CH25/

MEETING WITH MR PURDEY

From: T E D Eddy
Date: 9 February, 1994
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To: Mr Wilesmith - CVL
Mr Bradley - CVL
Mr Jackman - CVL
Mr Livesey - CVL
Mr Austin - CVL
Dr J Hope - AFRC/MRC Neuropathogenesis Unit, Edinburgh
Mr Salahud Din - PSD
Dr Marrs - DH
Dr Woodward - VMD
Mr K Taylor - ACVO
Mr Howard - AH(DC) B

I attach a draft note of the meeting we had recently with Mr Purdey. I would be grateful for any comments please. I am already grateful to Mr Bradley for casting his eye over an earlier draft. I am inclined to send Mr Purdey a copy of the final version minus the critique in square brackets about negative BSE cases and would appreciate any views you have on this.

T E D EDDY

NOTE OF A MEETING BETWEEN OFFICIALS AND MR PURDEY AND HIS BROTHER

From: T E D Eddy
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To: Mr K Taylor
Dr Matthews
Mr Howard
+ those present

On 17 January the following met Mr M Purdey and his brother Mr N Purdey to discuss Mr M Purdey's hypothesis about a possible role for organophosphates (OPs) in the BSE epidemic.

Mr T E D Eddy (Chairman) - MAFF
Mr J Wilesmith - CVL
Mr R Bradley - CVL
Mr R Jackman - CVL
Mr C Livesey - CVL
Mr T Austin - CVL
Dr Hope - AFRC/MRC Neuropathogenesis Unit
Mr Salahud Din - PSD
Dr T Marrs - DH
Dr K Woodward - VMD

The meeting started by reviewing the current status of the meat and bone meal hypothesis. Mr Wilesmith explained that the initial epidemiological studies had resulted in identification of contaminated meat and bone meal as the most likely cause of the disease and had led to the ruminant feed ban in July 1988. As a result of the ban the disease had declined, first in two year olds, then three year olds and now four year olds. This is the pattern that would be expected in a long incubation period disease when the source of infection has been cut off. We are currently around 300 cases a week down on the level of last year and all the evidence supports the meat and bone meal hypothesis as the major and perhaps only causative factor. Mr Eddy

pointed out that he understood that Mr Purdey and MAFF both agreed that the recent epidemiological finding was consistent with meat and bone meal being the source of infection from early 1980s until July 1988. Mr Purdey said that he viewed the meat and bone meal hypothesis as symbiotic with his own views namely that once the disease had been started by OPs, recycling of animal protein had indeed helped to concentrate disease if sheep dip poisons were being concentrated in the brain. He agreed that the source of infection had been removed in the late 1980s and thought that this was due coincidentally to the ruminant protein ban and to changes in the chemical treatment for warble fly when the impure forms of Phosmet were removed in the late 80s combined with the end of compulsory sheep dipping in 1992. He saw meat and bone meal as a factor for the spread of the disease by concentrating material in the "prion pyramid" but not the trigger factor. In answer to Dr Marrs he stated that he thought that the organophosphates had started the disease off by mutating the PrP gene or by action at transcription or translation. Dr Marrs pointed out that other countries such as Australia and New Zealand also used OPs and had large sheep populations. Mr Bradley pointed out that these countries did not have scrapie or BSE and there was careful surveillance. Mr Purdey felt that this was due to the fact that Phosmet was not used in New Zealand on cattle and that sheep were only dipped once a year. Dr Marrs pointed out that in South Africa sheep were very frequently dipped but Mr Purdey suggested this might be due to natural resistance of the sheep population there to scrapie.

Dr Hope pointed out that there was no evidence for any change in the DNA or RNA in these diseases and no evidence for any models based on conventional mutation. He asked whether Mr Purdey had therefore considered that the OPs might be acting in another way. Mr Purdey then drew attention to a report in Dr Hope's review of a possible link between acetylcholinesterase and PrP which could explain how organophosphates worked because they do act on acetylcholinesterase. However Dr Hope pointed out that his review had been written some time ago and had merely

quoted this as an idea from another researcher which had subsequently been shown to be non-tenable and had now been dropped. Mr Purdey did not seem to want to take this point on board and drew attention to the fact that one of the things found in the disease was disturbance of the cholinergic transmitter system. Dr Hope agreed that this was the case but pointed out that there was no link between PrP and the acetyl choline system. Mr Purdey however felt that there was but Dr Hope pointed out that this was an assertion and that there was no evidence of such a link.

Mr Purdey then went on to refer to the treatment of his cattle with magnesium and paradoxime which he felt supported the role of acetylcholine because he felt they might be blocking an oversupply of acetylcholine in the BSE animals. Dr Marrs felt that it was very unlikely that they would be acting to reactivate acetylcholinesterase such a long time after the initial exposure which caused the disease. Paradoxime could be acting directly on the receptor and benefiting the animal that way.
Mr Austin pointed out that there were clear indications of an effect on post synaptic membranes in BSE but that the pre synaptic membranes were relatively unaffected. However it looked as though all types of membranes were affected and there was no evidence of a selective effect on either the serotonin, acetylcholine or indeed the GABA systems and it does not suggest that there is a specific neuro chemical problem but a broadly based membrane problem in BSE cattle. The one receptor which may not be cut back is the one for N-methyl D aspartate and if this is blocked there is some evidence that you can delay the neurodegeneration. Mr Purdey asked if that was the NO pathway. Mr Austin confirmed that it was but said that these problems were common to many neurodegenerative diseases. The fact that magnesium helped to alleviate the symptoms in BSE cattle is not surprising since it was a common treatment for excitatory disease in ruminants. Mr Purdey persisted in saying that he thought that it showed that magnesium disruption was an indicator of a toxic chemical causing the disease. Mr Austin said that this might indeed be the case but the magnesium deficit might be endogenous and not exogenous. Mr Livesey pointed out

that one of the reasons hypomagnesaemia was a common problem in cattle was because there is no natural store in the animal, unlike calcium which is stored in the skeleton. One of the problems with BSE is an upset in the digestive physiology and this might well lead to a decline in magnesium occurring in the animal as effect rather than cause. Mr Purdey felt that the fact that in some cases the blood levels of magnesium were normal suggests that any magnesium deficit would have to be at the cellular membrane level and that the effect of increasing the magnesium levels was to depress excitability. Mr Austin pointed out that at the very high levels of magnesium Mr Purdey appeared to be using to treat his cattle there would indeed be a general depression of central nervous system activity.

Mr Purdey then went on to suggest that in some way the magnesium might be helping to restore a frame shift mutation which had been caused by the BSE. Dr Hope agreed that there was a hypothesis from Dr Wills in New Zealand suggesting that the BSE agent could work through a frame shift mistranslation as in some viruses. There was indeed a pseudoknot in the PrP gene which acted as a pause signal and frame shift translation would not need to occur at a very great frequency to cause a significant effect. There were differences in the PrP genes between species and in fact the human PrP gene had the best pseudoknot. Dr Hope's group had been doing experiments to investigate the frame shift hypothesis, but had not been able to detect frame shift translation in a system which was capable of picking this up at a rate of 1 in 100,000. In some of the viruses where it occurred, it occurred at a rate of 1 in 3, so it rather looked as though this was not the mechanism.

Mr Purdey then suggested that organophosphates might be acting on a protein folding chaperone to alter the folding of the PrP protein. Dr Hope agreed that the two proteins PrPC and PrPSc did indeed seem to be folded differently. There was a system in normal cells which screened out for destruction proteins which were not properly folded and it was feasible that something goes wrong with this system in the

prion diseases. PrP can act as a lymphocyte activating factor but we do not know whether it has this role in nature or indeed what role PrP plays in the normal animal. Experimental mice have been developed which are devoid of the PrP gene and thus PrP and they are perfectly normal, though this could be due to a compensating mechanism. Mr Purdey felt that there might be a role for PrP in the interferon system and pointed out that there was a lack of interferon in some forms of scrapie. Mr Austin pointed out that there were many biochemical deficits in these chronic and progressive degenerative diseases and it was very difficult to separate what is the primary effect and what is an associated result of the general decline in condition of the animal.

Mr Purdey then discussed his views about a possible link between nerve growth factor and PrP. Dr Hope said that the only link was the fact that nerve growth factor injection caused the induction of PrP messenger RNA but there was no evidence to link PrP with nerve growth factor (NGF) receptor . There was certainly no evidence to suggest that PrP is an NGF receptor. Mr Purdey felt that if the mRNA was stimulated by NGF and if there were a mutation, then if NGF were released in the body in times of stress then the BSE symptoms could be switched on because this would increase the production of the mRNA carrying the mutation. He felt that NGF was indeed naturally released in stress situations and Dr Hope agreed. Mr Purdey felt that is related to his view that in some way there was an oncogenic effect from the PrP. Dr Hope pointed out that the stimulation of m RNA production by NGF was not specific or surprising since there was a general increase in translational activity in neurones as a result of exposure to NGF and this was simply a result of the cell proliferation which was stimulated. The was no evidence of an oncogene. Mr Purdey still felt that there was but Dr Hope pointed out that the problem with this approach was that there was no specific effect, there was simply a general effect because of the induction of the proliferation of nerve cells by NGF.

Dr Hope felt that we were all in agreement that the role of PrP was central and that if Mr Purdey could tie in any link between organophosphates and PrP then that would be a reasonable basis for his hypothesis but the evidence which had been presented in his paper and discussed so far was either superficial rather than real or based on a misconception in reading the literature. The basic concept for the cause of the disease seems to be a factor binding to PrP and converting it to PrPSc. In the prion theory this is PrPSc itself and in other theories either a virus or other particle. Clearly in one version of Mr Purdey's hypothesis it could be the organophosphate. The argument essentially is what is the factor binding to trigger the change from PrP to PrPSc and there was no evidence that it was a member of the organophosphate group. Dr Marrs pointed out that the organophosphates were not a homogenous group and that they were not all, for instance, immuno-toxic. Their only common property was that they all affected acetyl cholinesterases. Mr Nigel Purdey pointed out that he did not feel that scientists had currently met Koch's second postulate in isolating the transmissible agent in BSE. Dr Hope felt that a transmissible factor had been isolated as shown by the transmissibility work into mice and this was associated with protein but in the current state of knowledge it was not clear whether the protein was associated with a virus, nucleic acid or another molecule. Mr Mark Purdey pointed out that GSS had also been transmitted experimentally and thought that Alzheimer's Disease had also been transmitted (note: only amyloid plaques with disease had been transmitted to marmosets). Mr Austin pointed out that GSS seems to be related again to PrP and due to a change in the gene. It did seem that the mutant form of the PrP protein in GSS was sufficient to spread the disease and there is certainly no suggestion of a role for organophosphates in the propagation of that disease or for any post-translational change; it is a classical gene mutation.

Mr Eddy asked, given the fact that Mr Purdey's hypotheses implied a mutagenic role for organophosphates, whether they were in fact mutagenic. Dr Marrs said that he felt that they were not probably mutagenic <u>in vivo</u> and Mr Livesey pointed out that

there were certainly far more powerful mutagens found naturally in the environment. Dr Hope felt that mutagenesis was only a necessary part of Mr Purdey's hypothesis in relation to the frame shift ideas of Dr Wills.

Mr Purdey reverted to the earlier discussion about the origins of the disease. He felt this was due to a change in the organophosphate use. In 1984 Lindane had been banned and the use of organophosphates increased as a result. The withdrawal period for Fenthion had been increased to levels which made it uneconomic for dairy farmers and thus it led to increased use for the other warblecides which were subsequently withdrawn in the late 1980s. This tied in reasonably well with the development and later decline of the disease. He also felt that there had been other changes in animal feed particularly the growing use in cereal screenings and citrus pulp. There had been a particular problem with screenings and Isophenphos which had caused chronic OP problems in pigs. Mr Livesey pointed out that the problem with Isophenphos had been a one-off accident due to the use in animal feed of seed grain material which had been intended for planting and had therefore been treated with different pesticides. The disease caused had been quite unlike BSE in cattle or experimental pig BSE and was typical of OP poisoning. Mr Purdey pointed out that there had in fact been no cases of BSE reported in organic farms on home bred animals. Mr Nigel Purdey pointed out that these could have been exposed to meat and bone meal as, under the Soil Association rules, organic farms are able to use up to 20% of this in their rations. Mr Salahud Din asked Mr Purdey whether he felt the problem with OPs was related to its use on crops or its use in veterinary medicines only. Mr Purdey felt that the problem related to both uses and that the disease had been caused because the overall exposure of the animals from all sources had exceeded the tolerance of the animal to detoxify these chemicals which had then built up in the animal and triggered the disease. This was why organophosphates had not caused similar problems in other countries like New Zealand where cattle were fed less intensively. Mr Eddy pointed out that other countries such as The Netherlands and Denmark fed cattle very intensively but still did not seem to have BSE problems.

Mr Purdey said that he felt there were indeed cases of BSE in The Netherlands which were being covered up. He then went on to say that there had been 4 BSE cases on the Liscombe EHF in the low-input herd where no meat and bone meal was used. But there had been no cases in the wholly-organic herd. Only the low-input herd had been treated with warblecides. He felt that there were at least some born after the ban cows where the farmers had said that they had never used animal protein. Mr Wilesmith pointed out that it was always very difficult to be clear that meat and bone meal had never been used.

Mr Purdey then went on to suggest that as we had exported meat and bone meal to other countries he did not understand why BSE had not occurred in those countries too. Mr Wilesmith pointed out that most of our exports had gone to countries such as Saudi Arabia where they were used in poultry feed. There had been a small number of cases in France and in particular Switzerland which seemed to be related to UK exports of meat and bone meal for cattle but this had been a relatively small trade. Mr Purdey seemed to accept this as he agreed that the incidence of disease was related to the dose.

Mr Livesey went back to Mr Purdey's point about the use of cereal screenings and said that if there was a suggestion that there were high levels of organophosphates in this material then we needed to know. Mr Salahud Din said that there was no evidence from the surveys of total organophosphate levels in cereals of any cases being above the limit and this did not seem to be a significant source. Mr Livesey then asked about citrus pulp. Mr Purdey felt that one of the problems with citrus pulp was the natural amounts of D carbone which used up liver cytochrome P450 oxidase activity and therefore made the liver less capable of detoxifying organophosphates and other toxins. Mr Livesey pointed out that there were many P450 pathways and you could not say that the pathway was being depleted unless you could show that it was the same pathway. Mr Purdey moved to another point and felt that one had to

look not only at the levels of organophosphates in chemicals in individual products but at the whole challenge facing the animal.

Dr Marrs then discussed the effects of long term organophosphate exposure. He pointed out that everyone accepts that in cases where there are convulsions there is indeed long term damage to the central nervous system but this damage is static and it does not lead to degenerative disease; in contrast BSE is progressive and fatal.

Mr Purdey said that most of these studies related to long term effects of acute high level exposure and not to effects of continued low dose exposure. Dr Marrs felt that there had been some studies on this and they did show some changes in receptor populations but that was probably a homeostatic response. Mr Purdey pointed out that there were also receptor changes in BSE. Mr Austin pointed out that none of the descriptions he had ever read showed that OP toxicity syndrome was at all like BSE.

Mr Livesey then went back to ask about the evidence that suggests that the pattern of exposure to organophosphates could in any way be shown to be similar to the pattern of origin and subsequent decline of BSE. This was really necessary to really show even circumstantial evidence for any link. Mr Purdey agreed but felt that the evidence did support that. He felt that the disease had occurred in the UK because we had a particularly high level of exposure to OP residues. Mr Livesey felt that there was no evidence to suggest that this was the case and Mr Salahud Din agreed and pointed out that the use of organophosphates was now going down. Mr Purdey pointed out that the incidence of BSE was also going down. Mr Salahud Din agreed but pointed out that the decline in organophosphate use had started in 1983. Mr Purdey felt that this simply showed the fact that the disease was one with a very long incubation period.

Mr Eddy then went on to discuss possible ways in which the OP theory could be tested. Given the fact that there was no evidence for mutagenesis the most reasonable version of the hypothesis would be that organophosphates might interfere in some

way with the folding of PrPC to generate PrPSc but it would be very difficult to test that because no one had yet developed a system to study the change in vitro. Dr Hope felt however that other ways of examining the hypothesis might be more fruitful, particularly because of the difficulties of reproducing the effect which might be more related to long term, low level dose than an acute, high dose. Biochemistry has now become a definitive diagnostic and the detection of PrPSc is now used as proof of SEs. One might predict from Mr Purdey's hypothesis that PrPSc would be detectable in those suffering from chronic OP poisoning. Dr Hope felt that the discussion had not shown that there was any link between organophosphates and this disease. One of the major problems was that Mr Purdey's hypothesis was not sufficiently defined to be testable. Mr Purdey said that at this stage he did not feel that his hypothesis could be further defined because of such a large range of OPs in use and the possibility that it was not OPs themselves but a contaminant or combination which had caused the problem in the UK when animals' detoxification systems had been so stressed by the overall high level of exposure to these chemicals. This contaminant may no longer be present which would explain the subsequent decline in the disease.

Mr Purdey then raised the question of chemically-induced scrapie like diseases. Dr Hope pointed out that none of the artificially induced diseases were PrP related so this was not particularly helpful to Mr Purdey's hypothesis. Mr Livesey pointed out that in any case the relative restriction of BSE to the UK suggested that it could not be OPs in general. Mr Purdey then returned to his idea of the batch with an impurity. He went on to say that several types of impurity had been known to arise particularly sulphatec. Dr Mans agreed that Diazinone always was contaminated with sulphatec and monotec and this causes about 10% of the overall reported toxicity. Mr Eddy pointed out the difficulty of ever testing this hypothesis since we would never know which the mystery contaminant was, particularly if it was suggested that this had only arisen by accident in the middle 1980s when the disease started and was no longer

around. Mr Eddy went on to ask what was wrong in Mr Purdey's view with the meat and bone meal hypothesis as an explanation. Mr Purdey queried why the bone meal hypothesis would lead to the start of the disease in 1985. Mr Wilesmith explained that this was due to a change in rendering practice which would have led to an increased titre of the disease agent getting through and also explained the lower incidence of the disease initially in Scotland where rendering practices were not changed. Mr Purdey was sceptical and drew attention to an apparent recent rapid increase in the disease in Shropshire but Mr Wilesmith said that this was not happening. Mr Bradley asked Mr Purdey what he thought would now happen to the disease. Mr Purdey agreed with the Department that it would trail off because the vector of PrP scrapie entering animal food had been removed through the ruminant feed ban. He also felt that the decline in the use of Phosmet for warble treatment had also been partly responsible together with the ending of compulsory dipping.

Mr Bradley asked why, in that case, we had seen no major increase in the level of scrapie in the 1980s. Mr Purdey felt that this was because there was relatively little recycling of protein back to sheep and the disease was largely sub clinical in sheep. His hypothesis did not deny the importance of the meat and bone meal in spreading the disease but was more related to the role of organophosphates in the initiation of the disease working through a frame shift or some other mechanism. Mr Bradley said in that case if there was a country with no scrapie or BSE, but the sheep were treated with organophosphates in a similar fashion to the UK and then fed to cattle, then Mr Purdey's hypothesis suggested that BSE should occur in the cattle. Mr Purdey agreed that this was so but it would depend on when sheep had been dipped since he felt that the effects were likely to be most potent at particular stages of pregnancy and when the brain of the foetus was susceptible to toxicity caused by OPs. Going back to warble treatments he pointed out that some countries exempted pregnant cows from warble treatment and he felt that this was one reason why BSE had not occurred there. He felt that it was probably in utero exposure to organophosphates, possibly at

a particular stage of pregnancy, which caused the PrP change in the embryo which eventually led to the development of the disease in the offspring when it became an adult animal. He felt that this was in some way related to teratogenesis. Mr Bradley pointed out there is no correlation between any of this and teratogenesis and Mr Wilesmith pointed out that if there was a general problem with organophosphates acting on embryos of cattle then you would expect this to be thrown up with an increase in malformed calves and other evidence of mutation and teratogenesis which was not happening. Mr Purdey felt that this was not necessarily so because the BSE effect occurred at lower doses than the other effects.

Mr Livesey went back to probing the general organophosphate hypothesis. He felt that it had been agreed and established in the meeting that the problem cannot lie with the use of organophosphates in general and we have to assume that it was due to a rare ingredient or contaminant. The problem then arises as to how likely it is for such a rare contaminant to trigger the disease on the widespread scale on which it arose. This seems unlikely and has to be contrasted with the fact that there is, in the meat and bone meal hypothesis, a model which currently fits the facts and seems inherently more probable. Mr Purdey did not rise to this and drew attention to a paper produced by Dr Ishihawa in Japan about chronic organophosphate poisoning which he claimed affected the same areas of the central nervous system as BSE. Mr Livesey pointed out that in his view the paper did not show that the same areas were affected and that in any case Dr Ishihawa's paper dealt with humans and it was already known that the areas of the brain affected by spongiform encephalopathies in different animal species were not the same so nothing could be drawn from a comparison between humans and cattle. Mr Bradley pointed out that the pathology in chronic OP poisoning seemed to relate to the tracts and the white matter whereas BSE related predominantly to the nucleii and neurones in grey matter. Going back to chemically-induced scrapie cuprisone again affected the tracts not the nucleii however Mr Bradley did agree to review again this particular Japanese paper if Mr Purdey

would send it to him. Mr Purdey later indicated that it was not a paper but a letter to him. Mr Purdey felt that might indeed be the case but that the pattern of brain degeneration was perhaps like that found in BSE negative cases. [Comment: Mr Purdey seems to be under the misapprehension that all BSE negative cases are caused by a common syndrome which he again ascribes to organophosphate poisoning. When defeated on showing that chronic OP toxicity causes positive BSE he switched to the idea that perhaps it caused negative BSE. Furthermore there is a range of different lesions in different BSE negative cases and some (just over half) show no morphological change.]

Mr Austin pointed out that histopathologically BSE and OP delayed neuro toxicity syndromes were very different and in particular OP toxicity affected the peripheral as well as the central nervous system. Mr Purdey pointed out that the OP work had been done on high doses and his concern was about low doses over a longer time frame. Mr Austin felt that nevertheless it was difficult to square this with the fact that the peripheral nervous system does not seem to be morphologically affected in any of the TSEs. He then went on to ask Mr Purdey what he had experienced on his own farm in treating his animals with magnesium and atropine. Mr Purdey concentrated on magnesium where he said the he felt that it was interesting that injection of magnesium did not work within one hour as is normal with hypomagnesaemia but took between 24 and 48 hours to have its effect. Two of the four animals he treated had recovered but the other two had later declined and BSE was confirmed. Unfortunately he confirmed to Mr Livesey that no blood tests had been done for hypomagnesaemia. Mr Livesey felt that the symptoms of BSE in its early stages and hypomagnesaemia were very similar. Mr Austin agreed and felt that if an animal were both hypomagnesaemic and in the early stages of BSE then the hypomagnesaemia might help to show up the BSE symptoms at an earlier stage but treatment for the former would then lead to recovery until the BSE degeneration had reached the stage where the symptoms would come through properly. Mr Livesey

pointed out that metabolic diseases were a balance between inputs, stores and outputs. If anything upset this, as could happen as one of the side effects of BSE on digestion, then this might lead to a greater tendency to metabolic disease, but that would be cause and not effect. Mr Purdey accepted that it might well be cause and not effect but nevertheless felt that the magnesium benefit was telling us something fundamental about the disease and problems at the membrane level. He felt that OPs, by putting pressure on the serotonin system, were depleting intra-cellular levels of magnesium and this was leading to many of the problems through mutation, starting the accumulation of PrP. He drew attention to problems on the island of Guam with human disease where magnesium deficiency had been a potentiating factor in making the population more susceptible to a toxin in their food which had led to a very high incidence of neurodegenerative diseases in the population. Mr Austin pointed out that there was no evidence of a primary role for magnesium. It clearly has a pharmacological role in treatment of animals and the fact that this is true of BSE and other neurological problems in cattle did not necessarily show that there was a primary effect. There was then a rather complicated discussion about changes in the acetylcholine and serotonin receptor systems which seem to conclude that the evidence was by no means clear and that in some models there was an increase in acetylcholine transferase levels and in some a decrease. It was always difficult in major neurodegenerative diseases to be able to isolate cause and effect when a whole galaxy of transmitter systems were changing either as a cause of or as a result of the major changes going on in the structure of the animal's central nervous system.
Dr Hope pointed out in particular that changes in the serotonin system came late in the disease and it was therefore unlikely to be a primary trigger for the onset of disease. Mr Purdey however felt that chronic OP exposure could agonise the serotonin system and this could perhaps switch on the susceptibility to the onset of symptoms in the animal. There seemed to be two stages at which OPs were involved. Firstly at the level of protein folding or translation and secondly at a later stage to trigger the onset of symptoms through the serotonin system. Mr Purdey went back to

his earlier assertion which Dr Hope had already said was wrong, that PrP was in some way related to cholinesterase and suggested that there was a specific effect on the PrP from OPs because of this link. Mr Purdey felt that there was some sort of mutagenic effect. Dr Hope pointed out that all neurones expressed the PrP gene and Mr Livesey pointed out that OPs were not particularly known to be mutagenic in general and that it was very unusual, if not unknown, for a mutagen to be specific to a particular gene in the way implied by Mr Purdey.

Mr Wilesmith pointed out that BSE had been known to occur in some animals which had never been exposed to organophosphates in veterinary medicines and Mr Purdey's hypothesis would therefore have to relate solely to low level chronic exposure to organophosphates from other sources. Mr Purdey accepted that there might be such cases and that in those cases the BSE would be due to OP residues picked up in the field, either through animal feed, pesticides, or for instance, through the disposal of sheep dip. Mr Livesey pointed out that the problem with this approach would be the very low potential dosage that these animals might be exposed to from such routes. Mr Purdey felt that animals were in fact exposed to reasonably high doses through feedingstuffs and said for instance that his own feed merchant would not handle cereal screenings because of the risk of pesticide residues. Mr Livesey pointed out that if this was the sole trigger then it was surprising that the disease did not occur in other countries where these materials were also used.

Mr Eddy then moved the discussion on to the situation in the two Channel Islands and the evidence from that. Mr Wilesmith pointed out that warble treatments had not been required on either Island and it was not possible to relate the high level of disease on Guernsey and low level of disease on Jersey with the use of organophosphates. But it correlated reasonably well with the pattern of meat and bone meal usage. Mr Purdey felt that animals on the Islands had been exposed to organophosphates, for instance through the use of potatoes in animal feed which in

Jersey had been sprayed with Phosmet. Mr Wilesmith pointed out that Jersey had particularly low level of BSE but Mr Purdey disputed that and suggested that many potential suspect animals had been slaughtered in the early stages of the disease for "nervousness". Mr Livesey was worried at the suggestion that organophosphates in cereal screenings were at such levels as to cause serious risks and felt that it was misleading to draw this conclusion from the isolated incident with Isophenphos, which had already been discussed. Mr Purdey drew attention to a New Scientist paper showing that pesticides bind to grain and cannot be detected by chemical analysis. He said that in Jersey pourons had been used voluntarily for lice control and that they were on sale in the shops. Mr Wilesmith pointed out that there were big differences in the incidence of disease between Jersey and Guernsey which could not be explained by the OP hypothesis or the use of non MBM material in feeds such as citrus pulp which were used in both islands. But Guernsey with its high rate of BSE had had a very high level of meat and bone meal and this did explain the difference in rates. Mr Purdey argued that the BSE rates in Jersey were now going up and that some of them were born after the ban cases. Mr Wilesmith said that there had been no rise in the level in Guernsey which was now going down as was the rate in Jersey. Mr Wilesmith also disputed the fact that the Jersey rate had been hidden by other slaughter of cattle since an awful lot of cattle would have had to have been slaughtered in this way to explain the difference in the disease incidence. Mr Purdey felt that reports from the Island had been problematic and referred to a feed merchant who had told him that no organophosphates were used when he subsequently found an advertisement from the same merchant for Holstathion.

Mr Purdey alleged that the usage of citrus pulp had doubled since 1980 and that use of this material had also led to problems of teratogenesis. Mr Austin was surprised and felt as teratogenicity in cattle was very rare there was no evidence of a significant increase. Mr Livesey did however undertake to examine the figures on cereal

screenings and citrus pulp to see if the usage figures did in any way relate to the pattern of BSE.

Mr Austin felt that Mr Purdey had not established any tangible evidence for a link between organophosphates or an ingredient of organophosphates and PrP changes. Mr Purdey felt that this was simply because no-one had tried to do the research. Mr Livesey pointed out that this was because the research was difficult and there was no a priori evidence to suggest that it was a reasonable hypothesis. Given that the disease was now on the decline it was difficult to see how Government could justify expenditure in this area to test what was not at first sight the most likely hypothesis. Mr Purdey felt that research should be done to make sure that the initiating agent had been removed from the environment as he was concerned that this might not be the case and disease could reoccur either in the UK or elsewhere.

Dr Hope discussed work which his unit was undertaking to look at the expression of the PrP gene in mouse and now sheep embryos. This has shown that the gene is expressed at day 10, half way through mouse development and at day 100, about two-thirds of the way through sheep development. Mr Wilesmith asked Mr Purdey what he thought the defect was which caused BSE and Mr Purdey said that he felt that this was probably mis-folding caused either by a direct effect of organophosphates in translation at the ribosomes or frame shifting and an effect via depletion of magnesium. This might come about through effects of OPs on agonising the serotonin system and the consequent diversion of intracellular magnesium to serotonin production. Dr Hope corrected a misapprehension on Mr Purdey's part that PrP was found in particularly high levels in the embryo and pointed out that the level was 10 times less by weight than in the adult animal.

Mr Purdey pointed out that his paper was soon to be published in February in the Journal of Nutritional Medicine. In answer to Mr Bradley he confirmed that he

agreed with MAFF's view on the very remote risk of transmissiblity of BSE to humans and that meat and bone meal is clearly one vector in the transmission of the disease, although he feels that it is not the initiating factor. He also considered the MAFF controls now in place were sufficient to protect human and animal health in regard to BSE. What he would like to see from MAFF was research to show whether or not organophosphates had a role in the initial triggering of the disease which would have to be done by work in vivo by exposure of animals to realistic levels of low OPs over a considerable time. He would also like the mutagenic effects of OPs and other veterinary medicines and pesticides to be screened for, on a regular basis, to ensure that they did not affect PrP or the expression of the PrP gene, both in isolation and in combination as the latter was more realistic in real life. This would have to include chronic low level exposure trials.

Mr Purdey also said that there had been no recorded case of BSE in homebred animals on organic farms. He told us that there were only some 35 registered organic cattle farms.

Mr Livesey felt that it was unlikely to be a mutagenic effect of organophosphates since we know that they are not particularly mutagenic and the pattern of OP usage in the UK is not markedly different from other countries where BSE had not arisen. If, as has been suggested the problem was an impurity which may not now be present then it would be very difficult to do any reasonable experiments. There also seemed to be some confusion over whether low levels of magnesium were a cause or an effect. If low magnesium was part of the trigger mechanism which induced the initial mis-folding then hypomagnesaemia would have to be pre-existing for animals to develop BSE. Mr Purdey agreed but pointed out that low levels of magnesium would not necessarily need to be in the animal as a whole but only on an intra-cellular basis. This could come about by the operation of organophosphates either on the serotonin system or on the MNDA receptor. Mr Austin said there was no effect on the MNDA

receptor. Mr Purdey said that he thought there was an effect of OPs on glutamate decarboxylase but Mr Austin pointed out that that is nothing to do with the MNDA receptor. In his view there was no evidence of any link between OPs and the MNDA receptor and no evidence that OPs as a class could do anything other than affect esterases. Mr Livesey pointed out that some but not all OPs could alkalate but that the alkalating OPs were generally not in use because alkalation caused other toxic side effects.

Mr Bradley asked Mr Purdey whether in the light of his hypothesis there were any tests which he thought we might look at to diagnose BSE or apply on BSE cattle to help prove his hypothesis. Mr Purdey felt that it might be helpful to look at liver enzymes to see if there was a greater incidence of damage to these enzymes in BSE cattle than other cattle which might indicate a higher level of exposure to OPs. Mr Livesey asked which enzymes and Mr Purdey said the cytochrome P450 in general and the transferases. Basically all the enzymes linked to degradation of OPs. He thought that we would find there was a decline in activity of these in BSE cattle. Mr Livesey pointed out that this was a large number of enzymes and it would not be easy.

In conclusion Mr Purdey said that his object had been to try and stimulate MAFF to take an interest in his theory and to consider possible research. He did not have any particular research in mind.

Further action:

Mr Livesey has agreed with Mr Purdey that he will look at citrus pulp and cereal screenings to see if there is any correlation between usage and the pattern of the BSE epidemic. I think he also undertook to try to find out whether we know anything about the OP load in these particular animal feeds, and I would be grateful if he could

please try to trace the New Scientist article claiming that analysis results for pesticide residues in grain were too low because of binding of pesticide to the grain.

I would also be grateful if Mr Wilesmith could look into the question of the Liscombe BSE cases and examine the records for the 35 registered organic farms to see if it is indeed the case that none of these farms have had BSE in homebred cattle.