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This study performed under a Memorandum of
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LEGAL NOTICE

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This work was done as part of a U. S. Public Health Service radiation surveillance study of wildlife ruminants in the Nevada Test Site environs under a Memorandum of Understanding, SF 54 373, between the Atomic Energy Commission and the U. S. Public Health Service, Las Vegas, Nevada.

ABSTRACT

The percent calcium of bone ash found in hock joints of 63 mule deer (Odocoileus hemionus), 14 white-tailed deer (Odocoileus virgianus), 14 elk (Cervus canadensis), 13 desert bighorn sheep (Ovis canadensis nelsoni), 9 antelope (Antilocarpa americana), and 7 buffalo (Bison bison) is reported. The average calcium content of hock joints in the 6 species from 18 different areas throughout the United States was 37.6% of bone ash. Differences between species, regardless of geographic locations, were small when present.

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INTRODUCTION

The assessment of ^{90}Sr levels in bones of mammals has been expressed in various units, e.g., pCi ^{90}Sr/gm calcium, pCi ^{90}Sr/gm bone ash, pCi ^{90}Sr/gm wet bone (fresh bone), pCi ^{90}Sr/gm dried bone. A great many of the earlier reports list ^{90}Sr levels in bone as pCi of ^{90}Sr/gm of calcium (strontium units). However, when reported in pCi ^{90}Sr/gm bone ash, calcium content is not normally determined. In comparing data presented in strontium units with that in pCi of ^{90}Sr/gm bone ash where the percent calcium is not given, one must use an assumed constant percent (Ca/gm bone ash) to convert either unit to the other. Values reported in the literature for wildlife ruminants indicate that bone ash contains from 37.4% to 38.8% calcium depending on what bone of the skeleton is analyzed and from what species the bone is collected. (Table 1).

Table 1. Percent calcium of ash bone reported in literature.

Species	Number of Samples	Bone Specimen	% Ca in Bone Ash	Reference
Deer	Not given	Hock Joint	38.7	Lindberg[1]
Animals*	Not given	Not given	38.8 $\pm$ .50**	Holtzman[2]
White-tailed deer	18	Mandible	37.4 $\pm$ .16***	Shultz[3]
White-tailed deer	18	Antler	36.2 $\pm$ .28***	Shultz[3]

*Species not given. **Explanation of plus or minus value not reported in original reference.
***One standard error of the mean.

The primary objective of this study was to determine the calcium content in hock joints of various wildlife species and to determine if these values were species or geographically dependent or both. This information is necessary to aid in determining whether our ^{90}Sr values are a reflection of true fallout, species differences, analytical error, or calcium deficiency and to enable comparison of our findings with those reported in the literature as strontium units.

Note: pCi = pico curie.

MATERIALS AND METHODS

During the fall and winter hunting season of 1965, 120 wildlife ruminant hock joints were received from various conservation agencies throughout the United States as well as from interested individuals. The samples were obtained from the Northeastern United States, Oklahoma, Montana, Nebraska, Kentucky, Nevada, Utah, Minnesota, Oregon, Idaho, Kansas, Arizona, and California.

The samples were taken from the right or left leg of the animal by cutting the bone at a point four inches above and below the hock joint. All skin and attached muscular tissues were removed prior to analysis.

Calcium analysis was performed by the United States Public Health Service, Southwestern Radiological Health Laboratory, Las Vegas, Nevada. The technique developed by Johns[4] is included in detail because it is a modification of the classic calcium oxalate technique[5].

The bone sample is ashed at 500°C in an electric furnace. A 1.00 gram portion of the ground bone ash is then dissolved in 6N HCl. Fifty ml of saturated oxalic acid solution is added and the pH adjusted to 3.0 with 6N ammonium hydroxide to precipitate calcium oxalate. The solution is allowed to stand for 12-24 hours to permit the precipitate to settle. The supernatant is removed by filtration and the calcium oxalate precipitate washed several times with 0.5% ammonium oxalate. The precipitate and filter paper are placed in a muffle furnace and heated to 500°C for conversion of calcium oxalate to the calcium oxide. The ash is then dissolved in dilute nitric acid and diluted to 250ml with deionized water. A 5ml subsample is diluted to approximately 50ml, to which 5ml 6N potassium hydroxide is added, and the solution allowed

to stand for at least 3 minutes. One-tenth gm (Cal-Red Indicator Dilute*) is then added, and the solution titrated with 0.1M ethylenediaminetetraacetate disodium salt (EDTA Salt). The EDTA Salt solution is standardized against a $CaCO_3$ primary standard using the same buffering and titration procedure.

Percent calcium in bone ash is calculated according to the following formula:

$$\% \text{ calcium in bone ash} = \frac{\text{ml EDTA Salt} \times \underline{M} \text{ EDTA Salt} \times 40.1 \times 100}{\text{Sample weight in grams} \times 1000}$$

*Registered Trade Mark, U. S. Patent Office, Washington, D. C.

RESULTS AND DISCUSSION

The average calcium content of six species from 18 different geographic areas are arranged in descending order (Table 2). The highest and lowest mean value, a difference of 2.2%, consisted of only one sample each. If these two are disregarded, the difference between the buffalo(Montana) and the mule deer(Southeast Oregon) is 0.9%. A statistical test of the difference between mean values failed to show a significant difference among species from various locations.

Table 2. Percent calcium in bone ash by species and geographical location.

Species	Location	$\overline{X}$% Ca in Bone Ash	Range	No. of Samples
Buffalo*	Oklahoma	38.8		1
Buffalo	Montana	38.1	37.5-38.8	6
Elk	Montana	38.1	37.7-38.4	4
White-tailed deer	Nebraska	38.0	37.3-39.0	6
White-tailed deer	Kentucky	38.0	37.7-38.2	4
Elk	Oklahoma	38.0	36.9-39.3	6
Antelope	Montana	38.0	37.7-38.4	5
Elk*	Southern Nevada	37.8	37.3-38.2	2
White-tailed deer	New England states	37.7	37.3-38.4	4
Mule deer	Utah	37.7	36.9-38.1	6
Mule deer	Nebraska	37.6	36.7-38.1	3
Mule deer	Southern Nevada	37.5	36.4-38.4	12
Mule deer	Minnesota	37.5	36.9-37.9	6
Elk*	Northeastern Nevada	37.5	37.3-37.7	2
Antelope	Northern California	37.5	37.1-38.4	4
Desert bighorn sheep	Arizona	37.4	36.6-37.9	5
Mule deer	Idaho	37.4	36.9-37.7	6
Mule deer	New Mexico	37.4	36.9-38.2	6
Mule deer	Southern California	37.4	36.9-38.4	4
Mule deer	Northern Nevada	37.4	36.7-38.0	8
Mule deer	Northwest Oregon	37.4	35.8-37.9	6
Mule deer	Southeast Oregon	37.2	36.7-37.7	5
Desert bighorn sheep*	Southern Nevada	37.2	36.2-38.0	2
Mule deer*	Northeast California	36.6		1

*Inadequate number of samples to be included in statistical analysis.

The percent calcium in bone ash in various species regardless of location are listed in Table 3. The buffalo, white-tailed deer and elk have the highest values while the antelope, mule deer and desert bighorn sheep have the lowest. It is interesting that, in general, the buffalo, white-tailed deer and elk are animals from higher rainfall regions. Generally it is assumed that areas of calcium deficiency occur in areas of high rainfall where the calcium carbonates have been leached from the soil. Our data show that the calcium content in the hock joints of animals browsing in these areas do not have lower values than those animals grazing in areas of low rainfall.

Table 3. Percent calcium/bone ash in various species regardless of location.

Species	$\bar{X}$ % Ca in Bone Ash	s*	No. of Samples
Desert bighorn sheep	37.3	0.536	13
Mule deer	37.4	0.529	63
Antelope	37.8	0.488	9
Elk	37.8	0.441	14
White-tailed deer	37.9	0.476	14
Buffalo	38.2	0.453	7

*standard deviation

The average calcium content in the mule deer hock joints is identical for both male and females (Table 4). Perhaps this is a reflection of the calcium drain on the body of the male mule deer during antler formation, which under certain conditions may be equal to the calcium drain of the lactating female mule deer. Females show a greater variance (Table 4) than do the males probably because both lactating and non-lactating does were sampled.

Table 4. Percent calcium in bone ash found in hock joints of mule deer by sex.

Sex	$\bar{X}$ % Ca in Bone Ash	Range	s*	No. of Samples
Male	37.4	36.7-38.4	0.504	27
Female	37.4	35.8-38.1	0.579	31
			Total	58**

* standard deviation
**total hock joints that were identified by sex.

The calcium content in bone ash of mule deer hock joints is arranged by age class in Table 5. Examination of the mean values indicates an apparent increase in calcium content from the 0.5 year age class (fawns) to the 3.5 year age class (maturity), then a decrease in animals above that age class. However, it was not possible to verify the increase statistically due to the large variation of % Ca values within each age group (as indicated by the ranges shown). The factors contributing to this variation, in order of importance, are the grouping of animals into half-year age groups, the natural animal to animal variation and the error of the method of chemical analysis.

Table 5. Percent calcium in bone ash found in hock joints of mule deer by age class.

Age	$\bar{X}$ % Ca in Bone Ash	Range	No. of Samples
0.5 years	37.0	35.8-37.9	7
1.5 years	37.2	36.4-38.1	12
2.5 years	37.6	36.7-38.2	7
3.5 years	37.7	36.9-38.4	7
4.5 years*	37.4	36.6-38.1	22
			Total 55**

* 4.5 years or greater.
**total hock joints that were identified by age.

It is apparent that by increasing the number of samples of certain species from several locations, little would be gained as no significant differences were noted in calcium content among species, location or sexes where there was an adequate sample size for statistical analysis. The data obtained during this study will serve as background as well as an aid in comparing our data with that of others.

SUMMARY

The average calcium content of hock joints in six species of wildlife ruminants from 18 different areas was 37.6% of bone ash. Differences between species, regardless of geographic locations, were small when present.

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