Effect of Vehicle Viscosity and an Anticholinergic Agent on Bioavailability of a Poorly Absorbed Drug (Phenolsulfonphthalein) in Man

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Abstract □ The purpose of this study was to determine if an increase in the viscosity of the solution vehicle, or premedication with an anticholinergic agent, can increase the bioavailability of a poorly absorbed drug in man. The absorption of phenolsulfonphthalein by healthy adults was decreased significantly during the 1st hr. when the drug was administered in very viscous sodium alginate solution or when the subjects had been premedicated with propantheline. Sodium alginate had no effect on the total amount of phenolsulfonphthalein absorbed; pretreatment with the anticholinergic agent increased the total amount of phenolsulfonphthalein recovered in the urine from an average of 16 to 24% of the dose. This effect is attributed to a decrease of the GI transit rate.

Keyphrases □ Drug absorption—effect of vehicle viscosity and anticholinergic agent on bioavailability of poorly absorbed drugs (phenolsulfonphthalein), man □ Phenolsulfonphthalein absorption—effect of vehicle viscosity and anticholinergic agent, man □ Anticholinergic agents—effect on bioavailability of poorly absorbed drugs (phenolsulfonphthalein) in man □ Viscosity of solution vehicle—effect on bioavailability of poorly absorbed drugs (phenolsulfonphthalein) in man □ Bioavailability of poorly absorbed drugs (phenolsulfonphthalein)—effect of vehicle viscosity and anticholinergic agent, man

Some orally administered drugs, particularly those that are almost completely ionized in the pH range of GI fluids and have a relatively high molecular weight, are not completely absorbed. It is of interest to determine if the bioavailability of such drugs may be increased by pharmaceutical or pharmacological means. Previous studies with riboflavin, a vitamin absorbed by a specialized transport process, showed that administration in a very viscous sodium alginate solution vehicle or premedication with the anticholinergic drug propantheline increases the total amount of riboflavin absorbed upon oral administration of a large and incompletely absorbed dose (1, 2). This increased absorption was presumably due to prolonged retention of the vitamin at intestinal absorption sites as a result of decreased intestinal transit rate. Extrapolation of these results to drugs may be limited by the rather unique absorption characteristics of riboflavin (3, 4). The previous studies were extended by determining the effect of vehicle viscosity and premedication with propantheline on the bioavailability of a poorly absorbed drug, phenolsulfonphthalein (phenol red).

EXPERIMENTAL

Eight healthy male volunteers received 20 mg. phenolsulfonphthalein USP[1], dissolved in 50 ml. of aqueous vehicle, in the morning on an empty stomach. For the control experiments, the drug was dissolved in about 2 ml. water with the aid of 20 mg. sodium bicarbonate, and this was added to 48 ml. of a vehicle containing 1% citric acid, 0.1% sodium saccharin, 0.01% terpeneless lemon oil, and distilled water.

To determine the effect of viscosity on phenolsulfonphthalein absorption, 2% sodium alginate[2] was incorporated in the solution. The rheologic characteristics of the alginate solution were similar to those described previously (1).

To determine the effect of an anticholinergic agent, the subjects received 30 mg. propantheline bromide[3] the night before and again 1 hr. before the experiment. Subject T received only one-half this dose because of his low body weight. Phenolsulfonphthalein was administered in the same vehicle as was used in the control experiments.

The three types of experiments were carried out in random order on each subject at least 1 week apart. No food was permitted until 4 hr. after phenolsulfonphthalein administration, at which time the subjects ate lunch. Urine was collected hourly for the first 12 hr. and then at longer intervals for a total of 24 hr. The subjects drank sufficient water to maintain an average urine flow of 50–100 ml./hr. Phenolsulfonphthalein in the urine was determined colorimetrically as described by McLeod *et al.* (5), except that the pH and capacity

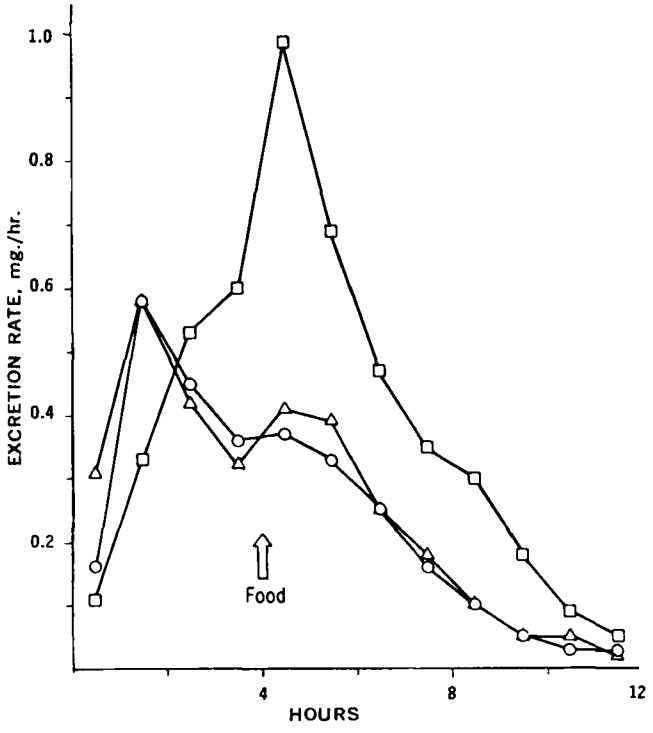

Figure 1—*Mean urinary excretion rates of phenolsulfonphthalein after oral administration of 20 mg. in solution to eight normal adult subjects. Key: △, control; ○, in sodium alginate solution; and □, after pretreatment with propantheline. Some of the individual data were obtained by interpolation.*

[1] Lot No. 851p, Allied Chemicals Corp.

[2] Sodium alginate XRD-1000, Marine Colloids, Inc., New York, N.Y.

[3] Pro-Banthine tablets, 15 mg., Searle.

Table I—Effect of Sodium Alginate and Propantheline on Phenolsulfonphthalein Absorption in Man

Subject	Age, years	Weight, kg.	Amount of Phenolsulfonphthalein Excreted, mg. 1 hr. Control	1 hr. Sodium Alginate	1 hr. Propantheline	24 hr. Control	24 hr. Sodium Alginate	24 hr. Propantheline
A	36	84	0.21	0.20	0.21	2.9	3.9	3.9
G	23	66	0.26	0.09	0.05	3.7	3.2	5.7
K	27	68	0.27	0.20	0.14	3.3	2.7	5.9
L	37	64	0.42	0.33	0.11	4.1	4.1	4.8
O	28	75	0.42	0.18	0.03	4.3	2.5	3.3
S	27	83	0.24	0.08	0.09	3.3	3.2	5.5
T	29	55	0.20	0.01	—[a]	1.7	1.6	4.1
Y	26	68	0.42	0.19	—[a]	2.7	2.9	5.4
Mean			0.30	0.16	0.10	3.2	3.0	4.8
Relative *SD*			32	61	62	26	26	20
Statistical significance[b] *versus* control				$p < 0.005$	$p < 0.01$		N.S.	$p < 0.01$

[a] These subjects were unable to void until after the 1st hr. [b] Paired *t* test.

of the alkaline buffer were increased to avoid underestimation of phenolsulfonphthalein concentrations in particularly acidic urine samples. The modified buffer solution consisted of 38.0 g. $Na_3PO_4 \cdot 12H_2O$, 26.8 g. $Na_2HPO_4 \cdot 7H_2O$, and enough distilled water to make 1 l. (pH approximately 11.5). The samples were centrifuged at $1000 \times g$ for 10 min., and part of the supernatant phase was removed for determination of the absorbance at 560 nm., with water in the reference cell. Blank determinations were made in the same manner, except that the alkaline buffer was replaced by 0.1 *N* acetic acid. The blank values averaged 0.38 mg. apparent phenolsulfonphthalein/24 hr. (0.09 mg. *SD*).

Binding of phenolsulfonphthalein to sodium alginate was determined by equilibrium dialysis at 37° using 5 ml. of the sodium alginate solution (without phenolsulfonphthalein) employed in the absorption study and 5 ml. 0.9% NaCl in 0.01 *N* HCl in the cellophane bag and using 30 ml. of a solution of 0.0015% phenolsulfonphthalein and 0.9% NaCl in 0.01 *N* HCl as the outer phase. The external phase was assayed at 24 hr. and both phases were assayed at 48 hr. The concentrations of phenolsulfonphthalein in the external phase were identical at 24 and 48 hr., indicating that equilibrium had been reached in less than 24 hr.

RESULTS

Administration of phenolsulfonphthalein in sodium alginate solution or premedication with propantheline decreased significantly the amount of phenolsulfonphthalein excreted 1 hr. after administration of the dye (Table I). Equilibrium dialysis showed that there was no measurable binding of phenolsulfonphthalein by sodium alginate under the experimental conditions. Phenolsulfonphthalein excretion continued for less than 24 hr.; the total amount excreted in 24 hr. was not significantly different from the control when phenolsulfonphthalein was administered in sodium alginate solution. On the other hand, premedication with propantheline increased the total recovery of phenolsulfonphthalein from 16 to 24% of the dose, a statistically significant difference (Table I). The time course of phenolsulfonphthalein excretion in the control, sodium alginate, and propantheline experiments is shown in Fig. 1. The averaged data in the figure are a good reflection of the individual results and are not distorted by the averaging process. A secondary excretion maximum was noted in all three experiments. It occurred immediately after lunch, *i.e.*, 4 hr. after phenolsulfonphthalein administration.

DISCUSSION

Phenolsulfonphthalein, a weak acid with a molecular weight of 354, is almost completely ionized at pH above 1. About 85% of a 6-mg. dose is recovered in the urine within 6 hr. after intravenous administration to normal subjects (5). About 3% of the dose is eliminated in the bile (5). The dye is excreted partly by renal tubular secretion, with a transport maximum of 35.8 mg./min./1.73 $m.^2$ (6). Intestinal perfusion studies in rats have shown that phenolsulfonphthalein is equally well absorbed in the proximal and distal regions of the small intestine (7). In the 0.5–20-mg./l. concentration range, phenolsulfonphthalein absorption from the intestinal tract of the rat occurs mainly by passive diffusion (8). At lower concentrations, there is an appreciable contribution by a specialized transport process which can be inhibited by *p*-aminohippuric acid (9). Oral administration of 6, 30, 60, and 120 mg. phenolsulfonphthalein to normal human subjects resulted in the recovery of 13.2, 8.0, 6.5, and 5.3% of the dose in the urine, respectively (5). A double reciprocal plot of maximum excretion rates or amounts recovered in the urine in 24 hr. *versus* the oral dose shows a good linear relationship suggestive of a specialized transport process, but similar results could be obtained by other mechanisms (such as partial precipitation of phenolsulfonphthalein due to interaction with a component of the GI fluids or nonlinear binding to mucosal proteins as the first step in absorption). Two colectomized patients absorbed phenolsulfonphthalein as well as normal subjects, suggesting that absorption of orally administered phenolsulfonphthalein occurs mainly in the small intestine (5).

The decreased excretion of phenolsulfonphthalein in the 1st hr. after administration in sodium alginate solution and after premedication with propantheline may be attributed to slower gastric emptying. The sodium alginate solution was slightly thixotropic and had a viscosity of more than 6000 cps. at a shear rate of 5.14 $sec.^{-1}$ and a temperature of 37°. It formed a stiff gel when acidified with 0.01 *N* hydrochloric acid. This should result in appreciable slowing of phenolsulfonphthalein transfer from the stomach to the intestines. Binding or complexation of phenolsulfonphthalein with sodium alginate could also reduce the rate of absorption of the dye, but this unlikely possibility (both are weak acids) was ruled out by equilibrium dialysis studies. Propantheline is a potent anticholinergic agent and inhibits gastric emptying by pharmacological means. Intravenous injection of propantheline has been reported (in abstract) to retard significantly gastric emptying and to decrease the rate, but not the extent, of acetaminophen absorption from *tablets* by human subjects (10).

Administration of phenolsulfonphthalein in sodium alginate solution did not increase the total amount of phenolsulfonphthalein absorbed relative to the aqueous control (Table I). On the other hand, pretreatment with propantheline increased phenolsulfonphthalein recovery by 50%, presumably due to longer retention of the dye at intestinal absorption sites because of slower transit of intestinal contents. The secondary excretion rate peak after lunch is most probably not due to enterohepatic cycling since very little phenolsulfonphthalein is excreted in the bile. It may reflect transfer of residual phenolsulfonphthalein from the stomach to the intestines or enhanced absorption due to discharge of bile into the intestinal lumen.

The effect of propantheline on phenolsulfonphthalein absorption is very similar to that on riboflavin absorption (2). Pretreatment with the anticholinergic agent delayed the absorption of both substances but increased appreciably the total amount absorbed. The variability of the 1-hr. excretion data was increased and that of the total amounts excreted was decreased with both substances by propantheline.

The results of this study show that the initial rate of absorption of a poorly absorbed substance such as phenolsulfonphthalein *from solution* can be decreased by pharmaceutical and pharmacological

means and that an anticholinergic drug can increase appreciably the bioavailability of such a substance.

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Effect of Various Steroids and ACTH on Plasma Levels of Zoxazolamine and Dicumarol

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Abstract □ Comparative experiments were performed on rats given pregnenolone-16α-carbonitrile, estradiol, progesterone, triamcinolone, hydroxydione, or ACTH (corticotropin) to study correlations between the *in vivo* conditioning effects upon resistance to zoxazolamine and dicumarol and the plasma concentrations of these drugs. The conditioners were classified as catatoxic when a decrease in toxicity was associated with increased blood clearance of the drug, and they were classified as syntoxic when *in vivo* protection was accomplished without a simultaneous fall in the plasma drug level. With these criteria, pregnenolone-16α-carbonitrile was markedly protective and catatoxic whereas triamcinolone and ACTH were moderately protective and syntoxic against zoxazolamine. The remaining steroids elicited very mild changes in zoxazolamine paralysis and blood clearance. Of all the conditioners tested, only estradiol protected against dicumarol intoxication and accelerated plasma clearance of this drug. Hence, only estradiol exhibited a manifest catatoxic effect against this anticoagulant.

Keyphrases □ Steroids (pregnenolone-16α-carbonitrile, estradiol, progesterone, triamcinolone, and hydroxydione)—effect on plasma levels of zoxazolamine and dicumarol □ ACTH—effect on plasma levels of zoxazolamine and dicumarol □ Zoxazolamine, plasma levels—effect of various steroids and ACTH, resistance–plasma concentration correlations □ Dicumarol, plasma levels—effect of various steroids and ACTH, resistance–plasma concentration correlations

It is well known that certain pharmacological agents, including steroidal hormones, can reduce the biological half-life of others by the induction of drug-metabolizing enzymes (1). During the past few decades, numerous observations have confirmed that, in addition to their classic hormonal functions as regulators of reproduction and general metabolism, steroids also play a decisive role in determining the resistance of the body against the most varied types of injury (2). These adaptive steroids can be classified according to their mechanism of action into two main groups: (*a*) "syntoxic" steroids, which improve host-tissue tolerance by permitting coexistence with the substrate (*e.g.*, by suppressing nonspecific inflammatory or allergic reactions against it); and (*b*) "catatoxic" steroids, which enhance the detoxication of endogenous and exogenous toxicants *via* induction, activation, decreased degradation of drug-metabolizing enzymes, and/or accelerated substrate elimination from the body. To obtain the best catatoxic effect, the steroids are usually administered 2–3 days before the toxicant. However, recent findings (3) indicate that even posttreatment facilitates protection against certain intoxications.

Systematic studies (2, 4) in these laboratories revealed that, among more than 1200 steroids tested, pregnenolone-16α-carbonitrile and its close derivatives exert the greatest prophylactic effect *in vivo*. Biochemical investigations (5) on rats showed that pregnenolone-16α-carbonitrile stimulates catatoxic mechanisms. On the other hand, steroids such as estradiol protect *in vivo* only against a limited number of drugs, and it is not known whether their actions are syntoxic or catatoxic (6, 7).

In view of these observations, an attempt was made to investigate the correlation between the *in vivo* effects of steroids and the plasma concentrations of zoxazolamine and dicumarol after treatment with pregnenolone-16α-carbonitrile, estradiol, triamcinolone, progesterone, hydroxydione, or ACTH. The steroids were selected on the basis that the syntoxic effects are virtually limited to glucocorticoids, whereas the catatoxic properties appear to be independent of any other known steroid