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Elevated silver, barium and strontium in antlers, vegetation and soils sourced from CWD cluster areas: Do Ag/Ba/Sr piezoelectric crystals represent the transmissible pathogenic agent in TSEs?

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Summary High levels of Silver (Ag), Barium (Ba) and Strontium (Sr) and low levels of copper (Cu) have been measured in the antlers, soils and pastures of the deer that are thriving in the chronic wasting disease (CWD) cluster zones in North America in relation to the areas where CWD and other transmissible spongiform encephalopathies (TSEs) have not been reported. The elevations of Ag, Ba and Sr were thought to originate from both natural geochemical and artificial pollutant sources — stemming from the common practise of aerial spraying with 'cloud seeding' Ag or Ba crystal nuclei for rain making in these drought prone areas of North America, the atmospheric spraying with Ba based aerosols for enhancing/refracting radar and radio signal communications as well as the spreading of waste Ba drilling mud from the local oil/gas well industry across pastureland. These metals have subsequently bioconcentrated up the foodchain and into the mammals who are dependent upon the local Cu deficient ecosystems. A dual eco-prerequisite theory is proposed on the aetiology of TSEs which is based upon an Ag, Ba, Sr or Mn replacement binding at the vacant Cu/Zn domains on the cellular prion protein (PrP)/sulphated proteoglycan molecules which impairs the capacities of the brain to protect itself against incoming shockbursts of sound and light energy. Ag/Ba/Sr chelation of free sulphur within the biosystem inhibits the viable synthesis of the sulphur dependent proteoglycans, which results in the overall collapse of the Cu mediated conduction of electric signals along the PrP-proteoglycan signalling pathways; ultimately disrupting GABA type inhibitory currents at the synapses/end plates of the auditory/circadian regulated circuitry, as well as disrupting proteoglycan co-regulation of the growth factor signalling systems which maintain the structural integrity of the nervous system. The resulting Ag, Ba, Sr or Mn based compounds seed piezoelectric crystals which incorporate PrP and ferritin into their structure. These ferrimagnetically ordered crystals multireplicate and choke up the PrP-proteoglycan conduits of electrical conduction throughout the CNS. The second stage of pathogenesis comes into play when the pressure energy from incoming shock bursts of low frequency acoustic waves from low fly jets, explosions, earthquakes, etc. (a key eco-characteristic of TSE cluster environments) are absorbed by the rogue 'piezoelectric' crystals, which duly convert the mechanical pressure energy into an electrical energy which accumulates in the crystal-PrP-ferritin aggregates (the fibrils) until a point of 'saturation polarization' is reached. Magnetic fields are generated on the crystal surface, which initiate chain reactions of deleterious free radical mediated spongiform neurodegeneration in surrounding tissues. Since Ag, Ba, Sr or Mn based piezoelectric crystals are heat

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resistant and carry a magnetic field inducing pathogenic capacity, it is proposed that these ferroelectric crystal pollutants represent the transmissible, pathogenic agents that initiate TSE.

Introduction

Exceptionally high levels of Ag, Sr and Ba were measured in deer antlers, vegetation, soils sourced from chronic wasting disease (CWD) cluster areas in Colorado, Wisconsin, Saskatchewan, whilst levels were 3-fold, 2.5-fold and 3-fold less (for Ag, Sr, Ba, respectively) in CWD-free areas of Alberta/UK. Ag was virtually undetectable in antlers sampled from CWD-free deer herds in the UK.

These observations were recorded as part of an extensive comparative analytical study of the levels of 46 metals in the soils, water and vegetation of CWD cluster and CWD-free regions conducted across North America. This work represented the North American perspective of a three year globally orientated project designed to establish whether any abnormal mineral profile or abnormal magnetic/radioactive/oxidative capacity is a common characteristic of transmissible spongiform encephalopathie (TSE) cluster ecosystems around the world, and, if so, whether that abnormality plays a primary role in the pathogenesis of TSEs.

Since the primary origins of TSEs are unknown, this study was designed to challenge the theory based upon previously amassed data [1—3] that high levels of specific metals, such as manganese (Mn) or Ag, in combination with low levels of Cu in the environment may bring about a rogue metal replacement at vacant Cu ligands on the cellular prion protein (PrPc) — a Cu binding protein [4] whose misfolded isoforms hallmark the TSE diseased brain [5].

The Hypothesis: Pt 1, the healthy function of Cu—PrP

Does a network of Cu—PrP—Cu-proteoglycan conduits conduct a relay of electric signals that regulate the auditory/circadian associated circuitry of the biosystem?

Since PrPc 'knock out' mice develop symptoms (sleep disorders, abnormal EEG, etc.) that indicate an underlying disturbance in the regulation of the nocturnal—diurnal rhythm [6,7] and GABA inhibitory currents at synapses [4,5], it has been suggested that PrPc performs some metabolic role in mediating the circadian diurnal—nocturnal rhythm and other external stimuli. In this respect, it has been proposed that the paramagnetic Cu [8] component of the PrP molecule [4] performs a role as a conductor of electrical signals [2,3] that mediate the neuronal response to external diurnal —nocturnal, auditory, tactile stimuli, etc.; whereby the electrical signals that are transduced from the incoming energy of these external stimuli, are relayed to the synapses for regulating the GABA inhibitory amino acids/excitatory amino acids that ultimately modulate the neuronal response. It is interesting that PrPc and copper are highly concentrated at the synapse/end plates of select neuronal tracts in the CNS [4], whilst PrP has been shown to influence GABA-type inhibitory currents [4].

Ultraviolet, acoustic and other sources of incoming energy enter the organism and are transduced at the melanin granules (located in the retina, skin [9] and cochlear cells [10]) into electric signals which are conducted via Cu atoms onto PrP's octapeptide repeat metallo binding domains, whereupon that electrical energy is relayed from prion protein to prion protein — 'domino-style' — to synapses/end plates along the auditory, vestibular and circadian associated circuitry for modulating the GABA type inhibitory currents, which, in turn, modulates the overall neuronal response to the original external stimulus. In this respect, these electrical signals perform a key communicative role in connecting the external diurnal/nocturnal, auditory, tactile stimuli with a broad diversity of circadian/auditory effector tissues/organs throughout the biosystem.

The Paramagnetic Cu co-partners of PrP could be viewed as conducting the electrical signals into the synapses/endplates in order to modulate the wide array of physiological processes under auditory, tactile, diurnal—nocturnal circadian regulation — e.g., sleep/wake rhythms, sexual cycles, mood/behaviour, heart beat, immune response, gastrointestinal rhythms, growth and repair of cells (particularly during embryonic/early develop-

ment), including the growth of tumour cells [11–14].

Interestingly, PrPc is intensively localised/expressed in these neural/extra neural tissues that are directly associated with the circadian/auditory circuits and/or their target tissues – e.g., in the cochlear [15], retina, pineal, hypothalamus, visual cortex, pituitary, medulla, glial cells, sympathetic neurones, spleen, lymphatic, tonsils, appendix, myocardial cells, nerve growth factor (maintaining growth and repair) mediated tissues [5,16–18].

It is possible that the Cu component of the PrP delivers its electrical 'cargo' to a copper centred sulphated proteoglycan molecule [19,20] and that these two molecules may play some broad ranging 'two way' co-operative role in the conduction of electrical signals around the biosystem; where the Cu components on PrP deliver their 'spark' to the sulfated proteoglycans (heparin) – growth factor co-receptors [21]; which, in turn, regulate the all important signalling system that maintains/modulates the growth and structural integrity of the nervous system [21]. It is interesting that proteoglycans are known to bind with copper [19] as well as PrPc [5,22,23] in the healthy mammalian biosystem, and that the successful binding of PrP to proteoglycans is disrupted in the TSE diseased organism [23].

Further support for these ideas on PrPc's healthy role can be gleaned from the clinical and pathological profiles that emerge from the disruption/loss of PrP function in TSEs. For instance, the initial lesions in TSEs tend to erupt in the retina [24,25], the skin and the cochlear [15] – e.g., cell lines where the melanin 'transducer stations' are intensively expressed [10,11] – involving tissues that are in the front line of receiving incoming light, tactile and sound stimuli. Many of the more distal regions of the auditory, optic and vestibular tracts are also lesioned in TSEs [26]. Furthermore, one of the key early stage clinical features in BSE suffering cattle involves a pronounced 'hypersensitivity' to sound, touch and light. UK vets actually exploited the symptom of hyperacusis in BSE, by applying a simple 'handclap' startle response test as the best means of diagnosing clinical BSE.

Interestingly, genetically engineered mutations in the melanocyte cells (the melanin producing cells [11]) of experimental animals have been shown to induce spongiform encephalopathy [27]; suggesting that a disruption at any point along these putative conduits of electrical signalling could be associated with the initiation of TSE pathogenesis.

The Hypothesis: Pt 2, metal nucleated crystals and the unhealthy dysfunction of PrP

Ag, Ba, Sr or Mn nucleated crystal-PrP-ferritin complexes disrupt these conduits of electrical signalling throughout the CNS. A primary prerequisite for the pathogenesis of TSEs? (see Fig. 1)

In this respect, the pathogenesis of TSE could be initiated by a disruption at any point along these putative PrP-proteoglycan 'conduits' of electrical conduction; ultimately resulting in the overall collapse in both the GABA mediated modulation of neuronal response at the synapses [4] and the proteoglycan regulated anti-oxidant/growth factor signalling systems [21,28].

Environmental analytical observations in TSE cluster zones suggest that a foreign metal replacement binding on the native Cu/Zn domains on PrPc [4] and/or the proteoglycans [19,20] could be implicated here. This would involve a substitution by a rogue ferrimagnetic or diamagnetic metal species that disrupts the normal paramagnetic conductive capacity of PrP's Cu co-partner. It is also possible that a radioactive metal species could be involved as the rogue metal replacement here.

So once the vacant Cu domains on the prion protein/proteoglycans have been substituted by a rogue reactive metal species, it is easy to envision how the brain could be subjected to a steady, self perpetuating state of deleterious free radical 'melt down'. For these foreign substitute metals will fail to act in the overall best interests of the organism and conduct electric signals in a balanced way.

Rogue metals chelate sulphur and inactivate S-proteoglycans?

Chronic exposures to the highly reactive Ba, Sr salts are known to invoke various other metabolic disturbances [43] that are evident in the pathogenesis of TSEs – such as the hyperactivation of calcium and potassium channels [5]. The fact that Ba, Sr, Ag and Mn will readily conjugate with sulphur in the biosystem [43] could be very pertinent to TSEs, since this would deprive the endogenous sulphated(S)-proteoglycan molecules of their crucial sulphur component, thereby disrupting the co-operative role of the S-proteoglycans in the fibroblast growth factor mediated signalling system that maintains the overall growth and structural integrity of the nervous system [21]. Disrupted S-proteoglycan signalling systems are a consistent

feature of several neurodegenerative diseases, such as TSEs [21—23]. Furthermore, the demonstration of a metabolic association between the S-proteoglycan molecules and the cellular prion protein [22,23] suggests that the disruption of S-proteoglycan mediated signalling systems — observed in the pathogenesis of TSEs — might perform a pivotal role in the origins of TSEs.

High intensities of low frequency acoustic shock waves, a secondary prerequisite for the pathogenesis of TSEs? (see Fig. 1)

It is proposed that these Ag, Ba, Sr or Mn pollutants act as founder nuclei which seed piezoelectric crystals [29] that incorporate PrP and ferritin protein into their structure. Loss of S-proteoglycan activity would deprive the biosystem of one of its key endogenous molecules that inhibits the formation of crystals; thereby enabling these rogue crystals to multireplicate unimpaired, which chokes up the formerly viable PrP-proteoglycan conduits of electrical conduction throughout the CNS. This compromises the ability of the brain to process and protect itself against incoming bouts of high energy sonic shockbursts from the external environment — such as the intensive pressure waves that radiate from sources of low frequency infrasound — low flying jets, military explosions, earthquakes, thunderstorms, etc. Interestingly, one or other of these eco-phenomena have been consistently observed as a key characteristic of every global TSE cluster environment that has been studied by the author to date [2,3].

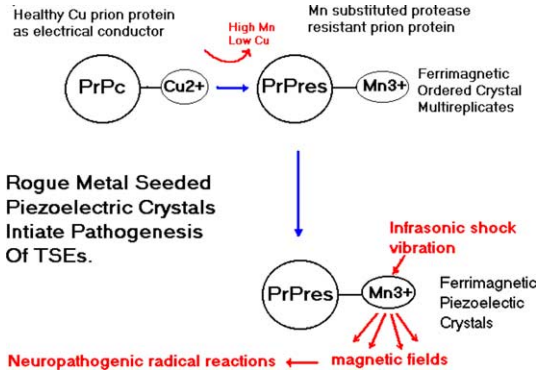


Figure 1

The piezoelectric crystal component captures the sound

Incoming mechanical shock waves of energy are absorbed by the ferrimagnetically ordered 'piezoelectric' crystals, and duly transduced into an electrical/magneto energy [30] which largely accumulate within the crystalline metal-PrP-ferritin aggregates [31] (e.g., the 'prion fibrils') until a point of saturation polarization is reached.

In this respect, the whole TSE disease process can be likened to a battery on continuous charge; whereby the incoming energy from the environment is ultimately captured within these ferroelectric crystal pollutants lodged in the brain. Electric signals and Magnetic fields are generated on the crystal surface. These upset the magneto-electrical homeostasis of the CNS and initiate chain reactions of deleterious free radical [32] mediated spongiform neurodegeneration, leaving a 'halo' of neuronal vacuolation around the crystal particles (see Fig. 2). Interestingly, some of the barium seeded crystals have a tendency to develop into 'flower' shaped structures. In this respect, the whole putative concept of the pathogenic crystal as a cause of TSE could explain the presence of the large 'Florid plaques' surrounded by a halo of spongiform neurodegeneration — the neuropatho-

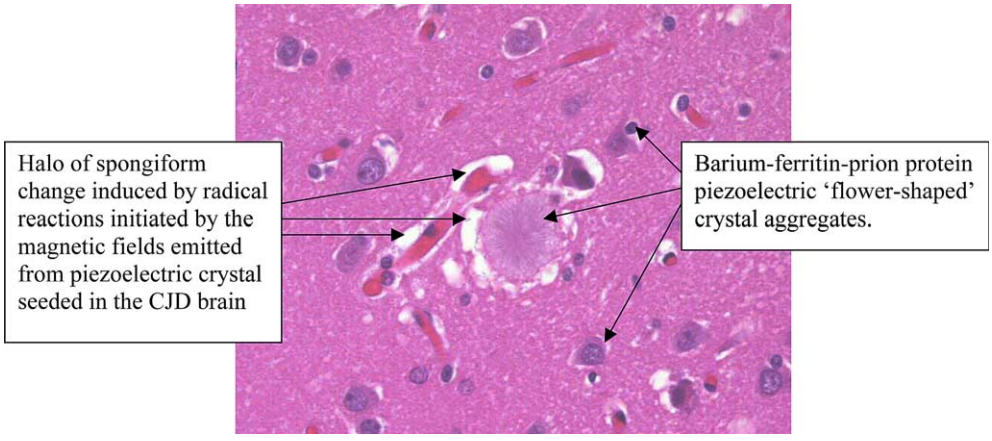


Figure 2 Barium seeded piezoelectric crystals theory explains the presence of florid plaques surrounded by a halo of spongiform change in the cortex of vCJD brain (Courtesy of CJD Surveillance Unit, Edinburgh, UK).

logical hallmarks of the brains of vCJD, Kuru and CWD casualties (see Fig. 1).

The capacity of the metal based piezoelectric crystal to transduce and store up incoming sound energy is well illustrated in several industrial applications; such as the use of chromium, iron or Mn 3+ doped crystals in audio music tape material that stores up a record of sound energy in ferromagnetic form. Other examples involve the use of Ba in sound proof boarding for recording studios, or in the microphone; where the incoming pressure waves of sound are duly converted by the microphone's piezoelectric crystals into electrical signals.

The TSE diseased brain could be described as having a million mini 'microphone' contaminants lodged within it — yet without any loud speaker system to dissipate the energy that has been transduced from the sound. Each ferroelectric crystal accumulates that energy until a point of explosive saturation is reached.

The additional effects of sonic shockwaves on protein conformation

Furthermore, the bombardment of the biological system at large by shock waves from the external environment will also invoke an intensive burst of 'molecular motion' in the tissues, where proteins, for instance, are jiggled around by the heat energy that results from the actual pressure of the incoming shock wave pulse. In more extreme circumstances, the protein molecules will adopt an abnormal conformational shape as a result of the pressure stimuli, but will rapidly refold back into their normal shape providing those proteins are attached to their correct metal co-partners. But in the individual whose metallo proteins are conjugated onto rogue replacement metals — as is postulated in the case of the rogue metal contaminated, TSE susceptible brain — any sonic shock induced conformational derangement of those proteins could remain as a permanent 'fixture' — as evidenced by the presence of the stable misfolded PrP isoform in TSE diseased brain [5].

Interestingly, PrPc cell cultures which have been challenged by sound waves have demonstrated a 10-fold increased expression of PrP [33], This provides some evidence that PrPc expression responds to incoming challenges of acoustic energy.

Ag, Sr, Ba or Mn nucleated piezoelectric crystals, the pathogenic transmissible agent in TSEs?

The theory of the proteinaceous prion particle as the pathogenic agent in TSEs has not been validated in the experimental or epidemiological context. It is proposed that the protein moiety of the metallo-prion complex merely serves as an innocuous *vehicle* that transports the toxic metal causal agent; much like a trojan horse galloping around the synapses of auditory, circadian circuits of the brain carrying its lethal cargo of metallic missiles on board — a magnetic field inducing 'fire power' capacity that is potentially capable of detonating a deleterious chain reaction of free radical mediated neurodegeneration — a progressive pathogenesis that can be generated by the magnetic fields and/or radioactive decay that are emitted by any rogue magnetically ordered or radioactive metal piezoelectric crystals that are successfully seeded in biological tissue.

The magnetic fields proliferate, progressively corrupting the key circadian/acoustic/vestibular circuits (areas that are lesioned in TSEs [5,15,26]), inducing a contagious domino-like aggregation of metallo-crystal PrP molecules that multireplicate themselves into crystalline 'fibril' tombstone deposits in the diseased brain.

Much of the epidemiological history surrounding the major epidemic of BSE in the UK, indicates that the protein only 'prion' hypothesis on the origins of BSE fails to fulfil Koch's postulates [2]. For instance, $\approx$10—25% of the cattle that have been slaughtered each month under the UK government's BSE order for exhibiting the full profile of BSE symptoms had failed to demonstrate the presence of prions at post-mortem [34]. The fact that these so called 'BSE-negative' cows shared the same idiosyncratic clinical profile and spatial-temporal distribution as the BSE positive cows, suggests that these *prion negative* cases were suffering from the same disease as the *prion positive* cases.

Since transmission experiments using TSE affected brain homogenate have indicated that the causal agent remains 'pathogenic' after heating up to temperatures in excess of 800° [35], then the theory of Ag, Ba, Sr or Mn crystals as the TSE causal candidate fulfils this prerequiste for pathogenicity well. For the piezoelectric capacity of most ferroelectric crystal structures will remain stable until the crystals are heated up to their respective 'curie point' temperatures around the 1000° mark; at which point the orientation of the domains and the alignment of the dipoles is destroyed, whereupon the crystal is instantly depolarised and drained. At higher temperatures, the melting point of the crystal is exceeded [29]. Likewise, each ferrimagnetically ordered metal atom within the crystal structure would hold onto their remnant magnetic charge until they are heated to temperatures above their respective curie point temperatures

(500–600°) [8], whence the thermal agitation is sufficient to instantly drain the charge. Such factors as the piezoelectric and magnetic field inducing capacity of the crystal, as well as its self replicating properties, determine the pathogenic potential of the crystal, thus its ability to induce TSE.

It is therefore proposed that these ferroelectric crystal pollutants represent the transmissible, heat resistant, pathogenic agents that cause TSE. These crystals can be readily transmitted via any inoculum of TSE contaminated brain homogenate into a healthy laboratory animal, which, in turn, reintroduces the TSE crystal nucleating agent into the healthy recipient organism.

Methods

Soil sample collection/analysis method

Each soil sample comprised a 300 g sample drawn from a mix of 20 columns of dry soil bored with a stainless steel auger; each column having been bored at equidistant spaces along a W shape spanning an area of ≈10 acres, the area being representative of the region grazed by the CWD affected deer under study. Each column was drawn from the top soil to a depth of 6 in. having taken care to avoid inclusion of root material/surface organic matter and collection of samples near to gateways, roadsides, animal dung, disturbed/excavated or polluted terrain. The 20 columns were collected into a plastic bag, then mixed into an even homogenate, from which a further sample of no more than 300 g was drawn and placed into a small polythene bag, then sealed, labelled and transported to the laboratories at the Department of Geology, Royal Holloway, University of London, Egham Hill, Surrey TW20 0EX, where samples were dried after arriving at the laboratory in forced air flow cabinets. The temperature was maintained below 32 °C during the 12 h drying period and the air was constantly dehumidified. The soil samples were then ground to pass a 2 mm mesh using a hammer mill. The mill was flushed between samples using a small portion of the next sample. Each sample was analysed by standard Mass Spectrometer analytical procedure.

Vegetation sample collection/analysis

Each plant tissue sample comprised a 200 g sample representing tissue collected from ≈10 pickings taken at equal spacings in a W shape across an area of ≈10 acres that was representative of the region grazed by the CWD affected deer/elk under study. Samples were picked dry and at an appreciable distance from roadsides, gateways, animal manure, mechanically disturbed or 'spot' polluted terrain. The tissue was packed directly into plastic bags, lightly sealed, labelled, refrigerated and then transported to the laboratories of the Department of Environmental Sciences at Derby University, Kedleston Road, Derby, DE22 1GB, UK. Each sample was placed in a plastic sieve and thoroughly washed in deionised water. After removal of any roots or soil, the samples were spread evenly on a drying tray and dried in a 90 °C oven to constant weight, and then ground by Christy Norris mill, a small portion of the next sample being used to flush the mill, before collection of the ground material. The samples were then prepared for analysis by dry ashing for non-volatile elements and wet digestion in aqua/regia for volatile elements (e.g., selenium). Analyses was by standard ICP scan.

Antler collection and analysis

Antlers from 2 to 3 years old free ranging or farmed cervidae were collected during April–June 2003 across the regions/farms where the most intensive outbreaks of CWD had been officially identified (DNR 2003). Samples were batched according to CWD cluster/CWD-free region and sent to the University of London at the Royal Holloway, Egham, Surrey, UK for chemical analysis. The samples for analysis were ignited to 600 °C to remove organic material and then powdered in an agate mortar and pestle. They were then dissolved in hydrofluoric and nitric acids and analysed by Inductively Coupled Plasma Atomic Emission and Inductively Coupled Plasma Mass Spectrometry.

Results and discussion

High silver, possible environmental sources and modes of uptake

High levels of Ag and low levels of Cu were recorded in the antler material, soils and deer browse vegetation drawn from CWD affected zones (see Tables 1 and 2, Graphs 1 and 2). These results represent the first time that Ag has been detected in antlers, whilst adding some support to the proposal [2,3] that high

Table 1 Levels of metals in antler from CWD cluster and CWD-free zones across North America

Matrix	Sampling zone	CWD status	Ca0 (%)	Ba (ppm)	Cu (ppm)	Sr (ppm)	Ag (ppm)	Mn (ppm)
Antler	Fort Collins, Co.	CWD+	25.25	138	2	130	3.9	0 (w)(t)
Antler	Fort Collins, Co.	CWD+	25.56	125	2	137	3.4	0 (w)(b)
Antler	Mt Horeb, Wi	CWD+	25.78	63	3	42	2.1	0 (w)(t)
Antler	Mt Horeb, Wi	CWD+	25.43	60	3	42	3.0	0 (w)(b)
Antler	N.Manitou, Sk	CWD+	25.59	206	2	117	4.7	0 (w)
Antler	N. Manitou, Sk	CWD+	25.64	202	2	130	2.7	0 (w)
Antler	N. Manitou, Sk	CWD+	25.29	280	2	114	9.6	0 (w)
Antler	Lloydminster, Sk	CWD+	24.98	88	76	136	3.1	0 (F)elk
Antler	Lloydminster, Sk	CWD+	25.20	77	3	120	2.7	0 (F)elk
Antler	Lloydminster, Sk	CWD+	25.35	156	2	130	4.6	0 (F)elk
Antler	Manitou, Sk	CWD+	24.17	184	2	121	0.7	4 (w)
Antler	Manitou, Sk	CWD+	23.96	179	3	122	0.9	4 (w)
Antler	Manitou, Sk	CWD+	23.98	253	3	140	3.1	8 (w)
Antler	Manitou, Sk	CWD+	24.12	258	3	141	1.0	8 (w)
Antler	Manitou, Sk	CWD+	23.29	269	4	145	0.8	8 (w)
Antler	Manitou, Sk	CWD+	24.28	263	3	143	9.6	8 (w)
Antler	Manitou, Sk	CWD+	23.71	185	3	122	0.8	4 (w)
Antler	Manitou, Sk	CWD+	24.04	191	3	124	5.2	4 (w)
Antler	Manitou, Sk	CWD+	24.32	197	2	115	2.8	8 (w)
Antler	Manitou, Sk	CWD+	24.27	200	2	117	2.8	8 (w)
Antler	Manitou, Sk	CWD+	24.50	202	2	116	2.5	8 (w)
Antler	Manitou, Sk	CWD+	24.59	202	2	117	0.8	4 (w)
Mean CWD antler			24.78	181	5.8	119	3.2	3.4
Antler	Alberta	CWD-free	25.98	56	2	77	2.4	0 (F)(t)
Antler	Alberta	CWD-free	25.35	52	2	71	4.2	0 (F)(b)
Antler	Alberta	CWD-free	25.10	72	2	38	2.0	0 (w)(t)
Antler	Alberta	CWD-free	24.73	69	3	36	3.4	0 (w)(b)
Antler	Somerset UK	CWD-free	24.15	38	3	42	0.0	8 (w)
Antler	Somerset UK	CWD-free	24.57	43	3	57	0.1	19 (w)
Antler	Somerset UK	CWD-free	23.84	74	3	45	0.1	12 (w)
Antler	Somerset UK	CWD-free	24.08	66	2	47	0.0	23 (w)
Antler	Somerset UK	CWD-free	24.20	42	2	42	0.0	4 (w)
Antler	Somerset UK	CWD-free	24.18	43	3	42	0.0	43 (w)
Mean CWD-free antler			24.61	55	2.5	49	1.2	11
Reference mammalian bone			25	5	13	52	0.01	0.2

(w) = Antler from wild deer herd, (F) = antler from farmed deer herd, (t) = section from tip of antler, (b) = section from base of antler.

Ag and low Cu in the environment may bring about an Ag replacement of vacant Cu ligands on the cellular prion protein (PrPc).

Particularly interesting is the fact that Ag was virtually undetectable in antlers collected from CWD-free herds in the UK. However, antlers collected in the CWD free areas of Alberta (which adjoins the CWD cluster zones along the Alberta/Saskatchewan borders) revealed marginally elevated levels of Ag, which may indicate that these areas are approaching the threshold of 'high risk' for hosting outbreaks of clinical CWD in their deer populations in future.

Ag is potentially highly toxic [36], exerting a stronger competitive binding affinity for specific Cu ligands on cuproproteins [37] than for Cu itself. The degree of intoxication encountered following Ag exposure is controlled by the overall Ag/Cu ratio within the biosystem.

Apart from the naturally occurring sources of Ag in soils — well renowned in the Colorado CWD cluster area — possible routes of Ag exposure in the CWD cluster ecosystems could stem from routine feeding of Ag contaminated concentrated feed pellets to captive and wild deer herds. In this respect, Ag was measured at 2.2 ppm in the feed

Table 2 Levels of metals in soils and vegetation sampled across CWD cluster and CWD-free zones

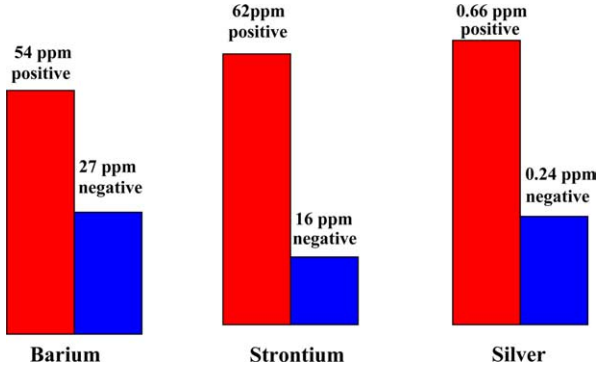
Matrix	Sampling zone	CWD status	Ca0 (%)	Ba (ppm)	Cu (ppm)	Sr (ppm)	Ag (ppm)	Mn (ppm)	S (%)	
Soil	Colorado	CWD+	2.65	568	18	192	0.35	619	0.27	(40)
Soil	Wisconsin	CWD+	1.28	477	16	114	0.21	915	0.13	(40)
Soil	Saskatchewan	CWD+	1.14	905	24	193	0.27	853	NR	(8)
Soil	*Mean*	*CWD+*	*1.69*	*650*	*19*	*166*	*0.27*	*795*	*0.20*	
Soil	Vermont	CWD−	1.99	474	22	98	0.08	757	NR	(20)
Soil	Alberta	CWD−	3.21	537	17	124	0.30	550	NR	(17)
Soil	*Mean*	*CWD−*	*2.60*	*505*	*19*	*111*	*0.19*	*653*		
Soil mean reference			1.00	250	30	80	0.07	750	0.30	
Veg	Colorado	CWD+	10,192	56	13	61.4	0.459	196	0.19	(40)
Veg	Wisconsin	CWD+	10,288	56	16	57.0	0.858	122	0.30	(40)
Veg	Saskatchewan	CWD+	11,295	50	4	68.1	NR	60	NR	(6)
Veg	*Mean*	*CWD+*	*10,590*	*54*	*11*	*62.1*	*0.658*	*126*	*0.24*	
Veg	Vermont	CWD−	7400	24	25	16.2	0.242	111	0.47	(20)
Veg	Alberta	CWD−	6271	31	5	16.4	NR	102	NR	(2)
Veg	*Mean*	*CWD−*	*6835*	*27*	*15*	*16.3*	*0.242*	*106*	*0.47*	
Pasture mean reference			5000	10	20	20	0.05	50	0.35	
Concentrated feed pellets			1.83	16	55	25	2.2	222		

Analyses was performed by MS. Measurements relate to total levels of element recorded as ppm on dry basis. (20) = number of sample sites (covering approx 10 acres for each site) involved in the constitution of each mean level of metal displayed above. NR = not recorded.

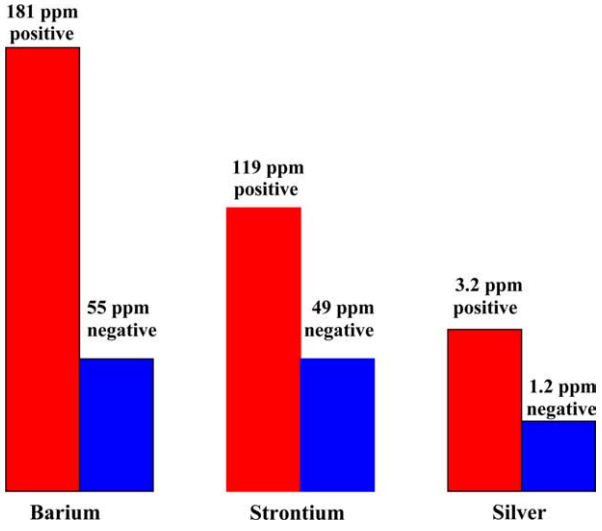
samples collected from deer farms across North America during this study (see Table 1).

Ag use in cloud seeding weather modification

Another significant source of Ag contamination in the drought prone regions where CWD has emerged, stems from the extensive aerial spray application of silver iodide crystals used as founder nuclei in cloud seeding 'rainmaking/snowmaking' operations [38]. The resulting Ag contaminated rain permeates the local vegetation, as well as the growing crops that are incorporated into the concentrated feeds for the deer.



Graph 1 Comparative levels of metals in the pasture of CWD and CWD-free regions. (Mean averages drawn from 86 and 22 samples, respectively.)



Graph 2 Comparative levels of metals in the antlers of deer from CWD and CWD-free regions. (Mean averages drawn from 22 and 10 antler samples, respectively.)

Whilst airborne Ag can be absorbed directly into the brain via the nasal-olfactory route of inhalation [39], the 'fall out' from atmospheric Ag contamination will bio-accumulate in bryophytes (lichens/

mosses concentrate Ag up to 9 ppm [38]) and other vegetation (see Table 2), which are subsequently ingested by the local deer/elk populations.

It is interesting that the practise of cloud seeding is largely contained within the North American continent — the area which has hosted virtually all cases of TSE in wild animals — whereas the application of silver ions as a broad spectrum biocide [40] in food production, etc., has been viewed with greater caution by the US authorities.

Ag use as a biocide

There is a greater use of Ag for its biocidal potential within Europe; where it has been increasingly used over the last two decades as a water purifier and sterilising agent in establishments like the London zoo, Rendering plants, Hospitals (for sterilising surgical implements, etc.), dentists (also used as a component of amalgam fillings), catering establishments, dairy farms, etc. Establishments which have been associated with high incidences of TSEs. Ag biocides are also used in air conditioning, waste water treatment, aquaculture, food and beverage treatment, swimming pools and surface cleaning in many applications.

It is interesting that the use of Ag ions to curb salmonella escalated in the UK poultry industry after the 'Edwina Curry' salmonella crisis hit UK poultry farms in 1988; and the subsequent bioaccumulation of Ag through the farm animal food chain (via use of waste poultry meat and bone meal and manure as both a feed and fertiliser) could have been contributory to the UK's BSE epidemic that peaked in 1992 [34] (see Fig. 3) — where simultaneous exposure to the exclusive compulsory high dose use of the Cu chelating organo dithiophosphate for warble control of UK cattle [1] had deprived PrPc of its Cu co-partner, rendering the protein vulnerable to an Ag replacement.

ENVIRONMENTAL PREREQUISITES UNDERPINNING UK's BSE EPIDEMIC;

1. Compulsory high dose use of systemic organo dithiophosphate insecticides;

a. chelates copper, starving prion protein of its copper co partner.
b. Increases permeability of b/b/brain barrier – increasing uptake of rogue metal crystal founder nuclei into the brain

2. Exposure to Ba, Ag, Mn, Sr crystal nuclei;

* **Ba** *in fishmeal derived from North Sea oil fields?*
* **Ag** *in MBM due to use as salmonella biocide in poultry units ?*
* **Sr90** *from Chernobyl fall out ?*
* **Mn** *as high dose mineral inclusions?*

3. Exposure to Low Frequency shock waves;

* *Low flying military jet practise, Concorde and other civilian jet take off overflights ,etc.*

Figure 3

High incidence clustering of BSE has consistently existed amongst cattle pastured in the main poultry/turkey producing region of Norfolk since BSE first erupted [34], where surrounding farmland has been generously fertilised by Ag and Mn rich poultry manure for many years.

High barium and strontium, possible environmental sources and routes of uptake

The other unusual observation resulting from this study implicates the elevation of Ba/Sr and low levels of sulphur in the antlers, vegetation and soils of the CWD affected deer (see Tables 1 and 2, Graphs 1 and 2).

Since an insufficient number of studies have been conducted on the levels of these metals in antlers [41,42], the mean reference levels of Ba/Sr in bone material have been used in this report as the best alternative for providing mean reference ranges of Ba/Sr in antlers. In respect of the reference levels of 5 ppm Ba and 52 ppm Sr in bone matrix [43—45], the mean levels of 181 ppm Ba and 119 ppm Sr recorded in the CWD antlers in this study could be regarded as 'elevated'. Levels of 55 ppm Ba and 49 ppm Sr in CWD-free antlers collected in both the UK and Alberta were consistently more than 3× and 2.5× lower than mean Ba/Sr levels in CWD + antlers.

The mean levels of Ba and Sr were significantly higher in the vegetation of the CWD cluster environments at 54 ppm Ba and 62.1 ppm Sr in relation to levels of 27.5 Ba and 16.3 Sr recorded in CWD-free control areas. The levels in CWD clusters were also several fold higher than their mean reference levels of 10 ppm Ba and 20 ppm Sr [44,46] for vegetation. A high Ba/Sr and low sulphur mineral profile has also been recorded by the author in TSE cluster ecosystems in Southern Italy, Sardinia, Japan and Iceland.

Sources of radioactive Sr90 in TSE clusters

Raised levels of Sr have been recorded previously in a study on antlers [47] where exposure to atmospheric contamination by radioactive Sr90 was considered to be responsible. Radioactive counts are currently being executed on the antler material collected in this study, but are not yet complete.

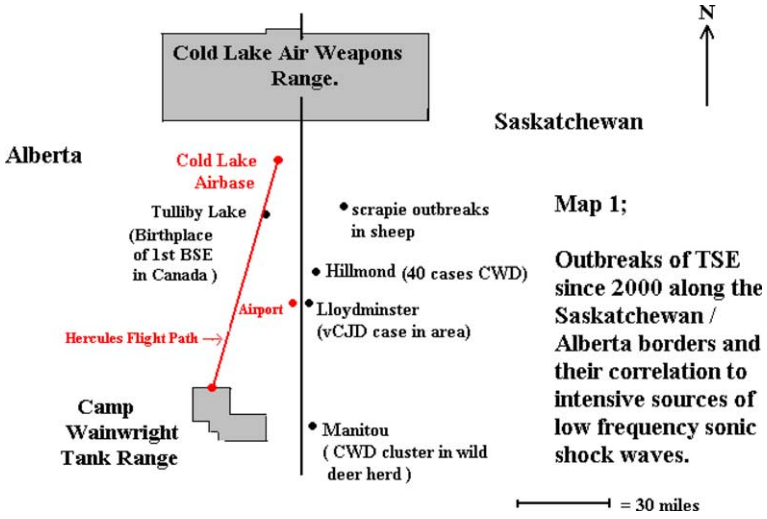
If the high Sr (119 ppm mean) recorded in the antlers in this study turns out to stem from a ra-

dioactive Sr90 source, rather than the stable Sr 88 form, then the contamination of the Northern Hemisphere by Sr90 as a 'fall out' legacy of the 1986 Chernobyl accident and the 1960s/1970s nuclear weapons testing [48,49] could provide an explanation for the source of the contamination. Furthermore, the fall out from a more local source involved the long term atmospheric leak of radioactive metals from the Rocky Flats Nuclear Weapons Plant during 1967 [50,51]. This was environmentally monitored and found to contaminate [53,54] the precise region where the first recorded cluster of CWD emerged in north eastern Colorado in 1968 [52]. The first deer to develop CWD in this outbreak [52] had actually originated from the same pens at the Fort Collins wildlife facility that had been involved in a raft of experiments to monitor the effects of exposure to various radioactive metals (Pu, Cs137, Sr90), which included transporting and grazing the deer on the intensively contaminated pastures around the Rocky Flats Plant itself [53–55].

In this respect, it is interesting that the majority of TSE clusters in North America have emerged near to significant military munitions production/storage/testing facilities such as the White Sands missile range [3], a missile factory in Tucson [57], The Rocky Flats Nuclear weapons factory [58], a battery of 'cold war' and modern missile silos scattered between NE Colorado/SE Wyoming/SW Nebraska and the Cold Lake Air Weapons Range/Camp Wainwright on the Alberta–Saskatchewan borders [58] (see Fig. 4, Map 1) where radioactive

Location	*State*	*TSE type*	*Military connection*	*sonic source*	*Ba*	*Sr*	*Ag*	*Cu*
Tucson	AZ	CJD cluster	Missile factory staff	?				
Fort Collins	CO	CWD cluster Wild/captive deer.	Missile silos/ LF jets Rocky Flats nuclear weapons factory.	Quarry explosions Guns / LFjets Tectonic rift line	56	61	.46	13
Mt Horeb*	WI	CWD cluster	Hercules flightpath LF jets / Badger Munitions site	New Road blasting Guns / LF jets Quake epicentre	56	57	.85	16
White Sands * Missile Range	NM	CWD cluster	Missile test range	Missile explosions	83	35	1.42	10
Mission	TX	Scrapie cluster	Former Airbase (now private)	Under take off flight path	not sampled			
Allenstown	PA	CJD cluster	International Airbase	LF jets.	not sampled			
Garden State	NJ	CJD cluster	Fort Dix military camp MacGuire Airbase	LF jets / Guns Shell explosions	not sampled			
Mabton *	WA	1st BSE	Hanford Nuclear Pl	LF jets	not sampled			
Spokane *	WA	1st vCJD	Yakima Military train Camp / Othello Airbase	Shell explosions				
Namao *	AL	Ist CWD farmed deer	Namao Airbase	Under take off flight path	61	40		9
Leduc *	AL	US BSE cow reared.	Leduc International Airport- mainly civilian	Under take off flight path	83	72		8
Tulliby Lake *	AL	Ist BSE	Cold Lake airbase Hercules flight path	Under LF jet practise circuit / Hercules flight path	45	100		4
Hillmond *	SA	CWD cluster farmed elk	Hercules flight path	Under Lloydminster airport take off path Gas well pumping	55	98		10
Manitou *	SA	CWD cluster wild deer	Camp Wainwright tank range	Tank shelling. Shooting range	64	53		3
Lloydminster *	SA	Ist vCJD	midway Cold Lake/ Camp Wainwright	Under Hercules flight path / tank shelling.	50	41		1.8

Figure 4 Location of the most renowned long standing and recent TSE clusters/outbreaks in USA/Canada in respect of their metal profile and proximity to major sources of low frequency sonic shock waves. LF = low flight. * = TSE case/s emerged since 2000.



Map 1

metal based materials are known to have been used.

Sr90 could represent a rogue metal candidate that potentially initiated the intensive outbreak of BSE across NW Europe in November 1986 — due to the fall out of this metal in the rainstorms which immediately followed the Chernobyl nuclear reactor accident in April 1986 [48]. This could be relevant to the many anecdotal reports by UK vets and farmers that cite a prevalence of osteoporotic-like bone wastage conditions and protracted episodes of atypical hypocalcaemia 'milk fever' (that failed to respond to standard therapeutic doses of Ca) in cattle that later went on to develop BSE [59]. This could indicate a case of successful Sr or Ba substitution at Ca binding domains [43] throughout the biosystem.

Ferritin protein is an integral component of the prion fibril [31] as well as acting as the specific chelating agent for treatment of Sr90 poisoned mammals [56]. If radioactive Sr90 binding to PrP/proteoglycan/ferritin could be experimentally achieved in cell culture models, then it would be tempting to postulate a causal association between chronic exposure to the more reactive Sr90 species and the more short lived, aggressive 'new strains' of TSE; where the additional pathogenic complication of radioactive decay emitted from the Sr90—PrP-ferritin crystalline complexes would exacerbate the intensity of neuropathogenic free radical chain reactions, creating an overall acceleration of the standard duration of the clinical phase of the conventional TSEs. This would invoke clinical disease in younger as opposed to more elderly mammals.

Use of Ba, Sr and Ag in conventional munitions

The toxic common denominator that underlies this correlation between the close proximity of military bases to TSE clusters, may actually relate to the contamination of their surrounding environments with the more conventional, non-radioactive metals that stem from the use of Ba, Sr or Ag in military munitions and other applications.

Furthermore, the previously reported 'sonic shock' prerequisite that has been observed in every significant global TSE cluster visited by Purdey [2,3] is also evident at the majority of the military installations that are contiguous to these TSE clusters in North America [2,3] (see Map 1).

For example, many of these TSE affected animal/human populations had been found to be living beneath low fly jet flight paths or the 'take off' flight paths coming out of military or civilian airbases like Namao and Leduc on the north and south sides of Edmonton, respectively.

Use of Ba ions in aerosol applications employed by the military and geophysical researchers

It should also be noted that aerosols containing the Ba ion — such as the ferroelectric Ba stearate or Ba strontium titanate compounds [60,61] — are discharged along jet flight paths/low flight practise areas and around munitions production/storage/guided missile testing facilities as a means of enhancing/refracting ducting radio/radar signalling communications for maintaining a reliable measure of security and rapid communication around the curvatures of the earth. Ba ions have also been widely discharged into the atmosphere since the

mid 1970s as a means of conducting geophysical explorations of the ionosphere [62].

Thus any foodchain that is sited around these top security military locations, flight paths or beneath these areas of ionospheric exploration, could find itself subjected to the toxic 'fall out' from this mode of atmospheric metal pollution.

Naturally occurring geochemical and bioconcentrated sources of Ba/Sr

The elevated levels of Ba in the North American CWD clusters may also partly derive from the dolomite/limestone and Cambrian granitic mica schist soil types of the CWD cluster areas [43]. These light, low organic matter soil types are naturally high in Ba and Sr [43], whilst being notoriously low in sulphur and copper. The low sulphur perspective exacerbates the problem of Ba/Sr toxicity in the mammal who is dependent upon these foodchains, in that an available source of free sulphur in the soil will conjugate with Ba and Sr, thereby locking up those minerals and acting as a 'toxic sink'/preventative against Ba intoxication [45].

The customary spreading of spent barium drilling mud across farmland (a waste product of the fast expanding oil and gas well industry in the CWD areas) has compounded the problem; with subsequent uptake of Ba into the pasture and hay crops which are ingested by local cervidae populations. Cultivated plants such as alfalfa/soy bean, as well as the wild 'locoweed' flora are prevalent in the CWD areas and are also ingested by the cervidae populations. These species are renowned to bioconcentrate Ba and Sr to high levels [43,44,63].

Drought conditions exacerbate the uptake of Ba/Sr into the ruminant

The author has observed that conditions of prolonged drought precede the outbreaks of CWD in North America. This correlation could be linked to several eco-influences that surface during drought conditions; such as the aforementioned use of Ag/Ba as cloud seeding nuclei during dry seasons. Drought conditions also exacerbate the problem of metal bioconcentration in grazing deer, in that the resulting shortages of pasture cause malnourished cervidae populations to consume abnormally high intakes of pine, juniper and locoweed as a substitute for their normal rations — a phenomena that is widely reported by hunters and ranchers operating in the CWD environments. These plants bioconcentrate Ba and Sr [43]. Furthermore, when overpopulated deer are forced to compete for the dwindling reserves of close cropped, drought-parched pasture, their intake of topsoil — and the metals contained therein — is dramatically increased [51,64]. Interestingly, increased amounts of soil, sand and grit have been observed in the digestive tracts of CWD positive slaughtered deer in relation to CWD-free deer [52].

It should also be noted that some of the concentrated feeds that were fed on deer farms during the drought periods contained Ba and other crystal nuclei in the 'bentonite' clay material that is added to the feed pellet as a binder. Bentonite was also used at a high inclusion rate in cattle concentrated feeds manufactured in the UK, where BSE erupted at epidemic levels.

Conclusion and future research

Irrespective of any role that elevated levels of Ag, Ba and Sr may play in the pathogenesis of TSEs, the novel observation of Ag in antler might suggest that the antler acts as a hitherto unrecognised toxic 'sink' for storing excess intakes of Ag, Ba, Sr and other metals in cervidae who are thriving off foodchains that have bioconcentrated these metals. The toxic load is conveniently shed along with the antler on an annual basis. Or, alternatively, Ag and/or Ba could perform some metabolic role as an electrical superconductor for mediating the rapid growth of the antler.

Whilst previous field studies had observed high levels of Mn and low levels of Cu in Icelandic and Slovakian TSE cluster environments [1,2], these more recent studies — which analysed for a broader spectrum of metals — have found elevated Ag/Ba/Sr and low Cu in the North American TSE clusters. These latest advancements indicate that the lab experiments conducted by Dr. David Brown [65] — which successfully generated 'de novo' protease resistant PrP formation after introducing Mn into Cu deprived PrP cell cultures — need to be extended in order to test the impact of these additional cations in the Cu depleted PrP/proteoglycan cell culture models. The rogue metal loaded cells should be exposed to low frequency sonic shock waves as a second stage challenge. Furthermore, It would be interesting to run cell culture tests to see if any radioactive metals, like Sr90, will bond onto PrPc/proteoglycans in the absence of Cu/Zn.

These environmental observations indicate that the high risk TSE foodchains need to be fortified with copper and/or zinc additives, administered in feed supplement or fertiliser form. These could putatively act as a preventative against the emergence of TSEs in susceptible individuals. Such measures would guarantee an optimum dietary in-

take of Cu and Zn, enabling adequate Cu/Zn binding to PrPc's octapeptide metallo-domains or proteoglycan centres, thereby protecting these molecules against competitive substitution by rogue replacement metals, that could act as founder nuclei for seeding crystals and initiating the pathogenesis of TSE.

The therapeutic use of compounds which can cross the blood brain barrier and inhibit the propagation of metal nucleated crystals by chelating sulphate, silicate, etc., could be tested as a means of arresting clinical TSE [66]. In this respect, it is interesting that anti-malarial quinine molecules, which have been shown to arrest the development of TSEs, are also recognised to chelate alkali earth metals, like Ba and Sr [67].

Since environmental exposures to the soluble sources of Ba will chelate sulphur in the biosystem [43] — thereby disrupting the viable synthesis of the sulphur dependent proteoglycan molecules — it is interesting that the therapeutic use of the S-proteoglycan heparin molecules is having a positive effect in human victims of vCJD. These beneficial effects could merely result from the reintroduction of viable sulphated proteoglycan molecules into the proteoglycan depleted biosystem; where the therapeutic arrest of the progression of the disease could be linked to the reactivation of proteoglycan mediated growth factor signalling, combined with the ability of the restored proteoglycans to inhibit any further multireplication of crystals. Whilst the loss of sulphate from S-proteoglycans could be metal induced in sporadic TSEs, there is evidence that this loss is genetically induced in some strains of scrapie [68].

More extensive and detailed analytical studies need to be performed on these environmental perspectives of TSEs in order to reach a more concise and conclusive consensus on these preliminary observations within this interesting area of TSE research.

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References

[1] Purdey M. Ecosystems supporting clusters of sporadic TSEs demonstrate excesses of the radical generating divalent cation, manganese, and deficiencies of antioxidant co factors Cu, Se, Fe, Zn: does a foreign cation substitution at Prp's Cu domain initiate TSE? Med Hypotheses 2000;54(2):278—306.
[2] Purdey M. The manganese loaded/copper depleted bovine brain fails to neutralise incoming shockbursts of low frequency infrasound; the origins of BSE? Cattle Practice 2002;10(4):311—35.
[3] Purdey M. Does an infrasonic acoustic shock wave resonance of the Mn 3+ loaded/Cu depleted prion protein initiate the pathogenesis of TSE? Med Hypotheses 2003;60(6):797—820.
[4] Brown DR. Prion and prejudice; normal protein and the synapse. Trends Neurosci 2001;24(2):85—90.
[5] Collinge J, Palmer MS, editors. Prion diseases. Oxford: Oxford University Press; 1997.
[6] Tobler L, Gaus E, Beboer T, Achermann P, Fischer M, Ruliche T, et al. Altered circadian activity rhythms and sleep in mice devoid of PrP. Nature 1996;380:639—42.
[7] Cagampang F, Whatley S, Powell J, Mitchel A, Campbell I, Cohen W. Circadian regulation of PrP messenger RNA in rat forebrain; a widespread and synchronous rhythm. Neuroscience 1999;91:1201—4.
[8] Orchard AF. Magnetochemistry. Oxford: Oxford University Press; 2003.
[9] Devlin TM. Textbook of biochemistry with clinical correlations. third ed. New York: Wiley-Liss; 1992.
[10] Prasher D, Luxon L. Biological effects of noise. In: Advances in noise research, 1. 19b Compton Terrace London, N1 2UN, UK: Whurr Publishers Ltd.; 1998.
[11] Dusenbery DB. Sensory ecology. New York: WH Freeman and Company; 1992.
[12] Scheving LE, Burns ER, Pauli JE. Circadian variation and cell division of the mouse alimentary tract, bone marrow and corneal epithelium. Anat Rec 1978;191:479—86.
[13] Bartlet P, Haus E, Tuason T. Circadian rhythm in number of erythroid and granulocytic colony forming units in culture in bone marrow of BDFI male mice. In: Haus E, Kabat HF, editors. Proceedings of the 15th International conference on Chronobiology. S. Krager: Basel; 1982.
[14] Dubocovich ML. Melatonin receptors in the retina, brain and pituitary. In: Foldes A, Reiter R, editors. Advances pineal research, vol. 6. John Libby and Co.; 1991. p. 131—9.
[15] Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, et al. Transmissions to mice indicate that new variant CJD is caused by the BSE agent. Nature 1997;389(2 October):498—501.
[16] Bastian F, CJD and other transmissible encephalopathies. Mosby Year book, St Louis; 1991.
[17] McKinley MP. Nerve growth factor induces gene expression of PrP and amyloid protein precursor in the developing hamster CNS. Prog Brain Res 1990;86:227—38.
[18] Hope J, Baybutt H. The key role of the nerve membrane protein PrP in scrapie-like diseases. Sem Neurosci 1991;3:165—71.
[19] Grant D, Long WF, Williamson FB. Infrared spectroscopy of heparin-cation complexes. Biochem J 1987;244:143—9.

[20] Grant D, Long WF, Williamson FB. A potentiometric titration study of the interaction of heparin with metal cations. Biochem J 1992;285:477–80.
[21] Kan M, Wang F, Kan M, To B, Gabriel JL, McKeehan WL. Divalent cations and heparin/heparan sulphate cooperate to control assembly and activity of the fibroblast growth factor receptor complex. The Am Soc Biochem Mol Biol 1996;271(42):26143–8.
[22] Perez M, Wandosell F, Colaco C, Avila J. Sulphated glycoaminoglycans prevent neurotoxicity of a human prion protein fragment. Biochem J 1998;335:369–74.
[23] Shyng S-L, Lehmann S, Moulder KL, Harris DA. Sulfated glycans stimulate endocytosis of the cellular isoform of the prion protein in cultured cells. The Am Soc Biochem Mol Biol 1995;270(50):30221–9.
[24] Fowler M, Robertson A. Observations on kuru 111: pathological features in 5 cases. Aust Ann Med 1959;8:16–26.
[25] Barnett K, Palmer A. A retinopathy in sheep affected with Natural Scrapie. Res Vet Sci 1971;12:383–5.
[26] Wells GAH. A Novel Progressive Spongiform Encephalopathy Vet Record 1987;121:419–20.
[27] He L, Lu XY, Jolly AF, Eldridge AG, Watson SJ, Jackson PK, et al. Spongiform degeneration in mahoganoid mutant mice. Science 2003;299(5607):710–2.
[28] Perriman N, Bernfield M. Nature 2000;404:725–8.
[29] Robinson WR, Odom JD, Holtzclaw HF. Chemistry; concepts and models. Lexington, USA: DC Heath and Co.; 1992.
[30] Becker RO, Selden G. The body electric; electromagnetism and the foundation of life. New York: Quill, William Morrow; 1985.
[31] Cho HJ, Grieg AS, Corp CR, Kimberlin RH, Chandler RL. Nature (London) 1977;267:459–60.
[32] Halliwell B, Gutteridge JMC, editors. Free radicals in biology and medicine. second ed. Oxford, UK: Clarendon Press; 1989.
[33] Gabriela P. Sensitive detection of pathological PrP by cyclic amplification of protein misfolding. Nature 2001;411(June):810–3.
[34] MAFF, BSE in Great Britain. A Progress Report. June 1998, MAFF, London, UK; 1998.
[35] Brown P, Rau EH, Johnson BC, Bacote AE, Gibbs C, Gajdusek DC. New studies on the heat resistance of hamster adapted scrapie agent: threshold survival after ashing at 600 °C suggests an inorganic template of replication. PNAS 2000;97(7):3418–21.
[36] Petering HG. Pharmacology and toxicology of heavy metals: silver. Pharmac Ther A 1976;1:127–30.
[37] Ghandour W, Hubbard JA, Deistung J, Hughes MN, Poole RK. The uptake of silver ions by *Escherichia Coli* K12; toxic effects and interactions with copper ions. Appl Microbiol Biotechnol 1988;28:559–65.
[38] Cooper CF, Jolly WC. Ecological effects of silver iodide and other weather modification agents: a review. Water Resources Res 1970;6(1):88–98.
[39] Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, et al. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Persp 2001;109(4):547–51.
[40] Gupta A, Silver S. Nature Biotech 1998;16(10):888–90.
[41] Bubenik AB. Geweihe und ihre biologische funktion. Nature Med 1971;8:33–51.
[42] Hyvarinen H, Kay RN, Hamilton WJ. Variations in the weight, specific gravity and composition of the antlers of red deer. Br J Nutr 1977;38:301–12.
[43] WHO. Environmental Health Criteria 107. WHO, Barium, IPCS, Geneva; 1990.
[44] Mineral tolerance of domestic animals. Barium pp. 54–9, Strontium pp. 459–65. National Academic Press: Washington, DC; 1980.
[45] Pais I, Benton Jones J. The handbook of trace elements. Florida: St Lucie Press; 1997.
[46] Underwood EJ. Trace element in human and animal nutrition. fourth ed. New York: Academic Press; 1977.
[47] Gelbke W. Radioaktivat in rotwild-stangen und menschliche strontium 90 aufnahume. Z Tierphysiol Tierernah Futtermittelkunde 1972;29:178–95.
[48] Eisenbud M, Gesell T. Environmental radioactivity. fourth ed. London: Academic Press; 1997.
[49] Whicker FW, Farris GC, Dahl AH. Concentration patterns of Sr 90, Cs 137, I 131 in a wild deer population and environment. In: Aberg B, Hungate FP, editors. Radioecological concentration processes. New York: Pergamon Press; 1966. p. 621–33.
[50] Hiatt GS. Plutonium dispersal by mule deer at Rocky Flats, Colorado. MS thesis. Colorado State University, Fort Collins; 1977. p. 143.
[51] Arthur WJ, Alldredge AW. Soil ingestion by mule deer in North Central Colorado. J Range Manage 1979;32.
[52] Williams ES, Young S. Spongiform Encephalopathy in cervidae. Rev Sci Tech Off Int Epiz 1992;11(2):551–67.
[53] Arthur WJ 111. Plutonium intake by mule deer at Rocky Flats, Colorado. MS Thesis. Colorado State University, Fort Collins, Co.; 1977. p. 123.
[54] Little CA. Plutonium in a grassland ecosystem. PhD thesis. Colorado State Universty, Fort Collins, Co.; 1979. p. 170.
[55] Hakonson TE. Tissue distribution and excretion of Cs134 in the mule deer. MS thesis. Colorado State University, Fort Collins; 1967. p. 121.
[56] Raymond KN, Telford JR. Bioinorganic chemistry: an inorganic perspective of life. NATO ASI series. Dordrecht: Kluwer Academic Publishers; 1995 [pp. 25–37].
[57] Crom R. Cluster of CJD cases in Tucson. Report; The epidemic Intelligence Service of the Arizona Department of Health Services, Pheonix; 1990.
[58] Purdey M. An Ecodetective's Journey to the Centre of Neurodegenerative Disease 2003. Available from: www.redflagsdaily.com/conferences/mad_cow/index.html.
[59] Purdey M. Are organo-phosphate pesticides involved in the causation of BSE. J Nutrit Med 1994;4(1):43–82.
[60] Paine TO. NASA Barium ion cloud; Patent US 3813875, Barium release system to create ion clouds in upper atmosphere. Application Number; US 1972000248761. Patent issue date; June 4th 1974.
[61] Dorsch J. Electronic News; January 11 1999.
[62] Heppner JP, Miller MB, Pongratz MB, Smith GM, Smith LL, Mende SB, et al. The cameo barium releases: e-parallel fields over the polar cap. J Geophys Res 1981;86:3519 [chapter 3].
[63] Shacklette A. In: Oehme FW, editor. Toxicity of heavy metals in the environment Part 1. New York: Marcel Dekker, Inc.; 1978. p. 56–9.
[64] Mayland HF, Florence AR, Rosenau RC, Lazar VA, Turner HA. Soil ingestion by cattle on semi-arid range as reflected by titanium analysis of feces. J Range Manage 1975;28(6):448–52.
[65] Brown D, Hafiz F, Glassmith L, Wong BS, Jones I, Clive C, et al. Consequences of manganese replacement of copper for prion protein function and protease resistance. EMBO J 2000;19(6):1180–6.
[66] Coveney PV, Davey R, Griffin JLW, He Y, Hamlin D, Stackhouse S, et al. J Am Chem Soc 2000;122:11557–8.

[67] Oliphant CM, Green GM. Quinolenes; a comprehensive review. Am Family Physician 2002;65:455–64.

[68] Leteux C, Chai W, Nagai K, Herbert C, Lawson A, Feizi T. 10E4 antigen of scrapie lesions contains an unusual nonsulfated heparin motif. J Biol Chem 2001; 276(16):12539–45.