Effects of Vitamin D on Phosphate Transport and Incorporation into Mucosal Constituents of Rat Intestinal Mucosa*

SZLOMA KOWARSKI AND DAVID SCHACHTER

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

SUMMARY

The effects of vitamin D on transport of inorganic phosphate into and across rat intestinal mucosa in vitro were studied, and the results used to interpret observations on incorporation of 32P-labeled phosphate into mucosal lipid, protein, and nucleic acids. Vitamin D given to intact rats increases phosphate transport in vitro selectively in the direction mucosa to serosa. The sterol in vivo acts directly in the intestine with no prior activation in other organs. 32Pi transport is via restricted channels in the mucosa, as indicated by the observation that the specific radioactivity of transported 32P-labeled phosphate is miscible with approximately one-third or less of the mucosal Pi pool. Because of this compartmentalization specific radioactivities of individual mucosal precursor pools of 32P, are difficult or impossible to estimate. Consequently, effects of vitamin D on incorporation of 32P-labeled phosphate into mucosal constituents cannot be interpreted definitively in terms of rates of synthesis. The relative effects of vitamin D on calcium as compared to phosphate transport in various segments of intestine, and other evidence, suggests that the sterol influences separately these two intestinal transport mechanisms.

Vitamin D is required for normal intestinal absorption of calcium (1), and in recent years a number of investigators have obtained evidence that the sterol is required to maintain a specific calcium transport mechanism in the small intestine (2-5). The calcium transport is via an active cation pump which can be shown directly in vitro with a specific calcium electrode (6) or inferred from experiments in vivo (7). Vitamin D is also required for optimal transport of inorganic phosphate by rat intestinal segments in vitro (8, 9).

Inasmuch as the biochemical basis for the preceding effects remains unknown, a report by Thompson and DeLuca (10) that prior treatment with vitamin D markedly increases incorporation of 32P into the phospholipids of rat intestinal mucosa in vitro was of considerable interest. Unfortunately, the authors did not estimate the specific radioactivity of 32P in the mucosal precursor pool. Hence their results do not distinguish between absolute increases in rates of biosynthesis of phospholipid-phosphate versus increased transport of 32Pi into the mucosa and higher specific radioactivity of the precursor pool. The present experiments were designed, therefore, to examine these alternatives. In addition, a number of features of the phosphate transport mechanism were studied further in relation to vitamin D and to calcium transport. It was established that vitamin D acted directly in the intestine to increase phosphate absorption, and biochemical evidence was obtained to show that the transport occurs across restricted channels in the mucosa.

MATERIALS AND METHODS

Animals—To induce vitamin D deficiency, weanling albino male rats, 40 to 50 g, of the Sherman strain, were maintained on the U.S.P. Rachitogenic diet No. 2 (Nutritional Biochemicals) for 4 to 5 weeks. Cages were shielded from light. Vitamin D repletion was with 20,000 i.u. of vitamin D3 given subcutaneously in ethanol-propylene glycol (1:5) 18 hours before experiments. Rats on the diet above, as well as normal males, 100 to 200 g, maintained on a stock laboratory diet (11), were fasted for 18 hours in metabolism cages with free access to water before experiments.

Gut Sac Experiments—In typical experiments from five to seven rats were killed and a single everted sac, approximately 5 cm long, was prepared from the proximal duodenum or the midjejunum (or both) of each rat as previously described (2). The serosal compartments were filled with 0.5 ml of incubation medium composed of Krebs-Ringer bicarbonate (12) containing 20 mm fructose and no phosphate or CaCl2. Groups of five to seven sacs were suspended together in flasks containing 20.0 ml

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of the incubation medium. Potassium phosphate, pH 7.4 (final concentration 1.2 mm), trace quantities of NaH$_2$PO$_4$ and CaCl$_2$ (final concentration 1.6 mm) were added to the medium bathing the mucosal surfaces, and the sacs were then agitated gently at 37° in an atmosphere of 95% O$_2$-5% CO$_2$ with constant gassing. At appropriate times sacs were removed, drained, and the mucosal tissue was scraped from the underlying coats with a spatula (11). Mucosal scrapings were weighed and homogenized in 5 volumes of ice-cold 145 mm NaCl-4 mm KCl.

**Chemical Estimations**—To estimate the specific radioactivity of $^{32}$P$_1$, samples of medium or homogenate were treated with 3% TCA$^1$ for 5 min at 2-5° and centrifuged for 5 min in a clinical centrifuge. The supernatant solutions were immediately neutralized with 1.0 n NaOH and samples were taken for precipitation of inorganic phosphate by magnesium mixture (13). The final precipitates were washed, dissolved in 10 n H$_2$SO$_4$, and aliquots were taken for estimation of phosphate by the method of Fiske and Subbarow (14). Samples were also added to Bray's solution (15) and the $^{32}$P counted in a liquid scintillation spectrometer. (Corrections for quenching, which amounted to less than 5%, were not necessary.) For transport studies, 5% TCA extracts of the media were prepared and phosphate was estimated as described above (14); calcium was estimated by titration with magnesia mixture. The values plotted are means for three experiments and are expressed relative to the specific radioactivity of the initial mucosal medium. Range of values was ±12.5% or less.

**FIG. 1. Effect of vitamin D on specific radioactivity of $^{32}$P$_1$ in duodenal mucosa.** Groups of five to seven duodenal sacs were incubated as described in the text with $^{32}$P$_1$-labeled phosphate added to the mucosal medium alone initially. Mucosa was subsequently scraped from the underlying coats, homogenized, and P$_1$ precipitated with magnesium mixture. Values plotted are means for three experiments and are expressed relative to the specific radioactivity of the initial mucosal medium. Range of values was ±12.5% or less.

**Incorporation of Inorganic Phosphate**—In initial experiments the effects of vitamin D on the specific radioactivity of mucosal inorganic phosphate, the precursor pool for incorporation into phospholipid and other constituents, was examined. Everted sacs of duodenum prepared from vitamin D-deficient and -repleted rats were incubated in Krebs-Ringer bicarbonate (see "Materials and Methods") with $^{32}$P$_1$ in the mucosal medium alone initially. At various intervals five or more sacs were removed, drained, and the mucosa was scraped off and homogenized. Specific radioactivity of the mucosal $^{32}$P$_1$ was estimated after precipitation with magnesium mixture (13) and expressed relative to the initial values in the mucosal medium. Results were similar in each of three experiments, and the mean values are plotted in Fig. 1. After 20 min of incubation, the specific radioactivity in the vitamin-treated segments was approximately twice that in the depleted ones. Thereafter, values for the deficient sacs approached and finally equalled those for repleted intestine at approximately 60 min. The mean values for specific radioactivity in the 0- to 60-min interval were equal to the final values at 60 min times 0.93 for vitamin-repleted or 0.83 for vitamin-deficient segments. These factors, estimated from the curves in Fig. 1 and reproducible within 12%, were subsequently used to calculate the mean specific radioactivity of the precursor pools in the following experiments.

To determine the incorporation of $^{32}$P$_1$-labeled phosphate into mucosal phospholipids, duodenal sacs prepared from depleted and treated rats were incubated for 1 hour as described above; the mucosa was scraped, homogenized, and samples were taken for extraction of phospholipids and for precipitation of inorganic phosphate. Both $^{32}$P and total phosphate were estimated in all fractions, and the results of four experiments are summarized in Fig. 2. The total pool of mucosal phospholipid phosphate was identical, within experimental error, for vitamin-depleted and -treated segments. In agreement with Thompson and DeLuca (10), incorporation of $^{32}$P$_1$-labeled phosphate into phospholipid, not corrected for specific radioactivity of the precursor pool, was increased by vitamin D in each experiment, with a mean increment of 32.0%. (In eight further observations similar

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$^1$ The abbreviation used is: TCA, trichloroacetic acid.
results were obtained for an over-all significance of \( p < 0.01 \). However, the incorporation of total phosphate into phospholipid, calculated as \( (32P_i \text{ incorporated})/(\text{mean specific radioactivity of mucosal } 32P_i) \), was in each experiment decreased by vitamin D \( (p < 0.01) \). When expressed in absolute units (micromoles of phosphate incorporated per g of mucosa per hour), vitamin D decreased the incorporation by 31.9\% (range, 24.5 to 40.2\%), and when expressed as a fraction of the mucosal lipid phosphate pool, vitamin D decreased incorporation by 31.9\% (range, 18.7 to 42.7\%). Very similar results were obtained in parallel observations with everted sacs of jejunum. Jejunum differed from duodenum only in more rapid uptake of \( 32P_i \) from the ambient mucosal medium, both by vitamin D-deficient and -repleted segments (see below).

Effects of vitamin D on incorporation of \( 32P_i \)-labeled phosphate into mucosal constituents other than phospholipid were studied with duodenal sacs incubated for 1 hour as described above. Mucosal homogenates were then prepared and phospholipid, total TCA-precipitable material, total nucleic acid, and protein fractions were obtained (see "Materials and Methods"). The incorporation for each fraction was expressed as the ratio (specific radioactivity \( 32P_i \)-labeled phosphate in fraction)/(mean specific radioactivity of \( 32P_i \) in mucosa). Vitamin D consistently lowered the ratio for all fractions studied, with mean decreases of 34.8\% (range, 23.8 to 42.8, four experiments) for lipid phosphorus, 51.1\% (range, 49.8 to 57.1, five experiments) for TCA-precipitable material, 44.5\% (range, 19.5 to 66.6, four experiments) for nucleic acids, and 44.8\% (range, 26.7 to 62.7, four experiments) for proteins. The effect of vitamin D in lowering these ratios was significantly greater for TCA-precipitable material than for lipid phosphorus \( (p < 0.01) \). Although the results could be interpreted to mean that vitamin D decreases the rate of incorporation of phosphate into major constituents of intestinal mucosa, two further lines of evidence suggest an alternative explanation. Incorporation of \( ^{14}C \)-uridine into mucosal nucleic acids and of \( ^{1}C \)-L-leucine into mucosal proteins were examined with duodenal gut sacs from vitamin-deficient and -repleted rats incubated with the \( ^{14}C \)-precursors added to the mucosal medium. No significant effect of the vitamin either on mucosal uptake or incorporation of \( ^{14}C \)-uridine or \( ^{1}C \)-L-leucine was found in three experiments. Evidence will be described below which indicates that \( 32P_i \) transport dependent on vitamin D occurs via restricted channels in the intestinal mucosa. If nonequilibrating or partially equilibrating pools of \( P_i \) exist in the mucosa, values for the over-all specific radioactivity of mucosal \( 32P_i \) are clearly not valid for specific precursor pools. No conclusions concerning effects of vitamin D on rates of incorporation of phosphate seem justified, therefore, until methods are available to assay the specific precursor pools.

**Vitamin D and Phosphate Transport**—Prior investigations have shown that optimal intestinal transport of phosphate in *vivo* is dependent on vitamin D, occurs maximally in jejunal segments, and has the features of an active transport (8, 9). Inasmuch as vitamin D also influences calcium transport, it was pertinent to ask whether the vitamin has two separate actions or only one, and whether the phosphate and calcium transfer mechanisms are coupled to each other. Accordingly, initial experiments were designed to explore similarities or differences in the action of vitamin D on calcium as compared to phosphate transport. A proximal duodenal and midjejunal everted sac was prepared from each of five to seven vitamin D-deficient or -repleted rats. In six experiments, groups of sacs were incubated with potassium phosphate, \( 32P_i \), and \( \text{CaCl}_2 \) in the mucosal medium alone initially; net transfer of phosphate and calcium to the serosal surface \( (S_{net}) \) and uptake and unidirectional transfer of phosphate at the mucosal surface \( (M_{net}) \) were estimated as described under "Materials and Methods." In three additional experiments, groups of duodenal sacs from deficient and repleted rats were incubated with potassium phosphate and \( 32P_i \)-labeled phosphate added initially to the serosal medium alone. \( S_{net}, m_{+}, s_{-} \), and \( s_{+} \) for phosphate were determined, and the results of all nine experiments are summarized in Table I. Similar to its effects on calcium transport (19) vitamin D increased net uptake of phosphate at the mucosal surface and net transport to the serosal surface, and the effects resulted entirely from increases in \( m_{+} \) and \( s_{+} \) with no significant change in \( m_{-} \) or \( s_{-} \). Thus, as for calcium, the vitamin acted specifically on transfers in the direction mucosa to serosa. (Net movement of phosphate at the mucosal surface was out of the tissue, as previously reported (20), in both vitamin-deficient and -repleted segments. Net uptake into mucosa has, however, been observed in the presence of elevated concentrations of \( K^+ \) (5).) The effects of the vitamin on calcium versus phosphate transport differed markedly, however, in jejunal as compared to duodenal segments. Thus the vitamin-dependent increments in \( S_{net} \) for phosphate and calcium, respectively, were \( +0.60 \) and \( +0.50 \) pmole per gut sac for duodenum (ratio \( \Delta \text{phosphate to } \Delta \text{calcium} = 1.2 \)) whereas the corresponding values for jejunum were \( +0.84 \) and \( +0.12 \) (ratio \( \Delta \text{phosphate to } \Delta \text{calcium} = 7.0 \)).
TABLE I

Effects of vitamin D on phosphate and calcium transport across duodenal and jejunal gut sacs

Gut sacs were prepared and incubated as described in the text. Transport parameters are defined under "Materials and Methods."

<table>
<thead>
<tr>
<th>Transport mechanism</th>
<th>Transfer across duodenal sacs</th>
<th>Transfer across jejunal sacs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin D-deficient (a)</td>
<td>Vitamin D-repleted (b)</td>
</tr>
<tr>
<td></td>
<td>µmoles/sac/hr</td>
<td>µmoles/sac/hr</td>
</tr>
<tr>
<td>For phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta P )</td>
<td>0.73</td>
<td>2.06</td>
</tr>
<tr>
<td>( \Delta \mu )</td>
<td>3.78</td>
<td>4.03</td>
</tr>
<tr>
<td>( \Delta S )</td>
<td>-3.05</td>
<td>-1.97</td>
</tr>
<tr>
<td>( \Delta S_{\text{net}} )</td>
<td>1.21</td>
<td>+1.81</td>
</tr>
<tr>
<td>For calcium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \Delta P )</td>
<td>0.43</td>
<td>0.03</td>
</tr>
<tr>
<td>( \Delta \mu )</td>
<td>1.16</td>
<td>1.47</td>
</tr>
<tr>
<td>( \Delta S )</td>
<td>0.68</td>
<td>0.73</td>
</tr>
<tr>
<td>( \Delta S_{\text{net}} )</td>
<td>0.48</td>
<td>+0.74</td>
</tr>
</tbody>
</table>

The results seem best explained by separate actions of the vitamin on two distinct transport mechanisms. Additional evidence that the mechanisms are distinct includes the following. Phosphate transport was greater in jejunum as compared to duodenum (Table I), both in vitamin-deficient and -repleted rats, and significant differences were noted in \( \Delta P \), \( \Delta \mu \), \( \Delta S \), and \( \Delta S_{\text{net}} \) (\( p < 0.001 \)). Under standard incubation conditions, e.g., Table I, \( \Delta S_{\text{net}} \) in duodenum is negative for phosphate but positive for calcium (3). Prior studies have shown that marked variations in phosphate concentration influence calcium transport across duodenal sacs in vitro very little (20). Finally, the effects of calcium on phosphate transport were studied by incubating groups of duodenal sacs from vitamin-deficient and -repleted rats with 1.2 mM potassium phosphate, trace \( ^{32}P \) and calcium, and with or without 1.6 mM CaCl\(_2\) added to the mucosal medium. In three experiments no significant effect of added calcium on phosphate transport was observed with either vitamin-depleted or -repleted segments.

Channelized Phosphate Transport—In the experiments illustrated in Fig. 1 the specific radioactivity of \( ^{32}P \) was estimated at various times in the mucosa and in the media bathing the mucosal and serosal surfaces of the everted duodenal sacs. Sacs from vitamin-deficient and -repleted rats were incubated with \( ^{32}P \)-labeled phosphate added initially to the mucosal medium alone. The values for relative specific radioactivity in all three compartments are illustrated in Fig. 3. Throughout 60 min of incubation, values for the mucosal medium, although declining, remained considerably above those in the other compartments. In vitamin-repleted sacs values for mucosal tissue were approximately constant in the 20- to 60-min interval, whereas those for the serosal medium increased progressively to exceed the mucosal tissue values by approximately 80% at 1 hour. The results indicate that \( ^{32}P \) transported across the mucosa was miscible with only a portion of the tissue \( P \), i.e., phosphate transport was via restricted channels. Results with the vitamin-deficient sacs were similar except that the specific radioactivity of \( ^{32}P \) in the serosal medium exceeded that in the mucosal tissue by only 24% at 1 hour. The fraction of mucosal \( P \) which is miscible with \( ^{32}P \) transported across the mucosa can be calculated as \( A_m/A_s \), where \( A_m \) is the mean specific radioactivity of \( ^{32}P \) (counts per min per pmole) over a given time in

An increase in calcium transport across duodenal sacs in vitro on addition of \( Ca^{2+} \) to the medium has been described by Helbock, Forte, and Saltman (21). Their results are not directly comparable to the present studies inasmuch as they used older rats weighing 400 g. We have demonstrated (3) that calcium transport is quite depressed in these older animals, and the active transport is often not detectable. The effects of phosphate might then result from complexing of calcium ion with resultant changes in passive permeability and over-all flux rate.
mucosal tissue, and $A$ is the corresponding value for specific radioactivity of $^{32}\text{Pi}$ transported across the mucosa. Whereas $A_m$ can be determined directly, $A$ can be estimated only indirectly by sampling the serosal medium. The specific radioactivity in the serosal medium is less than true $A$, because endogenous, nonradioactive Pi is transferred from mucosal cells, particularly early in the course of incubation, and probably from other cells of the gut wall. Moreover, some equilibration of transported $^{32}\text{Pi}$ labeled phosphate with pools in the submucosal gut layers seems probable. Accordingly, two methods of approximating $A$ were used. In the first, $A$ was defined for the increment in $^{32}\text{Pi}$ transport dependent on vitamin D, according to the relation

$$A = \frac{P_d - P_e}{(P_d - P_e) / (A_d / A_e)}$$

Here $P_d$ and $P_e$ equal the $^{32}\text{Pi}$ (counts per min) transferred to the serosal surfaces of vitamin-repleted and -deficient sacs, respectively, incubated in parallel. $(P_d$ and $P_e$ were found to equal total $^{32}\text{Pi}$ in the serosal media by isotope dilution experiments. Alqouts of the media were mixed with large quantities of carrier inorganic phosphate and precipitated with magnesia mixture. The final specific radioactivity was estimated and indicated that essentially all the radioactivity was precipitable.) $A_d$ and $A_e$ equal the specific radioactivity of $^{32}\text{Pi}$ (counts per min per pmole) in the serosal media of the vitamin-repleted and -deficient sacs, respectively. These calculated values of $A$ should be independent of possible errors, e.g. equilibration with submucosal gut layers, common to vitamin-deficient and -repleted segments. Values for the fraction of mucosal Pi miscible with transported $^{32}\text{Pi}$, i.e. $A_m/A$, where $A_m$ was determined for vitamin-repleted sacs and $A$ was calculated as above, ranged from 0.17 to 0.29 in four experiments (Table II). A second method of calculating $A$ was based on the observation (Fig. 3) that the specific radioactivity of $^{32}\text{Pi}$ in serosal fluid increased progressively with time of incubation, indicating that dilution of transported $^{32}\text{Pi}$ was greatest in the early time periods. Consequently $A$ was calculated for the increment in transport of $^{32}\text{Pi}$ with time, i.e.

$$A = \frac{(P_d - P_t)}{(P_d - P_t) / (A_d / A_t)}$$

where subscripts 1 and 2 refer to serosal media obtained at successive time points, and $P$ and $A$ are defined in the preceding calculation. Values for $A_m/A$ calculated for vitamin-depleted and -repleted segments ranged, respectively, from 0.30 to 0.68 and 0.22 to 0.33 in three experiments. Finally, Table II also lists values for $A_m/A$ calculated for experiments in which net transport of $^{32}\text{Pi}$ labeled phosphate was from serosa to mucosa. Duodenal sacs from vitamin-deficient and -repleted segments ranged, respectively, from 0.30 to 0.68 and 0.27 to 0.36.

Direct Action of Vitamin D on Phosphate Transport—A direct effect of vitamin D added in $\textit{vitro}$ on calcium transport across vitamin-deficient intestinal segments has not been established. Nonetheless, an experimental method was devised (22) to show that the sterol acts directly in the duodenum to affect calcium transport, without prior activation in other organs. In the following experiments a similar method was used to show a direct intestinal action on phosphate transport. Rats depleted of vitamin D were anesthetized with ether, and two midjejunal segments, separated by 3 cm of gut, were used to prepare closed loops, each about 5 cm long. Loops were washed clean with isotonic NaCl and in half the animals adjacent loops were filled with 0.25 ml of a control solution, 0.004 K sodium taurocholate in 0.85% NaCl. In the remaining rats one loop was filled with the control solution and the other with the same containing 400 i.u. of vitamin D; vitamin was instilled into the proximal or distal loop alternately in successive animals. (Vitamin D$_3$ was added to the control solution in a minimal amount of ethanol, and the final preparation was a clear micellar solution. Identical amounts of ethanol were added to the solution for control loops.) Abdominal incisions were closed and rats awoke approximately 10 min after onset of anesthesia. After 5 hours the loops were removed, washed, everted, a single sac prepared from each, and groups of seven sacs were incubated in $\textit{vitro}$ to study $^{32}\text{Pi}$ transport from mucosa to serosa ("Materials and Methods").

If vitamin D acts directly in the intestine, a greater increment in phosphate transport should be observed in the jejunal loop filled with the sterol as compared to the adjacent loop in the same rat. If the vitamin must first be absorbed and its action initiated in another organ, both loops, where one has been filled with vitamin D, should show similar increments in transport.

<table>
<thead>
<tr>
<th>Direction of transfer</th>
<th>Mode of calculation</th>
<th>Time interval (min)</th>
<th>$^{32}\text{Pi}$ transferred to mucosa (A time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosa to serosa</td>
<td>$\Delta$ vitamin D</td>
<td>0-60</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-60</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-80</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0-80</td>
<td>0.17</td>
</tr>
<tr>
<td>$\Delta$ time</td>
<td>Vitamin-deficient</td>
<td>40-80</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40-80</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Vitamin-repleted</td>
<td>40-80</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20-60</td>
<td>0.33</td>
</tr>
<tr>
<td>Serosa to mucosa</td>
<td>$\Delta$ time</td>
<td>30-60</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Vitamin-deficient</td>
<td>30-60</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-60</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>Vitamin-repleted</td>
<td>30-60</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Table II: Fraction of mucosal pool of inorganic phosphate miscible with inorganic $^{32}\text{Pi}$ transported across duodenal segments in $\textit{vitro}$

Values listed are for $A_m/A$, which is calculated and defined in the text. Values calculated from the increment in phosphate transport with vitamin D are listed as $\Delta$ vitamin D, and those calculated from the increment in net transport with time are listed as $\Delta$ time.
Differences between left- and right-hand values are indicated. Units for $S_{net}$, $m_1$, and $m_2$ are micromoles per gut sac per hour. $S/M$ refers to the final concentration ratios serosal medium to mucosal medium.

as compared to rats receiving control solution alone. Fig. 4 illustrates the results of four experiments in which the effects on five parameters of phosphate transport were expressed as absolute differences between adjacent loops. Direct instillation of vitamin D significantly increased phosphate transport as compared with the adjacent control loop. The final concentration ratios serosal medium to mucosal medium for $32P$ ($p < 0.01$) and phosphate ($p < 0.02$), as well as $S_{net}$ ($p < 0.01$) and $m_1$ ($p < 0.01$) and $m_2$ ($p < 0.01$) were increased, whereas $m_2$ was not significantly affected, in accord with results above. Moreover, the control loops adjacent to vitamin D-filled segments showed no significant increase in phosphate transport as compared to control loops in rats given no vitamin D.

**DISCUSSION**

The experimental results indicate that $32P_1$ transported across rat intestinal mucosa is miscible with only one-fifth to one-third of the mucosal pool of $32P$, i.e. phosphate transport is via restricted channels or compartments. Before considering the implications for studies of incorporation of $32P$-labeled phosphate, one possible objection to this conclusion should be discussed. It might be argued that the relatively low values observed for specific radioactivity of $32P_1$ in mucosal tissue, as estimated in homogenates by precipitation with magnesia mixture, results from breakdown of very labile esterified phosphate in the course of the chemical estimation. Although efforts were made to avoid this by using only 3% TCA, maintaining the acid-treated mixtures at 2-5°C, and working as rapidly as possible, the possibility of breakdown exists. However, the following quantitative considerations indicate that the results cannot be explained wholly in this way. In the experiments with vitamin D-repleted duodenal sacs illustrated in Figs. 1 and 3, the average values over 1 hour of incubation for total $32P$-labeled phosphate in mucosal tissue (counts per min per g of mucosa) and mean specific radioactivity of $32P$, in serosal fluid and in mucosal tissue (counts per min per mole of phosphate), respectively, are $25 \times 10^4$, $5 \times 10^4$, and $0.9 \times 10^4$. Thus the quality of hypothetical labile ester phosphate in mucosa which would have to break down to dilute the specific radioactivity of serosal fluid to the value observed for mucosa is: $(25 \times 10^4)(0.9 \times 10^4) - (25 \times 10^4)(5 \times 10^4) = 23 \mu$moles per g of mucosa. Now, small intestine contains a total of approximately 32 $\mu$moles of phosphorus per g (23), of which 6.5 is nucleic acid phosphate (23), 7.0 is lipid phosphorus (see above), and at least 5.0 is present as $P_1$ in the experiments under discussion. (The last value is calculated from the total counts per min of $32P$-labeled phosphate in the mucosa and the mean specific radioactivity of $32P$ in the mucosal medium during incubation. Isotope dilution studies with magnesium mixture indicated that >90% of the mucosal $32P$-labeled phosphate was precipitable in these studies.) Therefore, the mucosal phosphate available as hypothetical labile ester would not exceed $32 - (6.5 + 7.0 + 5.0) = 13.5 \mu$moles per g, clearly inadequate to explain the results. Moreover, it seems improbable that all the mucosal phosphate not accounted for in the three pools listed above would be in this labile pool. We conclude, therefore, that the possibility of breakdown of tissue extre phosphate does not invalidate the general conclusion of channelization of phosphate transport, although it might influence the size of the channels as estimated above.

The present studies do not identify the site of the phosphate channels. They could presumably be within or between mucosal cells generally, or they might represent a specialized subpopulation of mucosal cells along the villus. Likewise as the tips of the villi might have preferential access to materials in the luminal bathing media, it is noteworthy that channelization of phosphate transport was also observed when the transfer was from serosal surface to mucosal surface.

The demonstration that $P_1$ is compartmentalized in the mucosa implies that the specific radioactivity of $32P_1$ estimated for the mucosa as a whole need not be representative of individual precursor pools for incorporation into specific cellular constituents. We are unable, therefore, to conclude that vitamin D influences directly the rates of incorporation of $32P$-labeled phosphate into mucosal constituents. Although we could confirm the observation of Thompson and DeLuca (10) that the vitamin increases total incorporation of $32P$-labeled phosphate into phospholipid, our results indicate this could be secondary to increased mucosal uptake and a greater specific radioactivity of precursor $32P$-labeled phosphate. Indeed, vitamin D decreased the ratio of specific radioactivity in lipid-phosphorus to specific radioactivity in mucosal $32P_1$ as well as the corresponding ratios for total TCA-precipitable material, nucleic acids, and proteins. The decreases can be ascribed to compartmentalization of phosphate transport, i.e. the increment in mucosal $32P$-labeled phosphate with vitamin D is not necessary available as precursor for incorporation. This seems reasonable, particularly because the
total incorporation of $^{32}$P-labeled phosphate into nucleic acid, protein, or total TCA-precipitable material was not influenced by the vitamin when expressed relative to tissue weight, and no differences in incorporation of $^{14}$C-uridine or $^{14}$C-leucine were noted. However, total incorporation of $^{32}$P-labeled phosphate into lipid phosphate was increased by vitamin D when expressed relative to either tissue weight or incorporation into total TCA-precipitable material. Although in accord with an increased rate of synthesis of lipid phosphate, this observation, too, could result from channelized transport, on the reasonable assumption that the phosphate channels are lined with membrane containing phospholipid, e.g., the endoplasmic reticulum. Hence the increased mucosal $^{32}$P$_1$ resulting from the action of vitamin D might yield additional precursor for phospholipid but not for nucleic acid synthesis.

Although the biochemical mechanism by which vitamin D increases intestinal transport of phosphate remains unknown, the effect seems to be distinct from that on calcium transport. Calcium transfer is a cation, active transport (3, 6) is maximal in the duodenum (3), and relatively independent of phosphate added in vitro (20). Phosphate transport is maximal in the jejunum (8) and relatively independent of calcium added to the medium, as demonstrated above. Moreover, the effects of vitamin D on calcium as compared to phosphate transfer are approximately equal in duodenum, but much greater on phosphate in jejunum. Finally, recent studies have shown that the effect of vitamin D on calcium transport is associated with the appearance of a calcium-binding protein in intestinal mucosa of chickens (24) and rats (6). In rats the calcium-binding protein activity also correlates with the activity of the calcium transport mechanism as a function of distribution in the intestine (i.e., mainly in duodenum and quite low in more distal intestine), age of the rat, pregnancy, and level of dietary calcium (6). If the effect of vitamin D on phosphate transport were secondary to its effect on calcium transfer, as proposed by Harrison and Harrison (8), one might predict, on the basis of the distribution of the calcium-binding protein, rather little influence on phosphate transport in jejunum as compared to duodenum. Yet the observed effects are approximately equal in the two segments. The evidence of the prior authors (8) that EDTA decreases the increment in phosphate transport in vitro observed with vitamin D seems inconclusive, because the chelating agent is known to inhibit other intestinal transport mechanisms, e.g., uptake of the vitamin B$_12$-intrinsic factor complex (25), and may act nonspecifically.

The present results demonstrate that the actions of vitamin D on calcium and phosphate transport are similar in at least two ways. In both mechanisms the sterol increases selectively the unidirectional transfer from mucosa to serosa, and the vitamin acts directly in the intestine without prior activation in another organ. Further studies are needed to determine if the action on phosphate transport, like that on calcium transfer, is associated with the biosynthesis or maintenance of a specific protein (or proteins) which may play an essential role in the mechanism.

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Effects of Vitamin D on Phosphate Transport and Incorporation into Mucosal Constituents of Rat Intestinal Mucosa

Szlama Kowarski and David Schachter


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