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# SUGAR TRANSPORT BY INTESTINE

# ESCAPE OF GALACTOSE FROM PRELOADED MUCOSA OF HAMSTER JEJUNUM

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## SUMMARY

Everted hamster jejunum was loaded with D-galactose and then escape into an initially galactose-free mucosal solution was followed. Mucosal anaerobiosis greatly increased the rate of escape, an effect which might have been caused by inhibiting reuptake from the unstirred layer and/or by augmenting the ease of unidirectional efflux across the brush border membrane. The former effect was expected because of our previous results from influx studies, and the main object here was to find out if the ease of efflux is affected by anaerobiosis. With phlorizin present in the mucosal solution during escape, information about unidirectional efflux was obtainable. We estimated that $10^{-4}$ M phlorizin inhibited the ease of efflux via the phlorizin-sensitive pathway by about 65%. Apparently the reason why mucosal phlorizin accelerates escape of sugar from loaded mucosa, an effect which has been reported previously by others, is that it inhibits unidirectional efflux less effectively than it inhibits reuptake from the unstirred layer. Residual efflux via the phlorizin-sensitive pathway was markedly increased by mucosal anaerobiosis. This increase did not require an elevation of intracellular $Na^+$ concentration. These results, together with those of our previous study, show that mucosal anaerobiosis abolishes uphill transport of galactose across the brush border of hamster jejunum by inhibiting unidirectional influx and by increasing the ease of unidirectional efflux. Neither of these effects requires a rise in intracellular $Na^+$ concentration.

## INTRODUCTION

We have studied some characteristics of galactose escape across the luminal border of preloaded jejunal mucosa. The results help answer the following questions about sugar transport: (1) Does mucosal anaerobiosis, a condition which was previously shown to inhibit galactose influx [1], influence the ease of galactose efflux

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across the brush border? (2) Is the effect of mucosal anaerobiosis on galactose efflux mediated by a rise of intracellular $Na^+$ concentration? (3) Does phlorizin immobilize the sugar carrier so that it can no longer mediate efflux?

## METHODS

Adult male golden hamsters were used. They were allowed food and water ad libitum. Each animal was anesthetized with ether, and a segment of upper jejunum was cut from the mesentery and immediately placed in oxygenated Krebs-Henseleit solution [2] at 37 °C (pH 7.4; 287 mosM), in which it remained for nearly all the subsequent manipulations. The segment was well rinsed inside and out and everted with a stainless steel rod. It was then tied to the glass apparatus described in a previous publication [1]. The apparatus was immersed to the top of the jejunal segment in a test tube containing 14.0 ml of Krebs-Henseleit solution with 5.0 mM D-galactose and a trace of D-[1-$^{14}$C]galactose (New England Nuclear). The segment was then filled with 3.4 ml of the same solution. Both mucosal and serosal solutions were continuously and vigorously gassed with $O_2/CO_2$ (95 : 5, v/v). The inside (serosal) solution circulated because of the bubble lift effect. The gut and incubation media were maintained at 37 °C during the entire procedure.

Incubation with 5.0 mM galactose on both sides continued for 10 min. During this "loading period" the absorptive cells accumulated labeled galactose. Then the glass apparatus with attached intestine was quickly dipped into two changes of galactose-free Krebs-Henseleit solution (at 37 C) to remove most extracellular galactose from the mucosal surface, and then immersed in a fresh galactose-free mucosal solution (14.0 ml) from which 50-$\mu$l samples were taken at appropriate intervals for $^{14}$C counting (in a liquid scintillation counter) in order to measure the rate of galactose escape into the mucosal solution. In some experiments mucosal gassing was switched from 5 % $CO_2$ in $O_2$ to 5 % $CO_2$ in $N_2$ after 8.3 min of escape. The serosal solution was not changed and during the escape period always contained somewhat over 5 mM galactose.

After the escape period (24 min) the segment was cut from the apparatus, slit open, rinsed briefly in ice-cold 5.07 % mannitol solution, blotted on filter paper, placed in a tared centrifuge tube and weighed. It was then extracted overnight with 3.0 ml of 70 % ethanol, and samples taken for $^{14}$C counting as described previously [1]. Tissue dry weight : wet weight ratios were determined in preliminary experiments by drying segments to constant weight at 110 °C. The change in volume of the mucosal solution was determined gravimetrically and was assumed to be a linear function of time during the escape period. In some experiments the segment was removed after escape had occurred for either 6 or 22 min; after the usual rinsing and blotting, the mucosa was scraped off and extracted as above.

Several experiments were also performed with non-everted jejunum. The technique was essentially the same as above except that following the 10-min loading period, mucosal rinsing was done by aspirating and reinjecting with a syringe. The initial volume of the mucosal solution was 3.4 ml; its final volume was roughly measured volumetrically after aspiration into a syringe.

Thin-layer chromatograms of samples of extracts and final mucosal and serosal solutions revealed no evidence for radioactivity in anything but galactose.

The above methods were used in Galveston. A completely independent series of escape studies was performed in Indianapolis with the following modifications: A 20-min rather than a 10-min loading period was employed; the serosal solution contained 5 mM glucose as a metabolic substrate; the volume of the mucosal solution was 30 ml instead of 14 ml; escape was allowed to continue for 42 min. In the Indianapolis series, the tissue was rinsed and blotted at the end of the escape period, and then the mucosa was scraped off with a glass slide and weighed and homogenized in 4.5 ml of distilled water. The homogenate was dried and then digested in $HNO_3$; $Na^+$ content was determined by atomic absorption. During the loading period the mucosal medium was always Krebs-Henseleit solution with 5 mM galactose. During the escape period the mucosal medium was initially galactose free and sometimes contained only 25 mequiv/l $Na^+$ instead of the usual 143 mequiv/l (accomplished by substituting mannitol isosmotically for the NaCl). Sometimes this mucosal solution also contained phlorizin ($10^{-4}$ M), and it was sometimes gassed with 95 % $N_2$ instead of $O_2$.

## RESULTS

If mucosal oxygenation was maintained, the rate of escape reached a steady value in about 8–10 min (Fig. 1). Within 10 min of substituting $N_2$ for $O_2$ in the mucosal solution, the rate of escape markedly increased. The average rate of galactose escape during the interval between 20 and 24 min was 0.10 $\mu$mol/min per g with mucosal $O_2$ and 0.48 $\mu$mol/min per g with mucosal $N_2$. Thus, mucosal anaerobiosis produced a 4.8-fold increase in the rate of escape.

Phlorizin in the mucosal solution at a concentration of $10^{-4}$ M strongly

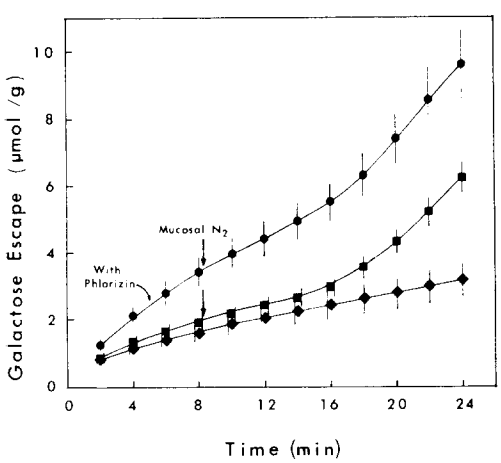


Fig. 1. Galveston studies. The lowest curve represents control experiments in which the serosal and mucosal solutions were gassed with $O_2$ during the entire escape period. In the other two sets of experiments the mucosal solution was gassed with $N_2$ beginning at 8.3 min. The top curve is from experiments in which $10^{-4}$ M phlorizin was present in the mucosal solution for the entire escape period. Each point represents the mean from five experiments. Standard errors are indicated by vertical lines. At the end of the escape period (24 min) the amount of galactose remaining in the tissue, in $\mu$mol per g of wet intestine, was 5.5 $\pm$ 0.3, 5.1 $\pm$ 0.6, and 4.3 $\pm$ 0.2 for the control, mucosal $N_2$, and phlorizin plus mucosal $N_2$ experiments, respectively.

augmented galactose escape (Fig. 1). Between 4 and 8 min escape averaged 0.33 $\mu$mol/min per g with phlorizin and only 0.14 $\mu$mol/min per g without phlorizin (combined data from both sets of experiments without phlorizin). In the presence of $10^{-4}$ M phlorizin in the mucosal solution, mucosal anaerobiosis still had a stimulatory effect (about 2.2-fold) on the rate of galactose escape.

It would be conceivable that mucosal anaerobiosis blocks movement of galactose into the serosal solution, thereby maintaining a higher tissue pool of galactose which could lead to increased escape into the mucosal solution. However, the data on tissue content of galactose (see legend to Fig. 1) made it apparent that this was not the case; consequently, we can assume that the effect of mucosal $N_2$ was on the apical surface of the epithelium.

Table I shows the concentrations of galactose in mucosal tissue water after either 6 or 22 min of escape in the presence of phlorizin in the mucosal solution, and after 22 min of escape without phlorizin. It is apparent that in spite of large variations in rate of escape, the galactose concentration in the mucosa did not vary much among the three conditions of incubation. This result is attributable to the large pool of galactose remaining in the serosal solution, the concentration of which averaged 6.5 mM at the end of the escape period.

Results from two experiments with non-everted jejunum are shown in Fig. 2. There was no appreciable change in mucosal volume during the escape period (except that caused by sampling); thus, the rate of change in mucosal concentration reflects the rate of transport. Again, there was no question about the effect of mucosal anaerobiosis. Escape increased rather promptly and reached a new steady value within 5 min. In eight experiments done with non-everted gut the 4–10-min escape averaged 0.020 ± 0.002 $\mu$mol/min per g, a value which is only about 15 % of that obtained with everted gut. We do not know why there should be so much difference. The effect of mucosal anaerobiosis was studied in four non-everted preparations; the average increase in rate of escape was 4.5-fold. The effect of mucosal anaerobiosis on galactose escape was fully reversible, as illustrated in Fig. 3. In the particular experiment used for Fig. 3, the original rate of galactose escape from the tissue was restored

TABLE I

CONCENTRATION OF GALACTOSE IN MUCOSAL TISSUE WATER

| Phlorizin in mucosal solution during escape period | Escape time (min) | Gassing | Galactose concentration* (mM) |
|---|---|---|---|
| 0 | 22 | $O_2$ | 9.00 ± 1.08 |
| 0 | 22 | $N_2$** | 8.55 ± 0.56 |
| $10^{-4}$ M | 6 | $O_2$ | 14.35 ± 1.98 |
| $10^{-4}$ M | 22 | $N_2$** | 7.80 ± 1.06 |

* Mean ± S.E. Number of animals was four in each case. For comparison, at the beginning of the escape period the concentration of galactose in mucosal tissue water was 19.6 and 21.8 mM in two determinations.

** Gassing with $N_2$ started at 8.3 min.

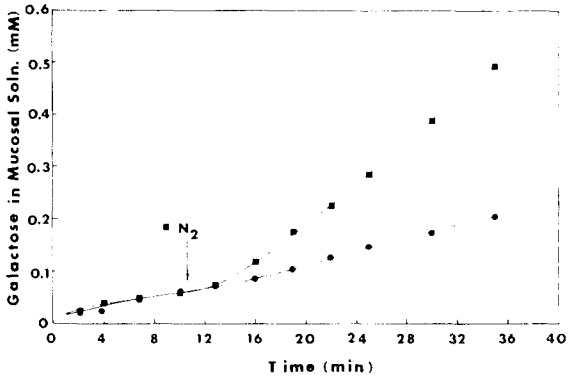


Fig. 2. Galactose escape in two typical experiments done with non-everted jejunum. In the experiment indicated by squares mucosal gassing was switched from $O_2$ to $N_2$ at 10.6 min. In the other experiment the mucosal solution was oxygenated the entire time.

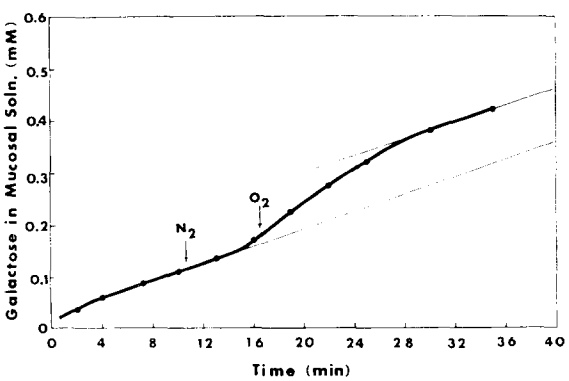


Fig. 3. This experiment using a non-everted segment of jejunum illustrates that the effect of mucosal anaerobiosis is reversible. The straight lines represent the slopes before the effect of $N_2$ and after reversal of this effect and are essentially parallel.

within 12 min after switching back to mucosal $O_2$. This time required for reversing the effect of mucosal $N_2$ was rather variable.

We next attempted to investigate the influence of tissue $Na^+$ concentration on galactose escape from preloaded jejunum. These are the Indianapolis studies referred to in Methods. The results are shown in Figs 4 and 5, and in Table II we list the amount of galactose escape during the 12–22-min portion of the escape period. During the first 12 min (with $O_2$ and no phlorizin, Fig. 4) escape was somewhat slower into the medium containing only 25 mequiv/l $Na^+$ than into the medium containing 143 mequiv/l $Na^+$. This result was unexpected and contrasts sharply with the observation of Robinson [3] that escape of $\beta$-methylglucoside from preloaded guinea pig ileum is markedly accelerated by removing $Na^+$ from the medium. We do not know the reason for this discrepancy. Robinson's [3] result was expected because reuptake of galactose from the unstirred layer (see below) ought to be inhibited in the low $Na^+$ medium and during this early time period there is probably not much change

in tissue $Na^+$ concentration. This inhibitory effect of the low $Na^+$ medium was not significant in the presence of phlorizin and was abolished by mucosal $N_2$. After the first 12 min there was no appreciable difference in rate of escape between the high and low $Na^+$ experiments.

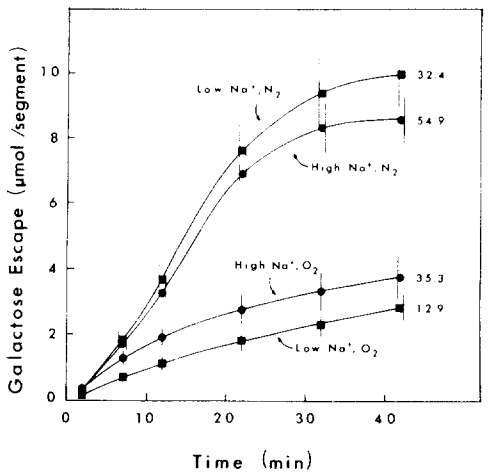


Fig. 4. Indianapolis studies without phlorizin. Each point represents the mean from nine experiments. The vertical lines indicate standard errors. "High $Na^+$" signifies 143 mequiv/l in the mucosal solution during escape, "low Na" signifies 25 mequiv/l. $N_2$ and $O_2$ designate how the mucosal solution was gassed. The serosal solution always contained 143 mequiv/l $Na^+$ and was gassed with $O_2$. The numbers to the right of the curves are the respective $Na^+$ contents of the mucosal tissue (in mequiv per kg of wet mucosa) at the end of the escape period. The relative standard errors of the $Na^+$ values were under 10 %.

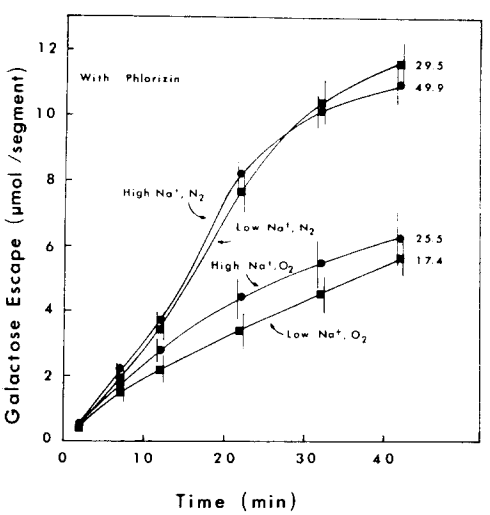


Fig. 5. Indianapolis studies with $10^{-4}$ M phlorizin in the mucosal solution during the escape period. Each point represents the mean from nine experiments. See legend to Fig. 4 for further explanation.

TABLE II

GALACTOSE ESCAPE DURING 12–24-MIN PORTION OF ESCAPE PERIOD*. EFFECTS OF PHLORIZIN, MUCOSAL ANAEROBIOSIS, AND A LOW $Na^+$ MEDIUM

| $Na^+$ in mucosal solution (mequiv/l) | Phlorizin | Gassing | Galactose escape 12–22 min (μmol/segment) |
|---|---|---|---|
| 143 | 0 | $O_2$ | 0.86 |
| | 0 | $N_2$ | 3.64 |
| | $10^{-4}$ M | $O_2$ | 1.61 |
| | $10^{-4}$ M | $N_2$ | 4.57 |
| 25 | 0 | $O_2$ | 0.68 |
| | 0 | $N_2$ | 4.00 |
| | $10^{-4}$ M | $O_2$ | 1.18 |
| | $10^{-4}$ M | $N_2$ | 4.21 |

* Indianapolis studies.

The effect of mucosal anaerobiosis was about the same as it was in the Galveston studies. For example, with 143 mequiv/l $Na^+$ in the mucosal solution (but no phlorizin), 4.2 times as much galactose escaped between 12 and 22 min with mucosal $N_2$ as escaped with mucosal $O_2$; with phlorizin present in the mucosal solution the effect of mucosal anaerobiosis was a 2.8-fold increase in escape. Phlorizin in the mucosal solution caused a 1.9-fold increase in galactose escape between 12 and 22 min (with mucosal $O_2$ and 143 mequiv/l $Na^+$); this is very close to the 2.4-fold effect of phlorizin reported above. The "Indianapolis studies" demonstrate that approximately the same effects of mucosal anaerobiosis and of mucosal phlorizin could be obtained when the mucosal solution initially contained only 25 mequiv/l $Na^+$.

The final tissue $Na^+$ contents shown in Figs 4 and 5 should be compared to a value of $28.2 \pm 2.0$ mequiv/kg which was the mean $Na^+$ content of eight mucosal tissues obtained after the loading period, but before the escape period. It is evident that during the escape period tissue $Na^+$ rose when the mucosal solution contained 143 mequiv/l of $Na^+$ and was made anaerobic. This rise during anaerobiosis was prevented by lowering the concentration of $Na^+$ in the mucosal solution to 25 mequiv/l. The important point is that even when tissue $Na^+$ content did not rise appreciably, mucosal anaerobiosis still had its usual stimulatory effect on galactose escape; this was true both in the presence and in the absence of phlorizin in the mucosal solution. Obviously, the rate of escape was influenced far more by anaerobiosis than it was by tissue $Na^+$ content.

*Unidirectional efflux and the complication of reuptake from the unstirred layer*

The rate of galactose escape from preloaded mucosa into the mucosal solution is, in reality, the net flux ($J_L$) across the unstirred aqueous layer covering the epithelial surface. During a quasi-steady state

$$J_L = J_{21} - J_{12} = J_{32} - J_{23} \qquad (1)$$

where each $J$ with numbered subscript represents a unidirectional flux, and the

subscripts (1, 2 and 3) represent, respectively, the bulk mucosal solution, the unstirred solution just at the outer surface of the brush border membrane, and the intracellular solution just at the inner surface of the membrane. The donor and recipient locations are given from left to right in the subscripts. Thus, $J_{32}$ is unidirectional efflux from the epithelium.

From the data shown in Fig. 1, we found that $J_L$ was 0.10 μmol/min per g (between 20 and 24 min with mucosal $O_2$). At this time, the galactose concentration in mucosal tissue water averaged 9.0 mM (Table 1). In our previous paper [1] using essentially the same technique we demonstrated that $J_{32}$ (called $J_{cm}$ in ref. 1) was 1.25 μmol/min per g when tissue concentration was 24.0 mM. Therefore, with a tissue concentration of 9.0 mM, $J_{32}$ would be at least 0.47 μmol/min per g, and perhaps higher if the relation between $J_{32}$ and tissue concentration were hyperbolic rather than linear over this concentration range. Consequently, $J_{23}$ (i.e. reuptake from the unstirred layer) must have been at least 0.37 μmol/min per g. It is clear from this example that the rate of escape from preloaded tissue is not a valid measure of unidirectional efflux because of reuptake from the unstirred layer. Although the data in Figs 1–5 clearly show that mucosal anaerobiosis greatly increased $J_L$, we cannot directly conclude that $J_{32}$ was increased. This effect could be explained by a large decrease in $J_{23}$ [1].

*Estimation of unidirectional efflux via the phlorizin-sensitive pathway*

It can be assumed that the unidirectional fluxes, $J_{32}$ and $J_{23}$, each consist of two components: (1) flux via phlorizin-insensitive pathways, and (2) flux via the mediated phlorizin-sensitive pathway, $J^P$. If we further assume the phlorizin-insensitive pathways to have no directional preference

$$J_L = J_{32}^P - J_{23}^P = \alpha (C_3 - C_2) \quad (2)$$

Where $\alpha$ is the coefficient for galactose flux via the phlorizin-insensitive pathway(s), and $C_3$ and $C_2$ are the galactose concentrations at the inner and outer surfaces, respectively, of the apical membrane.

Assuming $J_{23}^P$ is negligible in the presence of $10^{-4}$ M mucosal phlorizin (see Discussion),

$$J_{32}^P = J_L - \alpha(C_3 - C_2). \quad (3)$$

In the Galveston experiments (Fig. 1) with phlorizin, $J_L$ averaged 0.33 μmol/min per g during the 4–8-min period (mucosal $O_2$) and 0.55 μmol/min per g during the 20–24-min period (mucosal $N_2$). Values for $\alpha$ are obtained from ref. 1, assuming diffusion kinetics and no directional preference for the phlorizin-insensitive pathway(s): these values are 0.0089 $\mu mol \cdot min^{-1} \cdot g^{-1} \cdot mM^{-1}$ with mucosal $O_2$ and 0.0137 $\mu mol \cdot min^{-1} \cdot g^{-1} \cdot mM^{-1}$ with mucosal $N_2$. We feel it is legitimate to use these values from our previous paper since they were obtained from the same intestinal preparation as employed here. Values for $C_3$ are found in Table 1. Values for $C_2$ are calculated from Eqn 3 of ref. 1 using average $C_1$ values measured at the appropriate times and the same values for $l$, $A$ and $D$ used in ref. 1. We estimate that $C_3 - C_2$ was 13.8 mM during the 4–8-min period (mucosal $O_2$) and 6.6 mM during the 20–24-min period (mucosal $N_2$). Consequently, using Eqn 3, we estimate that $J_{32}^P$ was 0.21 μmol/min per g during the 4–8-min period with mucosal $O_2$ and 0.46 μmol/min per g during the

TABLE III

ESTIMATED EASE OF GALACTOSE EFFLUX VIA THE PHLORIZIN-SENSITIVE PATHWAY ($J_{32}{}^{P}/C_3$)

| Mucosal gassing | $J_{32}{}^{P}/C_3$ ($\mu$mol · min$^{-1}$ · g$^{-1}$ · mM$^{-1}$) | | Inhibition by phlorizin |
|---|---|---|---|
| | Without phlorizin* | With phlorizin | |
| $O_2$ (4–8 min period) | 0.043 | 0.015 | 65 % |
| $N_2$ (20–24 min period) | 0.129 | 0.059 | 54 % |
| Increase by $N_2$ | 3.0-fold | 3.9-fold | |

* Derived from date in ref. 1.

20–24-min period with mucosal $N_2$. Efflux via the phlorizin-sensitive pathway apparently increased 2.2-fold during mucosal anaerobiosis, even though the concentration of galactose in the donor compartment ($C_3$) was only about one-half what it was during mucosal aerobiosis. We conclude that the ease of efflux via the phlorizin-sensitive system is increased by mucosal anaerobiosis.

In Table III we show the apparent permeability coefficients for galactose efflux via the phlorizin-sensitive pathway ($J_{32}{}^{P}/C_3$) with either mucosal $O_2$ or mucosal $N_2$. The values obtained with phlorizin present are calculated from the $J_{32}$ values derived in the preceding paragraph and the $C_3$ values in Table I. For comparison we also list in Table III the $J_{32}{}^{P}/C_3$ values that can be derived from the data in our previous paper [1]; these data were from experiments without phlorizin in the mucosal solution. The relative increase in $J_{32}{}^{P}/C_3$, caused by mucosal $N_2$, was roughly the same in both cases.

## DISCUSSION

In our previous paper [1] we showed that the ratio between unidirectional efflux across the brush border and tissue concentration of galactose was increased 2.8-fold by mucosal anaerobiosis. It was concluded that the ease of efflux was augmented by mucosal anaerobiosis. However, this conclusion depended on the assumption that efflux was linearly related to tissue concentration since the concentration of galactose in the tissue was much lower in the experiments with mucosal $N_2$ than in those with mucosal $O_2$. We pointed out that if this relationship were actually hyperbolic over the pertinent concentration range, the effect of mucosal anaerobiosis on ease of efflux would have been overestimated. Naftalin and Curran [4] have provided evidence that in rabbit ileum unidirectional efflux of galactose is approximately a linear function of cellular concentration up to about 30 mM. But the present data provide reason for thinking that efflux may not be linearly related to tissue concentration in hamster jejunum. With maintained mucosal oxygenation, escape over the 4–8-min period was only 1.18 times what it was over the 20–24-min period (data for bottom curve in Fig. 1) even though the tissue concentration was about twice as great. Because of this

uncertainty in interpreting our previous results, we felt that a new approach was necessary.

In the present paper it is demonstrated that escape of galactose from preloaded jejunum was markedly augmented by mucosal anaerobiosis even when the concentration of galactose in the tissue was about the same as during mucosal oxygenation. However, another problem in interpretation arises: escape may have been increased, not by increasing unidirectional efflux, but by inhibiting unidirectional influx (i.e. reuptake from the unstirred layer). In fact, we have previously demonstrated that unidirectional influx of galactose is strongly inhibited by mucosal anaerobiosis [1].

In an attempt to reduce the complication of re-uptake and examine efflux directly, we repeated the experiments with phlorizin present in the mucosal solution during the escape period. The concentration of phlorizin used ($10^{-4}$ M) should have been high enough, relative to the concentration of galactose, to block completely the mediated influx (reuptake) of galactose [1, 5]. We have demonstrated that with this concentration of phlorizin mucosal anaerobiosis no longer inhibits galactose influx [1]. Consequently, the observed augmentation of galactose escape by mucosal anaerobiosis in the presence of phlorizin must have been caused by increased efflux rather than by decreased reuptake from the unstirred layer.

We showed that the rate of galactose escape is markedly increased by mucosal phlorizin, thus confirming previous observations [3, 6–8]. The explanation for this effect is now clear; phlorizin inhibits both efflux and reuptake from the unstirred layer, but inhibits the latter the more effectively. The values for $J_{32}^{P}/C_3$ (i.e. the apparent ease of galactose efflux via the phlorizin-sensitive pathway) shown in Table III serve as the basis for this conclusion. Phlorizin strongly, but not completely, inhibited $J_{32}^{P}/C_3$ in the presence of either mucosal $O_2$ or mucosal $N_2$. Admittedly, a number of assumptions are involved in deriving these values and the degree of inhibition should be regarded as a rough estimate. However, it seems clear that inhibition of $J_{32}^{P}$ is far from 100 % even when enough phlorizin is present in the mucosal solution to block $J_{23}^{P}$ completely.

Of course, a considerable inhibition of $J_{32}^{P}$ by phlorizin was expected because of the evidence that the phlorizin-carrier complex is relatively immobile and cannot transport phlorizin into the cells at an appreciable rate [9]. It might be expected that phlorizin simply ties up the carrier so it cannot operate in either direction. The observation that phlorizin did not totally abolish $J_{32}^{P}$ indicates that it does not completely immobilize the carrier. Perhaps some carrier is made available to facilitate $J_{32}$ because of actual movement of phlorizin into the cells, or because of carrier-mediated movement of glucose produced from phlorizin by the action of phlorizin hydrolase [10, 11].

The Indianapolis experiments (Figs 4 and 5, and Table II) demonstrate that the rate of escape was not affected much by moderate changes in tissue $Na^+$ concentration. For the experiments done without phlorizin interpretation is complicated by the possibility that, when the $Na^+$ concentration in the mucosal solution was reduced to 25 mequiv/l, reuptake from the unstirred layer was inhibited, an effect which could have masked an inhibition of efflux. However, when $J_{23}^{P}$ was blocked with mucosal phlorizin, there was still no appreciable effect of tissue $Na^+$ concentration on $J_L$. In this circumstance $J_L$ is given by Eqn 3. We assume no effect of $Na^+$ on $\alpha$; therefore, there was apparently no effect of these changes in tissue $Na^+$ concentration on $J_{32}^{P}$.

Of course, we cannot rule out effects with larger changes of tissue $Na^+$. It is interesting that Naftalin and Curran [4], using a completely different method of measuring the ease of efflux in rabbit ileum, were also unable to demonstrate an inhibition when cellular $Na^+$ concentration was decreased. In fact Naftalin's more recent data indicate an increase in the ease of galactose efflux when tissue $Na^+$ concentration was reduced [12].

Is the effect of mucosal anaerobiosis on $J_{32}{}^P$ mediated by a rise in intracellular. $Na^+$ concentration? This important question seems to be answered by the data in Fig. 5. If the curve labeled "High $Na^+$, $O_2$" is compared to the two upper curves, it is clear that mucosal anaerobiosis increased escape and, therefore, $J_{32}{}^P$ just as much when tissue $Na^+$ did not rise appreciably as it did when tissue $Na^+$ doubled. This observation does not prove that $J_{32}{}^P$ is directly inhibited by metabolism, but it does seem to rule out mediation via the inside $Na^+$ concentration.

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