

Papers and Articles

Inactivation of the bovine spongiform encephalopathy agent by rendering procedures

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Bovine brain infected with the bovine spongiform encephalopathy (BSE) agent was used to spike material processed in pilot scale facsimiles of 12 rendering processes which are used within the European Union, and three which are not. The raw materials for experimental rendering represented those used in practice, and consisted of appropriate proportions of BSE-infected brain tissue, bovine or porcine intestine, and bovine bone. Meat and bone meal, and tallow were produced from the rendered tissues. Suspensions of all the meat and bone meal samples were assayed in inbred mice for BSE infectivity, and two of the tallow fractions were tested similarly. Four of the 15 processes produced meat and bone meal with detectable BSE infectivity. Neither of the tallow samples had detectable infectivity.

THE suspicion that the epidemic of bovine spongiform encephalopathy (BSE) in cattle in the United Kingdom might be dietary in origin (Dickinson and Taylor 1988, Morgan 1988) was substantiated by epidemiological data which implicated meat and bone meal as the source of the infection (Wilesmith and others 1988, 1991). It was established that meat and bone meal, which is manufactured by the rendering industry from animal offal and carcasses, principally from ruminants, was a common protein supplement in commercial cattle rations (until it was banned in Great Britain in 1988). It was also recognised that some rendering procedures had changed during a period when the UK sheep population had expanded, and the level of the sheep disease, scrapie, was considered to have increased. The conclusion was that these factors had resulted collectively in the production of meat and bone meal containing sufficient scrapie infectivity to establish the infection when it was fed to cattle. A key component was considered to be the large scale abandonment of solvent extraction procedures by the rendering industry in Great Britain during the late 1970s and early 1980s. These extraction procedures had typically been applied to greaves, which are the solid materials that remain after the primary cooking process, to increase the yield of tallow.

Their exposure to the solvents, and to the heating processes used to remove the residual solvent, could have provided suffi-

cient additional inactivation of the scrapie agent to keep its level in meat and bone meal below the threshold at which it could infect cattle, but nothing was known about the inactivating potential of the solvents used or about the use of heat under these particular circumstances (Taylor 1989). The timescale of the cessation of solvent extraction was significant with regard to the emergence of BSE in 1985/86, because the disease occurs most commonly in four- to five-year-old cattle, and the range of the incubation period can for practical purposes be taken as the distribution of the age of animals at the clinical onset of the disease (Wilesmith and others 1992).

The difficulty of inactivating the unconventional agents which cause transmissible degenerative encephalopathies such as scrapie is well recognised (Taylor 1991a). A number of studies of the thermal inactivation of scrapie and analogous agents such as the agent of Creutzfeldt-Jakob disease have been conducted, but none mimicked the conditions used in rendering processes (Taylor 1989). Although BSE was considered to represent the transmission of scrapie from sheep to cattle via meat and bone meal, at least initially, samples of the BSE agent from cattle in different parts of Great Britain have produced identical patterns of incubation periods in mice of different genotypes; this contrasts with the variety of patterns of incubation periods and distributions of brain lesions which are produced when samples of scrapie agent from different sheep sources are injected into the same strains of mice (Bruce and others 1994). These observations suggest that BSE is caused by only one strain of the agent, which may have an enhanced capacity to withstand the rigours of at least some rendering procedures. Strain differences in thermostability are recognised for the scrapie agent (Dickinson and Taylor 1978, Kimberlin and others 1983). It is also considered that a significant factor in the perpetuation and expansion of the BSE epidemic was the recycling of infection from BSE-infected bovine carcasses before the feeding of ruminant-derived protein was prohibited on July 18, 1988 (Wilesmith and others 1991). As a result, in planning the experiments to determine the inactivating effect of rendering processes on scrapie-like agents, the priority was to use BSE-infected bovine brain as the spiking material.

From surveys of rendering practices in the UK (Wilesmith and others 1991) and other European Union countries (European Renderers Association [EURA], personal communication) it was apparent that although these involve a relatively limited range of equipment, the equipment is used in many different ways. In designing the pilot-scale spiking experiments it was not practicable to test every method in precise detail; protocols were therefore designed to mimic both the average and the minimal conditions for each generic process (Table 1). Two additional hyperbaric procedures (identified as R and S in Table 1) which do not mimic BSE agent, the main experimental design resulted from discuss-

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TABLE 1: Summary of the experimental protocols used to mimic commercial rendering processes

Process	Code	Particle diameter (mm)	End temperature (°C)		Time (min)
			Planned	Achieved	
BSE brain titration	A	Homogenate	NA	NA	NA
Batch atmospheric	B	150	120	121	150
Continuous atmospheric	C	30	100-125	112	50
(natural fat)	D	30	125	123	125
	E	30	100-140	122	50
	F	30	140	139	125
Continuous atmospheric (high fat)	G	30	140	136	30
	H	30	140	137	120
Continuous vacuum (high fat)	I	10	125	120	20
	J	10	125	121	57
Continuous wet rendering (natural fat)	K	20	100-120	101	120
	L	20	120	119	240
	M	20	70	72	240
Batch pressure (natural fat)	Q	50	133	133	30*
	R	30	136	135	28*
	S	30	145	145	28*

NA Not applicable

* Includes 10 minutes for heat penetration

of Agriculture, Fisheries and Food (MAFF), the Agricultural and Food Research Council Institute for Animal Health and the BSE/Rendering Processes Subgroup of the European Commission's Scientific Veterinary Committee. Comments from individual rendering companies were also taken into consideration.

Materials and methods

Collection and preparation of the spiking material

The brains from 861 cattle with suspected BSE were obtained between August and November 1990 from five MAFF veterinary investigation centres throughout England. The brains were placed in individual polythene bags and stored at $<-20^{\circ}\text{C}$, after portions of the brain stems had been removed to permit subsequent histological examination for spongiform encephalopathy. To facilitate maceration during the preparation of the brain pool, the brains were removed from cold storage and allowed to reach approximately 4°C . To ensure the homogeneity of the pool, the brains collected from the five veterinary investigation centres were unwrapped and placed randomly in a presterilised stainless steel bin, 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 further by remincing, and were again collected as six equal lots. From these, appropriate amounts were weighed into double polythene bags for the individual experiments (Table 2). Approximately 10 g of each aliquot was collected and pooled to provide a sample for measuring the titre of infectivity by bioassay in mice. All the aliquots were held at $<-20^{\circ}\text{C}$ until required.

Selection and mixing of raw materials

For each process the mixtures of raw materials (Table 2) were prepared on the day of the experiment. For most protocols the ratios of brain:intestine:bone were 1:3:6 but to obtain a representative end-product in protocols K and L the ratios were 1:5:4. The mixtures were processed through a mincer, using extrusion plates of appropriate sizes (Table 1). To maximise the homogeneity of the mixtures, aliquots of the bone, intestine and brain were fed

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

Protocol	Frozen BSE brain (thawed to 4°C)	Normal intestine (bovine or porcine)	Normal bone (bovine)	Added fat (production tallow*)
I,J	5	15	30	200
G,H	10	30	60	100
C,D,E,F	20	60	120	0
B,Q,R,S	25	75	150	0
K,L,M	50	250	200	0

* From a combination of bovine, ovine and porcine sources

TABLE 3: Temperature and pressure (pounds per square inch gauge pressure) for the raw materials processed by hyperbaric procedures

Protocol	Heat-up time (min)	Holding time (min)*	Temperature ($^{\circ}\text{C}$)	Steam pressure (lb/in 2)	Cool down time to 100°C (min)
Q	12	30	133	30	16
R	14	28	135	34	20
S	9	28	145	58	20

* Includes 10 minutes for heat penetration

Experimental rendering procedures

During the cooking experiments, temperatures were recorded either with appropriately positioned thermocouples (Pyrometer Services; Stockport) connected to a DPR 3000 chart recorder (Honeywell) or with a hand-held type 8476-081-010 B digital thermometer (Honeywell). The times and the terminal temperatures attained in the different protocols are summarised in Table 1. Where relevant, moisture content is referred to in the text; the chamber steam-pressures are shown in Table 3.

At the end of each experiment, unless described otherwise, the free-run tallow was collected in stainless steel containers, and samples were taken for cold storage and bioassay as appropriate; each stored sample (approximately 2 litres) consisted of a pool of five aliquots collected randomly from the free-run tallow. Solid material was transferred to specially fabricated solid bottomed cylinders, the walls of which consisted of strips of wedgewire metal with 2 mm gaps (Prosper De Mulder). Using a hydraulic press (Prosper De Mulder), excess fat was expressed through the cylinder apertures, leaving a low-fat residue for pulverising.

The pressed material was pulverised in a hammer-mill (Christy Norris), to produce meat and bone meal with a maximum particle diameter of 2.2 mm which was mixed in a revolving drum-mixer. Samples for bioassay were collected in polythene bags; each sample (approximately 2 kg) consisted of a pool of five aliquots collected randomly from the bulk meal. From these, aliquots were placed in sterile containers for a microbiological assay in a MAFF-authorized laboratory before they were bioassayed.

The size of the pilot scale equipment and the batches of raw material was such that a 1:20 representation of full scale production was achieved for batch processing; for the other processes the scale was 1:100.

Batch processing (protocol B). - This protocol was designed to simulate 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 40°C and at the end of the process after 150 minutes its temperature was 121°C ; the temperatures at intermediate stages were 112°C after 30 minutes, 118°C after 60 minutes, 119°C after 90 minutes and 121°C after 120 minutes.

and R). - These protocols were designed to simulate the condi-

TABLE 4: Temperature profiles (°C) of raw materials with natural fat content processed continuously

Protocol	Minutes							
	0	30	40	50	60	90	120	125
C	32	99	112	112(end)				
D	29	100	NT	NT	108	115	122	123(end)
E	38	108	116	122(end)				
F	31	100	NT	NT	107	121	138	139(end)

NT Not tested

minimum (C and E) and average temperatures (D and F). The raw materials were processed at atmospheric pressure in a model 1VA Rotadisc drier (Stord International). This had a steam-jacket and a rotating central shaft with agitator paddles that was also steam-heated. The paddles effected a unidirectional horizontal flow of the solid materials during cooking. The temperatures attained during the various cycles are shown in Table 4.

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 a vacuum to be applied optionally from a pump. At the start of the process the temperature of the raw material was 82°C; after 30 minutes the temperature had reached 136°C, and an aliquot was removed to provide samples of meat and bone meal and tallow to represent protocol G. The run was continued for a total of 120 minutes after which the temperature was 137°C; the temperatures at intermediate stages were 103°C after 15 minutes, 136°C after 60 minutes and 137°C after 90 minutes. The meat and bone meal 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 58°C, and it was 72°C after four minutes at 0.8 bar; the pressure remained constant throughout the first stage which lasted nine minutes, after which the temperature was 71°C. One minute later, with the temperature still at 71°C, the vacuum was adjusted to 0.38 bar for the duration of the second stage; this adjustment took five minutes during which the temperature increased to 118°C. The run was continued to give a total time of 20 minutes at which point the temperature was 120°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 59°C. After applying vacuum at 0.8 bar throughout the first stage, the temperature was 70°C after 10 and 20 minutes. Stage one was completed after 30 minutes when the temperature had reached 75°C. After 31 minutes, with the temperature still at 75°C, the vacuum was adjusted to 0.4 bar for the second stage and took six minutes to stabilise, by which time the temperature was 115°C. After 47 minutes the vacuum had increased to 0.38 bar, when the temperature was 122°C, and was maintained until the end of the cycle at 57 minutes when the temperature was 121°C.

Continuous wet rendering (protocols K and L). — These protocols were designed to simulate the average and minimal conditions for approximately 20 minutes and the maximum temperature of the

a model 13A twin-screw press (Stord International); 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 84°C and its moisture content was 44 per cent. After 120 minutes the temperature was 101°C, with 32 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 by which time the temperature had reached 119°C, and the moisture content of the product was 3 per cent. The temperatures at intermediate stages were 98°C after 30 minutes, 100°C (39 per cent moisture) after 60 minutes, 101°C after 90 minutes, 103°C after 150 minutes, 108°C (16 per cent moisture) after 180 minutes and 113°C after 210 minutes.

Continuous wet rendering (protocol M). — The purpose of this procedure was to produce starting material for solvent rendering protocols. To minimise the loss of BSE infectivity, the temperatures were the lowest which could be used to produce a physically suitable endproduct. The raw material was passed continuously through the Rotadisc drier. The transit time was approximately 30 minutes and the maximum temperature on exit was 80°C. The material was then fed into the Stord twin-screw press. The tallow/water fraction was discarded because it was unrepresentative of any normal production method, and the press-cake was transferred to the Atlas cooker for drying under vacuum at 0.85 bar for 240 minutes; the maximum temperature attained was 72°C, and the product contained 2.5 per cent moisture, 18.2 per cent fat, 44.6 per cent protein and 33.2 per cent ash.

Pressure cooking (protocols Q, R and S). — All of these procedures were conducted in the Iwel cooker in which the raw materials were heated initially to 100°C. As steam was generated, air was displaced by venting for 10 minutes. The venting valve was then closed and the chamber pressure was permitted to increase to the appropriate pressure. Holding times were started when the required temperature was reached on the surface of the raw materials. The exposure times included 10 minutes to permit heat to penetrate into the particles. The process conditions for these protocols are shown in Table 3. Steam was generated from the raw materials by applying heat from the steam jacket and beater shaft, as is the practice in full-scale production in Germany. Because of the finite quantity of moisture present the maximum venting period which could be used was 10 minutes. In one case (protocol S) this resulted in the trapping of some residual air, as is indicated by the discrepancy between the temperature and relative pressure in relationship to pure steam (Table 3).

Procedures for decontamination and prevention of cross contamination

In the absence of data for the BSE agent at the time the experiments were conducted, the procedures for chemical decontamination were based upon what was known about the agents of other transmissible degenerative encephalopathies, particularly scrapie, which is the model for the group (Taylor 1991b).

When possible, fresh sodium hypochlorite solution yielding 20,000 ppm available chlorine was prepared each day from a stock 14 to 15 per cent solution (Hay's Chemicals). When the use of hypochlorite was impractical, 2M sodium hydroxide was prepared daily from a stock 32 to 35 per cent solution (Hay's Chemicals).

The experiments were conducted at three separate locations on a single site. The movement of materials between these locations was accompanied by the decontamination of containers. Personnel also changed their protective clothing except for wellington boots which were scrubbed with hypochlorite. When there was a possibility, and between the usage of the various items of cook-

TABLE 5: Dilutions of the samples injected into mice

Sample from protocol	Material	Dilutions injected (negative log ₁₀)
A	BSE brain pool	1,2,3,4
I,S	Tallow	1
C,E,G,I,K	MBM*	0
B,D,F,H,J,L,Q,R,S	MBM*	0,1,2
M	MBM*	0,1,2,3

* Supernate from a centrifuged 30 per cent (w/v) homogenate of meat and bone meal

would be delivered were cleaned and decontaminated; after scrubbing with detergent these apertures were sprayed repeatedly with sodium hypochlorite so that they remained moist for an hour before they were washed with water. The decontaminated exit points were protected from contamination by shrouding them with disposable polythene sheeting. In the case of the Atlas cooker the flow of cooked material was controlled by a ball-valve; because this valve might have been corroded if it had been exposed repeatedly to strong sodium hypochlorite, this discharge line was decontaminated, after scrubbing it with detergent, by allowing 2M sodium hydroxide to flow freely through the line while rotating the ball-valve several times. After closing the valve the line was filled with 2M sodium hydroxide and left overnight; the sodium hydroxide was then released and the line was rinsed thoroughly with water.

To prevent corrosion from repeated applications of sodium hypochlorite, the metal bins used to catch the cooked samples were decontaminated initially and after each usage by filling them with 2M sodium hydroxide. The holding time was usually overnight but never less than two hours, and was followed by thorough rinsing with water. For the same reasons the disassembled components of the press cylinders, the hydraulic press, the drum-mixer and the mill were decontaminated in the same way after each usage.

Bacteriological screening

Although scrupulous measures were adopted to prevent cross-contamination of the experimental samples with BSE agent, it was recognised that the starting materials would be contaminated with conventional microorganisms. The meat and bone meal samples were screened bacteriologically by Q Laboratories, and the brain macerate and tallow samples were screened at the Neuro-pathogenesis Unit.

Bioassay of BSE infectivity

Weanling RIII/FaDk-ro mice were used for the bioassay because they had been shown to have shorter incubation periods with the BSE agent than other mouse genotypes (Fraser and others 1988); they were injected with 0.02 ml intracerebrally and 0.5 ml intraperitoneally.

To assay its titre of infectivity, the pooled BSE brain macerate was homogenised in sterile antibiotic solution containing 5000 iu penicillin and 5000 µg streptomycin/ml (Northumbria Biologicals) in a Griffiths tube to give a 10⁻¹ w/v preparation. Three further 10-fold dilutions (v/v) were made in antibiotic solution to 10⁻⁴. Each dilution was injected into weanling RIII mice.

Each sample of meat and bone meal was homogenised in sterile distilled water to give a 30 per cent (w/v) homogenate in a Virtishear homogeniser (Virtis); the homogeniser probes were decontaminated between each usage by porous-load autoclaving at 136°C for 18 minutes, followed by washing and re-sterilisation by autoclaving. The homogenates were centrifuged at 500 g for 10 minutes and the supernates were injected into groups of weanling

TABLE 6: Titration in mice of the infectivity in pooled BSE-infected brain

Dilution group	Number affected/number injected*	Mean (SE) incubation period (days)	Infectivity titre/g of tissue
10 ⁻¹	13/13	415 (10)	
10 ⁻²	9/14	480 (26)	
10 ⁻³	4/16	508 (29)	10 ^{2.7} ID ₅₀
10 ⁻⁴	0/13		

* Excludes intercurrent deaths

(Table 5). The single exception was sample M which was diluted in antibiotic solution because it was known to be contaminated with bacteria.

Because the proportion of BSE brain in the mixtures of raw materials was 10 per cent, the maximum titre which could have been present in the samples of meat and bone meal was 10-fold less than in the BSE brain-pool. It was assumed that the cooking methods would produce at least a 10-fold loss in infectivity. Consequently, when the infectivity of meat and bone meal was titrated, the highest dilution tested was a 100-fold more dilute than for the BSE brain pool titration. The single exception was with the meat and bone meal produced in protocol M by deliberately cooking at a low temperature to minimise the loss of infectivity, because it was to be used as the starting material for other protocols; in this case one additional 10-fold dilution was tested (Table 5).

The initial dilution of meat and bone meal in sterile distilled water, 3/10, was calculated on the basis that the product represented approximately 30 per cent of the weight of the initial raw materials; this makes it possible to compare directly the infectivity titres in the end materials with those in the starting materials, on the assumption that all the infectivity partitioned with the meat and bone meal fraction.

Because meat and bone meal is considered to have been the vehicle of BSE infection only the tallow samples I and S, representing one low temperature and one high temperature process, were tested for infectivity. These samples were injected into mice before and after they had been filtered through diatomaceous earth, as used in commercial practice. They were filtered through a 13 mm bed of Kenite 1000 (Diacel) aided by vacuum. Before the tallow samples were injected 10 per cent w/v homogenates were prepared in sterile physiological saline by means of glass/teflon tissue-grinders.

After they had been injected, the mice were observed and scored for the development of clinical neurological disease for up to 904 days, according to a protocol developed for scrapie in mice (Dickinson and others 1968). The brains from all the mice were immersion-fixed in 10 per cent formal saline. Sections stained with haematoxylin and eosin were prepared from five coronal sections chosen to represent the whole brain (Fraser and Dickinson 1968), and examined microscopically for the presence of spongiform encephalopathy.

Results

The brain pool macerate contained moderate numbers of *Bacillus* species, *Streptococcus faecium* var *durans* and *Hafnia alvei*. All the samples of meat and bone meal were negative bacteriologically except that from protocol M which, as has been discussed, was an atypical and unrepresentative low temperature procedure designed to provide BSE-infected material for other protocols; this sample contained 20 colony-forming units of clostridia/per gram. No bacteria were detected in the tallow samples I and S.

Table 6 shows that the infectivity titre of the BSE-infected brain-pool macerate was 10^{2.7} ID₅₀/g, calculated by the method of

TABLE 7: Numbers of mice dead or culled without neurological disease after being injected with meat and bone meal (MBM) or tallow

Sample code	Number of mice injected	Days after injection		
		301-450	451-650	651-904
B, MBM	24	7	7	4
D, MBM	24	4	10	7
F, MBM	24	8	9	4
G, MBM	24	3	8	4
H, MBM	24	3	8	3
K, MBM	24	6	7	8
L, MBM	24	5	9	7
M, MBM	24	0	7	7
Q, MBM	24	2	11	5
R, MBM	24	6	4	11
S, MBM	24	4	7	8
I tallow*	48	11	11	2
S tallow*	48	11	18	2

* Unfiltered plus filtered

weight of the solid material remaining after rendering was 30 per cent of that of the starting material, the maximum titre in the resulting meat and bone meal would have been $10^{1.7}$ ID₅₀/0.3 g (or per ml of 30 per cent suspension). For the protocols which produced meat and bone meal and tallow (two tested) which failed to transmit disease to mice, the survival data are shown in Table 7. Table 8 demonstrates that the meat and bone meal produced by protocols C, E, I and J was infected, and that the titre of infectivity surviving protocol J was $10^{1.6}$ ID₅₀/ml 30 per cent meat and bone meal suspension. The samples of meat and bone meal from protocols C, E and I were not titrated.

Discussion

These results establish that the pilot equipment had performed similarly to full-scale plant in microbiological terms.

At $10^{2.7}$ ID₅₀/g, the infectivity titre of the pool of BSE-infected brain was less than might have been expected from the results of other assays (Taylor and others 1994). This was due partly to the fact that 15 per cent of the constituent brains was subsequently shown to be either negative or inconclusive for a BSE diagnosis; this proportion is similar to that recorded for the brains from all BSE suspects submitted during the same period and throughout the rest of 1990 (MAFF, unpublished data). A further contributory factor was that the portions of brain stem removed for histological examination were much larger than was subsequently shown to be necessary; in BSE-affected bovine brain, the medulla has been shown to contain the highest level of the disease-specific form of PrP protein, designated PrP^{Sc} (Scott and others 1990), which is considered to be associated with infectivity. In the hamster scrapie model PrP^{Sc} accumulates progressively with the titre of infectivity and reaches its highest concentration at about the time of onset of the clinical disease (Czub and others 1986). The precise relationship between the titre of infectivity and the accumulation of PrP^{Sc} is, however, unclear, because the treatment of the host with amphotericin B can depress PrP^{Sc} production but not the titre of infectivity (Xi and others 1992). Nevertheless, under normal circumstances, quantitative differences in PrP^{Sc} levels are regarded as reasonable indicators of differences of titre.

Given the titre of the brain pool, and its 10 per cent inclusion level in the raw materials, the bioassays were able to detect slightly less than a 100-fold reduction in titre. It cannot, therefore, be concluded that the procedures which produced meat and bone meal in which no infectivity was detected were necessarily completely effective. However, four of the procedures produced infected material, and in the case of protocol J, it had a titre of infectivity comparable to that of the untreated raw materials. As a result, these methods in the EU have been revised (European Commission 1994). The limits of extrapolation from the pilot scale to full scale

TABLE 8: Presence of spongiform encephalopathy in mice injected with 30 per cent suspensions of meat and bone meal

Sample code	Number affected/number injected*	Mean (SE) incubation period (days)	Infectivity titre /ml
C	15/16	521 (23)	
E	10/14	566 (17)	
I	7/15	520 (44)	
J undiluted	9/11	440 (63)	
1/10	10/15	417 (69)	
1/100	4/13	531 (52)	$\sim 10^{1.6}$ ID ₅₀

* Excludes intercurrent deaths

In continuous rendering systems it is difficult to know what the range of particle residence times is, because some particles can be accelerated through the system by 'leap-frogging' while others can be retarded through 'back-tracking'. As a preliminary to defining the minimum conditions for continuous rendering in the pilot-scale studies, manganese dioxide was used as a marker in flow-rate studies in some continuous rendering systems (Woodgate 1991). In the experiments involving natural fat continuous systems, the minimum exposure-time protocols C, E and K did not achieve the same temperatures as the average-time protocols D, F and L (Table 1). This was because, while water remains the heating medium at atmospheric pressure, the temperature remains at around 100°C, but when sufficient water has evaporated, fat becomes the heat-transfer medium, and the temperature rises towards the expected end-temperature. Because the minimum-time samples were taken before all the water had evaporated, the final temperature would be expected to have been between 100°C and the maximum temperature. In contrast, in the experiments involving added fat (protocols G, H, I and J), there was little difference between the temperatures achieved in the minimum-time protocols (G and I) and the average-time protocols (H and J), because fat was the heating medium.

As discussed earlier, protocol M was an atypical low temperature procedure (72°C for 240 minutes) devised to produce infected greaves for solvent extraction studies; these studies have not been described because the greaves produced by protocol M had no detectable infectivity. This was surprising, given the known thermostability of BSE and scrapie agents (Taylor 1992, Taylor and others 1994) but underlines the fact that these types of agent have not previously been tested under the conditions found during rendering. The thermostability of the BSE agent in protocol M may have been due to the fact that much of the fat was removed from the raw materials before they were processed. Other laboratory studies on the inactivation potential of solvent extraction procedures are in progress.

Each mouse was injected intracerebrally and intraperitoneally because the normally greater efficiency of intracerebral challenge with mouse-passaged scrapie agent does not hold for bovine material infected with BSE, or sheep material infected with scrapie, when they are injected into mice (Kimberlin 1993, Taylor and others 1994). The mean incubation periods for bovine BSE agent in RIII mice have been shown to range from around 360 days for a high titre inoculum to around 500 days at limiting dilutions (H. Fraser, personal communication). The shortest single incubation period which has been observed with BSE in RIII mice during tissue transmission studies is 317 days (H. Fraser, personal communication); any mice which died less than 300 days after being injected in this study have therefore not been included in the results. The maximum single incubation periods which have been recorded have been around 600 days (Taylor and others 1994, H. Fraser, personal communication), and RIII mice used in tissue transmission studies are observed for up to 650 days after injection. However, heating has been found to modify the dose-response curve of the scrapie agent, and results in extended incubation periods (Fraser and others 1992). Mice injected with heated BSE agent were therefore observed for up to 904 days after injection. The maximum single incubation period for untreated

form encephalopathy after being injected with the heated BSE agent, seven had incubation periods ranging from 624 to 703 days. This result demonstrates that the dose-response curve for the heated agent is delayed compared with the untreated agent, as has been described previously with the rodent-passaged ME7 and 263K strains of the scrapie agent (Dickinson and Fraser 1969, Taylor and others 1994).

The processes which led to the survival of BSE infectivity in this study attempted to mimic the continuous rendering processes which were responsible for the production of increasing proportions of meat and bone meal during the early and mid 1970s. The timescale over which these changes in production methods occurred is not consistent with the onset of the effective exposure of the cattle population to BSE in 1981/82 (Wilesmith and others 1988).

However, the rapid decline in the use of solvent extraction as an adjunct to rendering processes which occurred during the late 1970s and early 1980s in Great Britain has been identified as consistent with the estimated onset of the exposure of cattle to sufficient scrapie-like agent to cause BSE which occurred in 1981/82 (Wilesmith and others 1988). It is postulated that the solvent extraction processes may have reduced the infectivity of meat and bone meal either as a direct effect of the solvents or because the solvents were stripped out of the defatted rendered material by the direct application of live steam.

It therefore appears that the effective exposure of cattle to sufficient scrapie-like agent to cause disease could have been the result of a two-stage process involving the cumulative effect of the two major changes in rendering practices which occurred during the 1970s and early 1980s.

These experiments have examined the effect of rendering procedures on the BSE agent, but renderers are also interested to know how effective their processes are with the scrapie agent; field strains of scrapie which are more thermostable than BSE may exist. Similar experiments, using scrapie-affected sheep brains, are in progress.

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Abstracts

Welfare of sows in different housing conditions

SIXTY-FIVE sows from a single source were allocated when they were about nine months of age, and in the seventh week of their first pregnancy, to three types of accommodation. Twelve were placed in good quality unstrawed stalls, three groups of five were put in strawed pens with individual feeding stalls, and 38 were kept in a strawed yard with an electronic sow feeder. They all returned to the same conditions after periods in farrowing and service accommodation. In terms of a wide range of welfare indicators the stall-housed sows had more problems than the group-housed sows, and these problems had become worse by the fourth pregnancy, when the stall-housed sows spent 14 per cent of their time behaving stereotypically, and a further 50 per cent on activities which were sometimes stereotyped, such as 'drinking' or rooting and chewing at the pen fittings. In contrast, the comparable figures for the group-housed sows were 3.7 and 8.1 per cent, respectively, and there was little difference between the sows in the two types of group accommodation. The stall-housed sows were also more aggressive and weighed less. However, there were no differences between the groups in terms of physiological or immunological tests, or measures of reproductive output.

BROOM, D. M., MENDEL, M. T. & ZANELLA, A. J. (1995) *Animal Science* 61, 369

Treatment of urinary tract infections in dogs

ONE-HUNDRED-AND-FOUR dogs with clinical signs of a urinary tract infection were selected by 15 practitioners for a comparative, randomised study of different antibiotic treatments. The clinical diagnosis was confirmed by bacteriological tests on urine. Each dog then received either marbofloxacin (2 mg/kg orally once a day or 4 mg/kg subcutaneously every four days) or amoxicillin-clavulanic acid tablets (12.5 mg/kg twice a day) for 10 or 28 days, depending on the clinical diagnosis. Each case was examined and analyses of urine were made at least three times, and possible side effects were looked for carefully. Marbofloxacin and amoxicillin-clavulanic acid both gave good bacteriological cure rates (96 and 85 per cent, respectively) and clinical cure rates (83 and 70 per cent). There were fewer relapses among the dogs treated with marbofloxacin. A few mild side effects were recorded with both treatments.

COTARD, J. P., GRUET, P., PECHEREAU, D., MOREAU, P., PAGES, J. P., THOMAS, E. & DELEFORGE, J. (1995) *Journal of Small Animal Practice*

Report of Findings of Directed Inspections of Sheep Rendering Facilities

Executive Summary

The Food and Drug Administration (FDA) carried out a survey of current practices in the United States (U. S.) for rendering or otherwise disposing of adult sheep carcasses and parts, specifically heads, brain and spinal cord. Limited inspections of rendering plants were conducted to: 1. assess compliance by U. S. renderers with the industry imposed voluntary ban on rendering adult sheep for cattle feed, 2. identify rendering plant practices concerning adult sheep, and 3. determine if rendered adult sheep protein by-products are sold or labeled for use as feed or feed components for cattle. Fifteen plants were identified and investigated that were estimated to process greater than 85% of the adult sheep rendered in the U. S..

The 15 plants rendering adult sheep carry out a variety of management schemes. Eleven render adult sheep with heads, 7 render sheep separately from other species, and 4 render sheep that have died of causes other than slaughter. The rendering industry voluntary ban does not appear to be fully implemented since 6 of the 11 renderers processing adult sheep with heads are selling rendered protein by-products to cattle feed producers.

Industry should be made aware of these shortcomings, and improvements should be monitored by FDA. The Center for Veterinary Medicine recommends that a regulation be prepared to restrict the use of rendered adult sheep proteins in the feed of cattle. Publication of this proposal would be decided at a later date.

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