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ACKNOWLEDGMENTS AND ADDRESSES

Received from the *College of Pharmacy, University of Iowa, Iowa City, IA 52240*

RESEARCH ARTICLES

Effect of Ethanol on Intestinal Absorption of Theophylline

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Abstract □ The purpose of this investigation was to determine directly the effect of ethanol on the absorption of theophylline from the small intestine of the rat. In the first part of the investigation, a 50-mg % solution of theophylline containing 0, 5, 10, or 20% ethanol was placed in a ligated segment of small intestine of anesthetized rats and sampled at intervals. Theophylline absorption was significantly increased by 5% ethanol and decreased by 20% ethanol. There was a positive rank-order correlation between theophylline absorption rate from the four solutions and water net flux. In the second part of the investigation, a segment of small intestine of anesthetized rats was perfused with theophylline solutions containing 0 or 2% ethanol. The concentration of ethanol was kept constant by continuous infusion of ethanol into the circulating perfusion solution. Theophylline absorption and water net flux were significantly increased by 2% ethanol. Intravenous infusion of ethanol at a rate sufficient to produce the same plasma concentration of ethanol as was observed upon intestinal perfusion with a solution containing 2% ethanol had no effect on theophylline absorption from the small intestine. The intestinal clearance of theophylline was independent of concentration in the 10–200-mg % range. Ethanol solutions without theophylline produced the same changes in water net flux as did the theophylline–ethanol solutions. It is concluded that the effect of ethanol on the intestinal absorption of theophylline is probably due to solvent drag, secondary to a change in water flux induced by alcohol.

Keyphrases □ Ethanol—effect on intestinal absorption of theophylline, rat small intestine □ Theophylline—effect of ethanol on intestinal absorption, rat small intestine □ Absorption—effect of ethanol on theophylline intestinal absorption, rat small intestine

The relatively slow and erratic absorption of theophylline from solid oral dosage forms has led to the development of various liquid dosage forms of this drug, including hydroalcoholic solutions (1). It has been claimed that the ethanol in hydroalcoholic solutions of theophylline enhances the absorption of the drug, but there have been conflicting reports.

A solution of theophylline containing 20% ethanol produced a significantly larger increase in the vital capacity of asthmatic patients than did the same dose of theophylline in aqueous solution (2). On the other hand, in a study of theophylline concentrations in the blood of two subjects, after oral administration of theophylline in solution, a more rapid absorption was found from aqueous solution by one subject and a more rapid absorption from the hydroalcoholic solution by the other (3). Flora (4) observed no significant differences in plasma theophylline concentrations of human volunteers receiving theophylline in aqueous solutions containing either 1.43 or 8.44 ml ethanol [15 ml of a 9.5% (v/v) and 42.2 ml of 20% (v/v) solution, respectively]. Studies on rabbits (5) showed more rapid absorption of theophylline from a solution containing 20% ethanol than from an aqueous solution without ethanol.

The purposes of this investigation were to deter-

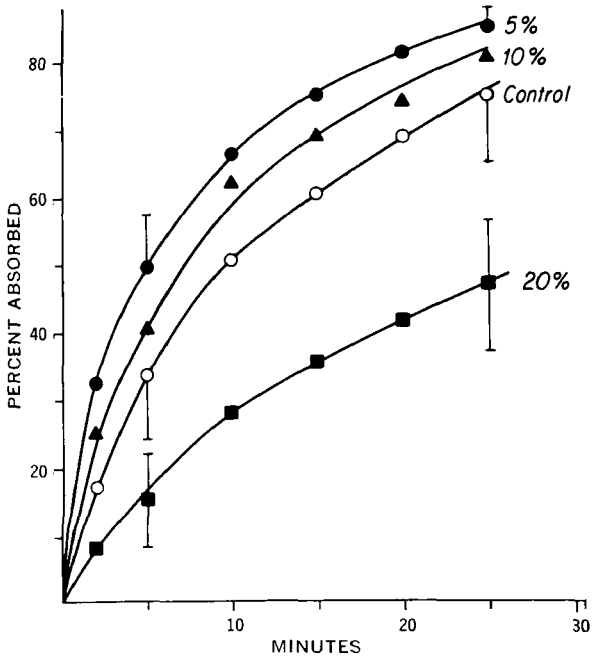

Figure 1—*Effect of 0, 5, 10, and 20% (v/v) ethanol on the absorption of theophylline from a 50-mg % solution instilled into a cannulated segment of small intestine of anesthetized rats (average of six animals per group). Vertical bars indicate 1* SD *in each direction.*

mine directly the effect of various concentrations of ethanol on the absorption of theophylline from the small intestine of the rat and to establish the mechanism by which ethanol might modify theophylline absorption.

EXPERIMENTAL

Male Sprague-Dawley rats, weighing 220–350 g and fasted for about 15 hr, were used. Each animal was anesthetized with urethan, 1.5 g/kg ip.

***In Vivo* Intestinal Loop**—Drug absorption was investigated by the *in situ* intestinal loop technique of Doluisio *et al.* (6) with certain modifications as described by Hayton and Levy (7). A solution of 50 mg theophylline in 100 ml Sørensen buffer, pH 6.4, made isotonic with sodium chloride and containing 0, 5, 10, or 20% (v/v) ethanol, was instilled into the lumen of the cannulated segment of small intestine[1], and 0.2-ml samples were removed periodically for assay. The volume of drug solution in the intestine was kept constant by addition of 0.9% sodium chloride solution before each sample removal. The difference between the total volume of saline solution added during the experiment and the volume of solution removed for assay served as a measure of net water absorption and will be referred to as water net flux. Positive flux refers to net absorption from the intestine; negative flux designates net flow into the intestinal lumen. The percent of drug remaining in the lumen of the intestine segment was determined as a function of time. All experiments were carried out in randomized order.

To confirm the effect of ethanol on the absorption of theophylline, the drug concentration in the plasma was determined in one group of rats receiving 50 mg % theophylline in buffer solution and in another group receiving a solution of 50 mg % theophylline and 5% (v/v) ethanol in Sørensen buffer. Eight milliliters of drug solution/275 g body weight of animal was instilled into the cannulated segment of small intestine, and blood was taken from the aorta 5 min later. Plasma theophylline concentrations were determined in duplicate.

[1] The length of the cannulated segment of intestine was 100.6 ± 7.0 cm (mean ± *SD*, $n = 47$).

Solutions of 0, 5, and 20% (v/v) ethanol in Sørensen buffer (but without theophylline) were used to determine the effect of ethanol alone on water net flux.

Perfusion Method with Constant Concentration of Ethanol in Perfusion Solution—The intestinal perfusion setup and other pertinent experimental details of the surgical and perfusion procedure were described previously (8). The control group of animals was perfused with a solution of 50 mg theophylline in 100 ml isotonic Sørensen buffer, pH 6.4, at a rate of 2 ml/min. The other group was perfused at the same rate with the theophylline solution also containing 2% (v/v) ethanol. An infusion pump delivered 23% (v/v) ethanol in normal saline solution into the reservoir of the perfusion setup at a rate of 0.0216 ml/min. The concentration of ethanol in this solution required to maintain the initial concentration of ethanol in the perfusion solution was calculated from its absorption rate constant (determined in preliminary experiments) and the amount of ethanol in the intestine at the beginning of the experiment, for the particular infusion rate used.

The perfusion and infusion pumps were started simultaneously; additional drug solution was then added to the reservoir, which was immersed in a 37° water bath, until the system (pump, rat intestine, reservoir, and tubing) was filled to capacity, approximately 9.0 ml. The drug solution was circulated through the intestine for 5–10 min before the zero-time sample was removed. From then on, 0.2-ml samples were obtained periodically from the reservoir for theophylline assay and 0.1-ml samples were obtained for ethanol assay. The volume of the perfusion solution was kept constant throughout the experiment by adding 0.9% sodium chloride solution intermittently to the reservoir from a buret to replace fluid loss due to sampling and water absorption from the intestine.

The apparent first-order absorption rate constant for theophylline was determined from the slope of the line, fitted by the method of least squares, of semilogarithmic plots of the percent of theophylline remaining in the intestine *versus* time. The rate con-

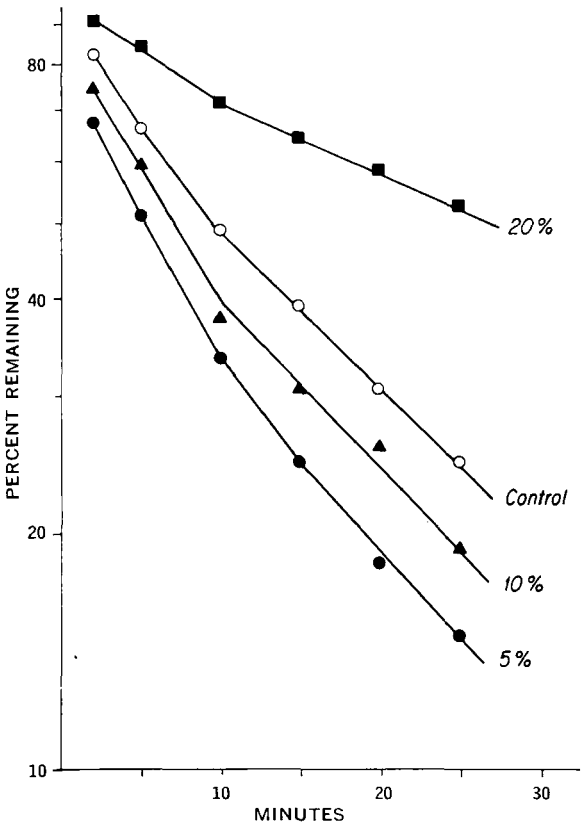

Figure 2—*Semilogarithmic plot of the time course of theophylline absorption from a 50-mg % solution also containing 0–20% (v/v) ethanol, which was instilled into a cannulated segment of small intestine of anesthetized rats (average of six animals per group).*

Table I—Statistical Analysis of Effect of Ethanol on Theophylline Absorption

Condition	Ethanol Concentration, %	Statistically[a] Significantly Different from 5 min	Statistically[a] Significantly Different from 20 min
A	0	B, D	B, D
B	5	A, C, D	A, C, D
C	10	B, D	B, D
D	20	A, B, C	A, B, C

[a] $p < 0.05$ by t test.

Table II—Effect of Ethanol on Theophylline Absorption from Rat Small Intestine as Reflected by Plasma Theophylline Concentrations[a]

50 mg % Theophylline		50 mg % Theophylline and 5% Ethanol	
Rat Number	Plasma Concentration, μg/ml	Rat Number	Plasma Concentration, μg/ml
113	16.42	114	24.25
116	16.35	115	27.14
117	17.80	120	19.06
119	17.60	121	25.08
122	14.49	123	24.72
125	17.56	124	29.60
Mean	16.70		24.98
SD	1.25		3.51
Statistical difference	$p < 0.001$		

[a] Five minutes after instillation of drug solution into the intestinal tract. The body weight of the control animals was 288 ± 31 g (mean ± *SD*) and that of the animals in the ethanol group was 304 ± 16 g.

stant was corrected for drug removed in the samples and was converted to clearance per centimeter by multiplying the rate constant by the volume of drug solution and dividing it by the length of perfused intestine.

To determine the effect of concentration on theophylline absorption and water net flux, solutions of 10 and 200 mg % theophylline in Sørensen buffer were circulated through the rat intestine and sampled as already described.

Perfusion Method with Concomitant Intravenous Infusion of Ethanol—In this series of experiments, a concentration of approximately 130 mg % ethanol was maintained in the plasma. Based on preliminary experiments, a loading dose of 1.05 g ethanol/kg body weight was given intravenously over 5 min as a 19% (v/v) solution. Then an ethanol infusion of 0.5–0.8 g/kg/hr into the tail vein was started at a rate of 0.0216 ml/min of a solution containing 19% (v/v) ethanol in normal saline. The control animals received normal saline solution at the same rate. After 30 min of intravenous infusion to attain steady state, perfusion of the intestine with 50 mg % theophylline in buffer solution at a rate of 2 ml/min was started. Samples of perfusate were obtained periodically from the reservoir, and the volume of solution was kept constant as already described. A blood sample for determination of ethanol concentration was obtained at the end of the experiment.

Assay—Theophylline concentrations in the perfusion solution and in plasma were determined by the UV spectrophotometric procedure of Schack and Waxler (9). Recovery of theophylline averaged 91% from plasma and 97% from perfusion solution. The blank apparent theophylline concentration was 2 μg/ml in plasma and 1.1 mg % in the perfusion solution.

Ethanol concentrations were determined by chromatography, using a modification of the method of Cooper (10). A gas chromatograph[2] with a flame-ionization detector and a column packed with Parapak Q[3], 80–100 mesh, was employed. The column temperature was 160°, and the injection port and detector temperatures were approximately 210°. *n*-Propyl alcohol was added to the samples as an internal standard. Helium was used as the carrier gas at a flow rate of 30 ml/min; the flow rates of hydrogen and air were 30 and 350 ml/min, respectively. A plot of the area of the ethanol peak divided by the area of the internal standard peak as a function of ethanol concentration was linear and passed through the origin. Protein in the plasma samples was precipitated by the method of Cooper (10) after addition of the internal standard. The average recovery of ethanol from plasma was 100%.

[2] Perkin-Elmer model 811.

[3] Waters Associates, Inc., Framingham, Mass. The metal column was 1.83 m (6 ft) long and 0.31 cm (0.125 in.) in diameter.

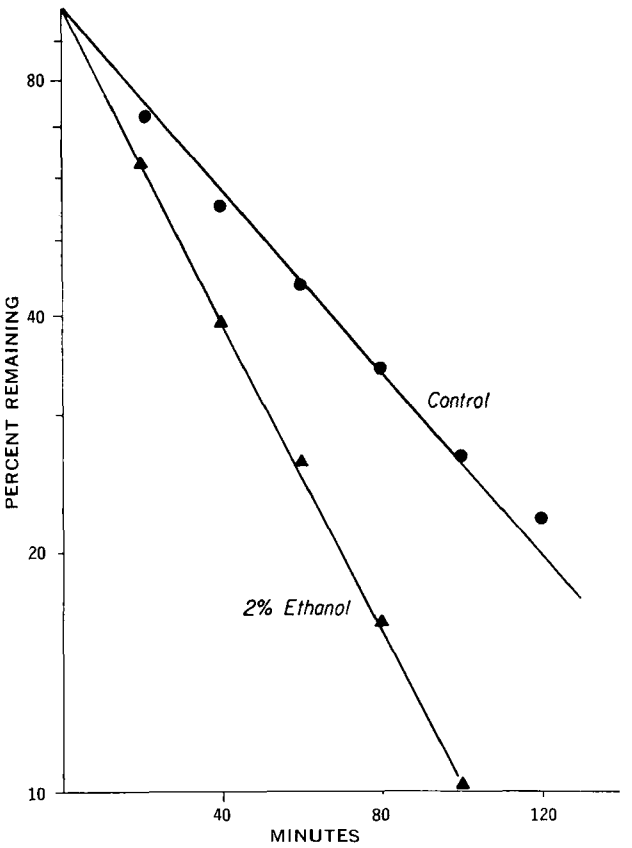

Figure 3—*Semilogarithmic plot of the time course of theophylline absorption from a perfused segment of small intestine of anesthetized rats (data from one animal each). The perfusion solution contained 50 mg % theophylline and 0 or 2% (v/v) ethanol. The concentration of ethanol was maintained constant by continuous infusion of ethanol into the circulating perfusion solution.*

RESULTS

The effect of ethanol on the absorption of theophylline from a solution instilled into a cannulated segment of small intestine of anesthetized rats is shown in Figs. 1 and 2. Solutions containing an initial concentration of 5% ethanol enhanced theophylline absorption significantly, while solutions that initially contained 20% ethanol had a significant retarding effect on theophylline absorption (Table I). The absorption-enhancing effect of 5% ethanol was verified by determining the concentration of theophylline in the plasma of additional groups of rats 5 min after instillation of theophylline solution, with and without ethanol, into the segment of cannulated intestine. The results obtained (Table II) are in excellent quantitative agreement with the results obtained from measurements of theophylline disappearance from the intestinal solution (Fig. 1). There were significant differences in intestinal water net flux observed with the different solutions. Theophylline solutions with 5% ethanol significantly increased water absorption from the intestinal lumen, while solutions containing 20% ethanol actually caused net flow of water into the intestinal lumen (Table III). There is a perfect positive rank-order correlation between the water net flux associated with the instillation of

Table III—Effect of Ethanol on Water Net Flux in Rat Small Intestine

Composition of Solution	Water Flux[a], ml/cm/25 min	Statistical Difference	Body Weight, Mean ± *SD*, g
50 mg % theophylline (control)	+ 0.012 (0.008)[b]	—	304 ± 36
50 mg % theophylline and 5% ethanol	+ 0.050 (0.010)	$p < 0.001$	276 ± 17
50 mg % theophylline and 10% ethanol	+ 0.015 (0.013)	N.S.	291 ± 26
50 mg % theophylline and 20% ethanol	−0.016 (0.012)	$p < 0.005$	279 ± 42

[a] Mean of six rats. Positive sign: water net flux from intestinal lumen; negative sign: flux into intestinal lumen. [b] Standard deviation in parentheses.

Table IV—Effect of Ethanol in Perfusion Solution on Theophylline Absorption from *In Situ* Perfused Rat Small Intestine

	Control		2% Ethanol
Theophylline clearance, ml cm^{-1} min^{-1} × 10^3	5.27 (0.62)[a]	$p < 0.001$	8.33 (1.09)
Water net flux, ml cm^{-1} hr^{-1}	0.048 (0.017)	$p < 0.001$	0.116 (0.013)
Ethanol concentration in perfusion solution, % v/v	—		1. 93 (0.35)
Ethanol concentration in plasma, mg %	—		131 (19)
Body weight, g	297 (41)	N.S.	296 (29)

[a] Mean of six animals each. Standard deviation in parentheses.

Table V—Effect of Intravenous Infusion of Ethanol on Theophylline Absorption from *In Situ* Perfused Rat Small Intestine

	Control		Ethanol
Theophylline clearance, ml cm^{-1} min^{-1} × 10^3	5.92 (0.90)[a]	$p > 0.1$	6.92 (1.32)
Water net flux, ml cm^{-1} hr^{-1}	0.085 (0.012)	$p > 0.6$	0.082 (0.016)
Ethanol concentration in plasma, mg %			125 (27)[b]
Body weight, g	282 (25)	N.S.	281 (21)

[a] Mean of six animals each. Standard deviation in parentheses. [b] Not significantly different ($p > 0.5$) from ethanol-perfused animals.

the four different theophylline solutions into the rat intestine (Table III) and the rate of theophylline absorption from the intestine (Figs. 1 and 2).

Another set of experiments was carried out by perfusing a segment of small intestine with a recirculating solution of theophylline or theophylline and 2% ethanol, with the ethanol concentration being kept constant during the 120-min experiments by infusing additional ethanol at a constant rate, equal to the rate of ethanol absorption, into the system. Unlike the results obtained in the previous experiments (Fig. 2), these experiments yielded data that could be described by first-order kinetics (Fig. 3). The presence of 2% ethanol caused a significant increase in the absorption of theophylline and also in water net flux (Table IV). Similar experiments with 5% ethanol could not be carried out for 120 min since the animals expired after 80–105 min, apparently due to ethanol intoxication; the average intestinal clearance of theophylline in three such experiments was 8.10 (range 7.60–9.00) ml cm^{-1} min^{-1} × 10^3.

Six rats were each perfused intestinally with theophylline solution and intravenously with either saline solution or ethanol in saline at a rate sufficient to produce the same plasma concentration of ethanol as was observed upon intestinal perfusion with solutions containing 2% ethanol. Intravenous ethanol had no statistically significant effect on theophylline absorption and water net flux (Table V).

Perfusion of rat small intestine segments with solutions containing 10 and 200 mg % of theophylline showed no difference in absorption kinetics and yielded almost identical clearance values to those obtained previously with solutions containing 50 mg % theophylline (Table VI). There was also no difference in water net flux in experiments with 10- and 200-mg % theophylline solutions.

The effect of ethanol alone on intestinal water net flux was determined by instilling solutions of 0, 5, and 20% (v/v) ethanol in Sørensen buffer into cannulated small intestine segments of anesthetized rats. As observed previously with theophylline solutions containing the same concentrations of ethanol (Table III), water net flux was significantly increased by 5% ethanol and was actually reversed (*i.e.*, there was net flow of water into the intestinal lumen) when the initial ethanol concentration was 20% (Table VII).

DISCUSSION

The results of this investigation show directly that low concentrations (<10%) of ethanol can increase the rate of absorption of theophylline from the small intestine. The usual therapeutic dose of theophylline hydroalcoholic solution contains about 15 ml ethanol (2). Israel *et al.* (11) found that a 40-g oral dose of ethanol yielded concentrations of up to 3% in the small intestine of normal human subjects and that ethanol concentrations above 1% were maintained there for 30–60 min. The concentrations of ethanol that enhanced theophylline absorption in this investigation are, therefore, similar to those that may be expected in the small intestine fluids of patients who take therapeutic doses of theophylline in hydroalcoholic solution.

As in a previous study on the effect of amides on steroid absorption (7), a decreasing effect of ethanol on theophylline absorption with time was encountered during experiments involving instillation of drug solutions into cannulated *in situ* small intestine segments (Fig. 2). This effect was due to the decrease of ethanol concentrations with time as a result of ethanol absorption. When the ethanol concentration was maintained constant and

Table VI—Effect of Concentration on Theophylline Absorption from *In Situ* Perfused Rat Small Intestine

Theophylline Concentration, mg %	Clearance, ml cm^{-1} $min^{-1} \times 10^3$	Water Net Flux, ml cm^{-1} hr^{-1}	Number of Animals	Body Weight, g
10	5.35 (1.02)[a]	0.069 (0.010)	7	270 (32)
50	5.27 (0.62)	0.048 (0.017)	6	297 (41)
200	5.33 (0.47)	0.062 (0.024)	3	279 (10)

[a] Standard deviation in parentheses.

Table VII—Effect of Ethanol in Buffer Solution on Water Net Flux in Rat Small Intestine

Ethanol Concentration, % v/v	Water Flux[a], ml/cm/25 min	Statistical Difference from Control	Body Weight, g
0	+0.021 (0.002)[b]	—	278 (18)
5	+0.054 (0.006)	$p < 0.001$	266 (35)
20	−0.027 (0.004)	$p < 0.001$	277 (23)

[a] Mean of four rats. Positive sign: water net flux from intestinal lumen; negative sign: flux into intestinal lumen. [b] Standard deviation in parentheses.

the system was preequilibrated in perfusion-intestinal infusion experiments, the kinetics of theophylline absorption became apparent first order (Fig. 3). The theophylline absorption-enhancing effect of ethanol could be demonstrated with both techniques. It was not possible to use ethanol concentrations above 5% in the perfusion-intestinal infusion experiments due to toxic effects resulting from absorption of large amounts of the alcohol. In the intestinal loop experiments, a high concentration (20%) of ethanol actually retarded theophylline absorption and also reversed water net flux so that water was actually flowing into the intestinal lumen. This was presumably due to the osmotic pressure of the 20% ethanol solution. The fact that intravenous infusion of ethanol had no significant effect on theophylline absorption shows that the absorption-enhancing action of ethanol is not due to a systemic mechanism.

There is a perfect positive rank-order correlation between the absorption rate of theophylline (Fig. 1) and water net flux (Table III). This suggests, but does not prove, a cause-effect relationship. A close association between solute absorption and water flux has been noted in a number of reports, summarized recently by Ochsenfahrt and Winne (12); these investigators also pointed out that changes in water net flux can affect solute concentration at the surface of the intestinal mucosa and modify blood flow rate in the capillaries near the intestinal epithelium in addition to causing solvent drag effects in a more restricted sense. Negative water net flux produced by hypertonic solutions in the intestinal lumen decreased the absorption of urea, antipyrine, and aminopyrine while hypotonic solutions had the opposite effect (12, 13). Kinetic analysis revealed that this was not due to changes in blood flow rate through capillaries near the epithelium but only to water–solute interactions within the epithelial membranes (12). Other investigators (14) demonstrated similar effects of changes in intestinal water flux, due to changes in osmotic pressure, on the absorption of salicylic acid. Kojima *et al.* (15) noted that the effect of hypotonicity on sulfaethidole absorption could be accounted for by correcting the absorption rate constant for changes in solution volume and intestinal surface area but that these changes did not account for the effect of hypertonic solutions.

It is necessary to consider certain possible alternative or additional mechanisms for the absorption-enhancing effect of ethanol. Thakkar *et al.* (16) and Kirschbaum (17) found that theophylline can undergo self-association in aqueous solution, forming dimers, trimers, and tetramers. If diffusion of theophylline to the epithelial surface were absorption rate limiting or influencing under the conditions of the present experiments, and if ethanol were to inhibit self-association of theophylline, then an absorption-enhancing effect of ethanol could be rationalized on physical-chemical grounds.

Another possibility involves a pharmacological interaction of theophylline and ethanol. Pierce *et al.* (18) found that superior mesenteric artery infusion of theophylline induced net secretion of water and electrolytes from the jejunum of dogs. They speculated that this could be a systemic effect, perhaps due to elevation of cyclic AMP levels resulting from inhibition of phosphodiesterase, or a direct effect on the intestinal mucosa. The latter possibility is supported by observations that 10 m*M* theophylline applied directly to viable isolated stripped rabbit ileal mucosa produces inhibition of sodium absorption (19). Ethanol also inhibits phosphodiesterase, but in addition it inhibits adenyl cyclase, the enzyme responsible for the formation of cyclic AMP (20). An antagonistic effect of ethanol on theophylline with respect to intestinal water flux (which, in turn, affects theophylline absorption) is, therefore, a possibility.

The assumptions underlying both of these possible mechanisms, physical-chemical and pharmacological, have been explored by determining the concentration dependence of theophylline absorption over a range of 10–200 mg % (Table VI). Kirschbaum's (17) studies showed that monomers of theophylline predominate at the lower concentration while dimers predominate at a concentration of 200 mg %. It may also be expected that pharmacological effects, such as those affecting water flux, should vary in intensity over such a wide concentration range. The lack of any effect of theophylline concentration on the absorption of this drug and on water net flux (Table VI) suggests that the two mechanisms are not operative and that a direct effect of ethanol, rather than a physical-chemical or pharmacological interaction of ethanol with theophylline, may be involved.

Evidence for a direct effect of ethanol was obtained by determining the relationship between ethanol concentration in the intestinal lumen and water net flux (Table VII). As with the solutions containing both ethanol and theophylline, 5% ethanol increased and 20% reversed intestinal water net flux. It appears, therefore, that low concentrations of ethanol enhance theophylline absorption by increasing water net flux which, in turn, increases theophylline absorption by a solvent drag effect. The mechanism by which ethanol increases water flux is not determinable from this investigation. It is known that ethanol inhibits several specialized intestinal absorption processes (11, 21), possibly by lowering the ATP content of the small intestine. Ethanol in concentrations above 0.5–1 *M* has also been shown to increase gastric mucosal permeability in dogs (22). Interestingly, ethanol in concentrations of 1–10% enhanced the absorption of barbiturates from the rat stomach, but 0.5–2% ethanol had no effect on the absorption of barbiturates and some other drugs from the perfused small intestine of the rat (23). Magnussen (23) also stated that the volume correction factor (a reflection of water net flux) was not affected by ethanol concentration, but no data were given. His study differed from the present one in some technical details, including the ionic composition of the perfusion solution, and additional studies have been initiated in this laboratory to resolve this question. The specificity of the ethanol effect as well as the effect of other agents capable of affecting intestinal water flux is also being investigated.

Finally, in considering the relationship of the results of this investigation to the clinical situation, it must be recognized that this investigation was designed to determine directly the effect of ethanol on theophylline absorption from the intestine. Ethanol also inhibits gastric emptying; 120 ml (4 oz) of whiskey 15 min before a test meal increased the half-life of gastric emptying from a mean of 105 to 204 min in eight healthy men (24). It is possible, therefore, that, under clinical conditions, the enhancing effect of ethanol on the intestinal absorption of theophylline may be modified by the effect of ethanol on gastric emptying.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 23, 1973, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication January 17, 1974.

Supported in part by Grant GM 20852-01 from the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD 20014

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GLC Determination of Meprobamate in Water, Plasma, and Urine

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Abstract □ A GLC method for the determination of meprobamate in water, plasma, and urine is described. The procedure is based on alkaline hydrolysis of meprobamate followed by silylation of the hydrolysis product to give a trimethylsilyl derivative. Quantitation of the drug was effected using a homolog of the hydrolysis product as an internal standard, which is also silylated during the procedure. The method is specific, sensitive, and reproducible and has been used for the routine analysis of biological samples obtained from meprobamate-treated dogs and humans.

Keyphrases □ Meprobamate—GLC analysis in water, plasma, and urine □ GLC—analysis, meprobamate in water, plasma, and urine

The quantitative determination of meprobamate (2-methyl-2-propyl-1,3-propanediol dicarbamate) is of interest from a toxicological standpoint because of its continued widespread use as a tranquilizing agent and as a muscle relaxant. The availability of meprobamate in a large number of generic forms as well as recent awareness of questionable bioavailability (1) has increased the amount of effort devoted to determining the quantity of product in solution following dissolution studies (2) as well as the quantity found in plasma and urine. Several spectrophotometric methods (3–9) and GLC procedures (10–21) were reported for the qualitative and quantitative analysis of this drug. Many reported colorimetric methods are adequate for the determination of the drug in dissolution studies, but they lack either specificity or sensitivity for the measurement of meprobamate levels following therapeutic drug doses. Most GLC methods presently available were developed for toxicological purposes and involve direct GLC, with the inherent problem of thermal decomposition at the injection port (20). The need for an analytical procedure useful in pharmacokinetic studies of meprobamate in humans and animals treated with therapeutic or subtherapeutic doses led to the development of the method presented in this report.

EXPERIMENTAL

Reagents—The following reagents were used: stock solutions of meprobamate[1] containing 5, 10, 15, 20, and 25 μg/100 μl in water; stock solution of internal standard (2-methyl-2-ethyl-1,3-propanediol[2], I) containing 75 μg/ml in water; sodium acetate buffer (pH 5.0) (22); 0.25 *M* K_2HPO_4 (pH 7.2); β-glucuronidase[3]; *N,O*-bis-(trimethylsilyl)acetamide[4]; and reagent grade anhydrous ether.

Instrumentation—A gas chromatograph[5] equipped with a hydrogen flame-ionization detector, a recorder[6], and a glass column [1.5 m × 0.63 cm (5 ft × 0.25 in.)] packed with a 3% SE-30 on 80–100-mesh Chromosorb W[7] was employed. A gas chromatograph peak identifier[8] coupled to a recorder[9] was employed for peak identification by mass spectroscopy.

The GLC operating conditions were: column temperature, 115°; injector temperature, 175°; and detector temperature, 175°. The gas flow rates were: carrier gas (helium), 50 ml/min; hydrogen, 30 ml/min; and air, 300 ml/min.

Procedure—*Water Standards*—To 1 ml of water contained in a 15-ml Pyrex test tube, fitted with a Teflon-lined screw cap, were added 100 μl of aqueous meprobamate stock solution, 100 μl of stock solution of I, and 1 ml of 50% KOH. The hydrolysis of meprobamate was carried out at 100° for 10 min. The tubes were cooled under running water, and 3 ml of ether was added. The

[1] Wyeth Laboratories, Philadelphia, Pa.
[2] K & K Laboratories, Hollywood, Calif.
[3] Calbiochem, Los Angeles, Calif.
[4] Tri-sil/BSA, Pierce Chemical Co., Rockford, Ill.
[5] Varian model 1400, Varian Aerograph, Walnut Creek, Calif.
[6] Varian A-25, Varian Aerograph, Walnut Creek, Calif.
[7] Varian Aerograph, Walnut Creek, Calif.
[8] Finnigan Corp., Sunnyvale, Calif.
[9] Visicorder, Honeywell, Denver, Colo.