Vitamin K₁ intestinal absorption in vivo: influence of luminal contents on transport

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Hollander, Daniel, Elena Rim, and K. S. Muralidhara. Vitamin K₁ intestinal absorption in vivo: influence of luminal contents on transport. Am. J. Physiol. 232(1): E69-E74, 1977 or Am. J. Physiol.: Endocrinol. Metab. Gastrointest. Physiol. 1(1): E69-74, 1977. — Intestinal absorption of [³H]phylloquinone was investigated in the unanesthetized rat by the use of a technique of recirculating perfused isolated intestinal segments. Apparent saturation kinetics were found as the concentration of the vitamin in the perfusate was increased in a stepwise fashion from 15 nM to 300 μM. Alkalization of the perfusate or the addition of 2.5 mM linoleic acid to the perfusate caused a significant (P < 0.05) decrease in the absorption rate of phylloquinone. Modulations in the perfusate concentration of sodium taurocholate, the substitution of a nonionic detergent (Pluronic F-68) for sodium taurocholate, the addition of medium- and long-chain saturated fatty acids, or the addition of vitamins K₂ and K₃ to the perfusate did not alter the absorption rate of the vitamin. Decreasing the thickness of the unstirred water layer by increasing the perfusion rate caused a significant increase in phylloquinone absorption rate. In vivo absorption of vitamin K₁ appears to be mediated by an energy requiring saturable transport mechanism. The composition of the perfusate, its pH, and its rate of flow are all important determinants of vitamin K₁ absorption rate.

MATERIALS AND METHODS

Materials. 12-³Hmethylphylloquinone (41.4 μCi/μmol) and nonradioactive phylloquinone (Nutritional Biochemicals Corp., Cleveland, Ohio) were purified by silicic acid column chromatography using increasing concentrations of benzene in hexane (20). The purity of the compounds was checked by thin layer chromatography on Silica Gel-G developed in 10% methanol in benzene (4). The compounds were repurified if chromatography disclosed the presence of more than 5% impurities. Phylloquinone remained 98% pure on storage at -20°C in the dark for 4 mo. Purified sodium taurocholate (K & K Laboratories, Plain View, N.Y.), tested by thin layer chromatography (10), was found to have less than 3% impurities consisting primarily of cholic acid. Butyric, octanoic, stearic, oleic, and linoleic acids (95-99% purity) were obtained from Sigma Chemical Co., St. Louis, Mo. Menaquinone-9 (vitamin K₉ of bacterial origin) was obtained through the generosity of the Hoffman-LaRoche Company, Basle, Switzerland, and menadione (vitamine K₃) was purchased from Sigma Chemical Co. Menaquinone purity was assayed by ultraviolet spectrophotometry (extinction coefficient of 246 at a wavelength of 248 μM in hexane) and thin layer chromatography (14). Menadione purity was checked by Silica Gel-G thin-layer chromatography developed in acetone water 50:50 mixture (13). When necessary the compounds were repurified using silicic acid column chromatography with increasing concentrations of benzene in hexane (20). [¹⁴C]Inulin (Amersham Searle, Arlington Heights, Ill.) with specific activity of 12.9 mCi/nmol was used as a nonabsorbable marker (6). Intestinal perfusate solution was prepared in Krebs phosphate buffer at pH 7.40 with dextrose concentration of...
fluid absorption was found to take place over the 55 min of perfusion. Therefore, all experimental values reported in this communication were not corrected for fluid absorption.

Experimental methods. Male Sprague-Dawley rats weighing 180–220 g were fed regular chow and tap water ad libitum and were not fasted prior to experimentation. The abdomen was opened under ether inhalation anesthesia, and a proximal inflow polyethylene catheter (6 mm OD) was inserted into the lumen of the small bowel 1 cm distal to the insertion of the common bile duct. An outflow catheter was placed 10 cm distally to the inflow catheter. The catheters were secured by encircling ligatures that passed between the parallel end vessels. Uterine care was taken not to obstruct blood vessels or lymphatics with the ligatures. A distal small bowel segment of 10-cm length was similarly isolated with the outflow catheter being placed 2 cm proximal to the ileocecal junction. Both intestinal segments were flushed with phosphate buffer in order to remove any residual contents and the segments were replaced in the peritoneal cavity, which was closed surgically. The animal was allowed to awaken and was placed in a Plexiglas restraint cage which allowed minimal mobility but prevented dislodgement of the cannula. A forced-air heating device and a feedback temperature controller (Thermistemp model 74, Yellow Springs Instrument Co., Yellow Springs, Ohio) monitored the animal's body temperature via a rectal probe and maintained the animal's body temperature at 38°C. The cannulas were connected to two separate reservoirs, with the outflow cannula being allowed to drain into the reservoirs by gravity. The inflow proximal and distal cannulas were passed through a totally occlusive roller pump (Buchler Instruments, Inc., Fort Lean, N.J.) which controlled the flow rate of fluid from the reservoirs into the cannulated segments. The intestinal segments were perfused at a constant rate of 0.5 ml/min unless indicated otherwise. Both the reservoirs and tubing were immersed in a constant temperature bath maintained at 38°C. The cannulas were passed through a totally occlusive roller pump (Buchler Instruments, Inc., Fort Lean, N.J.) which controlled the flow rate of fluid from the reservoirs into the cannulated segments. The intestinal segments were perfused at a constant rate of 0.5 ml/min unless indicated otherwise. Both the reservoirs and tubing were immersed in a constant temperature bath maintained at 38°C. The proximal and distal intestinal circuit reservoirs were filled with 10 ml of perfusate that contained phylloquinone and other components as specified. Samples of 50 μl each were taken out of the reservoirs in triplicate in order to assay the initial specific activity of the vitamin. Absorption of phylloquinone was monitored by taking 50-μl samples of the constantly recirculating perfusate at 5-min intervals. Assessment of the rate of disappearance of the vitamin from the perfusate allowed us to calculate the absorption rate of phylloquinone. An initial perfusion period of 30 min was necessary in order to reach a steady rate of absorption of the vitamin from the perfusate. Thereafter, 50-μl aliquots were taken out of the perfusate at 5-min intervals for a 25-min period. The nonabsorbable indicator [14C]julin was used to monitor fluid shifts (21). Less than 5% net fluid absorption was found to take place over the 55 min of perfusion. Therefore, all experimental values reported in this communication were not corrected for fluid shifts. Less than 1% adsorption of phylloquinone to the tubing and reservoirs was noted in base line experiments as well as experiments in which major changes in absorption rate were found (e.g., experiments with linoleic acid addition or experiments at pH 4.5).

At the end of the experiment, the rat was killed by an overdose of ether inhalation and the intestinal segments were removed. The length of each intestinal segment was measured after attaching a 10-g weight to its dependent portion and suspending the segment for 24 h at room temperature. This procedure insured measurement of intestinal lengths under conditions of constant stretch. The weight of the segments was measured after a 24-h period of drying under vacuum when a constant weight was reached. The conclusions did not change when the results were expressed per unit length or weight. Therefore, experimental results throughout this communication are reported per unit intestinal length.

Radioactivity determinations. Aliquots of intestinal perfusate were placed in scintillation counting vials containing a dioxane-based scintillation solution (13). Radioactivity was measured in a Beckman LS 200 liquid scintillation counter with automatic quench calibration at ambient temperature to a counting error of ±1%.

Statistical calculations. The data were analyzed with the aid of a digital computer. The computer program was based on a modification of the Student t test (17) and applied regression analysis theories (5).

RESULTS

Phylloquinone absorption rate at different perfusate concentrations. Intestinal perfusate solution consisting of 10 mM sodium taurocholate, 2.5 mM oleic acid, and 2.5 mM monoolein in the Krebs phosphate buffer was sonicated to form micelles. Sonication was performed for 5 min at 60 W of power (Artek 150, Artek Corp., Farmingdale, N.Y.) and resulted in a mixed micellar solution that remained optically clear upon standing at 20°C for 24 h. After sonication, radioactive as well as nonradioactive phylloquinone were added to the micellar solution by homogenization in a glass Teflon tissue homogenizer for 60 s in order to avoid structural changes in the phylloquinone molecule that could have been induced by sonication. The micellar solution containing phylloquinone was passed through a UM-2 filter (Amicon Corp., Lexington, Mass.) in order to test the distribution of radioactive phylloquininone between the micellar and free monomeric forms of the vitamin. Less than 2% of the radioactivity passed through the membrane, indicating that 98% of the phylloquinine molecules were solubilized within the micellar particles. Radioactively labeled phylloquinone was added in a 3-nM concentration. Unlabeled phylloquinone was added to the perfusate in varying amounts as specified. Absorption experiments were performed at phylloquinone concentrations ranging from 10 nM to 300 μM. Three to six different animal experiments were performed at each concentration. The mean ± standard error values for absorption from all experiments at each concentration were derived by linear regression analysis of the slope of absorption of the vitamin. These values were plotted against the concentrations in the nanomolar range (Fig. 1) and the
micromolar range (Fig. 2). In the nanomolar range of concentrations (Fig. 1) using the least-squares method, the data fitted best ($r = .98$) to a linear plot. In the micromolar range of concentrations (Fig. 2) using the least-squares method, the data fitted best ($r > .92$) to a rectangular hyperbolic plot with apparent saturation kinetics being described both by the proximal and distal small bowel segments. An apparent maximal velocity of absorption was reached both by the proximal and distal small intestinal segments between 15 and 20 nmol/min per 10 cm of small bowel. Both in the micromolar and micromolar ranges of concentrations, the rate of absorption of phyloquinone by the proximal and distal small bowel were not significantly different ($P > 0.05$).

**Influence of perfusate pH on phyloquinone absorption.** The effect of variations in the perfusate pH on phyloquinone absorption in vivo was investigated in the next series of experiments. The perfusate was composed of 225 μM phyloquinone, 10 mM sodium taurocholate, 2.5 mM oleic acid, and 2.5 mM monoolein. The pH of the infusate was varied by changes in the relative amounts of the monobasic and the dibasic phosphates in the buffer. When the pH was raised from 7.4 to 8.0, a decrease in the absorption rate of phyloquinone was observed in the proximal and distal intestinal segments (Table 1). When the perfusate pH was decreased from 7.4 to 4.5, an increase in phyloquinone absorption rate was noted (Table 1). Changes in the pH of the perfusate did not cause precipitation of the vitamin from the micellar solution as monitored by comparing the specific activity of the vitamin in the perfusate prior to and following experiments at the various pH points studied. Likewise, at all hydrogen ion concentrations studied, no visual changes could be detected in the clarity of the micellar solution.

**Effect of saturated fatty acids on phyloquinone absorption in vivo.** Under physiological conditions, the intestinal lumen contains fatty acids of varying chain lengths and degrees of saturation. The effect of the concomitant presence of the various fatty acids on phyillowquinone absorption was also assessed at sodium taurocholate concentrations of 5 and 10 mM. After each series of experiments, the small intestine was examined by light microscopy to look for possible damage to the absorptive cells. No morphological changes were found. Phyloquinone was found to be absorbed at the same rate ($P > 0.05$) by the proximal and distal small intestine in the presence of Pluronic F-68, 5 or 10 mM taurocholate (Table 2).

**TABLE 1. Effect of progressive alkalinization of infusate on vitamin K₁ absorption in vivo**

<table>
<thead>
<tr>
<th>pH</th>
<th>No. of Animals</th>
<th>Absorption, nmol/min per 10 cm</th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>3</td>
<td>22.4 ± 2.63</td>
<td>&lt;0.01</td>
<td>23.4 ± 3.8</td>
</tr>
<tr>
<td>6.0</td>
<td>3</td>
<td>25.2 ± 2.35</td>
<td>&lt;0.05</td>
<td>25.7 ± 4.0</td>
</tr>
<tr>
<td>7.4</td>
<td>4</td>
<td>15.6 ± 0.21</td>
<td>&lt;0.01</td>
<td>15.7 ± 0.5</td>
</tr>
<tr>
<td>8.0</td>
<td>3</td>
<td>11.8 ± 0.38</td>
<td>&lt;0.01</td>
<td>14.2 ± 0.59</td>
</tr>
</tbody>
</table>

Values are means ± SE. Influence of changes in the perfusate pH on phyloquinone absorption. Experimental methods and infusate composition as described in the text. Statistical analysis of the data was performed by comparing the absorption rate at each pH to the rates obtained at pH 7.4 using the Student t test. Five data points were obtained from each animal.

**TABLE 2. Effect of changes in bile salt concentration on vitamin K₁ absorption in vivo**

<table>
<thead>
<tr>
<th>No. of Animals</th>
<th>Absorption, nmol/min per 10 cm</th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluronic F-68</td>
<td>3</td>
<td>13.8 ± 1.4</td>
<td>13.0 ± 1.0</td>
</tr>
<tr>
<td>NTC, 5 mM</td>
<td>4</td>
<td>14.9 ± 1.9</td>
<td>11.6 ± 0.2</td>
</tr>
<tr>
<td>NTC, 10 mM</td>
<td>6</td>
<td>14.8 ± 0.6</td>
<td>12.0 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. The influence of changing the bile salt concentration or its complete replacement by a nonionic detergent on phyloquinone absorption. Materials and methods of experimentation as described in the text. No statistical difference ($P > 0.05$) was found between absorption rates under the different experimental conditions. Five experimental observations were obtained from each animal.

**FIG. 1. In vivo absorption rate of phyloquinone by proximal and distal small intestinal segments in nM range of concentrations. Each point represents mean ± SE absorption rate from 3 to 6 animals with 5 observations per animal. Experiments were performed and calculated as described in text.**

**FIG. 2. In vivo absorption rate of phyloquinone by proximal and distal small intestine in μM range of phyloquinone concentrations. Each point represents mean ± SE absorption rate from 3- to 6-animal experiments with 5 observations per animal. Experiments were performed and calculated as described in text.**
loquinone absorption was examined in this series of experiments. As a base line value, phylloquinone absorption rates by the proximal and distal small bowel segments were examined in the presence of 10 mM sodium taurocholate micelles without fatty acids or monoglycerides. The specific fatty acids studied were added in a 2.5 mM concentration to the sodium taurocholate powder prior to sonication. After the sonication procedures the resultant mixed micellar solution remained optically clear for 24 h at 20°C. The additions to the infusate of separate groups of animals of 2.5 mM saturated fatty acids—butyric (C4:0), octanoic (C8:0), and stearic (C18:0)—changed the absorption rate of phylloquinone only in the presence of butyric acid (Table 3).

**Influence of unsaturated fatty acids on phylloquinone absorption.** Phylloquinone absorption was tested in the presence of mono- and polyunsaturated fatty acids in the micellar perfusate. When compared to absorption rates of the vitamin in the presence of 10 mM sodium taurocholate micelles only, no differences in phylloquinone absorption were found when monounsaturated oleic acid was added to the infusate at 2.5 mM concentrations. However, the addition of the polyunsaturated linoleic acid (C18:2) to the taurocholate micellar solution caused a significant decrease ($P < 0.01$) in the absorption rate of phylloquinone by the proximal and distal intestinal segments (Table 4).

**Effect of perfusion rate on phylloquinone absorption.** Absorption rate of the vitamin was tested at intestinal perfusion rates of 0.5, 1.0, and 5.0 ml/min. The perfusate was composed of 225 $\mu$M phylloquinone, 10 mM taurocholate, 2.5 mM oleic acid, and 2.5 mM monolein solution. The additions to the perfusate on vitamin K absorption was studied under a wide range of concentrations in vivo. When the concentration of the vitamin in the infusate was increased from 30 to 1,000 nM (Fig. 1), a linear relationship was found between the concentration and absorption rate. The concentration of the vitamin was increased further to 300 $\mu$M, least-squares analysis of the data delineated a relationship between the concentration of the vitamin and absorption rate which fitted best with that of a rectangular hyperbola (Fig. 2). No differences ($P > 0.05$) were found between absorption rate of the vitamin by the proximal and distal small bowel. The apparent saturation kinetics observed in the micromolar range of concentrations confirmed the in vitro observations which

### Table 3. Effect of addition of saturated fatty acids on vitamin K$_1$ absorption in vivo

<table>
<thead>
<tr>
<th>Fatty Acid Added</th>
<th>No. of Animals</th>
<th>Proximal</th>
<th>P</th>
<th>Distal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6</td>
<td>14.8 ± 63</td>
<td>&gt;0.05</td>
<td>12.0 ± .66</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Butyric</td>
<td>5</td>
<td>17.2 ± 78</td>
<td>&gt;0.05</td>
<td>15.5 ± .75</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Octanoic</td>
<td>5</td>
<td>15.3 ± 62</td>
<td>&gt;0.05</td>
<td>10.5 ± .50</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Stearic</td>
<td>6</td>
<td>14.6 ± 97</td>
<td>&gt;0.05</td>
<td>11.6 ± .21</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Influence of addition of saturated fatty acids of varying chain lengths to the infusate on phylloquinone absorption. Experimental methods are described in the text. The data were analyzed statistically using the Student $t$ test by comparing experimental values with fatty acids to absorption values in the absence of fatty acids. Five experimental observations were obtained from each animal.

### Table 4. Effect of addition of unsaturated fatty acids on vitamin K$_1$ absorption in vivo

<table>
<thead>
<tr>
<th>Fatty Acid Added</th>
<th>No. of Animals</th>
<th>Absorption, nmol/min per 10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6</td>
<td>14.0 ± 76</td>
</tr>
<tr>
<td>Oleic</td>
<td>3</td>
<td>14.0 ± 78</td>
</tr>
<tr>
<td>Linoleic</td>
<td>6</td>
<td>9.3 ± 24</td>
</tr>
</tbody>
</table>

Values are means ± SE. Influence of addition of unsaturated fatty acids to the infusate on phylloquinone absorption. Experimental data were compared to absorption rates of the vitamin in the absence of fatty acids in the infusate using the Student $t$ test. Five experimental observations were obtained from each animal.

### Table 5. Effect of perfusion rate on vitamin K$_1$ absorption in vivo

<table>
<thead>
<tr>
<th>Perfusion Rate, ml/min</th>
<th>No. of Experiments</th>
<th>Proximal</th>
<th>P</th>
<th>Distal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4</td>
<td>15.6 ± .21</td>
<td>&gt;0.05</td>
<td>15.7 ± .50</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>16.6 ± .80</td>
<td>&gt;0.05</td>
<td>25.7 ± .80</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5.0</td>
<td>4</td>
<td>24.6 ± .98</td>
<td>&lt;0.01</td>
<td>25.4 ± .41</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Influence of perfusion rate on the absorption of phylloquinone. Absorption rates were compared statistically to the absorption rate at the next lower perfusion rate using the Student $t$ test analysis. Five data points were obtained from each animal experiment.
indicated that the vitamin is absorbed by a saturable energy requiring absorption process (12). Whether the saturable energy requiring step is due to coupling to a specific cytosol-binding protein similar to the fatty acid-binding protein (24) or whether the energy requiring step is connected with intracellular events required for exit of the vitamin from the enterocyte into the lymphatic circulation is not clear. A similar energy-mediated uptake of palmitic acid by the proximal small bowel of the hamster has been demonstrated (22) and may indicate the presence of energy-mediated uptake for a number of lipid compounds. The possible presence of binding proteins for lipid uptake in the luminal cell membrane or the cytosol could well account for the apparent specificity and selectivity in the absorption of steroid molecules (9) or the saturation kinetics found in the intestinal absorptive mechanism of retinol in vivo (unpublished observations).

A decrease in the absorption rate of vitamin K$_1$, was found as the infusate pH was raised above 7.4, whereas phylloquinone absorption increased as the pH was lowered below 7.4 (Table 1). It is unlikely that changes in the perfusate pH would modify the aqueous solubility of phylloquinone, which is negligible to begin with. Similarly, lowering the pH to 4.5 would not modify the aqueous solubility of sodium taurocholate that has a $pK_a$ of 1.8 (2). It is possible that increased absorption of phylloquinone observed at low pH (Table 1) could be due to the diminished negative surface charge of the micelle in the presence of high $H^+$ concentration. Normally, at high pH (e.g., pH 8.0), the micelle is negatively charged, resulting in resistance to its diffusion towards the negatively charged luminal cell membrane (18, 27). Changes of the physical characteristics of the fatty acid component of the micelle could also account for some of the changes in the rate of absorption of phylloquinone observed at pH above 7.4.

Neither the replacement of bile salts by a nonionic detergent (Pluronic F-68) nor modification in the sodium taurocholate concentration from 5 to 10 mM (Table 2) influenced the absorption rate of vitamin K$_1$, in vivo by either the proximal or distal small bowel. It is possible that a greater increase in sodium taurocholate concentration up to 20 mM could have influenced the rate of absorption of the vitamin. However, recent studies regarding the effect of sodium taurocholate on the small bowel have indicated that taurocholate in concentrations higher than 10 mM may change the mucosal integrity (7, 25). If one can extrapolate from what is known regarding the ratio between sodium taurocholate and cholesterol molecular ratios (130:1) in a micellar solution (1) to vitamin K$_1$, micellar solution, it could be inferred that, because of the large molar amounts of sodium taurocholate needed to solubilize small amounts of vitamin K$_1$, even an increase of taurocholate concentration from 5 to 10 mM would not appreciably change the number of phylloquinone molecules present in the micellar phase.

Addition to the perfusate of saturated medium- and long-chain fatty acids (Table 3) did not change the absorption rate of phylloquinone. An increase in the absorption rate was observed after the addition of butyric acid (Table 3). The mechanism responsible for the effect of butyric acid on phylloquinone absorption is unclear. Short-chain fatty acids have been previously shown to be absorbed by an active transport mechanism. They are absorbed rapidly and primarily into the portal vein rather than into the lymphatic circulation (16). How these unique pathways of absorption of the short-chain fatty acids could modify the absorption of an insoluble nonswelling amphiphile such as phylloquinone is unclear. The monounsaturated fatty acid, oleic acid, caused no change in the absorption rate of vitamin K$_1$, (Table 4). However, the polyunsaturated fatty acid, linoleic, caused a marked decrease in the absorption rate of phylloquinone both by the proximal and distal small bowel (Table 4). Similar inhibition of fat soluble vitamin absorption by polyunsaturated fatty acids has been observed with vitamin A$_1$ (unpublished observations) and vitamin E (8). Interaction between phylloquinone and polyunsaturated fatty acids could occur in any of the steps involved in the pathway of lipid absorption. These steps could range from interactions within the micelle itself (27), the unstirred layer (30), the absorptive cell membrane (3), or within the enterocyte itself. We propose that the fatty acid-binding protein described by Ockner and Manning (24) could be the site of interaction between phylloquinone and linoleic acid. The fatty acid-binding protein has been shown to bind unsaturated fatty acids with greater affinity than saturated or medium chain fatty acids (23, 24). Because phylloquinone intestinal uptake has been demonstrated both in vivo and in vitro (12) to display saturation kinetics, it is possible that the fatty acid-binding protein may be a carrier for phylloquinone as well. Similar findings for retinol absorption have also been described (unpublished observations). If this were the case, then competitive binding of the polyunsaturated fatty acids to the binding protein may explain the present experimental observations of decreased phylloquinone absorption in the presence of linoleic acid (Table 4). A phylloquinone-binding protein in the intestinal cell membrane or cytosol would have to be characterized in order to confirm this hypothesis.

Because vitamin K$_1$, is absorbed by an active energy requiring process (12), it was interesting to investigate whether its analogues that are structurally related would interfere with the absorptive process. Vitamin K$_2$ (menaquinone 9) is synthesized by intestinal bacteria and is distinguishable from phylloquinone by differences in the structure of the side chain at the 3 position. When menaquinone was added to the infusate containing vitamin K$_1$, no change in the absorption rate of vitamin K$_1$, both by the proximal and distal small bowel was observed. Vitamin K$_2$, which consists of the structural nucleus of vitamin K$_1$, and does not have a phytol side chain in the 3 position, also had no effect on phylloquinone absorption. The lack of changes in phylloquinone absorption after the addition of its analogues could be due to either a greater affinity of phylloquinone for its carrier or may indicate that the phytol side chain is a required structural feature for association with the carrier.

In order to obtain an indirect estimate as to the influ-
ence of the unstimulated water layer on phylloquinone absorption, the thickness of the layer was decreased by increasing the perfusion rate of the vitamin through the small bowel (19). As the perfusion rate was increased from .5 to 5 ml/min, proximal intestinal absorption rate increased significantly (Table 5). Similarly, an increase in the absorption rate of phylloquinone was observed when the perfusion rate was increased from .5 to 1 ml/min in the distal small bowel (Table 5). The somewhat earlier increase in absorption rate of phylloquinone observed by the distal small bowel is most likely due to the smaller diameter of the distal small bowel when compared to the proximal small bowel. The difference in diameter would result in faster rate of actual flow distally at equivalent infusion rate. The resultant increase in the absorption rate observed at higher flow rates indicates that the unstirred water layer is a significant barrier to diffusion of the vitamin under physiologically unstimulated conditions.

Because vitamin K is essential for mammalian survival, the delineation of its absorptive characteristics under a variety of intraluminal conditions is of great importance. These observations are also interesting from the point of view of our understanding of absorption of lipids and lipid-soluble substances in general. Because of the similarity in absorptive characteristics between vitamin K and retinol (unpublished observations) and the possible connecting link between these two compounds and the fatty acid-binding protein (24), further investigation into intracellular events of protein binding of these compounds is warranted. Information gained from such studies may increase our overall understanding of absorption of lipids and lipid-soluble compounds and may provide us with some unifying concepts and principles regarding their intestinal absorption mechanisms.

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