Vitamin D and Phospholipid Metabolism*

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(Received for publication, September 19, 1963)

Although it has been known for many years that the primary physiological action of vitamin D is to improve calcium absorption from the small intestine (1,2), studies on calcium metabolism alone have thus far failed to elucidate the biochemical mechanism of action of vitamin D. By using everted intestinal sacs, Schalitter and Rosen (3) have shown that vitamin D increases the transport of $^{48}$Ca against a concentration gradient in the proximal portion of the small intestine. Harrison and Harrison have confirmed these findings (4) and have also reported that vitamin D increases the rate of diffusion of calcium across the entire length of the intestinal wall. In this laboratory, DeLuca et al. (5,6) have demonstrated an action of vitamin D on the behavior and morphology of kidney mitochondria. More recently, Engstrom and DeLuca (7) have reported that vitamin D, in vitro or in vivo, stimulates the release of calcium from isolated rat kidney mitochondria. Studies in this laboratory on the subcellular distribution of vitamin D in rat kidney by biological assay (8) and by use of tritium-labeled vitamins D$_2$ and D$_3$ have demonstrated that vitamin D is associated with all membrane fractions. These findings suggest that the action of vitamin D at the cellular level may be closely associated with a function of the cellular membranes.

Hokin and Hokin (9) have studied the secretion of proteins, polypeptides, and other hydrophilic material and have found that an increase in the secretion of these materials is accompanied by an increase in the incorporation of $^{32}$P-orthophosphate into the phospholipids. Further work (10) on the synthesis of phosphatic acid from diglyceride and adenosine triphosphate led to a possible scheme for the participation of phosphatic acid in the transport of hydrophilic secretory materials across lipoprotein membranes. This scheme has more recently been modified to account for the number of sodium ions transported per ATP used (11). Several other investigators have proposed that phospholipids might function in the movement of cations and other hydrophilic material across cellular membranes (12,13). Karnovsky and Wallach (14) have reported that incorporation into various phosphatides is increased during phagocytosis.

Studies indicating that (a) vitamin D is involved in the transport of calcium across biological membranes, (b) vitamin D is associated with and has an effect on lipid membranes, and (c) phospholipids are involved in the secretion of hydrophilic material and have been implicated in the transport of cations across lipid membranes stimulated an investigation of the effect of vitamin D on phospholipid metabolism.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported by Grant AM05800-02 NTN from the United States Public Health Service. We are indebted to Merck Sharp and Dohme for some of the vitamins used in this study.


EXPERIMENTAL PROCEDURE

Preparation of Rats—Male albino rats of the Sprague-Dawley strain, weighing 50 to 70 g, were housed in individual hanging wire cages and given food and distilled water ad libitum. The rats were fed a modified version of the adequate calcium and phosphorus diet of Steenbock and Herting (15), except that vitamin-free casein plus 0.2% L-cystine replaced egg white as the protein source. Fat-soluble vitamins A, E, and K were given orally in a cottonseed (Wesson) oil solution three times a week. In each experiment, except in the case of the response studies, one-half of the animals received no vitamin D, while the other half received 75 i.u. of vitamin D$_2$ in cottonseed oil three times a week. In experiments concerned with the response to vitamin D, rats were killed 3 or 6 hours after an oral dose of 2000 i.u. of the vitamin.

Isotopes—$^{32}$P, carrier-free, was obtained from Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Glycerol-1,3-$^{14}$C (specific activity, 1.0 mc per mmole) was purchased from Nuclear Research Chemicals, Inc., Orlando, Florida.

DL-Serine-3-$^{14}$C (specific activity, 6.0 mc per mmole) was obtained from Volk Radiochemical Company, Skokie, Illinois.

Incubations—After the rats had been on the diet for 21 to 28 days, two vitamin D-deficient and two vitamin D-fed rats were killed by a sharp blow on the head followed by decapitation. The upper one-half (approximately 30 cm) of the small intestine was quickly removed, slit open, and placed in ice-cold 0.9% NaCl solution. The intestine was rinsed three times in cold NaCl solution, blotted on filter paper, and laid on a flat glass surface. The mucosa was removed by scraping with a microscope slide (16) and then weighed on a torsion balance. Mucosa (2 g) was placed in 20 ml of Krebs-Ringer-bicarbonate buffer (17), and the cells were suspended by drawing them up several times in a serological pipette. The suspensions were shaken for 5 minutes at 37° for thermal equilibration before initiation of the reaction by the addition of 100 mc of $^{32}$P$_1$ (yielding a specific activity of 5.2 mc per amole of phosphorus) plus NaCl solution (isotonic NaCl-KCl, 25:1, total volume of 4 ml) or, in some cases, 4 mc of $^{14}$C-labeled glycerol or serine plus NaCl solution. The cells were incubated with shaking for 1 hour at 37° with 0.5% O$_2$-5% CO$_2$ flowing continuously as the gas phase. Samples of 3.2 ml were removed by pipette at various times and added to 12 ml of cold chloroform-methanol, 1:2, to stop the reaction.

In experiments with kidney and liver slices, the tissues were sliced with a hand microtome (17), and 250 mg of tissue were placed in 2.5 ml of Krebs-Ringer-bicarbonate buffer in a 25-ml Erlenmeyer flask. Samples were initially incubated for 5 minutes at 37°, after which 10 mc of $^{32}$P$_1$ plus 0.9% NaCl solution (in a total volume of 0.5 ml) were added to each flask, and the
The column was allowed to stand overnight before the sample recovery of the radioactivity was obtained with an "aged" column.

Lipid extraction procedure was determined by the method of Martin and Doty (21). In this procedure the inorganic phosphate is complexed by molybdate and becomes soluble in an aqueous phase. An aliquot of this aqueous phase was plated on butanol-benzene phase, leaving only the organic phosphate in the chloroform phase. This was shaken, the silicic acid was allowed to settle, and 1 ml was applied. Preliminary experiments had indicated that better phospholipids, 2 ml of the lipid extract were removed and to this was added 2 ml of chloroform and 0.5 g of silicic acid (see below). The mixture was filtered through glass wool into a graduated centrifuge tube, and the phases were allowed to separate. An aliquot of the chloroform layer was removed and plated on an aluminum planchet to determine lipid-associated radioactivity. All radioactivity measurements were made in the Geiger region with a Nuclear-Chicago automatic counting device.

When 14C-labeled glycerol was added to the incubation medium, total lipid-soluble radioactivity was determined in the manner described above. For separation of the neutral and phospholipids, 2 ml of the lipid extract were removed and to this were added 2 ml of chloroform and 0.5 g of silicic acid (see below). This was shaken, the silicic acid was allowed to settle, and 1 ml of the chloroform was plated for radioactivity determination. The radioactivity remaining in the chloroform was taken to be associated with neutral lipids; the radioactivity of phospholipid was determined by difference.

Organic phosphate in the aqueous-methanol phase from the lipid extraction procedure was determined by the method of Martin and Doty (21). In this procedure the inorganic phosphate is complexed by molybdate and becomes soluble in a butanol-benzene phase, leaving only the organic phosphate in the aqueous phase. An aliquot of this aqueous phase was plated for radioactivity measurement.

Chromatography—Columns, 0.8 cm x 10 cm, were dry-packed with 4.0 g of Bio-Rad (Calbiochem) silicic acid (minus 325 mesh; especially prepared for the chromatography of lipids). The silicic acid had previously been placed in a vacuum desiccator for 6 to 8 hours, then activated at 135° for at least 24 hours prior to use. Chloroform (Analytical Reagent) was run through the column under slight (approximately 5 pounds) pressure until the silicic acid became clear (approximately 75 ml of chloroform). The column was allowed to stand overnight before the sample was applied. Preliminary experiments had indicated that better recovery of the radioactivity was obtained with an "aged" column.

RESULTS

Fig. 1 illustrates the effect of vitamin D on the incorporation of 32P into the lipids of intestinal mucosa. The stimulation by vitamin D was apparent even within the first 15 minutes of incubation, and was greater than 3-fold at the end of 1 hour. These results were quite reproducible as long as the animals used were of the same age. This incorporation of 32P into the phospholipids is totally dependent on oxidative metabolism. The incorporation was completely abolished when 95% N2-5% CO2 was the gas phase, and the results were variable when the samples were not gassed with 95% O2-5% CO2 throughout the incubation period. The effects of several metabolic inhibitors on 32P incorporation into the lipids were studied. The results are shown in Fig. 2. The incorporation was almost completely blocked in the presence of 10-3 M sodium cyanide, 7 X 10-5 M dicumarol, 5 X 10-5 M antimycin A, and 10-3 M phosphorin, and was partially blocked by 10-3 M gramicidin and 10-4 M oligomycin. Iodosaccharate (10-4 M) and 10-4 M p-chloromercuribenzoate3 showed no inhibition. The incorporation was also insensitive to dinitrophenol at a concentration of 2 X 10-4 M.

In an effort to determine whether or not vitamin D was stimulating the general conversion of inorganic phosphate to organic...
demonstrates that the increase due to vitamin D is not merely a
reflection of increased growth, since it has been previously ob-
served that vitamin D depresses growth with diets of a high Ca:P
ratio (15).

Further evidence that the stimulation due to vitamin D is not a
result of increased growth is the increased lipid-\(^{32}\)P incorpora-
tion as early as 3 hours after administration of an oral dose of
2000 i.u. of vitamin D (Fig. 4). The radioactivity in the samples
(B and B') from rats that received vitamin D 3 hours before
death was significantly greater than that in the samples from the
vitamin D-deficient controls (A and A') after 30 minutes (p < 0.05)
and 60 minutes (p < 0.01) of incubation. The response 6
hours after the administration of vitamin D (C and C') was
obviously highly significant. In some experiments, the vitamin
D-deficient controls were given 0.2 ml of pure Wesson oil at the
same time that the others were given vitamin D; no difference
was noted between these controls and those not given oil.

To ascertain whether or not specific components of the cells
were affected by vitamin D, the distribution of the lipid-\(^{32}\)P in
the subcellular fractions was studied. The whole cells were
incubated for 60 minutes with \(^{32}\)P and then fractionated. The
results are given in Table 1. The method for fractionation of
intestinal mucosa cells was not totally satisfactory because of
the difficulty in breaking the cells. Further, the difficulties
were multiplied when attempts were made to fractionate cells
which had been incubated previously. The stimulation by
vitamin D of \(^{32}\)P incorporation into the lipids was associated with
all of the cell fractions, but in general the difference was greater
in the mitochondrial and microsomal-supernatant fractions than
in the nuclear-debris fraction.

The effect of vitamin D on the incorporation of \(^{32}\)P into the
lipids of kidney and liver slices was studied. The kidney slices
(Fig. 5, left) showed a very large incorporation during the first 5
minutes of incubation. This initial phase was unaffected by
vitamin D, but in the 30- and 60-minute samples the relative
incorporation was increased by vitamin D. In liver slices (Fig.
5, right), a stimulation due to vitamin D was not observed.

Experiments conducted with \(^{14}\)C-labeled phospholipid pre-
cursors showed that there was no effect of vitamin D on the
incorporation of \(^{32}\)P into the lipids of intestinal mucosa from a vitamin D-defi-
cient rat. Incubation was carried out in Krebs-bicarbonate
buffer at 37° for 30 minutes as described in the text. The samples
were incubated for 10 minutes with the inhibitor before the addi-
tion of the \(^{32}\)P. The concentrations of inhibitors were: 10\(^{-3}\) m
NaCN, 2 \(\times 10^{-3}\) m dinitrophenol (DNP), 10\(^{-2}\) m oligomycin B,
10\(^{-2}\) m gramicidin S, 7 \(\times 10^{-3}\) m Dicumarol, 5 \(\times 10^{-7}\) m antimonycin
A, 10\(^{-3}\) m phlorizin, 10\(^{-4}\) m iodoacetate, and 10\(^{-4}\) m p-chloromer-
curibenzoate (PCMB).

Because of the well established relationship between vitamin D
and calcium metabolism, the effect of omitting calcium from the
incubation medium was studied, and it was found that the omis-
sion of calcium has no significant effect on the labeling of the
intestinal mucosa lipids from vitamin D-deficient or vitamin D-
fed rats.

The stimulation of \(^{32}\)P incorporation due to vitamin D has
been observed in rats fed high calcium rachitogenic diets as well
as in those fed the nonrachitogenic diet routinely used. This
demonstrates that the increase due to vitamin D is not merely a

Fig. 2. The effect of metabolic inhibitors on the incorporation of \(^{32}\)P into the lipids of intestinal mucosa from a vitamin D-defi-
cient rat. Incubation was carried out in Krebs-bicarbonate
buffer at 37° as described in text. The samples
were incubated for 10 minutes with the inhibitor before the addi-
tion of the \(^{32}\)P. The concentrations of inhibitors were: 10\(^{-3}\) m
NaCN, 2 \(\times 10^{-3}\) m dinitrophenol (DNP), 10\(^{-2}\) m oligomycin B,
10\(^{-2}\) m gramicidin S, 7 \(\times 10^{-3}\) m Dicumarol, 5 \(\times 10^{-7}\) m antimonycin
A, 10\(^{-3}\) m phlorizin, 10\(^{-4}\) m iodoacetate, and 10\(^{-4}\) m p-chloromer-
curibenzoate (PCMB).

Fig. 3. Lack of an effect of vitamin D on \(^{32}\)P incorporation into
nonlipid organic phosphate. Intestinal mucosa was incubated
in Krebs-bicarbonate buffer at 37° as described text. Organic
\(^{32}\)P was determined by the method of Martin and Doty (21).
\(\circ - \circ\), vitamin D-deficient; \(\bullet - \bullet\), vitamin D-fed. The 30-
and 60-minute values are averages of five experiments ± standard
deviation. Other points are averages of two experiments.
The commercial preparations were found to be quite impure and had to be purified further by thin layer chromatography and elution of the main component. This was subsequently confirmed by Galliard and Hawthorne (20), who identified the compound as diposphoinositol. The data given in Table III show that vitamin D had no effect on the rapid labeling of the mitochondrial lipids.

Distribution

The effects were indicated by Roman numerals I to VII in Fig. 6. The peaks were identified by comparison with commercial preparations and standards for the thin layer chromatography were purified either by silicic acid column chromatography or by previous thin layer chromatography and elution of the main component. The commercial preparations were found to be quite impure when first examined by thin layer chromatography. This heterogeneity has also been commented on by another investigator (27). The peaks from the silicic acid columns in most cases gave only a single spot on thin layer chromatography. The two major components, Peaks IV and VII, have been identified as phosphatidylethanolamine and lecithin, respectively.

TABLE I

Distribution of lipid-32P in subcellular fractions of intestinal mucosa from vitamin D-deficient and vitamin D-fed rats

Intestinal mucosa was incubated in Krebs-bicarbonate buffer at 37° for 1 hour as described in the text. In Experiments 1 to 3, the cells were broken by homogenizing without diluent in a Potter-Elvehjem homogenizer; in Experiments 4 to 6, by grinding with sand in a chilled mortar. The results are expressed as counts per minute per mg of mucosa, and each number is an average of four experiments ± standard deviation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Whole cells</th>
<th>Nuclei + debris</th>
<th>Mitochondria</th>
<th>Microsomes + supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-D</td>
<td>+D</td>
<td>-D</td>
<td>+D</td>
</tr>
<tr>
<td>1</td>
<td>34.6</td>
<td>176</td>
<td>41.2</td>
<td>103</td>
</tr>
<tr>
<td>2</td>
<td>75.5</td>
<td>312</td>
<td>27.8</td>
<td>85.6</td>
</tr>
<tr>
<td>3</td>
<td>64.0</td>
<td>314</td>
<td>16.7</td>
<td>35.9</td>
</tr>
<tr>
<td>4</td>
<td>31.0</td>
<td>72.0</td>
<td>13.5</td>
<td>12.7</td>
</tr>
<tr>
<td>5</td>
<td>82.8</td>
<td>160</td>
<td>41.2</td>
<td>35.0</td>
</tr>
<tr>
<td>6</td>
<td>52.9</td>
<td>220</td>
<td>38.5</td>
<td>83.7</td>
</tr>
</tbody>
</table>

Incorporation of glycerol-1,3-14C and serine-3,14C into phospholipids of intestinal mucosa

Intestinal mucosa was incubated in Krebs-bicarbonate buffer at 37° as described in the text. The reaction mixture contained either 0.27 mM DL-serine-3-14C (0.17 μC per ml, Volk Radiochemical Company) or 0.17 mM glycerol-1,3-14C (0.17 μC per ml, Nuclear Research Chemicals). Results are expressed as counts per minute per mg of mucosa, and each number is an average of four experiments ± standard deviation.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>14C-Glycerol</th>
<th>14C-Serine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-D</td>
<td>+D</td>
</tr>
<tr>
<td>4</td>
<td>4.8 ± 1.3</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>10</td>
<td>9.3 ± 2.0</td>
<td>8.0 ± 3.1</td>
</tr>
<tr>
<td>30</td>
<td>11.0 ± 3.2</td>
<td>9.2 ± 2.1</td>
</tr>
<tr>
<td>60</td>
<td>14.7 ± 3.9</td>
<td>11.2 ± 1.9</td>
</tr>
</tbody>
</table>

In Fig. 5, Effect of vitamin D on the incorporation of 32P into lipids of kidney slices (left) and liver slices (right). Incubation was carried out in Krebs-bicarbonate buffer at 37° as described in the text. Each point is an average of four to six experiments ± standard deviation. O—O, vitamin D-deficient, •—•, vitamin D-fed. In the left-hand graph, O (fed) > O (deficient) (p < 0.01) at 30 and 60 minutes. No significant differences were found at the 95% level in the experiments with liver slices (right).
intestinal mucosa were not removed from the silicic acid column with chloroform, but were eluted with 2% methanol in chloroform.

Neither the total amount of phospholipid present in intestinal mucosa nor the amount of any particular phosphatide was affected by vitamin D. Table IV gives the percentage of the total phospholipid found in each of the peaks shown on the chromatogram (Fig. 6). The percentage found in Peaks V and VI is given as a single figure because of the variability and lack of separation of the two. This variation occurred because the amount of solids present in the fractions was in the lower range of at least 14 experiments ± standard deviation. All experimental values are corrected for a blank.

**Table III**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No vitamin D</th>
<th>Plus vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2775 ± 485 (8)</td>
<td>2940 ± 600 (8)</td>
</tr>
<tr>
<td>Kidney</td>
<td>3090 ± 1150 (6)</td>
<td>3110 ± 1350 (6)</td>
</tr>
</tbody>
</table>

**Fig. 6.** Silicic acid column chromatography of phospholipid extract from 500 mg of intestinal mucosa. The conditions are described in the text. Identification of peaks: I (30 to 50 ml), unidentified; II (50 to 85 ml), cardiolipin, phosphatidic acid; III (65 to 90 ml), unidentified; IV (110 to 150 ml), phosphatidylethanolamine; V (150 to 180 ml), phosphatidylserine; VI (180 to 230 ml), phosphatidylglycerol, VII (250 to 350 ml), lecithin.

**Table IV**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Vitamin D-deficient</th>
<th>Vitamin D-fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8.3 ± 2.8</td>
<td>7.9 ± 2.5</td>
</tr>
<tr>
<td>II</td>
<td>6.4 ± 1.4</td>
<td>5.1 ± 1.3</td>
</tr>
<tr>
<td>III</td>
<td>8.9 ± 1.9</td>
<td>7.5 ± 1.3</td>
</tr>
<tr>
<td>IV</td>
<td>25.2 ± 4.5</td>
<td>26.7 ± 4.7</td>
</tr>
<tr>
<td>V + VI</td>
<td>13.2 ± 4.3</td>
<td>13.4 ± 3.0</td>
</tr>
<tr>
<td>VII</td>
<td>38.0 ± 4.3</td>
<td>39.4 ± 3.6</td>
</tr>
</tbody>
</table>

**Fig. 7.** Specific activity of phosphatide Components I to VII (as shown in Fig. 6) from intestinal mucosa samples that were incubated for 60 minutes. Solid black columns, vitamin D-fed; cross-hatched columns, vitamin D-deficient. The height of each column is the mean of four values.

of the sensitivity of the determination. Phosphatidylethanolamine (IV) and lecithin (VIII) constitute 65% of the phospholipid.

Fig. 7 shows the specific activities ([counts per minute per mg of lipid] × 10^(-2)) of the individual phosphatides from samples which had been incubated for 60 minutes. In every case except one (Peak I), vitamin D increased the specific activity approximately 3-fold. The small amount of radioactivity present in Peak I was thought to be contamination from Peak II. Thus it appeared that vitamin D, by increasing the incorporation of ^32P into each of the phospholipids, has a general effect on phospholipid metabolism rather than influencing a single compound.

The pattern of ^32P incorporation into the individual phosphatides differed widely. The incorporation into Peak II was rapid initially and leveled off; the specific activity of phosphatidylethanolamine increased linearly throughout the 1 hour of incubation; and lecithin was labeled very little during the first 20 minutes of incubation, but increased rapidly during the remaining 40 minutes. These patterns are similar to those observed by Karnovsky and Wallach (14).

**DISCUSSION**

The results show that vitamin D stimulates the incorporation of ^32P into the phospholipids of intestinal mucosa. The observance of a stimulation only 3 hours after the administration of 2,000 i.u. of vitamin D is of considerable importance. This is the earliest time at which a response to vitamin D has been observed when a dose of this magnitude has been given. No effect of vitamin D on calcium transport in everted intestinal sacs was detected 4 hours after administering 1,000 i.u. (4); however, Schachter has reported an increase in calcium transport only 1 hour after a 50,000-i.u. dose of vitamin D (29). Doses of this magnitude have not been studied with respect to phospholipid metabolism.

Attempts to stimulate the incorporation of ^32P into lipid by the addition of vitamin D in vitro were unsuccessful; however, other workers have also failed to produce effects in vitro when whole cell systems were involved (6). Effects in vitro have been produced in mitochondrial systems used for studying citrate oxidation (14) and calcium translocation (10).

The incorporation of ^32P into the phospholipids is totally dependent on oxidative metabolism. Anaerobiosis completely prevented labeling of the lipids. This is in agreement with observations of other investigators (30, 31). The effects of the
inhibitors agree closely with those found by Strickland (30). It is also of interest to note that at a concentration of $10^{-8}$ M, no inhibition of phospholipid labeling in brain slices by 2,4-dinitrophenol was observed by Strickland, while this concentration did stimulate oxidation. At concentrations of $10^{-4}$ and $10^{-3}$ M, dinitrophenol did inhibit the labeling of phospholipid in brain slices.

Several different compounds have been reported to stimulate $^{32}$P incorporation into the phospholipids of various tissues: acetylcholine in salt gland and brain slices (9, 32), potassium in brain slices (32), estrogen in the uterus (33), and insulin in rat diaphragm (34). The stimulation in the small intestine due to vitamin D appears to be unique in the magnitude of the effect and the number of compounds affected. The only other general effect reported was that of estradiol in the uterus, and this appeared to be due to a large increase in the net synthesis of phospholipid. A stimulation of the incorporation of $^{32}$P occurs in the system due to vitamin D appears to be unique in the magnitude of the effect and the number of compounds affected. The only other general effect reported was that of estradiol in the uterus, and this appeared to be due to a large increase in the net synthesis of phospholipid.

Several experiments were tried in an attempt to examine the effect of vitamin D on phospholipids in subcellular systems, but these were not successful. Homogenates, mitochondria, and mitochondrial supernatant of intestinal mucosa failed to incorporate $^{32}$P in any of the systems tried. Incorporation into kidney and liver mitochondria did occur, but the major phospholipids did not become labeled. Other in vitro experiments (35, 36) have reported the inability of homogenates, mitochondrial, and microsomal systems to bring about an appreciable incorporation of $^{32}$P into lecithin and phosphatidylethanolamine, yet considerable incorporation did occur in the polyglycerol phosphatides and phosphatidic acids. In any case, no difference due to vitamin D was observed in the incorporation into kidney and liver mitochondria.

Because the stimulation by vitamin D in the intestine and kidney, but not in the liver, this phospholipid effect may be a primary action of vitamin D, possibly directly related to calcium transport. The lack of an effect due to calcium in the medium is not particularly disturbing to this postulation. The effect on the phospholipids may not require the presence of calcium, or, if calcium is required, sufficient calcium may have been present in the tissue.

**SUMMARY**

The addition of vitamin D to the diet of rats caused a 3-fold increase in the incorporation of $^{32}$P-orthophosphate into the phospholipids of intestinal mucosa, while the incorporation into nuleolipid organic phosphate was not altered by the vitamin. Similar, but much smaller, stimulation of $^{32}$P incorporation into the lipids of kidney slices, but not of liver slices, was observed. The incorporation of $^{14}$C-labeled glycerol and -serine into phospholipids of intestinal mucosa was not stimulated by vitamin D, nor was the total amount of phospholipid present in intestinal mucosa altered by vitamin D. A stimulation of the incorporation of $^{32}$P into the lipids could be observed 3 hours after the administration of 2000 i.u. of vitamin D. The effect of the vitamin was not dependent upon the presence of calcium in the medium.

The incorporation of $^{32}$P into the phospholipids was totally dependent on oxidative metabolism. Complete inhibition was caused by anaerobicosis, or by the addition of cyanide, Dicumarol, antimony A, and phlorizin to the medium, and partial inhibition was caused by geomicidin and oligomycin. Dinitrophenol at a concentration of $2 \times 10^{-4}$ M failed to inhibit.

Silicic acid column chromatography of the intestinal mucosa phospholipids indicated that vitamin D increased the specific activity of each of the phosphatides. Further identification of the phosphatides was accomplished by thin layer chromatography. The major phosphatides were found to be lecithin and phosphatidylethanolamine, comprising 30 and 26% of the total phospholipid, respectively.

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