Chapter 76

Morphologic Studies of Selenosis in Herbivores

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Introduction

Spontaneous selenosis in grazing animals has been historically divided into two distinct clinical entities: “alkali disease” and “blind stagsgers” (Olson, 1978; Beath, 1982). Surprisingly, although alkali disease (AD) was recognized as a distinct disease by livestock producers before the turn of the century (Franke and Potter, 1934), there are few morphologic studies of either AD or blind stagsgers (BS). There are no published histologic descriptions of the integumentary lesions of herbivores with AD, although Se-induced lesions have been characterized in the central nervous system of intoxicated swine and, to a lesser extent, in porcine integument (Wilson et al., 1983). Neither are there any detailed pathophysiologic studies of selenosis in domestic herbivores due to selenomethionine (SEMET), the predominant form of Se in seleniferous grains and forage. The purpose of this report is to summarize 6yrs of morphologic investigation into experimental and spontaneous selenosis in horses and cattle.

Caveat Tinctor: the Search for a Reliable Means of Identifying Se in Tissues

Specimens taken from field cases or from chronic feeding trials in horses and cattle often reveal a variety of lesions, many of which are probably incidental to Se toxicity. Histochemical association between a given lesion and locally increased Se concentrations, while not absolute proof, would be strong evidence of a causal relationship. Autometallographic localization of Se in histological preparations was
introduced by Danscher (1982) as a modification of the intravital Timm sulfide silver technique for detecting heavy metals. This method was used by the originator to demonstrate the cellular and subcellular distribution of Se in tissues of rats, and was later used to demonstrate Se in the tissues of acutely intoxicated sheep (Smyth et al., 1990).

To determine whether the Danscher procedure was a reliable adjunct to chemical analysis prior to using it in pathophysiologic studies of chronic selenosis, adult male rates were dosed with four concentrations each of three different forms of Se: Na₂SeO₃, L-selenomethionine (L-SEMET) or selenocystine (SECYS) for 28d. Samples of liver, kidney, heart and brain were taken for autometallography and chemical analysis by fluorometry (Raisbeck et al., 1996). Metallic silver precipitation was most frequent in kidneys of rats given SECYS, where it was concentrated in the apical cytoplasm of epithelial cells of the proximal convoluted tubules. There was no correlation between staining intensity and renal Se concentrations. Two of 24 Se-treated rats had silver precipitation in the hippocampus and dorsal cochlear nucleus of the brain, but the Se concentrations in these brains were among the lowest of the Se-treated rats. Hepatic Se concentrations were similar to the renal levels, but silver precipitation was absent in this organ (O'Toole et al., 1995). A similar lack of correlation was found between tissue Se concentrations and autometallographic “staining” in cattle fed Na₂SeO₃, L-SEMET or SECYS grass hay and in mice fed Na₂SeO₃, L-SEMET or SECYS. Thus, this technique is not a reliable means of localizing Se in chronically exposed animals.

Elemental Se was identified by electron probe X-ray microanalysis as a component of silver-containing particles in tissues from human patients suffering from argyria (Matsumura et al., 1992), but we are not aware of successful detection of more common physiologic forms, such as protein-bound Se, at a light or electron microscopic level. It was recently attempted to characterize the subcellular distribution of Se in Se-poisoned mallards by semiquantitative x-ray microanalysis. The instrument (JEOL 35CS, NORAN, Boston, MA) was insufficiently sensitive to detect biologically high Se concentrations of 30-50µg/gm (w/wt) in liver and kidney (O'Toole and Raisbeck, unpublished data).

**Integumentary Lesions in Horses and Cattle**

The most distinctive gross lesions of chronic selenosis in horses and cattle involve the integument (Raisbeck et al., 1993). A typical example involves a steer given 0.8mg Se/kg as L-SEMET daily by gavage for several months as part of a chronic feeding study (O'Toole and Raisbeck, 1995). After receiving SEMET for 96d, this steer became moderately lame and exhibited slight selling and erythema proximal to the coronary band of both front legs. The swelling and erythema subsided, but 10d after the initial signs, a hairline crack appeared parallel to and 0.5cm distal to the coronary band and the steer became markedly lame. After approximately 100d on L-SEMET,
Fig. 76.1. Coronary papillae from a horse with spontaneous alkali disease. a. Transverse section to papillae at their distal tips, near a site of gross cleavage in hoof wall. Early changes include nucleated keratinocytes in inter-tubular matrix (parakeratosis; *), and multiple circumferential microfractures at the interface of incipient horn tubules with intertubular horn (>). H and E stain. Bar: 100μm. b. Higher magnification of a papilla, sectioned near the tip. Marked ballooning degeneration of keratinocytes (>), with karyorrhexis. Bar: 50μm.

hair on the distal 6cm of the tail (the tail switch) became detached at the surface of the skin. Hoof separation and lameness increased in severity until, by 120d, the steer refused to walk to feed and water, and had to be hand fed. By the time the steer was euthanized at 134d, all hooves had cracks that extended through to the sensitive laminae (Fig. 77.4, Chapter 77). New, normal tubular horn was growing between dystrophic horn and the epithelium. In field cases, the dystrophic hoof wall is eventually displaced by new growth and is shed or, if it remains partially attached, results in an elongated, deformed hoof (“slipper toe”).

Histologically, hoof damage begins as ballooning degeneration and necrosis of keratinocytes in the stratum spinosum of coronary papillary epidermis (Figs. 76.1 and 76.2) and near the tips of the primary laminae of the stratum internum covering the third phalanx. Similar degenerate changes occur in stratum spinosum of nail and beak of adult ducks with selenosis (O'Toole and Raisbeck, 1997a). Neutrophils and dyskeratotic cellular debris accumulate around epidermal papillae of the stratum corneum and, as cells are displaced from the papillae, in the lumen of horn tubules. The latter are dilated (300-500μm diameter vs. less than 100μm in normal hoof) and there is concomitant loss of intertubular keratin (Fig. 76.3). The normally highly organized spiral of keratinocytes around the tubule is disrupted. In more chronic cases of AD, injury within the epithelium of the hoof may be more extensive, involving more of the laminar epithelium and tubular horn papillae of the sole in addition to the
coronary area. Moderate to severe hyperplasia, acanthosis and parakeratosis occur at the tips of the epithelial laminae (Fig. 76.2b, c).

Parakeratotic ridges of keratin derived from the tips of the laminae are interspersed with islands of relatively normal orthokeratotic keratin from the lateral walls of the laminae. In severe cases, there may be milder, more diffuse changes in the lateral laminar walls. “Keratin columns” of the stratum internum of normal orthokeratotic keratin between adjacent laminae are narrowed (Fig. 76.2b, c). In areas where changes are marked, the columnar germinal epithelium near laminar tips
becomes disorganized and attenuated, and dermis underlying the affected epithelium may be edematous (O’Toole and Raisbeck, 1995; 1997b).

Not surprisingly, chemical analysis of dystrophic hooves reveals that elevated Se concentrations are spatially correlated with the dystrophic hoof lesions (Fig. 77.3b, Chapter 77). In the past, several authors including ourselves speculated that substitution of Se for S in hard keratin results in weakened inter- and intraprotein disulfide bonds and thus softened keratin, which shears parallel to the grain of the hoof from the concussive forces of walking or running. However, the cytotoxic damages demonstrated in these animals suggests an alternate hypothesis. The marked tubular dilation demonstrated in Fig. 76.3b results from substitution of necrotic cellular debris, which has little or no structural strength, for a significant portion of the intertubular keratin normally present in a cross-section of stratum medium. Similarly, the tubule itself is weakened by the disorganization and thinning of keratinocytes in the tubular wall. These changes are concentrated in a small area parallel to the coronary band by virtue of the fact that tubular keratin formation is a continuous process, with cellular damage occurring simultaneously in all papillae when local Se concentrations reach a critical peak. Hooves are designed to divert dangerous proximally-directed cracks (along the plane of horn tubules) in a lateral direction (along the plane of intertubular material) (Bertram and Gosline, 1986).
Dilation of horn tubules combined with microfissures in the intertubular material of the coronary area (Fig. 76.1a) probably exaggerate this tendency, resulting in focused circumferential cracks in the proximal part of the hoof wall. This pathogenic mechanism may explain the failure of sheep to develop typical alkali disease hoof lesions. In spite of some excellent experimental studies of the systemic lesions of selenosis in sheep (Glenn et al., 1964), ovine hooves have never been examined histologically for evidence of dystrophic hoof growth. The smaller body mass of sheep results in proportionately less stress on damaged hoof wall and decreases the likelihood of structural failure.

Selenium-induced alopecia most frequently affects the nape of the neck (mane) and the tail, but in severe cases may also involve other anatomic sites. At a histologic level, alopecia results from atrophy of primary hair follicles. Minimal or no changes are seen in secondary (non-medullated or undercoat) follicles. The ratio of atrophic to nonatrophic follicles in denuded areas may be as great as 1:3 as compared to less than 1:10 in normally haired areas. Most atrophic follicles are collapsed and lack a hair shaft (Fig. 76.4). The inner root sheath is atrophic or absent, the outer sheath contains poorly laminated or dyskeratotic (or apoptotic) keratinocytes (Fig. 76.4c). The hyaline membrane and connective tissue sheath surrounding the follicle are thickened. Follicles that are less severely affected may contain small dystrophic shafts. Accessory follicular structures such as sebaceous glands and arrector pili muscles are unaffected.

Other Lesions of Selenosis

To our knowledge a primary encephalopathy has never been reproduced as a direct effect of selenosis in cattle, sheep or horses, although “polioencephalomalacia” was reported recently in pigs fed a high-selenium diet containing Astragalus bisulcatus (Panter et al., 1996). This term suggests that lesions in the brain were severe, since polioencephalomalacia implies a significant degree of necrosis and, in many instances, grossly evident changes (Gould, 1997). Selenium-intoxicated pigs can develop histological lesions in pontine, olivary, facial, reticular and motor trigeminal nuclei, in addition to well-characterized lesions in spinal intumescences (Wilson et al., 1983; Wilson et al., 1989). However, unlike the spinal lesions, which are responsible for clinical signs of ascending paralysis, lesions in the thalamas and brainstem do not generally result in grossly evident malacia. “Polioencephalomalacia” in intoxicated pigs does not appear to involve the cerebral cortex, unlike sulfur-induced polioencephalomalacia of ruminants.

Although the condition referred to as BS is often attributed to chronic selenosis in reviews and textbooks, only two original reports describe the condition (Draize and Beath, 1935; Rosenfeld and Beath, 1946). These reports attribute a virtual laundry list of lesions to Se intoxication. Despite a standard necropsy protocol that includes examining all major organ systems and histopathologic examination of at least 75 separate tissue specimens, none of the animals we have examined to date has shown
any clinicopathologic or morphologic evidence of the lesions attributed to chronic selenosis by Beath's group (O'Toole et al., 1996). The possible exception is myocardial necrosis. A single 16mo-old Angus-cross heifer fed 1mg Se/kg/d as Na₂SeO₃ for 81d developed disseminated myocardial necrosis and fibrosis, in addition to the integumentary lesions outlined above. This heifer developed a conditioned aversion to Se-treated diets early in the course of the experiment and, as a result, consumed the full Se dose somewhat irregularly. Myocardial necrosis is also prominent feature of acute Se intoxication in sheep (Maag and Glenn, 1967; Blodgett and Bevill, 1987), and there are numerous anecdotal reports of myocardial disease ("dishrag heart") in cattle with chronic selenosis. It thus seems reasonable that, under appropriate conditions of exposure, it is possible for an animal to sustain repeated subclinical toxic insults which culminate in a pattern of organ damage similar to that
seen in acute Se poisoning. Nonetheless, the most distinctive and characteristic lesions of chronic selenosis in large ruminants involve the integument.

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References


