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What is This?
Dental Caries in Albino Rats Fed Diets Containing Different Amounts of Calcium and Phosphorus with the Same Ca/P Ratio

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In earlier experiments it was found that the cariogenicity of a high-sucrose diet was reduced when the Ca/P ratio of the diet was changed by the addition of phosphate to the diet without altering the calcium content.1 Later it was shown that the diet could likewise be made less cariogenic when the Ca/P ratio was changed by adding calcium to the diet and keeping the phosphorus at a constant level.2 In the first experiment a progressive decrease in the number of carious lesions and the caries score occurred up to a certain point with a progressive decrease in the Ca/P ratio, whereas in the second experiment this relationship was reversed: a decrease in caries conduciveness of the diet was associated with an increase in the Ca/P ratio. These findings were taken to indicate that the changes induced in the cariogenic properties of our high-sucrose diet, by altering the calcium and phosphorus content, were due to differences in the actual calcium and phosphorus content of the diets and not to differences in their Ca/P ratios. The evidence for this deduction, however, was admittedly indirect, inasmuch as in each experiment the Ca/P ratio was altered when either calcium or phosphorus was added to the diet. In the present study our conclusion drawn from our previous experiments was submitted to a direct test by increasing the calcium and phosphorus of the diet without changing the Ca/P ratio.

EXPERIMENTAL METHODS

Sixty albino rats of the Emory-Wistar strain were selected at weaning in groups of four from the same litter and were sialoadenectomized. The fifteen quadruplicate groups from time of weaning were fed four diets that differed from each other only in their calcium and phosphorus content. The basic high-sucrose diet employed in these experiments is described elsewhere.3 Diet No. 1 contained 0.25 per cent calcium and the same amount of phosphorus. The other diets contained, respectively, 2, 4, and 6 times as much calcium and phosphorus as did diet No. 1. The Ca/P ratio was therefore the same in all four diets. The calcium and phosphorus were increased by adding calculated amounts of CaCO3 and H3PO4 in the preparation of the salt mixture, which has been described elsewhere.4 Whenever necessary, adjustments were made until the desired calcium and phosphorus content could be verified by analysis. After a feeding period

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of 80 days on the experimental diets, the animals were sacrificed, and the teeth were examined and scored for caries by our usual procedure.³

RESULTS

Increasing the calcium and phosphorus content of the diet from 0.25 to 0.50 and 1.00 per cent resulted in a progressive decrease in the number of carious lesions and in the caries score, which, as shown in Table 1, was statistically significant. Further increase of the calcium and phosphorus to 1.5 per cent did not produce an additional decrease in the caries-conduciveness of the diet.

### TABLE 1

**DENTAL CARIES IN ALBINO RATS FED SAME HIGH-SUGAR DIET WITH DIFFERENT AMOUNTS OF CALCIUM AND PHOSPHORUS IN SAME RATIO**

<table>
<thead>
<tr>
<th>No. Animals</th>
<th>Diet</th>
<th>Per Cent Ca</th>
<th>Per Cent P</th>
<th>Av. No. Carious Lesions</th>
<th>Av. Caries Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1</td>
<td>0.25</td>
<td>0.25</td>
<td>24 ± 2.4</td>
<td>47 ± 15</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>0.50</td>
<td>0.50</td>
<td>21 ± 1.4</td>
<td>33 ± 11</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>1.00</td>
<td>1.00</td>
<td>18 ± 3.5</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>1.50</td>
<td>1.50</td>
<td>17 ± 4.0</td>
<td>20 ± 4</td>
</tr>
</tbody>
</table>

### STATISTICAL EVALUATION

<table>
<thead>
<tr>
<th>Diet</th>
<th>Av. No. Carious Lesions</th>
<th>t</th>
<th>P*</th>
<th>Av. Caries Score</th>
<th>t</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>4.0</td>
<td>&lt;0.01</td>
<td>47</td>
<td>2.4</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>3.2</td>
<td>&lt;0.01</td>
<td>33</td>
<td>3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>0.1</td>
<td>&gt;1.0</td>
<td>21</td>
<td>0.5</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Probability of a significant difference.

As the diet became less cariogenic by the addition of calcium and phosphorus while the Ca/P ratio remained constant, the results of these experiments confirm the conclusion deduced from indirect evidence in previous experiments, namely, that the cariogenicity of our high-sucrose diet is influenced by its calcium or phosphorus content and not by the Ca/P ratio. It is of interest to note that Harris and Nizel⁵ also obtained high and low caries scores with the same Ca/P ratio in the diet.

In our earlier experiments it was found that when calcium was kept at a constant level, the addition of phosphorus, up to approximately 1 per cent concentration, rendered the diet less conducive to caries. Beyond this point, no additional effect was produced by further increment in the phosphorus content. Likewise, a concentration of approximately 1 per cent in calcium appeared to be the critical level for maximum effectiveness in reducing the cariogenicity of the diet.

Upon comparison of the results of the present and our previous experiments, it was noted that when calcium and phosphorus were increased from 0.25 to 0.50 and 1.00
per cent, the caries scores were very close to those obtained when only the calcium or the phosphorus was increased from 0.25 to 0.50 and 1.00 per cent. The effects obtained by increasing either the dietary calcium or the phosphorus were therefore not additive when the concentration of both elements was increased simultaneously—for this we are unable at present to offer an explanation.

**DISCUSSION**

A review of the literature leads one to the general conclusion that the cariogenicity of experimental diets fed to rats or hamsters after tooth eruption may be influenced by the phosphorus or calcium content of the diet. There are, however, certain discrepancies that remain to be resolved.

**Phosphorus.**—It appears to be well established that Na$_2$HPO$_4$ is effective in rendering synthetic diets less conducive to dental caries. In the experiments of Constant, Sievert, Phillips, and Elvehjem, the addition of 2 per cent Na$_2$HPO$_4$ to their cariogenic diet retarded the development of fissure caries in the cotton rat. Similar results were obtained in our laboratories with the albino rat. Increasing the phosphorus content of a high-sucrose diet without changing the calcium concentration led to a reduction in the incidence and extent of fissure caries. The extra phosphorus was provided by using a larger amount of a mixture of NaH$_2$PO$_4$ and Na$_2$HPO$_4$ in making up the salt.

The severity of smooth-surface caries also and the number of carious teeth were reduced by the addition of 1.5 or 2.0 per cent Na$_2$HPO$_4$ to the wheat-flour diet which produces this type of caries.

However, when phosphorus is added to the diet in the form of insoluble salts, it is apparently not effective against cariogenesis. In McClure's experiments, CaHPO$_4$ was not cariostatic. In keeping with these results are those of Buttner and Muhler, who found that the insoluble calcium pyrophosphate (Ca$_3$P$_2$O$_7$) or the very slightly soluble dicalciumphosphate dihydrate (CaHPO$_4$·2H$_2$O) did not reduce the incidence of fissure caries when added to a coarse corn-particle diet. On the other hand, in unpublished data (cited by McClure), Strålfors observed a reduction in the development of dental caries in Swedish school children when CaHPO$_4$ was incorporated in their noon lunch by way of phosphated bread flour and sugar. These results, however, as noted by McClure, were obtained on a rather limited caries examination and only a 9-month period of examination.

When NaCl was added to CaHPO$_4$ in McClure's experiments, a definite cariostatic effect was observed, although NaCl of itself proved to be ineffective in this respect. McClure believes that the inability of CaHPO$_4$ to produce cariostasis may be attributed to the insolubility of this salt and that its effectiveness when NaCl was added may have been due to a solubilizing action of the latter.

From the evidence available at the present time, one may conclude that soluble phosphates in the diet are cariostatic in rats and hamsters after the teeth have erupted and are fully calcified. This conclusion is further supported by the experiments of Mitchell, Helman, and Chernausek, who found a reduction in hamster caries when either Na$_2$HPO$_4$ or (NH$_4$)$_2$HPO$_4$ was added to a caries-producing diet, and by those of Nizel, Keating, Sundstrom, and Harris, in which the addition of metaphosphoric acid to the diet resulted in a marked reduction in the caries scores of hamsters.

**Calcium.**—In the experiments of Constant et al., previously referred to, a definite
decrease in fissure caries in the cotton rat resulted from the addition of 1 per cent CaCO$_3$ to the diet. This cariostatic effect of CaCO$_3$ became more pronounced when the concentration was increased to 2 and 3 per cent. Conversely, decreasing the calcium level from 0.56 to 0 per cent caused an increase in the incidence and extent of caries.$^{14}$ In experiments reported from our laboratory,$^2$ an increase in the calcium content of the diet from 0.29 to 0.57 and 1.01 per cent by the addition of CaCO$_3$, while the phosphorus content was kept at a constant level, led to a progressive decrease in the caries score. McClure,$^7$ however, found that CaCO$_3$ added to a wheat-flour diet was not cariostatic for smooth-surface caries. The discrepancy between McClure's results and those obtained in the Wisconsin and Emory laboratories may perhaps be accounted for by the fact that McClure was studying smooth-surface caries, whereas we, as likewise Constant and her associates, were concerned with fissure caries. Different mechanisms may possibly be involved in the production of these two types of caries and influenced differently by dietary factors. This supposition is supported by the fact that many diets which produces fissure caries do not produce smooth-surface caries.

There are, however, other discrepancies in the literature which cannot be explained in this way. Dalderup$^{15}$ found that the addition of CaCO$_3$ to a coarse corn ration or to a ricket-inducing ration was ineffective in reducing dental caries. In the experiments of Barnard and Johansen$^{16}$ the addition of 2 per cent CaHPO$_4$ to relatively low cariogenic diets effected a significant reduction in dental caries, whereas the same salt added to a severely cariogenic diet was ineffective in reducing the cariogenicity of the diet. In later experiments reported from Johansen's laboratory,$^{17}$ the addition of 2 per cent CaCO$_3$ to a cariogenic diet actually increased its cariogenicity, whereas the same amount of calcium acetate made the diet less conducive to caries. The discrepancies and apparent contradictions in the results obtained in different laboratories by adding calcium, particularly in the form of carbonate, to cariogenic diets pose a puzzling problem which can be solved only by further experimentation.

SUMMARY

The calcium and phosphorus concentration of our high-sucrose diet fed to albino rats was increased in equal amounts, thereby maintaining a constant Ca/P ratio.

With an increase in the calcium and phosphorus from 0.25 to 0.50 to 1.00 per cent, there was a progressive decrease in the number of carious lesions and the caries score.

The caries conduciveness of the diet was not affected by a further increase in the calcium and phosphorus concentration to 1.5 per cent.

These results confirm the conclusion deduced from previous observations, namely, that the cariogenicity of our high-sucrose diet is influenced by the actual amount of calcium and phosphorus in the diet and not by the Ca/P ratio.

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