SEASONAL CALCIUM FLUX IN MOOSE

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Seasonal mobilization of calcium in relation to antler growth has focused on the site of new bone growth, but systemic changes have not been fully studied. Several theories on the role of various steroids in activating the transport of calcium to the site of growth have been postulated, but little attention has been paid to theimpact on body stores of this essential element. We have analyzed hair calcium levels as an indicator of mineral status and have noted seasonal changes in both males and females. Hair calcium levels in the May and June samples were markedly elevated over the other 10 months in both males and females. To follow up on these observations, we monitored blood serum calcium and serum hydroxyproline as indicators of calcium flux. Serum calcium was significantly higher in June through October than in winter and spring. The flux of calcium as indicated by hydroxyproline was markedly higher in May through August than in a later winter sample, February. Although both males and females demonstrated this change the magnitude of the male response was greater.

Franzmann and LeResche, (1978) have shown that multiple factors influence the level of calcium in the serum of moose Alces alces gigas. Age, season of the year, condition class and geographic location of the animal have all been related to changes in the balance of this essential element. All of these changes eventually indicate the availability of calcium in the diet and absorption across the gastrointestinal tract (Simeson, 1970). The report of Franzmann and LeResche (1978) on a large sampling (N=1506) provided an overall arithmetic mean of 10.34 $\pm$ 1.17 mg/dl of calcium in serum as a point for comparisons (normal Range 8.00 - 12.68 mg/dl). The means of most groups were within the normal range, but summer

and fall serum calcium levels were significantly higher than winter or spring values in all age classes. Hair calcium analysis, another indicator of calcium balance from moose at the Kenai Moose Research Center (MRC), demonstrated seasonal variations quite different from serum calcium findings (Franzmann and LeResche, 1978). Moose hair calcium representing May and June were markedly elevated over the remainder of the year showing dramatic deposition of calcium in hair at a time when serum calcium was generally low. Both serum and hair calcium indicate significant changes in calcium metabolism were taking place, but no clear picture of the systemic trends was evident.

Overlaying on the seasonal/nutritional impact on calcium is the annual development of new bone in antler growth in males. A number of studies have focused on the role of steroid hormones on growth of antler bone (Bubenik, et al., 1975; Goss and Schmidt, 1930; Lincoln, 1975). Bubenik, et al., (1975) used an anti-androgenic compound in describing the effects of depletion of androgenic steroid hormones on antler growth in white-tailed deer. The mineralization processes of the bone matrix were blocked and antlers grow throughout the year. Plasma levels of calcium did not differ from control values with the use of the blocking agent. Changes in the systemic handling of calcium during antler growth or when such new bone growth is impeded have not been reported in moose.

Our preliminary data indicated significant changes in calcium metabolism in the spring of the year in both female and male moose. Since studies on steroid manipulated male cervids did not demonstrate any significant changes in plasma calcium, we developed a study to specifically analyze the critical period of spring and summer in calcium metabolism in both female and male moose. Hair calcium, serum calcium and serum hydroxy-

proline were measured as various markers of systemic calcium metabolism.

METHODS

Moose were sampled during tagging and movement studies, from hunter-kill, and from regular collections at the MRC. Figure 1 depicts areas of collection. Forty-three percent of samples were from the MRC, which consists of four 2.6 km^2 enclosures located in the area of the 1947 Kenai burn, 35 km northeast of Soldotna, Alaska. Twenty-two fenceline traps (LeResche and Lynch, 1973) are strategically located (13 within and 9 outside the enclosures) to facilitate capture and immobilization (Franzmann and Arneson, 1974) of moose.

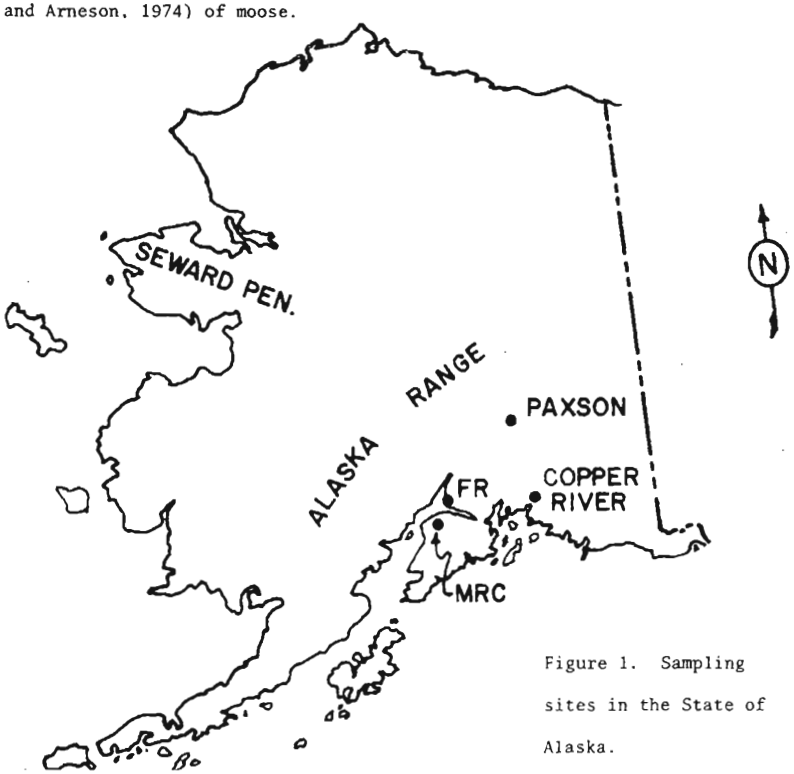

Figure 1. Sampling sites in the State of Alaska.

Hair samples were obtained by plucking hair from the shoulder hump of moose (Franzmann, et al., 1977). Samples were stored and shipped in plastic containers and analyzed on a semi-automated Perkin-Elmer Model 503 Spectrometer adapted for automated dilution with a Hamilton Precision Dispenser. Prior to analysis, hair samples were washed twice with diethyl ether to remove surface particulate matter without leaching elements from the hair structure. A 200 mg sample of hair was digested in 10.0 ml of 24% methanolic tetramethyl ammonium hydroxide for 2 hours at 55°C (Gross and Parkinson, 1974) as described previously (Franzmann, et al., 1977).

Serum samples were collected by venipuncture from the jugular vein into evacuated tubes with no anticoagulants. Serum was separated from the red blood cell clot and stored frozen until analyzed. Serum calcium was analyzed by colorimetric methods as a part of a multicomponent analysis (using a SMA-18 analyzer). Serum hydroxyproline was assayed by colorimetric methods utilizing Ehrlich's reagent (Dabew and Struck, 1971).

We collected a subsample of frozen stored serum from both female and male moose from the Kenai Moose Research Center to further analyze for serum calcium and hydroxyproline. Serum samples were analyzed for the months of February (10 females, 10 males), May (13 females, 4 males), June (13 females, 7 males), July (11 females, 9 males) and August (10 females, 9 males).

RESULTS

The multi-tissue analysis of calcium related markers indicated that systemic changes were occurring that were not reflected in blood serum calcium levels. The hair results (Fig. 2) demonstrate that calcium is dramatically elevated in May and June and is fairly comparable for the remainder of the year (with perhaps a rebound effect being seen in the

August sample). The deposition of calcium in the hair during spring months may indicate an increased availability or mobilization of calcium. Results shown in Table 1 indicate that summer/fall levels of serum calcium are high, whereas winter and spring values are generally lower. This information fits with the concept that serum calcium levels reflect dietary availability of calcium, but are in contrast with the hair results.

TABLE 1

SEASONAL SERUM CALCIUM LEVELS BY AGE GROUP IN ALASKAN MOOSE

Age Group (Months)	Season of Sample (N) Summer/Fall	Early Winter	Late Winter/ Spring
Calf (0-12)	10.56 ± 0.94 (38)[a]	9.21 ± 2.15 (46)	9.40 ± 1.58 (9)
1-2 Years (13-36)	10.47 ± 0.82 (76)[a]	9.51 ± 1.86 (45)	9.97 ± 1.26 (87)
Adult (37+)	10.48 ± 0.76 (286)[b]	10.09 ± 1.21 (170)[c]	10.38 ± 0.76 (237)

Mean ± S.D.

[a]Significantly different from both other groups ($p < 0.01$).
[b]Significantly different from early winter levels ($p < 0.01$).
[c]Significantly different from late winter/spring levels ($p < 0.01$).

Serum calcium values (Fig. 3) for male and female moose demonstrated that males were lower in May, but very comparable to females in February and June through August. Serum hydroxyproline (Fig. 4) demonstrated quite different patterns. As will be discussed later, elevated serum hydroxyproline is generally a marker of bone degradation. Both female and male levels of serum hydroxyproline are slightly elevated over the February values in May, with a further increase in June. Female levels of hydroxyline then begin to decline and come down in both July and August. Male serum hydroxyproline values continued to increase through July and decrease

in August.

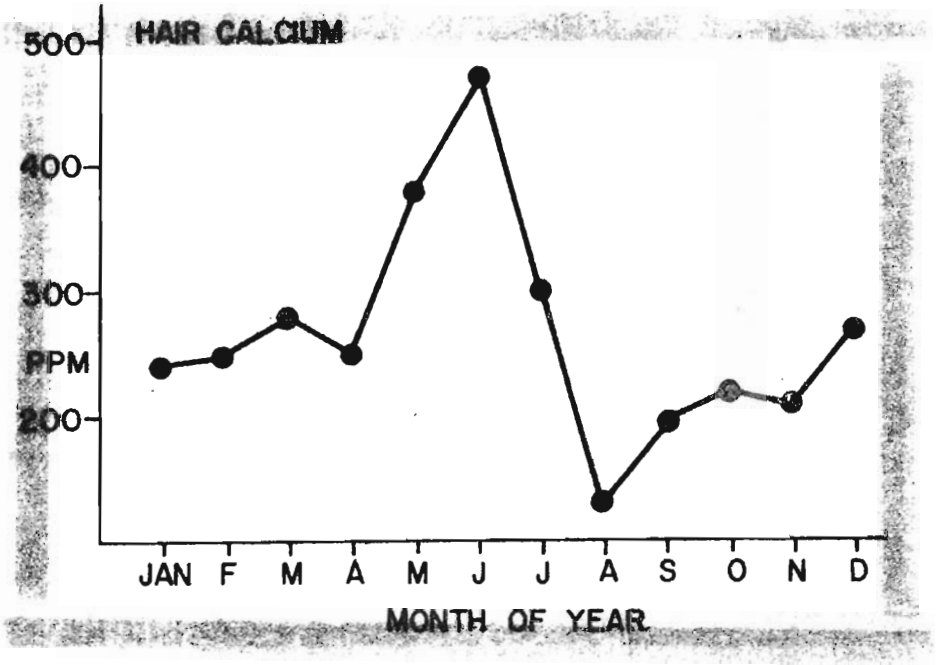

Figure 2. Seasonal hair calcium levels from female and male moose at the Kenai Moose Research Center, 1973-1794 (N=232).

The importance of recognizing geographical subpopulation differences when comparing calcium levels in both serum and hair is noted in Table 2. Comparison of samples from the Copper River Delta with samples from the Moose Research Center demonstrate the potential for marked differences in the mineralization of bone stores.

Seasonal sampling of serum calcium in various age groups of moose over several years provides results that are quite different. Results of

serum measurements in calves, 1-2 year olds and adults (Table 1) from the MRC on the Kenai Peninsula, Alaska indicated significant ($p < 0.05$) differences in both serum and hair calcium. From these two groups, the serum and hair calcium variation appears to be inversely related. These differences suggest that population studies must be well controlled to avoid misinterpreting subpopulation differences as common trends of all moose and seasonal differences by sampling at single times. With regard to calcium metabolism, much relevant data would be missed by studying single subpopulations of moose.

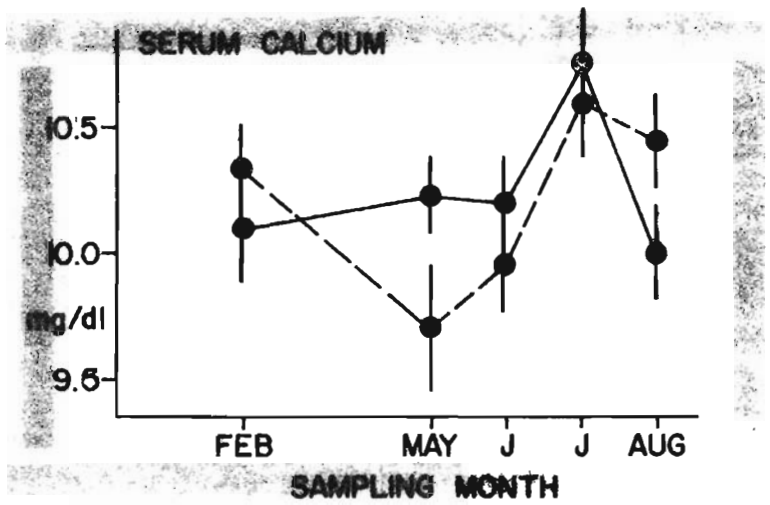

Figure 3. Serum calcium levels from female (●——●) and male (0----0) moose sampled in late winter, spring and summer at Kenai Moose Research Center (N=96).

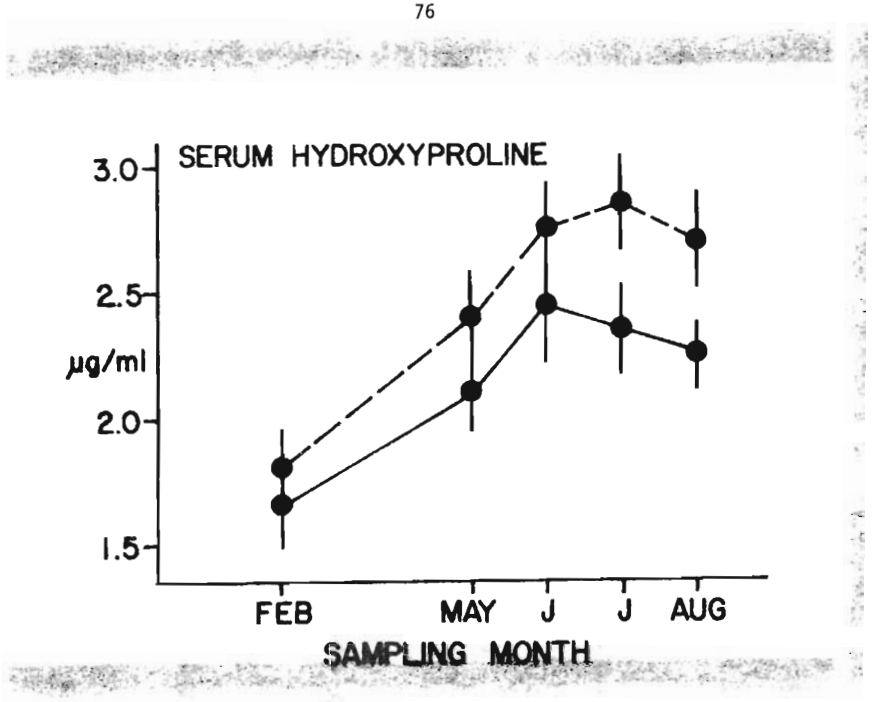

Figure 4. Serum hydroxyproline levels from female (●——●) and male (0-----0) moose sampled in late Winter, Spring and Summer at the Kenai Moose Research Center (N=96).

TABLE 2

GEOGRAPHICAL EFFECTS ON BLOOD AND HAIR CALCIUM IN ALASKAN MOOSE

Sampling Site	Serum Calcium (N)	Hair Calcium (N)
Cooper River Delta (March)	10.38 ± 0.74[a] (44)[b]	132.1 ± 63.2 (50)
Moose Research Center (Feb-Mar-April)	9.81 ± 0.64 (39)	241.8 ± 42.2 (23)[b]

[a] Mean $\pm$ S.D.

[b] Significantly higher than comparable group ($p < 0.05$).

DISCUSSION

The differences in growth of new bone and calcium flux in female and male moose provide an excellent model for numerous calcium related imbalances in other species and man. Annual bone growth in antlers may involve the formation of up to 8-10% ot total body weight in new bone. The dietary availability of calcium must be sufficient to support this growth or body stores are utilized. The skeleton is the major reserve for calcium and it may be released from bone when the body is subjected to calcium deficient diets or other calcium challenges (Rasmussen, 1977). Ramberg, et al., (1976) have shown that this process varies greatly with age and species. The ability to draw calcium from bone reserves may, however, result in as much as a 50 percent loss in ash content of skeleton in other species (Rasmussen, 1977). The dynamics of calcium metabolism in Alces alces has not been thoroughly studied yet provides a unique model of hormone interaction with dietary/metabolic availability of calcium.

Seasonal variation in hair and serum calcium in moose at the Kenai MRC provide a unique picture of contrasts in two calcium markers. Hair calcium levels point to increased availability and deposition of calcium in hair in the spring samples from May and June. Serum calcium during this same seasonal period is among the lowest (comparable with winter values). If serum calcium is indicative of dietary availability then it is in direct contrast with the hair calcium findings. Age of the animal has been shown to influence serum calcium concentrations. Our age distribution of serum calcium trends with season were very similar with older animals having slightly higher serum calcium values. Similar trends have been reported in dairy cattle by Ramberg, et al., (1976), which was related to a decline in calcium clearance and decrease in plasma parathyroid hormone concentrations.

The geographical differences in hair and serum calcium concentrations at the same season of the year provide some clues that hair and serum calcium may be opposite indicators of dietary calcium availability. The dissimilarity in food availability and diet quality between the Copper River Delta and the Kenai Moose Research Center are marked. The yearly flooding of the Copper River Delta and depositing of alluvium provide rapid, new growth and animals in the best condition classes. The Moose Research Center is situated in a regrowth area approximately 30 years after the last major burn, with animals in all condition classes. The geographically related hair and serum calcium results would indicate that serum calcium and not hair calcium is the better positive indicator of the calcium state of the animal. Sex differences, including antler growth and lactation, must also be fit into this complex scheme which led us to consider further studies utilizing serum calcium and hydroxyproline.

Evans, et al., (1976) demonstrated that in cattle, serum hydroxyproline was an index of calcium homeostasis. The basis for this conclusion is that elevated serum hydroxyproline is an indicator of collagen degradation. Since collagen is the organic matrix of bone, its degradation would be related to bone resorption (Lutwak, et al., 1974). Our findings indicate that there was a degradation of collagen in both females and males in early spring, but in males this condition continued until July. The magnitude of the hydroxyproline changes was greater in males and may relate to antler growth and the need for greater calcium mobilization. This finding in males presents somewhat of a paradoxical situation where there is new bone growth and existing bone degradation. The balance between the two states may be indicated by the hydroxyproline trends which indicate the existing bone degradation may be greater than new bone formation. The comparison

with the female population may indicate that dietary calcium may not be sufficient until June or July and interferes with new bone growth in males.

Mechanisms involved in the mobilization of calcium (both from gastrointestinal absorption and existing bone stores) in male moose with antler growth are not fully described, but can have a significant impact on the basic physiology of many states where calcium imbalance is noted. Bubenik (1975) has provided an interesting model of neurohormonal regulation of pedicle and antler growth that relates to testosterone stimulated calcium receptors in the forming bone matrix of the pedicle. The chemical configurations of the steroid stimulated receptors would provide insight into a number of syndromes that may involve new calcium receptor sites. Antler growth and calcium metabolism in moose provide a unique model of the balance between the utilization of calcium stores and new bone growth that need to be explored.

REFERENCES

Bubenik, A.B. 1975. Taxonomic value of antlers in Genus Ranifer, H. Smith pp 41-50 in J.R. Luick, P.C. Lent, D.R. Klein and R.G. White, Eds. Proceedings of the 1st International Reindeer and Caribou Symposium.

Bubenik, G.A., A.B. Bubenik, G.M. Brown and D.A. Wilson. 1975. The role of sex hormones in the growth of antler bone tissue. I: Endocrine and metabolic effects of antiandrogen therapy. J. Exp. Zool. 194: 349-358.

Dabew, D. and H. Struck. 1971. Microliter determination of free hydroxyproline in blood serum. Biochem. Med. 5: 17-21.

Evans, J.L., R.E. Fish and Z.B. Lelkes. 1976. Hydroxyproline in serum as a homeostatic index for calcium in cattle. J. Dairy Sci. 59: 1838-1841.

Franzmann, A.W. and P.D. Arneson. 1974. Immobilization of Alaskan moose. J. Zool. Anim. Med. 5: 26-32.

Franzmann, A.W. and R.E. LeResche. 1978. Alaskan moose blood studies with emphasis on condition evaluation. J. Wildlife Manage. 42: 334-351.

Franzmann, A.W., A. Flynn and P.D. Arneson. 1977. Alaskan moose hair element values and variability. Comp. Biochem. Physiol. 57A: 299-306.

Goss, H. and C.L.A. Schmidt. 1930. Calcium and phosphorus metabolism in rats during pregnancy and lactation and the influence of the reaction of the diet thereon. J. Biol. Chem. 86: 417-432.

Gross, S.B. and Parkinson. 1974. Analyses of metals in human tissues using base (TMAH) digests and graphite furnace atomic absorption spectrophotometry. Interfact 3: 10.

LeResche, R.E. and G.M. Lynch. 1973. A trap for free ranging moose. J. Wildl. Managmt. 37: 87-89.

Lincoln, G.A. 1975. An effect of the epididymis on the growth of antlers of castrated red deer. J. Reprod. Fert. 42: 159-161.

Lutwak, L.M., F.R. Singer and M.R. Urist. 1974. Current concepts of bone metabolism. Ann. Intern. Med. 80: 630-644.

Ramberg, C.F., G.P. Mayer, D.S. Kronfeld and J.T. Potts. 1976. Dietary calcium, calcium kinetics and plasma parathyroid hormone concentrations in cows. J. Nutr. 106: 671-679.

Rasmussen, P. 1977. Calcium deficiency, pregnancy and lactation in rats. Calc. Tiss. Res. 23: 87-94.

Simeson, C.E. 1970. Ruminant Nutrition. Grune and Stratton, New York. p. 284.