Drug Transport IV: Influence of Hypotonic and Hypertonic Solutions on Passive Drug Transfer across the Everted Rat Intestine

Keyphrases ☐ Drug transfer, passive—everted rat intestine ☐ Hypotonic, hypertonic solutions effect—passive drug transfer ☐ Everted rat intestine—passive drug transfer

Sir:

Research efforts in our laboratory have been concerned with factors affecting the intestinal absorption of drugs. We showed previously that certain cations may significantly alter drug transport across the isolated everted rat intestine (1, 2). For example, replacing Na^+ with K^+ in a Krebs bicarbonate buffer markedly inhibited the mucosal-to-serosal transfer of a number of ionized (cationic and anionic) and nonionized compounds (1–3). Several recent literature reports (4–6) support our initial observations. In addition, glucose and xylose have also been found to decrease the apparently passive transfer of certain drugs across the everted intestine, whereas mannitol was without effect (7). It appears that the inhibition of drug transfer is related to the degree of intestinal tissue fluid uptake induced by these various inhibitors (2, 7). To examine further the nature of this relationship, experiments were conducted to evaluate the influence of hypotonic and hypertonic solutions on the intestinal transfer of certain compounds.

Sprague-Dawley rats, weighing approximately 250 g., were fasted 20–24 hr. prior to the experiment. Water was allowed *ad libitum*. The experimental method for preparing the everted rat intestine preparation was described previously (2). After severing the intestine at the pyloric junction, the first 15 cm. of intestine was discarded and the following 20 cm. was divided into two 10-cm. segments. The most proximal segment was designated Segment 1, and the distal portion was designated Segment 2.

A modified physiologic Krebs bicarbonate buffer,[1] pH 7.4, was prepared to contain a total cation concentration of 154 m*M*. Buffer solutions were also prepared in which the NaCl concentration was reduced to 18 m*M* (hypotonic solution, about 100 mOsm) or increased to 218 m*M* (hypertonic solution, about 500 mOsm). Two milliliters of buffer was placed inside the sac (serosal solution), and the entire preparation was placed into approximately 100 ml. of buffer solution containing the drug (mucosal solution) which was continually gassed with O_2–CO_2 (95:5, v/v). In any given experiment, the mucosal and serosal solutions were identical in composition except for the presence of drug in the mucosal solution. The serosal compartment was quantitatively sampled every 10 min. during the entire 2-hr. experiment, and the solution was assayed for drug content as previously described (2). The concentrations of the respective compounds in the mucosal solution remained essentially constant (*viz.*, 20 mcg./ml. riboflavin, 2.0 mg./ml. salicylate, and 100 mcg./ml. sulfanilamide) throughout the experiment, due to the large volume of mucosal solution.

Table I—Influence of Hypotonic and Hypertonic Solutions on the 30-Min. Cumulative Transfer of Several Drugs across the Everted Rat Intestine

		Segment 1		Segment 2		
Drug	Experiment	Amount Transferred in 30 Min. ± *SD*	Percent Control	Amount Transferred in 30 Min. ± *SD*	Percent Control	Comparison and Level of Significance[a]
Riboflavin[b]	Control	1.9 ± 0.2 mcg.	—	1.2 ± 0.1 mcg.	—	
	Hypotonic	0.7 ± 0.2 mcg.	37	0.4 ± 0.1 mcg.	33	2 *vs.* 1: $p < 0.01$[c]
	Hypertonic	2.0 ± 0.4 mcg.	105	1.8 ± 0.3 mcg.	150	3 *vs.* 1: $p < 0.01$[c]
Salicylate[b]	Control	0.7 ± 0.1 mg.	—	0.7 ± 0.1 mg.	—	
	Hypotonic	0.5 ± 0.1 mg.	71	0.4 ± 0.04 mg.	57	2 *vs.* 1: $p < 0.01$
	Hypertonic	0.8 ± 0.04 mg.	114	0.8 ± 0.06 mg.	114	3 *vs.* 1: n.s.
Sulfanilamide[d]	Control	23.5 ± 0.9 mcg.	—	23.2 ± 5.7 mcg.	—	
	Hypotonic	13.9 ± 1.9 mcg.	59	10.6 ± 2.4 mcg.	46	2 *vs.* 1: $p < 0.01$
	Hypertonic	26.2 ± 0.8 mcg.	111	23.4 ± 2.8 mcg.	101	3 *vs.* 1: n.s.

[a] Using a 2 × 2 ANOVA; n.s. = not significant: $p > 0.05$. [b] The values reported represent the mean ± standard deviation of the mean for five determinations under each experimental condition. [c] In these cases, the *F*-ratio values for segments and interactions were also significant ($p < 0.05$). [d] The values reported represent the mean ± standard deviation of the mean for four determinations under each experimental condition.

Table I summarizes the results of these experiments in terms of the cumulative amount of drug transferred in 30 min. In all cases, the hypotonic solution significantly inhibited intestinal transfer. Inhibition was most marked in the case of riboflavin. Statistically significant inhibition of drug transfer in the presence of the hypotonic solution was also observed upon comparison of the cumulative amounts transferred after

[1] KCl, 5 m*M*; KH_2PO_4, 1 m*M*; $NaHCO_3$, 26 m*M*; and NaCl, 122 m*M*.

1 and 2 hr. In contrast, the hypertonic solution increased the amount of drug transferred compared to control values. This increase, however, was statistically significant only in the case of riboflavin.

Measurements of intestinal tissue fluid uptake in the hypotonic and hypertonic buffers were made to test the previously observed relationship between gut fluid uptake and inhibition of drug transfer. Experimental details were reported (2). Essentially, 5-cm. segments of everted rat intestine were incubated for 20 min. in the buffer solution, and fluid uptake was determined as the difference between final and initial tissue weights. The results, in terms of milligrams fluid per gram initial wet weight plus or minus the standard deviation of the mean, were as follows: control buffer (isotonic solution), 52 ± 40 (11); hypotonic buffer, 97 ± 31 (11); and hypertonic buffer, −60 ± 28 (12). The numbers in parentheses represent the numbers of gut segments used. In the case of the hypertonic buffer, a net loss of fluid from the tissue was observed. The results of the gut fluid uptake studies clearly support the apparently general relationship that factors which increase tissue water uptake also inhibit passive intestinal transfer. This has been shown to be the case with glucose, xylose, or K^+ in the buffer medium and now with hypotonic solutions.

Kipnis and Parrish (8) suggested that both hypotonicity and elevated K^+ content of the buffer result in marked expansion of the intracellular volume of rat diaphragm tissue, with concomitant contraction of the extracellular space. The inulin space of the intact tissue, determined after 1 hr. of incubation, was reduced from about 16 ml./100 g. wet weight in control buffer to about 10 ml./100 g. in either high K^+ buffer or hypotonic buffer. Under both conditions, there was a significant increase in total tissue water compared to control levels. Similarly, Fox *et al.* (9), using kidney cortex, and Tews and Harper (10), using rat liver slices, reported a marked decrease in the extracellular space in high K^+ buffer. More recently, Jackson and Cassidy (11) found that glucose-induced fluid uptake by everted sacs of rat intestine was associated with considerable cellular swelling. It is plausible to consider that such swelling occurs at the expense of intercellular space in the mucosal barrier. If this is the case, then intercellular space may play an important role in the transfer of certain compounds across the everted rat intestine.

(1) M. Mayersohn and M. Gibaldi, *J. Pharm. Sci.*, **58**, 1429 (1969).

(2) M. Mayersohn and M. Gibaldi, *Biochim. Biophys. Acta*, **196**, 296(1970).

(3) M. Mayersohn and M. Gibaldi, unpublished data.

(4) J. H. Caldwell, J. F. Martin, S. Dutta, and N. J. Greenberger, *Amer. J. Physiol.*, **217**, 1747(1969).

(5) J. H. Caldwell, T. G. Halpin, and N. J. Greenberger, *J. Lab. Clin. Med.*, **71**, 43(1970).

(6) L. Z. Benet, J. M. Orr, R. H. Turner, and H. S. Webb, presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington, D. C. meeting, May 1970.

(7) M. Mayersohn and M. Gibaldi, to be published.

(8) D. M. Kipnis and J. E. Parrish, *Fed. Proc.*, **24**, 1051(1965).

(9) M. Fox, S. Thier, L. Rosenberg, and S. Segal, *Biochim. Biophys. Acta*, **79**, 167(1964).

10) J. K. Tews and A. E. Harper, *ibid.*, **183**, 601(1969).

(11) M. J. Jackson and M. M. Cassidy, *Experientia*, **25**, 492 (1969).

Michael Mayersohn*
Milo Gibaldi†
Department of Pharmaceutics
School of Pharmacy
State University of New York at Buffalo
Buffalo, NY 14214

Received September 28, 1970.
Accepted for publication October 22, 1970.

* Fellow of the American Foundation for Pharmaceutical Education, 1969–1970. Present address: Faculty of Pharmacy, University of Toronto, Toronto 5, Ontario, Canada.
† To whom requests for reprints should be sent.

Flash Methylation and GLC of Diphenylhydantoin and 5-(*p*-Hydroxyphenyl)-5-phenylhydantoin

Keyphrases □ Diphenylhydantoin, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin—determination □ Plasma, urine—diphenylhydantoin and metabolites, determination □ GLC, flash methylation—analysis

Sir:

The development of rapid, sensitive, and simple assay methods for determining blood and urine levels of diphenylhydantoin and 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (major metabolite of diphenylhydantoin) is most imperative for studying the interactions of diphenylhydantoin with other drugs and for more effective control of seizures in patients. We have been able to simplify the GLC analysis of diphenylhydantoin and 5-(*p*-hydroxyphenyl)-5-phenylhydantoin by converting them to nonpolar, volatile, methylated derivatives in the injection port of a gas chromatograph with trimethylanilinium hydroxide (1, 2).

The use of trimethylanilinium hydroxide as a methylating reagent offers the following advantages over *N,N*-dimethylformamide-bis(trimethylsilyl)acetamide, a reagent commonly used in preparing the trimethylsilyl derivatives of diphenylhydantoin: (*a*) trimethylanilinium hydroxide is a stable reagent that is easily prepared; (*b*) the methylation reaction with diphenylhydantoin and 5-(*p*-hydroxyphenyl)-5-phenylhydantoin occurs instantaneously in the injection port at temperatures of 300°, producing quantitative yields of methylated derivatives; and (*c*) it is not necessary to dry the purified plasma extracts at high temperatures or take time-consuming measures to exclude water from the extract or derivative-forming reagent *N,N*-dimethylformamide–bis(trimethylsilyl)acetamide.

Normal pooled plasma containing known amounts of drugs and internal standards and the patient's plasma, after adding the internal standards, were purified *via*