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Absorption and Excretion of Riboflavin in the Rat: An Unusual Example of Nonlinear Pharmacokinetics

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Abstract □ An estimation of the availability of riboflavin after oral administration of riboflavin-5′-phosphate is greatly complicated in the rat because of the occurrence of a complex and marked nonlinear, dose-dependent excretion of the vitamin. The total urinary recovery of riboflavin after intraperitoneal administration of riboflavin-5′-phosphate was found to be highly dose dependent in both normal and vitamin-deficient rats as well as in deficient rats with ligated bile ducts. The elimination of the vitamin involves at least two nonlinear processes occurring simultaneously and having opposite effects on the dose–total urinary recovery relationship. One process involves biliary excretion which increases disproportionately with increasing body levels of riboflavin. The other process appears to be a binding of the vitamin to tissues which function kinetically as deep compartments. Apparently, the higher the body level of the vitamin, the smaller is the fraction that can be "immobilized" in the compartments and the larger is the fraction that can be detected in the urine. Over the dose range studied, the nonlinear tissue binding phenomenon is predominant over the nonlinear biliary excretion in the riboflavin-deficient rat. The converse is true in the normal, nondeficient animal.

Keyphrases □ Riboflavin—absorption and excretion after oral administration of riboflavin-5′-phosphate, nonlinear pharmacokinetics, rats □ Pharmacokinetics, nonlinear—absorption, excretion of riboflavin, rats □ Absorption, riboflavin—nonlinear pharmacokinetics, rats □ Excretion, riboflavin—nonlinear pharmacokinetics, rats

The extensive studies of Levy and Jusko (1, 2) with riboflavin revealed that the GI absorption of this vitamin in man deviates markedly from the classical principles of passive transport. For example, the urinary recovery of riboflavin as a function of dose after oral administration of riboflavin or riboflavin-5′-phosphate to fasted healthy humans shows that the absorption process is capacity limited and easily saturable. Urinary excretion data further suggest that the absorption of the vitamin is limited to the proximal small intestine. When the vitamin is administered with food, absorption of riboflavin is enhanced and saturation of intestinal absorption is evident only with doses exceeding 30 mg. The site specificity and capacity-limited characteristics of riboflavin absorption suggest that the vitamin is absorbed by a specialized transport process rather than by passive diffusion. More recently, Jusko *et al.* (3) suggested that bile may play a role in the absorption of riboflavin. After oral administration of a saturation dose of the vitamin (150 mg./m.^{2}) to children with biliary obstruction, the absorption of riboflavin was significantly impaired as compared to that observed in normal children of similar ages. Surgical correction of biliary atresia in two patients led to increased absorption of the vitamin.

The characteristics of riboflavin absorption in the rat appear to be quite different than those observed in man. For example, although riboflavin seems to be absorbed by a specialized transport process in man, Spencer and Zamcheck (4) observed that the isolated rat intestine fails to transport riboflavin against a concentration gradient. Studies in this laboratory indicate that transfer of riboflavin across the rat everted intestine is unaffected by classic inhibitors such as cyanide and dinitrophenol. More importantly, the marked capacity-limited absorption of riboflavin observed in man has not been seen in intact rats even at doses (on a weight basis) considerably larger than those employed in man. After oral administration of up to 8-

Table I—Blank Values Expressed in Terms of Apparent Riboflavin in Rat Urine Collected under Different Experimental Conditions

Experimental Conditions				
Number of Animals	Nutritional Status	Dietary State	Bile Duct	Amount Excreted, mcg.[a]
5	Riboflavin deficient	Fasted	Intact	11 ± 2
8	Riboflavin deficient	Fasted	Ligated	10 ± 2
5	Riboflavin deficient	Fed	Intact	15 ± 2
5	Normal	Fasted	Intact	69 ± 12
5	Normal	Fed	Intact	151 ± 36

[a] Mean amount of apparent riboflavin ± *SD* of the mean, in rat urine collected over a 72-hr. period.

Table II. Urinary Recovery of Riboflavin after Administration of Riboflavin-5′-phosphate Equivalent to 1 mg. Riboflavin

Experimental Conditions						
Number of Animals	Route of Administration[a]	Nutritional Status[b]	Dietary State	Bile Duct	Amount Excreted in 24 hr., mcg.[c]	Total Amount Excreted, mcg.[d]
10	g.i.	Deficient	Fasted	Intact	15 ± 9	44 ± 21
5	g.i.	Deficient	Fed	Intact	8 ± 6	44 ± 33
5	i.p.	Deficient	Fasted	Intact	404 ± 38	413 ± 36
11	g.i.	Deficient	Fasted	Ligated	18 ± 7	58 ± 26
8	i.p.	Deficient	Fasted	Ligated	657 ± 68	721 ± 74
6	g.i.	Normal	Fasted	Intact	65 ± 31	98 ± 40
7	i.p.	Normal	Fasted	Intact	490 ± 35	509 ± 49

[a] Key: g.i. = gastric intubation, i.p. = intraperitoneal injection. [b] Deficient denotes riboflavin-deficient rats. [c] Mean ± *SD* of the mean. [d] Mean ± *SD* of the mean, urine collected over a 72-hr. period.

mg./kg. doses of riboflavin-5′-phosphate to riboflavin-deficient Wistar rats, the urinary excretion was found to be proportional to the dose of the vitamin (5). However, unlike the situation in healthy humans where it is clear that urinary recovery of riboflavin is linearly related to the amount of the vitamin absorbed (6), the relationship between amount absorbed and urinary excretion has not been established in the rat.

In view of the reported differences between man and rat with respect to the apparent mechanism(s) of riboflavin absorption, it was of interest to study several additional aspects of riboflavin absorption in the rat—*viz.*, the influence of bile and the coadministration of food. It was also decided to examine the relationship between the availability of the vitamin and its urinary recovery in both normal and riboflavin-deficient rats.

EXPERIMENTAL

Male, Sprague-Dawley rats[1] weighing about 250 g. were used. The rats were divided into two groups; one group was maintained on a special riboflavin-deficient diet[2] while the other group received the routine laboratory diet[3]. Studies were initiated only after a rat had received a given diet for at least 2 weeks. Bile duct ligation was performed on randomly selected rats from the riboflavin-deficient group, using techniques described previously (7). These animals were allowed a 2-week recovery period before further studies were performed.

An amount of the sodium salt of riboflavin-5′-phosphate[4] equivalent to 0.2 or 1.0 mg. of riboflavin was dissolved in 1 ml. of water and administered either by gastric intubation or intraperitoneal injection. Usually, the rats were fasted 24 hr. before and during a given experiment. In some cases, however, food was not withdrawn. After receiving the vitamin, the animals were placed in individual stainless steel metabolism cages which permitted separate collection of urine and feces.

Urine was collected at 24-hr. intervals for a total of 72 hr. after administration. This period was sufficient in all cases to ensure complete recovery. Total riboflavin in urine was determined fluorometrically by methods described previously (1). The urinary excretion data were corrected for blank values on the basis of 72-hr. urine samples obtained from several rats under different experimental conditions before vitamin administration.

RESULTS AND DISCUSSION

The total amount of apparent riboflavin in blank urine collected over a 72-hr. period varied significantly under different experimental conditions. As shown in Table I, nutritional status was the single most important parameter influencing blank levels. The level of apparent riboflavin in urine from fasted normal rats was about six times that found in urine from fasted riboflavin-deficient animals. Continuous feeding of the standard diet to normal rats during urine collection resulted in considerably greater blank values. In fact, the high blanks found under this condition made it virtually impossible to determine the influence of feeding on the GI absorption of riboflavin in normal rats without resorting to tracer riboflavin. Blank values of urine from riboflavin deficient rats were relatively low under all conditions, on the order of 3–5 mcg. of apparent riboflavin per day. Bile duct ligation had no effect on the blank value, but allowing continuous access to the special diet during collection did produce a small but significant increase in the level of apparent riboflavin.

The urinary recoveries of riboflavin after oral or intraperitoneal administration of a 1-mg. dose of the vitamin under different experimental conditions are presented in Table II. After intubation, the total amount of riboflavin recovered from rat urine represents a very small fraction of the administered dose. Only 4% of the dose was recovered from the urine of riboflavin-deficient rats under both fasting and feeding conditions. Bile duct ligation resulted in a modest increase up to a level of about 6% of the dose. The greatest urinary recovery after gastric intubation was found with normal rats where the total amount of excreted riboflavin accounted for about 10% of the dose.

The urinary excretion data suggest not only the possibility of incomplete absorption of riboflavin from the rat gut but also an exceedingly low rate of absorption of the vitamin, particularly in the riboflavin-deficient animal. The amount of riboflavin in the urine collected during the first 24 hr. after gastric intubation of riboflavin to vitamin-deficient rats represented only 20–30% of that which was ultimately excreted. In all cases with the deficient rats, the peak excretion rate was noted during the second 24-hr. period after administration. In contrast, the amount of riboflavin in the urine collected during the first 24 hr. after gastric intubation of the vitamin to normal rats accounted for about two-thirds of the amount ultimately excreted. Inspection of the urinary excretion data obtained after intraperitoneal administration shows that the delayed excretion of the vitamin in riboflavin-deficient rats after gastric intubation was due to slow absorption from the GI tract. Almost all (91–98%) of the vitamin ultimately recovered in rat urine after intraperitoneal injection, under various experimental conditions, was found within 24 hr. after administration.

Urinary recovery of riboflavin after intraperitoneal administration of 1 mg. of the vitamin was substantial in all cases but nevertheless incomplete. About 40% of the dose was found in the urine after intraperitoneal administration of the vitamin to deficient animals. Bile duct ligation produced almost a twofold increase in recovery. This finding strongly suggests that biliary secretion plays a major role in the elimination of the vitamin under these conditions. Since riboflavin is virtually unmetabolized in the rat (8), the incomplete urinary recovery of the vitamin after intraperitoneal administration to bile duct-ligated, deficient animals suggests that a significant fraction of the dose is immobilized in "deep compartments" from which release is so slow as to produce total urine levels that are only negligibly higher than blank values.

As noted previously, Christensen (5) implied a linear relationship between the total recovery of riboflavin in the urine of vitamin-defi-

[1] Blue Spruce Farms, Altamont, N. Y.
[2] Nutritional Biochemicals Corp., Cleveland, Ohio.
[3] The Pasteurizable Diet 4 RF, Agway, Inc., Syracuse, N. Y.
[4] Sodium riboflavin 5′-phosphate, Hoffmann-La Roche, Inc., Nutley, N. J.

Table III—Urinary Recovery of Riboflavin after Intraperitoneal Administration of Riboflavin-5′-phosphate Equivalent to 0.2 mg. Riboflavin

Experimental Conditions			
Number of Animals	Nutritional Status	Bile Duct	Total Amount Excreted, mcg.[a]
5	Riboflavin deficient	Intact	59 ± 5
5	Riboflavin deficient	Ligated	57 ± 9
10	Normal	Intact	125 ± 28

[a] Mean ± *SD* of the mean, urine collected over a 72-hr. period.

Table V—Influence of Dose and Nutritional Status on Urinary Recovery of Riboflavin after Intraperitoneal Administration of Riboflavin-5′-phosphate

Number of Animals	Dose, mcg.	Nutritional Status	Percent of Dose Excreted[a]
5	200	Riboflavin deficient	29 ± 3
10	200	Normal	63 ± 14
5	1000	Riboflavin deficient	41 ± 4
6	1000	Normal	51 ± 4

[a] Mean ± *SD* of the mean, urine collected over a 72-hr. period.

cient rats and the amount absorbed. If this were the case, then several approaches would be available to estimate the bioavailability of riboflavin after oral administration under the various experimental conditions. However, considerable evidence indicates that this assumption is untenable. The urinary excretion data in Table II suggests the existence of two phenomena: biliary excretion and tissue binding, which are frequently nonlinear in nature. Najjar and Holt (9) reported that the urinary recovery of riboflavin after intravenous administration of 1 mg. of the vitamin is substantially less in human subjects with ariboflavinosis than in normal subjects. In contrast, Axelrod *et al.* (10), employing intravenous doses of 10–20 mg. of the vitamin, found no difference in the urinary recovery of riboflavin in normal and avitaminosis individuals. Presumably the larger doses so far exceeded the quantity that could be "utilized" or "immobilized" even by a deficient individual as to leave a great excess available for urinary excretion; consequently, the anticipated low excretion in subjects with avitaminosis did not occur. More recently, Nogami *et al.* (11) observed an unusual dose dependency in the biliary excretion of riboflavin in normal rats. Upon intravenous administration of 4 mg. riboflavin-5′-phosphate, about 30% of the dose was recovered from bile. On the other hand, only about 15% of a 0.04-mg. dose was found in the bile.

Further evidence that the pharmacokinetics of riboflavin elimination in both normal and deficient rats involve nonlinear processes is provided by the urinary excretion data obtained after intraperitoneal administration of a 0.2-mg. dose of the vitamin. As noted in Table III, urinary recovery of riboflavin after administration of this lower dose of the vitamin to deficient rats was only 29% of the dose. Bile duct ligation had no effect on urinary recovery. However, a substantially larger amount of riboflavin, on the order of 63% of the dose, was found in the urine of normal animals.

Table IV summarizes the data obtained after intraperitoneal administration of each dose level to deficient rats. The marked dose dependency in the urinary excretion of riboflavin is quite evident. At the 0.2-mg. dose, the total urinary recovery of riboflavin was identical in ligated and nonligated animals, suggesting that no net biliary elimination takes place at this dose level. A highly significant difference in urinary recovery of riboflavin from ligated and nonligated rats was observed at the 1-mg. dose. Net biliary secretion appears to play a considerable role in the elimination of the vitamin at the higher dose level. The data in Table IV indicate, in accord with the findings of Nogami *et al.* (11), that the higher the amount of riboflavin in the body the greater the contribution of biliary secretion to the overall elimination of the vitamin.

Conversely, if one focuses on this nonlinear phenomenon alone, it might be assumed that the higher the body level of riboflavin the lower the relative urinary recovery of the vitamin. Further inspection of the relationships in Table IV reveals, however, that this converse is not true. The urinary recovery of riboflavin expressed as a percent of dose in bile duct-ligated animals increases substantially when the dose is increased fivefold. The observation strongly implies that a second nonlinear process is also operative in the apparent elimination of riboflavin in deficient rats. This process appears to be a binding of the vitamin to tissues which function kinetically as deep compartments. By making the reasonable assumption that such binding would involve a capacity-limited process, it is clear that the higher the body level of riboflavin the smaller the fraction that can be immobilized and the larger the fraction that can be detected in the urine. Even in the nonligated rats, where both nonlinear processes are presumed to be operative, the urinary recovery increases (rather than decreases) significantly with increasing dose. As anticipated, the increase is not nearly as large as that observed in ligated animals. This finding simply indicates that, over this dose range in the riboflavin-deficient rat, the nonlinear tissue binding phenomenon is predominant over the nonlinear biliary excretion.

Table V summarizes the data obtained after intraperitoneal administration of each dose level to normal and riboflavin-deficient rats and illustrates the influence of nutritional status on the dose-dependent urinary excretion of the vitamin. At the low dose, the urinary recovery of riboflavin from normal rats was more than twice that obtained from deficient animals. However, at the 1-mg. dose, the urinary recovery of riboflavin was quite similar in both normal and deficient rats. These findings are analogous to the results in human subjects reported by Najjar and Holt (9) and Axelrod *et al.* (10). Urinary recovery of riboflavin can be used to differentiate normal and ariboflavinosis subjects only when an appropriately small dose is employed. As the dose is increased, tissue utilization or immobilization of riboflavin, which truly distinguishes between the normal and deficient subjects, becomes less and less important in the overall elimination of the vitamin.

As noted previously, the urinary recovery of riboflavin (expressed as percent of dose) in the deficient rats increases considerably as

Table IV—Influence of Dose and Bile Duct Ligation on Urinary Recovery of Riboflavin after Intraperitoneal Administration of Riboflavin-5′-phosphate to Riboflavin-Deficient Rats

Number of Animals	Dose, mcg.	Bile Duct	Percent of Dose Excreted[a]
5	200	Intact	29 ± 3
5	200	Ligated	29 ± 5
5	1000	Intact	41 ± 4
8	1000	Ligated	72 ± 7

[a] Mean ± *SD* of the mean, urine collected over a 72-hr. period.

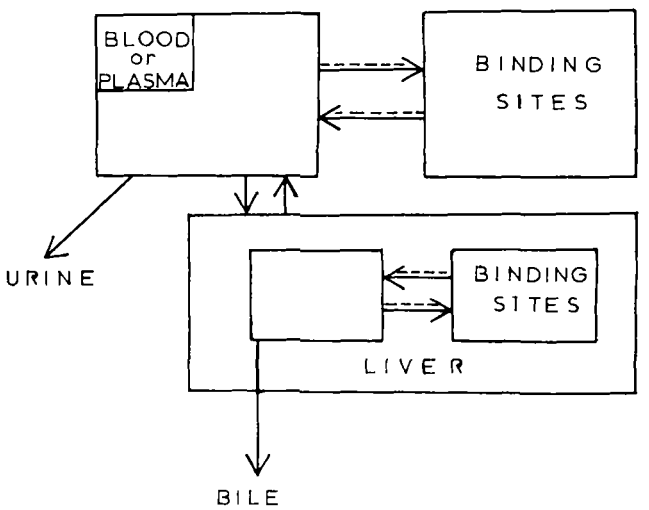

Figure 1—*Nonlinear multicompartment model which is compatible with the observed pharmacokinetics of riboflavin in the rat. The dashed arrows denote capacity-limited processes. See text for discussion.*

Table VI—Estimates of Availability of Riboflavin-5′-phosphate after Gastric Intubation of Dose Equivalent to 1 mg. Riboflavin to Riboflavin-Deficient Rats[a]

Number of Animals	Dietary State	Bile Duct	Amount Absorbed, mcg.[b]
10	Fasted	Intact	150 ± 70
5	Fed	Intact	150 ± 110
11	Fasted	Ligated	200 ± 90

[a] See text for discussion. [b] Mean ± *SD* of the mean (assuming variability in urinary recovery after intraperitoneal administration of 200-mcg. dose is relatively negligible).

the intraperitoneal dose is increased from 0.2 to 1 mg. However, in the normal rat the urinary recovery of the vitamin actually shows a modest decrease over this dosage range. Presumably, tissue binding is of much less significance in the normal rat than in the deficient animal and the decreased riboflavin recovery at the higher dose level reflects the nonlinear biliary excretion phenomenon.

A pharmacokinetic model such as that shown in Fig. 1 readily rationalizes the observed dose dependency in the urinary excretion of riboflavin in the rat. The stippled arrows denote capacity-limited processes, which may be quantitatively characterized by Langmuir-type isotherms as suggested by DiSanto and Wagner (12). It is clear that in the bile duct-ligated situation, the apparent urinary recovery expressed as a function of the dose of the vitamin increases with increasing dose.

It is also clear that in animals with intact bile ducts, the biliary clearance increases with increasing total levels of vitamin in the liver. Interestingly, the unusual nonlinear process characterizing the biliary excretion of riboflavin observed in the present study as well as by Nogami *et al.* (11) was also observed by Jusko (6) with respect to the renal elimination of riboflavin. Renal clearance values of riboflavin in man and dog increase with increasing serum concentrations of the vitamin. Despite the kinetic similarities, it is likely that the mechanisms of these processes are different. It is proposed in the present study that the nonlinear biliary excretion of riboflavin is due to tissue binding. On the other hand, Jusko (6) suggested that the dose-dependent renal clearance of riboflavin is due to saturable tubular reabsorption.

The assumption that urinary recovery of riboflavin in the deficient rat is linearly related to the available dose may introduce significant error in the estimation of bioavailability. Not only is the percent urinary recovery of riboflavin dependent on the available dose, but it is also likely to be dependent on the rate of absorption of the available dose. Despite the problems of interpretation, the urinary excretion data do permit some conclusions to be drawn as to the effects of various conditions on the GI absorption of riboflavin. The amount of riboflavin recovered in urine of ligated and nonligated deficient rats after intraperitoneal administration of 0.2 mg. of the vitamin is quite similar to the amount of riboflavin recovered in the urine of deficient rats under various conditions after gastric intubation of 1-mg. doses. Accordingly, one can make the following assumptions: (*a*) the 0.2-mg. i.p. dose of riboflavin is fully available, and (*b*) there is, within experimental error, a linear relationship between the urinary recovery of riboflavin and the available dose over an available dose range of 150–200 mcg.

Based on these assumptions, one can estimate the bioavailability of riboflavin after gastric intubation of 1-mg. doses under different experimental conditions. The fallacy in this approach is that the time course of absorption is quite different after intraperitoneal and oral administration. Hence, this type of calculation will only represent the minimum amount absorbed from the gut. These data, however, may be quite useful in a relative sense since the time course of absorption was quite similar under all conditions in deficient rats after gastric intubation. The estimates are shown in Table VI. The most noteworthy aspect of the data is that the absorption of riboflavin in the rat is quite variable. The minimum amount absorbed was virtually identical in fasting and feeding rats. The mean riboflavin availability estimate in the ligated rat was somewhat higher than that calculated in the nonligated animals, but differences were well within experimental variability. Hence, it was not possible in the present study to detect any influence of feeding or biliary insufficiency on the GI absorption of riboflavin in the vitamin-deficient rat.

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