RE-EVALUATION OF SELENIUM TOXICITY IN GRAZING MAMMALS[1]

M. F. Raisbeck, D. O'Toole, D. A. Sanchez, R. L. Simieon and J. W. Waggoner[2]

Abstract: The potential uptake and concentration of selenium by vegetation grown on abandoned coal mine lands has been the focus of many restrictions governing the reclamation of such sites. While there is little question that selenium is very toxic, recent research suggests that much of what has been anecdotally attributed to selenium in the past, is, in fact, due to other environmental factors. This presentation will summarize research undertaken by the authors during the last 6 years to more clearly define the effects of selenosis on herbivores in Wyoming.

Additional Key Words: selenosis, poisoning, antelope, cattle

Introduction

Selenium (Se) is a group VI element that is chemically very similar to sulfur. In North America, it is reportedly most abundant in Cretaceous shales and glacial drift of the Great Plains. Concentration by leaching, evaporation and/or plant uptake may result in forage concentrations which are potentially toxic to grazing mammals. The fact that these natural processes may be exacerbated by anthropogenic activities such as surface mining and irrigation resulted in increased control of such activities during the 1980's to prevent future Se toxicity to livestock and wildlife.

It has become fashionable to begin any paper on the toxic effects of Se by citing historical documents such as Marco Polo's journal as early reports of selenosis (Trelease and Beath, 1949a; Harris, 1991; James et al., 1990). In point of fact, however, the first conclusive evidence of naturally-occurring Se toxicity (selenosis) in mammals was contained in reports from the Wyoming and South Dakota Agricultural Experiment Stations during the 1930's (Beath et al., 1934; Franke, 1934a; Franke, 1934b; Franke & Potter, 1934). During the subsequent 2 decades, a model of selenosis emerged from experiments and field studies undertaken by these laboratories and the USDA Bureau of Animal Industries. According to this model, ingestion of toxic amounts of Se, either as purified inorganic salts or as accumulated in range plants, resulted in one of several distinct pathophysiologic entities: acute selenosis, experimental chronic selenosis, "blind staggers" and "alkali disease" (Trelease and Beath, 1949b; Rosenfeld and Beath, 1964).

The discovery in the 1950's that Se was, in addition to being highly toxic, an essential trace element (Schwartz and Foltz, 1957) produced a marked shift in emphasis in biomedical Se research. Relatively little original research into the toxicity of Se was conducted during the 1960's and 1970's; the dogma of the 1930's and 1940's became enshrined in the scientific literature by repetitive citation. Thus, it is not surprising that the popular press and, more importantly, regulatory personnel struggling with reclamation standards accepted this paradigm uncritically.

Acute selenosis is of limited relevance to range animals for two reasons. First, acute selenosis requires quantities of Se that animals are very unlikely to ingest voluntarily under range conditions. For example, an acutely lethal oral dose of Na_2SeO_4 for a 400 kg cow contains about 4 gm Se. Assuming that this dose was *evenly* distributed throughout a single day's forage intake, the final Se concentration would be about 500 ppm. While the so-called "accumulator" plant species may accumulate this much Se or more, the chemical form is such as to render the plant extremely unpalatable. As noted by Beath (1920), "when cattle were placed

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[2] From the Departments of Veterinary Sciences (Raisbeck, O'Toole, Sanchez and Simieon) and Range Science (Waggoner), University of Wyoming, Laramie, WY 82070.

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in enclosures on *astragalus* they would not touch the plant at all and after a few days could not be kept inside the fence". Second, many accumulator species such as two-grooved milkvetch (*A. bisulcatus*) contain other, more significant toxins such as swainsonine, which render the theoretical toxicity of any Se present moot.

The extent of selenosis in Wyoming

The popular perception that "Extensive livestock losses have occurred and continue today" (Boone, 1986) isn't consistent with the experience of animal health researchers and stockmen in the western United States. For example, a review of the records of the Wyoming State Veterinary Laboratory from its inception in 1947 through 1988 revealed no confirmed cases of selenosis (L.W. Woodard, 1988). Reportedly, Se intoxication in livestock is most commonly the result of over-supplementation rather than natural sources (Edmonson et al., 1993). Similarly, a prospective survey of selenosis in domestic livestock conducted by the University of Wyoming from 1988 through 1991 found only isolated instances of the disease (Raisbeck, et al, 1993).

In the latter survey, which is still ongoing, cases of suspected selenosis in livestock were solicited from veterinarians and producers by announcements in the Wyoming Veterinary Medical Association newsletter and in various statewide livestock publications. Originally, submissions were restricted to cases of illness or death in which one or more clinical signs were similar to those reported in the older literature (Table 1), and for which there was adequate clinical history to permit correlation between the disease process and environmental Se concentrations.

Table 1: Criteria for inclusion of cases in original prospective survey of selenosis in Wyoming.

History of possible exposure to seleniferous plants
Availability of appropriate tissue samples for diagnosis
Clinical signs
Neurological
Blindness
Head pressing
Circling
Ataxia
Tremors
Convulsions
Epithelial
Hair loss, especially of mane or tail
Lameness, especially if accompanied by hoof deformities
Sudden death
Reproductive
Poor conception rates not related to other causes
Male infertility

The response to this original solicitation was very limited. Thus, in early 1990, the survey was widened to include any incident of livestock disease that the attending veterinarian or livestock owner believed might be associated with elevated dietary Se from any source. As of June, 1994, our lab had investigated 116 accessions[3]. Of these, six equine and two bovine accessions were attributed to Se on the basis of tissue concentrations and elimination of other possible etiologies. Two additional wild bird accessions which had extremely high tissue Se concentrations were suspect, but were too decomposed to permit any further diagnostic workup.

The clinical signs observed or reported in confirmed cases were similar to those described as the "alkali disease" form of chronic selenosis in the older literature (Trelease and Beath, 1949a). Typically, 1 - 2 months after exposure to the suspect feedstuffs, owners report noting alopecia of the mane and tail. Soft tissue swelling in the region of the coronet and/or a very fine, horizontal crack in the surface of the hoof just distal to the coronet are also noted at this time or soon thereafter. Lameness at this stage is variable. The hoof lesion and attendant lameness

[3] An accession is a single incident of a disease occurrence reported to our laboratory. It might represent a single pet horse, or an outbreak in a herd of cattle.

progressively worsen over a period of several months until, finally, the toe separates from the hoof and is lost. None of the cases we have investigated thus far exhibited any evidence of other organ (e.g. liver) involvement. No affected animals have died. It is, however, easy to see how death from starvation, arthritis, and/or many of the other conditions attributed to alkali disease might have resulted from the severe lameness if animals had been left to fend for themselves under range conditions.

Twenty-three accessions met the clinical criteria of the condition described as "blind staggers" (Draize and Beath, 1935; Trelease and Beath, 1949b). Of these, 10 were diagnosed as lead poisoning on the basis of tissue lead concentrations, eight were histopathologically diagnosed as polioencephalomalacia and the remainder attributed to various pathogenic infections. Only one had elevated tissue Se concentrations, which were judged to be diagnostically insignificant given the extremely high lead concentrations.

Experimental studies: cattle

Much of the experimental data regarding selenosis in large domestic mammals is derived from exposure to inorganic salts of Se such as Na_2SeO_4 (Maag et al., 1960; Miller and Williams, 1940; Olson and Embry, 1973). However, the predominant forms of Se in forage plant species are the seleno analogs of sulfur-containing amino acids, especially selenomethionine (Olson, 1977). Published data about the physiological effects of this form of Se are variable; however, there is evidence in trout (Hamilton and Buhl, 1990), waterfowl (Heinz et al., 1988; Fairbrother and Fowles, 1990) and laboratory mammals (Waschulewski and Sunde, 1988) that the chronic toxicity of selenomethionine differs both quantitatively and qualitatively from that of inorganic Se salts. A similar comparison in grazing (ruminant) species is thus very relevant to mine reclamation in Wyoming.

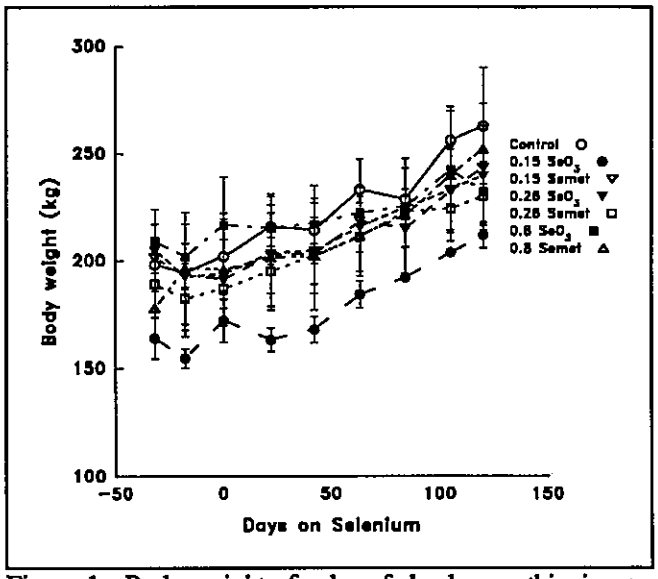

Figure 1: Body weight of calves fed selenomethionine or selenite at 0.15 mg Se/kg BW, 0.28 mg Se/kg BW or 0.8 mg Se/kg BW. Selenium was fed from day "0" through day 120. Each point represents mean ± standard error.

We undertook such a study with support from the Abandoned Coal Mine Land Research Project and the UW Agricultural Experiment Station during 1992-1993. Yearling steer calves obtained from the Medicine Bow area were fed a Se-adequate basal ration of grass hay typical of dryland pastures in the state. After a 45-day acclimation period, during which they were de-wormed and vaccinated for diseases common in Wyoming, steers were randomly assigned to either a "high", "medium" or "low" selenomethionine or $SeO_3^=$ group. Dosage rates (0.15, 0.28 or 0.8 mg Se/kg BW) were calculated to approximate 5, 10, or 30 ppm dietary Se under range conditions. Doses were prepared daily for each animal and thoroughly mixed with that day's ration of chopped hay. Rations for each animal were adjusted weekly to ensure that the entire dose was consumed. Control steers remained on the basal diet. Periodic blood samples were taken for Se, immunology and clinical pathology. After 120 days on Se, each steer was humanely killed and subjected to a complete necropsy with a detailed histopathologic review (Appendix 1).

Both experimental and control calves appeared clinically healthy throughout the duration of the experiment. All gained weight throughout the 120 day exposure to Se. Although there was no statistically significant difference between treatment groups by ANOVA ($P<0.05$), controls gained more than the high $SeO_3^=$ group at 84, 105 and 120 days (Figure 1). There were no variations attributable to Se in any of the clinical pathologic parameters measured (blood urea nitrogen, serum creatinine, serum creatinine kinase, serum alkaline phosphatase, serum aspartate aminotransferase, serum bilirubin, serum glucose, serum albumin, serum

total protein, red blood cell count, white blood cell count, hemoglobin, hematocrit, or differential white cell count). Of particular interest, and in contradiction to early reports (Franke and Potter, 1934; Draize and Beath, 1935), there were no changes in erythrocytic parameters that indicate anemia.

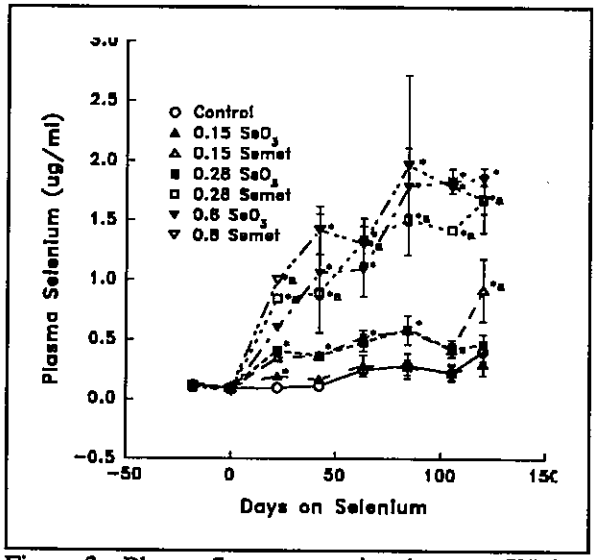

Figure 3: Plasma Se concentration (mean ± SE) in calves fed selenomethionine or selenite at 0.15, 0.28 or 0.8 mg Se/kg BW. "*" indicates significantly greater than controls, "a" greater than corresponding dose of SeO_3.

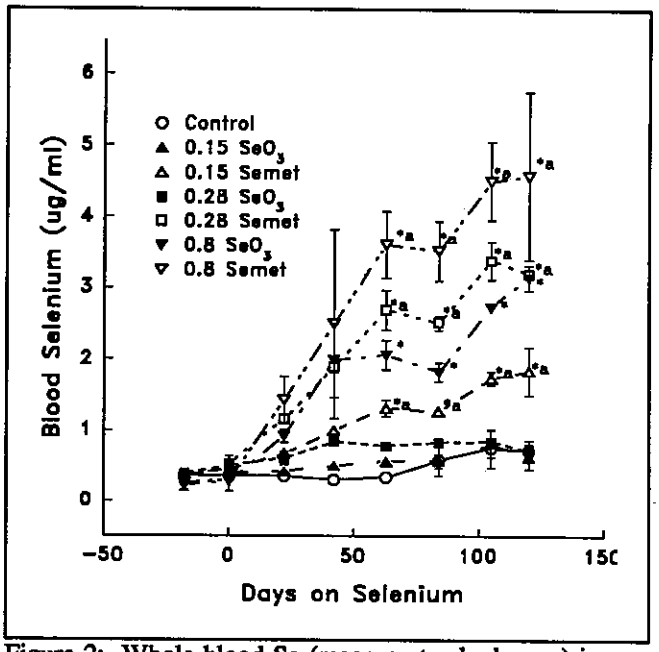

Figure 2: Whole blood Se (mean ± standard error) in calves fed selenomethionine or selenite. "*" indicates significantly greater than controls, "a" greater than corresponding dose of SeO_3.

Not surprisingly, blood and plasma Se concentrations increased in the steers fed Se (Figures 2 & 3). Plasma Se concentrations increased more rapidly and were generally more labile than whole blood Se. This reaffirms our long-standing position that blood is a better index of long term Se status than plasma or serum in mammals. With the exception of the high $SeO_3^=$ group, blood concentrations appeared to plateau between 60 and 80 days. Dietary selenomethionine resulted in significantly higher blood concentrations than did corresponding dosages of $SeO_3^=$ (Figures 2 & 3). *Post mortem* tissue Se concentrations, with the exception of liver, were also significantly greater with selenomethionine than with $SeO_3^=$ (Figure 4). This differs from the situation in waterfowl where selenomethionine resulted in significantly greater accumulation in liver (Heinz et al., 1988) and likely reflects greater metabolic turnover in mammalian liver.

One steer (#14) developed a conditioned aversion to the selenomethionine diet after 10 days on Se and was replaced in the experiment by a similar animal from a local herd. The aversion exhibited by this steer was so severe that he completely refused hay

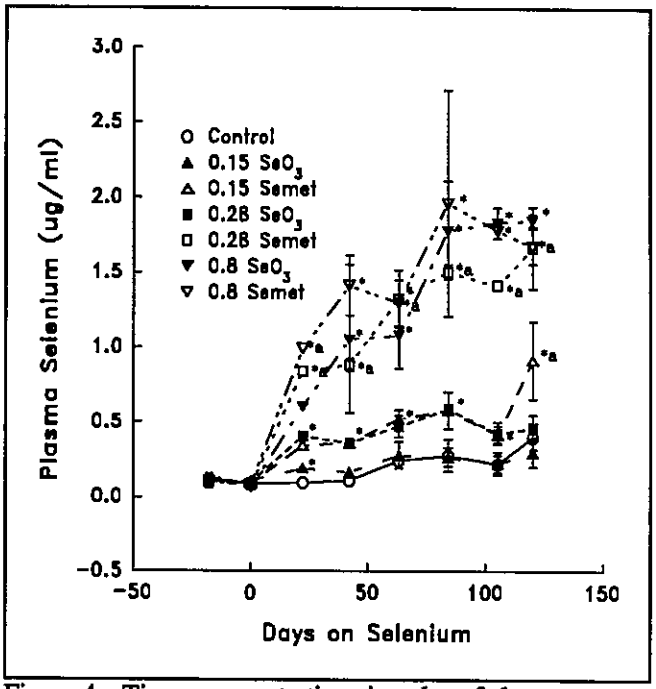

Figure 4: Tissue concentrations in calves fed selenomethionine or selenite at 0.15 mg, 0.28 or 0.8 mg Se/kg BW. "*" indicates significantly greater than controls, "a" greater than the corresponding dose of SeO_3.

for several days whether or not it was amended with Se. This steer was therefore given his daily dose by gavage with a gelatin capsule for the remainder of the experimental period. After being fed by stomach tube for 4 days, it began to eat grass and slowly regained weight, but never completely regained its original condition (Figure 5). We conclude that the difference in blood concentration and effects on body weight was due, in part, to the difference in bioavailability of a single daily gavage dose and continuous feeding.

On day 96, slight swelling and erythema just proximal to the coronary band developed in both front legs. Ten days later a hairline crack appeared parallel to and 0.5 cm distal to the coronary band and the steer became lame. Hoof separation and lameness both increased in severity until, by 120 days, it refused to walk to feed and water and had to be hand fed. At necropsy, new hoof growth was found underlying the separated horny tissue. Histopathologic examination of the juncture between new hoof growth and old revealed hyperplasia and acanthosis of the lamellar epithelium of the hoof.

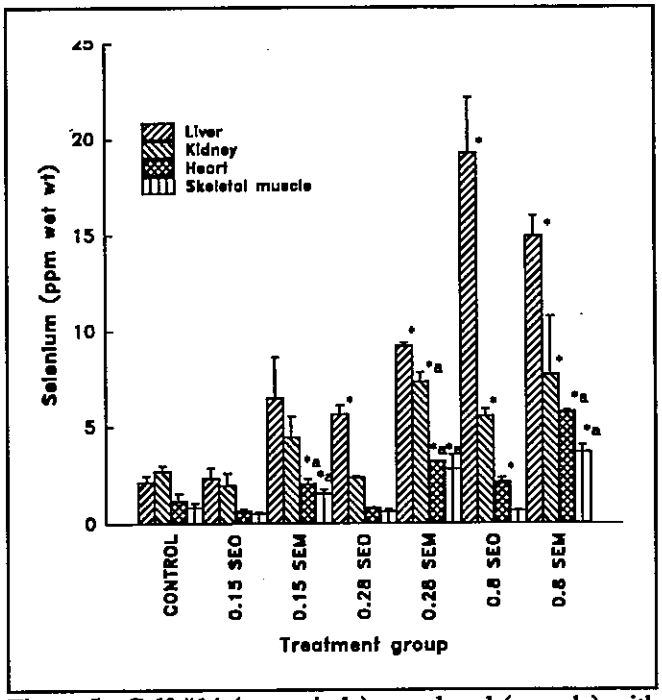

Figure 5: Calf #14 (open circle) was dosed (capsule) with 0.8 mg Se/kg BW as selenomethionine daily, calves in the 0.8 mg Se/kg BW dose group were fed spiked, chopped hay. The initial weight loss in #14 was probably due to starvation.

Although none of the calves *fed* Se exhibited any lameness or hair loss, histopathologic lesions similar to those seen in #14 were seen in one high SeO3, one medium selenomethionine and all high dose selenomethionine steers. The dystrophic keratin formation associated with this lesion is probably responsible for the hoof separation seen in alkali disease, and the doses (0.8 mg Se/kg BW as SeO3, 0.28 mg Se/kg BW as selenomethionine) which caused it probably represent a threshold or near-threshold of toxicity. For further description of the lesion and photomicrographs see O'Toole and Raisbeck (1994).

Experimental studies: pronghorn antelope

To validate the results of the steer experiment in a second target species, we undertook a similar study in antelope. Captive-raised male antelope (four yearling fawns, five adults) were housed in concrete-floored pens at the Wyoming Game and Fish Sybille Research Center. Water (less than 50 ppb Se) was provided via automatic waterers and the antelope had access to sheds for shelter from the weather. After a one month acclimation period, the antelope were divided into experimental (two fawns, three adults) and control (two fawns, two adults) groups. The experimental group received a finely chopped 60:40 mixture of 25 ppm Se native grass hay and locally grown alfalfa hay (approx. 0.3 ppm Se). The final Se concentration of the experimental diet was 15 ppm. The control group received a similar diet containing approximately 0.3 ppm Se. The experimental exposure period lasted 168 days.

Although the antelope initially ate the ground grass/alfalfa diet readily, feed refusal of both the control and experimental diets was a recurring problem from day 30 until the end of the study. During the last 60 days of the experiment the diet of fawns was supplemented with 200 gm per day of a corn-oats-barley mixture in order to avoid cachexia. Total Se consumption for the 168 day feeding period was approximately 4100 mg Se/kg BW. Feed consumption and weight gain (Figure 6) were less in antelope on the high Se ration, especially the fawns during the last 60 days of the experiment, which coincided with early Spring. Nonetheless, both groups appeared in good flesh and remained lively and alert throughout the experiment.

Specifically there were no signs of lameness, hair loss, vision impairment, or liver, kidney or cardiac abnormalities attributable to the high Se diet.

Clinical pathology and biochemistry testing did not reveal differences between groups, or deviations attributable to the high Se diet.

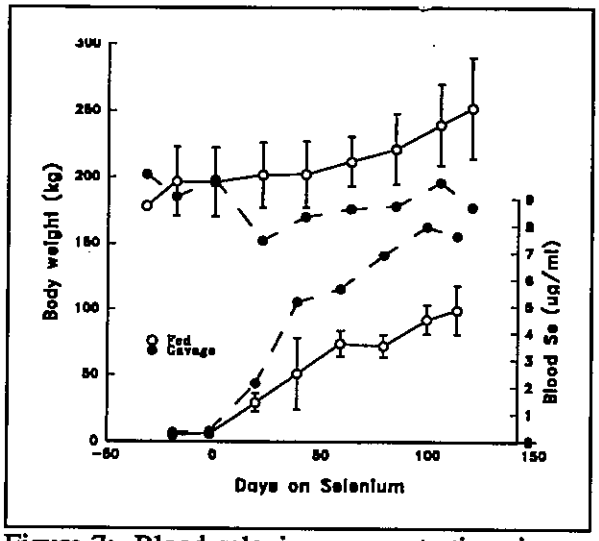

Figure 7: Blood selenium concentrations in antelope receiving 15 ppm hay. Each point represents the mean plus or minus standard error of the treatment group.

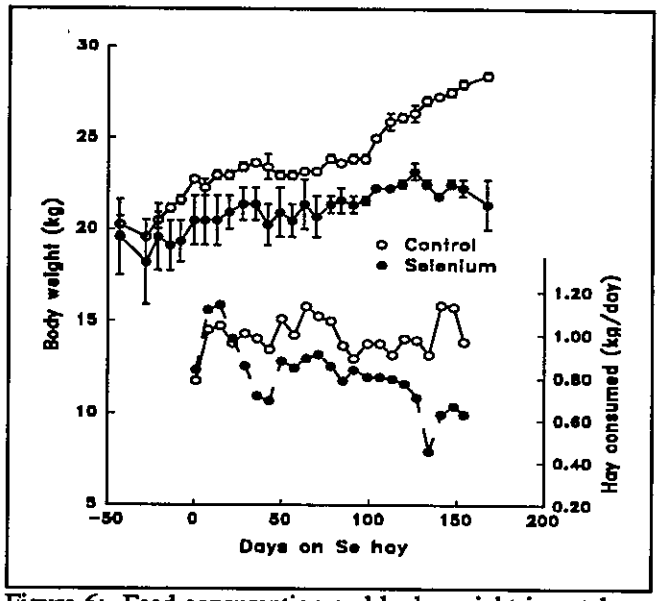

Figure 6: Feed consumption and body weight in antelope fawns on high Se hay. We believe that the lesser feed consumption in the Se hay group accounts for the smaller gain seen.

from historical normals. Blood Se concentrations, while elevated in the experimental group, were intermediate between the bovine $SeO_3^=$ group and the bovine selenomethionine group (Figure 7). Detailed histopathologic examination, similar to that used with the steers, of tissues from the antelope did not reveal lesions

Research techniques: caveats

An old graduate school saw indicates that "if the method worked as published, it probably didn't work." The following observations are included for other laboratories interested in Se toxicity and will perhaps spare them some of the aggravation we experienced.

At least part of the confusion about Se toxicity results from radical changes in analytical and biomedical technology during the half century since it was first reported. Studies in the senior author's lab (Dolan et al., 1985), and more recently, by Maas, et al. (1992) indicate that, while various methods for mammalian tissue Se analysis are generally in good agreement, they diverge at either end of the deficiency-toxicity spectrum. Thus, comparison of results between laboratories and, especially, between eras should be undertaken with caution.

In theory, drying samples before inorganic analysis yields more consistent results than running them "wet". However, in our hands, drying a tissue sample such as liver before analysis to obtain "dry weight" Se concentrations resulted in loss of Se. This effect tends to occur with especially high or low Se samples. Often there is insufficient sample to obtain a dry weight/wet weight ratio on a separate specimen, thus we, like most veterinary toxicology labs, routinely use a "live" or wet weight basis for calculating Se concentrations in tissues.

The histochemical technique commonly known as the autometallographic or "Danscher stain" purportedly marks Se in light and electron microscopic sections and has been used in previous reports of experimental Se toxicity (Smyth et al., 1990a, 1990b). Our studies indicate that, while the technique may detect Se in

some tissues, it is insufficiently specific for Se under conditions of chronic exposure for toxicology research (O'Toole et al. 1994a).

Discussion

The results of a 6-year, prospective study of naturally-occurring selenosis in Wyoming indicate that, while chronic selenosis does occur in domestic animals in the state, the incidence is much less than that alluded to by early investigators and the popular press. The clinical presentation of this condition is similar to that originally referred to as "alkali disease", which is characterized by damage to epithelial structures such as skin and hoof. Epidemiologic investigation and our experimental studies suggest that, most if not all, of the other pathologic syndromes attributed to chronic selenosis in domestic animals (eg. anemia, liver damage, cachexia and arthritis) are secondary to the profound lameness seen with selenosis and consequent starvation under range conditions.

To date, we have not verified a single case of the condition referred to as "blind staggers". It is noteworthy that the term "blind staggers" has been historically applied by stockmen to any disease condition of ruminants in which animals exhibit neurologic signs. It is also noteworthy that the signs ascribed by Beath et al., (Trelease and Beath, 1949b) to "blind staggers" are virtually identical to those of polioencephalomalacia, a condition now known to sometimes result from excess dietary sulfur (Raisbeck, 1982). The latter element frequently co-occurs with Se in Wyoming minelands. These results, plus a comprehensive review of archived research materials from the Beath group (O'Toole et al., 1994), lead us to conclude that the condition was mis-diagnosed and probably should have never been attributed to Se.

Our research indicates that higher Se tissue concentrations accumulate when ruminants are fed selenomethionine than when fed $SeO_3^=$. The significance of this fact is problematic in several ways. First, what is the diagnostic significance of "x" ppm Se in blood or tissue, when the chemical form ingested is unknown? This may account for some of the discrepancy in the biomedical literature as to what constitutes "confirmed" selenosis. The senior author has seen a case of subacute iatrogenic selenosis in swine in which blood concentrations ranged from 1 - 2 ppm, yet many of our calves tolerated such concentrations for several weeks with only minimal, if any effects. Thus tissue concentrations alone are not sufficient criteria for diagnosis. At present, our criteria are blood concentrations in excess of 1 ppm, *together with lesions characteristic of selenosis*, in the absence of other differential diagnoses. Tissue "levels", do not prove selenosis unless they are greatly (greater than 5 ppm wet weight) elevated. In our opinion, there is serious need for a more specific diagnostic test to confirm selenosis.

Second, what is the toxicologic significance of a given dietary concentration, if the dietary source was selenomethionine? It has been suggested for several years (De Marco and Di Girolamo, 1981; Stadtman, 1991) that dietary selenomethionine accumulates non-specifically in tissue proteins where it poses relatively little hazard. Indeed, Puls (1988) lists quite different diagnostic blood concentrations for "natural" (selenomethionine, presumably from feedstuffs) vs. "selenite" toxicity. On the other hand, results from laboratory rodent and waterfowl studies suggest that chronic dietary selenomethionine is actually more toxic than inorganic Se salts (Fairbrother and Fowles, 1990; Waschulewski and Sunde 1988). The dietary concentrations associated with hoof lesions in our study support the latter hypothesis, but larger scale experiments with more animals are necessary to be completely convincing. Perhaps, selenomethionine's greater bioaccumulation provides a larger pool of Se than e.g. $SeO_3^=$ to draw on for the metabolic formation of a proximate toxicant, thus making it *potentially* more toxic after sustained exposure.

The fact that tissue concentrations in antelope fed 15 ppm hay were somewhat lower than predicted by the steer experiment is also problematic, given that Se in grass hay is reported to be predominantly selenomethionine. There are several possible explanations for this observation. It may be that the Se species in our high Se hay was other than selenomethionine. It may be that protein-incorporated selenomethionine is toxicologically different from free selenomethionine. Finally, it is possible that the Se metabolism of

antelope differs slightly from that of cattle. We haven't yet distinguished between these possibilities because of the difficulty in reliably quantitating selenomethionine in hay.

If the difference in the chronic toxicity of selenomethionine and inorganic salts can be accurately quantified for the relevant species, a more precise, predictive reclamation standard for vegetation Se can be based on the proportion of various Se species present. In the meantime, our research suggests that the existing 5 ppm standard is extremely conservative under Wyoming conditions.

Acknowledgements

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Appendix 1: Tissues examined histologically for evidence of selenium-induced lesions

- Neurologic
  - Brain (11 standard levels)
  - Spinal cord (C3, C6, T3, T7, T12, L1, L4)
  - ulnar nerve
  - median nerve
  - ischiatic nerve
  - fibular nerve
- Skeletal muscle
  - biceps brachii
  - extensor carpi radialis
  - vastus lateralis
  - biceps femoris
  - tibialis cranialis
  - longissimus lumborum
  - psoas major
- Digestive
  - Salivary gland
  - Tongue
  - Esophagus
  - Reticulum
  - Rumen
  - Omasum
  - Abomasum
  - Duodenum
  - Jejunum
  - Ileum
  - Cecum
  - Colon
  - Liver
  - Gallbladder
- Endocrine
  - Pancreas
  - Thyroid
  - Pituitary
- Lung
  - anterior lobe
  - middle lobe
  - posterior lobe
- Cardiovascular
  - Heart
    - interventricular septum
    - right ventricle
    - left ventricles
  - Aorta
- Genitourinary
  - kidney
  - bladder
  - adrenal
- Blood forming organs
  - Spleen
  - Lymph node (mesenteric, hepatic)
  - Bone marrow
  - Thymus
- Eyes
- Integument
  - Skin
    - tail
    - right lateral thorax
    - dorsal midline cervical
  - Hoof
    - periople-coronary hoof
    - coronary-laminar hoof

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