Effect of Crystal Growth on the Comparative Fixation of Sr$^{89}$ and Ca$^{45}$ by Calcified Tissues*

R. C. Likins, A. S. Posner, Boris Paretzkin, and Ann P. Frost

From the National Institute of Dental Research, National Institutes of Health, United States Public Health Service, Bethesda 14, Maryland, and the American Dental Association Research Division, National Bureau of Standards, Washington 25, D. C.

(Received for publication, March 22, 1961)

In a previous paper (1) it was shown that discrimination against strontium as compared to calcium, in the formation of synthetic hydroxyapatite, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$, was greater in large, more slowly grown crystals than in small, more rapidly precipitated crystals. The exclusion of strontium as compared to calcium in the hydroxyapatite crystals increases as the preparation conditions approach equilibrium. Since the mineral portions of some calcified tissues differ in crystal size (2, 3), an experiment was designed to test whether crystal size and strain as related to rate of crystal growth affects the relative incorporation of Sr$^{89}$ and Ca$^{45}$ in vivo.

EXPERIMENTAL PROCEDURE

One hundred female Sprague Dawley rats, age 25 days, were divided equally into ten groups and treated by intraperitoneal injection of 0.5 ml of a solution containing trace amounts of Ca$^{45}$ and carrier-free Sr$^{89}$, plus 0.05 mg of calcium. At the end of 1 hour the rats were killed and the lower incisor and third molar teeth were extracted. One tibia was removed from each of the specimens. For the purposes of line broadening the 002 maximum was regarded as a pure instrument broadening by the method of Warren (8) and the intensity against angle 2 $\theta$ after making a correction for background. The width at half maximum was then corrected for crystal size and perfection, these results argue that the rate of crystal growth affects the relative inclusion of Sr and Ca in mineralized tissues. This relationship is better seen in Fig. 1 where the Sr$^{89}$:Ca$^{45}$ ratios in the different tissues are plotted against the x-ray line broadening values.

It is recognized that the radioisotope uptake is the result of both incorporation in newly formed crystals and surface exchange on existing crystals. Although the exact contribution of each of these processes cannot be stated with certainty there can be little doubt that new crystal formation and not surface exchange is primarily responsible for the differential uptake of Sr and Ca. This conclusion is apparent from the lack of correlation between radioisotope uptake and specific surface as obtained from the x-ray diffraction results. In addition, were

1 Ignoring strain, an estimate of the average crystal size, in the c-axis direction, for each sample can be obtained from the $\beta$ values as follows: Size in microns = $\frac{57.3 \times 10^{-4}}{\beta(2 \theta) \cos \theta}$, where $\lambda = 1.54$ A and
surface exchange the major factor in determining the discrimination against Sr as compared to Ca, one would expect the samples with the highest surface areas (i.e., largest $\beta$ values) to have the lowest $\text{Sr}^{40}:\text{Ca}^{45}$ ratio, and vice versa. Table I, again, shows that this is not the case.

These results are in accord with previous studies in which the final compositions of a series of synthetic hydroxyapatites were found to be related to the degree of crystal perfection (1). Synthetic hydroxyapatite crystals which gave essentially the same degree of x-ray diffraction line broadening as bone also showed little or no evidence of discrimination between Sr and Ca. Those preparations giving x-ray diffraction lines sharper than $\beta = 26.0^\circ 2\theta$. Since it is reasonable to assume that apatite crystals of the hard tissues studied are similar in shape, their specific surfaces are proportional to average crystal size. For example, the specific surface (in $\text{m}^2\text{per g}$) values for a cubic shape are: tibia end, 121.8; tibia shaft, 102.9; incisor dentin, 91.6; molar dentin, 73.4; and molar enamel, 41.0.

those of bone showed a discrimination against Sr during precipitation which increased with crystal perfection.

On the basis of x-ray line broadening alone one could expect a higher $\text{Sr}^{40}:\text{Ca}^{45}$ ratio in the tibia ends. However, factors other than crystal growth rate may affect the comparative retention of these ions. The exchange release of Sr relative to Ca from the ends of the tibia has been shown to be greater than from the shaft (4), probably as a result of their different metabolic activity. Indeed, the 1-hour experimental period was chosen to minimize the contribution of this effect on the $\text{Sr}^{40}:\text{Ca}^{45}$ ratios. However, differences due to metabolic activity in exchangeable loss of Sr and Ca cannot account for the comparative discrimination against Sr in dentin and enamel. This conclusion is apparent from the fact that the lowest $\text{Sr}^{40}:\text{Ca}^{45}$ ratios were found in dentin and enamel where metabolic activity was minimal as shown by their radio-calcium uptake (Table I). In addition, a threefold difference in radiocalcium uptake between the ends

---

**Table I**

Comparison of x-ray diffraction line broadening and uptake of $\text{Sr}^{40}$ and $\text{Ca}^{45}$ by rat enamel, dentin, and bone

<table>
<thead>
<tr>
<th>Sample</th>
<th>$% \text{ of dose/mg of ash}^b$</th>
<th>$\text{Sr}^{40}:\text{Ca}^{45}$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ends of tibia</td>
<td>$0.156 \pm 0.0089$</td>
<td>$0.164 \pm 0.0087$</td>
<td>$0.95 \pm 0.009$</td>
</tr>
<tr>
<td>Shaft of tibia</td>
<td>$0.053 \pm 0.0021$</td>
<td>$0.053 \pm 0.0022$</td>
<td>$1.00 \pm 0.018$</td>
</tr>
<tr>
<td>Incisor dentin*</td>
<td>$0.062 \pm 0.0016$</td>
<td>$0.067 \pm 0.0017$</td>
<td>$0.93 \pm 0.010$</td>
</tr>
<tr>
<td>Molar dentin*</td>
<td>$0.050 \pm 0.0039$</td>
<td>$0.060 \pm 0.0051$</td>
<td>$0.84 \pm 0.012$</td>
</tr>
<tr>
<td>Molar enamel</td>
<td>$0.034 \pm 0.0070$</td>
<td>$0.045 \pm 0.0072$</td>
<td>$0.76 \pm 0.022$</td>
</tr>
<tr>
<td>Incisor enamel</td>
<td>$0.022 \pm 0.0000$</td>
<td>$0.029 \pm 0.0083$</td>
<td>$0.70 \pm 0.025$</td>
</tr>
</tbody>
</table>

---

$^a$ Mean ± standard error for each sample calculated from the individual ratios.

$^b$ The width of the 002 x-ray diffraction reflection at half-maximum corrected for instrument broadening, expressed in degrees $2\theta$. Error estimated to be ±5%.

$^c$ Mean ± standard error for one tibia from each of 20 rats; dentin and enamel values based on ten pools of ten rats each; weight of each enamel ash calculated from the P content (see text).

$^d$ Proximal third of lower incisor.

$^e$ Third molar.

$^f$ Insufficient sample for x-ray analysis.

---

**Fig. 1.** A plot of the $\text{Sr}^{40}:\text{Ca}^{45}$ ratios against the corresponding values for the width at half-maximum, $\beta$, of the 002 X-ray diffraction peaks of a series of calcified tissues. In order of ascending $\beta$ value the points correspond to: molar enamel, molar dentin, incisor dentin, tibia shaft, and tibia end.
and shaft of the tibia can be related only to a maximal change of 5% of the Sr$^{99}:\text{Ca}^{44}$ ratio. It would appear, then, that crystal perfection is principally responsible for the comparative discrimination between Sr and Ca observed in all the tissues.

SUMMARY

The deposition of injected Sr$^{99}$ and Ca$^{44}$ was compared in several different calcified tissues of the rat. The results showed that discrimination against strontium relative to calcium increased with an increase in crystallinity as determined by x-ray diffraction line broadening analysis. A range in size or perfection in the crystal texture, or both, of different calcified tissues was demonstrated.

REFERENCES

Effect of Crystal Growth on the Comparative Fixation of Sr$^{89}$ and Ca$^{45}$ by Calcified Tissues

R. C. Likins, A. S. Posner, Boris Paretzkin and Ann P. Frost