Bile Salt Enhancement of Riboflavin and Flavin Mononucleotide Absorption in Man

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ABSTRACT The gastrointestinal absorption of riboflavin and flavin mononucleotide (FMN) was determined under control conditions and after oral administration of 600 mg sodium deoxycholate. When the bile salt is given prior to a 30-mg dose of riboflavin there is a 50 to 80% increase in total urinary recovery of apparent riboflavin. A similar, but less marked, enhancement is observed when the same dose of FMN is given with sodium deoxycholate. Urinary excretion data also suggest an unusually prolonged absorption of riboflavin in the presence of the bile salt. The possibility exists that the bile salt enhancement of riboflavin and FMN absorption may be due to changes in gastrointestinal motility or changes in the permeability of the gastrointestinal membranes to the transport of the vitamins, or both. Other possibilities are also explored.

Although the human nutritional requirement for riboflavin has been recognized for some time, speculation still exists concerning the gastrointestinal absorption of this vitamin. Morrison et al. (1) have suggested that riboflavin is absorbed high in the human intestinal tract based upon the observation that peak urinary excretion values were obtained within 2 hours after oral administration of riboflavin in solution or in sustained release form. Lane et al. (2) reported that the percentage of the administered dose of riboflavin recovered in the urine (which reflects the percentage of dose which is absorbed) following oral administration, decreased with increasing dose. The latter observation was confirmed by Stripp (3), who also showed that not more than 14 to 18 mg of riboflavin are excreted in the urine, independent of oral dose of riboflavin or flavin mononucleotide (FMN) when the dose exceeds 50 mg. More recently, Levy and Jusko studied the gastrointestinal absorption of riboflavin (4) and FMN (5) in man and suggested the existence of a specialized transport process for the vitamin in the upper region of the intestinal tract.

The absorption of several nutrients may be strongly influenced by bile salts, an important class of physiological surface active agents. Extensive research has indicated the importance of bile salts in lipid absorption (6–8). In addition, there have been several studies implicating bile salts in the absorption of water-insoluble vitamins. These reports demonstrate that the absorption of vitamins A (9–11), D (12–14) and K (15) is enhanced by the presence of various bile salts.

Bates et al. (16–18) have suggested, based on solubilization and dissolution studies of several water-insoluble drugs, that bile salts may influence drug absorption by enhancing dissolution rate. Cavallito and O’Dell (19) studied the influence of coadministration of sodium cholate and dehydrocholate on the pharmacologic response to a quaternary hypotensive agent in dogs. In each case an apparent increase in drug absorption was suggested by the enhanced pharmacologic response. Davenport (20) reported that sodium taurocholate and natural bile markedly disrupt the gastric mucosal barrier in dogs as evidenced by changes in ionic fluxes. Studies with isolated intestinal segments have shown the influence of bile salts on nutrient (21) as well as drug (22) transfer.

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A preliminary indication that bile salts may affect riboflavin absorption is the work of Onishi (23) who found that ursodeoxycholic acid significantly increases the absorption of esterified riboflavin as well as total riboflavin in excised dog intestine. The present study concerns the influence of an unconjugated bile salt, sodium deoxycholate, on the absorption of riboflavin and FMN in man.

EXPERIMENTAL

Five, apparently healthy, male volunteers, ranging in age from 24 to 29 years, served as test subjects. The urinary excretion of riboflavin was followed after oral administration of riboflavin or FMN under the following experimental conditions: 1) riboflavin, 30 mg, with and without 600 mg sodium deoxycholate (four subjects); 2) FMN, equivalent to 30 mg riboflavin, with and without 600 mg sodium deoxycholate (five subjects); and 3) riboflavin, 5, 10, 20 and 30 mg with and without 600 mg sodium deoxycholate (one subject).

All subjects ingested the vitamin in the morning following an overnight fast. In studies involving administration of sodium deoxycholate, the bile salt was first dissolved in 100 ml of water. Ten grams of an artificial orange juice concentrate were added to the solution to mask the taste of the bile salt and the resulting colloidal dispersion was ingested 0.5 hour prior to ingestion of the vitamin. In control studies, the equivalent amount of orange concentrate in 100 ml of water was administered 0.5 hour before the vitamin. Riboflavin or FMN was dissolved or partially dissolved in 200 ml of water prior to ingestion.

Urine was collected immediately after ingestion of the vitamin and every half-hour for 2 to 3 hours thereafter, at hourly intervals for at least the next 4 hours, and then at convenient intervals up to 24 hours. Where possible, on the day before the experiment a 24-hour control urine collection was made. At least two such collections were carried out in each subject. Glacial acetic acid (about 3 ml/100 ml urine) was added to each urine sample and the urine was immediately refrigerated (protected from light) until assayed within 24 hours after termination of urine collection.

Subjects were instructed to drink a sufficient quantity of water after each voiding to maintain adequate urine volumes. In addition, the subjects were asked to avoid ingesting foods known to contain appreciable amounts of riboflavin as well as any vitamin preparations and other drugs. No food was ingested until at least 2 hours after administration of the vitamin. All paired experiments (i.e., control versus sodium deoxycholate) were performed in a random manner and at least 1 week elapsed between experiments in any one subject.

Total riboflavin in the urine was determined fluorometrically according to Levy and Jusko (4), using a Turner fluorometer. Briefly, this method involves mixing 5 ml diluted urine with 1 ml of 1 N acetate buffer, pH 4.8. One milliliter of 4% potassium permanganate and, subsequently, 1 ml of 3% hydrogen peroxide are added. The fluorescence intensity of this solution is determined before and after reduction of riboflavin with sodium hydrosulfite. All data were corrected for blank values which averaged 0.5 mg/24 hours of apparent riboflavin. Apparent riboflavin excretion was unchanged during the 24-hour period following the oral administration of 600 mg sodium deoxycholate.

RESULTS

The influence of sodium deoxycholate on the 24-hour total urinary riboflavin recovery is summarized in table 1 and a representative plot is shown in figure 1. The control recovery values range from 13 to 20% of the 30-mg dose, in agreement...
TABLE 1
Total (24 hour) urinary excretion of riboflavin after oral administration of 30 mg riboflavin

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control</th>
<th>SDC</th>
<th>Ratio (SDC/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF</td>
<td>20.2</td>
<td>30.2</td>
<td>1.50</td>
</tr>
<tr>
<td>WJ</td>
<td>13.5</td>
<td>22.5</td>
<td>1.67</td>
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<tr>
<td>MM</td>
<td>23.1</td>
<td>38.7</td>
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</tr>
<tr>
<td>CN</td>
<td>15.0</td>
<td>26.7</td>
<td>1.78</td>
</tr>
</tbody>
</table>

1 Expressed as percentage of dose.
2 Sodium deoxycholate, 600 mg, administered 0.5 hour before the vitamin.

The results were statistically analyzed by the method of paired comparisons using Student’s t test (24). Table 3 shows the comparisons made and the resulting levels of significance. Total recovery of riboflavin after oral administration of riboflavin or FMN with bile salt is significantly greater than the respective control values. Although urinary recoveries of riboflavin after oral administration of either riboflavin or FMN did not differ significantly from each other, there was a high level of significance between urinary recoveries after riboflavin with sodium deoxycholate and FMN with sodium deoxycholate.

TABLE 2
Total (24 hour) urinary excretion of riboflavin after oral administration of flavin mononucleotide (FMN)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control</th>
<th>SDC</th>
<th>Ratio (SDC/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>27.9</td>
<td>31.6</td>
<td>1.13</td>
</tr>
<tr>
<td>SF</td>
<td>24.5</td>
<td>26.2</td>
<td>1.07</td>
</tr>
<tr>
<td>WJ</td>
<td>11.3</td>
<td>16.5</td>
<td>1.46</td>
</tr>
<tr>
<td>MM</td>
<td>14.0</td>
<td>24.3</td>
<td>1.74</td>
</tr>
<tr>
<td>CN</td>
<td>14.9</td>
<td>18.5</td>
<td>1.24</td>
</tr>
</tbody>
</table>

1 Expressed as percentage of dose.
2 Dose equivalent to 30 mg riboflavin.
3 Sodium deoxycholate, 600 mg, administered 0.5 hour before the vitamin.

Fig. 1 Cumulative amount of riboflavin excreted after oral administration of 30 mg riboflavin (subject WJ). (○) Control; (●) sodium deoxycholate, 600 mg, administered 0.5 hour before the vitamin.

Fig. 2 Cumulative amount of riboflavin excreted after oral administration of FMN, equivalent to 30 mg riboflavin (subject WJ). (○) Control; (●) sodium deoxycholate, 600 mg, administered 0.5 hour before the vitamin.

The results were statistically analyzed by the method of paired comparisons using Student’s t test (24). Table 3 shows the comparisons made and the resulting levels of significance. Total recovery of riboflavin after oral administration of riboflavin or FMN with bile salt is significantly greater than the respective control values. Although urinary recoveries of riboflavin after oral administration of either riboflavin or FMN did not differ significantly from each other, there was a high level of significance between urinary recoveries after riboflavin with sodium deoxycholate and FMN with sodium deoxycholate.
Figure 3 shows a typical semilogarithmic plot of excretion rate of riboflavin as a function of time after oral administration of riboflavin with and without sodium deoxycholate. Administration of riboflavin with sodium deoxycholate results in a marked increase in the peak excretion rate compared with control values. Similar results are observed after administration of FMN. The data in figure 3 also indicate a considerable shift in the peak excretion rate after bile salt administration. Peak excretion rate of riboflavin occurs within 1 hour after oral administration of riboflavin, but only after about 2 hours when the vitamin is given with sodium deoxycholate. This shift is suggestive of a prolonged absorption of riboflavin in the presence of the bile salt. Shifts in peak excretion rates between control and sodium deoxycholate experiments were not as evident after FMN administration.

The decline in body levels of total flavins in the postabsorptive phase, as manifested by the decreasing excretion rates of riboflavin some time after administration, may be characterized by a rapid elimination and slow elimination phase, as

<table>
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<tr>
<th>Comparison</th>
<th>1 mean % of dose excreted</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin(1) versus riboflavin-SDC(2)</td>
<td>18.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>FMN(1) versus FMN-SDC(2)</td>
<td>18.5</td>
<td>P &lt; 0.025</td>
</tr>
<tr>
<td>Riboflavin(1) versus FMN(2)</td>
<td>18.0</td>
<td>P &gt; 0.1</td>
</tr>
<tr>
<td>Riboflavin-SDC(1) versus FMN-SDC(2)</td>
<td>29.5</td>
<td>P &lt; 0.025</td>
</tr>
</tbody>
</table>

1 Paired comparison using Student’s t test.
2 Dose equivalent to 30 mg riboflavin.

Fig. 3 Urinary excretion rate of riboflavin after oral administration of 30 mg riboflavin (subject WJ). (○) Control; (●) SDC.
noted in figure 3. The biexponential elimination of riboflavin has been noted and discussed previously by Levy and Jusko (4). In all experiments with FMN or riboflavin, with and without sodium deoxycholate, the rapid elimination phase yielded half-lives of 0.5 to 2 hours, while the slow elimination phase yielded half-lives of 4 to 7 hours. Paired analysis of the half-life data clearly indicated that sodium deoxycholate had no significant effect on the elimination kinetics of riboflavin after the administration of either riboflavin or FMN. Hence, one may conclude that differences in the excretion rate of riboflavin in the presence of sodium deoxycholate are the result of changes in the gastrointestinal absorption of the vitamins when administered with the bile salt.

Figure 4 shows a dose-response-type plot of the total amount of riboflavin ultimately excreted in the urine after various oral doses of riboflavin, with and without sodium deoxycholate. It is evident that there is a considerably increased urinary excretion of riboflavin at all dose levels when the bile salt is coadministered with the vitamin. It is also apparent that both curves tend to plateau as the dose increases. Levy and Jusko (4) have postulated that the latter phenomenon may be due to a specialized intestinal absorption process which is capacity limited.

**DISCUSSION**

In agreement with previous work (2–5), the present results demonstrate that the percentage of administered dose of riboflavin which is absorbed after oral administration, decreases with increasing dose. This finding is consistent with the hypothesis that the absorption of riboflavin involves a specialized transport which is capacity limited (4). There is no evidence that renal excretion of riboflavin is saturable in the concentration range found in the present study (4). Furthermore, in the dose range employed, there is essentially quantitative urinary recovery of absorbed riboflavin or FMN, as riboflavin (3, 4). It follows, therefore, that a Lineweaver-Burk-type plot of the reciprocal of the amount excreted versus the recipro-

![Fig. 4 Total amount of riboflavin excreted as a function of oral dose of riboflavin (subject SF). (O) Control; (●) SDC.](image)
cal of the dose of riboflavin, should be linear (4). Evidence of this is shown in figure 5.

Dowd and Riggs (25), have noted that care must be taken in the interpretation of the usual Lineweaver-Burk-type plot since the double reciprocal plot offers the poorest estimate of the appropriate kinetic parameters. They propose that when dealing with values of "v" (which correspond to values of the amount recovered in the urine in the present study), where the error is unknown, it would be advantageous to plot "v" versus "v/S" (where S corresponds to the administered dose) to obtain the best values of K_m and V_max. An analogous plot using the riboflavin data is shown as an inset to figure 5. Unweighted least square regression analysis of both lines yields values of 12.1 and 11.3 mg as the maximum amount of riboflavin which can be absorbed from the gastrointestinal tract of the fasting subject, and values of 27 and 24.2 mg as the dose which yields an amount absorbed equal to one-half of the maximum, using the double reciprocal plot and the "total amount excreted versus total amount excreted per dose" plot, respectively. The two types of plots yield values which are in excellent agreement and tend to confirm the capacity-limited nature of intestinal riboflavin absorption. The urinary excretion data obtained after administration of various doses of riboflavin with sodium deoxycholate yielded poor fits in Lineweaver-Burk-type plots.

Previous studies in this laboratory suggest a number of possible mechanisms by which sodium deoxycholate may influence riboflavin and FMN absorption. If the absorption of a material is limited to the upper gastrointestinal tract, as is the case for both riboflavin and FMN (1, 2, 4), then the residence time of the material at the absorption sites is a critical determinant of the total amount of drug which can be absorbed. Feldman et al. (26)

![Figure 5](https://academic.oup.com/jn/article-abstract/98/3/288/4778549)

Fig. 5 Lineweaver-Burk-type plot of the riboflavin urinary excretion data as a function of oral dose, as discussed in the text (subject SF). Inset shows an alternate method of plotting the data.
and Feldman and Gibaldi (27) have recently shown that oral administration of sodium taurodeoxycholate and sodium deoxycholate markedly inhibits gastric emptying and proximal intestinal transit in the rat. Similar effects in man would result in prolonged and more complete absorption of the coadministered vitamins. This possibility is consistent with the data obtained after oral administration of riboflavin as shown in figure 3. The plot clearly suggests that the absorption of riboflavin is prolonged in the presence of sodium deoxycholate as evidenced by the shift in peak excretion rate. Levy and Jusko (4, 5) have shown marked increases in riboflavin and FMN absorption upon administration after a standard meal, in contrast to the absorption observed in fasting subjects. They attribute this enhancement to a decrease in gastric emptying due to the presence of food which causes the vitamins to be in contact with optimum absorption sites in the proximal region of the intestinal tract for a longer period of time.

Changes in the permeability of the gastrointestinal membranes due to sodium deoxycholate may also account in part for the marked increases in the absorption of riboflavin and FMN. The effects of bile salts on membrane permeability have been studied extensively (20, 22, 26, 28, 29). The possibility that administered bile salts increase the permeability of the gastrointestinal membranes to riboflavin is supported by current studies in this laboratory.10 Figure 6 shows a plot of the rate of transfer of riboflavin across the isolated everted rat intestine determined according to the method of Feldman and Gibaldi (22), in the presence and absence of 10 mM sodium taurodeoxycholate. A sig-

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Fig. 6 Mucosal-to-serosal riboflavin transfer across the everted intestine of the rat. (O) Control; (●) sodium taurodeoxycholate, 10 mM. Mucosal solution concentration maintained constant at 20 μg/ml riboflavin.

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10 Mayersohn, M., and M. Gibaldi, unpublished data.
significant increase in the transfer rate of riboflavin in the presence of the bile salt is apparent.

In vitro studies indicate that sodium deoxycholate can solubilize riboflavin in water. Approximately 156 moles of bile salt are required to solubilize 1 mole of riboflavin. Hence, the possibility must be considered that the detergent action of sodium deoxycholate simply enhances the solubility of riboflavin in the gastrointestinal tract as with certain fat-soluble vitamins. Though FMN is considerably more water soluble than riboflavin, it must first be dephosphorylated, and then the resultant riboflavin may require further solubilization before absorption.

Another possible effect of sodium deoxycholate could be on the intestinal microflora. Yang and McCormick present evidence to suggest degradation of riboflavin by the intestinal microorganisms. Inhibition of these microorganisms, for example by deoxycholate, may result in a larger fraction of drug absorbed.

A particularly interesting observation in the present study, which cannot be explained at present, is the difference in effect of sodium deoxycholate on riboflavin and FMN absorption. As shown in figures 1 and 2, and table 3, the effect of sodium deoxycholate on riboflavin absorption was significantly greater than its effect on FMN absorption. Levy and Jusko present evidence which suggests that riboflavin and FMN are absorbed by the same specialized transfer process. There is extensive evidence showing that FMN is rapidly and completely dephosphorylated to free riboflavin in the small intestine, which explains the similarity in the absorption of riboflavin and FMN noted by Levy and Jusko, and in the present study in the absence of bile salt. A possible explanation for the differences in absorption between riboflavin and FMN in the presence of sodium deoxycholate may be the influence of the bile salt on the dephosphorylating enzyme system. Unconjugated bile salts have been found to be very active inhibitors of a number of enzyme systems in the intestine (21), and may also depress the conversion of the phosphate ester to free riboflavin. A further possibility which cannot be ruled out is that significant amounts of FMN may be excreted in the urine after administration of deoxycholate. This would be the situation if a sufficient amount of bile salt reached the systemic circulation to inhibit dephosphorylation. Since the assay procedure is not specific for riboflavin, the results would suggest a lower amount of "apparent" riboflavin than the true amount of total flavins.

LITERATURE CITED


11 See footnote 10.