THE RELATIONSHIP OF SODIUM AND POTASSIUM TO CARBONATE IN BONE*

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Approximately a third of the total body sodium content of man, dogs, and rats is present in the inorganic portion of the skeleton (1-3). Although the magnitude of this fraction and its variability in acidosis and sodium depletion have been defined by direct analysis and by the use of isotopes, the precise position which sodium occupies in bone salt remains obscure. Harrison (4), pointing out the association of sodium with the carbonate of naturally occurring apatites, suggested that it might be present as carbonate. Neuman et al. (5), studying fluoride uptake by bone salt in vitro, concluded that their data could best be interpreted by assuming that some of the carbonate present shared a monovalent bond with calcium, and that the second carbonate valence was occupied by sodium, i.e. Ca—O—CO$_2$—Na.

In at least two instances substantial changes occur in the sodium and carbonate content of bone, namely, during growth and in acidosis. It has long been known that bone carbonate shows a gradual but significant increase with maturation (6), and increments of bone sodium with age have been more recently demonstrated (7). Irving and Chute (8) found decreased bone carbonate in rats undergoing oral HCl loads; acute and chronic sodium deprivation and acidosis have resulted in significant decrements of bone sodium. It therefore appeared desirable to measure both sodium and carbonate simultaneously in normal bone of varying age and in the bone of acidic animals in order to determine whether the changes observed might suggest a relationship helpful in determining the position of bone sodium. Since previous work (7) has shown that bone also contains significant amounts of potassium, this cation was included in the present study.

EXPERIMENTAL.

The method for bone analysis has been presented in detail elsewhere (7). Sodium, potassium, calcium, chloride, and carbonate were measured in the

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bones of normal rats of varying age and in rats depleted of sodium and made acidotic by intraperitoneal dialysis against ammonium chloride. Each bone sample comprised all of the long bones of an individual rat. The epiphyses were discarded and the shafts dried for 48 hours at 105° and ground in a mortar. Aliquots of the dry powder were used for subsequent analyses. Serum sodium, chloride, and water concentrations were determined at the time of sacrifice.

Chemical Methods

Bone

Sodium and potassium were determined by internal standard flame photometry (9). Samples of 100 to 200 mg. of powder were ashed in platinum crucibles at 550° and the ash dissolved in 10 per cent nitric acid. Calcium was precipitated as the oxalate after raising the pH to 8 to 9 (brom thymol blue) with concentrated NH₄OH. Since sodium is known to coprecipitate with calcium oxalate under these conditions (10), three precipitations were carried out, the supernatant fluid being removed in each instance and the precipitate redissolved. An appropriate amount of lithium was added to the pooled supernatant fluids before flame photometry was performed.

Calcium—The precipitate formed during the sodium and potassium analyses was washed with 2 per cent NH₄OH and dissolved in 12 N H₂SO₄. The resulting solution was titrated with KMnO₄.

Carbonate—An adaptation of the micro diffusion method of Conway was used. Approximately 20 mg. of dry bone powder were weighed into the outer well of a Conway diffusion chamber. 2.00 ml. of 0.025 N Ba(OH)₂ were placed in the center well. 2 ml. of approximately 12 N H₂SO₄ were added to the outer well; the chamber was then sealed and tilted gently. The unit was placed in a 65° oven for 30 minutes, after which titration of the center well was carried out with 0.100 N HCl delivered from a micro burette.

<table>
<thead>
<tr>
<th>Titrimetric</th>
<th>Volumetric</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.028</td>
<td></td>
</tr>
<tr>
<td>1.016</td>
<td></td>
</tr>
<tr>
<td>1.043</td>
<td>1.044</td>
</tr>
<tr>
<td>0.992</td>
<td>0.938</td>
</tr>
</tbody>
</table>

Average . . . . 1.019 0.991
Reagent blanks were run with each set of samples and carbonate was calculated as the difference between blank and sample titration figures. This procedure was checked against the volumetric method of Danielson and Hastings (11) and was found to be in close agreement (Table I).

**Serum**

Sodium was measured by flame photometry (9). Chloride was determined by the iodometric method of Van Slyke and Hiller (12). Serum water was ascertained by the use of micro pycnometers (13).

### Table II

*Data for Normal Rats of Varying Age and for Acidotic Adult Rats*

<table>
<thead>
<tr>
<th>Group</th>
<th>Fresh bone, m.eq. per kilo</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>Juvenile (8 rats)</td>
<td>108</td>
<td>34†</td>
</tr>
<tr>
<td></td>
<td>(8.4)*</td>
<td></td>
</tr>
<tr>
<td>Normal adult (14 rats)</td>
<td>173</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>(5.7)</td>
<td>(5.0)</td>
</tr>
<tr>
<td>Acidotic adult (13 rats)</td>
<td>119</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(6.7)</td>
<td>(1.9)</td>
</tr>
</tbody>
</table>

* Standard error.
† Data from four rats only.

### Calculations

Chloride was assumed to be extracellular. On this basis, bone water was divided into extracellular and intracellular compartments by using serum chloride concentrations (Donnan correction) and total bone chloride values. Total bone sodium was corrected by subtraction of the calculated extracellular water sodium, and the remainder was taken to represent "extra bone sodium" as originally defined by Harrison, Darrow and Yannet (1). A similar correction was made for intracellular potassium, which was estimated by arbitrarily assigning a value of 150 m.eq. per liter for cell water potassium concentration (13). The resulting corrected cation values were expressed as milliequivalents per kilo of fresh wet bone. Carbonate was not corrected, since the amount present in bone extracellular water (8 to 10 m.eq. per kilo of bone) is an insignificant fraction of the total bone carbonate content. Calcium was expressed as milliequivalents per kilo of wet bone. Since the degree of mineralization of bone varies widely not only with age but also with respect to the skeletal area sampled, it was felt that the best expression...
of relationship between sodium, potassium, and carbonate concentrations in bone salt could be obtained by using calcium as a standard of reference. Ratios of carbonate, sodium, and potassium to calcium were therefore calculated.

Results

Table II shows the values for each constituent in milliequivalents per kilo of bone and the ratios obtained. As in previous studies, carbonate and sodium increased with age, both in absolute amounts and in relation to calcium. The change in the ratio $\text{CO}_3^+:\text{Na} + \text{K}$ (4.81 to 5.58) was +14 per cent. In the acidotic adult rats, $\text{Na} + \text{K}:\text{Ca}$ decreased to 0.0165 from the normal adult value of 0.0246, a change of 0.008 m.eq. per m.eq. of Ca, or -33 per cent ($p = <0.01$). $\text{CO}_3^+:\text{Ca}$ decreased from 0.137 in normal to 0.121 in acidic adult rats, a decrement of 0.016 m.eq. per m.eq. of Ca, or -12 per cent ($p = <0.01$). The ratio of $\text{CO}_3^-$ lost to $\text{Na} + \text{K}$ lost is therefore 0.016:0.008, or 2:1 in milliequivalents (1:1 in millimoles). The ratio $\text{CO}_3^+:\text{Na} + \text{K}$ increased to 7.34 in the adult acidotic group from its control value of 5.58 in the normal adult group.

Discussion

The finding of equimolar losses of Na + K and CO$_3^-$ in acidosis is in accord with the hypothesis of Neuman et al. that sodium may be present as Ca–O–CO$_2$–Na, since liberation of Na and CO$_3^-$ would then proceed as follows:

$$\text{Ca–O–CO}_2^–\text{Na} + \text{HA}^- \rightarrow \text{CaA}^- + \text{NaHCO}_3$$

In the normal rats, $\text{CO}_3^+:\text{Na} + \text{K}$ is 5.58/1 or 100/18. According to the hypothesis of Neuman et al. (5), each 100 m.eq. of $\text{CO}_3^-$ (50 mM) would therefore be distributed as follows: 32 m.eq. of CaCO$_3$ + 18 mM of Ca–O–CO$_2$–Na (K) ($\text{CO}_3 = 100$ m.eq.; Na + K = 18 m.eq.).

Replacement of 32 per cent of the Na + K as measured in the present acidotic group would have the following result:

$$\begin{align*}
(2) & \quad 32\text{CaCO}_3 + 18\text{Ca–O–CO}_2^–\text{Na} (\text{K}) + 6\text{HA}^- \rightarrow \\
& \quad 32\text{CaCO}_3 + 12\text{Ca–O–CO}_2^–\text{Na} (\text{K}) + 6\text{CaA}^- + 6\text{Na} (\text{K})\text{HCO}_3
\end{align*}$$

(CO$_3$ = 88 m.eq.; Na + K = 12 m.eq.)

In this case, the ratio CO$_3^+:\text{Na} + \text{K}$ after acidosis would be 88/12 = 7.34. This ratio, as determined in the present study, was 7.34 (Table II). The decrease in CO$_3^-$ commanded by the mobilization of 32 per cent of Na + K according to Equation 2 would be 12 per cent (100 to 88 m.eq.). The decrease measured was 12 per cent (0.137 to 0.121, Table II). The close

$^1 A^- = \text{anion}.$
agreement between the results obtained and those predictable from the formula \( \text{Ca} - \text{O} - \text{CO}_2 - \text{Na} \) suggests that all of the \( \text{Na} + \text{K} \) present in bone could be accounted for by such a combination.

**SUMMARY**

1. The results of analysis of rat bone for \( \text{Na}, \text{K}, \text{Ca}, \) and \( \text{CO}_3 \) in normal animals of varying age and in acidotic animals are presented.
2. The ratios \( \text{CO}_3 : \text{Ca}, \text{CO}_2 : \text{Na} + \text{K}, \) and \( \text{Na} + \text{K} : \text{Ca} \) are indicated.
3. The changes observed following acidosis suggest that all of the \( \text{Na} \) and \( \text{K} \) in bone is present as \( \text{Ca} - \text{O} - \text{CO}_2 - \text{Na} \) (K).

It is a pleasure to acknowledge the assistance of Dr. William M. Wallace, whose suggestions initiated this work and whose guidance made possible its conclusion.

**BIBLIOGRAPHY**

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