lancet* **341**, 1098.
- BROWN, P., GIBBS, C.J., AMYX, H.L., KINGSBURY, D.T., ROHWER, R.G., SULMA, M.P. & GAJDUSEK, D.C. (1982a). Chemical disinfection of Creutzfeldt-Jakob disease virus. *New England Journal of Medicine* **306**, 1279–82.
- BROWN, P., ROHWER, R.G., GREEN, E.M. & GAJDUSEK, D.C. (1982b). Effects of chemicals, heat and histopathological processing on high-infectivity hamster-adapted scrapie virus. *Journal of Infectious Diseases* **145**, 683–7.
- BROWN, P., ROHWER, R.G., GREEN, E.M. & GAJDUSEK, D.C. (1983). The effect of chemicals, heat, and histopathologic processing on high-infectivity hamster-adapted scrapie virus. In *Virus non Conventioneels et Affections du Systeme Nerveux Central*, eds. L.A. Court & F. Cathala, pp. 156–63. Paris: Masson.
- BROWN, P., ROHWER, R.G. & GAJDUSEK, D.C. (1986). Newer data on the inactivation of scrapie virus or Creutzfeldt-Jakob disease virus in brain tissue. *Journal of Infectious Diseases* **153**, 1145–8.
- BROWN, P., LIBERSKI, P.P., WOLFF, A. & GAJDUSEK, D.C. (1990a). Resistance of scrapie agent to steam autoclaving after formaldehyde fixation and limited survival after ashing at 360°C: practical and theoretical implications. *Journal of Infectious Diseases* **161**, 467–72.
- BROWN, P., WOLFF, A. & GAJDUSEK, D.C. (1990b). A simple and effective method for inactivating virus infectivity in formalin-fixed tissue samples from patients with Creutzfeldt-Jakob disease. *Neurology* **40**, 887–90.
- BRUCE, M.E., MCBRIDE, P.A. & FARQUHAR, C.F. (1989). Precise targeting of the pathology of the sialoglycoprotein, PrP, and vacuolar degeneration in mouse scrapie. *Neuroscience Letters* **102**, 1–6.
- BRUCE, M.E., CHREE, A., MCCONNELL, I., FOSTER, J., PEARSON, G. & FRASER, H. (1994). Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. *Philosophical Transactions of the Royal Society B* **343**, 405–11.
- BRUCE, M.E., WILL, R.G., IRONSIDE, J.W., MCCONNELL, I., DRUMMOND, D., SUTTIE, A., MCCARDLE, L., CHRER, A., HOPE, J., BIRKETT, C., COUSENS, S., FRASER, H. & BOSTOCK, C.J. (199). Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Lancet* **389**, 498–501.
- CARP, R.I., MERZ, P.A., KASCSAK, R.J., MERZ, G. & WISNIEWSKI, H.M. (1985). Nature of the scrapie agent: current status of facts and hypotheses. *Journal of General Virology* **66**, 1357–68.
- CHESEBRO, M. (1998). BSE and prions: uncertainties about the agent. *Science* **279**, 42–3.
- DHSS (1984). Management of patients with spongiform encephalopathy Creutzfeldt-Jakob disease (CJD)]. DHSS Circular DA (84) 16.
- DICKINSON, A.G. (1976). Scrapie in sheep and goats. In *Slow Virus Diseases of Animals and Man*, ed. R.H. Kimberlin, pp. 209–241. Amsterdam: North-Holland.
- DICKINSON, A.G. & TAYLOR, D.M. (1978). Resistance of scrapie agent to decontamination. *New England Journal of Medicine* **229**, 1413–4.
- DIRINGER, H. & BRAIG, H.R. (1989). Infectivity of unconventional viruses in dura mater. *Lancet* **i**, 439–40.
- ERNST, D.R. & RACE, R.E. (1993). Comparative analysis of scrapie agent inactivation. *Journal of Virological Methods* **41**, 193–202.
- FARQUHAR, C.F., SOMERVILLE, R.A. & BRUCE, M.E. (1998). Straining the prion hypothesis. *Nature* **391**, 345–6.
- FRASER, H., BRUCE, M.E., CHREE, A., MCCONNELL, I. & WELLS, G.A.H. (1992). Transmission of bovine spongiform encephalopathy and scrapie to mice. *Journal of General Virology* **173**, 1891–7.

- GAJDUSEK, D.C. & GIBBS, C.J. (1968). Slow, latent and temperate virus infections of the central nervous system. In *Infections of the Nervous System*, ed. H.M. Zimmerman, pp. 254-80. Baltimore: Williams & Wilkins.
- GARD, S. & MAALOE, O. (1959). Inactivation of viruses. In *The Viruses*, vol. 1, eds. F.M. Burnet & W.M. Stanley, pp. 359-427. New York: Academic Press.
- HAIG, D.A. & CLARKE, M.C. (1968). The effect of B-propiolactone on the scrapie agent. *Journal of General Virology* **3**, 281-3.
- HUNTER, G.D. & MILLSON, G.C. (1964). Further experiments on the comparative potency of tissue extracts from mice infected with scrapie. *Research in Veterinary Science* **5**, 149-53.
- HUNTER, G.D. & MILLSON, G.C. (1967). Attempts to release the scrapie agent from tissue debris. *Journal of Comparative Pathology* **77**, 301-7.
- HUNTER, G.D., GIBBONS, R.A., KIMBERLIN, R.H. & MILLSON, G.C. (1969). Further studies of infectivity and stability of extracts and homogenates derived from scrapie affected mouse brains. *Journal of Comparative Pathology* **79**, 101-8.
- KIMBERLIN, R.H., WALKER, C.A., MILLSON, G.C., TAYLOR, D.M., ROBERTSON, P.A., TOMLINSON, A.H. & DICKINSON, A.G. (1983). Disinfection studies with two strains of mouse passaged scrapie agent. *Journal of the Neurological Sciences* **59**, 355-69.
- LATARJET, R. (1979). Inactivations of the agents of scrapie, Creutzfeldt-Jakob disease and kuru by radiations. In *Slow Transmissions of the Central Nervous System*, vol. 2, eds. S.B. Prusiner & W.J. Hadlow, pp. 387-408. New York: Academic Press.
- MANUELIDIS, L. (1997). Decontamination of Creutzfeldt-Jakob Disease and other transmissible agents. *Journal of Neurovirology* **3**, 62-5.
- MANUELIDIS, L. (1998). Cleaning CJD-contaminated instruments. *Science* **281**, 1961.
- MILLSON, G.C., HUNTER, G.D. & KIMBERLIN, R.H. (1976). The physico-chemical nature of the scrapie agent. In *Slow Virus Diseases of Animals and Man*, ed. R.H. Kimberlin, pp. 243-66. Amsterdam: North-Holland.
- MOULD, D.L., DAWSON, A.M. & SMITH, W. (1965). Scrapie in mice. The stability of the agent to various suspending media, pH and solvent extraction. *Research in Veterinary Science* **6**, 151-4.
- POCCHIARI, M. (Unpublished data cited by HORAUD, F.) (1993). Safety of medicinal products: summary. *Developments in Biological Standardization* **80**, 207-8.
- PRUSINER, S.B., MCKINLEY, M.P., GROTH, D.F., BOWMAN, K.A., MOCK, N.I., COCHRAN, S.P. & MASIAZ, F.R. (1981). Scrapie agent contains a hydrophobic protein. *Proceedings of the National Academy of Science* **78**, 6675-9.
- PRUSINER, S.B., MCKINLEY, M.P., BOLTON, D.C., BOWMAN, K.A., GROTH, D.F., COCHRAN, S.P., HENNESSEY, E.M., BRAUNFELD, M.B., BARINGER, J.R. & CHATIGNY, M.A. (1984). Prions: methods for assay, purification, and characterisation. In *Methods in Virology*, vol. viii, eds. K. Maramorosch & H. Koprowski. New York: Academic Press, pp. 293-345.
- ROSENBERG, R.N., WHITE, C.L., BROWN, P., GAJDUSEK, D.C., VOLPE, J.J., POSNER, J. & DYCK, P. (1986). Precautions in handling tissues, fluids, and other contaminated materials from patients with documented or suspected Creutzfeldt-Jakob disease. *Annals of Neurology* **19**, 75-7.
- STAMP, J.T., BROTHERTON, J.C., ZLOTNIK, I., MCKAY, J.M.K. & SMITH, W. (1959). Further studies on scrapie. *Journal of Comparative Pathology* **69**, 268-80.
- STEELE, P.J., TAYLOR, D.M. & FERNIE, K. (1999). Survival of BSE and scrapie agents at 200°C. *Abstracts of a Meeting of the Association of Veterinary Teachers and Research Workers*. Scarborough, 29-31 March 1999, p. 21.
- TAGUCHI, F., TAMAI, Y., UCHIDA, K., KITAJIMA, R., KOJIMA, H., KAWAGUCHI, T., OHTANI, Y. & MIURA, S. (1991). Proposal for a procedure for complete inactivation of the Creutzfeldt-Jakob disease agent. *Archives of Virology* **119**, 297-301.
- TAMAI, Y., TAGUCHI, F. & MIURA, S. (1988). Inactivation of the Creutzfeldt-Jakob disease agent. *Annals of Neurology* **24**, 466-467.
- TATEISHI, J., TASHIMA, T. & KITAMOTO, T. (1988). Inactivation of the Creutzfeldt-Jakob disease agent. *Annals of Neurology* **24**, 466.
- TATEISHI, J., TASHIMA, T. & KITAMOTO, T. (1991). Practical methods for chemical inactivation of Creutzfeldt-Jakob disease pathogen. *Microbiology and Immunology* **35**, 163-6.
- TAYLOR, D.M. (1986). Decontamination of Creutzfeldt-Jakob disease agent. *Annals of Neurology* **20**, 749.
- TAYLOR, D.M. (1991a). Spongiform encephalopathies. *Neuropathology and Applied Neurobiology* **17**, 345-6.
- TAYLOR, D.M. (1991b). Resistance of the ME7 scrapie agent to peracetic acid. *Veterinary Microbiology* **27**, 19-24.
- TAYLOR, D.M. (1993). Inactivation of SE agents. *British Medical Bulletin* **49**, 810-21.
- TAYLOR, D.M. (1995). Survival of mouse-passaged bovine spongiform encephalopathy agent after exposure to paraformaldehyde-lysine-periodate and formic acid. *Veterinary Microbiology* **44**, 111-2.
- TAYLOR, D.M. (1996). Transmissible subacute spongiform encephalopathies: practical aspects of agent inactivation. In *Transmissible Subacute Spongiform Encephalopathies: Prion Disease. IIIrd International Symposium on Subacute Spongiform Encephalopathies: Prion Diseases Paris, 18-20 March 1996*, eds. L. Court & D. Dodet, pp. 479-482.
- TAYLOR, D.M. (1999a). Transmissible degenerative encephalopathies. Inactivation of the causal agents. In *Principles and Practice of Disinfection Preservation and Sterilisation*, eds. A.D. Russell, W.B. Hugo & G.A.J. Ayiliffe, pp. 222-36. Oxford: Blackwell Scientific Publications.
- TAYLOR, D.M. (1999b). Inactivation of prions by physical and chemical means. *Journal of Hospital Infection* **43** (Suppl.), S69-76.
- TAYLOR, D.M. & BELL, J.E. (1993). Prevention of iatrogenic transmission of Creutzfeldt-Jakob disease. *Lancet* **341**, 1543-4.
- TAYLOR, D.M. & DIPROSE, M.F. (1996). The response of the 22A strain of scrapie agent to microwave irradiation compared with boiling. *Neuropathology and Applied Neurobiology* **22**, 256-8.

- TAYLOR, D.M. & MCCONNELL, I. (1988). Autoclaving does not decontaminate formol-fixed scrapie tissues. *Lancet* **i**, 1463-4.
- TAYLOR, D.M., FRASER, H., MCCONNELL, I., BROWN, D.A., BROWN, K.L., LAMZA, K.A. & SMITH, G.R.A. (1994). Decontamination studies with the agents of bovine spongiform encephalopathy and scrapie. *Archives of Virology* **139**, 313-26.
- TAYLOR, D.M., MCCONNELL, I. & FERNIE, K. (1996). The effect of dry heat on the ME7 strain of scrapie agent. *Journal of General Virology* **77**, 3161-4.
- TAYLOR, D.M., FERNIE, K. & MCCONNELL, I. (1997a). Inactivation of the 22A strain of scrapie agent by autoclaving in sodium hydroxide. *Veterinary Microbiology* **58**, 87-91.
- TAYLOR, D.M., BROWN, J.M., FERNIE, K. & MCCONNELL, I. (1997b). The effect of formic acid on BSE and scrapie infectivity in fixed and unfixed brain-tissue. *Veterinary Microbiology* **58**, 167-74.
- TAYLOR, D.M., FERNIE, K., MCCONNELL, I. & STEELE, P.J. (1998a). Observations on thermostable populations of the unconventional agents that cause transmissible degenerative encephalopathies. *Veterinary Microbiology* **64**, 33-8.
- TAYLOR, D.M., FERNIE, K., MCCONNELL, I., FERGUSON, C.E. & STEELE, P. (1998b). Solvent extraction as an adjunct to rendering; the effect on BSE and scrapie agents of hot solvents, followed by dry heat and steam. *Veterinary Record* **143**, 6-9.
- TAYLOR, D.M., FERNIE, K. & STEELE, P. (1999a). Boiling in sodium hydroxide inactivates mouse-passaged BSE agent. *Abstracts of a Meeting of the Association of Veterinary Teachers and Research Workers*. Scarborough, 29-31 March 1999; p. 22.
- TAYLOR, D.M., FERNIE, K., MCCONNELL, I. & STEELE, P.J. (1999b). Survival of scrapie agent after exposure to sodium dodecyl sulphate and heat. *Veterinary Microbiology* **67**, 13-6.
- WALKER, A.S., INDERLIED, C.B. & KINGSBURY, D.T. (1983). Conditions for the chemical and physical inactivation of the K.Fu. strain of the agent of Creutzfeldt-Jakob disease. *American Journal of Public Health* **73**, 661-5.
- WILSON, D.R. (1955). Unpublished work cited by Dickinson, A.G. (1976).

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