CALCIFICATION OF TEETH

III. X-RAY DIFFRACTION PATTERNS IN RELATION TO CHANGES IN COMPOSITION*

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PLATES 1 AND 2

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The purpose of the present investigation was to study the x-ray diffraction patterns of teeth in relation to changes in composition induced by diet.

Previous studies showed that the composition of the upper incisors of the albino rat can be influenced by the Ca:P ratio of the diet (1). In a typical experiment the mean values of the PO₄:2CO₃ ratios for enamel (used as an index of \( n \) in the apatite formula \([\text{Ca}_3(\text{PO}_4)_x(\text{CaCO}_3)]\)) varied from 3.71 on the high Ca:P diet to 7.72 for the low Ca:P diet, and for the corresponding dentins the mean values of these ratios varied from 5.47 to 9.31. There was no significant influence of the diet on the Ca:PO₄ ratios of the teeth. The residual Ca:PO₄ ratios of the enamel were higher and those of the dentin lower than the theoretical 1.50 in the apatite formula \([\text{Ca}_3(\text{PO}_4)_x(\text{CaCO}_3)]\).

In the present study, the compositions of the enamels and dentins obtained (Table I) are in essential agreement with results obtained previously; namely, for rats fed the high phosphorus-low calcium diet (Diet C), the PO₄:2CO₃ ratios of both enamel and dentin are higher than the corresponding ratios for rats fed the low phosphate-high calcium diet (Diet B) (1). The Ca:PO₄ ratios of all enamels are higher than for the corresponding dentins.

For purposes of comparison, enamel and dentin of young rats (of the same age as the experimental animals), raised on our stock diet (2), were analyzed (Table I). The mean value of the Ca:PO₄ ratio of the enamel in Experiment 1 is 1.47 and in Experiment 2, 1.53. This is distinctly lower than any ratio we observed for the enamel of rats on the experimental diets, both in the present study and in the previous investigation in which the relation of the composition of blood, teeth, and diet was established.

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(1). The Ca:PO$_4$ ratio for dentin is 1.20 for Experiment 1 and 1.21 for Experiment 2. These are also lower than the values obtained for the dentin of animals on the experimental diets (1).

Results show that prior to ignition the predominant x-ray diffraction pattern is that of apatite in all cases, the lines being more distinct in the enamel than in the dentin (Table I; Figs. 1 and 2). This distinctness of lines has been observed previously and is due to the lower percentage of organic matter in the enamel (1, 3–8). The fact that the predominant

<table>
<thead>
<tr>
<th>Table I</th>
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</table>

| X-Ray Diffraction Patterns of Teeth of Various Compositions |

<table>
<thead>
<tr>
<th>Diet</th>
<th>Specimen</th>
<th>PO$_4$:Ca$_2$</th>
<th>PO$_4$:Ca$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>molar ratio</td>
<td>molar ratio</td>
</tr>
<tr>
<td>B</td>
<td>Enamel</td>
<td>4.01</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Dentin</td>
<td>4.55</td>
<td>1.35</td>
</tr>
<tr>
<td>C</td>
<td>Enamel</td>
<td>5.40</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>Dentin</td>
<td>7.50</td>
<td>1.39</td>
</tr>
<tr>
<td>Stock. Ex-</td>
<td>Enamel</td>
<td>5.21</td>
<td>1.47</td>
</tr>
<tr>
<td>peri-</td>
<td>Dentin</td>
<td>6.15</td>
<td>1.20</td>
</tr>
<tr>
<td>ment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Enamel</td>
<td>8.34</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Dentin</td>
<td>12.01</td>
<td>1.21</td>
</tr>
</tbody>
</table>

**Composition of Diets**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Ca:P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>molar ratio</td>
</tr>
<tr>
<td>B</td>
<td>1.20</td>
<td>0.203</td>
<td>4.60</td>
</tr>
<tr>
<td>C</td>
<td>0.028</td>
<td>0.841</td>
<td>0.026</td>
</tr>
<tr>
<td>Stock. Experiment 1</td>
<td>0.369</td>
<td>0.398</td>
<td>0.72</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>0.282</td>
<td>0.380</td>
<td>0.58</td>
</tr>
</tbody>
</table>

The persistence of apatite as the main solid structure of teeth has been shown before (9, 10). This was true even when considerable changes in composition took place under the influence of sodium fluoride (11). The situation seems to be analogous to that of bone (12) in which, in spite of wide variations in composition, all x-ray evidence to date (9, 10) indicates that bone salts are present in an apatite structure.
There are a number of factors that may account for the predominance of the apatite structure in spite of wide variations in composition. Apatite may be considered a continuous series of solid solutions in which the composition of the solid reflects the composition of the liquid with which it is in equilibrium (9). Ionic exchange between the liquid and solid phase has been indicated (13) and may be a cause of further changes in composition. Adsorption is an added factor that must be considered in accounting for the variation in the composition of tooth salts (3, 14, 15). The experiments of Logan and Taylor (16) with inorganic models indicate that the calcium carbonate portion of the tooth may be adsorbed or at least be present in higher concentrations on the surface than in the interior of the tooth. Walden and Cohen (17) have shown that the constituents adsorbed on the surfaces of crystals cannot be detected by means of x-ray powder diffraction.

After ignition, the dentin of all groups and the enamel of the group on the stock diet showed \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \), while the enamel of animals on both the low calcium-high phosphorus diet and the high calcium-low phosphorus diet continued to give apatite patterns (Table I; Figs. 1 and 2). The occurrence of \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \) on ignition appears to be a function of the Ca:PO\(_4\) ratios. Hodge and coworkers (18), working with calcium phosphate precipitates of varying composition, showed that when the Ca:PO\(_4\) ratio was less than 1.50 the pattern on ignition was always, that of \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \). When the ratio was between 1.50 and 1.59, the pattern was predominantly that of \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \), and when above 1.59, the ignited specimen continued to give apatite patterns. If one reexamines the data of Dallemagne and Brasseur (19), it is seen that bone, which after treatment with KOH in glycerol had a Ca:PO\(_4\) ratio of 1.73,\(^1\) on ignition gave an apatite pattern. After treatment with hydrochloric acid (which removed the carbonate) and washing with water, the residue of the original bone had a Ca:PO\(_4\) ratio of 1.50 and on ignition gave a \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \) pattern. Similarly pure \( \text{Ca}_3(\text{PO}_4)_2 \), which they prepared with an actual ratio equal to the theoretical of 1.50, gave \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \) on ignition. When this substance was hydrolyzed with alkali, the resulting compound had a ratio of 1.67 and gave the apatite pattern on ignition. When this precipitate was treated with hydrochloric acid so that the residue once more had a ratio of 1.50, and then ignited, the \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \) pattern was again obtained. Hirschman and his coworkers (20) showed that when commercial apatite with a Ca:PO\(_4\) ratio of 1.67 was ignited the apatite pattern was obtained. When this was mixed with CaHPO\(_4\) so that the resulting mixture had a Ca:PO\(_4\) ratio of 1.51 and then ignited, the \( \beta \cdot \text{Ca}_3(\text{PO}_4)_2 \) pattern predominated. Thus it appears that the Ca:PO\(_4\) ratio of such compounds is the predominant factor

\(^1\) Weight ratios given by the authors are converted to molar ratios.
that decides whether on ignition one obtains a $\beta$-Ca$_3$(PO$_4$)$_2$ or an apatite pattern. The pattern of the ignited product may therefore be used as an indirect index of the Ca:PO$_4$ ratio.

In connection with the analyses obtained for the rats on the stock diet of Bills et al. (2), it may be worth referring to the discussion in our earlier paper (1), where it was postulated that, while the PO$_4$:CO$_3$ ratio of the blood serum will have an influence on the composition of the tooth on a given diet, the components of the diet, other than calcium and phosphorus, are likely to influence the type of relationship that will be found between blood and teeth. On comparing all results in Table I it is seen that although in Experiment 1, on the stock diet, there is a slight deviation, the PO$_4$:2CO$_3$ ratios of enamel and dentin change in the same direction as do serum PO$_4$:CO$_3$ ratios.

Both enamel and dentin of the group on the stock diet have distinctly lower Ca:PO$_4$ ratios than those obtained for the corresponding enamel and dentin in any of the experimental groups (Table I) (1). These differences cannot be due to the Ca:P ratio of the stock diet, since this ratio was covered by the range of ratios in the experimental Diets B, C, and D (1). They might be due to other differences between the experimental and the stock diets. The main ingredients of the Bills stock diet (dried milk, crude casein, whole yellow corn, alfalfa, and cottonseed meal) are different from those of the experimental diets (degerminated yellow corn-meal, wheat gluten, and brewers' yeast) (1, 2). Thus differences in Ca:PO$_4$ ratios for the enamel and dentin of the rats on the stock diet and those on the experimental diets (Table I) may be accounted for by differences between the composition of the stock diet and experimental diets other than calcium and phosphorus.

The difference in the PO$_4$:2CO$_3$ ratios of the enamel and dentin of the two groups on the stock diet raises a similar question. In the experimental diets the same batch of ingredients was used throughout a given experiment. Only the calcium and phosphate content was changed by the addition of salts. For the stock diet, however, different batches of the ingredients were used in the two experiments carried out 6 months apart. Each one of the main ingredients can undergo variation from batch to batch and the composition of two stock diets may therefore be different.

It must be added that the possibility that seasonal variations cause changes in the composition of teeth cannot be excluded. Blincoe et al. (21) have shown that the calcium and inorganic phosphorus of serum are influenced by environmental temperature.

A systematic study of the influence of not only the Ca:P ratios of the diet but also that of other components (vitamins, proteins, fats, carbohydrates, and trace minerals) may throw further light on the subject.
EXPERIMENTAL

Young rats, of an original Wistar strain, 21 to 23 days of age, kept on the Bills stock diet (2), were weaned and placed on one of the experimental diets. Diets B and C were essentially the same as that described before (1) except that the basal diet (used in making up the experimental diets) had 0.20 per cent phosphorus compared to 0.118 per cent in the previous basal diet (1). The stock group was fed the Bills diet (2). The calcium and phosphorus content of the diets is given in Table I.

Forty-eight animals were placed on Diet B, forty-nine on Diet C, and a total of 58 on the stock diet (twenty-two in Experiment 1, and, 6 months later, thirty-six in Experiment 2). The animals were sacrificed at the end of 45 days and the blood and teeth from the rats in each group were pooled, sampled, and analyzed as previously described (1). Mean values for duplicate analyses of replicate samples are reported in Table I.

Powder diffraction patterns were made on both ignited and unignited specimens, ground to pass a 250 mesh sieve, by means of a 57.3 mm. Debye-Scherrer camera (22). The samples were mounted in thin walled capillaries and nickel-filtered copper radiation was used. Exposures were of 3½ hours duration. Ignition was conducted in platinum crucibles for 2½ hours at 900°.

SUMMARY

1. X-ray powder diffraction patterns of enamel and dentin of widely varying composition gave apatite as the dominant pattern. The teeth used were the upper incisors of albino rats of the Wistar strain.

2. After ignition at 900°, the predominant pattern was apatite when the Ca:PO₄ ratio was more than 1.60 and was β-Ca₅(PO₄)₂ when the ratio was 1.53 or less. These findings are in harmony with those obtained by other investigators using inorganic calcium phosphate.

3. The pattern obtained after ignition at 900° appears to be an index of the Ca:PO₄ ratio.

4. It was again possible to show a relationship between PO₄:2CO₃ ratios of enamel and dentin and PO₄:CO₃ ratios of serum.

5. The differences in Ca:PO₄ ratios of the enamel and dentin obtained from animals on experimental diets and the animals on the stock diet suggest the importance of examining the influence of not only the Ca:P ratios of the diets but also that of the other components (vitamins, proteins, fats, carbohydrates, and trace minerals).

BIBLIOGRAPHY


**EXPLANATION OF PLATES**

**PLATE 1**

Fig. 1. X-ray diffraction patterns of teeth from rats on Diets B and C. A, Diet B; C, Diet C; D, dentin; E, enamel; I, ignited at 900°.

**PLATE 2**

Fig. 2. X-ray diffraction patterns of teeth from rats on stock diet. R, rat; E, enamel; D, dentin; I, ignited at 900°.
(Sobel, Hanok, Kirchner, and Fankuchen, Calcification of teeth)
CALCIFICATION OF TEETH: III.
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