VITAMIN A DEFICIENCY AND HEPATIC RETINOL LEVELS IN SEA OTTERS, ENHYDRA LUTRIS


Abstract: Vitamin A deficiency has rarely been reported in captive or free-ranging wildlife species. Necropsy findings in two captively housed southern sea otters (*Enhydra lutris nereis*) included irregular thickening of the calvaria characterized by diffuse hyperostoses on the internal surface. One animal also had moderate squamous metaplasia of the seromucinous glands of the nose. There was no measurable retinol in the liver of either sea otter. For comparison, hepatic retinol concentration was determined for 23 deceased free-ranging southern and northern (*Enhydra lutris kenyoni*) sea otters from California and Alaska. Free-ranging otters were found to have similar hepatic retinol concentrations (316 ± 245 mg/kg wet weight) regardless of their location and subspecies. All of these values were significantly higher than the levels in the affected animals. Consumption of a diet with very low vitamin A concentrations and noncompliance in daily supplementation are hypothesized as the causes of vitamin A deficiency in these two sea otters.

Key words: Deficiency, *Enhydra lutris*, hyperostoses, retinol, sea otter, vitamin A.

INTRODUCTION

Sea otters (*Enhydra lutris*) are marine mammals native to the eastern Pacific coastal zones of North America. Southern sea otters (*E. lutris nereis*) are found along the central California coast and northern sea otters (*E. lutris kenyoni*) are found along the coastline from the Aleutian Islands east and south to Washington state. Southern sea otters have been listed as threatened under the Endangered Species Act since 1977. Reproduction, physiology, and pathology of sea otters are all fields that have been explored extensively; however, little is known about the nutritional requirements for this species.

There are no specific dietary vitamin A requirements recommended to ensure optimal health in sea otters. In captive settings, sea otters are generally fed a mixed diet of frozen and thawed seafood. This mix often includes clams, mussels, crabs, squid, and shrimp. Flesh from these items are low in vitamin A (<2000 μg retinol/kg dry matter), with vitamin A contained primarily in the viscera. In addition, storage and processing may negatively affect the nutritional content. To assure a balanced diet in the face of these variables, captive otters are supplemented with a daily multivitamin. A commonly utilized vitamin contains 5,500 IU vitamin A (SEA TABS, Pacific Research Laboratories, La Jolla, California 92037, USA) and is usually given in half or quarter tablets to provide dosages of 1,375–2,750 IU/day. Subsequent to this investigation, the animals at this facility are now given a 10,000-IU tablet (Major Pharmaceuticals, Livonia, Michigan 48150, USA) once daily.

Hypovitaminosis A is widely acknowledged in various domestic animal species. In captive wildlife, the condition and lesions of vitamin A deficiency have been documented in the African lion, *Panthera leo*. The role of vitamin A in vision, the immune system, the reproductive system, epithelial differentiation, and bone remodeling produces a broad spectrum of lesions when insufficient vitamin A is ingested. These lesions may be found incidentally on necropsy or be the cause of clinical signs such as abnormal neurologic behavior, reproductive failure, immune depression, and irregular bone remodeling.

Two cases of vitamin A deficiency with hyperostoses of the calvaria and squamous metaplasia of the nasal seromucinous glands in captive southern sea otters are presented. Comparative data from free-ranging northern and
southern sea otters provides reference ranges for liver retinol concentration.

Case Report 1

A subadult female southern sea otter was found stranded, disoriented, and semicomatose on San Simeon Beach (San Luis Obispo County, California, USA) on 17 June 2002. The otter was transported to Monterey Bay Aquarium, where it was stabilized. On 16 May 2003, the animal was sent to SeaWorld San Diego for long-term care. The otter was subsequently introduced into a 196,000-L natural salt water single-species exhibit holding three other female sea otters. This animal was fed a diet of clam foot, crab legs, and decapitated shrimp fed in small increments five times a day. Total daily feed intake was approximately 4.5 kg. Vitamin A determinations subsequently performed on the diet as fed revealed levels of vitamin A below 0.2 mg/kg wet weight of diet. The diet did not include any vitamin supplementation because of dietary discretion/refusal. Idiopathic epilepsy was managed with daily oral phenobarbital (Hikma Pharmaceuticals PLC, London W1S 1HL, England; 150 mg p.o. q 12 hr) administration and the animal remained otherwise healthy, maintaining a stable weight for almost 3 yr.

On 2 February 2006, the otter was anesthetized with a combination of fentanyl (Spectrum Chemical, Gardena, California 90248, USA; 0.22 mg/kg i.m.) and midazolam (Bedford Laboratories, Bedford, Ohio 44146, USA; 0.07 mg/kg i.m.) for a routine annual physical examination. The animal had an adverse anesthetic reaction and did not recover. The otter was maintained on respiratory support for 4 days, but could not be revived and was euthanized with an injection of pentobarbital (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, Michigan 48126, USA; 2,340 mg i.v.).

Necropsy examination revealed an adult female southern sea otter in good body condition. The interior surface of the skull was diffusely and irregularly thickened. There were multiple anastomosing small bony 1–2-mm irregular proliferations lining the interior surface of the calvarium (Fig. 1). Proliferations merged to form irregular plateaus and mounds. Similar milary proliferations extended into the pia and arachnoid tissues. There was no secondary deformation or eventration of brain tissue. All other organs were within normal limits.

Representative tissue samples from several organs were fixed in 10% neutral-buffered formalin, routinely processed for paraffin embedding, sectioned at 5 μm, and stained with hematoxylin and eosin. Histologically, affected regions of the skull demonstrated multifocal to diffuse nodular hyperplasia of the lamellar bone, creating a generalized wavy profile. The bone contained variably sized lacunae; osteoclast activity was diminished. Histologic examination of the brain demonstrated mild scattered acute neuronal necrosis. No specific brain changes were identified that explained the clinical history of epilepsy.

Case Report 2

A 16-yr-old adult female southern sea otter was maintained in a single-species natural salt water exhibit for 12 yr. The diet consisted of clam foot, crab legs, and decapitated shrimp fed in small increments five times a day for a total ration of 4.5 kg/day with no vitamin supplementation. As in case report 1, the vitamin A content in these items as fed was below levels of detection at 0.2 mg/kg wet weight.

The animal had a 2-mo history of lethargy, anorexia, and interdigital pododermatitis with ulceration. Clinical blood work demonstrated neutrophilic leukocytosis. The animal was placed on dexamethasone (dexamethasone tablets USP, Roxane Laboratories, Columbus, Ohio 43216, USA; 3 mg p.o. q 24 hr for 54 days) and its response waxed and waned over the following 2 mo. Because of declining health in spite of aggressive medical therapy with injectable amikacin (Amiglyde-V, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA; 180 mg i.m. q 24 hr for 4 days), the animal was euthanized with an injection of pentobarbital (Fatal-Plus; 5,850 mg i.v.).

Necropsy examination revealed hepatomegaly with multifocal areas of liver pallor and necrosis.

---

**Figure 1.** Multifocal and coalescing hyperostoses on the internal surface of the calvarium from a female southern sea otter (case report 1).
Bacterial cocci were evident both extracellularly and occasionally within leukocytes. There was moderate splenic and lymph node enlargement. Hepatic aerobic bacterial cultures demonstrated a pure culture of *Streptococcus gallolyticus*. Examination of the skull revealed changes similar to those in case report 1. There was a moderate diffuse irregular thickening of the calvarium located on the cranial vault surface. There was generalized thickening characterized by anastomosing irregularly round, 1–2-mm bony projections on the interior surface of the skull. Similar irregular bony structures expanded the pia and arachnoid, especially along the falx cerebri.

Microscopically, there was mild to moderate acute necrotizing splenitis, hepatitis, and lymphadenitis. These findings, along with a pure culture for *S. gallolyticus*, suggest that this otter’s clinical signs were due to severe acute septicemia. The bones of the calvarium demonstrated hyperplasia of lamellar bone with a generalized wavy pattern of organization and irregular dilation of vascular channels (Fig. 2). Additionally, the nasal mucosa contained multifocal areas of rhinitis with neutrophilic infiltrates and squamous metaplasia and laminar keratinization of the seromucous glands (Fig. 3).

**Laboratory analysis**

Liver tissue was collected from both animals at the time of necropsy and frozen at −62°C (−80°F). These samples were submitted to the California Animal Health and Food Safety Laboratory (University of California, Davis, California 95616, USA) for retinol analysis using high-performance liquid chromatography (Model Alliance 2695 HPLC System, Waters Corporation, Milford, Connecticut 01757, USA, and Model 1100 HPLC System, Agilent Technologies, Santa Clara, California 95051, USA). The test revealed no detectable levels of retinol in either sample, with a minimum detection threshold of 0.2 mg/kg wet weight.

Because of a lack of easily accessible comparable liver retinol concentration data from sea otters, liver from 23 free-ranging sea otters (11 northern sea otters and 12 southern sea otters) was similarly analyzed to provide a control population (Table 1). The liver samples came from the Alaska SeaLife Center (Seward, Alaska 99664, USA) and the Marine Wildlife Veterinary Care and Research Center (Santa Cruz, California 95060, USA). The sea otters either were found dead, died in rehabilitation, or were euthanized in rehabilitation because of declining health. None of the comparison animals had liver disease; however, diet history was unknown. Liver tissue was collected at necropsy by the respective centers and held frozen at −70°C (−94°F) and protected from light until submitted for analysis. The mean ± SD for retinol liver concentration was 593 ± 1,353 mg/kg wet weight for all 23 otters. A separate mean was calculated after removing results from otter number EL0613, which was determined to be a statistically significant outlier using the Grubbs test. After removing the outlier, EL0613, the mean ± SD liver retinol concentration in these free-ranging otters was 316 ± 245 mg/kg wet weight. The mean ± SD hepatic
The mechanism of the hyperostotic lesions on the calvaria of these sea otters is not known. However, the negligible hepatic retinol in conjunction with the histologic lesions suggests hypovitaminosis A. The degree of skull formation and remodeling associated with the onset of low vitamin A may have impacted the amount of proliferative changes identified in these animals as compared to the published lesions in lion cubs. Vitamin A, or more specifically its metabolite all-trans-retinoic acid, plays a critical role in the process of bone remodeling. In a dose- and time-dependent manner, all-trans-retinoic acid enhances the bone resorption activity of osteoclasts by increasing protein synthesis by binding to nuclear retinoic acid receptors of osteoclasts. By contrast, vitamin A deficiency is associated with decreased bone resorption, disturbances in the homeostasis of osteoclasts and osteoblasts, and bone remodeling. Younger animals with vitamin A deficiency frequently exhibit defects in intramembranous ossification; the cranial vault does not properly remodel or increase in volume, resulting in compression of the growing nervous system and neurologic signs, blindness, and paralysis.

Hyperplastic skull changes are a described lesion of hypovitaminosis A in wild carnivores. Chandra et al. reported skull lesions in captive African lions from a Florida zoologic institution that were fed an all-meat diet with no vitamin A supplementation. In these lions, thickening of the bones of the cranial vault led to basisphenoid and tentorium cerebelli malformation. The lions demonstrated neurologic signs when the increase in intracranial pressure due to increased bony growth caused secondary cerebellar herniation. No cerebellar herniation was observed in either sea otter in this report.
The northern and southern sea otter skull normally undergoes age-dependent structural changes including irregular thickenings of the pia and arachnoid. These age-related changes differ from the proliferative skull change that these two otters exhibited. The age-related changes have not been analyzed related to vitamin A or retinol concentrations.

In case report 2, squamous metaplasia with hyperkeratosis was visualized in the ducts of the seromucous glands of the nasal mucosa. Numerous reports document the association between vitamin A deficiency and squamous metaplasia and keratin deposition. In most species, squamous metaplasia and keratin deposition in mucous-secreting epithelium is pathognomonic for vitamin A deficiency. Vitamin A works in a hormone-like fashion on nuclear retinoic acid receptors of epithelial cells to signal transcriptional proteins leading to cell differentiation. Squamous metaplasia is often first seen in the conjunctiva; however, it can occur throughout the body in the mucosal epithelium of the respiratory, gastrointestinal, and genitourinary tracts and pancreas. Epithelial metaplasia leads to altered mucosal function, resulting in decreased spermatogenesis, xerophthalmia, abortion, and a compromised immune function.

The S. gallolyticus bacterial infection diagnosed in case report 2 may have gained entry into the bloodstream to cause the severe septicemia from a damaged epithelial barrier around the pododermatitis, metaplastic epithelium in the nasal mucosa forming an inadequate barrier to bacteria, or because of compromised overall immune status from vitamin A deficiency. Infection may have been promoted by steroid administration or effects of the low tissue vitamin A. Hypovitaminosis A has direct negative effects on the immune system, as vitamin A is needed in the differentiation of various leukocytes. Vitamin A has been shown to be important in maintaining adequate numbers of T and B lymphocytes and macrophage function.

The eyes of the sea otters in these two cases were not examined histologically. However, microscopic examination of the eyes is a helpful aid in diagnosis of vitamin A deficiency. Williams et al. documented many cases of retinal dysplasia in free-ranging European otters (Lutra lutra) with low hepatic retinol concentrations. This vitamin A deficiency was linked to high tissue burdens of polychlorinated biphenyls (PCBs), which are capable of antagonizing liver vitamin A stores. PCBs have been widely used for industrial purposes, yet their use was recently curtailed due to the environmental stability of these compounds and the danger of trophic transfer to marine species and humans. In 2000, Simpson et al. analyzed hepatic PCB and vitamin A concentrations in European otters that were found dead in southwest England between 1988 and 1996. They found that higher hepatic PCB concentrations were associated with lower hepatic retinol concentrations. It is hypothesized that PCBs may decrease total liver vitamin A concentration by altering lipid metabolism and increasing destruction of vitamin A.

Hepatic PCB concentrations for the free-ranging sea otters were not determined, as their retinol concentrations were adequate. However if miliary hyperostoses are identified on the inner calvarium of a free-ranging sea otter, analysis of hepatic PCB and retinol concentrations should be considered.

Vitamin A deficiency has been associated with spinal degeneration in other species. The spines of these two animals were not examined histologically. However, clinical hind-limb deficits have been identified in this species. Future necropsy examinations should include routine collection and sectioning of the spinal cord as well as ocular tissue.

The hepatic retinol values not only were useful in providing a comparative picture for the two affected animals, but also provide baseline data for evaluating free-ranging otters. The mean hepatic retinol concentrations for the 22 control otters in this study was 316 ± 245 mg/kg wet weight. In 2009, The Marine Sanctuary Conservation Series (MSCS) reported a live, captured, free-ranging northern sea otter mean ± SD retinol liver concentration of 220.52 ± 344.41 mg/kg wet weight. The MSCS mean retinol concentration is within the range of the mean retinol concentration determined by the 22 otters in this study. The MSCS report also noted that there were significantly higher retinol levels in female otters than in male otters. Perhaps the difference, though not a significant difference, of the means between the Alaska and California populations found in this study was due to the sex selection of all male otters from the Alaska population. Each subspecies in this study had one sex selection of all male otters from the Alaska and California populations found in this study was due to the MSCS report.
specific diet preferences,\textsuperscript{10} and further correlations of hepatic retinol levels with diet should be evaluated. Liver concentrations for normal captive animals have not been determined. However, efforts to determine appropriate serum levels to assess vitamin A status are underway.

Sea otter prey selection is influenced by resource abundance,\textsuperscript{32} and learned behaviors are passed in a matrilineal fashion.\textsuperscript{11} Common food items of free-ranging southern sea otters are crabs, clams, abalone, marine worms, purple urchin, and mussels.\textsuperscript{31} Many of these items are marginal to deficient in vitamin A when compared to the known 1,000 μg/kg dry matter retinoic acid required for maintenance of an adult cat.\textsuperscript{23,24} Areas frequently inhabited by southern sea otters may have diminished food resources, leading to increased dietary specialization and reliance on these less nutritious food sources.\textsuperscript{32} A lack of dietary variety and increased reliance upon foodstuffs with a lower level of retinol could lead to a vitamin A deficient state. It is unknown whether or not sea otters have the ability to bioconvert ingested carotenoids. Though main food items in free-ranging sea otters have a low content of vitamin A concentrations, the substantial hepatic retinol concentration found in the 22 free-ranging sea otters measured in this study suggests that perhaps sea otters do have the ability to bioconvert β-carotene and use it as a vitamin A source.

Captive sea otters are commonly fed a mix of clams, squid, mussels, crab, abalone, octopus, scallops, and shrimp. Some facilities eviscerate these items prior to feeding to minimize concerns of domoic acid exposure (St. Leger, pers. obs.) because many of these food items are known accumulators of the biotoxin domoic acid.\textsuperscript{28,35} This practice was part of the feeding program for both of these otters. Evisceration lowers the concentration of vitamin A available as compared to consumption of whole prey items due to compartmentalized storage of vitamin A in the viscera of invertebrates (pers. obs.). The process may also remove β-carotene found in the viscera of the invertebrates fed to the sea otters, rendering it unavailable for conversion to vitamin A if indeed otters do have the ability to do so. This is a likely etiology as to how the two captive otters had a severely deficient retinol liver concentration compared with free-ranging otters on a similar diet.

Another management consideration is nutritional supplementation. Though it is recommended that captive sea otters receive a daily multivitamin supplement, acceptance and individual consumption varies.\textsuperscript{33} Sea otters are fastidious eaters and have a tendency to reject foods due to decreased palatability.\textsuperscript{19,33} Oral supplementation that improves acceptance and complete consumption will assure sufficient vitamin A levels in captive otters. Vitamin A supplementation for animals that may not ingest their needed amounts can also be administered via regular injections of vitamin A palmitate (Aquasol-A, Mayne Pharma Inc., Paramus, New Jersey 07652, USA). Studies on serum vitamin A concentration in normal and deficient otters may facilitate clinical evaluation and treatment of otters with hypovitaminosis A.

Acknowledgments: Additional efforts related to the care of these animals and the evaluation of wild animals was performed by Dr. Todd Schmitt, Dr. Tom Reidarson, the Sea World San Diego Animal Care Staff, Dr. Tim Tinker, and Erin Dodd.

LITERATURE CITED


Received for publication 15 January 2010