Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans\textsuperscript{1–4}

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ABSTRACT  The pharmacokinetics and utilization (flavocoenzyme synthesis) of orally and intravenously administered riboflavin in healthy humans were assessed. After the determination of circadian rhythms of riboflavin concentrations in blood plasma and urine of four males and five females (control period), each of these subjects received three different oral riboflavin doses (20, 40, and 60 mg) and one intravenous bolus injection of riboflavin (11.6 mg). Vitamins were administered in a randomized, cross-over design with 2 wk between each administration. Blood plasma and urine specimens were collected repeatedly over a period of 48 h after each administration. Concentrations of flavocoenzymes and riboflavin were analyzed in blood plasma; riboflavin was assayed in urine. During the control period, a small circadian variation was observed: plasma concentrations and urinary excretion of riboflavin were low during the afternoon ($P < 0.05$). Pharmacokinetics were calculated using a two-compartment open model. The maximal amount of riboflavin that can be absorbed from a single dose was 27 mg per adult. Half-life of absorption was 1.1 h. First-order rate constants describing distribution and elimination of riboflavin were significantly higher after intravenous than after oral administration ($P < 0.01$). Release of flavocoenzymes into plasma was low compared with the increase of riboflavin concentrations. 7α-Hydroxyriboflavin was identified in plasma. Clearance data indicated that urinary excretion of riboflavin contributes to one-half of the overall removal of riboflavin from plasma. No sex differences were observed for any of the pharmacokinetic variables ($P > 0.05$).  \textit{Am J Clin Nutr} 1996;63:54–66.

KEY WORDS  Riboflavin pharmacokinetics, human, intravenous riboflavin administration, oral riboflavin administration, flavins

INTRODUCTION

The limited former studies on the pharmacokinetics of riboflavin in humans dealt predominantly with data obtained from the urinary excretion of riboflavin or of its 5′-phosphate, flavin mononucleotide (FMN)\textsuperscript{(2)} after oral administration of riboflavin\textsuperscript{(1)}. These investigations indicated a saturation of absorption at doses of 30–50 mg riboflavin, whether administered as riboflavin or FMN\textsuperscript{(2)}. Urinary recoveries from doses lower than this saturation were in the range of 13–63% within 24 h (1–4). The extent of absorption was lower if the vitamin was administered to a subject with an empty stomach (1). A biexponential type of riboflavin elimination was described repeatedly, indicating the existence of a peripheral compartment of distribution (1, 3). Riboflavin excretion via urine seems to be enhanced by net tubular secretion in the case of high plasma concentrations (5, 6). Recently, studies were designed to assess the relative bioavailability of different formulations of riboflavin (7, 8). These studies were aimed at a comparison of maximal riboflavin concentrations, times to achieve these concentrations, and amounts of riboflavin in blood plasma. It was not intended in these studies to assess the amount of riboflavin absorbed, but they indicated a substantial effect of formulation factors on relative bioavailability from different preparations.

A complete pharmacokinetic model of riboflavin metabolism is not available because three aspects of the model have not been considered in detail until the present. These are 1) a comparison of the kinetics after different routes of riboflavin administration, 2) the dynamics of flavocoenzymes in blood plasma, and 3) circadian variations in riboflavin metabolism.

Regarding the first point, different routes of administration have to be considered because fundamental differences were described for the pharmacokinetics of vitamins when administered orally or intravenously (9). For intravenous administration, the first-pass effects of intestinal mucosa and liver passage are bypassed, i.e., the metabolic effect of the liver should be reduced. Because activities of flavokinase and flavin adenine dinucleotide (FAD) synthetase, responsible for converting riboflavin to the functional coenzymes, are high in liver (10), we expected pronounced differences in riboflavin kinetics depending on the route of administration of the vitamin. In the case of intravenous administration, the amount of riboflavin entering the peripheral circulation is known, i.e., it yields a standard for estimation of the availability of riboflavin given orally.

Regarding the second point, the flavocoenzymes FMN and FAD comprise the major part of riboflavin in blood plasma.
These bioactive forms of riboflavin are hydrolyzed to riboflavin before they enter cells, but riboflavin is accumulated in tissues by resynthesis of flavocoenzymes (12, 13). In the circulation, glomerular filtration of the vitamin is decreased by binding to plasma proteins (14). It is not possible from urinary data to determine the synthesis of flavocoenzymes after a riboflavin test dose because FMN and FAD are not eliminated in large amounts via kidney (15). Our intention was to describe the synthesis of flavocoenzymes and their liberation into blood plasma as caused by different doses and routes of riboflavin administration.

Regarding the third point, pharmacokinetic investigations deal with the quantitation of the metabolic fate of administered substances in the body. Vitamins exhibit baseline concentrations in body fluids that are not caused by the administered test dose; these concentrations depend on the regular dietary uptake. Baseline concentrations are usually taken into consideration by subtracting them from the concentrations observed after administration of the test dose. Circadian rhythms in concentrations of considered metabolites in body fluids would interfere with pharmacokinetic analyses because the baseline concentrations are usually measured only once just before the administration of the test dose. It is desirable to obtain data on such variations in body fluids, especially because it was shown that flavocoenzyme biosynthesis is regulated by thyroid hormones (16, 17). Concentrations of thyroxine and thyrotrophin exhibit pronounced circadian variations in blood serum and urine (18).

The lack of data on the pharmacokinetics of riboflavin and flavocoenzymes in humans encouraged us to conduct a study with the following objectives: 1) the workup of a pharmacokinetic model of riboflavin metabolism in healthy humans after oral and intravenous riboflavin administrations of different doses, 2) the consideration of the release of flavocoenzymes into blood plasma after riboflavin administration, and 3) the description of circadian rhythms in riboflavin concentrations in blood plasma and urine.

SUBJECTS AND METHODS

Subjects

Four male and five female subjects, recruited by poster solicitation, participated in this study (four were smokers). Although members of all ethnic groups were invited to participate, only white, non-Hispanic subjects gave their informed consent. Subjects were classified as healthy by physicians of the Emory University School of Medicine according to a general physical examination and the subjects’ medical histories. The use of medication was an exclusion criterion for participation. Subjects were instructed to avoid consumption of vitamin supplements 4 wk before participation in the study. Age and anthropometric characteristics of the subjects are summarized in Table 1. Study design as outlined below was approved by the University Human Investigation Committee and the General Clinical Research Center Advisory Committee of Emory University.

Study design

After the initial health examination, each subject received an oral multivitamin and mineral supplement for 7 d. This supplement provided 6 mg riboflavin/d for a total of 42 mg (Multibionta plus minerals; E Merck, Vienna). Other ingredients of the supplement were as follows (in mg per capsule): retinol palmitate, 1.32; thiamine nitrate, 2.0; riboflavin, 3.0; nicotinamide, 16.0; pantothenic acid, 8.0; pyridoxine hydrochloride, 3.0; cyanocobalamin, 0.003; ascorbic acid, 70.0; 2-amb-m-α-tocopherol, 12.0; folic acid, 0.1; biotin, 0.05; iron (as FeSO4), 20.0; magnesium (as MgO), 40.0; manganese (as MnSO4), 1.0; copper (as CuSO4), 1.0; and zinc (as ZnO), 1.5. All preparations were taken from the same lot (no. 765 0108) and two capsules were administered each day. The supplement was given to avoid any possible states of micronutrient deficiency. HPLC analysis of one supplement capsule revealed a content of 2.8 mg riboflavin per capsule, which meets the manufacturer’s declaration (3.0 mg).

On the last day of supplement use, the subjects collected a 24-h urine sample. After the supplementation period, a washout phase of 1 wk was introduced. This allowed the elimination of excessively supplemented vitamins. Thereafter, every subject was admitted to the Clinical Research Center (CRC) five times with 2 wk in between each admission. On the first admission, no riboflavin test dose was administered (control). The only source of riboflavin after an overnight fast was a standardized diet prepared by the Nutrition Unit of the CRC (discussed below). Blood samples were drawn in 2-h intervals between 0730 and 1930, ie, a period of 12 h was covered. Urine was collected as single spontaneous voids starting at 0730 over a period of 24 h. Urinary excretion of riboflavin on the control day was used for baseline correction on the days of riboflavin administration, because each subject received the same diet on all these occasions. The control group served also for measuring a potential circadian rhythm in riboflavin baseline concentrations in blood plasma and urine.

After each subject served initially as his or her own control, each subject was admitted to the CRC four more times in a randomized, crossover design. On these occasions each participant received the following test doses: three different oral riboflavin amounts [20 mg (53.1 μmol), 40 mg (106.3 μmol), and 60 mg (159.4 μmol)] and one intravenous, bolus injection of riboflavin [11.6 mg (30.8 μmol)]; ie, each subject received 131.6 mg (349.7 μmol) riboflavin within a period of 2 mo. The participants collected two 24-h urine samples for the days preceding each vitamin administration. These two additional samples were necessary because the standardized diet of the Nutrition Unit was consumed only for 24 h after the riboflavin administration. Because urine was collected for 48 h after each administration (discussed below), urinary excretion during the
second 24 h was corrected by the mean excretion of the 2 d before administration (subjects consuming self-chosen diets). (The procedure of baseline correction is explained in detail below.) On the day of riboflavin administration (oral or intravenous), a baseline blood sample was taken in the morning after subjects had fasted overnight (0 h, before vitamin administration). An additional sample for measurement of serum ethanol concentrations was drawn on this occasion.

The oral riboflavin dose (0 h) was administered with a standard breakfast at 0730. Thereafter, blood samples were drawn at 0.33, 0.67, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 11.0, 24.0, and 48.0 h. Urine was collected as spontaneous single voids during the 24 h after the riboflavin administration. On the second day (24–48 h), a 24-h urine sample was collected. The preparation used for oral riboflavin administration was Werdo 10 (Wörwag Pharma, Stuttgart, Germany). All preparations were taken from the same lot (no. 930503). According to the manufacturer’s declaration, one tablet contains 10 mg riboflavin. Our own spectrophotometric analysis at a wavelength of 447 nm (for which the molar extinction coefficient is $12.2 \times 10^3$; Spectronic 3000 Array Spectrometer, Milton Roy Company, Rochester, NY) revealed a content of 10.6 mg per tablet.

Riboflavin was administered intravenously as a bolus injection within 5 min in a forearm vein (end of injection = 0 h). Blood samples were withdrawn from the other arm at 0.05, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 11.0, 24.0, and 48.0 h. An additional sample for determination of the protein-bound fractions of riboflavin and flavoenzymes was drawn at 6.0 h. Urine was collected as described for the oral administrations. The sterile solution for injection was prepared by the Pharmacy of Emory Hospital according to the following procedure. Twenty milligrams of riboflavin (Sigma, St Louis) was weighed on an analytical balance. Riboflavin was dissolved in an appropriate volume of 0.9% (w:v) sodium chloride in water and filtered into sterile saline through a 0.22-$\mu$m filter for a final volume of 250 mL. Solutions were protected against light with brown bags or by wrapping the containers with aluminum foil. Before this preparation was used in our investigation, a sample was tested for pyrogenicity and sterility (Endosafe, Charleston, SC). An aliquot of each solution was analyzed spectrophotometrically at 447 nm for the determination of riboflavin concentration. Although 20 mg riboflavin was weighed initially, the amount finally injected into the volunteers was much less. This was because of incomplete solubilization of riboflavin, the use of 5 mL of each solution for spectrophotometric analyses, and an incomplete withdrawal of the solution from the storage bag before injection. The weight of the storage bag was obtained before and after the solutions were withdrawn. Because of these factors, the amount of riboflavin injected was calculated for every subject separately; it amounted to 11.6 ± 0.9 mg (30.8 ± 2.4 $\mu$mol) in a volume of 241.8 ± 2.2 mL (mean ± SD).

**Diet**

On the control day and the days of riboflavin administration, subjects consumed a diet prepared by the Nutrition Unit of the CRC. This diet yielded only a small amount of riboflavin per day, whereas energy supply, fat, carbohydrate, and protein were within usual ranges (Table 2). The same diet was consumed by all subjects on all days spent at the CRC. It was given at standardized times (0730, 1200, and 1800), yielding 0.10 mg riboflavin with breakfast, 0.15 mg with lunch, and 0.25 mg with dinner. The subjects had free access to drinking water, but no solid foods were allowed until the next morning. During the whole investigation, the participants were instructed to avoid alcohol and to not take any additional vitamin supplements. Self-chosen diets were consumed on the days in between each admission.

**Table 2**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>9382.1</td>
</tr>
<tr>
<td>Protein [g (% of energy)]</td>
<td>47.6 (8)</td>
</tr>
<tr>
<td>Fat [g (% of energy)]</td>
<td>61.4 (25)</td>
</tr>
<tr>
<td>Carbohydrates [g (% of energy)]</td>
<td>375.5 (67)</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*1 The composition was calculated using the DIET PLANNER, version 2.03 (General Clinical Research Center, San Francisco). This software contains the US Department of Agriculture nutrient database.

**Tests of compliance with the study protocol**

Analysis of the urinary riboflavin excretion (24-h urine sample) on the last day of the multivitamin supplementation served as a control of vitamin consumption during this period. Urinary creatinine excretion was measured on all days of urine collection to obtain information on the completeness of collection. On each of the 5 d of admission to the CRC, serum ethanol concentrations were measured to confirm abstinence from alcohol consumption.

**Collection of blood plasma and urine**

Blood was taken using an indwelling vein catheter on the fifth day of admission to the CRC. On all other occasions, blood samples were drawn by single vein punctures. Five milliliters of blood were drawn per withdrawal into tubes containing sodium heparin (Becton Dickinson, Rutherford, NJ). Tubes were put on ice and wrapped in aluminum foil to protect samples against light. Whole blood was centrifuged at 4°C to obtain blood plasma (2600 $\times$ g for 10 min). Plasma was frozen immediately at $-20^\circ$C. Samples for the determination of ethanol were drawn into tubes as required for serum analyses (Becton Dickinson). Serum was separated by centrifugation at 4°C ($2600 \times g$ for 10 min).

Urine was collected in brown bottles. It was preserved by adding crystalline oxalic acid dihydrate (Fisher Scientific, Pittsburgh) to each bottle before collection. Five grams of oxalic acid was added to the large bottles (3 L) for 24-h urine collection; 1 g was added to the small bottles (0.5 L) for collection of the single voids. In case urine was collected at home, subjects were instructed to keep the specimens in a refrigerator. The volume of urine was measured directly after voiding or after the subjects brought it to our unit. Aliquots were frozen at $-20^\circ$C until analyzed for riboflavin and creatinine.

Blood and urine samples were protected against exposure to light because of the strong light sensitivity of the flavins. The major part of samples was analyzed within 3 d after withdrawal.
for vitamin concentrations; no sample was stored longer than 5 d.

**Analyses of samples**

Samples were coded before analyses, ie, they were handled in a blinded fashion. Blood plasma was analyzed for its concentration of riboflavin and flavocoenzymes by HPLC as described previously (20). In brief, blood plasma was deproteinized by addition of 20% trichloroacetic acid (wt:vol). After centrifugation, the supernate was heated for 10 min in a water bath at 85 °C. By this procedure, FAD was hydrolyzed to yield FMN, ie, the flavocoenzymes FAD and FMN were analyzed in total as FMN. They are further referred to as flavocoenzymes. Flavocoenzymes and riboflavin were separated and quantified by reversed-phase HPLC. The hydrolysis step has the advantage of avoiding artificially reduced concentrations of FAD. This metabolite is hydrolyzed 31.5–84.8% in blood plasma within 10–40 min after blood withdrawal at 37 °C (21). FMN is relatively stable under these conditions (degradation < 2%). On average, 10–40 min elapsed in the present study from withdrawal of samples until they were frozen. In fact FAD was estimated to constitute 75.5% of flavocoenzymes in serum on a molar basis (11).

For the determination of the percentage of protein-bound riboflavin and flavocoenzymes in blood plasma, 750 μL plasma was filtered through a membrane filter with a cutoff at a molecular weight of 30 000 (MPS-1 Micropartition; Amicon, Beverly, MA) by centrifugation at 1200 g for 30 min at room temperature. The penetrant was analyzed as described for blood plasma and compared with a duplicate analyzed without prior filtration. Urinary riboflavin was determined by HPLC as described by Gatautis and Naito (22). Urinary creatinine was assessed by using the alkaline-picrate method according to Jaffe (23) (550 Express Clinical Chemistry Analyzer; Ciba Corning Diagnostics Group, Park Ridge, IL). Ethanol concentrations in blood serum were analyzed by using the alcohol dehydrogenase enzymatic assay according to Bonnichsen and Lundgren (24) (aca discrete clinical analyzer; DuPont Company, Wilmington, DE).

**Pharmacokinetic methods**

Baseline concentrations as observed in blood plasma and urine before administration of the test doses were subtracted from concentrations measured after administration. Our results regarding the circadian rhythm of riboflavin metabolites in body fluids showed that within-day variations were negligible (discussed below). For urine data, two different baseline values were chosen for correction of postdose concentrations: because the same diet was consumed by the subjects on each of the 5 d of admission to the CRC, the mean hourly riboflavin excretion of the control day was chosen for correcting the urinary riboflavin excretion on the days of riboflavin administration. The riboflavin excretion between 24 and 48 h after riboflavin administration was corrected by the mean excretion in the two 24-h urine samples collected before each administration.

Pharmacokinetic variables were calculated according to Gibaldi and Perrier (25). All data were calculated by using the individual curves instead of the mean curves. A two-compartment, open model was chosen for description of riboflavin kinetics. The areas under the vitamin plasma concentration versus time curves (AUCs) were calculated using the linear trapezoidal rule. For intravenous administration, this was done by using the extrapolated zero-time intercept for 0 h, as obtained by back-extrapolation onto the y axis (see below). AUCs were calculated for periods of 11, 24, and 48 h after the administration of the test doses. When the AUC was needed for calculation of other pharmacokinetic variables, the total AUC (AUC0–48h) was always used. The apparent first-order rate constants of disposition were calculated for blood plasma by nonlinear regression after the onset of the pure elimination processes. Best fits were obtained using a two-compartmental approach, ie, a biexponential function was used for computation of the elimination from the central compartment blood plasma (Eq 1). Half-lives of absorption (see below) and disposition were calculated from the apparent first-order rate constants of absorption and disposition (25).

\[
C_t = C_{01} e^{-kt} + C_{02} e^{-kt} \tag{1}
\]

where \(C_t\) is vitamin concentration in plasma at time \(t\) (nmol/L); \(C_{01}\), \(C_{02}\) are vitamin concentrations in plasma at time 0 (zero-time intercepts), obtained by extrapolation (nmol/L); \(k_t\) is the apparent first-order fast disposition rate constant (h\(^{-1}\)); \(k_p\) is the apparent first-order slow disposition rate constant (h\(^{-1}\)); and \(t\) is time (h).

Riboflavin elimination via urine was described assuming a one- or two-compartment open model, depending on the appropriateness to describe the individual curves. The slow β-phase of elimination was observed in urine in 17 of 36 cases, ie, we did not obtain it consistently. Presumably this was caused by an incomplete emptying of the bladder by the subjects. First-order rate constants for renal excretion were obtained by nonlinear regression of the mean hourly riboflavin excretion versus the mean time point of urine collection interval curves. The elimination rate constants of a one-compartment model were fitted according to Eq 2. In cases for which a two-compartment model was more appropriate to describe riboflavin excretion, rate constants were calculated according to Eq 3, but by using the mean hourly excretion instead of concentration values.

\[
C_t = C_0 e^{-kt} \tag{2}
\]

where \(C_t\) is mean hourly riboflavin excretion in urine at time \(t\) (nmol/h); \(C_0\) is mean hourly riboflavin excretion in urine at time 0 (zero-time intercept), obtained by extrapolation (nmol/h); \(k\) is the apparent first-order rate constant for renal excretion (h\(^{-1}\)); and \(t\) is the mean time point of the urine collection interval (h).

The renal clearance was calculated according to Eq 4, taking into account the fraction of riboflavin not protein-bound in plasma. Because only riboflavin was analyzed in urine, no renal clearance was computed for the flavocoenzymes. Values of clearance were transformed from units of h/L into the more common units mL/min by multiplying by 1000/60 or by expressing the AUC in units of nmol·min/mL instead of nmol·h/L. Clearance was corrected for a body surface area of 1.73 m\(^2\).

\[
Cl_t = X_t / \text{AUC}_0-48h \tag{3}
\]

where \(Cl_t\) is renal clearance (mL·min\(^{-1}\)·(1.73 m\(^2\) body surface area\(^{-1}\))\); \(X_t\) is amount of riboflavin excreted in urine (nmol); and \(\text{AUC}_0-48h\) is total area under the plasma concent-
tration versus time curve, corrected for protein binding (nmol·h/L).

The systemic clearance after intravenous riboflavin injection was calculated according to Eq 4. Values were transformed from units of L/h into mL/min as described for renal clearance. They were expressed referring to a body surface area of 1.73 m². The values obtained for the AUC were not corrected for binding of riboflavin to plasma proteins. It is not clear to what extent binding to protein affects uptake into tissues, which is part of the systemic clearance, but it probably decreases renal clearance of riboflavin.

\[ Cl_{sys} = \frac{D}{\text{AUC}_{0-\infty}} \]  

where \( Cl_{sys} \) is systemic clearance [mL·min⁻¹·(1.73 m² body surface area)⁻¹]; \( D \) is the dose of riboflavin injected (nmol); and \( \text{AUC}_{0-\infty} \) is total area under the plasma concentration versus time curve (nmol·h/L).

Values of the volumes of distribution of riboflavin were calculated from the intravenous injection data (25). The apparent volume of the central compartment, \( V_c \), is the proportionality constant relating the riboflavin concentration in blood plasma to the amount in the body immediately after administration. The apparent volume of distribution of the β-phase, \( V_β \), relates the riboflavin concentration in blood plasma to the total amount in the body during the terminal exponential phase in the plasma time curve. The principal shortcoming of this apparent volume of distribution is that \( V_β \) may reflect the degree of equilibration under dynamic conditions rather than its apparent distribution volume. This is because \( V_β \) is a function of the elimination kinetics of riboflavin. Therefore, it may be helpful to relate the amount of riboflavin in the body to its plasma concentration at steady state (\( V_{sat} \)).

The transfer of riboflavin between the central and the peripheral compartment was further characterized by calculation of first-order intercompartmental transfer rate constants. We have chosen a two-compartment, open model, in which elimination occurs from the central compartment (Figure 1). The first-order intercompartmental transfer rate constants and the apparent first-order elimination rate constant from the central compartment were calculated from the intravenous injection data (25).

According to our own results (discussed below), oral administration of 20 mg riboflavin seemed to be far enough below the saturation of the absorption capacity so that the apparent first-order rate constant of absorption could be obtained by applying first-order kinetics. It was calculated by nonlinear regression of the plasma concentration versus time curves; the previously calculated values for the rate constants of disposition as obtained from the intravenous injection were kept fixed for this purpose. No rate constant of absorption was calculated for the 40-mg and 60-mg doses, because in these cases Michaelis-Menten kinetics apply. The maximum amount of riboflavin that can be absorbed and the dose that yields an amount absorbed equal to one-half of the maximum were calculated and used to describe saturation kinetics of riboflavin absorption according to a suggestion of Mayersohn et al (3). Therefore, we plotted (analogously to Lineweaver and Burk) the reciprocal of the amount of riboflavin excreted unchanged in urine versus the reciprocal of the riboflavin dose administered. This procedure depends on the fact that renal excretion of riboflavin is not saturable in the concentration range considered (3). We observed neither a significant increase in the first-order rate constants for renal excretion with increasing doses nor a change in renal clearance.

Statistical methods

All statistic and pharmacokinetic analyses were computed with SPSS/PC 5.0.1 (SPSS Inc, Chicago). Medians (with 25th percentile to 75th percentile in parentheses) are reported unless indicated otherwise. Tests of significance comparing males and females were done using the U test of Mann-Whitney. Data obtained from males and females were pooled because no significant differences were found. The Wilcoxon test was chosen for comparing paired data. To determine the effect of dose on kinetic variables, the Friedman test with post hoc comparison was applied (26). Probability levels < 0.05 were considered to be significant.

RESULTS

Compliance with the study protocol

The subjects excreted 8.61 (7.46–9.79) μmol riboflavin per 24 h in urine on the last day of the multivitamin supplementation. This suggests that the supplement was used as requested by the study protocol. Even the subject with the lowest riboflavin excretion on this day (4.27 μmol/24 h) should have consumed the supplement. In all cases riboflavin excretion was far above the value indicative of a sufficient riboflavin status (≥ 120 μg/24 h), as suggested by Sauberlich et al (27). In no case was ethanol detected in blood serum, ie, none of the subjects consumed alcohol immediately before vitamin administration. Mean (± SD) urinary creatinine excretion was 14.0 ± 2.3 mmol/24 h for males and 10.3 ± 2.1 mmol/24 h for females (P < 0.001), which is within physiologic ranges (28). For one female subject we observed a pronounced reduction of creatinine excretion during the 24 h after the oral 60-mg riboflavin dose. Creatinine excretion on this day was ≈30% less than the subject’s mean excretion. This and other indexes of an incomplete urine collection (see below) led to the exclusion of the urinary recovery data of this subject for the 60-mg dose. The apparent first-order rate constant for renal excretion was not discarded in this case, because its calculation depends much less on a complete urine collection. In general, the subjects exhibited excellent compliance with the complex study protocol.
Circadian rhythm

Figure 2 illustrates variations in the plasma concentrations of riboflavin and flavocoenzymes between 0730 and 1930 on the control day, ie, when no supplemental vitamin was administered. The urinary excretion of riboflavin was examined over a period of 24 h on this day (Figure 3). For tests of significance, concentrations in plasma or excretion in urine at each time point were compared with the individual mean of the whole period of 12 or 24 h, respectively. The median plasma concentration of the nine subjects was 9.3 nmol riboflavin/L (8.3–11.1 nmol/L); the flavocoenzyme concentration was 79.2 nmol/L (70.0–80.7 nmol/L). Urinary excretion of riboflavin amounted to 58.2 nmol/L (16.3–85.2 nmol/L). Whereas no circadian variation was observed for flavocoenzymes, concentrations of riboflavin in plasma and urine were slightly lowered during the afternoon (Figures 2 and 3). Riboflavin in plasma was significantly diminished by 1.6–3.4 nmol/L, compared with median concentrations. Urinary riboflavin was 20.8–25.7 nmol/L lower than the median excretion. These decreases were negligible compared with the concentrations observed after riboflavin administration (see below), ie, no circadian rhythm had to be taken into consideration for pharmacokinetic data analyses. It was sufficient to correct the values after the intravenous or oral riboflavin administration by subtracting constant baseline concentrations, which were determined immediately before vitamin administration.

Riboflavin and flavocoenzymes in blood plasma

Baseline concentrations in blood plasma were 12.6 nmol riboflavin/L (8.2–20.0 nmol/L) and 78.9 nmol flavocoenzymes/L (62.2–96.5 nmol/L). The plasma concentration versus time curves of riboflavin after oral and intravenous doses are shown in Figure 4A. The peak concentration of riboflavin (Cmax) was higher after the 60-mg dose compared with the two lower oral doses (Table 3), but this difference was not significant. Peak concentrations were compared with the baseline concentrations obtained before each administration; the increase in the riboflavin concentration in blood plasma after oral administrations was 16.3-fold (7.2–27.2-fold) for the 20-mg dose, 16.3-fold (11.3–33.2-fold) for the 40-mg dose, and 23.2-fold (10.3–28.5-fold) for the 60-mg dose. For intravenous riboflavin administration, Cmax was calculated by back-extrapolation onto the zero-time intercept because the complete amount of the vitamin was in the body at this time (Figure 4A and Table 3). The peak concentration was 83.5-fold (67.9–99.0-fold) higher than the baseline concentration; this was significantly different from the oral administrations (P < 0.01). The times of maximal concentrations (tmax) were equal for all oral doses (Table 3).

The bioavailability of the administered riboflavin was further characterized by comparing the AUC obtained from blood plasma (Table 3). If the AUC is calculated over the whole observation period of 48 h, it is considerably influenced by the concentrations of riboflavin in the blood, as observed for the terminal time points of the AUC (29). This is because the area between these time points is calculated over large time intervals. Taking into consideration that the analytical error for these concentrations is higher than for the initial, much higher concentrations, it seemed reasonable to also provide data on the AUC for 11 and 24 h after the vitamin administrations (Table 3). The AUC of riboflavin in blood plasma calculated over 11, 24, or 48 h exhibited a similar pattern: the AUC calculated for the oral doses increased with higher doses, but the difference was not significant. There was no significant difference between the intravenous injection and the oral administrations, with the exception of a larger AUC (24 and 48 h) for the 60-mg dose compared with the injection (P < 0.05). Applying first-order kinetics for the lowest oral dose administered, the apparent first-order rate constant of riboflavin absorption was calculated to be 0.6370 h⁻¹ (0.4967–1.0000 h⁻¹). This equals an absorption half-life of 1.0881 h (0.6931–1.3955 h). The correlation coefficient for this regression analysis was 0.90822 (0.69230–0.94898). Data from eight subjects were considered for this calculation because the plasma data of one female volunteer did not allow an appropriate calculation (ka = 1.0000, r = 0.18628).
The response of flavocoenzymes in blood plasma was much less pronounced than that of riboflavin (Table 4, Figure 4B). Peak concentrations of flavocoenzymes were significantly lower after the 20-mg dose and the intravenous injection compared with the 60-mg dose (P < 0.05). No other significant differences were found for C_max, but the difference between the 40- and 60-mg doses was of borderline significance (P = 0.05). Relating C_max to the baseline concentrations, the increase of flavocoenzyme concentrations after the oral administrations was 1.3-fold (1.2-1.5-fold) for the 20-mg dose, 1.6-fold (1.2-2.1-fold) for the 40-mg dose, and 1.5-fold (1.5-1.6-fold) for the 60-mg dose. For the intravenous riboflavin injection, the flavocoenzyme peak concentration was 1.3-fold (1.2-1.3-fold) higher than the baseline concentration. Oral and intravenous doses were not significantly different regarding the increase over baseline concentrations. No significant difference was observed for t_max of flavocoenzymes between any of the riboflavin administrations (Table 4). According to the AUC, the secretion of flavocoenzymes into plasma was higher after the 40- and the 60-mg doses compared with the 20-mg dose or the intravenous injection. These findings depended somewhat on which of the calculated AUC values was chosen (Table 4).

After the oral but not the intravenous administrations, we observed the appearance of a further flavin metabolite in blood plasma (Figure 5). An artifact was excluded by the following observations. This metabolite was not detected after the intravenous vitamin injection, for which much higher riboflavin concentrations were observed. It was also not detected in any of the baseline samples, ie, before riboflavin administration. When a solution of the tablet used for oral administration was analyzed by HPLC, we observed only one peak, which was attributed to riboflavin by its retention time. The flavin metabolite was identified as 7α-hydroxyriboflavin (7-hydroxymethylriboflavin) by comparing its HPLC retention time, its chromatographic behavior in thin-layer chromatography, and its absorbance spectrum with an authentic (chemically synthesized) standard of the compound (30, 31).

Disposition of riboflavin from blood plasma was best described using a biexponential function, ie, a rapid phase of elimination was followed by a slower β-phase. Between 5 and 16 data points from the terminal slope of riboflavin in plasma were used for each regression analysis. The median correlation coefficients for these calculations were 0.99421 for the 20-mg dose, 0.99786 for the 40-mg dose, 0.99784 for the 60-mg dose, and 0.99956 for the intravenous injection. The rate constants of disposition and the corresponding half-lives are summarized in Table 5. Mean rate constants computed from the different oral doses are also provided (Table 5). Because three sets of data on oral administration were available for each subject, the precision of our calculations was improved. We pooled the oral data, although the rate constant of the α-phase of elimination was significantly lower after the 40-mg dose compared with the 20- and 60-mg doses (P < 0.05). No physiologic explanation was found for this observation. Additionally, the rate constants of the β-phase and the renal clearances (see below) were the same for the three oral doses. Therefore, we attributed the differing values during the α-phase to chance. The rate constants of disposition (kα and kβ) after the intravenous injection were significantly larger than the constants as calculated from the oral doses (P < 0.01). The concentration-time curves of flavocoenzymes in plasma did not allow us to calculate rate constants of disposition. The systemic clearance of riboflavin was 957.6 mL·min⁻¹·(1.73 m² body surface area)⁻¹ (637.9–1057.0 mL·min⁻¹·(1.73 m² body surface area)⁻¹) from the intravenous administration.

The volumes of distribution, as calculated from the intravenous riboflavin administration, are summarized in Table 6. Calculation of Vₚ yielded higher values than that of Vₚₚ (P < 0.01). Values calculated for the apparent intercompartmental transfer rate constants and the first-order rate constant of elimination from the central compartment are provided in Table 7 (see also Figure 1). Protein binding of riboflavin in blood plasma was determined to be 35.5% (13.1–60.6%). The proportion of protein-bound flavocoenzymes amounted to 54.8% (48.2–80.7%). The protein binding of riboflavin was investigated for the calculation of the renal clearance (discussed below).
Intravenous maximal administration increased plasma levels. Calculations of the 60-mg dose were performed after injection or oral administration. The rate constants for renal excretion and the corresponding half-lives are given in Table 8. From 36 available sets of data (27 oral and 9 intravenous administrations), a slow phase of elimination was observed in 17 cases. Rate constants of excretion during the fast and slow phase of elimination did not significantly differ on the dose given or the route of administration, although elimination during the α-phase seemed to be faster after the intravenous injection. Median correlation coefficients for the nonlinear regression analyses (rate constants) were 0.99478 for the 20-mg dose, 0.99502 for the 40-mg dose, 0.99895 for the 60-mg dose, and 0.99940 for the intravenous administration. Calculations were performed using 4–12 data points from each volunteer. Renal clearances of riboflavin are summarized in Table 8. They were calculated by using the fraction of riboflavin in blood plasma that was not protein-bound. Renal clearance was higher than the glomerular filtration rate (28), i.e., net tubular secretion took place.

The urinary excretion of riboflavin is presented in Table 9 for the different routes and doses of administration. The percentage of riboflavin recovered after oral administrations decreased with increasing dose (P < 0.05). With intravenous administration the amount reaching the circulation is exactly known so there is a reference for estimation of bioavailability from oral administrations. The amount reaching the circulation is known if the renal excretion kinetics for different routes of administration are similar. When the urinary recovery after the oral administrations was corrected by the recovery observed after the intravenous dose, the absorption of riboflavin was calculated to be 74.6% for the 20-mg dose, 43.3% for the 40-mg dose, and 36.4% for the 60-mg dose.

The Lineweaver-Burk type of plot of the amount of riboflavin excreted in urine versus the oral doses administered. Assuming a negligible dependency of urinary excretion from the observed concentrations, we calculated the maximum amount of riboflavin that can be absorbed as 71.2 μmol (26.8 mg). The dose saturating the absorption site by one-half was 48.9 μmol (18.4 mg) riboflavin.

Nonrenal elimination

The difference between the systemic clearance of riboflavin and its renal clearance was used to estimate its elimination by nonrenal pathways. The difference was 468.1 mL·min⁻¹·(1.73 m² body surface area)⁻¹. This is approximately one-half of the systemic clearance, i.e., 48.9% of riboflavin removal from blood plasma was caused by metabolism and nonrenal excretion.

**DISCUSSION**

The existence of circadian rhythms in riboflavin concentrations in blood plasma or riboflavin excretion in urine had not
been investigated until the present. Previous studies showed that no such variations existed in serum concentrations of thiamine (32) and vitamin B-6 (33, 34). In the present study, the dietary riboflavin supply was sufficiently low so as not to overlap the existent circadian rhythms of riboflavin in plasma and urine. Although the decrease in riboflavin concentrations during the afternoon was significant, it was small enough to be neglected for further analyses. The circadian rhythms should be seen in context with similar variations in serum or urinary concentrations of thyroxine (elevated during daytime) and thyrotrophin (elevated during night) (18). Although serum concentrations of triiodothyronine remained unchanged, thyroid hormones might contribute to the circadian rhythm in riboflavin concentrations. Flavocoenzyme biosynthesis in tissues is under control of thyroid hormones (16). A circadian rhythm of flavocoenzyme concentrations might exist in tissues, but their concentrations in blood plasma remained fairly constant.

Orally administered riboflavin was rapidly absorbed, as indicated by $t_{\text{max}}$ values in the range of 1.4–2.0 h. Riboflavin uptake takes place more efficiently in the proximal than in the distal small intestine (35). We characterized the absorption kinetics by two different approaches: zero-order kinetics were applied to calculate the maximum amount of riboflavin that can be absorbed ($\sim$26.8 mg) and the dose saturating the absorption site by one-half ($\sim$18.4 mg). This procedure was chosen because the amount of riboflavin absorbed did not increase in proportion to the doses given in the present study. Our finding for the maximum amount of riboflavin that can be absorbed agrees well with urinary excretion data: after the highest oral dose, 8.6 mg riboflavin was recovered in urine. Taking into consideration a urinary recovery of 39.3% according to the intravenous injection, the total excretion should amount to 21.9 mg flavin compounds. This is $>80\%$ of the maximum amount that can be absorbed.

To obtain more detailed information on the rate of absorption, we applied first-order kinetics to the lowest oral dose administered. This 20-mg dose half-saturated the absorption site. The apparent first-order rate constant of absorption $k_{\text{app}}$ was computed as 0.6370 h$^{-1}$, which equals an absorption half-life of 1.0881 h. According to this finding, absorption from gut lumen was $95\%$ complete within 4.4 h. The relatively low coefficient of correlation for the calculation of $k_{\text{app}}$ ($r = 0.90822$) was caused by the small number of data points available during the absorption of riboflavin. The rate constant obtained in this study should be a much better approximation than that extrapolated previously from urinary excretion data (4). In the study cited, $k_{\text{app}}$ was calculated to be 30 h$^{-1}$, but classified by the authors as not meaningful because it indicated that absorption was 95% complete in $\leq 0.10$ h (4). We assume that characterization of the absorption process by first-order kinetics provides a tool much closer to the low amount of dietary riboflavin supplied, compared with zero-order kinetics, which would only apply to unlimited (large amount) riboflavin. The latter is a consideration in the case of supplement use.

Three reasonable approaches were used to describe the extent of absorption from oral riboflavin formulations. These are the determination of $C_{\text{max}}$ and AUC of riboflavin in plasma, and its urinary excretion. Although $C_{\text{max}}$ is also influenced by the rate of absorption (36), it has been described to be suitable for estimation of extent as well (29). Peak concentrations of the three different oral doses did not differ significantly, but showed a tendency to increase with higher doses. The same was the case when $C_{\text{max}}$ was expressed as the increase above the baseline concentration, which makes this parameter more independent from the slight variations in predose concentrations.

A similar relation was observed when bioavailability was estimated by use of the AUC in blood plasma. No significant difference existed between the three oral doses, but there was a tendency to increase with larger amounts given. This finding remained unchanged, regardless of whether the AUC was calculated over periods of 11, 24, or 48 h. Both parameters, $C_{\text{max}}$ and AUC, indicated that riboflavin absorption becomes maximal at doses $\leq 40$ mg, as described previously (2).

The exact quantitation of absorption was possible by comparing the urinary excretion data. Thirty-nine percent of the intravenous injected dose was recovered in urine. Correction of the urinary recoveries after the different oral doses (14–29%) indicated that between 36% and 75% of the doses were actually absorbed. Again, absorption decreased with increasing dose. Recoveries of oral doses in the range of 13–63% were reported previously (1–4). This is not equivalent to the dose absorbed, because no correction was introduced for recovery after an
intravenous dose. The large variation of absorption in the studies mentioned depends on several factors. The lowest recovery (13–20%) was found when 30 mg riboflavin was administered to fasting subjects (3), i.e., a relatively high dose was administered under circumstances (fasting) that decrease absorption (1). Recovery was 61% for oral doses of 5–30 mg given together with breakfast (1) or 50% for doses of ~10 mg distributed over several hours (4). Wiegand et al (4) excluded the influence of pharmaceutical formulation factors by dissolving the riboflavin in water before administration. Together with altered availability from different pharmaceutical formulations (7, 8) and the large interindividual variation in riboflavin metabolism (1), these factors explain the variation in urinary recovery of oral riboflavin. Our results indicate that absorption of riboflavin, after correction for intravenous dose recovery, is higher than expected.

After its absorption or intravenous injection into the central volume of distribution, riboflavin equilibrated rapidly with its peripheral compartment. The exchange between both compartments was characterized by calculating the intercompartmental transfer rate constants. Uptake of riboflavin into the peripheral compartment was faster than release (k23/k21 = 2.7200:1.9520), which is in accordance with the two-compartment model chosen. Volumes of distribution can be assigned to anatomical compartments, e.g., plasma or extracellular water, only in cases when substances are not protein-bound (25). Because riboflavin is bound to various proteins in tissues and blood plasma (14), our data refer to apparent rather than real volumes of distribution (25). Because Vc (0.61 L/kg body wt) and Vf (0.94 L/kg body wt) exceed the theoretical maximum, i.e., total body water (~58% of body weight), it can be assumed that riboflavin is bound more tightly to tissue proteins than to plasma proteins (25).

The apparent volume of distribution at steady state was significantly lower than Vf, so for methodologic reasons, we prefer the former to describe the volume of distribution at equilibrium. Even though the data did not allow us to attribute the volumes of distribution to anatomical compartments, we speculate that distribution took place according to the following model: after absorption or injection, riboflavin spreads over its central volume of distribution, which we assume to be the extracellular water. Substances with a high molecular weight such as Evans blue (molecular weight = 960.83) or indocyanine green (molecular weight = 774.99) may even be restricted to the plasma volume for their distribution (25); the molecular weight of riboflavin is only 376.36. From its central volume of distribution, riboflavin enters its peripheral compartment, which can be attributed to total body water, i.e., extracellular water and cell water. The delayed uptake into tissues can be

### Table 5

<table>
<thead>
<tr>
<th>Riboflavin dose (mg)</th>
<th>kα</th>
<th>kβ</th>
<th>t1/2 (fast phase)</th>
<th>t1/2 (slow phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>h^-1</td>
<td>h^-1</td>
<td>h</td>
<td>h</td>
</tr>
<tr>
<td>20</td>
<td>1.3307^1 (1.0237–1.4273)</td>
<td>0.1811^1 (0.1235–0.2840)</td>
<td>0.5209^1 (0.4856–0.6771)</td>
<td>3.827^1 (2.4407–5.6125)</td>
</tr>
<tr>
<td>40</td>
<td>0.9388^1 (0.4953–1.1656)</td>
<td>0.0520^1 (0.0269–0.1372)</td>
<td>0.7383^1 (0.5947–1.3994)</td>
<td>13.398^1 (5.0521–25.7676)</td>
</tr>
<tr>
<td>60</td>
<td>0.9863^1 (0.6717–1.4728)</td>
<td>0.2027^1 (0.1108–0.2802)</td>
<td>0.7028^1 (0.4706–0.10319)</td>
<td>3.4196^1 (2.4738–6.2558)</td>
</tr>
<tr>
<td>Mean^2</td>
<td>1.2360^1 (0.6670–1.4040)</td>
<td>0.1740^1 (0.0670–0.2360)</td>
<td>0.5608^1 (0.4937–0.10392)</td>
<td>3.9836^1 (2.9371–10.3455)</td>
</tr>
<tr>
<td>Intravenous</td>
<td>11.6</td>
<td>6.8919 (4.1848–6.9745)</td>
<td>0.6856 (0.5896–0.9284)</td>
<td>0.1006 (0.0994–0.1656)</td>
</tr>
</tbody>
</table>

1 Values are medians (25th percentile–75th percentile); n = 4 males and 5 females. kα, apparent first-order fast disposition rate constant; kβ, apparent first-order slow disposition rate constant; t1/2f (fast and slow phase of disposition).

2 Significantly different from the 40-mg dose, P < 0.05.

3 Significantly different from intravenous administration, P < 0.01.

4 Significantly different from the 60-mg oral dose, P < 0.05.

5 Values obtained from the three oral administrations are also provided as mean of these trials (see text).

### Table 6

<table>
<thead>
<tr>
<th>Riboflavin dose (mg)</th>
<th>Vc</th>
<th>Vf</th>
<th>Vss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>L/kg body wt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.37</td>
<td>0.94^2</td>
<td>0.61</td>
</tr>
<tr>
<td>40</td>
<td>(0.32–0.40)</td>
<td>(0.83–1.04)</td>
<td>(0.59–0.67)</td>
</tr>
</tbody>
</table>

1 Values are medians (25th percentile–75th percentile); n = 4 males and 5 females. Vc, apparent volume of the central compartment; Vf, β-phase apparent volume of distribution; Vss, apparent volume of distribution at steady state.

2 Significantly different from Vss, P < 0.01.

### Table 7

<table>
<thead>
<tr>
<th>Riboflavin</th>
<th>k10</th>
<th>k12</th>
<th>k11</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.6 mg</td>
<td></td>
<td></td>
<td>h^-1</td>
</tr>
<tr>
<td>2.2250</td>
<td>2.7200</td>
<td>1.9520</td>
<td></td>
</tr>
<tr>
<td>(1.9230–2.6710)</td>
<td>(1.5310–3.4850)</td>
<td>(1.1580–2.8750)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are medians (25th percentile–75th percentile); n = 4 males and 5 females. k10, apparent first-order elimination rate constant from the central compartment; k12, apparent first-order intercompartmental transfer rate constant; k11, apparent first-order intercompartmental transfer rate constant, assigned to the transfer from the central to the peripheral compartment; k13, apparent first-order intercompartmental transfer rate constant, assigned to the transfer from the peripheral to the central compartment.
explained by the necessity of transport proteins for penetration of cell membranes (13).

Flavocoenzymes are synthesized in cells, but there is a modest increase of flavocoenzymes in plasma after vitamin administrations. Stripp (37) reported that FAD concentrations increased 1.7-fold in erythrocytes after the oral administration of 500 mg FMN to human subjects; concentrations of FMN and riboflavin remained unchanged. In the present study, flavocoenzyme release into blood plasma was weakly related to the different doses and routes of administration. It seems that liberation from cells into blood plasma is under strict control or that any surplus is rapidly cleared from plasma. Although the latter agrees with elimination characteristics observed after intravenous administration of FMN (15), the terminal phase of flavocoenzyme elimination from plasma should be slower than that of riboflavin. This would explain the higher concentration of the coenzyme forms compared with riboflavin. Until the present, this terminal, very slow phase of elimination had not been described, presumably because of methodologic reasoning (reproducibility of extremely small concentration differences over long periods of time). Our data on the urinary excretion of riboflavin suggest that the oral, 20-mg dose provided approximately the same amount of riboflavin as the intravenous dose. The AUC of flavocoenzymes after this lowest oral dose was somewhat higher than after intravenous administration, but this difference was of borderline significance (0.05 ≤ P ≤ 0.11). We cannot exclude that flavocoenzyme release into plasma depends to some degree on the first-pass effect after riboflavin absorption. Although the sources for flavocoenzymes in plasma have not been quantitated, high activities of flavokinase (38) and FAD synthetase (39) in liver suggest a contribution from this organ as well as from blood cells.

Rate constants of disposition calculated for riboflavin in plasma were larger after intravenous than after oral administration. The same observation was made for pyridoxine in humans (9) and biotin in cattle (40). Even though Frigg et al (40) explained the differences in elimination by lack of specificity in their microbiological assay, an underlying physiologic cause should not be excluded. This observation has now been reported for three water-soluble vitamins. In two of these cases, a specific HPLC method was used for plasma analyses. These

TABLE 8

<table>
<thead>
<tr>
<th>Riboflavin dose (mg)</th>
<th>k_a h⁻¹</th>
<th>k_B h⁻¹</th>
<th>t_1/2 (fast phase)</th>
<th>t_1/2 (slow phase)</th>
<th>Cl_r mL·min⁻¹·1.73 m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.5209</td>
<td>0.4100-0.6820</td>
<td>0.0744 (0.0381-0.1147)</td>
<td>1.3307 (1.0163-1.6906)</td>
<td>9.3165 (6.0431-19.1282)</td>
</tr>
<tr>
<td>40</td>
<td>0.5571</td>
<td>0.3818-0.6696</td>
<td>0.0713 (0.0114-0.3425)</td>
<td>1.2442 (1.0352-1.8155)</td>
<td>9.7216 (6.0238-60.8024)</td>
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<tr>
<td>60</td>
<td>0.5432</td>
<td>0.4070-0.6431</td>
<td>0.0498 (0.0489-0.0968)</td>
<td>1.2760 (1.0778-1.7031)</td>
<td>13.9168 (7.1606-17.1478)</td>
</tr>
<tr>
<td>Mean²</td>
<td>0.5610</td>
<td>0.3980-0.6320</td>
<td>0.0760 (0.0420-0.1510)</td>
<td>1.2356 (1.0968-1.7416)</td>
<td>9.1204 (4.5904-16.5035)</td>
</tr>
<tr>
<td>Intravenous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.6</td>
<td>0.9386</td>
<td>0.4198-1.1159</td>
<td>0.0700 (0.0472-0.0720)</td>
<td>0.7385 (0.6212-1.6511)</td>
<td>9.9021 (9.6270-14.6853)</td>
</tr>
</tbody>
</table>

¹ Values are medians (25th percentile–75th percentile). n = 4 males and 5 females. No significant differences were found between doses given or routes of administration. k_a and k_B, apparent first-order rate constants for renal excretion (fast and slow phase, respectively); t_1/2, half-life (fast and slow phase of excretion, respectively); Cl_r, renal clearance.

² Values obtained from the three oral administrations are also provided as mean of these trials (see text).

TABLE 9

<table>
<thead>
<tr>
<th>Riboflavin dose [mg (μmol)]</th>
<th>Excretion</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/48h</td>
<td>%</td>
</tr>
<tr>
<td>Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 (53.1)</td>
<td>15.56²</td>
<td>29.3⁻⁴</td>
</tr>
<tr>
<td>(14.03–17.31)</td>
<td>(26.4–32.6)</td>
<td></td>
</tr>
<tr>
<td>40 (106.3)</td>
<td>18.03²</td>
<td>17.0³</td>
</tr>
<tr>
<td>(15.83–24.84)</td>
<td>(14.9–23.4)</td>
<td></td>
</tr>
<tr>
<td>60 (159.4)</td>
<td>22.84²</td>
<td>14.3⁵</td>
</tr>
<tr>
<td>(18.75–26.60)</td>
<td>(11.8–16.7)</td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.6 (30.8)</td>
<td>12.73</td>
<td>39.3</td>
</tr>
<tr>
<td>(11.19–13.71)</td>
<td>(36.7–41.6)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Values are medians (25th percentile–75th percentile); n = 4 males and 5 females. Data were corrected by subtraction of riboflavin excretion before the administration of the test doses.

² Significantly different from the 40-mg dose, P < 0.05.

³ Significantly different from the 60-mg dose, P < 0.05.

⁴,⁵ Significantly different from intravenous administration: ⁴ P < 0.05, ⁵ P < 0.01.

FIGURE 6. Plot of the reciprocal of the amount of riboflavin excreted in urine versus the reciprocal of the riboflavin doses administered. Each data point represents the median of eight or nine subjects (four males and five females, P < 0.05).
findings suggest a fundamental influence of the first-pass effect of gut mucosa and liver on vitamin metabolism.

Intravenously administered riboflavin was < 40% recovered in urine. Other investigators reported a recovery of 72% within 24 h after an intravenous bolus injection of 5 mg riboflavin (1). The higher recovery in this study compared with our findings can be explained by the use of a less-specific fluorometric urine assay in the study cited. This assay might include urinary riboflavin metabolites other than riboflavin (see below). We showed that the bulk of the riboflavin was excreted within the first 24 h after vitamin administration. Net tubular secretion contributed substantially to the rapid elimination of the vitamin. Assuming a glomerular filtration rate of 124 ml/min for men and 109 ml/min for women (28), tubular secretion accounted for 75–80% of total urinary excretion after intravenous injection. This net tubular secretion depends strongly on the dose administered. Jusko and Levy (5) showed that both tubular reabsorption and secretion of riboflavin take place in kidney. Because the absorption is readily saturated, the high-capacity secretory process gains importance when there are elevated plasma concentrations (5). Our finding of tubular riboflavin secretion should not be extrapolated to situations of dietary riboflavin uptake that result in lower plasma concentrations. Although urinary excretion of unchanged riboflavin constitutes an important part of elimination, other routes contribute to flavin excretion. Elimination via bile in humans amounts to < 1% of an administered test dose, i.e., it is negligible (41).

Significant amounts of different riboflavin metabolites were found in human urine in previous studies. These are in the following quantitative order: 7α-hydroxyriboflavin, 8α-sulfonylriboflavin, lumiflavin, 8α-hydroxyriboflavin, and 10-hydroxyethylflavin (42). These metabolites amounted to 28–39% of total urinary flavins (42). Ohkawa et al (31) reported values of 31.1% for 7α-hydroxyriboflavin, 5.0% for 8α-hydroxyriboflavin, and 4.9% for hydroxyethylflavin, but only 25.6% of total urinary flavin was riboflavin. The percentages described for these metabolites can approximately explain the recovery after intravenous injection, though it should be kept in mind that some metabolites, such as 10-hydroxyethylflavin, are of microbiological origin (42), i.e., they should not contribute much to urinary excretion after intravenous injection, though the vitamin could displace a fraction present in tissue. Our present detection of 7α-hydroxyriboflavin in blood plasma indicates a contribution of further catabolites to flavin elimination. The urinary recovery of riboflavin we determined fits well with the clearance data obtained. Renal clearance of riboflavin was ~51% of systemic clearance, i.e., pathways other than renal excretion of unchanged riboflavin contributed to one-half of overall elimination.

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