VITAMIN D AND THE CONSERVATION OF CALCIUM IN THE ADULT

III. THE EFFECT OF VITAMIN D ON THE TEETH OF RATS*

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In spite of the fact that from earliest antiquity we have evidence of pathological changes in the teeth, data on variations in chemical composition are very limited. Outside of scattered references in various texts, the earliest data usable for our purpose date back only a few decades.

In 1906 Hinkins (1) analyzed the dentin and enamel of human teeth dried at 100°. The dried dentin was found to contain 71.3 per cent ash and the enamel 94.3 per cent. The calcium content was found to be 26.9 and 36.3 per cent respectively. Ulrich (2) found that the calcium oxide content of normal human teeth ranged from 50.8 to 55.0 per cent. Gassmann’s data (3) reveal a higher ash content in human wisdom teeth than in the others. The former contained 81.5 per cent ash while the latter ranged all the way from 71.2 to 78.5 per cent. It is very probable that some of the earliest values were affected by variation in the proportionate weight of crown to root. Howe (4) has found a higher calcium content in the crown. Kaushansky (5) separated the crowns from the roots of normal human teeth and found 31.5 per cent calcium in the crowns and 25.3 per cent in the roots. With carious teeth the values were 29.7 and 25.5 per cent, and with pyorrhetic teeth 30.2 and 25.4 per cent calcium. When analyzed from the standpoint of pathology, these figures do not reveal any significant

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variations due to disease. Perlzweig (6) in 1916 determined the effect of low calcium and low phosphorus diets upon the ash and calcium content of dog teeth. Although rickets was observed in the animals as a result of feeding these diets for 127 days, the teeth were found to contain an average of 76.5 per cent ash and 28.6 per cent calcium. This compares very favorably with the values obtained by Gassmann (3) who reported 74.0 per cent ash and 27.4 per cent calcium in the teeth of dogs. However, when Perlzweig (6) reduced the phosphorus content of a ration to one-half that contained in a normal ration, there occurred slight demineralization of the incisors of two puppies in 149 days. Mellanby (7) found that a very important factor necessary for the production of normal teeth in dogs was vitamin D. However, she reports values of calcium oxide ranging from 27.1 to 34.5 per cent of the wet weight of teeth which appeared to be entirely normal in appearance. Gies, Morgulis, and Perlzweig (8, 9) compared the calcium content of the teeth of rats from rachitic and normal animals. In the rachitic rats there was found only a slight reduction in the calcium content. With rats on a low calcium diet Perlzweig (6) found a material reduction in the calcium content. Miller and Gies (10) have reported considerable variations in the calcium content of incisors of twenty-four young rats on a stock ration. The values ranged from 20.8 to 28.5 per cent. It is, however, possible that their normal diet was not entirely satisfactory. Matsuda (11) found 24.4 per cent calcium in the incisors of rats raised to 150 days of age on a varied diet. Toverud (12) reported a much greater effect of feeding a diet low in calcium and vitamin D upon the bones and upon the teeth of rats. His data indicate that in the molars the ash content was decreased 2 per cent, in the incisors 3.5 per cent, and in the femurs 13.2 per cent.

From the foregoing survey it is evident that there are abundant opportunities for profitable study of the effect of the diet on the chemical composition of the teeth.

**EXPERIMENTAL**

Our experiments on the effect of vitamin D on teeth were carried out in two series. Both series were run on adult rats raised in our colony on a stock ration which had been used for many years. In the first series additional vitamin D was added to a diet which in
its essential features consisted of the stock ration. In these experiments the rats were subjected to the strain of reproduction and lactation to accentuate whatever shortcomings the ration might possess. In the second series of experiments the rats were put on a low calcium diet. Here there was no necessity for subjecting the rats to an unusual strain because it was expected that they would encounter considerable difficulty in meeting their calcium requirements for maintenance on this ration. The two series were primarily organized for studying the effect of vitamin D on the skeleton. The data on the analysis of the bones have already been reported (13, 14). We now wish to report our observations on the teeth.

For the lactation series of experiments the stock diet was modified to furnish additional amounts of vitamin B and to keep the intake of milk solids constant in proportion to the other ingredients. Specifically the diet consisted of the following ingredients: yellow corn 137 parts, oil meal 29, crude casein 10, alfalfa 4, bone ash 2, sodium chloride 1, dried yeast 9, skim milk powder 72, and butter fat 28. When vitamin D was added, it was supplied in the form of irradiated dried yeast at a level of 0.5 per cent of the weight of the ration. As this yeast contained 1 Steenbock unit of vitamin D per mg., it provided a liberal excess of this vitamin.

The low calcium diet for the maintenance experiments was composed of yellow corn 79 parts, wheat gluten 20, and sodium chloride 1. Instead of adding vitamin D to the ration in the form of irradiated yeast, vitamin D was synthesized in the ration by treating it with ultra-violet radiations. This was effected by exposing 200 gm. in each of four pans measuring 2 feet square at 18 inches from an Alpine Sun lamp. The exposure was continued for 30 minutes, the contents of the pans being mixed thoroughly after the first 15 minutes of treatment. 2 gm. of the ration contained approximately 1 rat unit.

In the lactation series there are reported data on three groups of animals. One group was kept as a control without being bred and without additions of vitamin D, a second group was bred but was not given vitamin D, and a third was bred and was given vitamin D as well. The vitamin D, however, was not added to the diet until parturition. In the maintenance series there are
reported data on three groups of animals. One was kept on the stock ration for control purposes, another was given the low calcium ration non-irradiated, and the third the low calcium ration irradiated.

The animals used for the experiments were raised specifically for this purpose. They were all young females which were growing actively, having reached a weight of approximately 220 gm. They were practically of the same age. Most of the animals on which analyses of femurs were reported were used for dental studies. In the lactation series there were nine to seventeen rats in each of the groups. They weighed from 188 to 247 gm., but they were so distributed among the different groups that the average weights ranged from 214 to 220 gm. In the maintenance low calcium series there were ten animals in each group. The extreme weights were 203 to 276 gm., but the average weights in each group ranged from 224 to 238 gm.

In the lactation series the animals were kept on clean pine shavings as litter. In the maintenance series they were kept on floors composed of wire screen containing 2 meshes to the inch. In all cases the rations were fed ad libitum except in the lactation series where it was found necessary to feed the females periodically during the day in order to prevent the young from eating their mothers' ration. Upon termination of the experiments the carcasses from the lactation series were dried at approximately 60° for preservation. Later the teeth were carefully dissected out after soaking the heads in water. Before analysis they were extracted in a Soxhlet extractor for 5 days with 95 per cent alcohol and then dried at 100°. In the maintenance series the entire heads were preserved in alcohol. From the preserved heads the incisors were later dissected out, then extracted with alcohol, and ashed in the usual manner. The jaws of two animals from each group were reserved for histological examination.

The results from the lactation series are presented in Table I. This also gives the exact number of animals used. Inspection of the data reveals a remarkable constancy in the percentage of ash, the weight of ash, and the weight of the extracted incisors. These range respectively from 72.4 to 73.6 per cent, 234 to 245 mg., and 323 to 335 mg. This stands in marked contrast to the results on the femurs already reported. The analyses of these showed a content
TABLE I
Analyses of Incisors from Lactation Series with and without Generous Amounts of Vitamin D Added to Stock Ration

<table>
<thead>
<tr>
<th>Ration No.</th>
<th>Weight of extracted incisors*</th>
<th>Weight of ash*</th>
<th>Ash*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of rats</td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>Controls</td>
<td>9</td>
<td>0.294-0.383</td>
<td>0.335</td>
</tr>
<tr>
<td>Lactation without vitamin D additions</td>
<td>11</td>
<td>0.270-0.364</td>
<td>0.323</td>
</tr>
<tr>
<td>Lactation with vitamin D additions</td>
<td>13</td>
<td>0.288-0.355</td>
<td>0.324</td>
</tr>
</tbody>
</table>

* Upper and lower incisors were analyzed together.

TABLE II
Analyses* of Incisors from Low Calcium Maintenance Series with and without Vitamin D

<table>
<thead>
<tr>
<th>Ration No.</th>
<th>Weight of extracted incisors</th>
<th>Weight of ash</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ration No.</td>
<td>Range</td>
<td>Average</td>
</tr>
<tr>
<td>Upper incisors</td>
<td>R-34, low calcium, non-irradiated</td>
<td>0.127-0.156</td>
<td>0.1445</td>
</tr>
<tr>
<td></td>
<td>R-35, low calcium, irradiated</td>
<td>0.205-0.231</td>
<td>0.2175</td>
</tr>
<tr>
<td></td>
<td>R-36, final control</td>
<td>0.188-0.236</td>
<td>0.2078</td>
</tr>
<tr>
<td>Lower incisors</td>
<td>R-34, low calcium, non-irradiated</td>
<td>0.123-0.145</td>
<td>0.1385</td>
</tr>
<tr>
<td></td>
<td>R-35, low calcium, irradiated</td>
<td>0.201-0.236</td>
<td>0.2197</td>
</tr>
<tr>
<td></td>
<td>R-36, final control</td>
<td>0.186-0.218</td>
<td>0.2011</td>
</tr>
</tbody>
</table>

* The analyses are those of eight rats in each group except for Ration R-35 where data on only six are presented. On this ration two of the rats had malocclusion of the incisors resulting in excessive wear of the upper incisors and lessened wear of the lower.
of 64.8 per cent ash in the controls, 60.9 per cent after lactation, and 61.4 per cent with vitamin D supplementation. It is evident from this that the actively growing teeth are far more resistant to decalcification than the femurs. Our stock ration apparently was too well supplemented with the elements necessary for calcification to allow any possible effect of vitamin D to be demonstrated on the teeth.

Table II, which presents the data on the low calcium maintenance experiments, reveals entirely different results. In all cases the ash analyses of both upper and lower incisors gave results surprisingly like those found with the femurs. As presented in detail in the previous publication, the femurs of the control animals contained 64.1 per cent ash; those of the animal on the low calcium non-irradiated ration contained 54.1 per cent and on the low calcium irradiated ration 57.4 per cent. For the data on the upper incisors, as an example for the purpose of discussion, the percentage of ash was 75.5, 66.6, and 73.7 respectively. Both the weight of the incisors and the weight of their ash constituents were increased by the addition of vitamin D.

That the teeth varied in composition in the different groups was obvious by the appearance of the lower incisors. On the non-irradiated low calcium ration the dentin was chalky in appearance and the enamel was distinctly yellow. On the irradiated ration the dentin was more translucent and the enamel was less highly colored. The appearance of the terminal pulp canals also varied. Without vitamin D they were incompletely filled with osteodentin. With vitamin D they were practically obliterated. On the control ration the dentin was definitely translucent and the enamel was colored a very light yellow. It should, however, be stated that while the appearance of the upper incisors was quite uniform throughout all groups, that of the lower incisors varied somewhat even in the same groups.

In general the molars showed fewer differences in appearance than the incisors. This probably was to be expected because of the well known fact that the molars cease growing early in life while the incisors normally continue to grow throughout life at a rate said to be approximately 2.5 mm. per week (15). Caries was found to occur in practically all the molars, but especially in the first two molars of the lower jaw. On the non-irradiated ration
caries was found to be present in 73 per cent of the lower molars and 6.6 per cent of the upper molars. On the irradiated low calcium ration these values were respectively 65.0 per cent and 3.3 per cent. On the control ration 40 per cent of the lower molars was involved, but the upper molars were found to be entirely normal. From the above it is seen that the introduction of vitamin D into the ration apparently increased the resistance of the molars to the incidence of caries, but it is evident that the protection was not complete and that even the control stock ration left much to be desired.

The macroscopic and chemical changes in the teeth led us to study some of the incisors and molars histologically. They were decalcified with 3 per cent nitric acid in 80 per cent alcohol, embedded in paraffin, and cut into sections 7 μ in thickness. With the incisors, the sections were made transversely; with the molars they were made longitudinally. They were stained with hematoxylin and eosin.

Microscopic examination revealed a pronounced improvement of dental structure with the irradiation of the ration, but in harmony with the gross appearance and chemical analysis, normal structure was not completely obtained. On the non-irradiated low calcium ration the calcification of the dentin was markedly reduced in density as compared with the normal. Calcification had taken place only in the outer one-third or one-half of the dentinal matrix. In some sections large uncalcified interglobular spaces lay between calcospherites. These were distributed throughout the dentin. In other sections a well calcified outer band merged into a zone of incompletely calcified interglobular spaces; and a wide band bordering the pulp cavity was wholly uncalcified. The odontoblasts were loosely packed and irregular in orientation. In some sections the odontoblastic layer was completely missing. The pulp cavity had a more fibrous appearance than normal, and the ameloblasts were poorly oriented and small in size.

On the irradiated low calcium ration the histological picture was much improved over that described above, but it still was not quite normal. About one-half to two-thirds of the dentinal matrix was well calcified. The calcospherites were more fully fused. The odontoblastic layer suffered only occasional interruptions in out-
line, while the pulp cavity appeared normal. The ameloblasts were somewhat reduced in height from the normal, and there was less crowding.

In general the data on the teeth correspond to those reported by other investigators using low calcium rations. They are also comparable to those reported by Becks and Ryder (16) on a high calcium-low phosphorus rachitogenic ration.

SUMMARY

A stock rat ration which was unable to maintain normal ash content of bone in female rats during lactation did not lead to depletion of minerals in the incisor teeth, and liberal additions of vitamin D correspondingly had no effect on the teeth. However, a ration low in calcium when fed to adults over a long period of time was unable to maintain normal composition of teeth or bone even without the strain of reproduction. The sole addition of vitamin D resulted in almost complete protection against mineral losses, as revealed by analysis for ash and histological examination. For complete protection the ration was probably too low in calcium.

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