acacia emulsions. Regardless of the emulsifier used, re-examinations of all emulsions showed no significant change in globule pattern or over-all mean diameters after 6 months on the shelf. These results were corroborated by the previously mentioned rheological stability of the emulsions after 6 months. If a general increase in particle size due to coalescence had occurred, the over-all viscosity of the emulsions would have decreased (12).

The above findings further contribute to the belief that thickness and strength of the emulsifier film adsorbed at the globule surface play a more important role in the stability of emulsions than does the initial particle size distribution.

SUMMARY

Mineral oil emulsions with a 4:6 oil to water ratio were prepared using various concentrations of a new polysaccharide.

The emulsions prepared with the new xantham gum demonstrated desirable rheological characteristics from a stability standpoint. The plastic nature of the xantham gum emulsions, as well as the corresponding yield values, were examined in detail. Their particle size distribution was broader in general than the emulsions prepared with acacia. Globule coalescence of the emulsions containing the xantham gum appeared to be minimal.

The emulsions containing more than 0.2% of the new gum did not exhibit creaming after 6 month's storage. The xantham gum did not leave a tacky film on the skin after drying. There appeared to be no evidence of bacterial decomposition in any of the emulsions, as evidenced by no significant change in pH or viscosity.

A much lower concentration of xantham gum than of acacia was required to produce stable emulsions. As a result of this investigation, it was felt that the new polysaccharide showed good potential as an emulsifier. For the mineral oil emulsions prepared in this study, the optimum concentration of the xantham gum appeared to be 0.4 to 0.5%.

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Effect of Probenecid on Riboflavin Absorption and Excretion in Man

By WILLIAM J. JUSKO and GERHARD LEVY*

An oral dose of probenecid given 1 hr. prior to oral administration of riboflavin or riboflavin-5′-phosphate (FMN) to normal humans produced a marked decrease in the excretion rate of the vitamin during the early excretion phase but had no significant effect during the later (post-absorptive) phase. Oral administration of probenecid prior to intramuscular injection of FMN increased the apparent "half-life" of the initial rapid phase of riboflavin excretion. The urinary recovery of riboflavin following oral or intramuscular administration of the vitamin was usually somewhat decreased by probenecid. These results are consistent with our earlier suggestion that riboflavin is excreted in part by renal tubular secretion. It appears that probenecid also inhibits the specialized transport process responsible for the intestinal absorption of riboflavin in man. The complex kinetics of riboflavin distribution and elimination in man are discussed and additional evidence is presented in support of enterohepatic cycling of the vitamin.

THE AUTHORS have shown recently that riboflavin and riboflavin-5′-phosphate (FMN) are absorbed from the gastrointestinal tract of man by a site-specific and saturable specialized transport process rather than by passive diffusion (1, 2). An analysis of published data has led also to the conclusion that the renal excretion of riboflavin occurs not only by glomerular filtration but apparently also by renal tubular secretion (3).

The purpose of the study to be described here was to determine the effect of probenecid on the excretion of riboflavin. Probenecid inhibits a

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number of specialized renal tubular transport processes (4, 5). An effect of this drug on the excretion of riboflavin would constitute additional support for the suggestion that riboflavin excretion involves active tubular secretion.

Some of the results of this study have bearing also on the absorption, distribution, and disposition of riboflavin and provide additional support for our earlier suggestion that riboflavin is subject to enterohepatic cycling (2).

EXPERIMENTAL

Seven healthy male volunteers, 24 to 38 years of age, served as test subjects. They received 1 Gm. of probenecid[1] (0.5 Gm. in one instance) in the morning after an overnight fast and then ate a standard breakfast. Exactly 1 hr. after probenecid administration, three subjects received 10 to 150 mg. riboflavin or FMN[2] dissolved in 200 ml. of water. Four of the subjects were given an intramuscular injection of approximately 30 mg. of riboflavin as FMN[3] 1 hr. after probenecid administration. The exact amount of FMN injected was determined by direct analysis of an equivalent volume of the parenteral solution measured in the same syringe used for the injection. The injected amounts averaged 28.4 mg. riboflavin equivalent. Similar experiments were carried out without preadministration of probenecid. The order of experiments was randomized with an interval of at least 2 weeks between them.

Urine was collected at frequent intervals for 24 to 48 hr. The standard breakfast, urine collections, and other details of the protocol were essentially the same as described in previous reports (1, 2).

Analyt cal Methods—Riboflavin and FMN in the urine were determined fluorimetrically by the method of Burch *et al.* (6) and by a modified U.S.P. assay procedure (7) using the Turner fluorometer, model 111, with primary filter 47-B and secondary filter 2A-12. The modifications of the two assays have been described in a previous publication (1).

At least two 24-hr. blank urine collections were carried out in each subject and all data were corrected for blank values. The data reported in the tables and figures are based on results obtained with the modified U.S.P. assay. Probenecid and probenecid metabolites (in urines collected after probenecid administration) were found to have no effect on the riboflavin assays.

RESULTS AND DISCUSSION

In the first part of this study, three subjects received various doses of riboflavin or FMN 1 hr. after oral administration of 1 Gm. probenecid. Typical examples of the effect of probenecid on the excretion of riboflavin after oral administration of FMN are shown in Figs. 1 and 2. Probenecid produced a pronounced decrease in the excretion rate of riboflavin during the early phase of riboflavin excretion, but had no detectable effect during the later (post-absorptive) phase of excretion. The effect of probenecid on the early excretion rates and on the total urinary recovery of riboflavin after oral administration of various doses of the vitamin is summarized in Table I for all experiments. In each instance, probenecid produced not only a marked decrease in the excretion rate of riboflavin during the first 2 to 3 hr. after administration of the vitamin, but caused also a statistically significant decrease in the total urinary recovery of the vitamin ($p < 0.05$, paired comparisons).

Bahal (5) found that 1 Gm. probenecid inhibited the elimination of sulfisomidine and procainamide for at least 8 hr. This is consistent with the reported plasma half-life of probenecid which, though

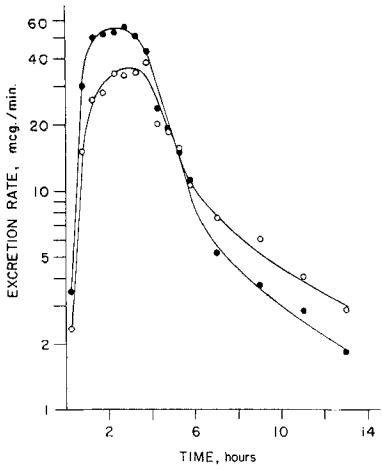


Fig. 1—Urinary excretion rate of riboflavin as a function of time after oral administration of FMN (equivalent to 30 mg. riboflavin) in aqueous solution with (○) and without (●) preadministration of 1 Gm. probenecid. Subject L.

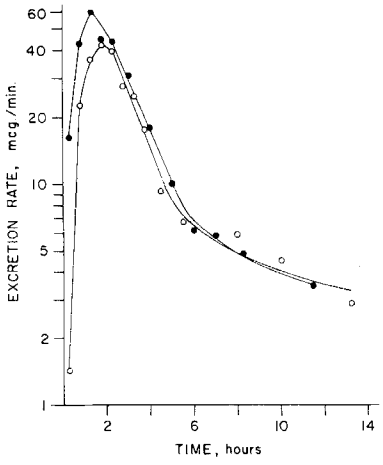


Fig. 2—Urinary excretion rate of riboflavin as a function of time after oral administration of FMN (equivalent to 30 mg. riboflavin) in aqueous solution with (○) and without (●) preadministration of 1 Gm. probenecid. Subject J.

[1] Benemid Tablets, Merck, Sharpe and Dohme, West Point, Pa.
[2] Sodium riboflavin-5′-phosphate, Hoffmann-LaRoche, Nutley, N. J.
[3] Hyrye Injection, Durst Co., Philadelphia, Pa.

TABLE I—EFFECT OF PROBENECID ON INITIAL EXCRETION RATES AND TOTAL URINARY RECOVERY OF RIBOFLAVIN AFTER ORAL ADMINISTRATION OF RIBOFLAVIN (FR) AND RIBOFLAVIN-5′-PHOSPHATE (FMN)

Subject	Drug; Dose, mg.[a]	Probenecid Dose, Gm.	Excretion Rate, mcg./min. Time, hr.: 0.5	1	1.5	2	2.5	% Urinary Recovery[b]
J	FR; 10	0	22	35	32	16	14	63[c]
		0.5	5	22	21	13	12	43
J	FR; 30	0	11	39	47	59	64	44
		1.0	5	35	34	32	33	36[c]
J	FMN; 30	0	16	42	59	45	43	44
		1.0	1	23	36	42	39	38
J	FMN; 150	0	18	58	71	75	73	15
		1.0	1	7	16	17	20	8
A	FMN; 30	0	5	28	44	37	42	37
		1.0	1	14	25	30	33	36
L	FMN; 30	0	3	30	50	51	53	49
		1.0	2	15	26	28	34	44

[a] FMN doses are expressed as riboflavin equivalents. [b] Thirty-six hour collection period, except where *Footnote c* designated. [c] Twenty-four hour collection period.

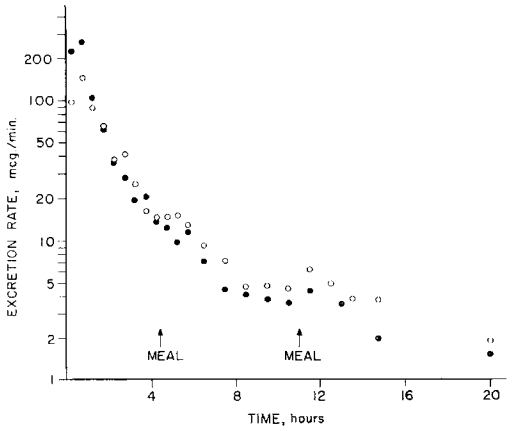


Fig. 3—Urinary excretion rate of riboflavin as a function of time after intramuscular injection of FMN (equivalent to 30 mg. riboflavin) with (○) and without (●) preadministration of 1 Gm. probenecid. Subject M. Note the secondary excretion rate maxima after each meal.

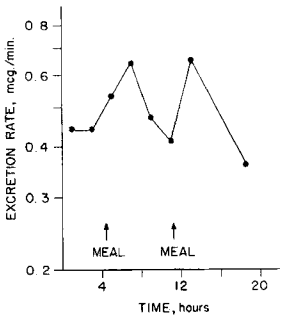


Fig. 4—Urinary excretion rates of riboflavin as a function of time in a subject (F) not receiving riboflavin except for the amount obtained from a normal diet.

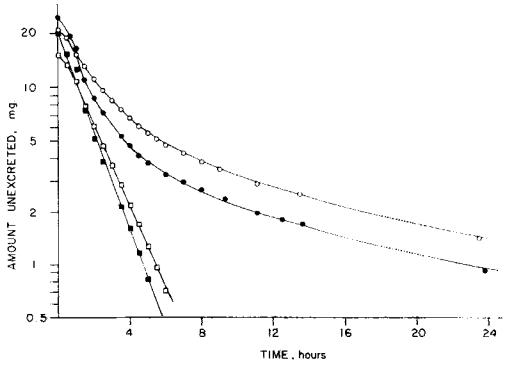


Fig. 5—Urinary excretion of riboflavin as a function of time after intramuscular injection of FMN (equivalent to 30 mg. riboflavin) with (open symbols) and without (solid symbols) preadministration of 1 Gm. probenecid. The lower curves (squares) are the residuals obtained by resolving the upper curves (circles) into two components by the "backward projection" technique.

apparently dose-dependent, is around 6 hr. for a 1-Gm. dose (8). The fact that riboflavin excretion was inhibited only during the first 2 to 3 hr. after administration of probenecid suggested to the authors that the latter inhibits the absorption rather than the excretion of riboflavin. While there appears to be no previous report of an intestinal absorption inhibiting effect of probenecid in man, it is of interest that Binder and co-workers have found recently that probenecid inhibits the active intestinal transport of certain amino acids in the hamster (9).

It was desirable, in view of the results obtained from oral administration of riboflavin, to determine the effect of probenecid on the elimination of parenterally administered riboflavin. A typical example of the effect of probenecid on the excretion rate of riboflavin as a function of time after intramuscular administration of FMN is shown in Fig. 3. The time course of riboflavin excretion observed in these experiments is similar to that found in previous studies (1, 2) where it was noted that the excretion of the vitamin exhibits an initial rapid and a subsequent slow phase. In addition, secondary excretion rate maxima are evident in Fig. 3. These maxima were found in about one-half of the experiments and have been noted also and consistently after oral administration of large doses of the vitamin (2). These secondary maxima are thought to

TABLE II—EFFECT OF PROBENECID ON URINARY RECOVERY AND INITIAL APPARENT HALF-LIFE OF RIBOFLAVIN AFTER INTRAMUSCULAR INJECTION OF FMN[a]

Subject	Injection Site[b]	% Urinary Recovery		Initial Apparent Half-Life[c]	
		Without Probenecid	With Probenecid	Without Probenecid	With Probenecid
J	L	68	66	1.1 (2.7)	1.8 (2.8)
N	A	88	75	1.2 (4.2)	1.4 (5.7)
M	A	102	79	0.8 (2.5)	1.0 (4.0)
F	L	59	83	0.9 (3.3)	1.7 (2.9)

[a] Average dose of 28.4 mg. riboflavin equivalent (range: 27.8 mg. to 29.0 mg.) based on an analysis of the parenteral solution at the time of administration. [b] L, vastus lateralis muscle (leg); and A, deltoid muscle (arm). [c] Figures in parentheses represent the number of "half-lives" during which the residuals yielded a straight line when plotted in semilogarithmic form.

be due to enterohepatic cycling of riboflavin. The possiblity that the secondary peaks observed in the previous study were due to delayed gastric emptying of a portion of the orally administered FMN is ruled out by the occurrence of secondary peaks in this study where the vitamin was administered parenterally. The secondary peaks are not due to absorption of additional riboflavin from dietary sources since the urinary excretion of riboflavin by subjects deriving the vitamin only from dietary sources is very much lower than that observed in the present study. It is of interest however that excretion rate maxima are observed after meals even in subjects receiving only dietary riboflavin (Fig. 4). The excretion rate maxima observed in the latter instance are at least one order of magnitude lower than those shown in Fig. 3. Similar pronounced secondary maxima have been observed recently in the serum levels of dipyridamole and have been ascribed also to enterohepatic cycling (10). The occurrence of such enterohepatic cycling precludes a rigorous pharmacokinetic analysis of riboflavin absorption, distribution, and elimination.

An effect of probenecid on riboflavin excretion after FMN injection becomes evident upon resolving a semilogarithmic plot of the amount of riboflavin unexcreted as a function of time after drug administration into two components by backward projection of the apparently linear terminal portion of the excretion curve. A good example of this is shown in Fig. 5. A semilogarithmic plot of the residuals was linear in all instances up to the time of occurrence of a secondary excretion rate maximum. As a matter of convenience, the rate of decline of the initial linear portion of the semilogarithmic plot of the residuals is described in terms of an "initial apparent half-life." It must be emphasized, however, that this designation is without rigorous pharmacokinetic basis since the actual kinetic model describing the distribution and elimination of riboflavin is considerably more complex than one involving only two parallel unidirectional exponential processes.

The initial apparent "half-lives" and urinary recoveries of riboflavin obtained with and without pre-administration of probenecid are listed in Table II. In all subjects, the initial apparent "half-life" of riboflavin was increased by probenecid but this difference was slightly short of statistical significance ($p > 0.05$; < 0.1). Also, in three of the four subjects, the urinary recovery of the vitamin was decreased by probenecid.

A comparison of the results obtained by the modified U.S.P. and Burch methods, which gives an indication of the relative concentrations of riboflavin and FMN in the urine (2), showed that probenecid had no noticeable effect on the metabolic fate of the vitamin. As noted in the previous study, the urine contained mainly free riboflavin regardless of the form or route of administration.

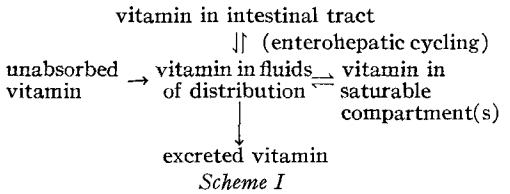
The results of the parenteral study suggest that probenecid does have an inhibitory effect on the renal excretion of riboflavin, presumably by inhibiting an active tubular secretory process (3). While the duration of this effect appears to be relatively short, it is believed unlikely to be due to a decreased rate of absorption of FMN from the site of injection since no such effect of probenecid is known to the authors. On the other hand, Rennick has found an active renal tubular secretory process for riboflavin in the chicken and noted that this process is inhibited by probenecid (11). She suggested that the vitamin shares the transport system for organic bases since the renal tubular secretion of riboflavin was inhibited also by tolazoline. In addition, Markkanen has found that probenecid decreases the basal riboflavin excretion in man and rabbits whose riboflavin intake was limited to that derived from the normal diet (12).

The incomplete recovery of riboflavin observed in most experiments in the present and in previous studies (1, 2), and the decreased urinary recovery of parenterally administered vitamin upon pre-administration of probenecid suggest that the elimination of riboflavin involves at least two parallel pathways.[4] One of these is the urinary excretion of riboflavin; the other process may involve biotransformation, extra-renal excretion, and/or a reversible association of the vitamin with proteins resulting in the formation of certain flavoproteins. The occasional complete urinary recovery of parenterally administered riboflavin (*Reference 13* and subject *M* in the present study) implies that biotransformation and extra-renal excretion may be either quantitatively unimportant or nonexistent. This is consistent with recent evidence that riboflavin is not degraded in rat tissues (14). The third suggested route, namely the reversible association[5] of riboflavin with certain proteins, must be treated kinetically as a bi-directional process involving a compartment of limited capacity. This concept is supported by the fact that nutritionally deficient subjects retain a large fraction of a small test dose of riboflavin (15) and that subjects in

[4] The various phosphorylation-dephosphorylation cycles to which riboflavin is subject in the body have been discussed in a previous publication (2) and are neglected in the present discussion.

[5] This refers to the formation of covalent bonds between riboflavin and proteins in extravascular tissues rather than complex formation with plasma proteins.

negative nitrogen balance (*i.e.*, during excessive catabolism of tissue proteins) show an increased basal excretion of the vitamin (16). These considerations lead to the kinetic model for riboflavin shown in Scheme I.



Scheme I

Even this model, though of considerable complexity, neglects certain additional processes such as phosphorylation-dephosphorylation cycles, formation of FAD, and synthesis of the vitamin by intestinal microorganisms. The time course of riboflavin elimination, as found in this and previous studies in this laboratory (1, 2), is qualitatively similar to that of bromosulfophthalein and rose bengal for which similar reversible multiple compartment models of limited capacity have been suggested (17, 18).

It is reasonable to assume that the saturable compartment is near capacity in subjects who are in exceptionally good nutritional status with respect to riboflavin. Subject *M* of the present study professed to have been taking 5 mg. of riboflavin a day in the form of multiple vitamin tablets four or five times weekly. He discontinued this regimen about 1 week prior to the first parenteral injection of riboflavin. At this time his basal excretion of riboflavin (1.4 mg./day) was indicative of an exceptionally good nutritional status with respect to the vitamin since the usual basal excretion rate is about 1.0 mg./day. (His basal excretion decreased to this level about 3 weeks after the first parenteral injection of riboflavin. During this time he did not use the dietary supplement.) This subject excreted 102% of the first intramuscular dose of FMN which was given without probenecid. It is conceded that this represents very limited evidence but it is noteworthy that none of the other subjects used vitamin preparations prior to the study and that the urinary recovery of riboflavin in these subjects was always incomplete.

The results of this study suggest that probenecid inhibits the urinary excretion of riboflavin. This is consistent with the previous conclusion that the renal excretion of riboflavin apparently involves active tubular secretion (3). It appears also that probenecid inhibits the active intestinal transport of riboflavin although the evidence is by no means unequivocal. This latter point must be established by appropriate animal experiments which await the development of suitable experimental methods since classical techniques used in active transport studies do not appear to be satisfactory for riboflavin (unpublished data). Efforts in this direction are presently being pursued in this laboratory.

ADDENDUM

Effect of Probenecid on Plasma Protein Binding of Riboflavin—Ultrafiltration studies, using human plasma containing 0.1 mg.% riboflavin or FMN alone and with 10 mg.% probenecid, respectively, showed that probenecid has no measurable effect on the plasma protein binding of the vitamin. Details of the binding of riboflavin and FMN by plasma proteins will be reported in a future communication. The concentrations of vitamin and probenecid used in this study reflect the maximum concentration in the plasma obtained upon administration of 30 mg. FMN intramuscularly and 1 Gm. probenecid orally (3, 8, 13). The lack of effect of probenecid on the plasma protein binding of riboflavin suggests that probenecid does not affect the distribution of the vitamin in the body.

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