Factors Affecting the Absorption of Riboflavin in Man

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The urinary recovery of riboflavin as a function of dose after oral administration to fasted normal humans shows that the process responsible for the absorption of this vitamin is saturable. The saturation effect is not evident when riboflavin doses as high as 30 mg. are administered after a meal. The enhanced absorption of riboflavin in the presence of food appears to be due to a decrease in intestinal transit rate which causes the vitamin to be retained at absorption sites in the small intestine for a longer period of time. The site specificity and saturability of riboflavin absorption suggest that the vitamin is absorbed by specialized transport rather than by passive diffusion. This conclusion is supported by kinetic and physical-chemical data, as well as by the results of studies of the effect of route of administration on the recovery of riboflavin in the urine.

SEVERAL GROUPS of investigators have found a linear relationship between the dose of riboflavin administered orally in solution or in other rapidly available forms and the urinary recovery of this vitamin (1–4). Melnick *et al.* (1) recovered an average of about 46% of the dose after administration of up to 10 mg. of riboflavin in solution. Brewer *et al.* (2) recovered about 50% in the dose range of 2 to 7 mg. but demonstrated that doses under 2 mg. are retained more completely. Morrison and Campbell (3) reported an average urinary recovery of 61% in the dose range of 1 to 20 mg., and Morrison *et al.* (4) recovered 56% of the dose after oral administration of 5 and 10 mg. of riboflavin. It has been suggested (5), on the basis of these observations, that riboflavin is absorbed by passive diffusion. In the course of a study of the effect of viscosity on gastrointestinal absorption in man (6), in which riboflavin was used as a model drug, results were obtained which appeared to be completely incompatible with the published observations of others. A thorough review of the data revealed only one potentially significant difference between the experimental designs employed by other investigators and that used in this laboratory: the other investigators administered riboflavin after a meal, while in this laboratory the vitamin was given on an empty stomach. Consequently, a formal study of the effect of time of administration (*i.e.*, before or after breakfast) on the urinary recovery of riboflavin was instituted. The study was designed to yield kinetic data as well, and was expanded subsequently to include an evaluation of the effect of route of administration on riboflavin excretion.

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EXPERIMENTAL

Absorption Study.—Four healthy male volunteers, ranging in age from 22–37 years, served as test subjects. Each subject received 5, 10, and 30 mg. of riboflavin U. S. P. in 100 ml. of 0.02 *N* acetic acid, followed by a small amount of water either on an empty stomach (after an overnight fast) or immediately after a standard breakfast consisting of 60 Gm. of cornflakes with sugar and 500 ml. of milk. One subject received also doses of 3 and 20 mg. of riboflavin on an empty stomach. Total urine collections were carried out at 0, 0.5, 1.0, 1.5, 2, 3, 4, and 6 hr. after riboflavin administration, and at convenient timed intervals thereafter up to 24 hr. Immediately after collection the urines were placed in brown opaque plastic bottles, glacial acetic acid (about 3 ml./100 ml. of urine) was added, and the bottles were placed in a refrigerator. The subjects were instructed to drink about 100 ml. of water every hour to maintain adequate urine output. When riboflavin was given on an empty stomach, breakfast was withheld for 2 hr. after the start of the experiment. The subjects were asked to avoid eating certain foods known to contain appreciable amounts of riboflavin and to refrain from taking vitamin preparations or drugs for at least 48 hr. prior to and during the experiment.

A similar protocol was followed when riboflavin was administered by other than the oral route. The vitamin was administered intravenously by rapid injection of a commercial preparation stated to contain 5 mg. of riboflavin (actual assay: 4.5 mg.) solubilized with nicotinamide (item No. 398, Eli Lilly & Co.). Rectal administration of riboflavin was carried out by means of a polyethylene syringe fitted with a 25-cm. catheter tube which was inserted fully in the rectum. The rectal solution consisted of 5 mg. riboflavin dissolved in 50 ml. of 0.5% methylcellulose (Methocel 4000 60 HG, Dow Chemical Co.) in water. This solution was retained in the colon.

At least two 24-hr. blank urine collections, with and without breakfast, were carried out in each subject. Control urine collections were carried out also after oral administration of 200 mg. of nicotinamide.

Analytical Methods.—Riboflavin in the urine was determined fluorometrically by the method of Burch *et al.* (7) as well as by a method based on the U. S. P. XVI assay procedure (8), using the Turner fluorometer, model 111, with primary filter 47-B and

secondary filter 2A-12. The Burch assay involves extraction of riboflavin into benzyl alcohol, while the modified U. S. P. XVI procedure does not require extraction. In the latter procedure, 5 ml. of suitably diluted urine is mixed with 1 ml. of pH 4.8 acetate buffer (1 *N*). One milliliter of 4% potassium permanganate and, subsequently, 1 ml. of 3% hydrogen peroxide are added. The fluorescence intensity of this solution is determined before and after reduction of riboflavin with sodium hydrosulfite. All data were corrected for blank values which averaged 0.6 mg./24 hr. by the Burch procedure and 1.0 mg./24 hr. by the modified U. S. P. procedure. Nicotinamide was found not to interfere in the assay of riboflavin, both *in vitro* (aqueous solutions) and *in vivo* (urines after nicotinamide administration).

Determination of Partition Coefficients.—Chloroform and 0.1 *N* hydrochloric acid or pH 7.0 phosphate buffer (0.1 *M*) were used as the organic and aqueous phases, respectively. The organic–aqueous phase volume ratio was 50:1, and the initial concentration of riboflavin in the aqueous phase was 5 mg./100 ml. The phases were shaken at 37° for 14 hr. The aqueous phase then was removed, centrifuged, adjusted to pH 4.8 with 0.1 *N* acetate buffer, and assayed fluorometrically by the modified U. S. P. method. Control experiments without riboflavin were carried out in parallel and yielded negligible fluorescence readings.

RESULTS

Both assay procedures employed in this investigation yielded essentially identical results. For example, the average urinary recovery after oral administration of 5 mg. of riboflavin after breakfast was 61.3% by the Burch assay and 60.5% by the modified U. S. P. procedure. The significance of these findings will be discussed in a subsequent report on the metabolic fate of riboflavin and riboflavin-5′-phosphate, respectively (9). All data presented in this report are based on the results obtained with the assay procedure of Burch *et al.* (7).

The urinary recoveries of riboflavin as a function of dose when given on an empty stomach and after a standard meal, respectively, are listed in Tables I and II. As shown in Fig. 1, there was a linear relationship between dose and the amount excreted when the vitamin was taken immediately after a standard breakfast. The average recovery of riboflavin was 62%, which is essentially identical to the 61% recovery obtained by Morrison and Campbell (3). When riboflavin was taken on an empty stomach, the per cent recovered in the urine decreased with increasing dose. In agreement with the observations of others [for example, Everson *et al.* (10)], there were consistently high and low excretors of riboflavin, respectively, regardless of dose and experimental conditions. The relationship between dose and urinary recovery of riboflavin given on an empty stomach is depicted in a Lineweaver-Burk-type plot (11) for the "high excretor," subject *M*, and the "low excretor," subject *J* (Fig. 2). The linear relationship obtained in each case indicates a limited capacity for riboflavin absorption under the experimental conditions.

Figure 3 depicts the excretion rate of riboflavin as a function of time after oral administration of 10 mg. either on an empty stomach or after breakfast. The plot on Cartesian coordinates shows the dif-

TABLE I.—EFFECT OF DOSE ON URINARY RECOVERY[a] OF RIBOFLAVIN GIVEN ON AN EMPTY STOMACH

Subject	Dose, mg. 3	5	10	20	30
J	48.0	35.8	24.5	19.5	10.0
L		33.4	22.2		13.4
A		52.7	22.8		18.2
M		67.9	49.8		21.1

[a] Per cent of dose.

TABLE II.—EFFECT OF DOSE ON URINARY RECOVERY[a] OF RIBOFLAVIN GIVEN AFTER A STANDARD BREAKFAST

Subject	Dose, mg. 5	10	30
J	49.8	61.0	52.4
L	59.2	54.6	50.0
A	60.1	68.9	59.9
M	76.1	68.4	80.4

[a] Per cent of dose.

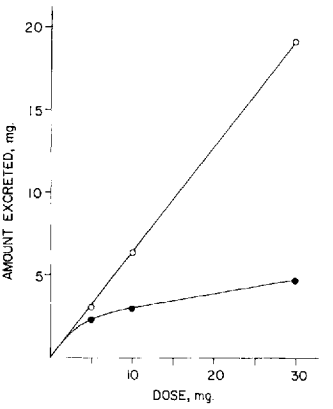

Fig. 1.—Urinary recovery of orally administered riboflavin as a function of dose when given on an empty stomach (●) and after a standard breakfast (○). Mean of 4 subjects.

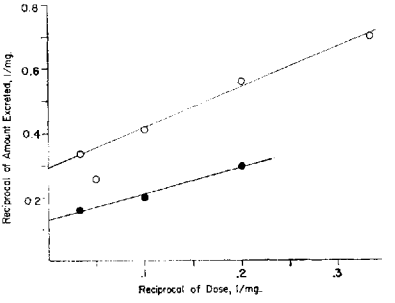

Fig. 2.—Lineweaver-Burk-type plot of the reciprocal of the amount of riboflavin recovered in the urine *vs.* the reciprocal of the oral dose given on an empty stomach. Key: ○, subject *J*; ●, subject *M*.

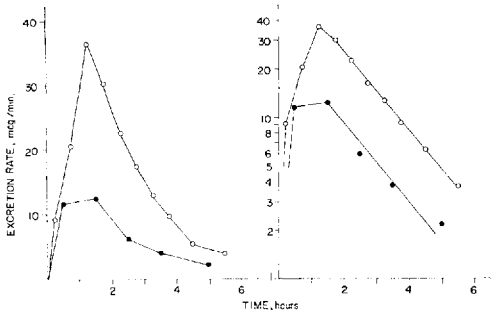

Fig. 3.—Excretion rate of riboflavin as a function of time after oral administration of 10 mg. of riboflavin in aqueous solution to 4 subjects on an empty stomach (●) and after a standard breakfast (○). Data are plotted on Cartesian coordinates (left) and in semilogarithmic form (right).

ference in the areas under the respective curves (which reflects the different amounts absorbed and excreted under the different experimental conditions) and indicates the very rapid absorption of the vitamin. The shapes of the 2 curves suggest that absorption under the 2 experimental conditions occurred at about the same rate initially, but continued for a longer period of time when the vitamin was given after breakfast. The semilogarithmic plot in Fig. 3 shows an exponential decline of excretion rate as a function of time, with an average half-life of 1.1 hr. (range of individual half-lives: 0.9 to 1.4 hr.). There was a consistent decrease in slope after 6 hr., when only a very small fraction of the total excreted amount remained to be excreted.

The effect of route of administration on riboflavin recovery is shown in Table III for subject *J*. The dose had to be restricted to 5 mg. to permit intravenous and rectal administration of the vitamin in solution. The viscosity of the rectal solution was increased by addition of methylcellulose to permit retention of the solution without leakage. The possibility of complex formation between riboflavin and the polymer was ruled out by equilibrium dialysis (6).

The kinetics of elimination of riboflavin after intravenous administration are depicted in Fig. 4. The experimental data could be resolved into a rapid and a slow component with half-lives of about 0.2 and 8 hr., respectively.

DISCUSSION

Site Specificity of Riboflavin Absorption.—Evidence which suggests that riboflavin is absorbed mainly in the proximal region of the intestinal tract has been reviewed by Campbell and Morrison (5).

TABLE III.—EFFECT OF ROUTE OF ADMINISTRATION ON URINARY RECOVERY OF A 5-mg. DOSE OF RIBOFLAVIN IN A FASTING SUBJECT

Rt. of Admin.	Recovery, %
Oral, empty stomach	35.8
Oral, after breakfast	49.8
Intravenous[a]	72.0
Rectal[b]	6.2

[a] Actual dose, 4.5 mg. [b] Retention enema.

For example, they point out that riboflavin absorption is decreased if the vitamin is administered in certain coated tablets or sustained-release preparations which do not release riboflavin relatively promptly. Moreover, administration of riboflavin in a number of sustained-release preparations having markedly different release characteristics has yielded excretion rate *versus* time curves which tend to drop off at about the same (early) times as curves obtained after giving the vitamin in rapidly available form.

Colonic absorption of riboflavin is practically insignificant (Table III). The present findings are in agreement with those of Everson *et al.* (10) and Campbell and Morrison (5). Najjar *et al.* (12) noted some absorption of riboflavin after administering 20 mg. as a rectal enema, but presented no quantitative data. It has been shown that rectally administered enemas and even suppositories spread in a retrograde manner at least to the mid-descending and frequently to the ascending colon (13, 14). In the present study, particular emphasis was placed upon introducing the riboflavin solution as high up the colon as possible by use of a 25-cm. catheter. The very low absorption of riboflavin after rectal administration is considered, therefore, to reflect an intrinsically poor absorbability of this vitamin in the colon, rather than being due to limited contact with colonic membranes.

It has been suggested (4) that the apparent site specificity of riboflavin absorption could be due to degradation of the vitamin in the lower bowel. This suggestion is based on a report by Selye (15) who stated, on the basis of very limited and indirect evidence, that riboflavin is rapidly destroyed in the large intestine of rats. The theory that the site-specificity of riboflavin absorption is due to degradation of the vitamin in the large intestine is rather untenable on kinetic grounds. If the degradation process is first order, a constant fraction of each dose, regardless of size, would be destroyed. If the process is apparent zero order (perhaps due to a limited metabolic capacity of a microbial population involved in this process), the fraction of a dose which will be destroyed would decrease with increasing dose. The experimental data (Table I) show exactly the opposite relationship. To explain this phenomenon on the basis of degradation in the lower gastrointestinal tract requires that one invoke such

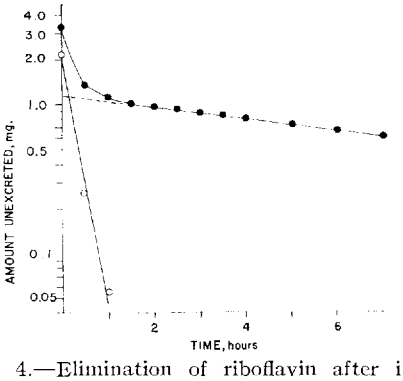

Fig. 4.—Elimination of riboflavin after intravenous administration (subject *J*). Key: ○, difference between experimental data; ●, extrapolated line.

possibilities as substrate inhibition of an enzymic system, or a dose-dependent effect of riboflavin on gastrointestinal motility. Neither of these possibilities is realistic in terms of present knowledge or in relation to the enhanced absorption observed when riboflavin is taken after a meal. Another remote possibility is that the decreased absorption of riboflavin with increasing dose is due to the dimerization of the vitamin at higher concentrations and that this is practically abolished in the presence of other interacting substances ingested as food. However, there is evidence that biologic membranes have a dissociating effect on homo-complexes and that dimerization will not affect absorption rate (28).

Saturability of Riboflavin Absorption.—The observation that the urinary recovery of riboflavin is decreased with increasing dose when the vitamin is taken on an empty stomach (Table I) suggests strongly that the process responsible for riboflavin absorption is saturable. There is no evidence that renal excretion of riboflavin is saturable in the concentration range encountered in the present experiments (*cf.* subsequent paragraph concerning elimination kinetics), and this possibility may, therefore, be ruled out as accounting for the experimental observations. Since given individuals appear to have relatively constant intestinal transit rates (16), the amount of an incompletely absorbed substance (given in solution) which is absorbed can be used as a relative measure of absorption rate in the individual. On this basis, Lineweaver-Burk plots have been prepared by plotting the reciprocal of the amount of riboflavin absorbed on an empty stomach against the reciprocal of the dose. Figure 2 shows such plots for a high and a low excretor of riboflavin. Each of these plots is linear and indicates an absorption maximum, as is characteristic of active transport processes (17). The magnitude of the extrapolated maximum values cannot be compared directly between different individuals, because they are a function also of intestinal transit rate and of the fraction of absorbed riboflavin which is excreted in the urine. These characteristics show appreciable intersubject variations. For example, the data listed in Table II show that subject *M* intrinsically excretes a larger fraction of riboflavin than does subject *J*.

The Effect of Food.—Food and viscous liquids slow gastric emptying and intestinal transit (18, 19). It is reasonable to assume that the presence of food causes riboflavin to be in contact with optimum absorption sites in the proximal region of the intestinal tract for a longer period of time, and thus, brings about the complete absorption of the vitamin over a wider dose range. The data in Table II indicate that riboflavin given after breakfast is absorbed either completely or that the same fraction of the dose is absorbed[1] in the dose range of 5 to 30 mg. While it would be desirable to demonstrate that saturation of riboflavin absorption is possible even when the vitamin is given after meals, results of such experiments would be equivocal since the limited solubility of riboflavin would require that the large doses necessary for such a study be given in suspension. If the amount of undissolved riboflavin is greater than that which can be dissolved relatively rapidly in gastric and upper intestinal fluids, the absorption kinetics would be apparent zero order for physicochemical rather than for physiologic reasons. However, if experiments now in progress in this laboratory show that riboflavin-5′-phosphate (FMN) has similar absorption characteristics as does riboflavin, large doses of the more water-soluble FMN will be given in solution after a meal to see if saturation effects can be demonstrated.[2]

It is of interest that apparently only Morrison and Campbell (3) have administered riboflavin also on an empty stomach. They used a 5-mg. dose only and reported "similar" urinary recovery to that obtained after breakfast, but did not present quantitative data. Their findings are explained readily by reference to Fig. 1, which shows that recovery of riboflavin given before or after breakfast is indeed "similar" at doses of 5 mg.; the differences become appreciable only when larger doses are given.

The Evidence for Specialized Intestinal Transport of Riboflavin in Man.—Two of the major characteristics of specialized intestinal transport processes are site specificity and saturability (20). These characteristics are evident in the absorption of riboflavin. The vitamin has a molecular weight of almost 400 which precludes rapid absorption by the pore route. Its chloroform–water partition coefficient, when the pH of the aqueous phase is either 1 or 7, is very small (< 0.001). Substances with such properties are not absorbed or only very poorly absorbed by passive diffusion (20). Yet riboflavin is absorbed very rapidly, as is evident from Fig. 3 and from the report by Wiegand *et al.* (21), who have estimated that the rate constant for riboflavin absorption (in doses of 5 mg. or less) is in excess of 30 reciprocal hr.! Although Wiegand *et al.* point out that a rate constant of this magnitude is not meaningful (it indicates that absorption is 95% complete in 6 min. or less), the value does reflect the very rapid absorption of the vitamin. It is inconsistent with present knowledge that such rapid absorption of a large, lipoid-insoluble substance can occur other than by some form of specialized transport.

Animal Experiments.—Spencer and Zamcheck (22) have studied riboflavin absorption with everted intestinal sacs from rats and hamsters and by the *in vivo* ligated loop technique in rats. They did not find any accumulation of riboflavin against a concentration gradient and noted that the intestinal sac appeared relatively impermeable to the vitamin. However, they used an almost saturated solution of riboflavin, and the high concentration alone can account for their inability to find serosal to mucosal concentration ratios greater than unity. The observed low permeability of the intestinal sac to riboflavin shows, regardless of other considerations, that the absorption of this substance by passive diffusion is at best very poor. Spencer and Zamcheck conclude that "the scant evidence available suggests that riboflavin may cross the intestine by diffusion rather than by specific transport," but add that "it is possible that in the presence of lower concentra-

[1] This would be the case if riboflavin is partially destroyed in the intestinal tract.

[2] After completion of this manuscript, the recent paper by Stripp [*Acta Pharmacol. Toxicol.*, **22**, 353(1965)] was received. He found that 50- to 500-mg. doses of FMN, given in solution after a meal, yielded the same riboflavin in blood levels and the same amounts excreted despite the 10-fold difference in dose. This demonstrates unequivocally the existence of an absorption maximum even when the vitamin is administered after a meal.

tions of the vitamin and with the admixture of food materials there may be other mechanisms of riboflavin absorption by the intestine" (22).

The significance of the study by Turner and Hughes (23), who used rat intestine preparations, is also limited by the high concentration of riboflavin used. They concluded that in rats all B group vitamins are absorbed by passive diffusion, but it is of interest that Spencer and Brody (24) have shown subsequently that at least one of these, biotin, is absorbed by specialized transport in the white mouse, hamster, and squirrel, though not in the rat, rabbit, and guinea pig. Thus the possibility of species differences alone limits the applicability of the results of animal studies to the elucidation of the mechanism of riboflavin absorption in man. A study by Middleton and Grice (25) is of interest because these workers administered riboflavin by stomach tube to intact rats. Their results show a relative site specificity for riboflavin absorption as well as definite apparent zero-order absorption kinetics which is compatible with the existence of a saturable transport process.

Kinetics of Riboflavin Elimination in Man.—Excretion of riboflavin after intravenous administration was very rapid initially and decreased after the first hour (Fig. 4). The experimental curve could be resolved into a rapid and a slow exponential component. Similar results are obtained upon graphical analysis of the data of Axelrod *et al.* (26), who administered intravenously 0.2 or 0.4 mg. of riboflavin per Kg. body weight to 4 subjects. They found that 30 to 40% of the dose was excreted within 1 hr. after injection. Evaluation of rate constants for their subject III yield essentially the same values as were obtained in the experiment depicted in Fig. 4. The data of Axelrod *et al.* (26) show riboflavin excretion rates of more than 6 mg./hr. and give no indication of possible saturation of excretory function.[3] The existence of an initial rapid elimination phase followed by a much slower phase is also evident from the study of Najjar and Holt (27), who administered 1 mg. riboflavin intravenously to 10 subjects after an overnight fast. They recovered 32 to 72% of the dose in the urine. Najjar *et al.* (12) established in a subsequent study that large doses of intravenously administered riboflavin do not cause an increase in fecal riboflavin output. This suggests that the incomplete recovery of absorbed riboflavin is due to biotransformation and/or metabolic retention of riboflavin, rather than to fecal excretion. All the available data suggest that riboflavin, when given intravenously, is relatively slowly distributed and therefore very rapidly eliminated initially. A variable fraction is either metabolized or retained in some form (probably as flavoprotein), and a small fraction is eliminated rather slowly.

The kinetics of riboflavin elimination after oral administration yield a somewhat different picture (Fig. 3). Excretion rate decreases exponentially with time, with an apparent half-life of about 1.1 hr. However, a slower elimination component was noted after 6 hr. in the present study and is evident also in the study of Morrison *et al.* (4). This slow component is probably identical to that found after intravenous administration and may reflect the elimination of riboflavin from a "deep" compartment. The more rapid early phase of riboflavin elimination after oral administration may reflect a combination of a distally decreasing intestinal absorption gradient and slow diffusion of the vitamin from blood into tissues, or it may represent an effect of route of administration on the distribution of riboflavin in the body.

[3] The same is evident from Fig. 3 of the present study.

REFERENCES

(1) Melnick, D., Hochberg, M., and Oser, B. L., *J. Nutr.*, **30,** 67(1945).
(2) Brewer, W., Porter, T., Ingalls, R., and Ohlson, M. A., *ibid.*, **32,** 583(1946).
(3) Morrison, A. B., and Campbell, J. A., *ibid.*, **72,** 435 (1960).
(4) Morrison, A. B., Perusse, C. B., and Campbell, J. A., *New Engl. J. Med.*, **263,** 115(1960).
(5) Campbell, J. A., and Morrison, A. B., *Am. J. Clin. Nutr.*, **12,** 162(1963).
(6) Jusko, W. J., and Levy, G., unpublished data.
(7) Burch, H. B., Bessey, O. A., and Lowry, O. H., *J. Biol. Chem.*, **175,** 457(1948).
(8) "United States Pharmacopeia," 16th rev., Mack Publishing Co., Easton, Pa., 1960, p. 907.
(9) Jusko, W. J., and Levy, G., to be published.
(10) Everson, G., Wheeler, E., Walker, H., and Caulfield, W. J., *J. Nutr.*, **35,** 209(1948).
(11) Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, **56,** 658(1934).
(12) Najjar, V. A., Johns, G. A., Medairy, G. C., and Holt, L. E., Jr., *J. Am. Med. Assoc.*, **126,** 357(1944).
(13) Matts, S. G. F., and Gaskell, K. H., *Brit. Med. J.*, **2,** 614(1961).
(14) Parker, J. C., and Siegelman, S., *Am. J. Digest. Diseases*, **10,** 463(1965).
(15) Selye, H., *J. Nutr.*, **25,** 137(1943).
(16) Levy, G., and Hollister, L. E., *J. Pharm. Sci.*, **54,** 1121 (1965).
(17) Schanker, L. S., and Tocco, D. J., *J. Pharmacol. Exptl. Therap.*, **128,** 115(1960).
(18) Bachrach, W. H., *Ciba Clin. Symp.*, **11,** 3(1959).
(19) Levy, G., and Jusko, W. J., *J. Pharm. Sci.*, **54,** 219 (1965).
(20) Wilson, T. H., "Intestinal Absorption," W. B. Saunders Co., Philadelphia, Pa., 1962.
(21) Wiegand, R. G., Buddenhagen, J. D., and Endicott, C. J., *J. Pharm. Sci.*, **52,** 268(1963).
(22) Spencer, R. P., and Zamcheck, N., *Gastroenterology*, **40,** 794(1961).
(23) Turner, J. B., and Hughes, D. E., *Quart. J. Exptl. Physiol.*, **47,** 107(1962).
(24) Spencer, R. P., and Brody, K. R., *Am. J. Physiol.*, **206,** 653(1964).
(25) Middleton, E. J., and Grice, H. C., *Can. J. Biochem. Physiol.*, **42,** 353(1964).
(26) Axelrod, A. E., Spies, T. D., and Elvehjem, C. A., *J. Clin. Invest.*, **20,** 229(1941).
(27) Najjar, V. A., and Holt, L. E., Jr., *Bull. Johns Hopkins Hosp.*, **69,** 476(1941).
(28) Levy, G., and Matsuzawa, T., *J. Pharm. Sci.*, **54,** 1003 (1965).