Bone Zinc Concentration in Range Cattle

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In range cattle the zinc concentration was significantly higher in the ash from caudal vertebra than from rib, humerus, femur, frontal bone, or thoracic vertebra. The concentration in ash from the frontal bone was lower than from the other bones studied. Bone zinc decreased with the age of the cattle. No effect of location or year of sampling was observed.

The importance of zinc in animal biochemistry is well known. Zinc deficiency diseases have been observed in several domestic and laboratory species and in man (*17, 18*). Zinc is felt to play a role in the calcification of bone (*12*). There are few data in the literature on the normal concentrations of zinc in bone, although the concentration of zinc in bone is greater than in most other organs. Only two reports of bone zinc concentration in cattle were found (*16, 19*).

This study was undertaken to determine the concentration of zinc in bone ash from cattle under normal western range conditions.

Methods

Purebred and grade Hereford cattle managed under range conditions were used for this study. Three range areas were used: Two in southern Nevada (DV and NTS) were typical of southwestern desert ranges and one in northeastern Nevada (KC) was typical of the Great Basin range. Cattle were slaughtered in late spring from these areas and bones taken for analysis. Before 1963, rib and femur samples and in 1963 and 1964 femur and caudal vertebra were routinely taken. In the spring of 1960, five animals from the KC location were used to study zinc distribution in six bones. The herds, locations, and sampling schedules have been described in detail (*4*).

The bone samples were dissected free of extraneous tissue, ashed overnight at 550° C. in porcelain containers, ground, and ashed again overnight at 550° C. The resulting white ash was stored in sealed containers until analysis. Tracer studies indicated quantitative recovery of bone zinc through this ashing procedure. Samples were prepared for atomic absorption spectrophotometry by dissolving 0.005 gram of ash by heating with a minimal quantity of 1*N* hydrochloric acid, filtering, and diluting to 100 ml. with distilled water.

A locally fabricated atomic absorption spectrophotometer similar to that described by Box and Walsh (*5*) was used. The analytical results were displayed on a recorder operated as an expanded scale readout with suppressed zero. Operating conditions were adjusted for maximum sensitivity with respect to flame composition, flame position, and other operational variables. The 2139-A. resonance line was used for analytical purposes. The determination of zinc was shown to be free from interferences at anticipated concentrations except for calcium, which enhanced absorption approximately 10%. Since the calcium content of bone ash is reasonably constant and the analysis was not sensitive to small variations in calcium content, the analytical standards were diluted with 0.18% calcium solution to correct for this interference. Under the operating conditions used, 0.01 p.p.m. of zinc in solution (2 p.p.m. of zinc in bone ash) deflected the recorder one scale division (1 mm.).

The standard curve was linear when plotted on semilogarithmic paper in the range of concentrations used for analysis. Statistical study of duplicate samples indicated a standard deviation of analysis of ±0.06 p.p.m. of zinc in solution or ±12 p.p.m. of zinc in bone ash.

Results

The distribution of zinc in the bovine skeleton was studied on five animals slaughtered in 1960 from the KC location. Eighth rib, head and half of the shaft of the femur, humerus, frontal bone, thoracic vertebra, and caudal vertebra were analyzed (Table I). Analysis of variance indicated that the differences between bones were significant, and Table I indicates which differences were statistically significant. The caudal vertebra contained significantly more zinc than other bones studied. The frontal bone tended to contain less zinc than the other bones, and these differences approached significance ($P < 0.10$).

The effect on zinc concentration of several environmental and physiological factors was studied. Samples were chosen to study the effect of location in the state (KC, DV, and NTS) and hence range type, age of the animals (yearling and mature cattle), year of sampling (1963 and 1964), and bone (femur and caudal vertebra). Three animals were used in each subclass and the data analyzed as a 3 × 2 × 2 × 2 factorial experiment replicated three times (72 samples or 36 animals). The results indicate that the difference between femur (165 ± 35 p.p.m.) and caudal vertebra (223 ± 43 p.p.m.) was the only significant factor studied. The means are given in Table II as Experiment 1. This difference was expected from the results of the study of the distribution of zinc in the bovine skeleton (Table I). The mean values found in these two sets of data were well within one standard deviation of each other. The effect of age approached significance ($P < 0.10$), and the means for each bone at each age are given in Table II.

To study further the effect of age of the animal on bone zinc concentration, another set of samples was chosen from samples collected in 1960 and through 1963. The factors studied were age of the animal (3 to 4 months old, yearling, and mature cattle), location in the state (KC and DV), and bone (rib and femur). Three animals were used in each subclass and the data analyzed as a 3 × 2 × 2 factorial experiment replicated three times (36 samples or 18 animals). The results are given in Table II as Experiment 2. The effect of age was highly significant. All bones studied showed a decrease in zinc concentration with age. As expected from the study of zinc distribution in the bovine skeleton, no significant difference was found between rib

Table I. Zinc Concentrations in Bovine Bone Ash

Bone	No. of Samples	Parts per Million
Frontal	5	139 ± 25[a,b]
Femur	5	172 ± 28[b,c]
Humerus	5	185 ± 37[b,c]
Rib	5	186 ± 34[b]
Thoracic vertebra	5	196 ± 26[b]
Caudal vertebra	5	271 ± 50[d]

[a] Standard deviation.
Figures with different superscripts ([b, c, d]) differ significantly at $P < 0.025$.

Table II. Effect of Age on Zinc Concentration in Bovine Bone Ash

	Femur				Rib		Caudal Vertebra	
	Experiment 1		Experiment 2		Experiment 2		Experiment 1	
Age	No. of samples	P.p.m.	No. of samples	P.p.m.	No. of samples	P.p.m.	No. of samples	P.p.m.
3 Months			6	275 ± 59[a]	6	230 ± 30		
Yearling	18	171 ± 34	6	172 ± 72	6	208 ± 56	18	236 ± 48
Mature	18	159 ± 27	6	170 ± 22	6	164 ± 28	18	210 ± 33

[a] Standard deviation.

and femur. The mean values were in good agreement with the data reported in Table I.

Discussion

The data reported herein for zinc concentration in cattle bone ash are generally consistent with those reported for other species. In the rat, higher (*1*), lower (*9*, *14*, *21*), and comparable (*13*) concentrations have been reported. In man, somewhat lower concentrations have been found (*1*, *13*, *15*, *20*). The concentrations in cattle were comparable to those reported in the chick (*7*, *10*), swine (*11*), and the domestic cat (*13*), but lower than those reported in the horse (*3*).

The insignificance of the difference in zinc concentration between rib and femur (Table I) is in agreement with the findings of Lutz (*13*) in human bones and is consistent with the ranges reported by Rusoff (*19*) in newborn calves. Taylor (*21*) reported no significant difference among the various bones of the rat, although the rib tended to contain less zinc than the femur and pelvis and the humerus more zinc. The zinc concentration of the frontal bone was lower and the caudal vertebra higher than other bones sampled in all cattle studied. The much greater zinc concentration of the caudal vertebra may reflect the later ossification of this bone.

The study of the effect of location on bone ash zinc concentration included any differences in nutritional levels of zinc at the locations studied. Alexander and Nusbaum (*1*) observed no effect of location on human bone ash zinc concentration, in agreement with this study on cattle. Range type, climate, and soil type were different between the northern (KC) and southern (DV and NTS) locations (*4*). There is at present no zinc mining in these areas. Dye (*6*) found no great differences in the zinc concentration of alfalfa in various irrigated valleys in the state, some of which were in close proximity to the ranges used in this study.

In cattle, bone ash zinc concentration decreased with age, especially during the first year of life (Table II). Alexander and Nusbaum (*1*) found no similar effect in rats or man. Taylor (*21*) found an increase of bone zinc with age in rats and Nusbaum *et al.* (*15*) found no correlation of bone zinc concentration and age in man. Bertrand and Vladesco (*2*) observed a decrease with age in whole-body zinc concentration in several species.

Our data are consistent, also, with the finding of Leonov (*11*) that skeletal zinc reached a maximum concentration in the human fetus at about the midpoint of gestation. Alexander and Nusbaum (*1*) reported that more zinc is present in the growing end than in the shaft of the femur. Haumont (*8*) observed histochemically a greater concentration of chelatable zinc in the calcifying portions of bone than in more mature areas. The present study indicated greater zinc concentration in the later ossifying caudal vertebra than in the thoracic vertebra. These studies generally indicated that the zinc concentration in bone is greatest during periods of active bone growth, possibly in response to greater biochemical need.

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