Absorption, Metabolism, and Excretion of Riboflavin-5′-phosphate in Man

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Evidence is presented which indicates that riboflavin-5′-phosphate (FMN) is absorbed from the gastrointestinal tract of man by specialized transport rather than by passive diffusion. Oral administration of the vitamin after a meal results in more extensive absorption which appears to be primarily due to a decrease in intestinal transit rate resulting in longer retention of FMN at absorption sites in the small intestine. Both FMN and riboflavin are excreted in the urine primarily, if not solely, as free riboflavin. The urinary recovery of riboflavin is the same when equimolar amounts of either form of the vitamin are administered orally in solution. The time course of urinary excretion of riboflavin after administration of high doses of FMN, together with data available in the literature, suggest that the vitamin is subject to enterohepatic cycling.

THE AUTHORS have recently found that the urinary recovery of riboflavin after oral administration to fasted normal humans decreases with higher doses and that absorption is limited mainly to the upper region of the gastrointestinal tract (1). The saturability and site-specificity of riboflavin absorption, together with other evidence summarized previously (1), led to the conclusion that the vitamin is absorbed mainly or solely by specialized transport rather than by passive diffusion. Unlike the results obtained when riboflavin was administered on an empty stomach, administration of the vitamin immediately after breakfast resulted in a constant per cent urinary recovery independent of dose. These results, which were obtained in the dose range of 5 to 30 mg., were interpreted as being due to decreased intestinal transit rate in the presence of food, resulting in prolonged retention of the vitamin at specialized absorption sites in the small intestine. If this interpretation is correct, it should be possible to observe saturation effects in the absorption of riboflavin even when the vitamin is administered on a full stomach, provided that sufficiently large doses are administered. The limited solubility of riboflavin itself made such a study unfeasible, since administration of the vitamin in suspension, rather than in solution, could lead to misleading results (1). This solubility problem is not encountered with riboflavin-5′-phosphate (FMN) and, provided that this form of the vitamin is subject to the same transport mechanism as is riboflavin itself, it should be possible to observe saturation effects when FMN is administered in sufficiently high doses after a meal. The purpose of the study to be described here was, therefore, to determine the mechanism of gastrointestinal absorption of FMN, and, if saturation effects become evident after oral administration of FMN on an empty stomach, to determine if similar saturation effects occur with sufficiently high doses when the vitamin is administered on a full stomach. In the course of the study, information has been obtained also concerning the metabolic fate of FMN and evidence will be presented which is indicative of the enterohepatic cycling of riboflavin.

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EXPERIMENTAL

Absorption Study.—Four healthy male volunteers, 22 to 37 years of age, served as test subjects. The same subjects participated in the previous study (1). Each subject received doses of sodium FMN[1] equivalent to 5, 10, and 30 mg. of riboflavin dissolved in 100 ml. of 0.02 *N* acetic acid. The container was rinsed with a small amount of water which was also ingested. The vitamin solution was administered either on an empty stomach (after an overnight fast) or immediately after a standard breakfast. Three of the subjects received sodium FMN also in doses equivalent to 150 and 300 mg. of riboflavin. The breakfast, urine collections, and other details of the protocol were essentially the same as in the study of riboflavin absorption (1). The subjects were permitted to eat their usual lunches at the normal time, except when it was desired to determine the effect of withholding lunch on the excretion of riboflavin.

In another phase of the study, the effect of volume of gastrointestinal contents on the absorption of riboflavin was determined. Three of the subjects ingested 600 ml. of water on an empty stomach, followed by 30 mg. of riboflavin in 100 ml. of 0.02 *N* acetic acid (plus about 40 ml. of water to rinse the container) to approximate the volume of food and drink ingested when the vitamin was administered after the standard breakfast (1).

At least two 24-hr. blank urine collections, with

[1] Sodium riboflavin-5′-phosphate, Hoffmann-LaRoche, Nutley, N. J.

and without breakfast, were carried out in each subject.

Analytical Methods.—Riboflavin and FMN in the urine were determined fluorimetrically by the method of Burch *et al.* (2) and by a modified U.S.P. XVI assay procedure (3) using the Turner Fluorometer, model 111, with primary filter 47-B and secondary filter 2A-12. The modifications of the two assays have been described in a previous publication (1).

All data were corrected for blank values which averaged 0.6 mg./24 hr. by the Burch procedure and 1.0 mg./24 hr. by the modified U.S.P. procedure.

RESULTS

The urinary recoveries of riboflavin as a function of the administered dose of FMN, given either on an empty stomach or after a standard breakfast, are shown in Fig. 1. The curves shown in the figure are those obtained with the same subjects after ingestion of equivalent doses of riboflavin, as described in a previous publication (1). It is evident that the relation between dose and urinary recovery is essentially the same for FMN and riboflavin. There is a linear relationship between the dose of FMN and the amount of riboflavin excreted when doses of FMN from 5 to 30 mg. were ingested after a standard breakfast. The average recovery of riboflavin after oral administration of FMN in doses ranging from 5 to 30 mg. was 63%. This is essentially identical to the 62% recovery found previously when riboflavin itself was ingested after breakfast. When FMN was administered on an empty stomach (after an overnight fast), the per cent recovery of riboflavin in the urine decreased with increasing dose. This apparent saturation effect is also very similar to that found previously with riboflavin itself.

Since the similarity in the gastrointestinal absorption characteristics indicates that both forms of the vitamin are absorbed by the same mechanism,

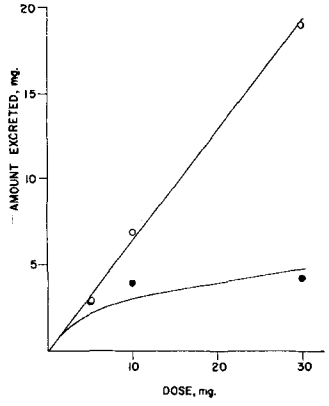

Fig. 1.—Urinary recovery of riboflavin after oral administration of FMN as a function of dose when given on an empty stomach (●) and after a standard breakfast (○). The curves are taken from Fig. 1 of *Reference 1* and represent data obtained after administration of riboflavin as such. Mean of four subjects. The two data points for the 5-mg. dose are superimposed.

TABLE I.—URINARY RECOVERY OF RIBOFLAVIN AFTER ORAL ADMINISTRATION OF FMN AFTER A STANDARD BREAKFAST

Subject	Urinary Recovery of Riboflavin, mg. Dose[a]: 5	10	30	150	300
J	2.08	5.21	20.1	22.8 16.2	22.5 (48.0)[b]
L	2.43	7.37	16.5	...	...
A	2.30	6.70	17.0	23.7	27.0 22.5[c]
M	4.77	8.09	22.4	22.5 23.5[c]	27.6
Mean	2.90	6.84	19.0	21.7	24.9
Dose, %	58.0	68.4	63.3	14.5	8.3

[a] Milligram riboflavin equivalent. [b] This value was not used in calculation of the average value. [c] Instances when subjects did not eat lunch.

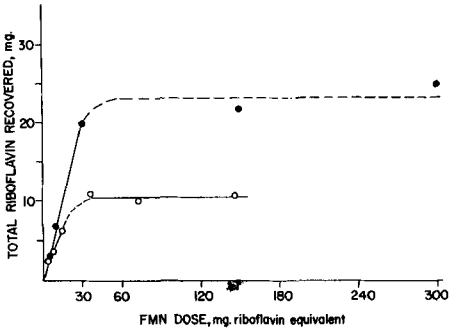

Fig. 2.—Urinary recovery of riboflavin after oral administration of FMN as a function of dose when given after a standard breakfast, showing the limited absorption capacity evident in the high dose range (●). Lower curve (○) taken from Fig. 2 of *Reference 4* (see text).

it was possible to take advantage of the greater solubility of FMN to determine if apparent saturation effects can be observed even if the vitamin is administered on a full stomach. Therefore, three subjects were given 150- and 300-mg. doses of riboflavin as sodium FMN after the standard breakfast. The relative urinary recovery decreased with increasing dose of FMN, demonstrating a definite upper limit in the gastrointestinal absorption of the vitamin (Table I, Fig. 2). This upper limit was somewhat above 30 mg. under the experimental conditions of the study. There was one unusually high recovery (48.0 mg. in subject *J* after a 300-mg. dose), but repetition of the experiment in the same subject resulted in a urinary recovery of 22.5 mg. The 48.0-mg. value was not included in the average data of Fig. 2 because of the large discrepancy relative to the remainder of the data. Figure 2 also includes data reported by Stripp (4) which appeared while the present study was in progress. Stripp's data also show a definite upper limit in the absorption of FMN. The absolute difference in the plateau values may be due to differences in the dietary regimen used in the respective studies.

The results of an experiment in which three subjects ingested 30 mg. of riboflavin (*a*) on an empty stomach, (*b*) after a standard meal, and (*c*) after

drinking a volume of water approximately equal to the volume of the standard breakfast are shown in Table II. The ingestion of 600 ml. of water before taking riboflavin increased the urinary riboflavin recovery from 15.0 to 21.6%, but the limited number of subjects does not permit determination of the significance of this difference. However, ingestion of riboflavin after breakfast resulted in an even greater recovery, namely 57.5%.

Analysis of the urines by both the Burch and modified U.S.P. methods permitted estimation of the respective amounts of riboflavin and FMN excreted after ingestion of either form of the vitamin. Riboflavin and FMN exhibit equal fluorescence intensities at equimolar concentrations in the modified U.S.P. assay, while the fluorescence intensity of riboflavin in the Burch assay is 3.4 times that of an equimolar concentration of FMN. The presence of significant amounts of riboflavin as FMN in the urine would result in appreciable differences between the results of the Burch and U.S.P. assays. There was no statistically significant difference in the urinary recovery of riboflavin as determined by the two assay methods. However, since the assay method is not sufficiently sensitive to permit determination of small amounts of FMN in the presence of considerably larger amounts of riboflavin, it can be concluded only that FMN is excreted largely, if not entirely, as free riboflavin.

All data presented in this report are based on the results obtained with the modified U.S.P. assay.

Figure 3 shows the time course of urinary excretion of riboflavin by subject *J* after oral administration of 30 and 300 mg. of riboflavin as FMN after breakfast. Maximum excretion of riboflavin after the 30-mg. dose occurred in the period 1 to 1.5 hr. after ingestion of the vitamin and was followed by an exponential decrease in the excretion rate with an apparent half-life of 1.1 hr. This was followed by a slower excretion phase after the 4th hr. The excretion curve obtained after ingestion of a 300-mg. dose of riboflavin as FMN after breakfast appeared to represent a plateau indicative of an upper limit in the absorption rate of FMN and the curve was drawn on the basis of that assumption. However, additional experiments in subject *J* and in other subjects have shown that the time course of urinary excretion rate after administration of large doses of FMN exhibits more than one maximum, and that the data for the upper curve in Fig. 3 represent two definite, although not very distinct, excretion rate maxima. More representative of the majority of results are the data shown in Fig. 4, where the excretion rate of riboflavin after administration of 150 and 300 mg. of FMN exhibited two distinct maxima.

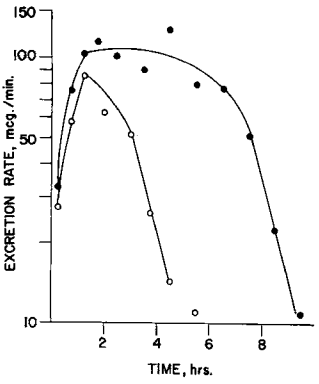

Fig. 3.—Urinary excretion rate of riboflavin as a function of time after oral administration of 30 mg. (○) and 300 mg. (●) of riboflavin as molar equivalents of FMN after a standard breakfast. Subject *J*.

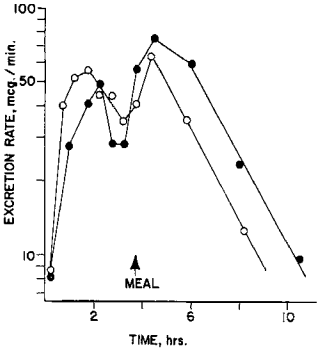

Fig. 4.—Urinary excretion rate of riboflavin as a function of time after oral administration of 150 mg. (○) and 300 mg. (●) riboflavin as molar equivalents of FMN after a standard breakfast. Subject *A*.

The second excretion maximum had a definite relation to lunch time in all of the experiments. When the experiments were repeated in two subjects with lunch being omitted, the second excretion rate maximum decreased appreciably in magnitude, but did not disappear entirely. This is shown in Fig. 5 for subject *A*. The amounts of riboflavin recovered in the urine after administration of large doses of FMN after breakfast were similar whether the subject ate lunch or omitted it (Table I).

TABLE II.—EFFECT OF TYPE AND VOLUME OF PREINGESTED MATERIAL ON URINARY RECOVERY OF RIBOFLAVIN AFTER ORAL ADMINISTRATION OF 30 mg. RIBOFLAVIN

	Conditions		
Subject	No Food	No Food, but 600 ml. Water	Std. Breakfast, Vol. Approx. 600 ml.
J	11.8	19.1	55.1
L	14.2	16.8	56.5
A	19.0	29.1	60.9
Mean	15.0	21.6	57.5

DISCUSSION

The results of the present study show that FMN has the same absorption and excretion characteristics as does riboflavin. The data suggest strongly that riboflavin and FMN are absorbed by the same specialized transport process which is saturable and located mainly or solely in the proximal region of the gastrointestinal tract. Just as with riboflavin, administration of FMN after a meal resulted in enhanced absorption of the vitamin,[2] and the post-

[2] The reader is reminded that this is a relatively specific effect and that food decreases the absorption of certain other drugs.

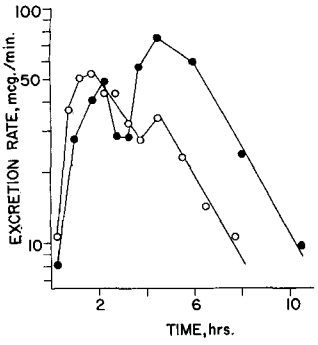

Fig. 5.—Urinary excretion rate of riboflavin as a function of time after oral administration of 300 mg. riboflavin as a molar equivalent of FMN after a standard breakfast with (●) and without (○) lunch. Subject *A*.

absorption excretion rate declined exponentially with a half-life of about 1.1 hr., followed by a slower component after about 4 hr. The latter may reflect the elimination of riboflavin from a "deep" compartment or it may be due to decreased renal clearance at low riboflavin concentrations, similar to the excretion characteristics of another B vitamin, pantothenic acid (5). It has now been possible to demonstrate by the use of large doses of FMN that saturation of the absorption process responsible for the gastrointestinal absorption of vitamin B_2 can be achieved even when the vitamin is administered after a meal. These results are consistent with the assumption that the more extensive absorption of riboflavin and FMN, when given on a full stomach, is due to prolonged retention of the vitamin at sites of absorption. The effect of food on the urinary recovery of riboflavin is not primarily a volume effect since the ingestion of an equivalent volume of water rather than food prior to riboflavin administration does not result in the marked increase in urinary recovery of riboflavin obtained when the vitamin is taken after a standard breakfast (Table II). However, the ingestion of a large volume of water may have a significant effect on riboflavin absorption, possibly by increasing the contact area of riboflavin with intestinal absorption sites.

The maximum amount that was recovered from the subjects under the experimental conditions was around 25 mg. as shown in Table I and Fig. 2, except for the one unusual value of 48.0 mg. The data reported recently by Stripp (4), which are also plotted in Fig. 2, show a lower maximum value. This difference in the maximum amount recovered is probably mainly due to the fact that a standard breakfast was ingested before administration of the vitamin in the present study, while the subjects in the Stripp study were "on an unrestricted diet."

The authors' previous data (1) and those of Stripp have shown that the upper limit in the urinary recovery of riboflavin is not due to a renal transport maximum, although there is evidence that riboflavin is excreted not only by glomerular filtration, but also by renal tubular secretion (6). Renal excretion rates over 450 mcg./min. (based on a 45-min. collection period) were observed by Stripp when 84 mg. of riboflavin was administered by intravenous infusion (4).

A review of the literature provides considerable information to explain the similarity in the absorption and excretion characteristics of FMN and riboflavin. There is extensive evidence showing that FMN is rapidly and almost completely dephosphorylated to free riboflavin in the small intestine. Okuda has found that FMN is rapidly decomposed enzymatically to free riboflavin in pancreatic juice, in homogenates of the small intestinal mucosa, and when injected into the lumen of the small intestine of rats (7, 8). Similar results were obtained by Turner and Hughes, who used isolated rat intestinal preparations (9). Okuda has found pronounced phosphomonoesterase activity in the jejunum and somewhat less in the duodenum and ileum of the rat (10). In this connection, it is of interest that betamethasone phosphate was found to undergo rapid dephosphorylation during gastrointestinal absorption and that only the free drug was found in the jugular blood of dogs after oral administration of this steroid ester (11).

Other studies reveal that riboflavin is eventually converted or reconverted to the phosphate ester (FMN) before it leaves the intestinal mucosa. Yagi and Okuda (12), Chen and Yamauchi (13), and Englard (14) have reported that riboflavin is phosphorylated enzymatically in the mucosa during absorption. Interestingly, Tedeschi and Canali (15, 16) find predominantly FMN in the *mesenteric* blood of rabbits after introduction of FMN or riboflavin into the intestine, while Stripp finds only the free vitamin in the *venous* blood of humans after oral administration of FMN (4). Thus, it appears that FMN is dephosphorylated in the intestinal lumen, rephosphorylated in the intestinal mucosa, transported in the blood to the liver as the phosphate ester, rapidly dephosphorylated in the liver, circulated as free riboflavin, and excreted mainly as such. This scheme represents the quantitatively important pathways in the metabolic disposition of large doses of the vitamin, and neglects the nutritionally more important but quantitatively minor biotransformation and retention of riboflavin in the form of FAD and various flavoproteins. The similarity in the absorption and excretion characteristics of riboflavin and FMN, respectively, can therefore be readily explained by the frequent and rapid interconversions of the two forms of the vitamin in the body. Such a pattern is by no means unique; Lawrence *et al.* have suggested that repeated hydrolysis and re-esterification in the intestinal mucosa occur during the absorption and storage of vitamin A (17).

Metabolism of Riboflavin and FMN.—Orally administered FMN was excreted in the urine mainly or solely as riboflavin. The same was true for riboflavin since no appreciable differences were found between the result of the Burch and modified U.S.P. assays of urines after oral administration of riboflavin (1). These findings are consistent with those of other investigators who could detect only riboflavin itself in the urine after oral or intravenous administration of riboflavin and FMN (4, 18, 19). However, Utsumi found that about 20% of an intravenous dose of FMN or riboflavin was excreted as FMN by ureter-cannulated patients with kidney disease (20). Since it is known that FMN is

hydrolyzed enzymatically in bladder urine (21), the results obtained by Utsumi may be due to by-passing of the bladder. It is of interest that Utsumi found FMN in the urine after intravenous administration of FMN as well as riboflavin (20). This may be due to some phosphorylation of riboflavin in the kidneys (22).

Apparent Enterohepatic Cycling of Riboflavin.—The second maximum in the excretion rate of riboflavin as a function of time (Figs. 3–5) appears to be due to the enterohepatic cycling of the vitamin. There is considerable evidence in the literature to support this hypothesis. In 1929, Makimura noted the presence of vitamins A, B, ascorbic acid, and vitamin D in the bile and proposed that enterohepatic cycling is responsible for the conservation of these vitamins (23). DePreux found riboflavin, mainly as FMN, in bile obtained by duodenal cannulation of healthy men (24). Tedeschi found mainly free riboflavin in the bile after introduction of FMN into the mesenteric circulation of rabbits (25). Yagi observed an accumulation of riboflavin in the small intestine of rats after subcutaneous injection of riboflavin and suggested that enterohepatic cycling was responsible (26). He provided additional data in support of this hypothesis in a recent study using ^{14}C-labeled riboflavin (27). Mizuhara (28) and Ono (29) perfused rabbit livers with blood containing riboflavin and dehydrocholic acid (the latter was used apparently to stimulate bile flow) and found that about 50% of the vitamin appeared in the bile.

Since Najjar *et al.* (30) found no increase in fecal riboflavin after intravenous injection of 5 to 30 mg. of riboflavin in man, and Stripp recovered 97% of an 84-mg. intravenous dose of riboflavin in the urine (4), it may be concluded that, in man at least, any riboflavin secreted into the bile is almost fully reabsorbed. It appears, therefore, that when large doses of riboflavin or FMN are administered, part of the absorbed vitamin is eliminated in the bile. When bile flow is stimulated by food, the vitamin enters the intestine and is reabsorbed, yielding the second peak in the excretion rate *versus* time plots obtained after riboflavin administration.

The possibility that the second peak in the excretion of riboflavin is due to delayed gastric emptying of a portion of the dose is ruled out by the similarity in the urinary recoveries of riboflavin in the experiments with and without lunch. Delayed gastric emptying of riboflavin would result in more extensive absorption of the vitamin in view of the saturability of its absorption mechanism.

In the two instances when the subjects repeated the experiments but did not eat lunch, the second excretion rate peak was considerably reduced in magnitude, but did not disappear entirely. Apparently, the thought of food at lunch time and the sight of others enjoying their lunch was sufficient to stimulate some flow of bile in the subjects. It is known that, while the emptying of the gallbladder normally follows the ingestion of certain foods, psychic influences will play a role. The sight and smell of food alone can produce a reflex stimulation of the gallbladder in both man and animals (31).

The second peak in the excretion rate of riboflavin was seldom seen with low doses (30 mg. or less) of riboflavin or FMN. While the reason for this is not readily apparent, it may be due to a concentration dependent relationship in the handling of riboflavin by the liver.

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