5. Diagnosis and therapy

5.1 The appearance from 1991 of BSE in cattle born after the introduction of the ruminant feed ban indicated that the ban, though achieving a considerable reduction in the incidence of BSE, had not been effective in eradicating it. This fact, combined with the long incubation period of BSE, again demonstrated that subclinical cases were entering the human or animal food chains. The development of a diagnostic test that could detect subclinical cases of BSE would help to prevent this from happening. The early diagnosis of vCJD would also be valuable in order to minimise the risk of transmission and allow possible therapy before the central nervous system is damaged.

5.2 This section describes key methodologies and techniques developed since 1986 that are relevant to the diagnosis and therapy of BSE and vCJD. First, we consider the method adopted by the UK, and more recently by the EU, to detect ruminant protein in animal feedstuffs; this was part of the package of policies implemented to halt the UK’s BSE epidemic. Second, we consider developments in post-mortem and ante-mortem diagnosis of BSE and vCJD. Finally, we consider development in treatments for transmissible spongiform encephalopathies (TSEs).

Detection of ruminant protein in animal feedstuffs

5.3 The ruminant feed ban came into operation in July 1988, and was the central element in the strategy to halt the BSE epidemic (see vol. 3: The Early Years, 1986–88 and vol. 5: Animal Health, 1989–96). However, in order to enforce the ban, a test was required which could detect ruminant protein and distinguish it from protein from other mammals and poultry present in cattle feed. The test developed to accomplish this was based on the ELISA technique. Enzyme-linked immunosorbent assays are widely used in laboratories as a sensitive and cheap method to measure the quantity of a substance by using antibodies to that substance. In this instance, an ELISA was developed using antibodies that recognised heat-stable proteins (ie, proteins present in rendered material) derived from a variety of ruminant tissues and organs.

5.4 In the following sections, we give a brief explanation of the technical basis of this ELISA, a full account of which is given by Ansfield.
and inoculating them into rabbits. The rabbits produce antibodies to these fragments which can subsequently be purified from their blood.

5.6 In the ELISA developed for the detection of ruminant protein, antibodies produced in this way are used to coat the surface of specialised plastic dishes. When suitably prepared samples of meat and bone meal (MBM) containing ruminant-derived protein are applied to these dishes, the specific antibodies recognise and bind the ruminant protein, but not protein from other species. The ruminant protein is therefore immobilised and bound to the plastic dish, and extraneous material can be washed away.

5.7 Further specific antibody is then added, although this time the antibody has been conjugated with an enzyme which catalyses the conversion of a colourless substrate to a coloured product. Thus, once the antibody has bound to the ruminant protein on the plastic dish, substrate is added and the enzymatic conversion of the substrate to a coloured product allowed to proceed. This is the detection step.

5.8 This method of carrying out an ELISA increases sensitivity as it incorporates an amplification step. This is because enzymes are capable of converting many molecules of substrate. Thus, one molecule of ruminant protein bound by one molecule of enzyme conjugate results in the conversion of many molecules of substrate.

5.9 The colour change in the substrate is proportional to the amount of ruminant protein in the sample. By testing known quantities of ruminant protein in parallel, it is possible to calculate the amount present in the MBM.

Development of the ELISA

5.10 Concern was first expressed in June 1988 by Mrs Elizabeth Owen (Food Standards Division, MAFF), that the ruminant feed ban would be unenforceable unless a test was developed that could distinguish between ruminant protein and protein from other mammals and poultry in cattle feed. 599

5.11 Mr Keith Meldrum (the Chief Veterinary Officer) invited comments on this matter in June 1988. 600 He received a reply from Dr Peter Dawson, his assistant, who was aware that staff at the Meat Research Institute of the Agricultural and Food Research Council were developing tests based on the ELISA to distinguish species differences in meat used in processed foods. 601 Following discussions between MAFF and the Meat Research Institute, it was agreed that a test based on the ELISA method would be useful for detecting ruminant protein in feedstuffs. It was also decided that the initial development of this ELISA test would involve detecting ruminant protein in rendered animal material before attempting detection in compound feedstuffs. 602 Work started on developing the test in March 1989, 603 but progress was hindered by a series of problems which included delays in the preparation of appropriate antibodies, 604 and the need to find methods of separating

599 YB88/6.10/8.1
600 YB88/6.27/2.1–2.3
601 YB88/6.27/2.1–2.3
602 YB88/9.7/1–1.4; YB88/12.12/7.1
603 YB89/4.63/1–3.10
604 YB89/7.21/8.1
gelatine from the samples prior to testing, as gelatine solidification interfered with the test. 605

5.12 By December 1990, the initial test for detection of ruminant protein in rendered animal material had been validated. 606 The test was then developed to include porcine protein in late 1993, and the method was finally patented and reported in 1994. 607

5.13 However, problems were encountered in applying this test to compound feedstuffs. These feedstuffs can contain protein from plant material and from various animal sources such as fishmeal and bloodmeal, not just rendered ruminant material. Several compound feedstuffs gave positive results even though there was no ruminant protein present in them. 608 Subsequent improvement in protein extraction procedures minimised the problems associated with compound feedstuffs. 609

5.14 On-farm testing of cattle feed began in June 1994 on farms where Born After the ruminant feed Ban (BAB) cases had arisen. 610 By 1996, 1,577 samples had been tested, of which only three gave positive results. 611 It was thought that these results were due to low-level cross-contamination of ruminant feed by ruminant protein in pig and poultry feed. 612

5.15 In November 1994 the feeding of cattle with any mammalian protein was banned in an attempt to prevent spread of disease, and to comply with direction from the EU. 613 The potential for cross-contamination, along with the continued fear that the ruminant feed ban was not being adhered to, led the European Commission, in 1995, to introduce a more widespread monitoring programme to test for the inclusion of ruminant protein in ruminant diets using the ELISA test. 614

5.16 In March 1996, the UK Government banned the use of mammalian MBM in feed for all farm animals and prohibited the production of pet food on any premises where such animal feed was prepared in order to prevent cross-contamination. 615

5.17 Full validation of the ELISA test for compound feedstuffs was not obtained until 1997 because of the increasing range of feed ingredients and specific EU requirements. 616 The results were published in 1998. 617 Delays in development of the test and the limited capacity of the MAFF laboratory to carry it out meant that it had little impact in monitoring the effectiveness of the ruminant feed ban. The development of the ELISA test for detection of ruminant protein in feed is discussed fully in vol. 5: Animal Health, 1989–96.
Diagnosis

5.18 Since its emergence, BSE has been diagnosed from clinical observations and confirmed by detailed histopathological examination of the brain following slaughter of the animal. The histopathological examination is based on the detection of characteristic vacuolation (sponginess) affecting the grey matter of the brain. This vacuolation in BSE is orderly, and usually distributed in a symmetrical bilateral fashion at the obex (see the full colour illustrations in vol. 16: Reference Material). The major target sites for examination are in the medulla oblongata of the brain, the lowest part of the brainstem, next to the spinal cord.618

5.19 Although significant advances have been made in the development of diagnostic tools since the emergence of BSE, clinical observations and histopathological examination of the brain are still the chosen form of diagnosis. Only recently has an ante-mortem test been developed which shows promise for the early diagnosis of BSE from peripheral blood samples.619 An alternative method is the DELFIA(R) technique, which is a fluoroimmunoassay for the analysis of PrPc in human blood and its components; PrPc was found mostly in plasma (68.5 per cent) and platelets (26.5 per cent).620

Developments in post-mortem diagnosis of BSE

5.20 In 1987 two techniques for diagnosing TSEs had been described and were in use at the Central Veterinary Laboratory (CVL). The first involved histopathological examination of brain tissue as described in paragraphs 3.6–3.10. The second technique was a test for scrapie-associated fibrils (SAFs) which detected the presence of SAFs in processed homogenates of brain tissue by electron microscopy.

5.21 Earlier, during 1985 and 1986, Dr Harash Narang and co-workers had developed a third diagnostic test for spongiform encephalopathies, in particular scrapie. The technique involved taking brain slices and placing carbon-coated grids onto the cut surface of the section.621 The grids were then soaked in a protein denaturing solution, fixed, stained and observed under an electron microscope. Positive diagnosis was made on detection of the presence of tubulofilaments, structures similar to but discernible from normal cell tubules. Dr Narang’s technique was likened to the ‘touch’ or ‘impression’ techniques used in haematological and other diagnostic studies. It was more rapid than the CVL’s SAF test, though required material taken straight from the brain. In contrast, the CVL’s SAF test used homogenised brain and was thus more suitable for macerated brains, which were unsuitable for histopathology. Dr Narang’s test, however, was not considered to have sufficient sensitivity and specificity to replace other methods. The work of Dr Narang and the appraisal of his touch test is considered in vol. 11: Scientists after Southwood.

618 YB93/8.19/1.3; Wells, G., Hancock, R., Cooley, W., Richards, M., Higgen, R. and David, G. (1989) Bovine Spongiform Encephalopathy: Diagnostic Significance of Vacuolar Changes in Selected Nuclei of the Medulla Oblongata, Veterinary Record, 125, 521–4
5.22 As discussed previously (see paragraphs 2.68–2.69), SAFs were found to be composed of the protease-resistant prion protein, PrP<sub>Sc</sub>.<sup>622</sup> PrP<sub>Sc</sub> became a marker for TSEs in general, and BSE in particular, and opened up a new avenue for research into a diagnostic test. Again it is helpful to note events after 1996. Several tests have been developed based on this marker by many groups, including the Neuropathogenesis Unit (NPU) and CVL, but progress has been slow. In July 1999, four tests were selected and evaluated by the European Commission.<sup>623</sup> The companies involved were:

i. E.G. & G. Wallace, UK;

ii. Prionics A.G., Switzerland;

iii. Enfer Technology Ltd, Ireland (licensee of Proteus International plc); and

iv. Commissariat à l’Energie Atomique (CEA), France.

5.23 All four tests involved the detection of protease-resistant PrP<sub>Sc</sub> in brain and spinal cord homogenates from confirmed BSE cases using material from healthy New Zealand cows as controls. The results indicated that post-mortem tests ii., iii. and iv. have ‘excellent potential’ for confirming clinical BSE or for rapidly screening dead or slaughtered animals.<sup>624</sup> Indeed, the Prionics test is currently being used routinely in Switzerland to detect BSE-infected animals at the abattoir to ensure that meat reaching the market is BSE-free. Validation of the Prionics test has recently been carried out using BSE cattle and controls. The authors observed that the test was highly sensitive, specific and reliable, and that sampling the correct area of the brain and the method of protein extraction are important for accurate diagnosis. Subclinical cases were also identified using this test. The authors concluded that it was a useful procedure for surveillance of cattle at the slaughterhouse.<sup>625</sup>

**Developments in ante-mortem diagnosis of BSE**

5.24 Work on ante-mortem tests began in the early 1990s. In 1991, Mr Roy Jackman of the CVL began development of the Electrochemical Urine Test for identification of BSE in cattle. This had initially been used to test scrapie-infected sheep. The method detected three urine metabolites, uric acid, catechol sulphate and an unknown, whose concentrations could be used diagnostically for BSE. However, false positive results were obtained in the initial trials.<sup>626</sup>

5.25 Further work, in an attempt to improve this test, took place in collaboration with Professor Barritault’s group in Paris, starting in August 1996. By 1998, the metabolite Marker T (thought to be the unknown marker previously identified at the CVL) had been isolated and identified in the urine of BSE-infected cattle. Plans

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<sup>624</sup> Ibid.


<sup>626</sup> M9 tab 44
have been made to characterise this metabolite and identify others, and to attempt to detect a marker in blood.627

5.26 The four tests evaluated by the European Commission in 1999 are able to detect low levels of PrPSc. This suggested that these tests could be used to detect infection in tissues or fluids before the development of clinical signs. However, there is little information available on the relationship between infectivity titres and PrPSc concentrations in different tissues and fluids throughout the incubation period. Furthermore, for a useful ante-mortem test, detectable levels of infectivity must be obtained in easily accessible fluids or tissues.

5.27 Both before and since March 1996, several protein markers for BSE other than PrPSc, such as 14-3-3 protein and S100 protein, have been detected in the cerebrospinal fluid (CSF) of BSE-affected cattle.628 These proteins are thought to originate from neuronal tissue and appear in the CSF as a result of the disease. However, none of the tests for these markers is sufficiently reliable for the diagnosis of TSEs.

5.28 In 1999 a promising test using capillary immunoelectrophoresis (CIE) was introduced for ante-mortem detection. It is claimed that the test can identify TSE agent in the blood of hamsters four weeks after parenteral inoculation. Also, white blood cell preparations (buffy coats) from lambs born of scrapie-affected ewes have been found to contain abnormal PrP one month after birth using the CIE test. The test depends on the complete removal of PrPc by enzyme digestion; incomplete removal can lead to false positive results. The CIE test is currently undergoing further evaluation. Another recently developed ante-mortem test for PrPSc is based on a paraffin-embedded tissue blot, which has been shown to detect disease in infected mice 30 days after inoculation and 145 days before the onset of clinical signs.629

Diagnosis of vCJD

5.29 Diagnosis of vCJD is initially based on clinical information (see vol. 8: Variant CJD). The clinical presentation of the disease is with behavioural and psychiatric disturbances. Patients sometimes complain of feeling extremely cold and of experiencing attacks of pain in the lower limbs. After several weeks or months, motor problems develop, and muscle coordination and balance maintenance are affected. This is followed by dementia, muscle paralysis and mutism. Other important information for diagnosis is the young age at onset and the prolonged clinical course. The median age in sporadic CJD at death is 66 years, compared with 29 years for vCJD.630 The median illness duration from the first symptom to death is 4 months in sporadic CJD but 14 months for vCJD.

5.30 The diagnosis is then confirmed by ante-mortem histopathological examination of a brain biopsy or by post-mortem histopathological brain examination. These examinations aim to detect the characteristics of vCJD: ‘florid

627 YB88/3.17/1.1
630 T24 p. 5
plagues’ in the cerebrum and cerebellum, abundant PrP deposition, spongiform change (especially in the basal ganglia) and severe glial cell proliferation in the thalamus.\textsuperscript{632}

5.31 However, the ante-mortem examinations of brain biopsies are not thought to be reliable. In oral evidence to the Inquiry, Dr Robert (now Professor) Will of the CJD Surveillance Unit (CJDSU) explained that the problem with biopsies is that only a very small piece of brain tissue is removed which may be ‘from an area of the brain that is not affected fully by the pathological process, and indeed you may sometimes sample an area that is normal, which may subsequently become abnormal. So a negative test does not exclude a diagnosis.’\textsuperscript{633} There are also inherent risks in the brain biopsy procedure, which means that it is only performed in the very late stages of disease. Therefore, the development of a reliable ante-mortem test for early diagnosis of vCJD has been considered to be very important.

5.32 Many of the tests already described for ante-mortem BSE diagnosis have been examined for their use in CJD diagnosis. Dr Narang reported detection of SAFs in the urine of patients with CJD but was unable to distinguish between sporadic and variant CJD.\textsuperscript{634} In 1997 the MRC funded an 18-month long evaluation at the CJDSU of Dr Narang’s urine test, which involved concentrating urine several thousand times and detecting ‘nemavirus’ particles (see paragraphs 2.59–2.61) using electron microscopy.\textsuperscript{635} Many problems were associated with the study and at the end of the 18-month period, no conclusions on the test’s reliability had been reached (see vol. 11: \textit{Scientists after Southwood}).\textsuperscript{636}

5.33 It was shown in work published in September 1996 that sporadic CJD could be diagnosed by the detection of 14-3-3 protein in CSF.\textsuperscript{637} However, it has not proved to be so useful in the diagnosis of the slowly progressing vCJD. More recently, the German National CJD Surveillance Unit suggested the measurement of S100 protein in serum as an early ante-mortem test for vCJD.\textsuperscript{638} However, it was not found to be sufficiently specific for CJD.\textsuperscript{639} Other protein markers studied by the German group were tau protein and neuron specific enolase (NSE).\textsuperscript{640}

5.34 Neuro-imaging techniques, such as single photon emission computed tomography (SPECT) analysis and magnetic resonance imaging (MRI) have also been recently proposed. These techniques are used when patients present with clinical symptoms suggestive of a neurological disorder. A reduction in the blood flow to the brain was detected in patients with vCJD by SPECT analysis.\textsuperscript{641} Although this finding was again not specific to CJD, it may help to raise initial suspicion of the possibility of this disease. Recent MRI studies in cases with suspected vCJD have revealed specific changes in the posterior thalamus (pulvinar).

\textsuperscript{631} The glial cells are the specialised cells that surround neurons
\textsuperscript{632} The thalamus is the mass of grey matter on either side of the third cavity in the brain
\textsuperscript{633} T24 pp. 71–2
\textsuperscript{634} S113 Narang para. 1.11
\textsuperscript{635} Ibid.; YB97/1.14/1.1
\textsuperscript{636} E2457
during the course of the clinical disease in 78 per cent of 36 confirmed cases and in none of 57 controls. The test promises to be of considerable value in the differential diagnosis of suspected vCJD.642

5.35 Studies carried out by Professor John Collinge’s group in 1999 indicate that detection of protease-resistant PrPSc in tonsil or appendix biopsy samples may provide a diagnostic tool for early ante-mortem identification of CJD.643 This process utilises the finding in animal studies that infectivity replicates in lymphoreticular tissue, including the tonsils, before neuroinvasion.644 Variant CJD can be distinguished from sporadic CJD by examining the glycoform patterns of PrPSc detected in the tissue sample (see paragraphs 4.8–4.9). These techniques are now being employed in the anonymous testing of tonsil and appendix tissue in three centres in the UK, in an attempt to determine the prevalence of vCJD (see paragraphs 4.22–4.23).645

5.36 With the increasing confirmation that the lymphoreticular system (LRS) is involved in agent replication comes the concern that infectivity could be present in the human bloodstream, suggesting that blood transfusions could therefore be a route of transmission. Although blood from guinea pigs and mice infected intracerebrally with CJD has been shown to be infective (paragraph 2.134), there is to date no evidence of disease transmission in humans from blood transfusions or blood products.646 However, BSE has been transmitted recently to a sheep via transfusion of 400 ml of blood. It is important to note that the blood came from an apparently healthy sheep, to which an oral dose of BSE had been administered. The donor sheep was in fact half way through its incubation at the time the blood was taken.647 Like patients with vCJD, infectivity in BSE-infected sheep is present in the LRS and so may provide a suitable model for assessing the risks of infection from human blood transfusion and also for evaluating preventive measures such as leucodepletion. This report gives support to suggestions that there may be risks from blood transfusions and emphasises the importance of developing a diagnostic blood test for vCJD. The Department of Health (DH) has initiated research in this area, under the leadership of Dr Eglin (Public Health and Laboratory Service, Leeds).648 Leucodepletion (removal of white cells) is currently being used to reduce the risk of transmission by blood transfusion.

5.37 Early detection of TSEs is important both for preventing disease transmission and for potential treatment. So far, the tests described above have only assayed a small number of samples, but with further study a specific and sensitive test may be obtained.


644 Ibid.

645 A newer PrPSc detection test, referred to as confocal dual-colour fluorescence correlation spectroscopy, now appears to be one order of magnitude more sensitive than western blotting analysis, and has detected a PrPSc–specific signal in cerebrospinal fluid from CJD patients. The authors consider that it provides the basis for a rapid and specific test for CJD and other prion diseases. See Beschiﬃe, J., Giese, A., Schulz-Schaeffer, W., Zerr, I., Poser, S., Eigen, M. and Kreitzschmar, H. (2000) Ultrasensitive detection of pathological prion protein aggregates by dual-color scanning for intensely ﬂuorescent targets, Proceedings of the National Academy of Science (USA), 97(10), 5468–73


648 YB99/9/27.1
5.38 Before the emergence of BSE, work had been carried out at the NPU into the effect of various pharmacological and immunomodulatory compounds on the incubation period of experimental scrapie.

5.39 In an important paper in 1986, Dr Christine Farquhar of the NPU reported the effect of a polyanionic glycan, dextran sulphate, on scrapie infectivity. Polyanionic glycans were tested because they were known to be active against viruses. At that stage, it was thought that the scrapie agent might be an unconventional virus. A single dose of dextran sulphate reduced the susceptibility of mice to scrapie. It had to be given within one month of infection, and the prolongation of the incubation period was directly related to dose. Treatment close to infection doubled the average survival time, an effect equivalent to a 90 per cent reduction in scrapie titre.

5.40 Dr Richard Kimberlin, also of the NPU, confirmed these results in a separate investigation in 1986. However, he added the proviso that for dextran sulphate to have any effect it must be administered before infection has established itself in the central nervous system.

5.41 Although the above results had been promising, and the emergence of BSE in cattle had suggested another use for polyanionic glycan therapy, advances in this area were slow. Certain independent scientists, notably Professor Lacey and Dr Dealler, recommended research into therapies early on in the epidemic. However, even after the Medical Research Council (MRC) Coordinating Committee on Spongiform Encephalopathies recommended in 1991 that research into therapeutic intervention should be considered a priority, very little progress was made.

5.42 In 1993 scientists at the National Institute for Allergy and Infectious Diseases in Montana (USA) also reported on the efficacy of polyanionic glycans for the inhibition of scrapie. In addition, they investigated the mechanism behind the effects, using a scrapie-infected neural cell line. They found that these compounds prevented the formation of PrP Sc , and that PrP Sc accumulation remained depressed even after the removal of the compound.

5.43 Scientists at the Washington University School of Medicine supported these findings in 1995. They investigated the mechanism of action further and suggested polyanionic glycans prevented PrP C from localising to the cell membrane, where it can be converted into PrP Sc . This then prevented the accumulation of PrP Sc.

5.44 In the early 1990s, other chemicals were identified that offered potential as treatment for TSEs. These included Congo red, a dye used to stain protein.
aggregates in diseases such as leprosy, tuberculosis and TSEs, and amphotericin B (an antibiotic).

5.45 Until the identification of vCJD cases, in 1996, few people raised concerns over the lack of research into TSE treatment. However, as possible human health implications arose, committees such as the MRC Coordinating Committee on Spongiform Encephalopathies put more emphasis on research in this area.

5.46 Since 1996, several groups have studied these chemicals further, and identified new chemicals which may have a role in the treatment of TSEs. These treatments can be grouped under two main headings: those that inhibit PrPSc production, deposition and accumulation; and those that prevent the neurotoxic effects of PrPSc and the amyloid plaques.

5.47 Suggested methods of inhibiting PrPSc production, deposition and accumulation would be by:

i. Stabilising the structure of PrPC, preventing its binding with PrPSc and thus preventing the transformation from PrPC to PrPSc; or even using chemicals which transform PrPSc back to PrPC. Examples of compounds which work in this way are dimethylsulphoxide, trimethylamine N-oxide and various polyols and sugars.

ii. Using antibodies to prevent PrPSc from binding to PrPC. Recently, one antibody has been produced, named 15B3, which is claimed to bind specifically to PrPSc. This would avoid any problems that might result from using antibodies directed against the normal PrPC, as these antibodies might inhibit any important functions that this normal protein could have in humans.

iii. Using nucleic acid molecules to prevent PrPSc from binding to PrPC.

iv. Inhibiting amyloid plaque formation with the use of non-steroidal anti-inflammatory agents and other compounds such as anthracycline.

v. Inhibiting the formation of the abnormal form of PrP by treatment with certain cyclic tetrapyroles, which have been shown to be effective in inhibiting TSE disease in experimental animals.

vi. Inhibiting the activity of the protein that is the precursor to amyloid with compounds carrying many negative charges, eg, dextran sulphate.

vii. Destroying PrPSc with branched polyamines.
A recent scientific paper proposes a rational approach to the discovery of drugs which inhibit PrP\textsuperscript{Sc} formation. Using this approach the authors identified two compounds (Cp-60 and Cp-62) which inhibited PrP\textsuperscript{Sc} formation in a dose-dependent manner and demonstrated low levels of toxicity.\textsuperscript{668}

5.48 It has recently been shown that treatment of protease-resistant PrP\textsuperscript{Sc} with synthetic β-sheet breaker peptides (iPrP13) can significantly reverse the protease resistance of the protein to a state similar to that of PrP\textsuperscript{C}.\textsuperscript{669} The effect was shown by incubating scrapie-infected material with the β-sheet breaker peptide for 48 hours and then testing the material by mouse bioassay. Incubation times were prolonged and the infectivity decreased by 90 to 95 per cent when compared with controls. This was confirmed by circular-dichroism analysis, which showed that the β-sheet content of the PrP\textsuperscript{Sc} sample was decreased from 41 to 8 per cent. These studies are important in that they show for the first time that conformational changes of the PrP molecule can be reversed \textit{in vitro}. They also confirm that the conformational change is an essential factor in the pathogenesis of TSEs. It remains to be seen whether β-sheet breaker peptides have a place in the treatment of these diseases.

5.49 Treatments based on preventing the neurotoxic effects of PrP\textsuperscript{Sc} and the amyloid plaques start with the assumption that PrP\textsuperscript{Sc} is intrinsically toxic and thus directly responsible for pathogenesis. Suggested methods of treatments are:

i. Using compounds that protect cells from destruction, eg, 1,4-benzoquinone derivatives and 1,2-hydroquinone derivatives.\textsuperscript{670}

ii. Using compounds, eg, antioxidants, that protect the cells from oxidative stress,\textsuperscript{671} which has an important role in the neurodegenerative changes associated with CJD.

5.50 However, the treatments mentioned above are based mainly on theory, \textit{in vitro} experiments and limited data from animal experiments, with no definitive evidence of efficacy or safety in human disease. This lack of progress is partly a result of many bodies, including the pharmaceutical industry, not being prepared to conduct substantial research and development programmes in relation to therapies because human TSEs are still so rare and progress so rapidly following the appearance of clinical symptoms, allowing little time for intervention.\textsuperscript{672} However, research into other human neurodegenerative diseases might produce results relevant to TSEs.

Summary

5.51 To the present day, BSE is diagnosed from clinical observations confirmed by histopathological examination of the brain post-mortem. Histopathological diagnosis is time-consuming, requires the expertise of skilled pathologists and is expensive. Reliable immunological tests that can identify BSE in samples of cattle.


\textsuperscript{671} An oxidative stress is a highly oxidised environment within cells

\textsuperscript{672} YB97/6.3/1.3
brain have recently been developed and are being applied in the UK to determine the incidence in over 30-month-old cattle going to slaughter, which is currently estimated as 0.5 per cent of those tested. In Switzerland the test is being used to guarantee that beef on the market is BSE-free. However, this test is insufficiently sensitive to be used ante-mortem on CSF or blood samples, and testing for pre-clinical disease in animals on their way to slaughter is not possible at the present time.

5.52 The diagnosis of vCJD likewise still depends on confirmation of clinical findings by histopathological examination of the brain post-mortem. Brain and tonsillar biopsies taken in the late stages of illness for the immunological detection of PrPSc can be diagnostic, but are less frequently used because of a high level of false negative results in the former and because both have a low level of acceptability by patients and their relatives. In any case, their use has been largely superseded by non-invasive MRI scanning, which reveals a positive ‘pulvinar sign’ in 78 per cent of affected patients. The availability of a reliable blood test would greatly aid clinical management but so far, no such test is available for clinical use.

5.53 With hindsight the early development of a test capable of detecting ruminant protein in animal feed would have had a dramatic effect on the size of the BSE epidemic. Such a test would have permitted the detection of cross-contamination in feed and the carry-over of existing stocks of cattle feed containing MBM after the introduction of the ruminant feed ban. An ELISA test for this purpose was eventually developed by 1994, but too late to have any real impact on the course of the epidemic.

5.54 A range of compounds have been investigated for their potential use in the treatment of TSEs, and some of these have shown a capacity to inhibit the production and accumulation of PrPSc. One compound, a synthetic β-sheet breaker peptide, has been shown to reverse the conformation of PrPSc back to PrPC and thus shows promise. However, none of these compounds has been assessed for its usefulness as an in vivo treatment for TSEs. The TSEs thus remain incurable and fatal diseases.