Trace element (nutritional) theory of "mad cow" disease:

Murray McBride, mbm7@cornell.edu

The outbreak of "mad cow" disease (BSE) in Britain had been connected epidemiologically to feeding of concentrates containing meat-bone meal (MBM) to dairy calves (Wilesmith et al., 1988, 1991, 1992a, 1992b). Beef cattle breeding herds (which generally do not have their rations supplemented with protein concentrates) have had a much lower BSE incidence in the UK, supporting this feed additive theory of BSE.

Since the practice of feeding animal protein is not new, the disease outbreak in the mid 1980's cannot be explained solely by the hypothesis of a disease agent in MBM. However, it has been hypothesized that the cessation of hydrocarbon solvent extraction of fat from MBM in the early 1980's at most rendering plants in England could have allowed an infective scrapie-like agent to pass into dairy feed (Wilesmith et al., 1991). This may not be the only possible explanation, as it was pointed out by Rhodes (1997) that another change in the cow's diet also could explain the epidemic, which was initiated by the higher prices for imported soy and fish meal in the early 1980's. This forced farmers to shift to greater use of the cheaper MBM. There is also reason to believe that the infective prion, reputed to be the cause of BSE, is sufficiently hardy that changes in the rendering plant processing of MBM may not have greatly affected infectivity. Taylor (1998) stated that "most of the rendering procedures used to manufacture meat and bone meal (MBM) throughout the European Union have been found to be incapable of inactivating BSE and scrapie agents". Even autoclaving at 132-138 C is not completely effective (Taylor, 1998). This observation, along with the fact that other countries have fed similarly processed MBM to dairy animals without causing an epidemic of BSE, suggests that an environmental or nutritional factor in certain regions of the UK is a predisposing or causative factor in the disease.

It is curious that the geographic occurrence of "mad cow" disease (number of cases per 1000 head) is not evenly or randomly distributed in the UK, but has tended throughout the epidemic to be highest in the southern and eastern counties (Wilesmith et al., 1992a). Several counties in this region are known to have widespread copper deficiencies in soils and crops (Thornton and Webb, 1980). These crop deficiencies could lead to copper deficiency in ruminants, a fairly well-recognized disease with specific symptoms, in those regions without copper supplements in rations. BSE has tended to have higher occurrence in particular herds, even though there is no definitive evidence that the disease can be transmitted animal-to-animal. Since consumption of MBM presumably varies from animal to animal, the impact on some animals could be much greater than on others.

One impact of a high-MBM diet could be to induce Cu deficiency, as feeds rich in protein, particularly soluble protein, decrease the efficiency of Cu absorption by ruminants (Rehbinder and Petersson, 1994; McDowell, 1985). One would expect heat-processed MBM to be high in soluble protein. Also, animal protein is high in sulfur content, and diets with as little as 0.4 % S can contribute to Cu-deficiency and even cause polioencephalopathy. (The presumed high Fe content of MBM due to blood also has a potentially negative effect on Cu availability). Another is the introduction of Pb, Cd and other toxic metals from organs and bone tissue of diseased "downer" cattle into the feed. On the basis of chemistry, the presence of soluble high-S protein in the rumen might solubilize lead from bone and other tissue, making the lead more bioavailable. The lead
concentration in bone tissue of cattle is high in contaminated areas (Milhaud and Mehennaoui, 1988). A similar argument suggests that high-S protein diets could mobilize certain toxic metals from the substantial quantity of contaminated soil that resides in the abomasum of cattle grazing contaminated land. (I recall a situation in which horses and cows were grazing lead-contaminated pasture, with only horses showing clinical signs of lead poisoning. However, when a chelating agent was administered to a cow from the pasture, she quickly developed lead toxicosis).

The use of animal protein, which increases nitrogen in the feed, could lead to a deficiency of essential fatty acids in the cell membranes, reducing membrane integrity, and making the animal more susceptible to encephalomalacia (Crawford et al., 1991). The fairly recent increased use of canola seed cake in animal rations in the UK could also contribute to this nutritional imbalance, as canola has a high sulfur content and can accumulate certain toxic metals from soils in the seed. Feeds which are high in molybdenum relative to copper are well-known to induce copper deficiency in ruminants (McDowell, 1985), and legume forages or soybean meal can have unacceptably high molybdenum content if grown on non-acid soils with more than 3 ppm molybdenum (McBride, unpublished data). This disease is referred to as molybdenosis, and is generally recognized by obvious symptoms such as changes in hair coat pigmentation.

Based on the number of references that can be found where Cu deficiency has been diagnosed in numerous ruminant species in the wild, as well as captive or in a farming environment, Cu deficiency appears to be common. It has been observed in cattle, moose, red deer, Sika deer, elk, muskoxen and goats (Mackintosh, 1998; Stafford, 1997; Blakley et al., 1998; Arnhold et al., 1998; Gogan et al., 1989), particularly in cases where wild animals have been captive or confined. Wapiti (elk) may be particularly susceptible to Cu deficiency, and the disease is reported frequently in red deer on farms (Blakley et al., 1992). Confining wild ruminants on farms appears to increase the risk of certain diseases, including copper and other trace element deficiencies (Mackintosh, 1998). Wild ruminants may be able to compensate for soil deficiency of particular micronutrients by obtaining a more varied diet than confined ruminants restricted largely to grass forage (Stafford, 1997). Interestingly, wild ruminants appear to be better adapted to low-Cu diets than most domesticated ruminants, as the necessary level of Cu in the liver tissue of domesticated sheep and cows (35 mg/kg dw) is higher than that for deer (10-20 mg/kg) (Arnhold et al., 1998). Domestic goats require even less Cu (8 mg/kg in liver), and it is interesting that the Cu level in the cerebrum is a more reliable indicator of Cu deficiency in goats than that in the liver (Arnhold et al., 1998). Cerebrum Cu concentrations in goats are generally less than 10 mg/kg (dw), levels considered to be marginal or low in sheep and cattle.

Neurological Symptoms in Copper Deficiency and TSE's

In sheep, copper deficiency has been recognized in the UK and elsewhere for a long time as the disease referred to as swayback. Neurological degeneration from swayback has generally been described as demyelination, but more recent investigations of the neuropathology note vacuolation of the white matter, neuronal necrosis, gliosis (Mohammed et al., 1995). Demyelination has been observed in deer with copper deficiency (Geisel et al., 1997; Yoshikawa et al., 1996). However, Yoshikawa et al. (1996) described the neuropathology as "spongy vacuolation and myelin deficiency in the white matter of the spinal cord and brain stem".

Chronic wasting disease in wild moose has become relatively common in Southern Sweden, and there is evidence that it is caused by Cu deficiency possibly induced by increased molybdenum in the forage (Frank, 1998). Neurological pathology associated with this disease is described as"
abiotrophy of the cerebellum characterized by a marked thinning and decreased cellularity of the granular layer and a severe loss of Purkinje cells, leaving empty 'baskets' as reminiscences". (Rehbinder et al., 1991; Rehbinder and Petersson, 1994).

Some researchers believe that neuronal degeneration in a number of diseases could have Cu deficiency as an etiological factor (Hartmann and Evenson, 1992). Menkes' kinky hair disease in infants and young children is a rare X-chromosome-linked genetic disorder of copper transport which appears to result from copper being trapped in certain tissues, especially the kidneys, by abnormal metabolism of metallothionein (Nooijen et al., 1981; Hart, 1983). This leads to copper deficiency, particularly in the brain, causing irreversible damage. The neurological degeneration in Menkes' disease is pathologically similar to that in Cu deficiency of sheep (swayback) (Tan and Urich, 1983), evidence that brain damage in Menkes' is substantially due to Cu deficiency. Neurological damage progresses in infants in spite of copper therapy (Johnsen et al., 1991), with copper accumulating in certain tissues including the kidney, and remaining low in the brain and liver.

Some descriptions of the pathology of central nervous system degeneration from Menkes' disease include:

"...neuronal destruction was widespread in the cerebral gray matter and in the cerebellum, and there was associated gliosis. The changes in the cerebellum were particularly severe, with neuronal loss in the internal granular cell layer. Many Purkinje cells were lost...." (Moon et al., 1987)

"... prominent vascular, cerebral and cerebellar degeneration." (Morgello et al., 1988)

"....marked neuronal loss and gliosis in most areas of the cerebral and cerebellar cortices, midbrain, pons and medulla. The spinal cord showed severe demyelination " (Uno and Arya, 1987)

" The cerebellum showed the most striking abnormalities: severe lack of internal granule cells. Purkinje cells with weeping willow pattern..." (Robain et al., 1988)

These descriptions bear a marked similarity to those noted above for Cu deficient ruminants, and, as will be discussed later, have considerable similarity to the neuropathology of the "transmissible spongiform diseases" of animals and humans.

Spongiform change itself does not appear to be particularly unique to prion diseases. For example, lead poisoning in dogs produced a neuropathology described as "cerebrocortical lesions comprising spongiosis, vascular hypertrophy and gliosis", as well as "spongiform changes" in the cerebellum with "spongiosis of the Purkinje cell layer and vacuolation of Purkinje cells" (Hamir et al., 1984). In cattle, Christian and Tryphonas (1971) observed that chronic lead poisoning produced "astrocytic swelling and development of focal status spongiosis" and "neuronal necrosis", and remarked that lead encephalopathy may be difficult to distinguish from polioencephalomalacia (PEM), especially in the acute stages. PEM was initially thought to be a thiamin deficiency, as the administration of thiamin often alleviated symptoms. However, recent evidence suggests that thiamin has the ability to counteract lead toxicosis (Gould, 1998).

Demyelination has been usually associated with Cu deficiency, for example, swayback disease in lambs. However, degeneration of myelin sheaths has also been reported in spongiform CNS disease in goats and mule deer (Obermaier et al., 1995; Guiroy et al., 1993), as well as in scrapie and Creutzfeldt-Jacob disease (Walis et al., 1997; El Hachimi et al., 1998). Copper deficiency is also
associated with neuronal degeneration and spongiform pathology, so again, we see evidence that the neuropathology of these presumed different diseases has similarity that may confuse diagnosis. Treatment of experimental animals with Cu-chelating compounds produces neural abnormalities including "spongiform changes in white matter" and "reduced myelin development" (Tanaka et al., 1993).

Given this unclear distinction in pathological symptoms of TSE's and other CNS diseases, one must question some of the conclusions that have been reached on the occurrence of TSE's in animals where disease transmission studies have not been done. Specifically, the neurological damage caused by Cu deficiency, and possibly exposure to neurotoxins such as lead, may not be easily distinguished from the damage from TSE's.

These unexplained facts would seem to suggest the existence of (as yet undiscovered) location-dependent environmental factors which may not actually cause TSE diseases, but predispose individuals to infection. For example, Agrimi and DiGuardo (1993) have proposed that the blood-brain barrier may be compromised in susceptible hosts, resulting in localization of metals such as lead in brain tissue, as has been shown in Alzheimer's disease. Heavy metals and/or Cu deficiency may damage the integrity of the blood-brain-barrier, increasing the chance of disease transmission.

Are Heavy Metals a Predisposing Factor in BSE?

In the UK it is possible that offal from some diseased cattle can contain high concentrations of lead, zinc or cadmium as soils of many regions are badly contaminated by centuries of mining and industrial activity. Thornton and Abrahams (1983) estimated that about 1,000,000 acres of agricultural land in the UK has been seriously contaminated by mining and smelting activities over the centuries, and subsequent dispersal by man and the elements. They found average daily intake of lead (Pb) to be higher than intake of Cu in all herds studied in Derbyshire and Cornwall, with Pb intake more than 10 times Cu intake where soil Pb was higher than 1000 mg/kg. Other references note that while clinical lead poisoning in grazing livestock in the UK is uncommon, subclinical lead poisoning and/or copper deficiency could be having significant and more widespread effects on animal health (Thornton and Webb, 1980). The rather common copper deficiencies which appear in sheep in the UK (swayback disease) appear to be exacerbated by lead toxicity (Suttle et al., 1975). Animals from some regions of the UK can have high levels of Pb in bone and other tissues, as well as high Cd in kidneys and liver. It is reasonable to assume that many of the "downer" cattle in the UK, which end up in MBM, will have higher than average concentrations of these toxic metals in their tissues and organs.

Contaminant metal binding to PrP in MBM may convert the PrP to the infective form; that is, a form that is not readily digested by proteases in the digestive tract, and that is able to cross membrane barriers into the blood stream and finally to the central nervous system (CNS). A recent study has shown that Cu ions can convert PrP to the infective disease form (McKenzie et al., 1998). Warren (1974) noted a long time ago that there was at least circumstantial evidence for a role of environmental lead in numerous CNS diseases. He pointed to evidence that suggested divalent metal cations alter membrane permeability, and that "heavy metal cations stimulate degradation of the phospholipids in membranes". Since copper deficiency leads to developmental abnormalities in the cerebellum and demyelination of the spinal cord in ruminants (Rehbinder and Petersson, 1994), and recently has been shown to bind with high specificity to PrP, one should also consider that copper deficiency or excess toxic metals might predispose animals to infection with TSE diseases.

Chronic Wasting Disease in Wild Ruminants- a TSE or Cu deficiency?
The above-described effects of feed quality on Cu status in ruminants could perhaps explain the incidence of "chronic wasting disease" in zoo animals and elk and deer confined on ranches. Kirkwood et al. (1993) reported incidence of spongiform encephalopathy in 5 of 8 greater kudu born since 1987 in a zoo in London, although 4 of 5 were thought not to have been exposed to feeds containing ruminant-derived protein. Numerous other cases of "spontaneous" TSE have been reported in the literature.

Generally, wild deer and elk have not shown this disease at high levels except in one region of Colorado and Wyoming. Outward symptoms in these animals are loss of body condition (wasting), behavioral changes, excessive drinking and urinating, salivation, incoordination, and tremors. Recent observations seem to put in question the belief that "chronic wasting disease" (CWD) of these wild animals is a prion disease at all. Sika deer on farms showed enzootic ataxia, with neuropathological lesions reported as spongy vacuolation in white matter of spinal cord and brain stem. The disease was attributed to copper deficiency (Yoshikawa et al., 1996). Moose in Sweden showed ataxia, wasting, and excessive salivation, with neuropathology reported as cerebellum abnormalities characterized by a marked thinning and decreased cellularity of the granular layer and a severe loss of Purkinje cells. The disease was again attributed to copper deficiency (Frank, 1998).

There are commonly reported incidences of Cu deficiency, diagnosed on the basis of very low blood and liver copper, in many regions of the world. These deficiencies often occur when wild deer, elk and other ruminants are confined on farms or ranches, and it is notable that CWD was observed in confined populations of deer and elk in Western North America for decades prior to the "outbreak" in wild populations of Colorado and Wyoming. There is evidence that confinement prevents animals from browsing for more Cu-rich plant material.

The occurrence of CWD in deer in the Western US, and no report (as yet) of the disease in the East, is consistent with the fact that soils of the West, particularly in the Colorado-Wyoming region and the arid Southwest, are prone to produce forages with high Mo content relative to Cu, potentially leading to Cu deficiency. Alfalfa hay is often fed to deer and elk on farms. Are we able to distinguish a prion disease from Cu deficiency solely on the basis of observations of symptoms in the field, or even cursory examination of brain tissue?

Potential Copper Involvement in Prion Infectivity

This proposed explanation for predisposition to BSE and other TSE's in ruminants, based on nutritional factors, obviously fails to explain the transmission of the disease from infected animals into experimental animals. Recently, however, Ebringer et al. (1997) have proposed that the development of neurospongiform pathology in the brains of experimentally infected animals is an autoimmune response. If this is correct, the validity of many of the reports of BSE transmission under experimental conditions is questionable.

Even if the prion-only theory of BSE proves to be substantially correct, copper and other trace metals may have a key role in controlling infectivity of this molecule. It now appears that the normal prion protein (PrP) of nerve cells in the brain could have a key role in the critical functions of copper in the brain. Recent work shows that copper rapidly and reversibly stimulates endocytosis of PrP from the cell surface (Pauly and Harris, 1998). This could mean that the normal prion acts as Cu sink, since it strongly chelates the metal, or functions as a carrier to deliver Cu into the brain cells. McKenzie et al. (1998) have shown that Cu restores infectivity of scrapie prion (PrPSc), increasing protease resistance after the scrapie prion had been denatured by guanidine.
A number of compounds, including tetrapyrroles (e.g., porphyrin), polyanionic sulfated glycans (e.g., dextran sulfate, pentosan sulfate), and Congo Red, have been shown to interfere with the development of scrapie in mice (Ladogana et al., 1992) and inhibit the formation of protease-resistant PrP in cells (Caughey et al., 1994). There were several studies done in the 1970's that showed a scrapie-like disease to be generated in laboratory animals by feeding them cuprizone, a Cu-selective chelating agent. Treatment of mice with triethylene tetramine dihydrochloride, a Cu-chelating compound, produces neural abnormalities their offspring, including "spongiform changes in white matter" and "reduced myelin development" (Tanaka et al., 1993). This suggests that the removal of Cu from neural cell PrP by soluble chelators could lead to the same pathological symptoms in the brain as caused by TSE disease. In effect, treatment of animals with cuprizone would induce severe Cu-deficiency and the concomitant neuronal degeneration.

References