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# Galactose Transport in Rabbit Ileum

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*Summary.* The four unidirectional fluxes of galactose across the mucosal and serosal borders of rabbit ileum have been evaluated as functions of galactose concentration on a single piece of tissue from simultaneous measurements of mucosal-to-serosal and serosal-to-mucosal fluxes using $^{14}C$- and $^{3}H$-galactose and the steady-state ratio of the two isotopes in the tissue. The effects of Na removal, ouabain and a hypertonic serosal solution on these fluxes was investigated. Galactose was actively transported across the mucosal membrane under control conditions; this process was inhibited by Na removal and ouabain as a result of a decrease in flux from mucosal solution to cell and an increase in flux in the opposite direction. Effective galactose permeability of the serosal membrane was comparable to that of the mucosal membrane. Galactose transfer across the serosal membrane did not appear to conform to simple diffusion and may involve a facilitated transfer mechanism although a weak active transport from serosal solution to cell cannot be ruled out. Galactose movement at the serosal side of the cell was not influenced by Na.

Unidirectional fluxes of sugars, amino acids and Na across the mucosal and serosal cell boundaries of intestine have been calculated from separate measurements of bidirectional transmural fluxes and of influx across the mucosal border [7, 16]. These unidirectional fluxes are thus derived from measurements made on three separate pieces of tissue and involve the assumption that the tissue is effectively a single compartment for the solutes. Recent studies of Frizzell and Schultz [5] have demonstrated a significant paracellular shunt pathway for ion movements in rabbit ileum for which corrections must be made in estimating fluxes across the individual cell boundaries. However, this shunt does not appear to play a significant role in the transepithelial fluxes of amino acids [13]. Recently, Bihler and Cybulsky [1] have employed isolated cells from hamster intestine in which the brush border transport mechanism has been inhibited in order to obtain informa-

tion on sugar movement across the serosal membrane. In the present study, we show that by making the above assumption that the tissue is, operationally, a single compartment, all unidirectional fluxes may be derived from direct measurements made on a single piece of tissue. We have used this technique to estimate the unidirectional fluxes of galactose across the mucosal and serosal membranes of rabbit ileum under a variety of experimental conditions.

## Theoretical Considerations

We assume that the distribution of the measured solute in rabbit ileum stripped of the serosa and most of the muscle layers is homogeneous. Hence, to a first approximation the ileum can be described in terms of a three-compartment system as illustrated in Fig. 1. Bidirectional transmural fluxes, $J_{13}$ and $J_{31}$, are measured with equal concentrations of the solute in the mucosal and serosal solutions and with different radioactive tracers, i.e. $^{14}C$- and $^{3}H$-galactose, in each bathing solution. Conditions are chosen such that during the experiment, the concentration of label in the trans solution never rises to a level sufficient to give a significant backflux.

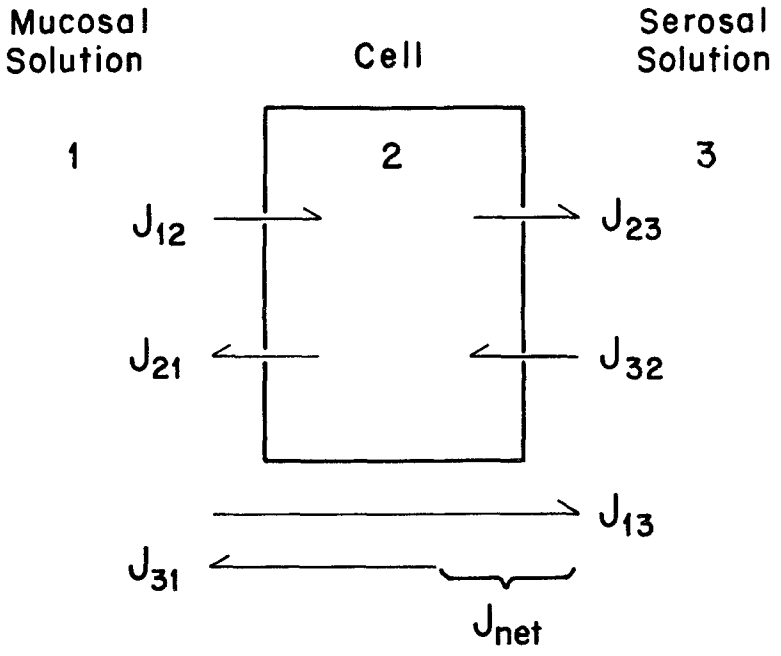


Fig. 1. Three-compartment system illustrating the measured and calculated unidirectional fluxes

Schultz *et al.* [16] have shown, following the approach of Ussing and Zerahn [20], that in such a system

$$J_{13}=\frac{J_{12}J_{23}}{J_{21}+J_{23}} \tag{1}$$

$$J_{31}=\frac{J_{32}J_{21}}{J_{21}+J_{23}} \tag{2}$$

in which the subscripts 1, 2 and 3 refer to the mucosal solution, cellular compartment and the serosal solution, respectively. $J_{ij}$ is the unidirectional solute flux from the compartment $i$ to compartment $j$. From Eqs. (1) and (2),

$$J_{13}/J_{31}=J_{12}J_{23}/J_{32}J_{21}. \tag{3}$$

If the solute is labeled with $^{14}C$ in the serosal solution and with $^{3}H$ in the mucosal solution,

$$\frac{dP_2^c}{dt}=J_{32}p_3^{*c}-(J_{21}+J_{23})p_2^{*c} \tag{4}$$

in which $P_2$ is total counts per minute (CPM) in compartment 2 and $p_i^*$ is specific activity (CPM/μmole). The superscript $c$ denotes $^{14}C$. In the steady state, $dP_2^c/dt=0$ and from Eq. (4),

$$p_2^{*c}=\frac{J_{32}p_3^{*c}}{J_{21}+J_{23}}. \tag{5}$$

Similar arguments apply to the $^{3}H$ label in the mucosal solution.

$$\frac{dP_2^T}{dt}=J_{12}p_1^{*T}-(J_{21}+J_{23})p_2^{*T} \tag{6}$$

in which the superscript $T$ denotes $^{3}H$. In the steady state $dP_2^T/dt=0$ and

$$p_2^{*T}=\frac{J_{12}p_1^{*T}}{J_{21}+J_{23}}. \tag{7}$$

Dividing Eq. (7) by Eq. (5) yields

$$\frac{P_2^T}{P_2^c}=\frac{J_{12}p_1^{*T}}{J_{32}p_3^{*c}}$$

since $p_i^*$ is $P_i$ divided by the cellular pool of solute. We then define a quantity $R$ by the relation

$$R=\frac{P_2^T/p_1^{*T}}{P_2^c/p_3^{*c}}=\frac{J_{12}}{J_{32}}. \tag{8}$$

If the $^3H$ and $^{14}C$ are assumed to be distributed homogeneously in compartment 2 and solute concentrations are identical in compartments 1 and 3,

$$R=\frac{(\text{CPM})_2^T}{(\text{CPM})_2^c}\times\frac{(\text{CPM/ml})_3^c}{(\text{CPM/ml})_1^T}.$$

Substitution of Eq. (8) into Eq. (3) yields

$$\frac{J_{13}}{J_{31}}=\frac{J_{23}R}{J_{21}}. \tag{9}$$

Introduction of Eq. (9) into Eqs. (1) and (2) then yields the simple relationships

$$J_{32}=J_{31}+J_{13}/R \tag{10}$$

$$J_{12}=J_{31}R+J_{13}. \tag{11}$$

In the steady state, the net solute flux across each individual barrier must be equal to the net flux across the tissue or

$$J_{12}-J_{21}=J_{23}-J_{32}=J_{13}-J_{31}.$$

Thus,

$$J_{21}=J_{31}(1+R) \tag{12}$$

$$J_{23}=J_{13}(1+1/R). \tag{13}$$

Consequently, the four unidirectional fluxes can be calculated from measured transmural fluxes, $J_{13}$ and $J_{31}$ and the ratio $R$. Accurate determination of $R$ requires only that the ratio of $^3H$ to $^{14}C$ in the tissue be measured, not the absolute amounts of the tracers. Thus, the tissue may be washed in unlabeled medium for a sufficiently long time to minimize the influence of extracellular isotope without affecting the ratio of isotopes within the cellular compartment. Clearly, it is not necessary that the solute concentrations be identical in the mucosal and serosal solutions to use this approach. A difference can be taken into account as indicated in Eq. (8).

## Materials and Methods

Male New Zealand white rabbits were killed by intervenous injection of sodium pentobarbital. A segment of ileum was removed rapidly, washed free of intestinal contents with ice-cold Ringer's solution and stripped of serosa and muscle layers as previously described [14]. The mucosal tissue was then mounted as a flat sheet between Lucite half-chambers with an area of 1.13 or 3.14 $cm^2$ in the apparatus described by Schultz and Zalusky [17]. The tissues were then exposed to Ringer's solution containing varying concentrations of D-galactose labeled with $^3$H-D-galactose (mucosal solution) and $^{14}$C-D-galactose (serosal solution). The Ringer's solution contained, in mM, 140 NaCl, 10 $KHCO_3$, 0.4 $K_2PO_4$, 2.4 $K_2HPO_4$, 1.2 $CaCl_2$, 1.2 $MgCl_2$ and was continuously bubbled with 95% $O_2$ – 5% $CO_2$. All experiments were carried out at 37 °C. In some experiments, all NaCl in the Ringer's solution was replaced by choline chloride.

One-ml samples were removed from the mucosal and serosal solutions for counting at 20- or 25-min intervals for 100 min. At the end of the experiment, the chambers were quickly disconnected from the gas lifts and the "hot" solutions were removed. Both sides of the chamber were washed rapidly with ice-cold Na-free Ringer's solution with the membrane still in place. The chambers were then opened; the exposed circle of tissue was cut out, washed with gentle shaking in cold Na-free solution for 1 min and blotted carefully on both sides to remove excess moisture. The tissue was weighed wet in a small tared flask and then extracted by shaking for 2 hr in 2 ml of 0.1 N $HNO_3$. Samples of the extract were taken for counting and for determination of Na and K by flame photometry. The extracted tissue was dried for 16 hr at 80 °C and the dry weight determined. Tissue water was calculated as the difference between wet and dry weights.

Radioactive samples were counted in a liquid scintillation spectrometer set to provide adequate discrimination between $^3$H and $^{14}$C. Appropriate standards were used to separate counts of the two isotopes in the various samples. Transmural unidirectional fluxes were calculated from the rate of tracer appearance on the "cold" side and the specific activity of the "hot" side. In most experiments, four pieces of tissue were studied simultaneously, each with a different concentration of galactose in the bathing solutions. An experiment was repeated 4 to 6 times for each of five conditions.

## Results

Fig. 2 shows the behavior of several experimental quantities as functions of external galactose concentration under control conditions. The normalized ratio of $^3$H to $^{14}$C in the tissue, $R$, declines as external concentration increases, falling from a value of 3.7 at 5 mM galactose to 1.05 at 40 mM. This behavior indicates that at 5 mM, nearly 4 times as much galactose enters the cells from the mucosal solution as from the serosal solution. At 40 mM, the steady-state level of cellular galactose is determined by approximately equal contributions from the two bathing solutions. In the range of concentrations tested, cellular galactose levels increase linearly with external concentration, but the accumulation ratio, $[gal]_c/[gal]_0$, varies from 4 at 5 mM to 1.8 at 40 mM. Mucosal-to-serosal flux $J_{13}$ shows a convex relationship to external galactose concentration while the serosal-to-mucosal flux $J_{31}$ appears to have a concave relationship, particularly in the concentration

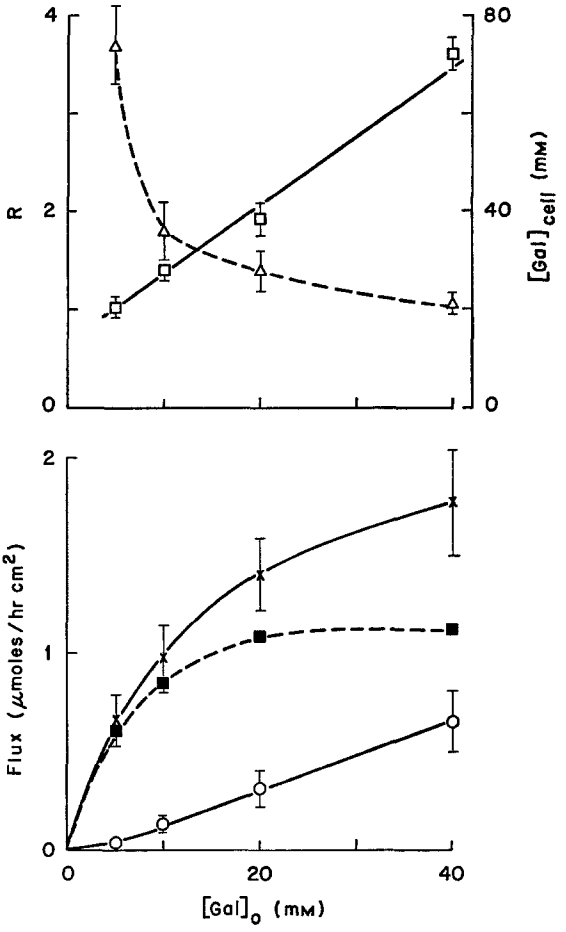


Fig. 2. Measured quantities as functions of external concentration of galactose under control conditions. △, $R$ (the normalized ratio of $^3H$ to $^{14}C$ galactose in the tissue); □, cellular galactose concentration; $X$, $J_{13}$; 0, $J_{31}$; ■, net galactose flux. Bars are ±1 SEM and the points are means of 5 or 6 experiments at each concentration

range of 5 to 20 mM. The net flux of galactose reaches a maximum value at approximately 20 mM external galactose.

The unidirectional fluxes across the individual cell boundaries, calculated as described in the theoretical section using the data in Fig. 2, are shown in Fig. 3 as functions of external or cellular galactose concentration. The flux $J_{12}$, from mucosal solution into cells, is convexly related to galactose concentration in the mucosal solution while the flux $J_{32}$ from serosal solution into the cells appears to be an S-shaped function of concentration in the serosal solution. However, further experiments at lower concentrations would be essential to establish this point clearly, and a linear relation be-

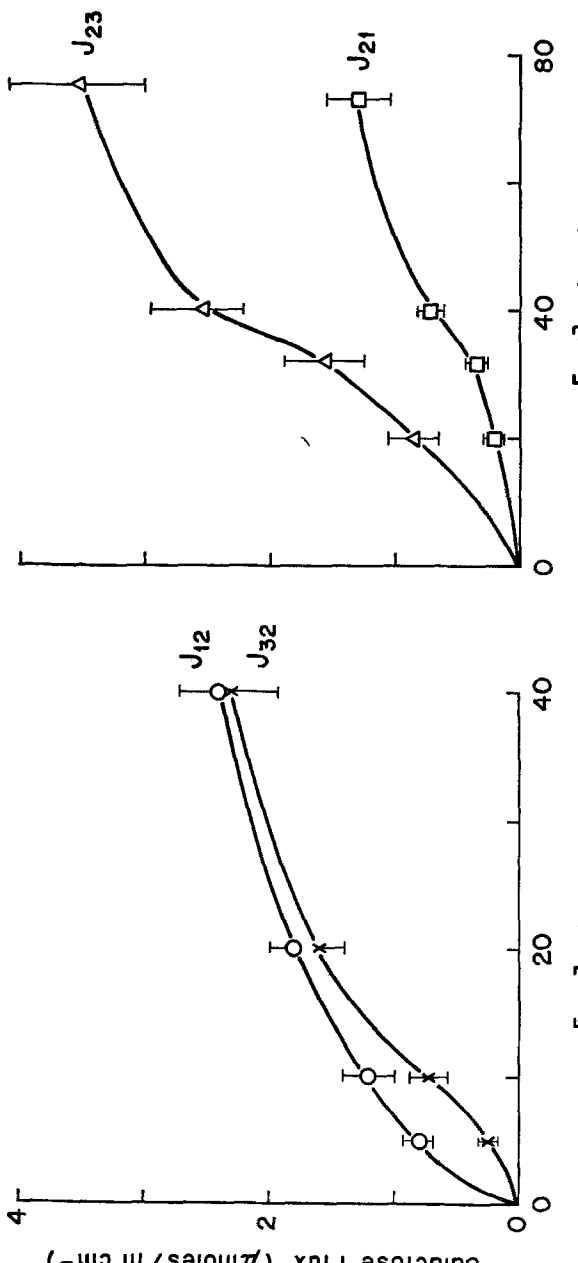


Fig. 3. Calculated unidirectional fluxes across the mucosal and serosal cell boundaries. The points for $J_{21}$ and $J_{23}$ are average values obtained at each of the 4 external galactose concentrations and are plotted against the corresponding average cellular concentration

tween flux and concentration cannot be excluded. The fluxes from the cells toward the external solutions, $J_{21}$ and $J_{23}$, are plotted against the average estimated tissue concentration calculated assuming an extracellular space of 30% of tissue wet weight. Because of the washing procedure used, the tissue concentration may be somewhat underestimated. In terms of these average values, both fluxes appear to bear an S-shaped relationship to cellular concentration. However, the errors in flux estimates and uncertainties in concentration again make it impossible to exclude a linear relationship (*see below*). There is a clear asymmetry of fluxes across the mucosal border of the cells. That is, the effective permeability coefficient for galactose movement from mucosal solution to cell, $P_{12}=J_{12}/[\text{gal}]_0$ is significantly greater ($p<0.001$) than the corresponding coefficient for flux in the opposite direction, $P_{21}=J_{21}/[\text{gal}]_c$. There is also some suggestion of asymmetry at the serosal boundary since $J_{32}$ is significantly larger than $J_{23}$ at 20 mM galactose (*see below*).

Experiments were carried out under four other conditions for comparison to the above control results. These conditions were: (a) replacement of all Na in the bathing solutions by choline; (b) addition of ouabain ($10^{-4}$ M) to the serosal solution; (c) addition of mannitol (100 mM) to the serosal solution; and (d) addition of both mannitol and ouabain to the serosal solution. Figs. 4 and 5 show effects of these treatments on measured quantities. Na-free conditions and mannitol plus ouabain reduce the ratio $R$ to values below unity indicating that under these conditions more than 50% of the cellular galactose comes from the serosal solution. Serosal mannitol alone does not change $R$ significantly from control levels while ouabain alone reduces $R$ significantly ($p<0.01$) below the control value at 5 mM galactose but not at higher concentrations. Cellular accumulation of galactose is unaffected by serosal mannitol alone but is virtually abolished by the three other treatments. Actually, the calculated cellular concentrations under these conditions were, in most cases, significantly higher than those in the external solutions but the differences were quite small.

As shown in Fig. 5, serosal mannitol produced values of $J_{13}$ consistently higher than control values but the differences were not statistically significant except perhaps at 40 mM galactose ($p<0.05$). The other three treatments reduced $J_{13}$ significantly below control levels. Under Na-free conditions, $J_{13}$ was a linear function of galactose concentration, but in the two ouabain-treated conditions some indication of saturation remained. The flux $J_{31}$ was not markedly altered by any of the experimental conditions. Exceptions occurred at 5 mM external galactose, where all conditions except serosal mannitol led to fluxes significantly higher than control values, and

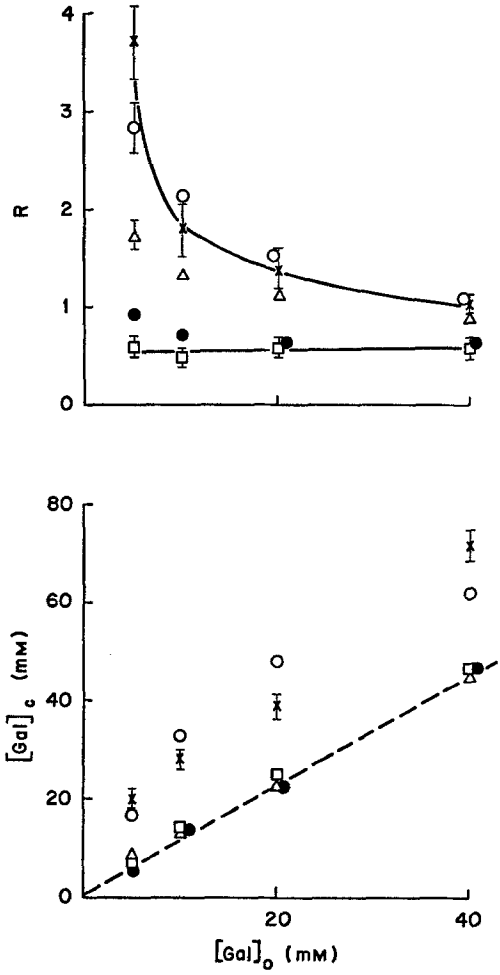


Fig. 4. The effect of experimental conditions on $R$ and cellular galactose concentration. $X$, control; 0, serosal mannitol; □, Na-free solution; △, ouabain; ●, serosal mannitol plus ouabain. Some points are displaced slightly along the X-axis for clarity

at 40 mM galactose where $J_{31}$ in the presence of serosal mannitol was significantly higher than the control value. There appeared to be a linear relation between flux and serosal concentration and the line in Fig. 5 has been drawn through the average value of all fluxes at each galactose concentration. Net galactose flux across the tissue was virtually abolished by Na-free solution and in the presence of ouabain or mannitol plus ouabain. Net flux in the presence of serosal mannitol did not differ significantly from that observed under control conditions.

The unidirectional fluxes across the individual cell borders calculated from these data are shown in Figs. 6 through 9 as functions of the appro-

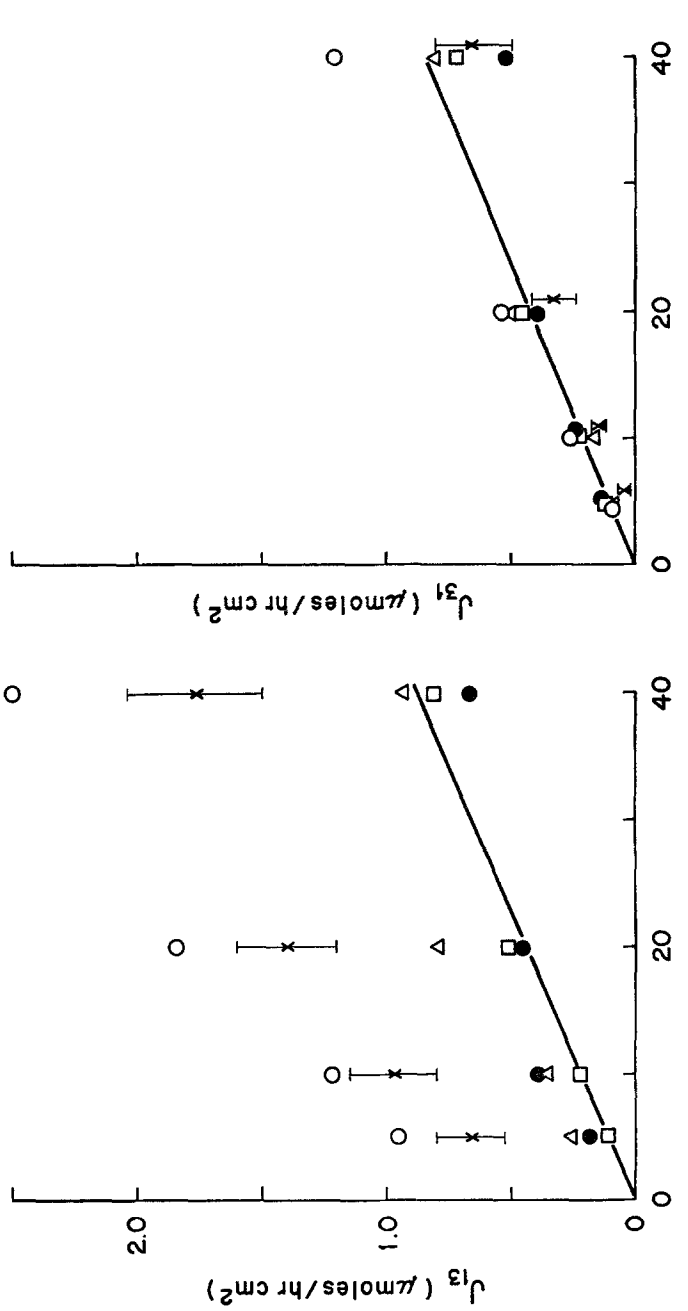


Fig. 5. Effect of experimental conditions on transmural galactose fluxes. Symbols as indicated in the legend to Fig. 4. For $J_{13}$, the line is drawn through the points obtained with Na-free solution; for $J_{31}$ through the mean flux for all conditions

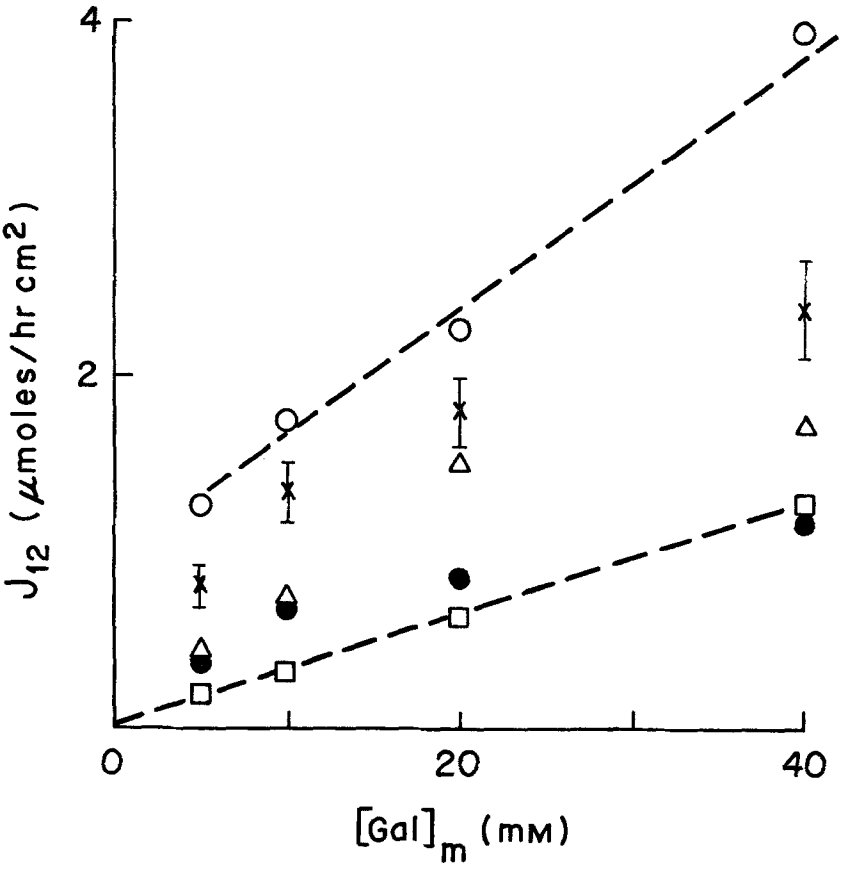


Fig. 6. $J_{12}$ under various experimental conditions. Symbols as indicated in the legend to Fig. 4

priate galactose concentrations. Fig. 6 summarizes flux from mucosal solution into the cells $J_{12}$. This flux was consistently higher in the presence of serosal mannitol than in controls but the difference is statistically significant only at 40 mM galactose. Between 5 and 40 mM galactose, $J_{12}$ was a linear function of concentration in the presence of serosal mannitol but not under control conditions. In the absence of Na, $J_{12}$ was markedly reduced and became a linear function of concentration. Similar behavior was observed by Goldner, Hajjar and Curran [6] for 3-O-methyl glucose in experiments in which $J_{12}$ was measured directly. Treatment of the tissue with ouabain, either with or without serosal mannitol, also caused a reduction in $J_{12}$. However with ouabain alone, the effect was clearly less than that caused by removal of Na from the bathing solutions.

Fig. 7 shows values of the flux from cell to mucosal solution, $J_{21}$, as a function of cellular concentration of galactose for control conditions and

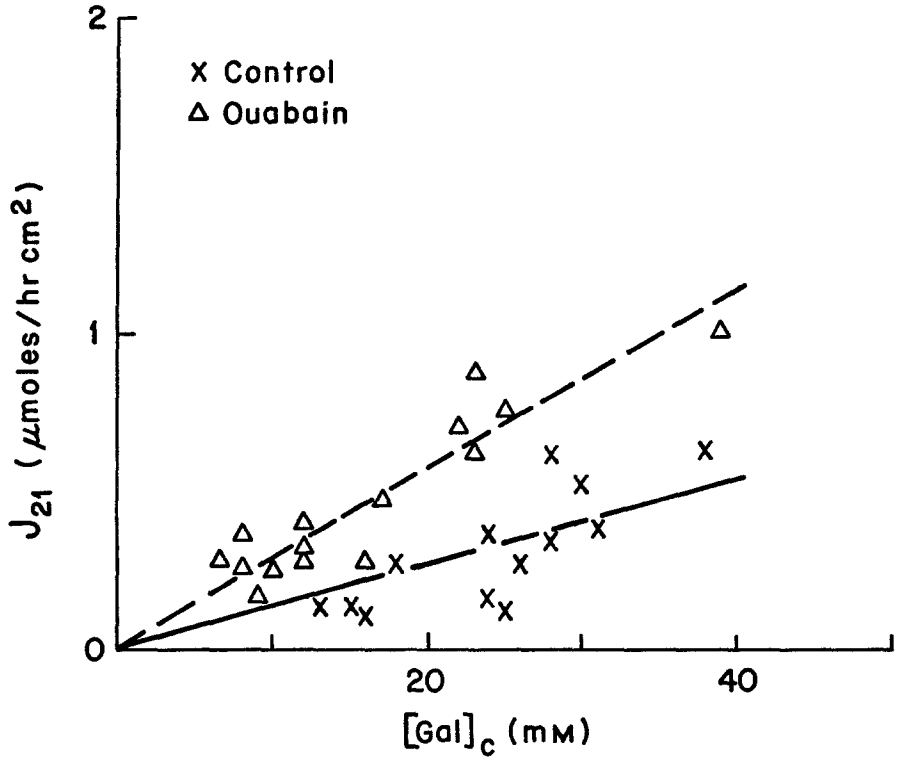


Fig. 7. $J_{21}$ under control conditions and in the presence of ouabain as functions of cellular galactose concentration. Lines were determined by least squares. Each point represents a determination on a single tissue

for tissues treated with ouabain. The flux appears to be linearly related to concentration, at least up to 40 mM and the slope of the line defines an effective permeability coefficient, $P_{21}$. The data obtained under control conditions and with serosal mannitol suggest that there may be an increase in $P_{21}$ at cellular galactose concentrations above approximately 40 mM. Consequently, only data obtained below 40 mM have been used to evaluate $P_{21}$ in order to have comparable information for all experimental conditions. Table 1 summarizes the resulting values of $P_{21}$. In all five conditions, the intercept of the least-squares line through the points did not differ significantly from zero. Consequently, slopes were determined by assuming that the lines pass through the origin. Ouabain causes a significant increase in $P_{21}$ in both the presence and absence of serosal mannitol and $P_{21}$ is also greater in the absence of Na than in its presence.

Fig. 8 shows the flux from cell to serosal solution, $J_{23}$, as a function of cellular galactose concentration for control conditions. The data show

Table 1. Permeability coefficients for $J_{21}$

| Condition | $P_{21}$ ($\pm$SEM) (cm/hr) |
|---|---|
| Control (14) | $0.015 \pm 0.002$ |
| Choline (14) | $0.027 \pm 0.003$ |
| Ouabain (15) | $0.029 \pm 0.002$ |
| Mannitol (12) | $0.017 \pm 0.002$ |
| Mannitol + ouabain (15) | $0.029 \pm 0.003$ |

Number of observations are given in parentheses.

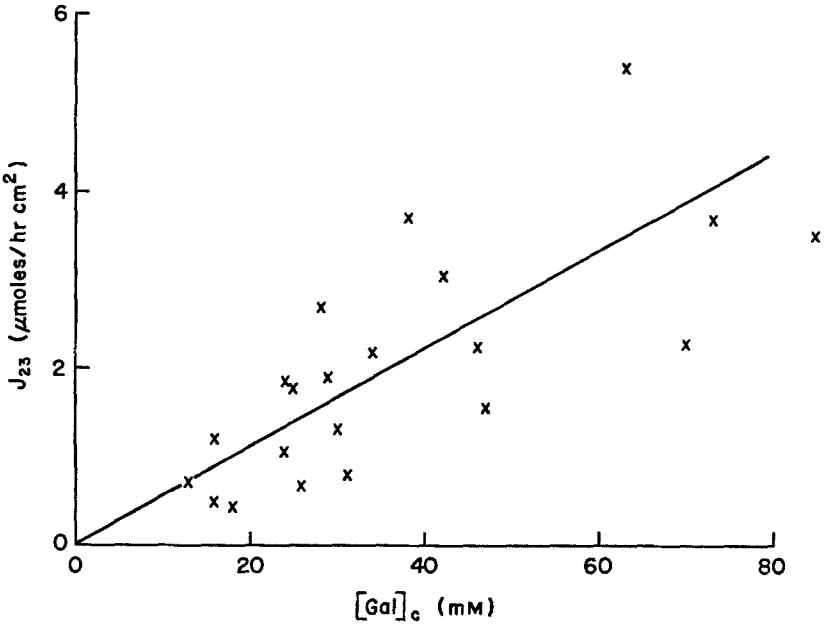


Fig. 8. $J_{23}$ as a function of cellular galactose concentration. Each point represents a single tissue. The line is the least-squares line fitted through the origin

considerable scatter but can be described reasonably well by a straight line; in this case the apparent permeability coefficient does not appear to increase at high cellular galactose concentrations. Similar results were obtained under the other experimental conditions and are summarized in Table 2 in terms of a permeability coefficient, $P_{23}$. Addition of mannitol to the serosal solution causes a significant increase in $P_{23}$ above control levels and addition of ouabain to the mannitol-containing solution reduces $P_{23}$ to the control level. Choline and ouabain alone do not alter $P_{23}$ from the control value.

Table 2. Permeability coefficients of the serosal membrane

| Condition | $P_{23}$ (cm/hr) | $P_{32}$ (cm/hr) | $P_{32}/P_{23}$ |
|---|---|---|---|
| Control (21) | 0.055±0.005 | 0.062±0.007 | 1.18+0.10 |
| Choline (18) | 0.052±0.003 | 0.064±0.004 | 1.27±0.03 |
| Ouabain (19) | 0.050±0.008 | 0.048±0.006 | 1.04±0.06 |
| Mannitol (21) | 0.069±0.005 | 0.087±0.005 | 1.36+0.14 |
| Mannitol (18)+ouabain | 0.045±0.005 | 0.052±0.005 | 1.12±0.05 |

Number of observations are given in parentheses. Errors are SEM.

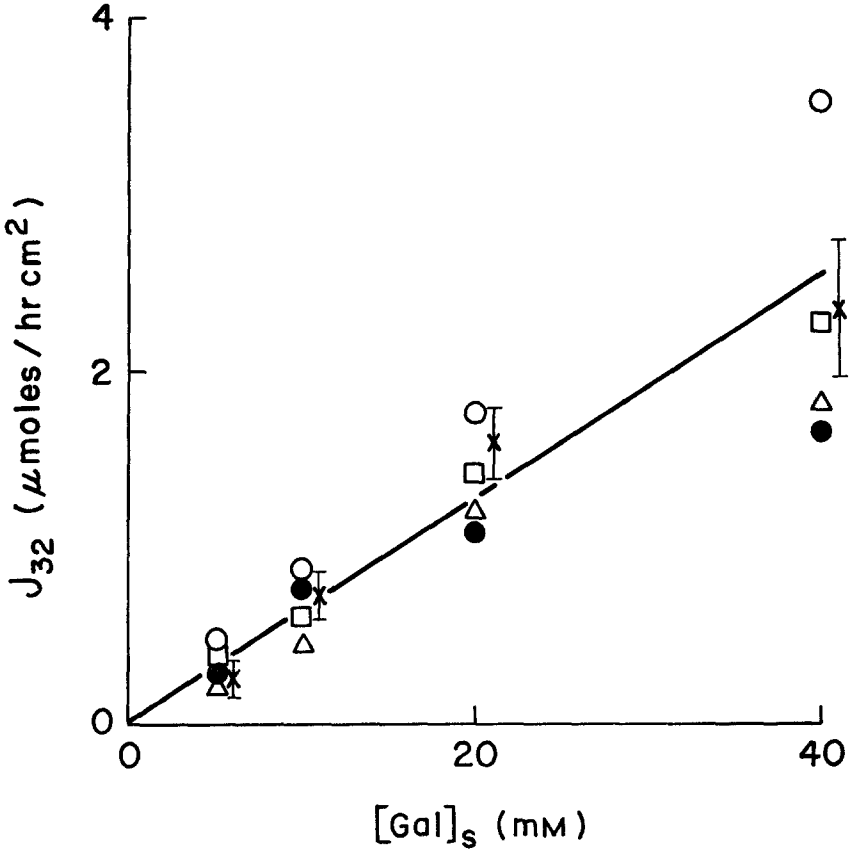


Fig. 9. $J_{32}$ as a function of external galactose concentration. Symbols are described in the legend to Fig. 4. Control values ($X$), ±1 SEM, are displaced along the X-axis for clarity. The line is the least-squares line through the control points

Fig. 9 shows the relationship between flux from serosal solution into the cells as a function of serosal galactose concentration. Within the limits of error there is a linear relationship between flux and concentration and the

calculated values of $P_{32}$ are summarized in Table 2. Addition of mannitol to the serosal solution increases $P_{32}$ significantly above control levels and this increase is abolished by addition of ouabain. Neither ouabain alone nor replacement of Na by choline alter $P_{32}$ significantly from the control levels. Thus, the effects of the various experimental conditions on the fluxes $J_{23}$ and $J_{32}$ are essentially identical.

Although the fluxes across the serosal barrier appear to be linearly related to concentration, the data suggest that they may not be the result of simple diffusion. As shown in Table 2, the ratio of $P_{32}/P_{23}$ is significantly greater than unity for all experimental conditions except treatment with ouabain. Thus, the flux ratio at this membrane is not equal to the ratio of concentrations in the serosal solution and the cell. The discrepancy may also be underestimated if the washing procedure has led to an underestimate of cellular galactose concentration.

## Discussion

The present analysis assumes that all radioactivity measured in the solutions and tissue represents galactose. There appears to be little information on metabolism of galactose by rabbit intestine but the assumption is entirely consistent with observations on rat small intestine. Galactose is metabolized by this tissue, but the rates of conversion to lactate [21], $CO_2$ [8] and galactose-1-phosphate [4, 15] could account for at most a few percent of the observed fluxes or tissue levels of radioactivity under our experimental conditions. In addition, Olsen and Rosenberg [12] have reported for rat intestine that after a 60-min incubation in very low concentrations of $^{14}$C-galactose over 90% of the tissue $^{14}$C is in the form of galactose. The effects of metabolism should be substantially less at the much higher concentrations used in the present study. Finally, we have obtained similar results in a few experiments on rabbit ileum. After 2 hr of incubation in Ringer's containing 20 mM galactose or Na-free choline medium containing 5 mM galactose no significant production of $^{14}$C lactate could be detected and well over 90% of tissue radioactivity migrated as galactose on paper chromatographs.

The method described in the theoretical section for obtaining unidirectional fluxes of galactose across the individual cell borders gives results that are consistent with those obtained earlier [6, 7] by direct measurement of influx across the brush border, $J_{12}$ in our notation. Goldner *et al.* [7] found that $J_{12}$ for galactose at a concentration of 20 mM was $2.3 \pm 0.5$ μmoles/hr

$cm^2$ while the value obtained by the present method was $1.8 \pm 0.2$ μmoles/hr $cm^2$. However, at first glance, there appears to be a serious discrepancy between the present results and those of Goldner *et al.* The relationship between $J_{12}$ and galactose concentration in the mucosal solution can be described reasonably well by a rectangular hyperbola having a half-maximal concentration $K_t$ of 18 mM. This value of $K_t$ is approximately 3 times higher than the value of 5.7 mM found by Goldner *et al.* on the basis of direct measurement of $J_{12}$ for galactose. This apparent discrepancy can be explained on the basis of the conclusion of Goldner *et al.* [7] that sugar influx across the brush border in the absence of Na can be ascribed to simple diffusion and does not involve a mediated process. The present results (Fig. 4) showing a linear relationship between $J_{12}$ and galactose concentration under Na-free conditions are entirely consistent with this conclusion. If we assume that $J_{12}$ can be described by a relationship of the form

$$J_{12} = \frac{J_{12}^m [G]_m}{K_t + [G]_m} + \alpha [G]_m$$

the data can best be fitted if $J_{12}^m = 1.6$ μmoles/hr $cm^2$, $K_t = 5.3$ and $\alpha = 0.025$ cm/hr. The value of $K_t$ agrees well with that given by Goldner *et al.* and the value of $\alpha$ is close to the value of 0.031 cm/hr obtained from the slope of the line relating $J_{12}$ to $[G]_m$ under Na-free conditions (Fig. 6). Thus, the present method appears to yield values of $J_{12}$ that agree reasonably well with those obtained by direct measurements.

Goldner *et al.* [7] did not estimate the other unidirectional fluxes of galactose but they did calculate the four fluxes of 3-O-methyl glucose at an external concentration of 20 mM. In rabbit ileum with intact serosal and muscle layers, the 3-MG fluxes across the mucosal boundary were appreciably greater than those across the serosal boundary. In the preparation used here, with muscle layers removed, galactose fluxes across the serosal cell border are similar to those across the mucosal border. Actually, $J_{12} \approx J_{32}$ and $J_{23} > J_{21}$. This apparent increase in fluxes across the serosal border upon removal of muscle layer is consistent with the observations of Hajjar, Lamont and Curran [9] on effects of muscle layers on alanine fluxes in rabbit ileum and with the finding of Smulders and Wright [19] that removal of the muscle layers from hamster intestine leads to a marked increase in galactose flux from mucosa to serosa. These results indicate that the muscle layers provide a significant barrier to diffusion of solute leading to a retardation of flux across the operationally defined serosal barrier. The assumption that the epithelium acts as a single compartment with respect to galactose

is common to the present approach and to other approaches to estimation of unidirectional fluxes. With the present technique, the errors inherent in the assumption of a single compartment are spread over all four fluxes. In the approach used previously, $J_{12}$ was measured directly so that any errors with respect to the assumption of a single compartment are confined to the three other fluxes[1]. However, the present method has the advantage of requiring only a single piece of tissue to estimate all four fluxes while the previous approach involved measurements on 3 or 4 separate pieces of tissue.

Certain aspects of the present results are similar to those reported recently by Bihler and Cybulsky [1] on galactose movement across the basal/lateral membranes of epithelial cells from hamster intestine. They blocked the brush border transport system with $HgCl_2$ then isolated cells and studied galactose uptake. Their results indicate that under control conditions with 0.5 mM galactose, 5 to 6 times as much sugar enters the cells from the mucosal side as from the serosal side while in the absence of Na, the reverse is true. These results are similar to those obtained in the present study (as indicated by the ratio $R$) and suggest that our assumption of a single pool for galactose is reasonable.

### *Effect of Serosal Hypertonicity on Galactose Fluxes*

Previous observations [11] have shown that there is a rectification of osmotic water flow in bullfrog intestine; hypertonic serosal solutions had a much greater effect on net water flow than the same degree of hypertonicity of the mucosal solution. Recently, DiBona [3] has shown in toad bladder that the lateral intercellular spaces enlarge following addition of hypertonic solutions to the serosal side and contract when the mucosal solution is made hypertonic to the serosal solution. Smulders, Tormey and Wright [18] have obtained similar results in rabbit gallbladder. In the present case, effects of a hypertonic serosal solution cannot be interpreted simply in terms of osmotic flow across a two-barrier series system. With ouabain present, there is virtually no net galactose flux when the serosal solution contains 100 mM mannitol indicating minimal solvent drag. In addition, mannitol in the serosal solution causes an increase in both bidirectional transmural fluxes of galactose with little change in net flux. This effect could be due to

1 If the tissue contains a compartment for galactose that is not involved in transmural transport, the estimate of $R$ for the transport compartment will be in error leading to errors in calculated unidirectional fluxes. However, because of the nature of Eqs. (10)–(13), errors in fluxes will be proportionally less than errors in $R$. A 10% error in $R$ will, under the most unfavorable conditions, lead to errors in calculated fluxes ranging from 2 to 7%.

an increase in volume of the lateral intercellular space with a concomitant increase in effective area of the lateral and serosal membrane leading to an apparent increase in effective galactose permeability. Alternatively, the lateral space itself could provide a significant resistance to galactose movement (*see*, for example, recent studies on gallbladder [18]) which is decreased with distension of the space.

The following experimental data support this hypothesis: (a) Mannitol increases the serosal membrane permeability to galactose both for entry and exit; (b) this increase is reduced by the action of ouabain; (c) ouabain has no significant effect on the serosal membrane permeability of control tissue, hence the efficacy of ouabain in reducing serosal membrane permeability to galactose is contingent on the presence of mannitol in the serosal fluid; (d) the serosal influx permeability to galactose, $P_{32}$, which is not significantly dependent on the concentration of galactose in the serosal fluid, correlates positively with net galactose flux across the tissue ($p < 0.01$) and this correlation is maintained even when net flux is reduced by ouabain ($p < 0.01$); there is no significant correlation between net flux and $P_{32}$ with mannitol present in the serosal fluid, but when the tissue transport activity is reduced by ouabain in the presence of mannitol the correlation between net flux and $P_{32}$ returns ($p < 0.01$). Mannitol also appears to increase the accessibility of the serosal membrane to ouabain since the inhibition of $J_{13}$ and $J_{12}$ caused by ouabain is significantly greater with mannitol present in the serosal solution.

These findings are consistent with the view that access to the serosal membranes of the intestinal epithelium from the serosal fluid is dependent on net galactose flux. Hypertonic serosal solution increases the accessibility of the epithelial membranes to a limit beyond which no additive effect of mannitol and net flux occurs. The increased accessibility of the epithelial serosal membrane caused by hypertonic mannitol appears to depend on the ouabain-sensitive transport activity of the tissue. A possible interpretation of these findings is that the paracellular space is expanded to a limit by the action of the tissue transport systems and this action is enhanced by mannitol. If net transport is reduced by an inhibitor, the space collapses and access to the serosal surface of the epithelium from the serosal fluid is reduced (*cf.* Smulders *et al.* [18]).

### *Effects of Choline and Ouabain*

The effects of addition of ouabain or complete removal of Na from the bathing solutions are, in many respects, quite similar to those obtained previously. Both treatments reduce net transmural galactose flux to near

zero and essentially prevent cellular accumulation of the sugar [6, 7]. The flux $J_{12}$ is reduced by both treatments but the effect of Na removal is substantially greater than that of ouabain. Similar results were obtained by Goldner *et al.* [6] for 3-O-methyl glucose in experiments in which $J_{12}$ was measured directly. The flux in the opposite direction across the mucosal membrane, $J_{21}$, was increased relative to control values by both treatments; i.e., the effective permeability coefficient, $P_{21}$, was increased significantly at cellular galactose concentrations below 40 mM. Addition of ouabain in the presence of serosal mannitol had a similar effect since $P_{21}$ (mannitol) $< P_{21}$ (mannitol + ouabain). These effects of ouabain might be expected on the basis of the "Na-gradient hypothesis" for sugar transport [2] since according to this hypothesis the elevation of cellular Na concentration caused by ouabain should lead to an increased efflux of sugar across the brush border membrane. The effect of choline is not, however, that expected on the basis of this hypothesis because the reduction in cell Na caused by incubation in Na-free solution should lead to a *decrease* in $P_{21}$ rather than an increase.

However, there are factors involved in these experiments that may complicate interpretation of fluxes across individual membranes in terms of mechanism and at least one of these may have a substantial effect on $J_{21}$. In their studies of efflux of alanine across the brush border of rabbit intestine, Hajjar *et al.* [9] found that $^{14}$C-alanine leaving the cell tended to be "recaptured" and hence appeared slowly in the mucosal solution. The effect was relatively large when the mucosal solution contained 140 mM Na and a low concentration of alanine but was minimal if all Na was removed from the solution or if alanine concentration was high. Although such experiments have not been done with sugars, similar effects might be expected and they should influence the transmural flux in the same manner. In fact such an effect would provide an entirely adequate explanation of the tendency toward a concave relationship between $J_{31}$ and external galactose concentration observed in control tissues and in the presence of serosal mannitol. The observation that this concave relationship disappears in the absence of Na would also be expected. Thus, while the measured $J_{31}$ is the "true" value for the experimental conditions, its interpretation in terms of membrane properties may be relatively complex. Since $J_{21}$ depends directly on $J_{31}$ [Eq. (12)], it is most susceptible of the four calculated fluxes to variations in $J_{31}$. For example, at 5 mM external galctose, under control conditions, doubling $J_{31}$ will double the calculated value of $J_{21}$ but will increase the values of $J_{12}$ and $J_{32}$ by less than 20%. Thus, a possible explanation of the observation that removal of Na increases the values of $P_{21}$ may be that this experimental maneuver markedly decreases the "recap-

ture" of isotope moving from cell to mucosal solution. Such behavior may make it possible to interpret the effects of Na replacement by choline in terms of the Na-gradient hypothesis. However, it may become necessary to find an alternative explanation of these data that requires a more radical departure from the existing theory.

### *Galactose Transport Across the Serosal Membrane*

As indicated in Table 2, the ratio of galactose fluxes across the serosal membrane is slightly but significantly greater than the concentration ratio suggesting that the process may not be due to simple diffusion. The difference may actually be underestimated since the washing procedure may lead to an underestimate of cellular concentration and, as discussed above, $J_{32}$ could be somewhat underestimated under some conditions. Both of these effects will tend to increase the ratios $P_{32}/P_{23}$ given in Table 2. Such an asymmetry in fluxes could be due to the presence of a facilitated transfer mechanism or an active transport system for the uptake of galactose at the serosal membrane. The present data do not permit a clear distinction between these possibilities, in part because calculated cellular galactose concentration was almost always greater than concentration in the serosal solution and net flux was down a concentration difference. However, in 6 out of 21 experiments under control conditions and in 7 of 21 experiments with serosal mannitol, sufficient $^{14}C$ entered the cells from the serosal side to elevate cellular concentration above that in the serosal solution. If all tissue $^{14}C$ is in the form of galactose, these results suggest that an active uptake of galactose at the serosal side might be possible. It is of interest to note the accumulation of $^{14}C$-galactose was not observed in the absence of Na or in the presence of ouabain. On the other hand, if there is an active uptake step at the serosal membrane, it does not appear to be strongly Na-dependent because neither $P_{23}$ nor $P_{32}$ are altered significantly by Na removal (Table 2). Bihler and Cybulsky [1] also found that sugar movement across the basal/lateral membrane of hamster mucosal cells was Na-independent but probably involves a mediated transfer process. They indicated, however, that in preliminary experiments they were unable to show that the system could cause cellular accumulation of sugars against a concentration difference. The concept of a ("weak") active uptake mechanism at the serosal side of the intestine is not unprecedented. Kinter and Wilson [10] proposed such a system nearly 10 years ago on the basis of their radioautographic studies of 3-O-methyl glucose and galactose transport by hamster intestine. These observations, plus the finding that the flux of galactose into the cells from

the serosal solution is of the same magnitude as influx from the mucosal solution, raise a number of questions regarding interpretation of studies of sugar transport by isolated intestinal cells or by tissue slices. In these situations in which the solute has access to both sides of the cell, effects of experimental manipulations cannot be assigned with certainty to one or the other cell border.

The present studies have provided results, in a single set of experiments, that appear to be reasonably consistent with conclusions regarding sugar transport drawn from a variety of other studies. In particular, the patterns of cellular accumulation of sugar and of influx across the brush border are similar to those previously reported for rabbit ileum. They also provide the first specific information regarding the other three unidirectional fluxes across the cell borders and suggest that this approach may be useful in obtaining more detailed information on the characteristics of various transport systems in epithelia.

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