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MINISTRY OF AGRICULTURE FISHERIES AND FOOD
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John

*Mr Keldy's min 9/12 refers - Mr Keldy - Thanks
cc PS Mrs Scrimgeour
PS from Sec*

From the Minister

Dr Harash K Narang *Mr Osborne*
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*Mr Caveman
Mr Meldrum
Mr Haddock
Dr Shannon
Mr K. Taylor
Mr Smith - inc
Mr Bradley
Dr Brown*

21 February 1996

Dr Mendler

Dr Harash K Narang

Thank you for your letters of 27 December and 5 January. As you know from my Private Secretary's replies to you, I have sought expert advice on the highly technical issues you raise in your letter. I attach a copy of the advice which has been sent to me. I am copying this to recipients of your letters.

I have to say, on reading the technical advice that I have been given, that I do not see any reason why we should depart from the long established mechanisms under which this Department funds research.

MAFF is planning to spend £6.4 million on research into BSE and related diseases in the coming financial year. Furthermore, we are not the only funding bodies. The BBSRC, MRC, private trusts and individuals also fund work and the SEAC is consulted. So far as MAFF research is concerned the Policy Division determines the field of research and the Chief Scientists Group organises the proposals and ultimately issues contracts. All proposals are carefully scrutinised by the Policy Division, CSG and sometimes others, including independent scientists. There is ongoing liaison with the Research Councils concerned with TSE research and we also hold quinquennial reviews (the last in early 1995) in which scientists independent from MAFF are referees. Feedback is constantly received from expert committees including the SEAC as projects are established and current ones come to an end.

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This system applies to all MAFF research and we are constantly refining the procedures to make them more efficient and to secure the best return for the tax

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payer. I reject completely the accusation that no real progress has been made. This is at variance with the views of all the major organisations in the world who have an interest in TSE research and include The World Health Organisation, The European Commission and the Office International des Epizooties. Two researchers at CVL, Mr Wilesmith and Mr Bradley have received important awards from national and international organisations for their research and other achievements in the TSE field. Most of the project leaders of work funded by MAFF are in national and international demand for presentations at scientific meetings.

I certainly agree that fairness is one criterion for awarding research funds but of equal or greater importance are the need for the work, the skill of the team, the resources required and available, the design of the study, its costs and priority in relation to other studies. Work with TSE is necessarily long term and usually demands a team approach in a centre of excellence though new groups have been encouraged to initiate work and some have creditable results.

I understand that any proposals from yourself have been examined in the same way as those from other individuals and institutes. If they have not been funded this is because they lacked sufficient merit or were not of sufficient priority.

In your letter of 27 December 1995 you appear to suggest that research grants may not be awarded fairly and as an example you state that Electrophoretics International Ltd has been given a contract to develop a live test without previously having experience in TSE research.

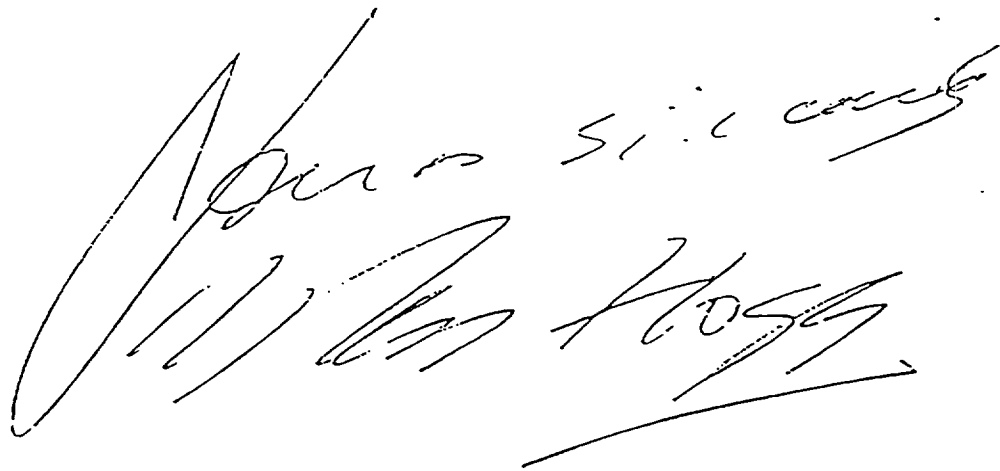
You are wrong to suggest this Company has no experience of TSE research. They have been previously working with blood and cerebrospinal fluid (csf) from patients with CJD using a technique known as 2 dimensional electrophoresis. They wish to explore the same line in cattle with BSE and to do this they need samples and access to the appropriate equipment. This can be provided at Weybridge where the staff have, for some time, been developing a similar method using funding provided by MAFF. However, Electrophoretics International Ltd are not being funded by MAFF for this work.

It is clear to me from reading the papers that this Department and the BBSRC have gone to considerable lengths to try to confirm some of the experiments which you claim underpin your theories about the agents causing BSE and scrapie. I have to say that, despite the expenditure of considerable sums of taxpayers' money the results remain disappointing and I see no justification and none of my scientific advisers have proposed that we should spend more money to develop tests based on theories which are not accepted by the majority of workers in the field and which are not substantiated by research findings. I know that this will be a disappointment to you and I am prepared to ask my

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scientific advisers to look again at the matter if you can give us sufficient technical background for a proper research proposal. However I am not prepared to ask them to depart from the normal procedures and to underwrite work which has only been reported in very general terms in the national press and not in sufficient detail in the peer reviewed scientific press and which you have subsequently refused to explain both to Departments and to the independent advisory committee, SEAC

You will also I think see from the scientific advice that I have been given that there is no evidence from Dr Prusiner's work, as reported in your letter of 5 January, to substantiate the nemavirus theory nor of course does Dr Prusiner himself and his co-workers suggest this explanation of their findings in the paper you sent to me.

A handwritten signature in cursive script, appearing to read 'Douglas Hogg', written in dark ink on a white background.

DOUGLAS HOGG

ADVICE TO THE MINISTER ON DR NARANG'S LETTERS OF 27 DECEMBER AND 5 JANUARY

The main thrust of Dr Narang's letters addresses two points; (a) the comparative blind study of brains from BSE-affected and control (unaffected) cattle using electron microscopy (two methods) and conventional histopathology. I will deal with this study below, including sufficient detail so that others reading this note are clear as to the aims, methods, results and conclusions, and (b) evaluation of Dr Narang's work in general.

(a) Comparative Blind Study

Historical

BSE is identified clinically and, being a notifiable disease in Member States of the European Union (EU), is reportable to the relevant authorities. Failure to do so is an offence. Since compensation at 100% of valuation (subject to minor adjustment) is payable in the UK, there is a strong incentive to report cases independently of the legal requirement to do so. Following notification, in a straightforward case, suspects are visited by a Ministry Veterinary Officer. If he or she believes BSE is the clinical diagnosis the animal is humanely killed, the carcass is taken to a secure site and the head removed for microscopic examination of the brain at a Veterinary Investigation Centre. The remaining parts of the carcass are incinerated.

Statutory Confirmation of BSE

This is done by microscopic examination of the brain by the standard techniques which are agreed by Member States of the European Union, The Office International des Epizooties and the World Association of Veterinary Laboratory Technicians. Veterinary neuropathologists from the EU and several countries round the world have been trained to a common standard using the methods developed at the Central Veterinary Laboratory, Weybridge. This method can be regarded as the 'gold standard'.

Alternative Methods in Use

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Detection of the disease-specific form of PrP (PrP^{Sc}) by immunoblotting (Western blotting) or by visualising scrapie-associated fibrils (SAF) using electron microscopy are methods supported for use in particular circumstances by the above-mentioned

organisations. Approved protocols exist. Immunocytochemistry is used by some laboratories and protocols are being developed for international approval and use. There is a sound correlation between the results derived by microscopic examination of the brain and SAF detection using these protocols though autolysis may affect the results from the former method in a small number of cases.

Other Methods

The 'Touch Method' (Narang, Asher and Gajdusek, 1987) has been used by Dr Narang in experimental scrapie in hamsters and rats, in experimental and natural CJD and in other species with transmissible spongiform encephalopathy (TSE), including cattle with BSE. Our own evaluation of the method is that it is specific for TSE, at least in regard to scrapie and BSE but that it shows no advantage over the conventional SAF method and is significantly less sensitive. It is however, a quicker method. This was one reason why the comparative study reported below was initiated in 1990/1991. The other was that, at that time, we did not know the comparative sensitivity between the conventional SAF method and Dr Narang's 'Touch Method'. No-one has, to our knowledge, published successful results from a peripheral tissue using the 'Touch Method'. This would be an essential requirement if it was to have any value as an *ante mortem* test in a clinically or pre-clinically affected animal. Mr Bradley did mention this important aspect to Dr Narang at the seminar held in London on 17 December 1992 and suggested he tried the method on spleen and lymph nodes of hamsters terminally affected with scrapie. We are not aware of any publications from Dr Narang on this aspect reporting work he may have done. We also point out that even if a more rapid and effective test was available to detect PrP in the brain (which it is not) the organs which are the most likely to contain infectivity in an infected animal (whether or not they are from an infected animal) are removed at source under the Specified Bovine Offals Order, so they can not enter any food or feed chain.

We know of no laboratory which uses the 'Touch Method' for the routine confirmation of TSE in any species. Indeed, when the protocols for the three standard confirmatory methods were circulated for comment to a number of laboratories round the world concerned with TSE diagnosis and research, none even mentioned the 'Touch Method'.

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Electrochemistry of urine as a means of clinical diagnosis of Alzheimer's disease and CJD was originally used by the French and has been developed further since, at CVL Weybridge. It is not yet developed to the stage where it can be used in the field.

Detection of SAF in urine has, so far as we are aware, only been reported by Michael Hornsby ('Times', 6 November 1995) referring to work by Dr Narang on the basis of information presumably supplied by Dr Narang. We know of no other published report on the subject and are disappointed that Dr Narang is unable to supply, even in confidence, any information to SEAC on the protocol or data on the validation of the method.

Detection of TSE-specific protein in cerebrospinal fluid is another method that is under investigation by various groups, but has not yet reached the stage of publication or validation.

The Aim of the Blind Study Involving Dr Narang

The aim was to determine the sensitivity and specificity of the 'Touch Method' by comparison with microscopic examination of the brain and with the conventional SAF detection method in a blind study. In this context 'blind' means that the operators (Dr Narang, PHLS Newcastle - ('Touch Method'); pathologists in the VI Service (microscopic examination of the brain); and electron microscopists at CVL Weybridge (conventional SAF method)) were unaware of the clinical status of the cows donating the brains or the results of tests by their opposite numbers until all the tests were complete. At this time the complete results from each study were communicated to all participants by the key holder, who played no part in the practical aspects of the study.

The Methods

Brain samples were supplied from ten cattle, five suspected to have BSE and five clinically healthy controls which were homebred, and came from farms without a history of BSE. No participant knew the history of any case.

Each test was carried out by the standard protocols of the laboratory concerned. Dr Narang reported the results to the SVIO, Newcastle VIC within half a day. Final results for all studies were tabulated and sent to each participant.

The Results

These are listed in Appendix 1. Mr Bradley wrote to Dr Narang on 20 March 1991 five days following communication of the results (Appendix 2). Dr Narang did not reply to this letter. However, he did point out an error to Dr Lightfoot on 20 March 1991 in which he noted that brain 4 had been received with brain 3 on 16 November, not 20 November as stated in the report. This is a simple error of transcription, not an error in the brain samples submitted to each participant. This has been confirmed both verbally and in writing by the SVIO, Newcastle VIC. The brain in question was negative in all three studies and supports this interpretation.

Comments of the Results

1. There was complete agreement between the results obtained from clinical examination, microscopic examination of the brain and the conventional SAF method.
2. Dr Narang's 'Touch Method' gave no false positive results, ie. there was complete agreement between all tests in all BSE-negative controls.
3. The 'Touch Method' correctly detected two BSE positive cases out of five. After re-examination Dr Narang subsequently changed one negative diagnosis to positive, but outside the agreed time interval.
4. The 'Touch Method' appeared, under the conditions of the study, to be specific for BSE. However, its sensitivity was inadequate since only two out of five (40%) or at best three out of five (60%) of positive BSE cases were correctly identified.
5. The only advantages of the 'Touch Method' over the conventional SAF method are speed (20 minutes versus 3 days at that time) and perhaps cost. The SAF method can now be completed in 3 hours.

Subsequent Developments

A recent paper by Stack *et al* (1995) has evaluated the 'Touch Method' using the cervical spinal cord and brains from 12 sheep with natural scrapie and six scrapie-negative controls, by comparison with the conventional (centrifugal extraction)

technique for SAF and microscopic examination of the brain. The conclusion of this study is that the 'Touch Method' did not offer any advantage over the conventional SAF method for the diagnosis of natural scrapie. Furthermore, the SAF method was clearly more sensitive since it gave consistently higher SAF scores in more brain regions than the 'Touch Method'. No false negative results were reported supporting the view that the 'Touch Method' is specific for TSE in sheep.

Overall Conclusion

Dr Narang's 'Touch Method' appears specific for natural TSE in sheep and cattle and in the limited studies undertaken (17 affected animals and 11 controls in total). However, to be of value in the field it must have a sensitivity, at least comparable to existing methods (eg. the conventional SAF method). Its sensitivity is substantially lower, and could not be used with confidence in a practical way to protect public or animal health when more sensitive methods are available.

The method might be of use in situations where speed of diagnosis is paramount (eg. in some research situations) but on the understanding that a negative result could be false and that the case in question may be infected and might be shown to be so by use of a more sensitive method.

We are convinced that there is no justification for conducting any further evaluation of the 'Touch Method' as it is inferior to methods already available, and protocols for which are adopted internationally. We reject Dr Narang's current accusation that the study was invalidated. If he thought this why did he write a draft paper for publication describing the study in 1991 using the table illustrated in Appendix 1 (but with case 7 altered to produce a positive result without comment)?

(b) Evaluation of Dr Narang's Research

Dr Narang's Papers

Dealing with the two files of papers not related to spongiform encephalopathy and the bibliography we note an impressive number of publications in refereed journals of repute and sometimes with eminent co-authors. Most of these papers relate to ultrastructural or electron microscopical studies in regard to conventional microbiological agents or diseases caused by them. Other than demonstrating a wide experience and a competence in the techniques, they are not directly relevant to

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spongiform encephalopathy (SE) research. We note that Dr Narang's main bibliography list contains 87 items which presumably includes all the 32 SE papers listed separately.

Examination of the 32 SE papers shows that 24 of them, and most of them since 1990, are single author papers. Some of the others, particularly the earlier ones are in co-authorship with scientists of distinction notably Dr E J Field, Dr R L Chandler and Dr D C Gajdusek. Certainly the papers are focused on two main areas, infection-specific particles as determined by electron microscopy and infection specific ssDNA and the connection between these two features. The greater majority of the SE papers are well known to the scientific colleagues I have consulted.

There are two original contributions that Dr Narang has made to the literature. Firstly the morphological description of nemavirus and secondly the evidence for the presence of homologous ssDNA. He has also contributed to the diagnostic technique known as the 'Touch Method' and this has been critically reviewed above.

Tubulovesicular Structures (TVS) and Tubulofilamentous Particles (TFP)

It is very clear that the particles we now know as TVS and first described in ultra-thin sections by David-Ferreira *et al* (1968) and by Bignami and Parry (1971) are genuine TSE-associated structures. Narang and/or others have extended these observations to more species and diseases including CJD and BSE. That is commendable. There now becomes a dispute between Dr Narang and other researchers when he claims that TFP, or nemaviruses, are TVS cut in the longitudinal plane. This is not supportable either on the grounds of size or shape. (For the arguments see Liberski P) (1995) *Acta Neurobiol Exp.* 55, 149-154).

His illustration of nemavirus (TFP) in more than one article, perhaps the best is Fig 2a in *Intervirology* (1993) 36, 1-10, has the morphological appearance of a doublet microtubule from 9+2 ciliary or flagellar axoneme (Chasey D (1994) *Intervirology* 37, 306). A possible origin for such structures is the ciliated epithelium of the ependyma. Cilia also occur in the grey matter of the spinal cord and brain. Such structures appear to be quite different from the intra-axonal tubulo-filamentous particles illustrated in his 1992 paper in *Intervirology* 34, 105-111. Similar structures to the latter have been found in myelinated axons and dendrites of scrapie-inoculated hamsters, in hamsters injected with a mercury salt and in sham, saline-inoculated control hamsters inoculated in a scrapie-free laboratory (Liberski P (1995) ref as

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above). Liberski concluded that TFP are swollen microtubules unrelated to TVS. We remain unconvinced that TFP (nemavirus), as described by Dr Narang are TSE-specific structures. The artistic drawings he has made are ingenious, but hypothetical and there is no morphological or immunological evidence to support their existence as TSE-related structures.

TFP and the Touch Method

I now turn to the use of the 'Touch or impression Method' and the identification of TFP on such grids. This would not be surprising either in the form of ciliary or flagellar axonemes or swollen microtubules. However, they would not be TSE-specific for the reasons stated above. However, we agree that TSE-specific fibrils can be observed on such grids, both in BSE and scrapie, from our own experience. We have no difficulty in accepting that the method is disease specific but are critical of its sensitivity when compared with the conventional SAF method.

Single Stranded DNA

I now pass on to the work on single-stranded DNA. As noted above, Dr Narang's model of a nemavirus (Fig 3 of Dr Narang's 1992 *Intervirology* paper or in his 1993 Brussels paper (VI/4131/94-EN) is ingenious. However, because we believe this to be based on the structure of a ciliary or flagellar axoneme, structures which are found in normal brain, the model is flawed. This does not itself exclude there being a disease-specific ssDNA in scrapie. Studies to investigate the existence of the specific ssDNA Dr Narang claims is part of the nemavirus, have been conducted by Dr L Bountiff in Professor Oxford's laboratory in association with Dr P Levantis. These studies could not substantiate Dr Narang's claim, even though his precise protocol was used under his guidance. The study was jointly funded by the BBSRC and MAFF and refereed by Professor J Almond (University of Reading) and Mr R Bradley (Central Veterinary Laboratory). Dr Helen Grant, a notable independent expert in the human disease CJD, was invited, at Dr Narang's request, to the final project meeting and she concurred with the results and conclusion namely that a scrapie-specific ssDNA could not be identified.

Dr Bountiff concluded specifically in her report to MAFF and the BBSRC as follows.

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"In the light of the data presented above", (*ie. in the report*) "using protocols that were as consistent as reasonably possible with those used by Dr Narang previously, and with due consideration of the criticisms made by Dr Narang, we" (*Professor J Oxford, Dr P Levantis and Dr L Bountiff*) "were unable to detect a scrapie-specific DNA 1.2kb in length.

Since the previous reports of detection were inconsistent, and have only been made in nucleic acids from scrapie infected animals at a very late stage of clinical disease, we conclude the 1.2 kb band is:

- (a) very unlikely to form the basis of a useful diagnosis test for scrapie;
- (b) very unlikely to cast any new light on the pathogenesis of scrapie."

The 'Touch Method' Re-Visited

Also at the London Hospital an opportunity was taken to examine brains of some of the scrapie-affected hamsters used in Dr Bountiff's study by the 'Touch Method' which was first described by Almeida and Howatson about 30 years ago. Nemavirus (TFP) was found in scrapie-affected and in control brain testifying again to the non-specificity of the method. The particles were considered to be microtubules.

Probe for ssDNA

Dr Narang has claimed to have isolated the presumptive scrapie-specific ssDNA and has prepared and patented a probe that he claims can specifically detect it. This was done some years ago. We understand he supplied some of this probe to an American institute, experienced in scrapie research and molecular biology, on numerous occasions. During this period Dr Narang spent some days at this institute and personally supervised the preparation of nucleic acids from coded brains from scrapie-infected and control (normal) animals used for hybridisation with his probe. Hybridisation experiments were performed numerous times with different preparations of his probe. These probe preparations were either brought to the institute by Dr Narang or were subsequently sent by him after his departure. When one of his probe preparations was used no hybridisation could be detected. With a different probe preparation the results were totally inconsistent with the brain samples tested; that is the probe hybridised to both normal brain and scrapie brain material and could not discriminate between them. In simple terms the probe did not work. His clone was requested but was not received. If the probe is as effective as he claims

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why had it not been used to detect infected animals and people? Indeed, instead of using his 'Touch Method' or the newly reported urine test, why is this probe not used even by Dr Narang? If a scrapie specific nucleic acid exists then it is probable that an effective test could be developed quite easily by using a specific probe and the polymerase chain reaction. It is incongruous that he claims to have such a probe and that it is not being used by him or anyone else so far as we can tell.

Urine Test

We are unable to comment upon Dr Narang's newly developed urine test since insufficient detail is published. We appreciate that he may wish to protect his discovery. This can be simply done at modest cost by patenting. However, if it is an effective test, we urge him to publish the protocol, and the validation procedures that have been completed (ie a blind study of urine from confirmed cases of SE and of urine from healthy controls determined by an approved method), in order that it can be used to benefit humans and animals as soon as possible. It is disappointing that he claims that his test could have contributed to the eradication of BSE much earlier, but he has kept the data to himself seeking to publish only the bare essentials in a daily newspaper rather than in a refereed scientific journal. If his test depends on the identification of nemavirus, the same questions will arise as those referred to in section (a) above. However, it is not clear what TSE-specific particles he claims to have discovered.

Protein 'X'

Dr Glenn Telling and his colleagues propose (in the paper Dr Narang kindly sent) that a species-specific (or host) macromolecule, they called protein X, participates in prion formation and may function as a molecular chaperone in the formation of the disease-specific form of PrP (PrP^{Sc}).

In Dr Narang's 1992 paper (*Res. Virol.* 143, 381-386) he mentions a non-host peptide "Accessory Protein" which is coded for by the putative ssDNA already discussed above. He indicates that the protein acts as an enzyme and described its (hypothetical) function.

There is thus a major difference in the two hypotheses. Telling *et al* (1995) are quite clear that the hypothetical protein X is a host protein. Furthermore, they state that, like the binding of PrP^C to PrP^{Sc} which is most efficient when the two isoforms have

the same sequence, the binding of PrP^C to protein X seems to exhibit the highest affinity when these two proteins are from the same species. By contrast, Dr Narang is equally clear that his unconfirmed ssDNA and the hypothetical 'Accessory Protein' are non-host and the former at least is scrapie-specific. The two observations are therefore quite independent and unrelated.

Telling *et al* (1995) developed the protein X hypothesis to explain an apparent interference phenomenon in transgenic mice carrying human and mouse *PrP* genes when challenged with CJD (human prions). Such mice did not develop CNS dysfunction any more frequently than non-transgenic controls. However, when the mouse *PrP* gene was ablated transmission resulted without difficulty and it was concluded that mouse PrP^C somehow inhibited conversion of human PrP^C into human PrP^{Sc} in the original transgenic model.

It is noteworthy that a study in the UK (Collinge *et al* 1995 *Nature* 378, 779-783) using the same transgenic mouse model (one containing both mouse and human *PrP* genes) CJD did transmit quite easily. Therefore it is quite probable there are other possible explanations which do not require the introduction of the protein X theory.

Decontamination Studies

Dr Narang's 1987 paper in the PHLS *Microbiology Digest* 4, 64-67 was an important paper because it included information on laboratory safety and agent decontamination and would be widely circulated within hospitals and laboratories, particularly those of the Public Health Laboratory Service (PHLS) in the UK. Unfortunately it was out of date and inaccurate. The issues in question relate to:

- (a) Listing four autoclaving procedures recommended by the DHSS in 1981 (though Dr Narang claimed this was 1984), two of which were suspended by new information resulting from research and published by the DHSS in 1984.
- (b) An incorrect statement relating to the effectiveness of steam at 132°C.
- (c) An overcautious statement in regard to the concentration of sodium hypochlorite required for effective decontamination of the scrapie and CJD agent.

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- (d) An incorrect statement relating to the concentration of sodium hypochlorite required for effective decontamination of the scrapie and CJD agent.

The full critique was in The *PHLS Microbiology Digest* (1988) 5 (1), and the author was Dr D M Taylor of the then AFRC/MRC Neuropathogenesis Unit (NPU) in Edinburgh.

Reference

STACK, M J, ALDRICH A M, KITCHING, A D AND SCOTT, A C (1995). Comparative study of electron microscopical techniques for the detection of scrapie associated fibrils. *Res. Vet. Sci.* 59, 247-254.