

Papers and Articles

Effect of rendering procedures on the scrapie agent

D. M. Taylor, S. L. Woodgate, A. J. Fleetwood, R. J. G. Cawthorne

Veterinary Record (1997) **141**, 643-649

A pool of scrapie-infected sheep brains was used to spike mixtures of porcine bone and intestine. These were processed in pilot-scale facsimiles of 12 rendering procedures that were in use within the European Union in 1991, and three that were not. Meat and bone meal, and tallow, were produced from the rendered tissues. Suspensions of all the meat and bone meal samples, and two of the tallow samples were assayed in mice for scrapie infectivity. Neither of the tallow samples had any detectable infectivity but the meat and bone meal samples were positive, except for those produced by processes involving exposure to hyperbaric steam. In addition, greaves were produced from the scrapie-spiked raw materials by an atypical low-temperature process and subjected to solvent extraction with hot heptane. The treated greaves were then exposed to steam to drive off residual solvent. Although the starting titre of infectivity in these greaves was low, there appeared to be no reduction in infectivity as a result of the treatments with hot heptane and steam. However, there was no detectable infectivity in the meat and bone meal prepared from the greaves produced by the atypical low-temperature process after it had been exposed to hyperbaric steam.

SCRAPIE is a fatal neurological disease that affects sheep and goats (and occasionally moufflon) in many parts of the world, and it has been endemic for at least two centuries among sheep in Great Britain and some of the countries of western Europe (Parry 1983). The disease is caused by an unconventional transmissible agent that is uncharacterised but has unusual properties, including a high resistance to inactivation (Taylor and others 1994). Bovine spongiform encephalopathy (BSE) is a scrapie-like disease of cattle, the first sporadic cases of which are suspected to have occurred in England in 1985 (Wells and others 1987). The disease has now affected more than 166,000 cattle in the United Kingdom, and smaller numbers of indigenous cattle in France, the Netherlands, Portugal, the Republic of Ireland and Switzerland. The suspicion that BSE might be dietary in origin (Dickinson and Taylor 1988, Morgan 1988) was confirmed by epidemiological

studies that demonstrated a correlation between the use of meat and bone meal (MBM) in cattle feed and the subsequent development of BSE (Wilesmith and others 1988). MBM is manufactured by the rendering industry, principally from tissues discarded by abattoirs and butchers, but also from fallen stock. Rendering procedures are essentially cooking processes that allow the molten fat fraction (tallow) to be collected, once the water has boiled off, the remaining solids (greaves) are pulverised to produce MBM. In 1988, a ban on feeding ruminant-derived protein to ruminants was introduced in Great Britain, and a similar ban was introduced in Northern Ireland in 1989. These restrictions were later extended to include all mammalian protein, and were applied throughout the EU (Commission Decision 1994a).

When BSE was first described, it was observed that the neuro-histopathological lesions were similar to those in sheep and goats with scrapie (Wells and others 1987). Although no natural scrapie-like disease of cattle had been recorded previously in Britain or elsewhere, there are those who consider that BSE was not caused by the scrapie agent from sheep but that it is a natural, previously undetected, disease of cattle. However, there is no evidence to support this hypothesis, and a survey of archived British cattle brains revealed no histopathological evidence of BSE in animals that had died before 1985 (Taylor 1995). It seems, therefore, that BSE is most likely to have been caused originally by the increased exposure of cattle to the scrapie agent which survived in rendered sheep tissues, and ended up in MBM. However, it was the recycling of BSE-infected bovine tissues through the rendering process that subsequently fuelled the epidemic (Wilesmith and others 1992).

The BSE infectivity from cattle in different parts of Great Britain has produced an identical array of incubation periods and brain lesion profiles in a panel of strains of inbred mice. This result contrasts with the variety of dissimilar incubation periods and brain lesion profiles produced when samples of the scrapie agent from different sheep sources are injected into the same strains of mice (Bruce and others 1994). This difference does not rule out the possibility that BSE was caused originally by the scrapie agent, because only a limited number of transmission studies to mice from sheep and goats with natural scrapie have been carried out. It may be that a previously uncharacterised single strain has an enhanced capacity to cross the species barrier into cattle. Alternatively, it is possible that a relatively thermostable strain has been able to withstand the rigours of at least some rendering procedures. Variations in thermostability are recognised among different strains of the scrapie agent (Dickinson and Taylor 1978, Kimberlin and others 1983). A number of studies on the thermal inactivation of scrapie and analogous agents, such as the Creutzfeldt-Jakob disease agent, have been conducted, but none have mimicked the conditions used by the rendering industry (Taylor 1989).

From surveys of rendering practices in the UK (Wilesmith and others 1991) and other EU countries (European Renderers Association, personal communication) it was apparent that

D. M. Taylor, FIBMS, FIScT, CBiol, MIBiol, PhD, BBSRC & MRC Neuropathogenesis Unit, Institute for Animal Health, West Mains Road, Edinburgh EH9 3JF

S. J. Woodgate, BSc, Beacon Research Ltd, Greenleigh, Kelmarsh Road, Clipston, Market Harborough LE16 9RX

A. J. Fleetwood, BVetMed, **R. J. G. Cawthorne**, BVM&S, DipBiol, PhD, MRCVS, Ministry of Agriculture, Fisheries and Food, Government Buildings (Toby Jug Site), Hook Rise South, Tolworth, Surbiton, Surrey KT6 7NF

Mr Fleetwood's present address is Central Research, Pfizer Ltd, Sandwich, Kent CT13 9NJ



TABLE 1: Summary of experimental protocols used to test the effect of rendering procedures on the scrapie agent

Process	Code	Particle size (mm)	End temperature (°C)		Time (min)
			planned	achieved	
Scrapie brain titration	A	Homogenate	NA	NA	NA
Batch atmospheric (natural fat)	B	150	120	114	150
Continuous atmospheric (natural fat)	C	30	100-125	102	50
	D	30	125	121	125
	E	30	100-140	100	50
	F	30	140	138	125
Continuous atmospheric (high fat)	G	30	140	134	30
	H	30	140	138	120
Continuous vacuum (high fat)	I	10	125	117	27
	J	10	125	126	61
Continuous wet rendering (natural fat)	K	20	100-120	103	120
	L	20	120	120	240
	M	20	70	72	240
Solvent extraction of greaves from protocol M	N	20	80	80	10
Steam treatment of greaves from protocol N	O	20	100	100	20
Steam treatment of greaves from protocol O	P	20	100	100	40
Batch pressure-raw material (natural fat)	Q	50	133	134	30
	R	30	136	136	18
	S	30	145	145	78
Batch pressure - meal (natural fat)	T	2.2	136	136	20
	U	2.2	145	145	20

NA Not applicable

although a relatively limited range of equipment is used, it is applied in many different ways. In designing the pilot-scale spiking experiments it was not practicable to test every commercial process, and protocols were therefore designed to represent both the average and the minimal physical conditions for each generic process (Table 1). Two additional hyperbaric procedures (identified as R and S in Table 1), which have never been used by the rendering industry, were tested because they were considered to be more likely to achieve a higher degree of inactivation of the BSE and scrapie agents than the traditional methods. These additional hyperbaric processes were also used to process infected MBM (protocols T and U in Table 1). Although the rendering industry has not traditionally treated MBM in this fashion, it was considered that such an approach might be used in the future to produce an end-product that is reliably free from BSE and scrapie infectivity. It would be a technically simple matter for renderers to add pressure cookers at the end of existing production lines, after the stage of MBM production. There would also be the possibility of transporting MBM to central facilities for steam sterilisation.

The final design of the experiments resulted from discussions between the European Renderers Association, the UK Renderers Association, the Ministry of Agriculture, Fisheries and Food (MAFF), the Agriculture and Food Research Council (now the Biotechnology and Biological Sciences Research Council) and the Medical Research Council's Neuropathogenesis Unit, and the BSE/Rendering Processes Sub-group of the Scientific Veterinary Committee of the European Commission. Comments from individual rendering companies were also taken into consideration.

An earlier study on rendering procedures used raw materials that were spiked with the brains of BSE-affected cattle (Taylor and others 1995). In that study it was shown that the BSE agent survived some of the rendering procedures used within the EU. Even though (for technical reasons) the input titre was not as high as it could have been, this finding validated the hypothesis that BSE had been caused by feeding MBM to cattle. As a consequence, the minimum conditions for rendering within the EU were revised (Commission Decision 1994b).

The evidence suggests that BSE is likely to have been caused initially by the survival of the scrapie agent derived from sheep tissues at a sufficiently high titre in MBM to represent an effective oral

TABLE 2: Weight (kg) of tissues used in the rendering experiments

Protocol*	Frozen scrapie brain (thawed to 4°C)	Normal intestine (pork)	Normal bone (pork)	Added fat
	I, J	5	15	30
G, H	10	30	60	100
C, D, E, F	20	60	120	0
B, Q, R, S	25	75	150	0
K, L	50	250	200	0
M	60	300	240	0
N, O, P	20 kg of greaves produced by protocol M			
T, U	100 kg of MBM produced by protocol M			

* See Table 1 for explanation of protocol codes

challenge for cattle. However, the question remains as to why it had not done so before the 1980s, given that MBM has been fed to cattle since the 1920s, and that scrapie has been endemic in the UK over this period. Wilesmith and others (1988) observed that some rendering procedures had changed over the period during which an already high UK sheep population had expanded, and that the incidence of scrapie might also have increased. The inclusion rate of sheep heads in material for rendering might also have increased (the highest titres of scrapie infectivity are present in the brain and spinal cord of affected animals). A key change was considered to be the abandonment of solvent extraction procedures by the UK rendering industry during the late 1970s and early 1980s, a period which corresponds extremely well with the emergence of BSE in 1985, given that the average incubation period is around five years and that the first exposure to dietary MBM is usually in calfhood. The solvent extraction procedures had been applied typically to greaves, the solid materials which remain after the primary cooking process, to increase the yield of tallow and produce a low-fat MBM which at one time attracted premium prices. Although it was considered that exposure to the solvents and/or the heating processes used to remove residual solvent might have provided sufficient additional inactivation of the scrapie agent for the resulting meal not to represent an effective oral challenge in MBM for cattle, nothing was known about the inactivating potential of either the solvents used or the application of heat under these circumstances (Taylor 1989). Although it had been planned to investigate the inactivating potential of solvent extraction in the earlier BSE-spiked rendering studies, this plan was precluded by the unexpected absence of detectable infectivity in the greaves (Taylor and others 1995). However, it proved possible to conduct such experiments with scrapie-spiked material in the study which is described here.

The rendering procedures which permitted the survival of the BSE agent (Taylor and others 1995) were those which were responsible for the production of an increasing proportion of MBM in Great Britain during the 1970s (Wilesmith and others 1991). It therefore appears that the effective exposure of cattle to sufficient scrapie agent to cause disease may have been influenced by two factors, the first being the introduction of new rendering procedures in the 1970s, and the second being the abandonment of solvent extraction by the early 1980s. This paper describes experiments in which scrapie-spiked raw materials were subjected to the various rendering processes that were in use within the EU in 1991. In addition, processes that have never been a part of normal rendering, but were anticipated to have an increased inactivation potential, were tested because they were considered to represent alternative options if the systems in use permitted the survival of infectivity.

Materials and methods

Collection and preparation of the spiking material

Brains from 2867 sheep with clinical signs of scrapie were obtained between October 1990 and August 1992 from 19 MAFF veterinary investigation centres throughout England, Scotland and Wales. They were placed individually in polythene bags and



TABLE 3: Changes in temperature (°C) of raw material with natural fat content during continuous processing

Protocol	0	30	40	Time (minutes)				
				50	60	90	120	125
C	30	98	99	102	(end)			
D	32	98	99	102	104	108	118	121 (end)
E	24	96	99	100	(end)			
F	25	96	99	100	101	108	134	138 (end)

stored below -20°C . To facilitate maceration during the preparation of the brain pool, the brains were warmed to 4°C immediately beforehand. To ensure that the pool was homogeneous, the brains were unwrapped and placed randomly in a presterilised stainless steel container, and then processed through a mincer fitted with a 10 mm extrusion plate to produce six equal aliquots in sterilised plastic dustbins. These aliquots were mixed by remincing, and were again collected as six equal lots. From these, appropriate amounts were weighed out into double polythene bags for the individual experiments (Table 2), after approximately 10 g of each aliquot had been collected and pooled to provide a sample for measuring the pool's titre of infectivity by bioassay in mice. All the aliquots were stored at below -20°C until required.

Selection and mixing of raw materials

The ratios of raw materials used in the different experimental mixtures are shown in Table 2, and the method of mixing was as for the BSE study by Taylor and others (1995). The only difference was that no bovine tissue was used because it was important to exclude BSE infectivity from the scrapie experiments; porcine bone and intestine were used instead.

Experimental rendering procedures

The procedures tested in the BSE study by Taylor and others (1995) were also tested in this scrapie study. In addition, a solvent extraction system was tested, and infected MBM was exposed to two hyperbaric steam processes. The instruments used to record times, temperatures and pressures were as described previously, as were the procedures for collecting tallow and greaves, and for MBM preparation. With few exceptions, the equipment and its mode of use were as described for the BSE study, as were the methods for decontamination and prevention of cross-contamination (Taylor and others 1995).

Batch processing (protocol B). – This protocol was designed to simulate the average conditions used in full-scale batch processing. The raw material was processed at atmospheric pressure in a cooker (Iwel) which had a steam-jacket and a rotating central shaft with agitator paddles that was also steam-heated. At the start of the process the temperature of the raw material was 22°C ; at the end of the process (after 150 minutes) the temperature was 114°C . The increasing temperatures achieved at intermediate stages were 95°C after 30 minutes, 100°C after 60 minutes, 100°C after 90 minutes and 114°C after 120 minutes.

Continuous processing with natural fat content (protocols C, D, E and F). – These protocols were designed to simulate the conditions for full-scale continuous rendering without added tallow at minimum (C and E) and average temperatures (D and F). The raw materials were processed at atmospheric pressure in a model IVA Rotadisc drier (Stord International) which had a steam-jacket and a rotating central shaft with agitator paddles that was also steam-heated. The paddles induced a unidirectional horizontal flow of the solid materials during cooking. The temperatures attained during the various cycles are shown in Table 3.

Continuous processing with high fat content (protocols G and H). – These protocols were designed to simulate the average and minimal conditions for full-scale continuous rendering at atmospheric

pressure with added tallow (1:1) pre-heated to 100°C . The raw material was loaded into a cooker (Atlas) which was kindly loaned by DAKA (Loesning, Denmark). The basic design of the cooker was similar to that of the Rotadisc drier except that a sliding valve arrangement permitted vacuum to be applied optionally from a pump. At the start of the process the temperature of the raw material was 78°C ; after 30 minutes the temperature had reached 134°C , and an aliquot was removed to provide samples of MBM and tallow to represent protocol G. The run was continued for a total of 120 minutes at which point the temperature was 138°C . The temperatures at intermediate stages were 101°C after 15 minutes, 143°C after 60 minutes, and 139°C after 90 minutes. The MBM and tallow end-products represented protocol H.

Continuous vacuum processing with high fat content (protocols I and J). – In protocol I the raw material was loaded into the Atlas cooker in which the added tallow had been pre-heated to 65°C . Vacuum was applied in two distinct stages to simulate the minimal conditions for the production-scale method. At the start of the process the temperature of the raw material was 48°C , and it was 76°C after four minutes, at a pressure of 0.7 bar; the pressure remained constant throughout the first stage which lasted for nine minutes by which time the temperature was 74°C . One minute later, with the temperature still at 74°C , the vacuum was adjusted to 0.35 bar for the duration of the second stage; this adjustment took eight minutes during which the temperature increased to 115°C . The run was continued for a total of 27 minutes at which point the temperature was 117°C .

In protocol J the raw material was loaded into the Atlas cooker in which the added tallow had been pre-heated to 65°C . Vacuum was applied in two distinct stages to simulate the average conditions for the production-scale method. At the start of the process the temperature of the raw material was 70°C . After applying vacuum at 0.68 bar, which was sustained throughout the first stage, the temperature was 72°C after 10 minutes and 71°C after 20 minutes. Stage one was completed after 30 minutes when the temperature had reached 71°C . After 31 minutes, with the temperature still at 71°C , the vacuum was adjusted to 0.25 bar for the second stage and took 10 minutes to stabilise, by which time the temperature was 99°C . After 51 minutes the vacuum had increased to 0.23 bar, and the temperature had increased to 122°C , and this pressure was maintained until the end of the cycle at 61 minutes when the temperature was 126°C .

Continuous wet rendering (protocols K and L). – These protocols were designed to simulate the average and minimal conditions for full-scale rendering by the low temperature wet method. The raw material was fed into the Rotadisc drier. The transit time was approximately 25 minutes and the maximum temperature of the material on exit was 89°C . The material was then fed directly into a model AB20 twin-screw press (Atlas); tallow was separated from the expressed tallow/water product, heated to 125°C and allowed to cool naturally. The press-cake was transferred to the Atlas cooker for processing at atmospheric pressure. At the start of the process the temperature of the press-cake was 79°C and the moisture content was 42 per cent. After 120 minutes the temperature was 103°C , with 29 per cent moisture, and an aliquot was removed to provide sample K. To produce sample L the process was allowed to continue for a further 120 minutes (total 240 minutes) by which time the temperature had reached 120°C , and the moisture content of the product was 4 per cent. The temperatures at intermediate stages were 100°C after 30 minutes, 103°C (38 per cent moisture) after 60 minutes, 104°C after 90 minutes, 103°C after 150 minutes, 106°C (18 per cent moisture) after 180 minutes and 111°C after 210 minutes.

Continuous wet rendering (protocol M). – The purpose of using this process was to produce infected greaves for the solvent extraction protocols (N, O and P in Table 1), and infected MBM for exposure to the hyperbaric steam protocols (T and U in Table 1). To minimise the loss of scrapie infectivity, the temperatures used were the lowest which could be used to produce a physically suitable end-product. The raw material was passed continuously



TABLE 4: Temperature and pressure conditions (pounds per square inch gauge pressure) for rendering protocols involving steam under pressure

Protocol	Material	Heat-up time (min)	Holding time (min)	Temperature (°C)	Steam pressure (psig)	Cool down time to 100°C (min)
Q	Raw	12	20	134	30	20
R	Raw	13	18	136	36	20
S	Raw	15	18	145	48	25
T	MBM*	10	20	136	38	12
U	MBM*	12	20	145	50	10

* Moisture content after processing was 15 per cent

through the Rotadisc drier. The transit time was approximately 30 minutes and the maximum temperature on exit was 75°C. The material was then fed into the Atlas twin-screw press to remove the tallow/water fraction which was discarded because it was unrepresentative of any normal production method. The press-cake was transferred to the Atlas cooker for drying under vacuum (0.8 bar) for 240 minutes throughout which the temperature remained at 72°C. The product had the following analysis: 4 per cent moisture, 19.5 per cent fat, 49 per cent protein and 31.8 per cent ash.

Solvent extraction (protocols N, O and P). – In full-scale production, solvent extraction is applied to raw material which has been cooked and from which a tallow fraction has already been extracted, thereby increasing the overall yield of tallow from the raw material, and producing MBM with a low fat content (less than 3 per cent compared with approximately 15 per cent with other methods). To test the effect of the solvent extraction process per se on scrapie infectivity it was necessary to produce spiked starting material which had already been rendered but which still contained scrapie infectivity. Such material was produced by protocol M in which the temperatures were kept to the minimum compatible with producing a physically suitable end-product.

Of the greaves produced by protocol M, 20 kg were evenly distributed into six nylon mesh-filter bags, 400 mm long, 150 mm wide and 40 mm deep; the pore size of the nylon mesh was 300 µm. Together with approximately 100 kg of porcine greaves, the bags were placed in the circular, metal centrifuge basket of the solvent extraction equipment (J. Lildal), which had 15 x 15 mm apertures, and was lined with a filter-cloth. Continuously recirculated heptane (Levy Brothers), maintained at a temperature of 80°C, was percolated through the raw materials for 10 minutes, during which the tallow-laden solvent was driven off by centrifugal force to a condenser where the solvent was evaporated. The nylon bags were then removed and allowed to drain at room temperature for four hours. Two of the bags were then used to prepare MBM sample N (Table 1), and two were exposed to steam at 100°C for 20 minutes, and were then used to prepare MBM sample O (Table 1). The remaining two bags were steam-treated at 100°C for 40 minutes and then used to prepare MBM sample P (Table 1).

Steam under pressure (protocols Q, R, S, T and U). – In the BSE-spiked rendering studies, 10 minutes were allowed for heat to penetrate into the particles during the processes involving steam under pressure. However, this period was subsequently considered to be irrelevant because the infectivity added from the brain-pool would be smeared on the surfaces of the particles of raw material, rather than contained within them. Consequently, in the scrapie-spiked studies, no such period was allowed. In this study, the raw materials were subjected to protocols Q, R and S, as shown in Table 4. In addition, MBM produced by protocol M, was exposed to the protocols T and U shown in Table 4. All of these procedures were conducted in the Iwel cooker. For protocols Q, R and S, steam was generated from the raw materials by applying heat from the steam-jacket and central shaft. As steam was generated, air was displaced by venting for 10 minutes. Because of the finite amount of moisture present, the maximum venting period which could be used was found to be 10 minutes. The venting valve was then closed and the chamber pressure was permitted to

TABLE 5: Bioassay of scrapie infectivity in the brain pool, MBM and tallow samples

Protocol	Material	Undiluted	Dilutions				Infectivity titre (ID ₅₀)
			10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	
	Brain pool	23/23*	23/23	12/13	2/5	NT	≥10 ⁴ 1/g
B	30% MBM	22/23	13/18	1/11	NT	NT	~10 ¹ 6/ml
C	30% MBM	16/16	NT	NT	NT	NT	Positive
D	30% MBM	5/11	5/5	2/8	NT	NT	~10 ¹ 5/ml
E	30% MBM	17/18	NT	NT	NT	NT	Positive
F	30% MBM	9/11	3/14	0/13	NT	NT	≤10 ⁰ 8/ml
G	30% MBM	13/13	NT	NT	NT	NT	Positive
H	15% MBM†	6/11	3/17	0/11	NT	NT	≤10 ⁰ 8/ml†
I	30% MBM	9/9	NT	NT	NT	NT	Positive
	tallow (unfiltered)	NT	0/12	NT	NT	NT	Negative
	tallow (filtered)	NT	0/13	NT	NT	NT	Negative
J	15% MBM†	8/9	9/15	1/5	NT	NT	~10 ¹ 5/ml†
K	30% MBM	16/18	NT	NT	NT	NT	Positive
L	30% MBM	12/14	4/5	2/3	NT	NT	~10 ¹ 1/ml
M	30% MBM	4/10	2/14	0/11	0/11	NT	≤10 ⁰ 3/ml
N	30% MBM	13/14	NT	NT	NT	NT	Positive
O	30% MBM	17/18	NT	NT	NT	NT	Positive
P	30% MBM	7/11	2/11	0/8	NT	NT	≤10 ⁰ 3/ml
Q	30% MBM	0/10	0/12	0/12	NT	NT	Negative
R	30% MBM	0/9	0/11	0/12	NT	NT	Negative
S	30% MBM	0/11	0/10	0/11	NT	NT	Negative
	tallow (unfiltered)	NT	0/19	NT	NT	NT	Negative
T	30% MBM	0/14	0/16	0/9	NT	NT	Negative
U	30% MBM	0/8	0/12	0/15	NT	NT	Negative

* Number of mice with scrapie/number injected for each dilution group (excluding intercurrent deaths)

† Samples were so absorptive that they could not be made up as 30 per cent suspensions

‡ Adjusted to be correct for a 30 per cent suspension

NT Not tested

increase to the appropriate pressure. The holding times at the required pressure started when the required temperature was reached on the surface of the raw materials. The methods were the same for protocols T and U, in which MBM was exposed to steam under pressure, except that steam was injected into the cooker chamber.

Bacteriological screening

Bacteriological screening of the MBM samples was performed by Q Laboratories. The brain macerate and tallow samples were also screened at the BBSRC and MRC's Neuropathogenesis Unit.

Bioassay of scrapie infectivity

For each experimental group, 24 weanling C57BL mice were injected by the combined intracerebral (0.02 ml) and intraperitoneal (0.5 ml) routes. These injection routes were used because the normally greater efficiency of intracerebral challenge with mouse-passaged scrapie agent does not apply when scrapie-infected sheep material is injected into mice (Kimberlin 1993). The mice were observed for the development of clinical neurological disease for up to 900 days after injection, according to the protocol of Dickinson and others (1968). Brains from all of the mice were immersion-fixed in 10 per cent formol saline. Sections were prepared from five paraffin-embedded, coronal tissue-blocks, and stained with haematoxylin and eosin. The blocks were chosen to represent different areas of the whole brain (Fraser and Dickinson 1968). The sections were examined microscopically for the presence of spongiform encephalopathy.

To assay its titre of infectivity, a sample of the pooled sheep brain macerate was homogenised in a sterile antibiotic solution

containing (Northum preparatio antibiotic the basis of brains of over six ID₅₀/g; of and other.

Each si in a Virtu homogen and J wh be reduc MBM pro bacteria (solution. utes, and ther serie cols whu cally in r and K in out. A q resented cesses (I tivity wa temperat to produ infected in Table

Altho has not and othe dures I i sented I commer (Kenite tion but viscous bed of I be inje homoge tissue gi

Results

The aimed r B poly phyicu (Table Microc. tallow Table I

The macera ≥10⁴ 1 ml of the raw of infe Bec ing wa mum t ml of infecti togeth These scrapie ing the steam The tocols in Tab



containing 5000 iu of penicillin and 5000 µg of streptomycin/ml (Northumbria Biologicals) in a Griffiths tube to give a 10⁻¹ w/v preparation. Further 10-fold (v/v) dilutions to 10⁻⁴ were made in antibiotic solution. The range of dilutions tested was selected on the basis of data showing that the average titre of infectivity in the brains of Suffolk sheep clinically affected with scrapie (averaged over six anatomical regions) is around 10⁵ mouse intracerebral ID₅₀/g; other breeds of sheep tend to have lower titres (Hadlow and others 1979).

Each sample of MBM was homogenised in sterile distilled water in a Virtishear homogeniser (Virtis) to give a 30 per cent (w/v) homogenate; the exceptions were the samples from protocols H and J which absorbed so much water that the MBM content had to be reduced to 15 per cent to provide sufficient fluid for bioassay. MBM produced by protocols M, O and P was contaminated with bacteria (see results), and was therefore homogenised in antibiotic solution. The homogenates were centrifuged at 500 g for 10 minutes, and the supernatants were injected either neat or after a further series of 10-fold dilutions, as shown in Table 5. For the protocols which represented the minimal conditions prevailing generically in rendering processes used within the EU in 1991 (C, E, G, I and K in Table 1), only a qualitative bioassay in mice was carried out. A quantitative assay was applied to the protocols which represented the average conditions used with the same generic processes (D, F, H, J and L in Table 1). In addition, the titre of infectivity was calculated for the MBM produced by the atypical low-temperature process (protocol M in Table 1) which was expected to produce infected greaves for the solvent extraction study, and infected MBM for exposure to hyperbaric steam (protocols T and U in Table 1).

Although tallow has been incorporated into ruminant diets, it has not been incriminated as a causal factor for BSE (Wilesmith and others 1988). Consequently, only two samples (from procedures I and S in Table 1) were assayed qualitatively; these represented higher and lower temperature processes. The normal commercial practice is to filter tallow through diatomaceous earth (Kenite 1000; Diacel). Sample I was tested before and after filtration but sample S was tested only unfiltered because it was too viscous to penetrate the filtration system, which was a 13 mm bed of Kenite aided by vacuum. To permit the tallow samples to be injected, it was necessary to prepare 10 per cent w/v homogenates in sterile physiological saline, using glass/teflon tissue grinders

Results

The macerate of pooled, scrapie-infected sheep brain contained moderate numbers of *Bacillus cereus*, *B. licheniformis*, *B. polymyxa*, *Micrococcus kristinae* and *Staphylococcus saprophyticus*. Only the MBM produced by processes M, O and P (Table 1) were contaminated (with a *Bacillus* species and *Micrococcus* species). No bacteria were detected in the two tallow samples which were bioassayed (from protocols I and S in Table 1).

The data for the calculation of the titre of infectivity of the macerated brain pool are shown in Table 5. The titre was $\geq 10^{4.1}$ ID₅₀/g, calculated by the method of Karber (1931). Because the raw materials contained 10 per cent of this macerate, the titre of infectivity of the spiked raw materials was $\geq 10^{3.1}$ ID₅₀/g.

Because the weight of the solid material remaining after rendering was 30 per cent of the weight of the starting material, the maximum titre in the resulting MBM would be $\geq 10^{3.1}$ ID₅₀/0.3 g (or per ml of 30 per cent suspension). The presence or absence of scrapie infectivity in the various MBM samples is shown in Table 5, together with the titres of surviving infectivity where appropriate. These data show that the MBM samples all contained detectable scrapie infectivity, apart from those produced by protocols involving the exposure of either spiked raw materials or infected MBM to steam under pressure.

The qualitative bioassays of the two tallow samples (from protocols I and S) failed to detect any infectivity. The data are shown in Table 5.

Discussion

Compared with the rendering study involving BSE-spiked raw materials, in which, for technical reasons, the level of BSE infectivity was disappointingly low (Taylor and others 1995), the titre of infectivity of the scrapie-infected sheep brain pool used in this study ($\geq 10^{4.1}$ mouse intracerebral ID₅₀/g) was less than 10-fold below the maximum that might have been achieved in a pool of infected brains (10⁵ mouse intracerebral ID₅₀/g) according to the data of Hadlow and others (1979). The only criterion for the inclusion of sheep brains in the pool for the study was based solely on the clinical assessment of the scrapie status of the sheep; there was no neurohistopathological confirmation. Although the level of scrapie infectivity in the spiked raw materials was 10^{3.1} ID₅₀/g, this may not have represented the worst-case conditions that might occur occasionally during rendering in practice. This is because, although it was necessary to mix the scrapie-infected brains thoroughly with the raw materials in the rendering experiments, infected brain tissue would not necessarily become evenly distributed throughout the raw materials during normal rendering. This is supported by the frequent occurrence of only one or two cases of BSE in herds of cattle, which suggests that the BSE infectivity occurs as pockets in MBM, rather than being distributed evenly throughout infected batches. The level of infectivity in the brains of individual sheep with scrapie can reach 10⁶ ID₅₀/g overall, and be as high as 10⁸ ID₅₀/g in specific areas of the brain (Hadlow and others 1979).

The input of scrapie infectivity in this study was 1.4 logs higher than the input of BSE infectivity in the rendering study by Taylor and others (1995) in which only four MBM samples had detectable infectivity. The survival of scrapie infectivity in all the MBM samples, apart from those derived from procedures involving exposure to hyperbaric steam, is therefore not necessarily surprising, given the known thermostability of the scrapie agent (Kimberlin and others 1983, Brown and others 1986, Taylor and others 1994). However, in this study with the scrapie agent, a loss of titre of around 1.5 logs was detected in the MBM produced by process J (Table 1), whereas the same process produced virtually no reduction in the titre of the BSE agent (Taylor and others 1995), suggesting that BSE infectivity may be more thermostable than the scrapie infectivity in the sheep brain pool. However, this cannot be determined with any degree of certainty because of the range of strains of scrapie agents that would have been present in the brain pool. It is possible that an extremely thermostable strain of scrapie agent might have been present in the brain pool, but at such a low level that its dilution by other strains did not permit its thermostability to be observed.

The titre of scrapie infectivity in the greaves produced by process M (Table 1) was not reduced from its starting titre of $\sim 10^{0.3}$ ID₅₀/ml by the solvent extraction process. This involved its exposure to heptane at 80°C for 10 minutes, and then to steam at 100°C for 20 minutes and again for 40 minutes. One of the most plausible explanations for the emergence of BSE in the UK has been the widescale abandonment of the solvent extraction system during the late 1970s and early 1980s (Wilesmith and others 1988), with BSE first occurring in 1985 (Wells and others 1987). Because BSE has an average incubation period of around five years, and because most infected cattle were exposed to infected MBM as calves, the period during which solvent extraction processes were abandoned within the UK has been considered to have a strong theoretical link to the emergence of BSE in 1985/86, particularly because the solvent extraction process is applied to greaves which have already been exposed to heat in the standard rendering processes. The results from the solvent extraction experiments with heptane do not support this hypothesis. Also, it had been observed that none of the solvents used by renderers had previously been tested for their effect on the scrapie agent, and that the effect of exposure to heat at 100°C would be minimal (Taylor 1989). Further laboratory studies on the effects of different solvents are in progress.

As a consequence of the results of this rendering study with scrapie-spiked raw materials, the regulations relating to rendering within the EU have been revised. Although there is already a



ban within the EU on feeding mammalian-derived proteins to ruminants (Commission Decision 1994a), it has still been permissible to feed MBM derived from ruminants to other species such as pigs and poultry, providing that it was produced by one of the various modified procedures approved in 1994 (Commission Decision 1994b). The revised position is that mammalian MBM may only be fed to such species if it has been produced by a hyperbaric process equivalent to protocol Q in Table 1 (Commission Decision 1996).

The maximum titre of scrapie infectivity that could have been present in the 30 per cent homogenates of MBM was $10^{3.1}$ ID₅₀/ml, and each mouse was injected with 0.52 ml; the absence of detectable scrapie infectivity in MBM produced by the 133°C hyperbaric cycle (protocol Q) therefore represents a $10^{2.8}$ -fold reduction in titre, and the same is true for the MBM produced by protocols R and S, in which there was also no detectable infectivity. However, a proportion of the infectivity in cattle brain containing more than $10^{5.2}$ ID₅₀/g of the BSE agent has been found to survive exposure to a hyperbaric steam cycle at 135°C for 18 minutes (Taylor and others 1994). Furthermore, the sensitivity of detection of any residual infectivity will be reduced because of the species barrier effect caused by the necessary use of mice for the bioassays; this reduction is known to be around 1000-fold for cattle compared with mice injected intracerebrally with the BSE agent. So, in theory, there may still be sufficient infectivity to infect ruminants in MBM processed by apparently effective methods. However, in the practical context of addressing the problem of a foodborne disease, the lack of sensitivity introduced by the species barrier is more than compensated for by the greater efficiency of the intracerebral, compared with the oral, route of challenge. Using BSE-infected bovine brain, it has been calculated that the transmission of BSE to mice is 200,000-fold more efficient by the intracerebral route than by the oral route (Kimberlin 1994). Nevertheless, given the evidence that the BSE agent is possibly more thermostable than scrapie agents in general, and that the input of infectivity in the BSE-spiked rendering studies was sub-optimal (Taylor and others 1995), it should not be assumed that the newly-recommended hyperbaric procedure is an absolutely reliable process for inactivating the BSE agent, although it may be inactivated sufficiently to reduce the titre of infectivity below that which can cause infection by the oral route.

No infectivity was detectable in either of the two tallow samples that were bioassayed. Although one of them was produced by protocol S, after which there was no detectable infectivity in the MBM, the other sample was produced by protocol I, after which all of the mice injected with the MBM developed scrapie. Much the same picture emerged during the BSE-spiked rendering studies by Taylor and others (1995). These data suggest that neither BSE nor scrapie infectivity partitions preferentially with tallow during rendering, and this conclusion is supported by data showing that in a poorly inactivating rendering process, the titre of the BSE agent in the MBM can be almost the same as the input titre (Taylor and others 1995). Collectively, these data support the conclusion of Wilesmith and others (1988) that, despite its use in cattle diets, there is no evidence that tallow has been a causal factor for BSE.

The failure thus far to detect any agent from sheep that has the phenotypic characteristics that the BSE agent has in mice does not rule out the possibility that BSE was caused originally by the scrapie agent. There have been only a few primary transmissions of scrapie from sheep to mice, and it is possible that a single, previously uncharacterised, strain of the scrapie agent has the capacity preferentially to cross the species barrier to cattle. Alternatively, it is possible that the strain of the scrapie agent that caused BSE happened to be selected out by the rendering process because it is more thermostable than other strains. In the scrapie-spiked rendering studies, infected MBM was produced by two processes that reached a temperature of 138°C (protocols F and H in Table 1), and experiments are underway with these two MBM samples to determine whether the pattern of incubation periods or brain-lesion profiles produced in five strains of mice is similar to that of the BSE agent, as described by Bruce and others (1994). Given that the scrapie-infected brain pool used as the spike came

from 2867 sheep distributed throughout England, Scotland and Wales, it seems likely that if BSE was caused originally by the scrapie agent, the strain responsible would have been present in the brain pool, even if only as a minor component.

The initial rationale for the rendering studies on BSE and scrapie-spiked raw materials was to test the effectiveness not only of the procedures in actual use, but also of other systems which might be used to provide a higher degree of inactivation of these agents. This policy was formulated collectively by scientists, the rendering industry, and regulators in 1991, and clearly signalled the opinion that MBM should continue to be available for use as a protein supplement in animal diets if it was safe to do so. Data from these studies have shown that the 133°C/20 minute cycle involving steam under pressure appears to be effective although, as already discussed, the margin of safety may be small. There are also higher pressure systems (protocols R and S) which have been validated and could also be used. However, the practical reality in the UK is that MBM is unlikely to be permitted to be used in any feedstuff for farm animals while there are any doubts about its safety. This conclusion stems from the putative link between BSE and a new variant form of the human disease, Creutzfeldt-Jakob disease (Will and others 1996) which, with one exception, has been confined entirely to the UK, albeit at a very low incidence (20 confirmed cases by October 1997). Nevertheless, it is intended to determine the effectiveness of new rendering processes which offer the potential of an even greater degree of reassurance regarding the safety of the MBM end-product. If these processes live up to expectations, there may be a defensible case for allowing MBM to be fed again to ruminants and (in the UK) to other farm animals.

Acknowledgements. – This work was funded jointly by the EU, MAFF and the rendering industry (Prosper De Mulder and EURA). Unless otherwise specified, equipment was loaned by Prosper De Mulder. The careful technical assistance of Miss M. Smith (MAFF State Veterinary Service, Leeds), Mr D. Blackham and Mr J. King (Prosper De Mulder), Mrs I. McConnell and Ms C. Ferguson (Biotechnology and Biological Sciences Research Council, Institute for Animal Health) is acknowledged gratefully. The authors are grateful to Mr R. Bradley, BSE Consultant, Central Veterinary Laboratory, Weybridge, for his critical assessment of the manuscript.

References

- BROWN, P., ROHWER, R. G. & GAJDUSEK, D. C. (1986) *Journal of Infectious Diseases* **153**, 1145
- BRUCE, M. E., CHREE, A., MCCONNELL, I., FOSTER, J. D., PEARSON, G. & FRASER, H. (1994) *Philosophical Transactions of the Royal Society, London, Series B* **343**, 405
- COMMISSION DECISION (1994a) *Official Journal of the European Communities* **L 172**, 23
- COMMISSION DECISION (1994b) *Official Journal of the European Communities* **L 172**, 25
- COMMISSION DECISION (1996) *Official Journal of the European Communities* **L 184**, 43
- DICKINSON, A. G., MEIKLE, V. M. H. & FRASER, H. (1968) *Journal of Comparative Pathology* **78**, 293
- DICKINSON, A. G. & TAYLOR, D. M. (1978) *New England Journal of Medicine* **299**, 1413
- DICKINSON, A. G. & TAYLOR, D. M. (1988) Proceedings of the 3rd World Congress on Sheep and Cattle Breeding. Vol 1. Paris, June 19-23. p 553
- FRASER, H. & DICKINSON, A. G. (1968) *Journal of Comparative Pathology* **78**, 301
- HADLOW, W. J., RACE, R. E., KENNEDY, R. C. & EKLUND, C. M. (1979) Slow Transmissible Diseases of the Central Nervous System. Vol 2. Eds S. B. Prusiner, W. J. Hadlow. London, Academic Press. p 3
- KARBER, G. (1931) *Archives of Experimental Pathology and Pharmacology* **162**, 480
- KIMBERLIN, R. H. (1993) Abstract of the 1Xth International Congress of Virology, Glasgow. p 318
- KIMBERLIN, R. H. (1994) Transmissible Spongiform Encephalopathies: A Consultation on BSE with the Scientific Veterinary Committee of the Commission of the European Communities, Brussels, September 14-15, 1993. Dordrecht, Kluwer Academics p 455
- KIMBERLIN, R. H., WALKER, C. A., MILLSON, G. C., TAYLOR, D. M., ROBERTSON, P. A., TOMLINSON, A. H. & DICKINSON, A. G. (1983) *Journal of Neurological Sciences* **59**, 355
- MORGAN, K. L. (1988) *Veterinary Record* **122**, 445
- PARRY, H. B. (1983) *Scrapie Disease in Sheep* London, Academic Press

TAY
TAY
TAY
TAY
WEL
C
V

Vete

Ser
abo
mat
bod
sera
355
sam
59
ser
11:2
15:5
tha
moi
exa
bas
a h
vidi
cas
anti
dan
cau

IN
non
init
bov
cau
Mu
dia
this
was
Thi
rep
and
and
of
Bar
for
Sut
of
and
lim

D. J
BS
EH
G.
Col
St I

