Drug Transport III: Influence of Various Sugars on Passive Transfer of Several Drugs across the Everted Rat Intestine

MICHAEL MAYERSOHN* and MILO GIBALDI

Abstract □ Depending upon the sugar (monosaccharide) present in the drug–buffer solution (isotonic Krebs bicarbonate, pH 7.4), one can effectively alter the passive transfer of several drugs across the everted rat intestine. A similar phenomenon was noted in a previous study concerned with the influence of various cations on transfer. Thus, the transfer of riboflavin, salicylate, and sulfanilamide is significantly decreased in the presence of glucose. Concentrations of glucose as low as 25 m*M* were found to inhibit riboflavin transfer significantly. Phlorizin (1 m*M*), which is known to inhibit active glucose transport, abolishes the glucose-stimulated inhibition of riboflavin transfer. A similar inhibition of riboflavin transfer was noted in the presence of xylose. Mannitol, on the other hand, a nonpenetrating sugar, had no effect on the cumulative transfer of these compounds over a 2-hr. period. As noted in the previous study, there appears to be a good correlation between the extent of tissue-fluid uptake under various experimental conditions and the inhibition of riboflavin transfer. Thus, materials causing tissue-fluid uptake (*e.g.*, glucose and xylose) inhibit riboflavin transfer; materials causing no tissue-fluid uptake (mannitol and glucose plus phlorizin) have no effect on drug transfer. Several plausible mechanisms are suggested to account for the observed decrease in passive drug transfer.

Keyphrases □ Drug transport, everted rat intestine—monosaccharide effect □ Glucose, xylose—passive-transport inhibition □ Phlorizin inhibition—glucose-retarded riboflavin transport □ Tissue fluid uptake relationship—drug transport

Much of the past work concerned with solute movement across biological membranes has assumed that the membrane is inert and behaves simply as a barrier to diffusion. This approach has served quite well for formulating basic physiocochemical laws describing the transfer process. However, it has become increasingly apparent that considering a biological membrane as a simple barrier to diffusion may be an undesirable oversimplification. For example, Barr and Riegelman (1, 2), showed that salicylamide is metabolized and stored by several intestinal tissue preparations; they suggested that a compartmental model rather than a barrier model should be used to describe transfer (or absorption) of certain drugs. A similar conclusion may be reached based on the work of Doluisio *et al.* (3), who suggested membrane distribution and storage during the absorption of several drugs in an *in situ* intestinal preparation.

Research efforts in this laboratory have been concerned with the evaluation of various factors that may influence the passive drug-transfer process. Previously reported results (4, 5) suggested that one is able to alter the membrane effectively and thereby influence the transfer process of drug molecules. Using an isolated intestinal preparation (everted rat intestine), the authors showed that the passive transfer of several drugs may be significantly influenced by the ionic content of the drug–buffer solution. For example, the transfer of several drugs is decreased when the major cation of the buffer solution is K^+ or NH_4^+. There appears to be a relationship between the extent to which these cations inhibit transfer and their ability to increase water uptake by the tissue. Recent literature reports supported these initial observations. Caldwell *et al.* (6, 7) noted that there is a significant decrease in the passive transfer of ouabain and digoxin across the everted rat intestine in the presence of K^+. In addition, there was direct verification of these observations concerning the influence of K^+ on salicylate transfer by Benet *et al.* (8).

Since several reports in the literature suggested that various sugars may alter membrane water uptake (9, 10), and since Nogami *et al.* (11) gave preliminary evidence that glucose decreased sulfanilamide transfer, it was decided to examine the influence of several of these sugars on the drug-transfer process and hopefully to elucidate further the nature of the inhibitory effects noted previously.

EXPERIMENTAL

Determination and Treatment of Drug-Transfer Data—Male, Sprague-Dawley strain rats,[1] weighing approximately 250 g., were fasted 20–24 hr. prior to the experiment. Water was allowed *ad libitum*. The animals were anesthetized with diethyl ether, and a midline abdominal incision was made. The intestine was isolated and severed at the ileo-cecal junction. The entire length of the intestine was removed and freed by cutting the intestine at the pyloric juncture. The intestine was placed immediately into normal saline at room temperature, and the lumen was washed with normal saline to remove any solid matter. An initial 15-cm. portion of the proximal intestine was discarded to ensure use of the jejunal region of the intestine. The intestine was then everted, and two 10-cm. segments were attached to individual glass cannulas, according to the method of Crane and Wilson (12). The 10-cm. segments were measured after stretching the entire intestine with an 11-g. weight. The initial, proximal segment was designated Segment 1 and the distal portion was designated Segment 2. A weight (7 g.) was attached to each segment and maintained during the course of the experiment. Both segments were then placed into test tubes containing 100 ml. of the mucosal drug solution, previously equilibrated at 37°. Due to the large solution volume, the mucosal concentration of drug remained essentially constant throughout the experiment. Two milliliters of buffer solution (devoid of drug) was then placed into the serosal compartment. A gas mixture of O_2–CO_2 (95:5, v/v) was constantly bubbled through the mucosal solution.

The serosal compartment was sampled every 10 min. during the entire 2-hr. experiment. The entire serosal volume was removed at the sampling time. Two milliliters of buffer solution was then introduced into the serosal compartment as a rinse, immediately removed, and added to the previous sample. Finally, another 2-ml. portion of buffer was placed into the serosal compartment and withdrawn at the next sampling interval.

Results of the transfer experiments are generally expressed in terms of the cumulative amount of drug transferred in 2 hr. How-

[1] Blue Spruce Farms, Altamont, N. Y.

Table I—Influence of Various Glucose Concentrations on the Transfer of Riboflavin across the Everted Rat Intestine

Glucose Concentration, m*M*	Segment 1 Amount Transferred in 2 hr., mcg. ± *SD*[a]	Segment 1 Inhibition, %	Segment 2 Amount Transferred in 2 hr., mcg. ± *SD*[a]	Segment 2 Inhibition, %	Level of Significance,[b] Control *versus* Glucose
0 (control)	14.2 ± 2.5	—	11.8 ± 0.8	—	—
25	12.3 ± 1.4	14	10.3 ± 0.7	13	$p < 0.05$
50	11.3 ± 1.7	21	8.6 ± 2.0	27	$p < 0.01$
100	8.1 ± 1.0	43	6.3 ± 0.6	47	$p < 0.01$
150	8.3 ± 1.3	41	6.2 ± 1.0	47	$p < 0.01$
200	7.1 ± 1.3	50	4.4 ± 1.5	63	$p < 0.01$
250	8.3 ± 2.3	41	6.0 ± 2.2	49	$p < 0.01$

[a] Represents the mean of five experiments ± standard deviation of the mean. [b] Using a 2 × 2 ANOVA. *F*-ratio values generated for segmental differences were significant in all cases ($p < 0.01$). *F*-ratio values for interaction were not significant ($p > 0.05$) in all cases.

ever, cumulative transfer for 0.5 and 1 hr. was also evaluated; usually, identical results were obtained when these intervals were chosen for comparison of effects. The statistical test employed to ascertain the significance of differences in experimental results was a twoway analysis of variance (ANOVA).

The data were also interpreted in terms of the percent of control transfer of drug as a function of time in the presence of various sugars or in terms of the percent inhibition of normal transfer as a function of time produced by the sugars. Percent of control transfer is the ratio of the cumulative amount of drug transferred to a given time under a test condition to that observed under control conditions to the same time, multiplied by 100. Percent inhibition is simply 100 minus the percent of control transfer. This method of analysis offers the advantage of canceling out all changes in the membrane that might be produced by any of a number of factors, except those changes resulting from the test material itself.

Determination of Tissue Fluid Uptake—The intestine was everted and the first 15 cm. discarded. The next 30 cm. of intestine was used to prepare six 5-cm. segments. Twelve segments (from two rats) were used per buffer solution. A ligature was attached to each segment; the segment was briefly and carefully blotted on Whatman No. 40 filter paper to remove any excess adhering fluid and then weighed. The weight of the ligature was subtracted from this initial weight of tissue. Each segment was then placed into 50 ml. of buffer solution in individual conical flasks maintained at 37°. A gas mixture of O_2–CO_2 (95:5, v/v) was then constantly bubbled through this solution. At the end of 20 min. the segments were removed, drained, and placed into weighing cups. Blotting the tissue at this point caused a significant loss of mucosal material. For this reason, blotting was considered inappropriate. Once the segment was placed into the weighing dish, there was formation of a small amount of fluid (*i.e.*, fluid adhering to the membrane). This fluid was wiped up, and the final tissue weight was determined. Fluid uptake was expressed in milligrams fluid per gram initial wet tissue. It was calculated by subtracting the initial tissue weight from the final tissue weight after 20 min. of incubation in the buffer solution, and dividing by the initial tissue weight.

Buffer Solutions—The buffer solution used was a modified Krebs bicarbonate buffer,[2] pH 7.4, after gassing with O_2–CO_2 (95:5). The pH never varied by more than ±0.2 pH unit before and after each experiment. In all cases, the total cation or cation equivalent concentration was 154 m*M*. Except when stated otherwise, the mucosal and serosal solutions were identical except for drug content. As a result, there was never an electrochemical or osmotic gradient existing between the mucosal and serosal solutions.

Whenever a buffered sugar solution was prepared, the sugar quantitatively replaced NaCl in terms of osmotic equivalents in the control buffer. Thus, a 250-m*M* sugar solution is equivalent (in osmotic terms) to 125 m*M* NaCl, since the sugar is unionized. The sugars examined were glucose,[3] xylose,[4] and mannitol.[5] In certain experiments, phlorizin[6] (1 m*M*) was added to the mucosal and serosal solutions.

Drug Solutions and Assay Procedure—The drugs examined and the concentrations used were riboflavin[7] (20.0 mcg./ml.), salicylate[8] (2.0 mg./ml.), and sulfanilamide[9] (0.1 mg./ml.). At pH 7.4, salicylate is completely ionized and sulfanilamide and riboflavin essentially nonionized. These compounds were chosen for investigation because they have a wide divergence in structure, physical–chemical properties, and presumably permeability characteristics. In addition, these materials are also presumed to be passively transferred across biological membranes. The passive nature of the transfer process for the compounds investigated here was inferred previously [sulfanilamide (11), riboflavin (13–15), and salicylate (16)].

Riboflavin was assayed fluorometrically using the Turner fluorometer, model 110,[10] based on the procedure reported by Levy and Jusko (17). Salicylate was assayed spectrophotometrically with the Hitachi-Perkin-Elmer model 139 spectrophotometer[11] by the method of Trinder (18). Sulfanilamide was assayed spectrophotometrically using the method of Bratton and Marshall (19).

RESULTS AND DISCUSSION

Influence of Glucose on Transfer—The effect on transfer rate of essentially replacing Na^+ from the buffer with glucose is shown in Fig. 1. The plot shows the percent of inhibition of transfer across Segment 1 for riboflavin and sulfanilamide in the presence of 250 m*M* glucose and for salicylate in the presence of 219 m*M* glucose. The influence of glucose on the transfer of salicylate and sulfanilamide is maximal within 10–20 min. and tends to remain essentially constant at approximately 18–20% inhibition. Glucose produces a much greater decrease in riboflavin transfer (40% inhibition), although a longer period of time is required to obtain a maximal effect, compared to the other two drugs. Similar differences between riboflavin transfer and salicylate and sulfanilamide transfer

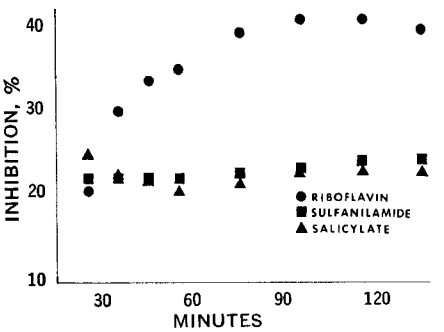

Figure 1—*Percent inhibition of drug transfer across intestinal Segment 1 as a function of time in the presence of glucose (250 m*M *for riboflavin and sulfanilamide, 219 m*M *for salicylate). Each data point represents the mean of five experiments with riboflavin (●) and salicylate (▲) and four experiments with sulfanilamide (■).*

[2] In m*M* concentrations: NaCl, 122; KCl, 5; KH_2PO_4, 1; and $NaHCO_3$, 26. With no additives, this solution is referred to in the text as the control buffer solution.
[3] Fisher Scientific Co., Fair Lawn, N. J., lot no. 791360.
[4] Fisher Scientific Co., Fair Lawn, N. J., lot no. 791758.
[5] Nutritional Biochemicals Corp., Cleveland, Ohio, lot no. 4654.
[6] Nutritional Biochemicals Corp., Cleveland, Ohio, lot no. 6398.
[7] General Biochemicals, Chagrin Falls, Ohio, lot no. 84134.
[8] Sodium salicylate, Fisher Scientific Co., Fair Lawn, N. J., lot no. 762784.
[9] Eastman Organic Chemicals, Rochester, N. Y.
[10] G. K. Turner Associates, Palo Alto, Calif.
[11] Perkin-Elmer Corp., Palo Alto, Calif.

Table II—Effect of Phlorizin (1 m*M*) on the Glucose Inhibition of Riboflavin Transfer

Experiment	Segment 1 Amount Transferred in 2 hr., mcg. ± *SD*[a]	Segment 1 Inhibition, %	Segment 2 Amount Transferred in 2 hr., mcg. ± *SD*[a]	Segment 2 Inhibition, %	Comparison and Level of Significance[b]
1. Control buffer	14.2 ± 2.5	—	11.8 ± 0.8	—	—
2. Control buffer + 1 m*M* phlorizin	12.9 ± 0.8	9	11.6 ± 1.1	2	2 *vs.* 1: n.s.
3. 25 m*M* Glucose	12.3 ± 1.4	14	10.3 ± 0.7	13	3 *vs.*1: $p < 0.05$
4. 25 m*M* Glucose + 1 m*M* phlorizin	13.4 ± 1.1	5	11.3 ± 1.4	4	4 *vs.*3: $p < 0.05$ 4 *vs.*1: n.s.
5. 25 m*M* Glucose—mucosal only	9.3 ± 1.0	35	8.2 ± 1.6	31	5 *vs.*1: $p < 0.01$
6. 25 m*M* Glucose—mucosal only + 1 m*M* phlorizin	12.7 ± 0.7	11	11.2 ± 1.1	5	6 *vs.*5: $p < 0.01$ 6 *vs.*1: n.s.

[a] Represents the mean of five experiments ± standard deviation of the mean. [b] Using a 2 × 2 ANOVA. *F*-ratio values for interaction for all comparisons were not significant (n.s.). *F*-ratio values for segmental differences were significantly different ($p < 0.05$).

were noted in the cation inhibition study (5). Although the data presented in the figures represent results from Segment 1, identical trends and similar values were obtained using Segment 2. When comparing the results of the glucose experiments shown in Fig. 1 to the control values, using an analysis of variance for the data obtained with both segments, there is a significant reduction in transfer ($p < 0.01$) for all drugs at each time interval evaluated.

Since glucose had the greatest influence on the transfer of riboflavin, additional experiments were conducted at lower glucose concentrations to examine further this inhibitory effect. The results of these experiments are summarized in Fig. 2 and Table I. Figure 2 is a plot of percent inhibition of cumulative riboflavin transfer over a 2-hr. period as a function of glucose concentration. An increase in inhibition is observed as the glucose concentration increases from 25 to 100 m*M*. Above 100 m*M* glucose, there appears to be a rather constant level of inhibition (40–50%). Table I summarizes all of the data for this series of experiments. At all glucose concentrations, there is a significant reduction in riboflavin transfer compared to the control. The reduction in cumulative transfer after 30 and 60 min. is also significant ($p < 0.01$) at all glucose concentrations.

After observing that 25 m*M* glucose on both sides of the membrane causes a decrease in riboflavin transfer, it was of interest to examine the influence of 25 m*M* glucose present initially only in the mucosal solution. In these studies the serosal solution was the normal control buffer, whereas the mucosal solution contained 25 m*M* glucose. Consequently, there was a concentration gradient with respect to glucose between the mucosal and serosal solutions. When glucose was initially present only on the mucosal side of the intestinal preparation, an even greater decrease in riboflavin transfer was noted compared to the experiment where glucose was present on both sides of the membrane. This can be seen in Fig. 3 and in Table II by comparing Experiments 3 and 5.

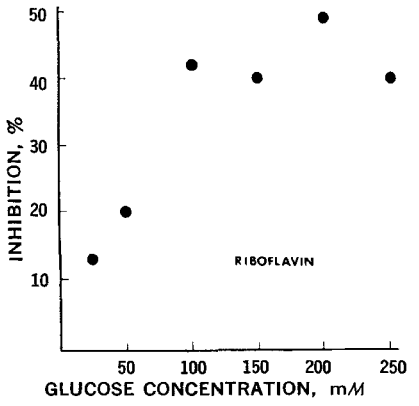

Figure 2—*Percent inhibition of riboflavin transfer as a function of glucose concentration* (mM). *Each point represents the mean of five experiments, based on the 2-hr. cumulative transfer across intestinal Segment 1.*

Influence of Phlorizin on Glucose Inhibition of Transfer—Since several reports in the literature (*e.g.*, 10 and 20) demonstrate that phlorizin can block the active uptake and transfer of glucose across the isolated rat intestine, it was decided to examine the influence of phlorizin on the glucose inhibition of riboflavin transfer. It was reasoned that if the uptake or transfer of glucose, or some function related to this process, was responsible for the decrease in drug transfer, one would expect that inhibition would be abolished or at least diminished in the presence of phlorizin. Thus, the influence of phlorizin (1 m*M* on both sides of the membrane) on the transfer of riboflavin in the presence of the lowest glucose concentration producing a significant decrease in transfer was evaluated. These results are summarized in Table II and Fig. 3. The data clearly demonstrate that when phlorizin is added to the glucose solutions, the inhibition of riboflavin transfer is markedly reduced. When phlorizin is added to the system containing glucose on both sides of the membrane, inhibition of transfer is reduced from 13–14% to 4–5% (Experiment 3 *versus* 4). The influence of phlorizin is even more striking when it is added to the system in which glucose is present initially only on the mucosal side. Whereas in the absence of phlorizin the glucose solution results in a 30–35% inhibition of transfer, in its presence inhibition is decreased to 5–10% (Experiment 5 *versus* 6).

Thus, it appears that phlorizin (1 m*M*) effectively prevents the glucose inhibition of riboflavin transfer, at least at the glucose concentration examined (25 m*M*). In addition, it may be inferred from these findings that with respect to active transport of glucose the tissue is apparently functional for the entire 2-hr. duration of the experiment.

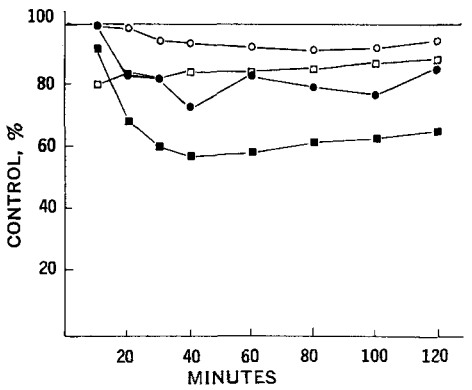

Figure 3—*Percent of control transfer of riboflavin as a function of time in the presence of 25* mM *glucose in the absence and presence of 1* mM *phlorizin. Each point represents the mean of five experiments for Segment 1. Solid horizontal line represents the control level. Key:* ●, *glucose present in both mucosal and serosal solutions;* ○, *glucose present in both mucosal and serosal solution + 1* mM *phlorizin;* ■, *glucose initially in mucosal solution only; and* □, *glucose initially in mucosal solution only + 1* mM *phlorizin.*

Table III—Influence of Xylose and Mannitol on Drug Transfer

Experimental Condition	Segment 1 Amount Transferred in 2 hr. ± SD[a]	Control, %	Segment 2 Amount Transferred in 2 hr. ± SD[a]	Control, %	Comparison and Level of Significance[b]
Riboflavin (mcg.)					
1. Control buffer	14.2 ± 2.5	—	11.8 ± 0.8	—	—
2. 250 m*M* Xylose	8.5 ± 0.9	60	7.6 ± 1.0	65	2 *vs.* 1: $p < 0.01$
3. 250 m*M* Mannitol	13.9 ± 0.9	98	11.5 ± 1.5	97	3 *vs.* 1: n.s.
Salicylate (mg.)					
1. Control buffer	3.6 ± 0.7	—	3.7 ± 0.5	—	—
2. 219 m*M* Mannitol	3.5 ± 0.2	97	3.3 ± 0.2	88	2 *vs.* 1: n.s.
Sulfanilamide (mcg.)					
1. Control buffer	131.2 ± 6.2	—	126.4 ± 23.7	—	—
2. 250 m*M* Mannitol	136.9 ± 17.6	104	134.3 ± 9.3	106	2 *vs.* 1: n.s.

[a] Represents the mean of five experiments ± standard deviation of the mean in the case of riboflavin and salicylate, and the mean of four experiments in the case of sulfanilamide. [b] Using a 2 × 2 ANOVA. *F*-ratio values for interaction were not significant (n.s.) ($p > 0.05$). *F*-ratio values for segmental differences were significant at $p < 0.05$ for riboflavin but not significant for salicylate and sulfanilamide.

Influence of Xylose and Mannitol on Transfer—The influence of two other sugars, xylose and mannitol, on drug transfer was also evaluated. Table III and Figs. 4 and 5 summarize the results. It is apparent that 250 m*M* xylose causes a significant decrease ($p < 0.01$) in the transfer of riboflavin compared to the control. The extent of inhibition of riboflavin transfer in the presence of xylose is identical to the inhibitory effect seen with glucose concentrations of 100 m*M* or higher.

Mannitol, on the other hand, had no effect on the cumulative transfer of riboflavin, sulfanilamide, and salicylate over a 2-hr. period, as shown in Table III. As can be seen in Fig. 5, which is a plot of percent control *versus* time in the presence of mannitol, there is initially (0–30 min.) a rather marked increase in the transfer of riboflavin over the control (horizontal line) but a decrease in the transfer of salicylate and sulfanilamide compared to control levels. After about 30 min., however, the values become quite constant and are maintained essentially at control levels. This unusual enhancement in transfer of riboflavin during the first 30 min. was not noted with any of the other sugars examined. However, examination of riboflavin transfer data obtained when either tromethamine[12] or Li^+ replaced Na^+ in the buffer also reveals an initial potentiation of riboflavin transfer over control levels. This potentiation essentially disappears in all cases after 30 min.

Tissue Fluid Uptake Study—Table IV lists the tissue fluid uptake values under various experimental conditions. As pointed out in a previous report (5), there is a great deal of variability associated with measurements of fluid uptake as is apparent from the standard deviation values shown in the table. Chauvenets' criterion (21) was applied to the values in each series of determinations; for this reason the mean may represent the average of 11 rather than 12 segments. At each of the glucose concentrations studied, there is a large increase in the mean fluid uptake compared to the control buffer (150–210 mg./g. *versus* 50 mg./g.). A similar effect is apparent when comparing the uptake in the presence of xylose to the control. Although the authors' studies have shown xylose to increase tissue fluid uptake by the rat intestine, Csaky and Esposito (22) suggested that xylose does not cause water uptake in the intestine of the bullfrog. Species differences as well as possible concentration differences with respect to xylose could account for this apparent discrepancy.

Several reports in the literature demonstrate that glucose can increase tissue hydration, *i.e.*, tissue water uptake (23, 24). Csaky and Esposito (22) suggested that active accumulation of a sugar in the intestinal tissue is associated with epithelial cellular swelling and discussed the possible correlation of this swelling with transmural electric potential. Jackson and Cassidy (25) measured gut fluid uptake using everted sacs of rat small intestine and demonstrated experimentally that this fluid uptake can be accounted for, at least in part, by epithelial cellular swelling. Thus, measurements of tissue fluid uptake in this and the previous study probably reflect swelling of the epithelial cells.

Since the method used in this study to determine tissue fluid uptake utilizes open intestinal segments, another approach was necessary to examine the influence of 25 m*M* glucose present initially only on the mucosal side. When using closed everted sacs of intestine, essentially no difference in tissue fluid uptake was noted when glucose was present either on both sides of the membrane or on the mucosal side alone. This observation was supported by the work of Lifson and Parsons (26), who found no difference in water absorption, which is related to tissue fluid uptake (9), when 29 m*M* glucose is present either in both serosal and mucosal solutions or only in the mucosal solution.

Of particular interest is the fact that when phlorizin is present in the 25 m*M* glucose solution, the fluid-uptake value is reduced from 210 mg./g. to about 50 mg./g., a level essentially identical to that observed under control conditions. Moreover, phlorizin also appears to inhibit tissue fluid uptake from the normal Na^+ control

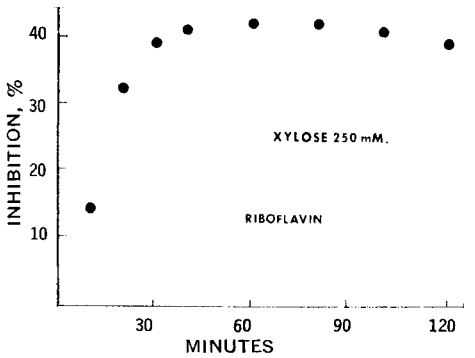

Figure 4—*Percent inhibition of riboflavin transfer as a function of time in the presence of 250 m*M *xylose. Each point represents the mean of five experiments for Segment 1.*

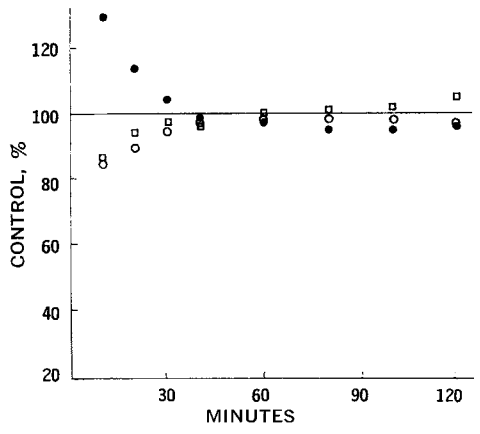

Figure 5—*Percent control transfer across intestinal Segment 1 as a function of time in the presence of mannitol (250 m*M *for riboflavin and sulfanilamide, 219 m*M *for salicylate). Each point represents the mean of five experiments with riboflavin (●) and salicylate (○) and four experiments with sulfanilamide (□).*

[12] TRIS.

Table IV—Tissue Fluid Uptake by Segments of Everted Rat Intestine under Various Experimental Conditions

Solution	Tissue Fluid Uptake, mg. H_2O/g. Tissue ± SD[a]
Control	52 ± 40 (11)
Control + 1 m*M* phlorizin	10 ± 29 (11)
Glucose 25 m*M*	209 ± 43 (12)
Glucose 25 m*M* + 1 m*M* phlorizin	50 ± 35 (11)
Glucose:	
50 m*M*	196 ± 48 (12)
100 m*M*	184 ± 50 (12)
150 m*M*	210 ± 47 (12)
200 m*M*	172 ± 40 (11)
250 m*M*	149 ± 39 (12)
Xylose 250 m*M*	148 ± 64 (12)
Mannitol 250 m*M*	27 ± 27 (11)

[a] The number of segments used are in parentheses.

buffer. In the presence of 1 m*M* phlorizin, there is a reduction in water uptake from 50 mg./g. to 10 mg./g. Parsons *et al.* (10) showed that 1 m*M* phlorizin can significantly decrease tissue fluid uptake by sacs of everted rat intestine, in addition to its ability to block glucose transfer. These authors suggested that phlorizin interferes with fluid uptake by disturbing glucose uptake and metabolism.

Since mannitol is known not to penetrate membranes to any significant extent (27), in contrast to glucose and xylose, it was expected that its presence would not cause swelling of the intestinal tissue. The important fact to be borne in mind here is that although the overall solution may be isoosmotic with respect to another solution, the penetrating ability of the various materials in the solution determines whether there will be a net flow of water across a membrane. The data in Table IV clearly support this hypothesis because the mean value for water uptake in the presence of mannitol is actually less than the corresponding value for the control.

Relationship between Tissue Fluid Uptake and Drug Transfer—The results of the present investigation clearly support the initial observation (5) that experimental conditions that modify the water content of intestinal tissue also modify drug-transfer rate. Thus, in the presence of glucose, there is a large increase in the mean tissue fluid uptake as well as a significant decrease in the transfer rate of each drug studied. Similarly, xylose significantly increases tissue fluid uptake and produces a marked inhibition of riboflavin transfer across the everted intestine. Mannitol, on the other hand, decreases gut fluid uptake and has little effect on the transfer of the drugs examined. Probably of greatest significance is the observation that concentrations of phlorizin that effectively block glucose-stimulated tissue fluid uptake also abolish the glucose inhibition of riboflavin transfer.

In the previous report (5), an excellent correlation was noted between the extent of inhibition of riboflavin transfer by various cations and the effect of these cations on tissue fluid uptake. By using these data as well as results from the present study, this correlation was extended and is presented in Fig. 6. The data points (representing both segments) were fitted by least-squares regression, and the equation of the line is $y = -0.41(x) + 115.19$, with a correlation coefficient of -0.940, which is highly significant ($p < 0.001$). If a correlation is attempted using the results of the mannitol, xylose, glucose (200 and 250 m*M*), and control experiments only, the equation of the line is $y = -0.38\ (x) + 113.30$, with a correlation coefficient of -0.966 ($p < 0.001$). A considerably poorer quantitative correlation is observed when one attempts to include the data from experiments using <200 m*M* glucose (*i.e.*, >50 m*M* Na^+). In view of the degree of inhibition of riboflavin transfer produced by these glucose solutions, their effect on fluid uptake is notably greater than predicted by the regression line.

Several plausible mechanisms might be suggested to explain the effects of cations and sugars on drug transport. The first of these considers the effective drug concentration in the intestinal tissue (*i.e.*, the epithelial cellular compartment). There is undoubtedly an increase in the compartmental cellular volume of the intestinal tissue in the presence of the various inhibitors. If this increase in cellular volume results in a decrease in the drug concentration in

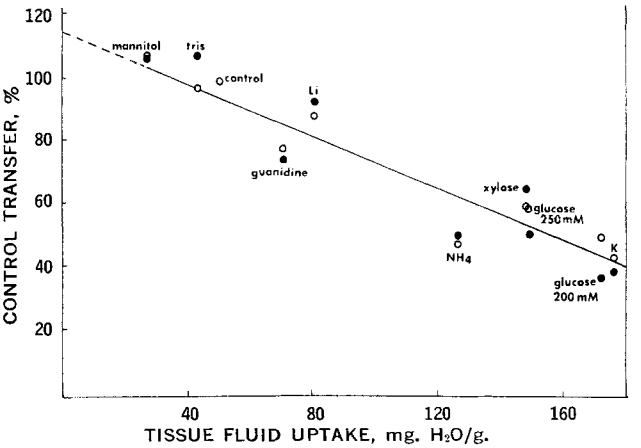

Figure 6—*Relationship between tissue fluid uptake (mg. H_2O/g. tissue) in the presence of various materials and percent of control transfer for riboflavin. Each point represents the mean of five experiments. Regression equation,* $y = -0.41\ (x) + 115.91$. *Correlation coefficient,* -0.940 ($p < 0.001$). *Key:* ○, *Segment 1; and* ●, *Segment 2.*

the cell, one expects that the concentration gradient existing between this compartment and the serosal solution would be decreased. If the transfer from the tissue to the serosal compartment is rate limiting, there would be a decrease in the driving force and a decreased rate of appearance of drug in the serosal compartment. Support for this mechanism can be sought only by a kinetic analysis of the changes of concentration and amount of drug in the tissue as a function of experimental conditions. Thus, if one finds that the same amount of drug is present in the tissue compartment when drug transfer is measured from the control buffer or any of the sugar or cation solutions examined, then there must be a concomitant change of the drug concentration due to volume changes of this compartment. Schultz *et al.* (28) showed that in the presence of K^+, which causes swelling of the intestinal tissue of rabbits, there is a large decrease in intracellular L-alanine concentration but virtually no change in the amount of this amino acid in the tissue. This effect was not a result of a specific inhibitory effect of K^+ on the transfer of L-alanine.

A second possibility requires one to consider the so-called pore route for solute movement across biological membranes. For example, Crone and Keen (29) suggested that pyridinium aldoximes traverse the isolated rat intestine by movement through aqueous channels or pores which exist between cells in the tissue. Kipnis and Parrish (30) reported that cellular swelling of rat diaphragm tissue in the presence of K^+ takes place at the expense of the extracellular volume. Thus, as the cells swell, there is a concomitant decrease in the space between the cells, which should result in a decrease in the effective pore or channel diameter. Two pathways may be available for the diffusion of drugs across the everted intestine: a cellular route which is associated with a lipoid barrier and an extracellular or pore route. If this is the case, then the degree of inhibition of drug transfer caused by cellular swelling is dependent upon the absorption pathway. For example, the transfer of a drug that diffuses essentially *via* the extracellular space would be markedly inhibited by cellular swelling.

REFERENCES

(1) W. H. Barr and S. Riegelman, *J. Pharm. Sci.*, **59**, 154(1970)
(2) *Ibid.*, **59**, 164(1970).
(3) J. T. Doluisio, W. G. Crouthamel, G. H. Tan, J. V. Swintosky, and L. W. Dittert, *J. Pharm. Sci.*, **59**, 72(1970).
(4) M. Mayersohn and M. Gibaldi, *ibid.*, **58**, 1429(1969).
(5) M. Mayersohn and M. Gibaldi, *Biochim. Biophys. Acta*, **196**, 296(1970).
(6) J. H. Caldwell, J. F. Martin, S. Dutta, and N. J. Greenberger, *Amer. J. Physiol.*, **217**, 1747(1969).
(7) J. H. Caldwell, T. G. Halpin, and N. J. Greenberger, *J. Lab. Clin. Med.*, **71**, 43(1970).
(8) 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.
(9) G. J. R. McHardy and D. S. Parsons, *Quart. J. Physiol.*, **42,** 33(1957).
(10) B. J. Parsons, D. H. Smyth, and C. B. Taylor, *J. Physiol.*, **144,** 387(1958).
(11) H. Nogami, M. Hanano, and M. Aruga, *Chem. Pharm. Bull.*, **14,** 166(1966).
(12) R. K. Crane and T. H. Wilson, *J. Appl. Physiol.*, **12,** 145 (1958).
(13) S. Christensen, *Acta Pharmacol. Toxicol.*, **27,** 27(1969).
(14) R. P. Spencer and N. Zamcheck, *Gastroenterology*, **40,** 794 (1961).
(15) R. P. Spencer and T. M. Bow, *J. Nucl. Med.*, **5,** 251(1964).
(16) L. S. Schanker, P. A. Shore, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **120,** 528(1957).
(17) G. Levy and W. J. Jusko, *J. Pharm. Sci.*, **55,** 285(1966).
(18) P. Trinder, *Biochem. J.*, **57,** 301(1954).
(19) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128,** 537(1939).
(20) H. Newey, B. J. Parsons, and D. H. Smyth, *J. Physiol.*, **148,** 83(1959).
(21) H. D. Young, "Statistical Treatment of Experimental Data," McGraw-Hill, New York, N. Y., 1962, pp. 78–80.
(22) T. Z. Csaky and G. Esposito, *Amer. J. Physiol.*, **217,** 753 (1969).
(23) R. B. Fisher and D. S. Parsons, *J. Physiol.*, **119,** 210(1953).
(24) *Ibid.*, **119,** 224(1953).
(25) M. J. Jackson and M. M. Cassidy, *Experientia*, **25,** 492(1969).
(26) N. Lifson and D. S. Parsons, *Proc. Soc. Exp. Biol. Med.*, **95,** 532(1957).
(27) R. Hober and J. Hober, *J. Cell. Comp. Physiol.*, **10,** 401 (1937).
(28) S. G. Schultz, R. E. Fuisz, and P. F. Curran, *J. Gen. Physiol.*, **49,** 849(1966).
(29) H. D. Crone and T. E. B. Keen, *Brit. J. Pharmacol.*, **35,** 304(1969).
(30) D. M. Kipnis and J. E. Parrish, *Fed. Proc.*, **24,** 1051(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 11, 1970, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication August 18, 1970.

Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Washington, D.C. meeting, April 1970.

* Fellow of the American Foundation for Pharmaceutical Education, 1969–1970. Present address: Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.

Dissolution of Slightly Soluble Powders under Sink Conditions II: Griseofulvin Powders

ISMAT ULLAH* and DONALD E. CADWALLADER†

Abstract □ Dissolution studies were carried out on micronized and regular milled griseofulvin powders. A three-compartment dissolution apparatus previously developed by the authors was used. A barrier was used in the dissolution medium to prevent the floating masses from entering directly into the sink phase. The individual, wetted particles of these powders could pass through the barrier pores. It was demonstrated that these particles do not enter and dissolve in the sink phase due to the presence of the aqueous layer around them. The presence of a sink phase and the introduction of samples in the form of suspensions were considered necessary to obtain appropriate rank order in the dissolution rates. A modified Hixon and Crowell cube root law was applied to obtain dissolution-rate constants for two different particle-size griseofulvin powders.

Keyphrases □ Griseofulvin powders—dissolution □ Dissolution, griseofulvin powders—sink conditions □ Particle-size effect—griseofulvin dissolution □ UV spectrophotometry—analysis

The importance of dissolution studies as an index of drug availability from solid dosage forms is widely accepted. The need to maintain appropriate sink conditions to conduct suitable dissolution studies for slightly soluble drugs was mentioned in a previous paper (1). In drugs with very low solubility, the dissolution process is controlled by the interfacial rate constant. Since the interfacial rate constant is the same for each drug species, the reduction of particle size is the common approach to increase the drug availability from drug formulations. Several investigators (2–5) showed an increased absorption rate for griseofulvin when the particle size of the drug was reduced. Similar effects from particle-size reduction were noted for chloramphenicol (6) and various salts of tetracycline (7). The relationship between particle size and drug availability is well recognized and, as a result, several excellent review articles were published in the last 5 years (8–11).

The general problems (flocculation, flotation, agglomeration, and wetting) encountered in the *in vitro* dissolution-rate studies of slightly soluble powders under sink conditions were discussed in the first article (1) of this series. Although dissolution studies under sink conditions might be generally desirable, they are almost necessary for poorly soluble drugs to obtain meaningful results. Rapid saturation of the dissolution medium may not allow the visualization of even major formulation differences. The limitations of other available sink methods (12, 13) for dissolution studies of powders were mentioned earlier (1). Although the use of a diffusion cell (14) or a large volume of dissolution medium (15, 16) could be used to avoid concentration build up, a method that utilizes an organic sink phase might be of value in the dissolution studies.

The objectives of this investigation were to demonstrate the use of a previously developed dissolution apparatus (1) to conduct dissolution studies of a very poorly soluble compound, griseofulvin, and to show