Metabolism and Mode of Action of Vitamin D

II. Storage of Vitamin D in Different Tissues in Vivo

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In order to learn more about the fate of vitamin D within the body tissues, and thus possibly to throw some light on its still obscure mode of action, studies were undertaken and have been reported (1) showing that when viosterol in oil was given by mouth to rabbits in a single dose of 20 cc.* detectable amounts of vitamin D were found to be circulating in the blood for from 2 to 3 months.

The object of the continuation experiments, the results of which are here reported, was to detect the duration of storage of vitamin D in different tissues under the same conditions that prevailed in the previous investigations.

Experimental

To twelve male rabbits, weighing from 3.5 to 5 kilos, 20 cc. of viosterol in oil were administered by stomach tube. The rabbits were killed at the end of 1, 3, 5, 6, 7, 8, 9, and 12 weeks by cutting the femoral vessels after the animals had been anesthetized with a small amount of ether. The animals were immediately placed in the refrigerator, and the tissues were removed not later than 10 hours after death.

The presence of vitamin D was determined in extracts of oxalated plasma, unwashed erythrocytes, brain, small and large intestines (free of chyme and feces), lungs, skin from abdominal region only (without hair and subcutaneous fat), kidney, and liver.

* The 20 cc. dose represents 200,000 U.S.P. units (United States Pharmacopoeia X, revised (1934)) or international units (corresponding to approximately 1.6 mg. of irradiated ergosterol).
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Method

The tissues were weighed fresh, ground through a food chopper or cut into small pieces with scissors, and treated for about 2 hours on the steam bath with 20 per cent potassium hydroxide in alcohol. In order to prevent coagulation while the solutions were cooling, ether was added carefully, with constant stirring, to the still hot but liquid material. 5-Fold ether extraction was then performed in Mojonnier flasks, with 300 to 600 cc. of ether. The ether extracts were washed with water, in separatory funnels, until the water remained colorless, and were slowly evaporated on the water bath. The residue from ether evaporation was then dissolved in Wesson oil in an amount equal to one-sixth of the original weight of the tissues. The concentration of the oil suspensions was thus kept comparable and constant for each single tissue.2

By pipette 0.1 cc. of the tissue extracts was fed daily for 10 days to rats which had been kept for 3 weeks on a rickets-producing diet (Steenbock Ration 2965). Roentgenograms of the lower extremities were made on the day the administration of the extracts was begun and on the 8th and 10th days following. After the last roentgenograms had been taken, the blood obtained from the rats receiving the same tissue extract was pooled, and serum phosphorus and calcium determinations were made by the titrimetric method of Samson (2).

Results

The results of these tests are recorded in Table I. The storage time of vitamin D in the tissues is shown in the last column. It must be realized that an excessive amount of vitamin D in the form of viosterol had been given. In all the tissues examined, the vitamin D was deposited and found present for at least 1 week.

2 The method was tested by adding known amounts of viosterol in oil to tissues (liver and muscle) from animals not used in the experiments. It was found possible in this way to recover vitamin D almost unit per unit. The efficacy of the method is evident also when the results given in this paper are compared with those previously reported (1). In the earlier experiments, blood serum from which vitamin D had not been extracted was injected in rachitic rats, with results that agree with those now obtained by the extraction method.
The order in which the vitamin D depots were depleted in vivo was as follows: brain, erythrocytes, small intestines, large intestines, skin, lungs, kidney, liver, blood plasma.

It is rather surprising to find that this fat-soluble vitamin disappeared first in the brain, which, on account of its chemical composition, would presumably be the most likely place of retention. This observation shows that other conditions than the purely chemical properties of the tissues are decisive in influencing the retention of vitamin D. This fact is also evident from the results obtained in all the other tissues; the length of time that vitamin D was stored by no means paralleled the fat or lipid content of the tissues.

The erythrocytes were apparently depleted completely of vitamin D after 6 weeks, whereas vitamin D was present in the blood plasma for as much as and possibly more than 3 months. In this connection it may be of interest to note that Hess (3) found that in the blood of cows fed irradiated yeast the plasma contained 4 times as much vitamin D per gm. as did the erythrocytes.

The time required for depletion of vitamin D in the skin, lungs, kidneys, and small and large intestines did not show much variation. It can only be stated that vitamin D was stored in all these tissues to about the same extent; that is, for about 5 to 8 weeks.

It seems as if the liver can hold vitamin D more tenaciously than can the other organs. The results obtained, however, are not sufficiently uniform and the difference in storage time in the liver, on the one hand, and in the skin, lungs, kidneys, and small and large intestines, on the other, is not sufficiently great to justify a more positive statement at this time. To have found that only the liver retained vitamin D for from 6 to 8 to 12 weeks is certainly suggestive of the hypothesis advanced by Gerstenberger (4) that the liver probably plays a decisive rôle in the functioning of vitamin D.

Coppens and Metz (5) reported that lungs and blood, when incubated in vitro, decomposed vitamin D. Their results do not agree with the findings presented here, obtained from experiments in vivo, nor is their assumption of the presence of an enzyme in lungs and blood which might lead to inactivation of vitamin D substantiated. On the contrary, it has now been established that
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blood plasma, \emph{in vivo}, contains vitamin D in active form longer than does any other tissue.

Considering the rather impressive length of time that vitamin D is stored in the body tissues, it can be assumed that if there is consumption of vitamin D within the tissues at all, it must be very slight. Investigations now in progress indicate that excretion is perhaps the only, certainly the chief means of depleting the body of vitamin D. That blood plasma contained vitamin D longer than did any other tissue is consistent with this conception. It might also be mentioned here that the protracted storage time of

\begin{table}[h!]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
Tissue extract & Healing in rats fed tissue extract from rabbits not given viosterol (controls) & & & & & & & & \\
\hline & x-Ray healing & P & Ca & x-Ray healing & P & Ca & x-Ray healing & P & Ca \\
\hline
Brain & 0 & 0 & ++ & 0 & 0 & 0 & 0 & 0 & 0 \\
Red blood cells & 0 & 0 & 5.3 & 10.4 & ++ & ++ & ++ & ++ & ++ & ++ \\
Small intestines & & & & & ++ & ++ & ++ & ++ & ++ \\
Large intestines & & & & & +++ & +++ & +++ & +++ & +++ \\
Skin & 0 & 0 & ++ & +++ & +++ & +++ & +++ & +++ & +++ \\
Lungs & 0 & +++ & +++ & + & ++ & +++ & +++ & +++ & +++ \\
Kidneys & & & & & & & & & \\
Liver & 0 & 0 & 4.4 & 10.3 & +++ & +++ & +++ & +++ & +++ & +++ \\
Blood plasma & 0 & 0 & 5.3 & 10.4 & +++ & +++ & +++ & +++ & +++ & +++ \\
\hline
No. of rabbits used & 1 & 1 & 1 & 1 \\
\hline
\end{tabular}
\end{table}
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vitamin D explains Harnapp's (6) recently reported cure of rickets in human beings with a large, single dose of vitamin D2.

**SUMMARY**

To twelve male rabbits was administered by stomach tube a single dose of 20 cc. of viosterol in oil (200,000 U.S.P. units of vitamin D, corresponding to approximately 1.6 mg. of irradiated ergosterol).

In order to detect the length of time that vitamin D is stored in the tissues, the animals were killed at the end of 1, 3, 5, 6, 7, 8, 9, 10, 11, and 12 weeks after the administration of the single dose of vitamin D.

<table>
<thead>
<tr>
<th>Vitamin D stored in tissues</th>
<th>6 wks.</th>
<th>7 wks.</th>
<th>8 wks.</th>
<th>9 wks.</th>
<th>12 wks.</th>
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</thead>
<tbody>
<tr>
<td>X-Ray healing P Ca</td>
<td>X-Ray healing P Ca</td>
<td>X-Ray healing P Ca</td>
<td>X-Ray healing P Ca</td>
<td>X-Ray healing P Ca</td>
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<tr>
<td>mg. per cent</td>
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<td>0</td>
<td>0</td>
<td>1-2</td>
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<tr>
<td>++</td>
<td>4.9</td>
<td>11.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>++</td>
<td>3.4</td>
<td>9.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>++</td>
<td>3.8</td>
<td>12.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ +</td>
<td>4.7</td>
<td>13.9</td>
<td>0</td>
<td>0</td>
<td>2.8</td>
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<tr>
<td>++</td>
<td>5.0</td>
<td>12.3</td>
<td>0</td>
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<td>+ +</td>
<td>7.0</td>
<td>12.5</td>
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<td>12.3</td>
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<td>+ +</td>
<td>3.4</td>
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<td>4.1</td>
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</tbody>
</table>

Which Had Received 1 to 12 Weeks before Death 20 Cc. of Viosterol in Oil by Stomach Tissue Extracts Had Been Started
9, and 12 weeks, and the tissues were removed. Extracts of the tissues were fed to rachitic rats. Under these conditions it was found that vitamin D was stored in the brain for 1 to 2 weeks, in erythrocytes for 5 to 6 weeks, in the small intestines for 5 to 8 weeks, in the large intestines for 6 to 8 weeks, in the skin for 6 to 8 weeks, in the lungs for 6 to 9 weeks, in the kidneys for 6 to 9 weeks, in the liver for 6 to 8 to 12 weeks, and in blood plasma for 8 to 12 weeks and more. It is assumed that consumption of vitamin D within the tissues, if it exists at all, must be very slight. It seems more likely that excretion is the chief means by which the body is depleted of its vitamin D depots.

BIBLIOGRAPHY

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