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# AMINO ACID TRANSPORT BY THE SMALL INTESTINE OF THE RAT. EFFECTS OF GLUCOSE ON TRANSINTESTINAL TRANSPORT OF PROLINE AND VALINE

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SUMMARY

The effects of galactose and glucose on transport of proline and valine by the rat small intestine have been studied using an everted sac preparation.

1. Two magnitudes of sacs were used; small sacs 7–8 cm long and large sacs 17 cm long.

2. Galactose inhibited the transport of both amino acids.

3. Glucose eliminated completely the inhibitory effect of galactose.

4. Glucose did not influence the degree of tissue accumulation or the serosal concentrations of proline or valine found at the end of a 60-min incubation.

5. Glucose increased markedly the net flux of water to the serosal fluid.

6. In the small sacs with relatively small increments of the serosal fluids the transport of amino acid was not significantly enhanced in the presence of glucose. But in the large sacs with a prodigious increase in serosal volume a highly significant enhancement was found.

7. The glucose effect on amino acid transport by the large sacs was closely simulated, when the glucose effect on the serosal fluid volume was simulated by different means. This, however, was not the case if the amino acid transport was subject to inhibition by galactose.

8. It is concluded that glucose enhances the transport of amino acids by the rat small intestine by:

(a) providing an energy supplement which allows the amino acid carrier to perform and maintain the control degree of epithelial accumulation in spite of a simultaneous expenditure of energy on sugar accumulation, and by:

(b) increasing the net transintestinal water flux, and so increasing the compartment with which the accumulating epithelium equilibrates.

## INTRODUCTION

Generally, intestinal amino acid transport is inhibited by actively transported sugars[1–3], although, as an exception to this rule, glucose has been found to enhance the transport of amino acids by everted sacs prepared from the small intestine of

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the rat[1]. This inhibitory effect of sugars on intestinal amino acid transport and the observation that amino acids are able to inhibit the transport of sugars[4,5] have been subject to different explanations. It has been proposed[2,4] that sugars and amino acids compete for a common mediator for their passage across the cellular membrane lining the microvilli. Although the sodium dependency of the active intestinal transport of both sugars and amino acids[6] appears to lend it support, this hypothesis has been disproved for both rabbit and rat small intestine. For the rabbit small intestine it has been shown[3] that sugars do not inhibit the mediated influx of amino acids across the brush border. For the rat small intestine[7] it has been demonstrated, that efflux of a previously accumulated amino acid out of an everted sac preparation can be induced by counterflow of other amino acids but not by actively transported sugars. Conversely efflux of an accumulated sugar can be induced by other sugars but not by amino acids. As an alternative hypothesis it has been proposed[1] that the ability of the epithelial cell to provide energy for transport processes is insufficient to allow both the amino acid and the sugar transport systems to function to their maximum capacity at the same time. According to this hypothesis glucose stimulates the amino acid transport of the rat small intestine by serving as a fuel even if another sugar is present at an inhibitory concentration. Small intestinal tissues from rabbit and hamster exhibit much lower glycolytic activities than that of rat intestine[8]. They therefore do not have the ability of the latter to derive energy from added glucose. So it is consistent with the proposal cited above, that in the small intestine of hamster and rabbit glucose like other actively transported sugars inhibits transport of amino acids[2,3]. This evidence supports the idea that limitation of available energy is the cause of sugar-dependent inhibition of intestinal amino acid transport, and that at least a part of the effect glucose has on the transport of amino acids by the rat small intestine depends on the utilization of glucose as a source of energy. However, results obtained in a recent study* of the effect of glucose on transport and metabolism of alanine by the rat small intestine indicated that the water flow induced by glucose through the intestinal wall, contrary to what has been claimed[9], might be of equal importance as a cause of the enhancement of amino acid transport. This notion is supported by the fact that an increase in the serosal volume would increase the amount of amino acid needed to raise its concentration to the point where the backflux to the mucosal fluid would equal the flux from this fluid. Additional support is gained if the effect of glucose on transmural water flow simulates the experimental conditions used by MATTHEWS AND LASTER[10], who during the linear phase of serosal accumulation of amino acids by everted sacs renewed the serosal fluid and by this means obtained transport rates far in excess of those ordinarily obtained with everted sacs. Therefore it was decided to study the relation between the effect of glucose on water movement and its effect on amino acid movement across the wall of the rat small intestine. For this purpose L-proline was chosen because preliminary experiments indicated that this amino acid is little if at all metabolized. L-Valine, which also was found to be only slightly metabolized by the rat intestinal tissues, was also used in the study, because it was desirable to repeat with everted sacs some of the previous experiments[11] that suggested an inhibitory effect of glucose on amino acid transport by a ring preparation of small intestine.

* B. G. MUNCK, unpublished results.

## MATERIALS AND METHODS

D-Sugars and L-amino acids of analytic grades were used. Uniformly labelled L-[$^{14}$C]valine and L-[$^{14}$C]proline were used as tracers. They were found to be chromatographically pure.

Male albino rats with a body weight of 120–150 g, fasted for 24 h, were the test animals. Everted sacs[12] either 7–8 or 17 cm long were prepared from the distal third quarter of the total small intestine. A test tube method similar to the one described by CRANE AND WILSON[13] was used to measure the amino acid transport. The sacs were incubated for 60 min at 37°; the medium bathing the mucosal surface was stirred and oxygenated by a stream of 100 % $O_2$. Samples were taken from reservoirs connected to the mucosal and serosal fluids, into which the fluids were raised by closing the air outlet. The $O_2$ stream vented through a trap for water droplets, which might escape from the test tube, through a known volume of 20 % NaOH in order to trap the $CO_2$ produced by the sac. Except when otherwise stated, the initial serosal volume was 1 ml. When the small (7–8 cm long) sacs were used the initial mucosal volume was 10 ml; with the large (17 cm long) sacs it was 40 ml. In all experiments amino acids and sugars were added to the mucosal fluid only. The incubation medium was KREBS phosphate buffer[14] with a pH of 7.4 modified so that the phosphate concentration was 8 mM. The final serosal volume was measured by emptying the sac into a preweighed test tube and reweighing the tube. The final mucosal volume was measured by weighing the tube used for incubation after removing the sac. Samples of intestinal tissue were extracted by placing them in boiling water immediately after terminating the incubation and boiled for 30 min. Liquid scintillation counting[15] was used to measure the radioactivity of samples from mucosal and serosal fluids and from the NaOH solutions. Samples from tissue dried overnight at 105° were combusted and the combustion products analyzed for radioactivity by liquid scintillation counting[15]. Samples of deproteinized tissue extracts and serosal and mucosal fluids were chromatographed by ascending chromatography on Whatman No. 1 paper. The solvent used was *sec.*-butanol–formic acid–water (75:15:10, by vol.) (ref. 16). The chromatograms were analyzed by a Packard radiochromatogram scanner in order to determine the distribution of radioactivity between the amino acids and their possible metabolites. Semi-quantitative estimates of the per cent distribution were obtained by measuring the area under the curve of each peak. As the degree of metabolism was found to be very low with both amino acids, the serosal and mucosal concentrations were calculated from the radioisotope assays without correction for metabolism. Student's *t*-test[17] has been used for evaluating the results statistically.

## EXPERIMENTS AND RESULTS

### *Metabolism of $^{14}$C-labelled proline and valine*

#### *Proline*

Samples of deproteinized serosal and mucosal fluids from the experiments given in Fig. 1 were analyzed chromatographically. It was found that radioactive metabolites did not appear in any of the fluids in detectable amounts; although it was found that 0.25 ± 0.04 % ($n = 8$) of the initial amount of [$^{14}$C]proline was recovered as $^{14}CO_2$.

TABLE I

SUPPLEMENTING DATA FROM THE EXPERIMENTS OF FIG. 1

| *Mucosal composition* | *Final serosal vol.* (*ml* ± *S.D.*) | *Final mucosal vol.* (*ml* ± *S.D.*) | *Final serosal concn.* (*mM* ± *S.D.*) | *Final mucosal concn.* (*mM* ± *S.D.*) | *Serosal/mucosal concn. ratio* ± *S.D.* | *Tissue dry wt.* (*mg* ± *S.D.*) |
|---|---|---|---|---|---|---|
| 10 mM proline (4) | 0.87 ± 0.02 | 9.20 ± 0.06 | 18.29 ± 1.01 | 6.47 ± 0.41 | 2.8 ± 0.3 | 105 ± 13 |
| 10 mM proline + 28 mM galactose (8) | 0.82 ± 0.04 | 9.09 ± 0.18 | 14.80 ± 2.45<br>$P < 0.05$ | 7.38 ± 0.45<br>$P < 0.01$ | 2.0 ± 0.5<br>$P < 0.02$ | 95 ± 9 |
| 10 mM proline + 28 mM glucose (5) | 1.12 ± 0.10 | 8.79 ± 0.17 | 17.65 ± 1.36 | 6.28 ± 0.75 | 2.9 ± 0.6 | 102 ± 28 |

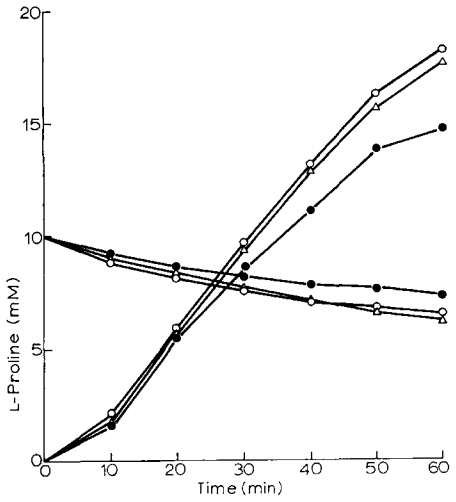


Fig. 1. 7–8-cm-long sacs incubated in 10 ml Krebs phosphate buffer for 60 min at 37° during aeration with 100% $O_2$. Initial serosal fluid, 1 ml Krebs phosphate buffer. Addition to incubation media: ○—○, 10 mM proline ($n = 4$); △—△, 10 mM proline *plus* 28 mM glucose ($n = 5$); ●—●, 10 mM proline *plus* 28 mM galactose ($n = 8$). Plots of serosal concentrations originate from zero, those of mucosal concentrations from 10 mM.

*Valine*

Samples of deproteinized tissue extract and serosal fluid from the experiments the data of which are given in Fig. 2 were analyzed chromatographically. For both of these sources a second peak of radioactivity was found immediately in front of the valine peak. The areas of these secondary peaks varied from below 1 up to 4 % of the areas of the corresponding valine peaks. For the serosal fluids ($n = 10$) the mean area was $2 \pm 2$ %. In these experiments ($n = 10$) the $^{14}CO_2$ production was $1.5 \pm 0.3$ % of the initial amount of radioactivity.

Neither in the proline nor in the valine experiments was the amino acid metabolism affected by the presence of glucose.

*Effects of sugars on proline transport*

*Small sacs* were incubated for 60 min with 10 mM proline, 10 mM proline *plus* 28 mM glucose, or 10 mM proline *plus* 28 mM galactose. Samples were taken every 10 min from both mucosal and serosal fluids (Fig. 1 and Table I). It is seen that glucose did not change the serosal concentration of proline reached during the incubation, nor did it influence the time course of the changes in serosal and mucosal concentrations of proline. By galactose, however, the proline transport was significantly inhibited. Judging from the changes in mucosal concentration this inhibition was significant below the 1 % level at the 30-, 50-, and 60-min samples and below the 2 % level at the 40-min measurement, whereas the effect of galactose on the serosal concentration was significant only at the end of the incubation. The lack of an effect of glucose and the inhibitory effect of galactose are also evident from the final concentration ratios (Table I) and from the estimates of tissue concentrations which are based on the data presented in the table. Assuming a 1:5 dry weight/wet weight ratio for the tissue, these concentrations are 34 mM in the absence of sugar, 37 mM in the presence of glucose, and 23 mM in the presence of galactose.

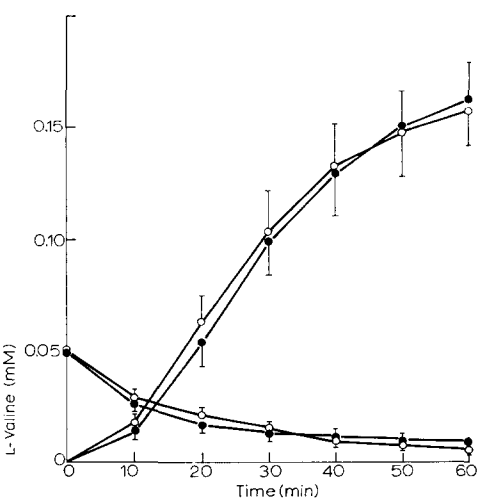


Fig. 2. 7–8-cm-long sacs incubated as stated for Fig. 1. Addition to incubation media: ●—●, 0.05 mM valine ($n = 5$); O—O, 0.05 mM valine *plus* 28 mM glucose ($n = 5$). The bars extending from the points indicate 1 × S.D. Plots of serosal concentrations originate from zero, those of mucosal concentrations from 0.05 mM.

*Large sacs* were incubated for 60 min with 10 mM proline or 10 mM proline *plus* 28 mM glucose. In these experiments samples were taken from the serosal and mucosal fluids only at the end of the incubation (Table II).

TABLE II

17-CM-LONG SACS INCUBATED IN 40 ml KREBS PHOSPHATE BUFFER FOR 60 min AT 37° DURING AERATION WITH 100% $O_2$

| *Mucosal composition* | *Initial serosal vol.* (*ml*) | *Final serosal vol.* (*ml* ± *S.D.*) | *Final serosal concn.* (*mM* ± *S.D.*) | *Proline disappeared from mucosal fluid* (*μmoles* ± *S.D.*) | *Proline disappeared from mucosal fluid* (*μmoles/g dry wt.* ± *S.D.*) |
|---|---|---|---|---|---|
| 10 mM proline (5)* | 1.00 | 1.08 ± 0.05 | 27.8 ± 2.9 | 81.0 ± 5.0 | 359 ± 46 |
| 10 mM proline + 28 mM glucose (6)** | 1.00 | 1.94 ± 0.20 | 28.1 ± 1.1 | 115.7 ± 10.5 $P < 0.001$ | 454 ± 58 $P < 0.02$ |
| 10 mM proline (4) | 1.90 | 2.03 ± 0.09 | 27.7 ± 2.0 | 102.0 ± 9.2 $P < 0.01$ | 509 ± 74 $P < 0.01$ |
| 10 mM proline (3) | 1.00 + 0.10 × 10 | 2.30 ± 0.21 | 23.0 ± 4.4 | 103.0 ± 12.9 $P < 0.02$ | 515 ± 94 $P < 0.02$ |

* Tissue $^{14}C$ activity: 1654 ± 60 counts/min per mg dry wt. (172 ± 6 μmoles/g dry wt.); $^{14}C$ recovery: 100 ± 1%.

** Tissue $^{14}C$ activity: 1669 ± 106 counts/min per mg dry wt. (173 ± 11 μmoles/g dry wt.); C recovery: 98 ± 1%.

The most striking observation from these experiments was that the serosal concentrations were the same whether glucose had been present or not. But since glucose caused a more than 90% increase in serosal volume, compared to a less than 10% increase during incubation with proline alone, the overall effect of glucose was by any criterion except that of serosal concentrations a significant enhancement of the proline transport. The data presented also show that the glucose-dependent increase in disappearance of proline from the mucosal fluid is very close to the amount of proline needed to fill the glucose-dependent increase in the serosal volume to the proline concentration achieved in the glucose-free experiments. As in the previous experiments the tissue uptake was unaffected by the presence of glucose.

*Effects of sugars on valine transport*

*Small sacs* were incubated for 60 min with 0.05 mM valine or 0.05 mM valine *plus* 28 mM glucose. Samples were taken at 10-min intervals from both serosal and mucosal fluids (Fig. 2 and Table III).

It was found that the transport and final tissue uptake of valine from this very low concentration were uninfluenced by the presence of glucose.

*Large sacs* were incubated for 60 min with 10 mM valine, 10 mM valine *plus* 28 mM glucose, 10 mM valine *plus* 28 mM galactose, or at 10 mM valine *plus* 28 mM galactose *plus* 28 mM glucose. Samples were taken at the end of the incubation (Table IV).

It was found that glucose significantly enhanced the transport of water and of valine, whether the transport of the amino acid was measured as μmoles or μmoles/g dry weight disappearing from the mucosal fluid, or as μmoles entering the serosal

TABLE III

SUPPLEMENTING DATA FROM THE EXPERIMENTS OF FIG. 2

| *Mucosal composition* | *Final serosal vol.* (*ml* ± *S.D.*) | *Final mucosal vol.* (*ml* ± *S.D.*) | *Final serosal concn.* (*mM* ± *S.D.*) | *Final mucosal concn.* (*mM* ± *S.D.*) | *Serosal/mucosal concn. ratio* ± *S.D.* | $^{14}C$ *activity in tissue* (*counts/min per mg dry wt.* ± *S.D.*) |
|---|---|---|---|---|---|---|
| 0.05 mM valine (5)* | 0.83 ± 0.02 | 9.19 ± 0.15 | 0.162 ± 0.017 | 0.008 ± 0.002 | 21 ± 6 | 8635 ± 1126 |
| 0.05 mM valine + 28 mM glucose (5)** | 1.08 ± 0.17 | 8.92 ± 0.13 | 0.157 ± 0.016 | 0.006 ± 0.002 | 29 ± 9 | 7794 ± 1582 |
| | | | | | | $P > 0.3$ |

* $^{14}C$ recovery: 82 ± 5%.
** $^{14}C$ recovery: 81 ± 4%.

TABLE IV

17-CM-LONG SACS INCUBATED AS STATED IN THE LEGEND FOR TABLE II

The initial serosal volumes were 1 ml or as stated in the table.

| *Mucosal composition* | *Final serosal vol.* (*ml* ± *S.D.*) | *Final serosal conc.* (*mM* ± *S.D.*) | *Valine appearing in serosal fluid* (*μmoles* ± *S.D.*) | *Valine disappeared from mucosal fluid* (*μmoles* ± *S.D.*) | *Valine disappeared from mucosal fluid* (*μmoles/g dry wt.* ± *S.D.*) |
|---|---|---|---|---|---|
| 10 mM valine (8) | 1.24 ± 0.13 | 30.66 ± 5.36 | 39.2 ± 9.5 | 103.6 ± 15.6 | 490 ± 70 |
| 10 mM valine + 28 mM galactose (6) | 1.14 ± 0.09 | 24.22 ± 2.50 | 27.8 ± 4.6 | 79.5 ± 9.4 | 374 ± 60 |
| | | $P < 0.02$ | $P < 0.02$ | $P < 0.01$ | $P < 0.01$ |
| 10 mM valine + 28 mM galactose + 28 mM glucose (5) | 1.86 ± 0.26 | 32.84 ± 2.41 | 61.6 ± 12.4 | 130.2 ± 16.4 | 599 ± 95 |
| | | | $P < 0.01$ | $P < 0.02$ | $P < 0.05$ |
| 10 mM valine + 28 mM glucose (5) | 2.05 ± 0.30 | 30.26 ± 5.42 | 64.1 ± 20.0 | 130.9 ± 32.1 | 599 ± 83 |
| | | | $P < 0.02$ | $0.1 > P > 0.05$ | $P < 0.05$ |
| 10 mM valine with initial serosal fluid: 1.90 ml (4) | 2.39 ± 0.16 | 33.94 ± 4.00 | 81.7 ± 15.3 | 151.0 ± 14.0 | 757 ± 176 |
| | | | $P < 0.001$ | $P < 0.001$ | $P < 0.01$ |
| 10 mM valine + 28 mM galactose, initial serosal fluid: 1.90 ml (2) | 2.09 ± 0.23 | 20.30 ± 1.62 | 42.6 ± 7.9 | 88.0 ± 5.0 | 411 ± 160 |

fluid. Galactose inhibited the valine transport, and the addition of glucose to the valine–galactose mixture restored valine transport to the level found in the presence of glucose. It was found, however, that glucose neither as only sugar present nor when counteracting the inhibitory effect of galactose raised the valine concentration above the level found in the absence of any sugar. It was also found that the glucose-dependent increase in valine disappearance from the mucosal fluid agreed well with the amount needed to fill the glucose-dependent increase in serosal volume to the concentration reached in the sugar-free experiments.

*Effects on amino acid transport of simulating the glucose effect on the serosal volume*

*With proline as substrate* this was done either by adding 100 $\mu$l amino acid-free Krebs buffer to an initial serosal volume of 1 ml at 5-min intervals for 50 min or by using an initial serosal volume of 1.9 ml. From Table II it is seen that these procedures increased the proline transport as did the presence of glucose.

*With valine as substrate* only the latter of these procedures was used, both in the presence and absence of galactose (Table IV). In the absence of galactose the effect on the valine transport was similar to the glucose effect. In the presence of galactose, however, the glucose-simulating effect was only partial, as the concentrating ability of the preparation was not restored to control value.

## DISCUSSION

The data presented here show that galactose inhibits the transintestinal transport of amino acids confirming results obtained by other investigators[1]. The possible mechanisms of this inhibition have been discussed[2,7,11,18,19]. Since galactose shares this inhibiting ability with other actively transported sugars, some of which are non-metabolizeable, and since evidence exists[3,7] that none of the inhibiting sugars tested competes with amino acids for mediated passage across the brush border, it seems most likely that the reason for this inhibiting effect consists in an inability of the intestinal preparation *in vitro* to supply the energy needed for the otherwise observable amino acid accumulation to be reached, when at the same time a sugar has to be accumulated[1].

The substitution of one of the inhibiting sugars by glucose or the addition of glucose to the amino acid–sugar-containing mucosal fluid will not decrease the saturation degree of the sugar carrier, neither will it then decrease the need of energy expenditure. When these changes in the sugar composition of the mucosal fluid nevertheless, as found in this study, restore to normal the amino acid accumulating ability of the rat small intestine, then it seems that the explanation as proposed by NEWEY AND SMYTH[1] must be that glucose serves as a fuel for the transport mechanisms for sugars and amino acids. However, in order to do so glucose has to enter the glycolytic pathway; this explanation therefore gains support from the observations that the tissues of the rat small intestine have a high glycolytic capacity compared to those of the rabbit and hamster small intestinal tissues[8], the amino acid accumulating capacities of which are inhibited by glucose as well as by other actively transported sugars[2,3].

Explained in this way the effect of glucose on the amino acid transport by the rat small intestine may be regarded as stimulatory, although being effective through

an increase in energy available for transport purposes it is a rather indirect kind of stimulation, which furthermore must apply to both the sugar and amino acid transport mechanisms. To determine, however, whether there is also a direct stimulation of the amino acid carrier by glucose, the uninhibited transport of amino acids must be used as control. Doing so it is seen that neither does glucose change the tissue concentrations of amino acids (Tables II and III), nor does it allow the sacs to accumulate proline or valine to concentrations above the control values or to reach these values faster. Further, it was found that glucose was unable to enhance the disappearance of proline from the mucosal fluid, when the experiments were done with small sacs, with which the glucose-dependent water transport although of a significant magnitude was very much less substantial than that observed for the larger sacs; and it was found that the effect glucose has on the transport of proline and valine by the large sacs could be closely simulated, if by other means the serosal volume of these sacs was increased. These results show that a major factor in determining the effect of glucose on the amino acid transport by the rat small intestine is the increase in transintestinal water flow induced by glucose and not a true stimulation of the amino acid carrier. However, the ability of sacs exposed to galactose to concentrate valine was not restored to control value by a simulation of the glucose effect on the serosal volume. This demonstrates that both the effect of glucose as a source of energy readily available to the rat small intestine and its effect on water transport are needed to produce the overall enhancement of amino acid transport.

How the water flow affects the amino acid transport and for which processes the energy is needed are questions open for speculation. Work by SCHULTZ and coworkers[20–23] and by CRANE[24] suggest that the processes for which energy is needed are those maintaining a normal intracellular ionic status. BROWN AND PARSONS[25] found that the $K^+$ concentration in mucosal tissue from rat jejunum was increased when everted sacs were incubated with glucose and decreased when incubated with other actively transported sugars, such as galactose. Assuming that a change in $K^+$ concentration is accompanied by an opposite change in $Na^+$ concentration close agreement is found between this observation, the interpretation of sugar inhibition of amino acid transport as a non-competitive phenomenon, and the above cited view on the connection between electrolyte and non-electrolyte transport, according to which this sugar inhibition results from an increase in efflux[3].

Inconsistency has been seen[18] between results obtained for the effect of glucose on amino acid transport by the everted sac[1] and by the ring preparation of rat small intestine[11]. The results reported here demonstrate that use of comparable parameters eliminates the part of this inconsistency which is seen in the inability of glucose to increase the amino acid accumulation by the ring preparation during incubation at 5 mM concentrations of different amino acids, as it was found (Tables II and III) that neither with the sac preparation was an increased degree of tissue accumulation part of the glucose effect on amino acid disappearance from the mucosal fluid or appearance in the serosal fluid. However, the results reported here on the effect of glucose on the ability of an everted sac to transport valine from a concentration of 0.05 mM differ from those obtained[11] with the ring preparation in that the tissue concentrations reached by the sacs were uninfluenced by the presence of glucose, whereas the accumulation by the rings was reduced in the presence of glucose. A cause of this remaining inconsistency may be found in the difference between the two types of

preparation, that the accumulative work performed by the epithelium of the sac is conserved in the compartment enclosed by the brush border part of the epithelial cell membrane but in the ring preparation lost, because diffusion from the subepithelial tissues back into the incubation medium will increase the gradient between the intraepithelial space and this tissue and thus reduce the epithelial accumulation. This reduction will be aggravated whenever the gradient between the epithelial cell and the subepithelial tissue is increased, as it must be for instance when the addition of glucose to the incubation medium causes an increase in water flow through the tissues. This aggravation may be sufficient to account for a significant decrease in the high and therefore easier disturbed distribution ratio obtained at a very low degree of saturation at 0.05 mM, but insufficient to alter significantly the lower distribution ratio reached at the 100 times higher concentration of 5 mM.

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