STUDIES ON THE METABOLISM OF THE ORAL TISSUES
INFLUENCE OF VITAMIN D ON RAT PALATAL MUCOSA RESPIRATION

by

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Recently the presence of a citric acid cycle has been described in rat palatal mucosa of both normal and rachitic animals [Mendelsohn, 1961a]. The action of phosphorylated vitamin D on the oxygen consumption of these tissues was then investigated [Mendelsohn, 1961b]. It was shown that phosphorylated vitamin D did not affect the basal oxygen quotient (QO2) of normal or vitamin D deprived tissue, nor did it influence the oxygen consumption of normal tissue on the addition of any of the members of the tricarboxylic acid cycle. However, in the case of mucosa from rachitic rats, the vitamin in the presence of components of the citric acid type, produced a further increase in the QO2, above that of the intermediate itself. Experiments were then performed to ascertain whether the in vitro response of rachitic tissue to vitamin D was in fact due to the action of the vitamin itself, or whether it was caused by a difference in handling and housing of the animals. In addition, investigations were carried out to determine whether the administration of non-phosphorylated vitamin D to rachitic rats could restore the metabolism of their oral tissues to normal. An attempt was also made to elicit the rapidity with which vitamin D could attain this effect. The results of these experiments will be reported in this communication.

MATERIALS AND METHODS

The materials and methods employed have been described previously [Mendelsohn, 1961a, b]. In order to determine whether the difference in behaviour of normal and rachitic oral tissue towards vitamin D was in fact due to the action of the vitamin itself, rats were subjected to the following dietary regime (control regime). The animals were maintained on a modified Sherman-Pappenheimer rachitogenic diet [Jephcott and Bacharach, 1926], which was supplemented with non-phosphorylated vitamin D. Each rat received a weekly oral dose of 32 I.U. vitamin D in arachis oil. These animals were also housed in darkened quarters impervious to sunlight. This control group of animals were thus treated in an identical manner to the rachitic rats except that vitamin D was administered to the control group. The treatment was continued for 21 days, after which time the animals were killed and their tissues investigated in a manner similar to those obtained from rats subjected to the curative dietary regime outlined below.

Curative dietary regime

Rats were placed on the above mentioned rachitogenic diet for 21 days when they weighed 60-80 g. One group of animals was killed and the long bones examined histologically for rickets [Coward, 1947]. The second group of rats received an oral dose of 32 I.U. vitamin D in arachis oil per os. Thereafter the animals in this group were further subdivided into two groups: (a) These animals were killed after the single dose of vitamin D. The tibiae, radius and ulnae were investigated histologically by means of the "line test". The action of phosphorylated vitamin D in vitro on the oxygen consumption of the palatal mucosa of these animals was also determined. (b) This group of rats was given a second dose of 32 I.U. vitamin D in arachis oil and killed seven days later. Their long bones were examined as in group (a).
In order to determine the rapidity of action of vitamin D on the metabolism of rat palatal mucosa, animals were placed on the same curative dietary regimen and their oral tissues examined 2 days after receiving a single dose (32 I.U.) of non-phosphorylated vitamin D.

RESULTS AND DISCUSSION

The results recorded in Table I indicate that the response of the palatal mucosa of animals reared on the control regime to phosphorylated vitamin D in vitro was identical with similar tissue obtained from animals fed the stock diet of the colony. In both cases, the in vitro addition of vitamin D caused no alteration in the $Q_{O_2}$ of these tissues in the presence of metabolites of the Krebs tricarboxylic acid cycle. No differences could be detected histologically between the epiphyses of the long bones of the control diet animals and those of normal animals.

<table>
<thead>
<tr>
<th>Metabolite added</th>
<th>No. of determinations</th>
<th>$Q_{O_2}$ without vitamin D added</th>
<th>Phosphorylated vitamin D added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>2.03 ± 0.38</td>
<td>2.3</td>
</tr>
<tr>
<td>0.02M Aconitate</td>
<td>4</td>
<td>2.1</td>
<td>2.3</td>
</tr>
<tr>
<td>0.02M Succinate</td>
<td>3</td>
<td>3.54</td>
<td>3.3</td>
</tr>
<tr>
<td>0.02M Malate</td>
<td>3</td>
<td>2.53</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The reaction mixture consisted of: 2 ml. Krebs-Ringer-phosphate solution, pH 7.4; either 0.05 ml. of a 0.5% aqueous phosphorylated vitamin D solution or 0.05 ml. distilled water (control); 20 to 30 mg. wet weight tissue. When substrate was added 1 ml. Krebs-Ringer-phosphate solution and 1 ml. substrate (final concentration indicated in the table) was used. The mean value of the observations is given followed by the standard deviation in the case of the control.

It would thus seem that the previously noted "D effect", i.e. the elevation of oxygen consumption by phosphorylated vitamin D on the addition of intermediates of the citric acid cycle above the increase due to the metabolite itself, was abolished by prior in vivo administration of vitamin D. It was therefore concluded that the action of phosphorylated vitamin D on the value of the $Q_{O_2}$ of rachitic oral tissue was directly due to the vitamin.

Histological examination of the tibiae, radii and ulnae of animals sacrificed seven days after receiving a single dose of vitamin D revealed healing of the induced rickets. Following a second dose of vitamin D, the epiphyses of the long bones were indistinguishable from normal ones. Metabolically, the non-phosphorylated vitamin D restored the animals to their normal condition, since the "D effect" previously found in rachitic oral tissues could no longer be detected in the oral tissues of animals after dosage with vitamin D. A similar result was obtained with the oral tissues of animals given two doses of vitamin D. Furthermore it was shown that in oral palatal mucosa obtained from animals sacrificed 2 days after receiving a single dose of vitamin D, the "abnormal" response of rachitic palatal mucosa to phosphorylated vitamin D could no longer be detected. These results are summarized in Table II.
The additions to each flask and the explanation of the table are the same as described in Table I. The figures in brackets indicate the number of observations. The "t" test showed that the addition of phosphorylated vitamin D in vitro did not significantly affect respiratory metabolism of oral tissues from animals maintained on the curative diet. Thus it would appear that the administration of non-phosphorylated vitamin D to rachitic rats was able to abolish the response of their oral tissues to the in vitro addition of phosphorylated vitamin D. This effect was apparent as early as two days after dosing the animals with vitamin D. A very rapid action of vitamin D has also been reported by Belanger and Migicovsky [1958]. They noted that two hours after the administration of vitamin D3 to rachitic chicks, signs of activity in the epiphyseal cartilage, as determined histologically, could already be observed.

**SUMMARY**

The administration of vitamin D3 to rachitic rats was able to cancel the previously noted in vitro action of phosphorylated vitamin D on the oxygen consumption of palatal mucosa obtained from vitamin D deficient animals. This action was apparent as early as two days after dosing the rats with the vitamin.

It has also been shown that the effect of phosphorylated vitamin D on the Qo2 of rachitic oral tissue was due to the action of the vitamin itself and not to differences in the handling and treatment of the animals.

**REFERENCES**


