Effect of Viscosity on Thiamine and Riboflavin Absorption in Man

ROBERT R. HEWITT and GERHARD LEVY

Abstract □ Thiamine and riboflavin are absorbed from the upper small intestine by specialized transport processes. It was thought that oral administration of these vitamins in viscous solution would affect their absorption, since a previous investigation in rats showed that viscous solutions decrease the absorption of drugs from the stomach and retard gastrointestinal transit. Contrary to expectations, oral administration of thiamine and riboflavin in 50 ml. of highly viscous methylcellulose solution (3400 cps. at a shear rate of 5.1 sec.$^{-1}$) did not affect significantly the rate and extent of absorption of the two vitamins in healthy adult volunteers.

Keyphrases □ Thiamine, viscosity—effect on absorption, man □ Riboflavin, viscosity—effect on absorption, man □ Viscosity—effect on riboflavin, thiamine absorption, in man

Previous studies in rats showed that the absorption of drugs and the gastrointestinal transit of a drug solution are decreased when the viscosity of the solution is increased by methylcellulose (1). Seager (2) found that the gastrointestinal absorption of nitrofurantoin in man is decreased significantly when the drug is administered as a *suspension* in 5% methylcellulose (viscosity grade not specified) rather than in water. Apparently, no formal study of the effect of viscosity on the gastrointestinal absorption of drugs in *solution* has been carried out in man. Therefore, the absorption of two water-soluble vitamins, thiamine and riboflavin, was studied upon oral administration in aqueous solution and in highly viscous methylcellulose solution.

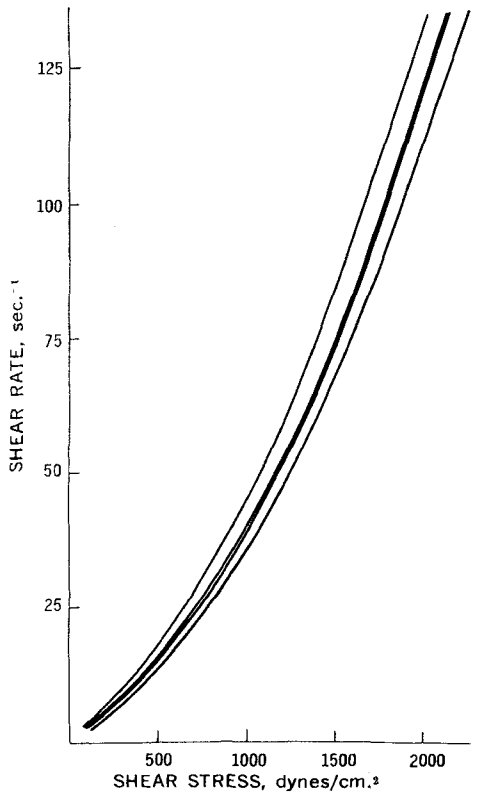

Figure 1—*Rheograms of the high viscosity (methylcellulose) vitamin solution used in the study. Each rheogram, obtained at 37°, represents one of the solutions prepared separately for each subject.*

Table I—Effect of Viscosity on Gastrointestinal Absorption of Thiamine and Riboflavin in Man

Subject	Thiamine		Riboflavin	
	Aqueous	Methylcellulose	Aqueous	Methylcellulose
	Percent of Dose Recovered in Urine			
SF	7.57	5.76	26.9	21.0
WJ	5.02	5.04	26.5	16.5
VS	9.62	6.92	20.8	19.8
WH	3.47	4.31	12.2	12.7
Mean	6.42	5.51	21.6	17.5

Thiamine and riboflavin are absorbed by specialized, saturable transport processes which are located in the proximal region of the small intestine (3–8). Oral dosage forms that remain at specialized absorption sites in the small intestine for longer periods of time than do ordinary aqueous solutions or tablets should increase the absorption of large, otherwise only partly absorbed, doses of the two vitamins. The administration of large doses of thiamine and riboflavin in highly viscous solution should, therefore, result in a decreased initial absorption rate but increased extent of absorption if the highly viscous solutions are emptied from the stomach and moved along the intestinal tract more slowly than ordinary aqueous solutions of these vitamins.

EXPERIMENTAL

Four young men, 24–28 years old, weighing 62–85 kg., served as test subjects. They received 41 mg. riboflavin-5′-phosphate·$2H_2O$ (equivalent of 30 mg. riboflavin) and 26.9 mg. thiamine hydrochloride (molar equivalent of 30 mg. riboflavin) in the morning on an empty stomach. The vitamins were dissolved in 50 ml. water or methylcellulose solution. The solutions were flavored with 1% citric acid and 0.06% sodium saccharin. Two of the subjects took the aqueous solution first and the methylcellulose solution several weeks later. The other two subjects took the solutions in the reverse order. The bottle containing the vitamin solution was rinsed with 30 ml. water which was also taken. The subjects had their normal lunch and dinner, but they were instructed not to take any vitamin preparation and drugs for at least 1 week preceding and during the study.

The subjects emptied their bladders immediately before taking the vitamin solution. Urine was then collected every 30 min. for 4 hr., every hour for the next 4 hr., every 2 hr. until bedtime, and at desired intervals thereafter for a total of 36 hr. Excretion was completed within that period of time. About 3 ml. glacial acetic acid was added to each 100 ml. urine immediately after collection, and the urine sample was placed in a refrigerator until assayed. All samples were protected from light. The subjects drank 50–100 ml. water after each

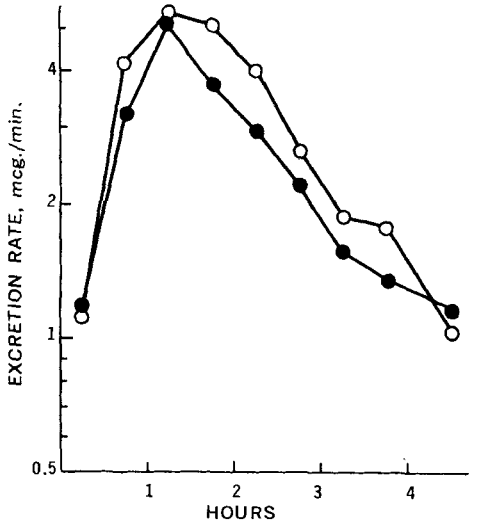

Figure 2—*Urinary excretion rate of thiamine as a function of time after oral administration of thiamine and riboflavin in water* (○) *and methylcellulose solution* (●). (*Average of four subjects.*)

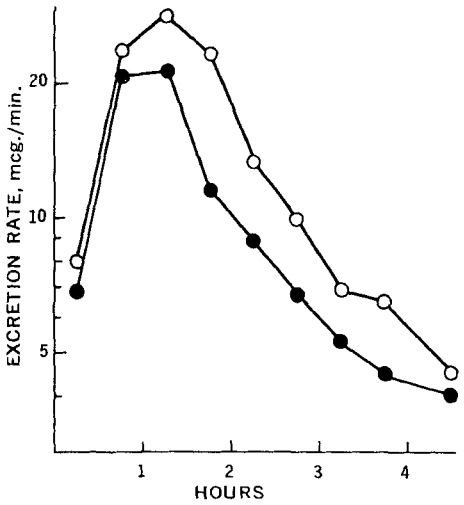

Figure 3—*Urinary excretion rate of riboflavin as a function of time after oral administration of riboflavin and thiamine in water* (○) *and methylcellulose solution* (●). (*Average of four subjects.*)

voiding to maintain an adequate urine output. Twelve-hour "blank" collections of urine were also carried out on each subject.

Riboflavin in the urine was determined fluorometrically by a modification of the USP XVI procedure (6). Thiamine was determined by the thiochrome method according to Mickelsen *et al.* (9), with minor modifications. Permutit was activated according to the procedure of the Association of Vitamin Chemists (10). Fluorescence was measured with the Turner fluorometer, model 111, with primary filter 7-60 and secondary filters 2A and 47B. Neither assay interfered to any measurable extent with the other, as determined by analysis of solutions containing known concentrations of riboflavin and thiamine. There was no evidence of degradation of the vitamins in acidified urine samples stored in the refrigerator for 2 weeks. All assays were performed within that time. Aliquots of the vitamin solutions were assayed together with the urine sample, and percent absorption was calculated relative to the vitamin content of the solutions as determined by the assay.

The methylcellulose solutions were prepared by dispersing methylcellulose[1] in hot water with a Waring Blendor. The vitamins and flavoring agents were dissolved separately in small volumes of water and added to the methylcellulose solution. The final concentration of methylcellulose in the solution was 2.5% w/w. The preparation was stored in a refrigerator. Rheograms were obtained after 3–4 days, and the solution was administered within 3–7 days after preparation. Fresh solutions were prepared for each experiment (*i.e.*, four solutions for the four test subjects).

Rheograms were obtained at 37° with the Epprecht Rheomat 15 viscometer, using measuring system C. The solutions were sheared initially at a rate of 5.14 sec.$^{-1}$ for 3 min. and then left undisturbed for 5 min. Shear stress determinations were made every minute at 15 shear rates, from the lowest to the highest rate and back to the lowest shear rate.

Five milliliters of vitamin solution containing methylcellulose was placed in Visking dialysis tubing and dialyzed at room temperature for 70 hr. against 50 ml. of vitamin solution without methylcellulose. Both solutions were assayed at 17 and 70 hr. for riboflavin and thiamine.

RESULTS AND DISCUSSION

The vitamin solutions with methylcellulose were so viscous that they had to be taken by spoon. They had the consistency of honey; rheograms of the solution are shown in Fig. 1. Since the solutions were pseudoplastic, their viscosity was shear rate dependent and averaged 3410, 2680, and 1560 cps. at shear rates of 5.14, 30.4, and 137 sec.$^{-1}$, respectively. Equilibrium dialysis of the vitamin–methylcellulose solution against vitamin solution without methylcellulose yielded thiamine and riboflavin concentration ratios of unity, showing that the macromolecule did not bind or complex with either vitamin. There was no significant difference in the total amount of thiamine and riboflavin excreted in the urine after oral administration of these vitamins in water and methylcellulose solution, respectively (Table I). There was also no significant difference in the initial excretion rates of the two vitamins (Figs. 2 and 3). The somewhat lower average excretion rate of riboflavin after the 1st hr. following administration of the viscous solution was primarily due to one of the four subjects (Subject WJ).

Similar results were obtained previously in this laboratory in an unpublished study in which 100-ml. solutions containing 10 mg. riboflavin or 10 mg. riboflavin and 1.5% methylcellulose were administered to four subjects in five experiments (11). The viscosity of the methylcellulose solution at low shear rates was about 370 cps., approximately one-tenth that of the more concentrated solution used in the present study. The average urinary recovery of riboflavin from the aqueous solution was 23.9% of the dose; that from the methylcellulose solution was 29.7%. This difference was not statistically significant.

The results of this study are not consistent with previous observations in animals (1) and with the results obtained by Seager in man with nitrofurantoin *suspensions* (2). It is possible that Seager's results were due to slower *in vivo* dissolution of the nitrofurantoin particles coated with methylcellulose rather than to slower gastric emptying of the suspension. Food retards the absorption of thiamine and increases the extent of absorption of riboflavin in man (12). Pathological conditions which decrease gastrointestinal motility increase the absorption of large doses of riboflavin (13).

The lack of effect of a relatively large volume (50 ml.) of highly viscous solution in the present study suggests that gastric emptying and intestinal transit were not affected significantly under the experimental conditions. Clearly, there is no justification for the assumption that a pronounced change in viscosity will invariably affect the gastrointestinal absorption of drugs from a solution.

[1] Methocel 4000, 60 HG, Dow Chemical Co., Midland, Mich.

REFERENCES

(1) G. Levy and W. J. Jusko, *J. Pharm. Sci.*, **54,** 219(1965).
(2) H. Seager, *J. Pharm. Pharmacol.*, **20,** 968(1968).
(3) G. Rindi and U. Ventura, *Experientia*, **23,** 175(1967).
(4) G. Levy, in "Importance of Fundamental Principles in Drug Evaluation," Raven, New York, N. Y., 1968, p. 141.
(5) A. D. Thomson, H. Baker, and C. M. Leevy, *J. Lab. Clin. Med.*, **76,** 34(1970).
(6) G. Levy and W. J. Jusko, *J. Pharm. Sci.*, **55,** 285(1966).
(7) W. J. Jusko and G. Levy, *ibid.*, **56,** 58(1967).
(8) A. D. Thomson, *Clin. Sci.*, **31,** 167(1966).
(9) O. Mickelsen, H. Condiff, and A. Keys, *J. Biol. Chem.*, **160,** 361(1945).

(10) Association of Vitamin Chemists, Inc., "Methods of Vitamin Assay," 3rd ed., Interscience, New York, N. Y., 1966, chap. 6.
(11) G. Levy and W. J. Jusko, unpublished data, 1964.
(12) G. Levy and R. Hewitt, to be published.
(13) G. Levy and M. MacGillivray, to be published.

ACKNOWLEDGMENTS AND ADDRESSES

Received October 21, 1970, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication December 14, 1970.

Supported in part by a grant from Hoffmann–La Roche Inc.

Degradation Products of Chloramphenicol

I. K. SHIH

Abstract □ Incubated aqueous solutions of chloramphenicol at various pH's (1–14) yielded detectable amounts of *p*-nitrobenzaldehyde (an oxidation product) and arylamine (a reduction product). Identical degradation products were also found in certain dosage forms (creams and capsules), although they were not found in ophthalmic ointment.

Keyphrases □ Chloramphenicol—degradation products determined □ *p*-Nitrobenzaldehyde—determined as degradation product in chloramphenicol □ Arylamine—determined as degradation product in chloramphenicol

The stability of chloramphenicol is known to be dependent in part upon two degradation pathways: amide hydrolysis at pH 2–7 and carbon–chlorine cleavage about pH 7 (1, 2). Amide hydrolysis leads to the formation of *p*-nitrophenyl-2-amino-1,3-propanediol. This compound was detected in dosage forms by Sahli *et al.* (3), Kassem *et al.* (4), Rousselet and Paris (5), and James and Leach (6). Other degradation products have not been reported in dosage forms of chloramphenicol below pH 7.0.

As suggested by Higuchi and Bias (2), the reactivity of chloramphenicol could lead to other degradation reactions. Initial studies in these laboratories revealed that aged chloramphenicol preparations contained arylamine and *p*-nitrobenzaldehyde. The present study was designed to illustrate the identification of these degradation products and to measure the concentrations of *p*-nitrobenzaldehyde in various chloramphenicol preparations.

EXPERIMENTAL

Materials and Apparatus—Reference standard chloramphenicol was used[1]. All other chemicals and solvents were of reagent grade. Silica gel sheets (chromatogram sheets, Eastman No. 6061) were used for TLC. The chromatographic solvent systems are shown in Table I. Spectrophotometric analyses were performed with the Bausch & Lomb 505 (visible) and the Beckman IR-8 (IR).

Spontaneous Oxidation and Reduction of Chloramphenicol in Aqueous Solution—Fourteen aqueous solutions (0.2%) of chloramphenicol were prepared in low actinic volumetric flasks ranging from pH 1 to 14, respectively. After standing at room temperature for 24 days or longer, 50 ml. of each sample solution was extracted with approximately 25 ml. ether. Evaporation of the ether extract yielded a residue. Tests were then performed for the presence of oxidation and reduction products by using the same methods as subsequently described for chloramphenicol dosage forms.

Identification of *p*-Nitrobenzaldehyde and Its Occurrence in Dosage Forms—The contents of 10 capsules, equivalent to 2.5 g. of chloramphenicol, were stirred with 400 ml. water for 0.5 hr. The suspension was filtered and the filtrate distilled. Approximately 300 ml. of distillate was collected and extracted with 50 ml. ether. Evaporation of the ether extract yielded a small amount of residue possessing the characteristic odor of *p*-nitrobenzaldehyde. The residue, Product A, was dissolved in 0.5 ml. methanol; 10–30 μl. was spotted on a thin-layer plate along with 5 μl. (containing 2 mcg.) of the reference *p*-nitrobenzaldehyde. After development, the TLC plates were sprayed with 0.5% alcoholic phenylhydrazine solution. A similar procedure was followed for chloramphenicol cream and ophthalmic ointment, except that a 20-g. sample was taken and a small amount of antifoam was introduced in the distillation step.

An alternate procedure was used as follows: the contents of five capsules were extracted with approximately 50 ml. ether and filtered. To the filtrate, a few drops of 0.5% alcoholic phenylhydrazine solution were added and the solution was evaporated. The orange-yellow phenylhydrazine derivative was extracted with 0.5 ml. of solvent mixture (two parts CCl_4 and one part $CHCl_3$). Then 10–30 μl. of this solution (Solution B) was spotted on a thin-layer plate along with 5 μl. (containing 2 mcg.) of a reference sample of *p*-nitrobenzaldehyde phenylhydrazone.

IR Identification of *p*-Nitrobenzaldehyde as Its Phenylhydrazone Derivative—The phenylhydrazone derivative was obtained as described previously. The orange-yellow spots of phenylhydrazone were cut out and extracted with methanol. Evaporation of methanol yielded a small amount of dark-orange residue, which was triturated with a small amount of potassium bromide. The IR spectrum of the sample in KBr pellets was compared to that of an authentic sample of *p*-nitrobenzaldehyde phenylhydrazone.

Estimation of *p*-Nitrobenzaldehyde in Chloramphenicol Dosage Forms—From the contents of 20 capsules, the powder equivalent to 1.0 g. of chloramphenicol, accurately weighed, was transferred to a 10-ml. volumetric flask and made to volume with ethanol. A filtered 5-ml. aliquot was mixed with 2 ml. of 0.5% alcoholic phenylhydrazine solution. After standing for 30 min., the absorbances of both the sample and standard solutions were measured against a reagent blank at 440 nm. A similar procedure was followed for chloramphenicol cream, except that a 5-g. sample was completely dissolved in an appropriate amount of ethanol and the cream base was separated by freezing. This ethanol extract was then treated as for capsules. The results are reported in Table II.

Identification of *p*-Nitrophenyl-2-amino-1,3-propanediol—The contents of four capsules were mixed in 40 ml. methanol and filtered. The filtrate was evaporated and the residue extracted with 2–3 ml. water. Then 30–60 μl. of the sample preparation (Solution C) was spotted along with 5 μl. (containing 10 mcg.) of reference *p*-nitrophenyl-2-amino-1,3-propanediol on a thin-layer plate. After development, the plate was sprayed with 0.2% alcoholic ninhydrin solution and heated in an oven for about 5 min. at 60°. For creams, a 5-g. sample was melted on a water bath, and 20 ml. of 95% ethanol was added. Most of the cream base separated on freezing, and the mixture was filtered through a cotton pad. The filtrate was evaporated to dryness, and the residue was treated as for capsules. A

[1] Obtained from Parke, Davis and Co.