Effect of Age on Intestinal Absorption of Riboflavin in Humans

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Abstract □ The development of gastrointestinal absorption function in humans was studied using riboflavin, a vitamin which is absorbed by a site-specific (proximal small intestine) and saturable transport process. Oral doses of 150 mg./m.² body surface area of riboflavin-5′-phosphate were administered in solution to subjects ranging in age from 0.25 to 40 years. The urinary recovery of the vitamin increased significantly (from 6 to 12% of the dose) over this age range. The ratio of maximum excretion rate to dose and the time of occurrence of the maximum excretion rate were independent of age. The kinetics of riboflavin elimination also did not show any appreciable change with age. These observations suggest that, in the age range studied, younger subjects retain the vitamin at intestinal absorption sites for a shorter period of time than do older subjects. This appears to be due to decreased intestinal transit rate with increasing age. Prompt release of drugs from pharmaceutical dosage forms seems therefore even more important in children than in adults in order to assure adequate absorption.

Keyphrases □ Riboflavin absorption—intestinal □ Age effect—riboflavin intestinal absorption □ Kinetic equations—urinary excretion parameters relationship, gastrointestinal absorption □ Elimination half-life, riboflavin—age effect

There is little biopharmaceutical information available to aid in the design of oral dosage forms for infants and children. Of the various physiologic characteristics which might change with age, those related to gastrointestinal absorption are among the least studied. Several recent reviews have pointed to the need for quantitative studies of intestinal absorption function at various ages (1–3).

In the course of investigations of factors affecting the gastrointestinal absorption of riboflavin (4) and riboflavin-5′-phosphate (5) in man, it was found that this vitamin is absorbed in the proximal small intestine by a saturable transport process. The absorption of riboflavin can therefore serve as an index of gastrointestinal transit rate since absorption only occurs while the vitamin is in the proximal small intestine. Since the gastrointestinal absorption of drugs administered in solid dosage forms can be impaired if the solid drug is not dissolved before or during passage through the small intestine (6), the results of absorption studies with riboflavin are applicable in general terms to other drugs inasmuch as their absorption is affected by the rate of gastrointestinal transit. The use of riboflavin is particularly advantageous in this study involving infants and children since the absorption of this vitamin can be assessed from urinary excretion data (4, 5).

THEORETICAL

In order to understand more clearly the relationship of urinary excretion parameters (time course of excretion rates and urinary recovery) to gastrointestinal factors involved in absorption by a specialized transport process (maximum absorptive capacity and intestinal transit rate), it is useful to evolve mathematical relationships for a simple pharmacokinetic model which is consistent with the proposed mechanism of intestinal absorption.

If a substance is absorbed by a saturable specialized process in the small intestine and the dose administered (X_D) is large enough to saturate the absorption process, then the rate of absorption can be considered to be zero order ($V_{max.}$). Assuming the body to be a single compartment and the rate of elimination to be first order (k_E), the appropriate pharmacokinetic model is:

$$X_A \xrightarrow{V_{max.}} X_B \begin{array}{l} \xrightarrow{k_e} X_U \\ \xrightarrow{k_m} X_M \end{array}$$

Scheme I

where X represents amounts of drug, subscripts refer to amount absorbed (A), amount in the body (B), and amount eliminated *via* urine (U), and by metabolism and/or extrarenal routes (M), and $X_D \gg X_A$. First-order rate constants for the elimination processes are represented by k_e and k_m and:

$$k_E = k_e + k_m \qquad \text{(Eq. 1)}$$

The differential equations which are applicable are:

$$-dX_A/dt = V_{max.} \qquad \text{(Eq. 2)}$$

$$dX_B/dt = V_{max.} - k_E \cdot X_B \qquad \text{(Eq. 3)}$$

$$dX_U/dt = k_e \cdot X_B \qquad \text{(Eq. 4)}$$

While absorption is occurring, solution of these equations for X_B yields:

$$X_B = (V_{max.}/k_E) \cdot [1 - \exp(-k_E \cdot t)] \qquad \text{(Eq. 5)}$$

and from Eqs. 4 and 5, it can be seen that:

$$dX_U/dt = (V_{max.} \cdot k_e/k_E) \cdot [1 - \exp(-k_E \cdot t)] \qquad \text{(Eq. 6)}$$

The maximum amount of drug in the body, $(X_B)_{max.}$, occurs when Eq. 3 equals zero or:

$$(X_B)_{max.} = V_{max.}/k_E \qquad \text{(Eq. 7)}$$

and the corresponding maximum urinary excretion rate, $(dX_U/dt)_{max.}$, from Eqs. 4 and 7 is therefore:

$$(dX_U/dt)_{max.} = V_{max.} \cdot k_e/k_E \qquad \text{(Eq. 8)}$$

The actual peak excretion rate (ER_p) will be proportional to $V_{max.}$ (Eq. 8) only after absorption at $V_{max.}$ has proceeded over a period of at least 4 elimination half-lives, *i.e.*, when:

$$\exp(-k_E \cdot t) \to 0$$

so that Eq. 5 reduces to Eq. 8. If the time of occurrence (t_p) of ER_p is $<4 \cdot t_{1/2}$, then $V_{max.}$ can be determined by rearranging Eq. 6 to yield:

$$V_{max.} = (ER_p \cdot k_E/k_e)/[1 - \exp(-k_E \cdot t_p)] \qquad \text{(Eq. 9)}$$

The amount of drug absorbed (X_A), from Eq. 2, is:

$$X_A = V_{max.} \cdot t_q \qquad \text{(Eq. 10)}$$

where t_q is the time during which drug absorption proceeds. This equation shows that two intestinal factors can modify the amount of drug absorbed under the conditions of the model: (*a*) the maxi-

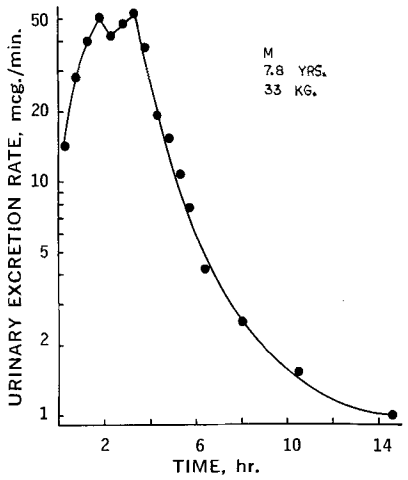

Figure 1—*Urinary excretion rate as a function of time after oral administration of 150 mg./m.² riboflavin to a human subject.*

mum absorption rate ($V_{max.}$), and (*b*) the residence time of drug at the absorption site (t_g). Since the urinary recovery of drug is proportional to the amount absorbed:

$$X_U^\infty = X_A \cdot k_e/k_E \qquad \text{(Eq. 11)}$$

it will also be dependent on the contribution of $V_{max.}$ and t_g to intestinal absorption of the drug.

Although the kinetics of absorption and excretion of riboflavin are more complex than the theoretical model, the mathematical relationships developed here will facilitate interpretation of the experimental results.

EXPERIMENTAL

Thirteen male and 10 female human subjects in apparent good health served as test subjects. The age of the subjects ranged from 3 months to 40 years. After an overnight fast, each subject received an oral dose of 150-mg. riboflavin, administered as riboflavin-5′-phosphate[1] (FMN), per square meter of body surface area. The surface area was calculated from the weight and height of each subject by the method of Dubois and Dubois (7). The vitamin was given in 15 to 40 ml. of aqueous solution immediately following a standard breakfast consisting of 30 g. cereal and 250 ml. milk or formula per square meter surface area. An equal volume of orange juice was usually mixed with the FMN solution to mask the taste. Urine was usually collected in older children and adults at 0.5-hr. intervals for 4 hr., 1-hr. intervals for the next 4 hr., and then at convenient intervals for a total of 36 hr. The subjects were moderately hydrated with oral fluids during the course of experiments to maintain adequate urine output. Studies on infants and children were carried out at the Children's Hospital of Buffalo under medical supervision. A plastic urine collection device was taped to the genital region of the infants and permitted quantitative collection of samples. The subjects were required not to take any drugs or vitamin preparations for at least 3 days prior to or during the experiments.

Total riboflavin in urine was determined fluorometrically by methods previously described (4, 5). An 18- to 24-hr. blank urine collection was obtained in each subject, and all data were corrected for blank values.

RESULTS

The typical time course of urinary excretion rate of riboflavin after oral administration of FMN is shown in Fig. 1. From such excretion data, the following parameters were determined for each subject: half-lives of elimination, urinary recovery, peak excretion rate, and time of occurrence of the peak excretion rate.

[1] Sodium riboflavin-5′-phosphate, Lot No. 414085, Hoffmann-La Roche, Nutley, N. J.

The results shown in Fig. 1 indicate that the decline in urinary excretion rate was biexponential but the initial "rapid" phase with a half-life of about 1.4 hr. accounts for elimination of most of the vitamin. A smaller fraction of the excreted vitamin was recovered in the urine during the terminal "slow" phase. To estimate the rapid and slow half-lives of elimination, semilogarithmic plots of amount of riboflavin remaining unexcreted as a function of time were constructed. Typical results obtained in four of the younger subjects are shown in Fig. 2. After the absorptive phase, which affects the first four hours of elimination, the decline in excretion was resolved into the rapid and slow phases by the method of residuals (8). The data are shown for the younger subjects because these were generally more variable than results obtained in older children and adults. This is partially due to the difficulty in ensuring complete urine voiding at the desired collection times for such subjects.

The relationship of the rapid and slow half-life of elimination to age of the subjects is shown in Fig. 3. In this and subsequent plots and statistical treatments, the logarithm of age was used. The rapid half-life of elimination exhibits a limited range of values, averaging 1.4 hr., and neither the least-squares regression nor the correlation coefficient shows any significant change of this parameter with age. The mean values, least-squares regression slopes and intercepts, correlation coefficients, and statistical calculations for this parameter, as well as others to be considered, are summarized in Table I. The slow half-life of elimination was quite variable but shows a barely statistically significant ($p = 0.05$) tendency to increase with age. Tests were repeated in five of the subjects as shown in Fig. 3. In four of these, where the age of the subject was similar in both tests, an average of the pairs of parameters was used with the mean of the logarithm of age in the statistical calculations.

There was a statistically significant ($p < 0.01$) increase in urinary recovery of riboflavin with age (Fig. 4), from a mean of about 6% at 0.25 years to about 12% at 40 years of age. Since the dose of riboflavin administered (150 mg./m.²) was greatly in excess of that

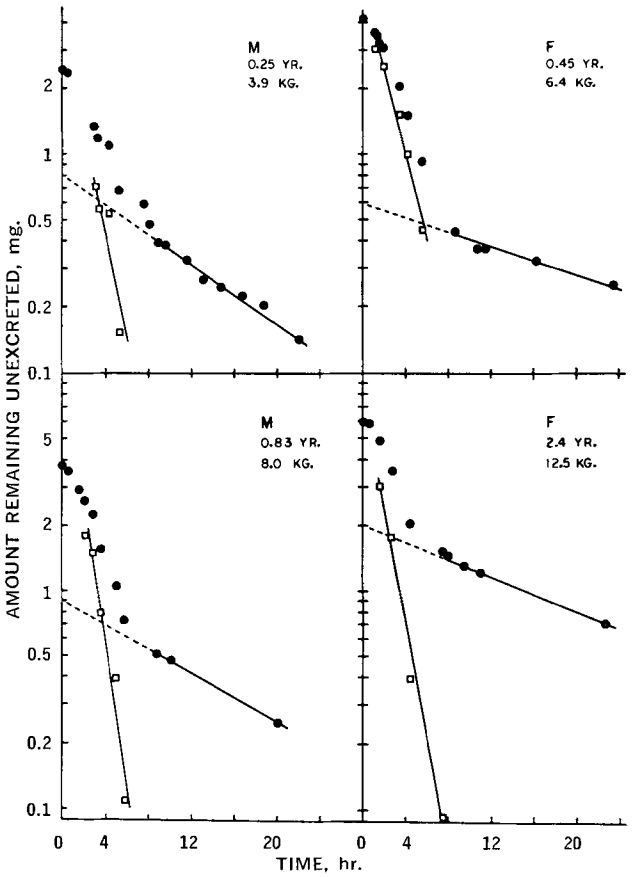

Figure 2—*Amount of riboflavin remaining unexcreted (●) as a function of time in four children. The rapid half-life of elimination was determined (□) from the postabsorptive data using the method of residuals by extrapolating (dashed line) the linear slow phase of elimination to the ordinate. Sex, age, and weight of the subjects are shown.*

Table I—Statistics of Various Riboflavin Absorption and Elimination Parameters as a Function of the Logarithm of Age

Parameter	Mean, SD	Least-Squares Linear Regression: Intercept[a]	Slope	95% Confidence Limits of Slope	Correlation Coefficient: r	t	Significance Level
Rapid half-life, hr.	1.35(0.60)	1.42	−0.197	−0.582 to 0.187	−0.161	0.82	50
Slow half-life, hr.	[b]	14.1	4.18	0.20 to 8.17	0.395	2.16	95
Percent urinary recovery	[b]	8.34	2.22	1.00 to 3.44	0.570	3.33	99
Peak excretion rate/dose	0.254(0.073)	0.245	0.032	−0.010 to 0.075	0.280	1.33	80
Time of peak excretion rate, hr.	2.18(0.89)	2.33	−0.458	−1.04 to 0.128	−0.280	1.27	75

[a] Intercept on parameter axis at age = 1 yr. [b] Significantly age dependent.

which can be absorbed (4, 5), the percent urinary recovery of the vitamin was correspondingly low.

The relationship of the peak excretion rate of riboflavin to the age of the subjects is shown in Fig. 5. The actual peak excretion rate was divided by the dose of riboflavin administered to permit comparison of the data over the entire age range. This parameter shows a slight and statistically insignificant (p = 0.2) tendency to increase with age. The value chosen for the peak excretion rate for the individual subjects is the average of the three highest excretion rates found. This method was chosen to reduce bias caused by occasional high excretion rate values resulting from incomplete voiding of an earlier sample by the younger subjects. The use of only the single highest values yielded a slope of essentially zero and indicated therefore no change with age.

The time of occurrence of the peak excretion rate is plotted as a function of age in Fig. 6 and shows a slight, but insignificant (p = 0.25), tendency to decrease with age.[2]

DISCUSSION

The intestinal absorption of riboflavin was studied as a function of age in normal subjects under conditions where maximum absorption of the vitamin will occur (*i.e.*, with food). The dose is at least five times greater than that needed to saturate the absorption process (4, 5) which accounts for the relatively low urinary recovery of riboflavin (Fig. 4). Because of the large dose employed, an assumption that riboflavin absorption occurs at a maximum rate ($V_{max.}$) during the early times after administration appears justified.

The urinary recovery of riboflavin shows a definite, though small, increase with age of the subjects (Fig 4). As shown in the *Theoretical* section, the increase in urinary recovery is a direct measure of the effect of age on intestinal absorption providing that the elimination kinetics of the vitamin are not also altered in the age range studied (Eq. 11). The kinetics of elimination of riboflavin are complex, but the data suggest that the renal excretion of the vitamin does not significantly change with age. A biexponential decline in elimination of riboflavin was found (Fig. 2), but it can also be seen (Fig. 1) that most of the vitamin is excreted during the phase characterized by a rapid half-life. This parameter remained essentially constant over the age range studied (Fig. 3). Although the slow half-life of elimination showed a tendency to increase with age, this phase contributed less than 30% to the overall urinary recovery of riboflavin. Furthermore, since the urinary excretion of the vitamin was only followed for 36 hr., it is probable that the age dependency of the slow half-life would cause the urinary recoveries of riboflavin to be even greater in the older subjects if urine was collected over a longer time period. Also, the length of the slow half-life relative to the duration of urine collections limits considerably the accuracy of this parameter.

Two factors should primarily affect the intestinal absorption of a substance such as riboflavin under the conditions of this study: the maximum absorptive capacity and the intestinal transit rate (Eq. 10). Since the peak excretion rate to dose ratio (Fig. 5), the time of its occurrence (Fig. 6), and the rapid half-life of elimination (Fig. 3) all seem unaffected by age, it may be concluded that the maximum absorptive capacity of riboflavin, when corrected for body surface area, is probably quite constant in the age range studied (Eq. 9). It therefore appears that an age-dependent increase in retention of the vitamin at intestinal absorption sites is responsible for the increased urinary recovery of riboflavin with age. This may be primarily due to decreased intestinal transit rate in older subjects. The possibility that the relative length of intestine capable of absorbing riboflavin increases with age cannot be ruled out although the age independence of $V_{max.}$ and t_p do not support this possibility.

Although the vitamin was administered as FMN in the present study, there is extensive evidence showing that FMN is rapidly and almost completely dephosphorylated to free riboflavin in the small intestine (5). The specialized transport process appears to involve subsequent rephosphorylation of riboflavin to FMN in the

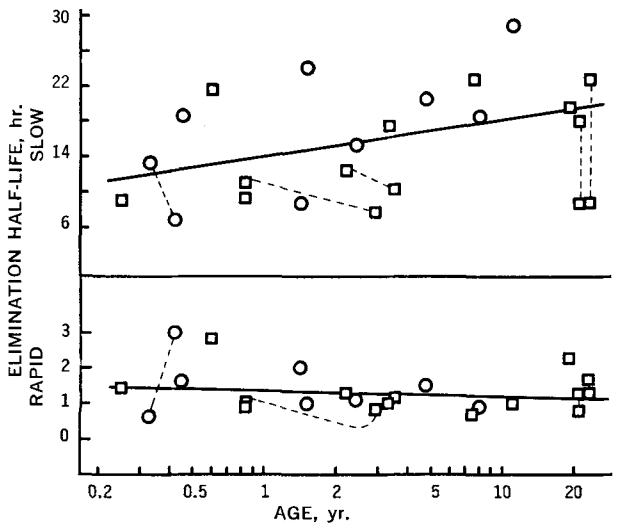

Figure 3—*Relationship between the rapid and slow half-life of elimination and the age (logarithmic scale) of the subjects. Squares and circles represent male and female subjects, respectively. Dashed lines connect repeated tests in the same subject and the solid line shows the least-squares regression fit of the data (Table I).*

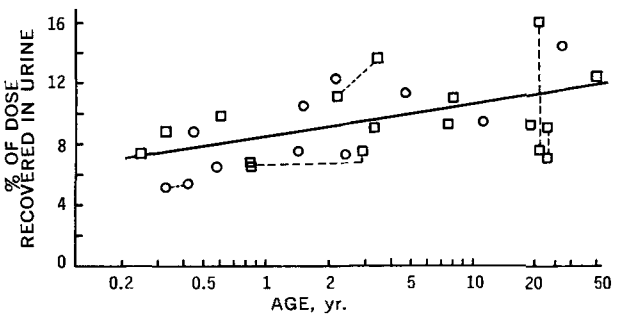

Figure 4—*Relationship between the percent urinary recovery of riboflavin and age of the subjects. Symbols are defined as in Fig. 3.*

[2] The difference in the number of data points shown in Figs. 3–6 is due to the lack of availability of some of the values, usually because of not frequent enough urine collections, particularly in the younger subjects.

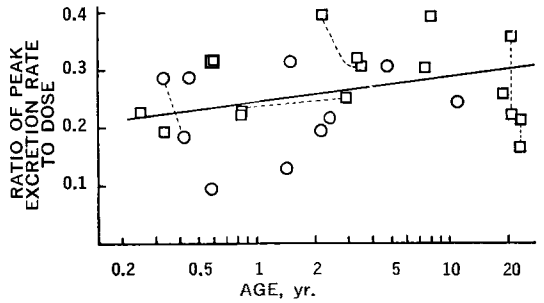

Figure 5—*Relationship between the ratio of peak excretion rate to dose and age of the subjects. Symbols are defined as in Fig. 3.*

intestinal wall (5, 9). The possibility that conversion of the oral dose of FMN to riboflavin could be rate-limiting in the absorption process appears to be ruled out by identical urinary excretion data obtained after oral administration of either form of the vitamin (4, 5). The lack of change in the peak excretion rate to dose ratio with age and the observation (9) that the activity of intestinal phosphatases decreases rather than increases with age (at least in the rat) further rule out the FMN dephosphorylation rate as a factor causing increased absorption of riboflavin with age.

The dependence of absorption on age and intestinal transit rate is not unique for riboflavin. D-Xylose, used commonly in clinical tests of intestinal absorption (10), exhibits absorption characteristics very similar to riboflavin. This sugar appears to be absorbed by a specialized process chiefly in the duodenum and proximal jejunum (10, 11). Lanzkowsky *et al.*, using both urinary excretion (12) and blood level (13) measurements, have shown a significant (twofold) increase in D-xylose absorption over an age range similar to that used in this study. Barreiro *et al.* (14) have further demonstrated a correlation of D-xylose absorption with intestinal transit time using direct measurements of intestinal motility in human subjects.

Many physiologic factors can influence drug absorption from the gastrointestinal tract (15), but little information is available on their role at various ages. A number of intestinal enzymes exhibit age-dependence in activity (1). Bender (16) has described a pronounced decrease in splanchnic blood flow in the elderly. Gastric emptying rate, though dependent on numerous factors and usually quite variable, is considered to be relatively constant throughout the childhood growth period (17). Intestinal secretions, the segmental activity of the bowel, the type of intestinal motility, the degree of vascularity, and the relative length of the intestine could also vary with age (1, 2, 9) and affect drug absorption. The increase in riboflavin absorption with age may, in part, be related to one or more of these factors which may also alter the apparent retention of the vitamin at intestinal absorption sites.

The results of this study should not be extrapolated to ages outside of the age range investigated. Bender (18) has cited possible factors which may account for decreased specialized intestinal absorption of several compounds in the elderly and it is known that many physiologic processes undergo rapid quantitative changes in the neonate (17). The results of the present study do suggest, however, that prompt release of drugs from pharmaceutical dosage forms to assure absorption is even more important in young children than in adults, in view of the apparently shorter residence time of such drugs at intestinal absorption sites.

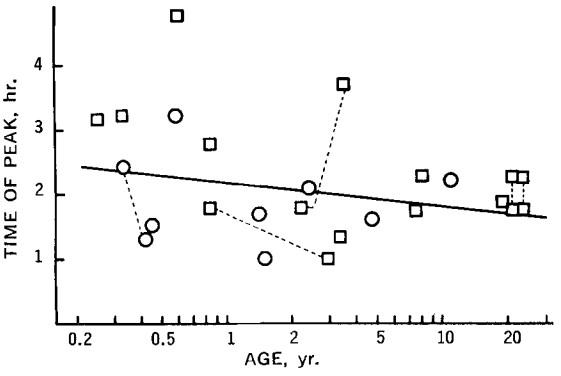

Figure 6—*Relationship between the time of occurrence of the peak excretion and age of the subjects. Symbols are defined as in Fig. 3.*

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