J. Physiol. (1979), **291**, pp. 291–303
With 5 text-figures
Printed in Great Britain

LYSINE TRANSPORT ACROSS RAT JEJUNUM: DISTRIBUTION BETWEEN THE TRANSCELLULAR AND THE PARACELLULAR ROUTES

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(*Received 17 October 1978*)

SUMMARY

Transport of lysine across the rat jejunum has been studied measuring transmural fluxes, J_{ms} and J_{sm}, under short-circuit conditions, influx across the brush-border membrane, J_{mc}, under open-circuit and voltage-clamp conditions, and steady-state uptake by the isolated mucosa.

1. J^{lys}_{mc} can be described as the sum of a saturable process with a K_t of 3 mM and a J_{max} of 2·25 μmole/cm^2.hr and a diffusional component corresponding to a lysine permeability of 0·014 cm/hr. Also J^{lys}_{ms} is well described as the sum of a saturable process and a diffusional contribution described by the same permeability as for J^{lys}_{mc}.

2. The effects of the transmural p.d. on J^{lys}_{mc} indicate that at 60 mM this flux includes a diffusional contribution, which corresponds to a lysine permeability of 0·014 cm/hr.

3. The passage of an electrical current across the gut wall changes the electrical conductance as expected for a cation-selective epithelium. The effect of a mucosa to serosa current on the J_{ms} value of mannitol provides confirmation of the expected current effect on transepithelial volume flow. These effects on conductance and solute flux, together with the electrostatic effect on lysine movements, suffice to account for the p.d. effects on J_{mc}, J_{ms}, and J_{sm} of lysine.

4. J^{lys}_{sm} is in a saturable manner stimulated by increasing concentrations of D-glucose. At higher (10 mM) concentrations of lysine this effect leads to a net secretion of lysine. Qualitatively and quantitatively these effects are consistent with the model of a glucose-induced fluid circuit between the mucosal solution and the lateral intercellular spaces.

5. All observations are consistent with a paracellular, transepithelial pathway for lysine, which includes the lateral intercellular spaces.

6. The transport of lysine across the basolateral membrane is analysed. Together the data on transcellular passage of lysine are very similar to those reported for rabbit ileum, except that more than one transport process could not be demonstrated.

0022-3751/79/3710-0714 $01.50

INTRODUCTION

It is evident that the paracellular route plays an important role in solute transport across the small intestinal epithelium (Frizzell & Schultz, 1972; Munck & Schultz, 1974; Desjeux, Tai, Powell & Curran, 1976), and that the passage of electrical currents across epithelia changes the permeability characteristics of ion-selective paracellular routes (Bindslev, Tormey & Wright, 1974; van Os, Michels & Slegers, 1976). It has also been shown recently (Munck, 1976; Munck & Rasmussen, 1977) that with currently used *in vitro* techniques the relative importance of the paracellular route across rat jejunum is much increased when glucose is added to the incubation media. With the aim of evaluating the paracellular and transcellular components of lysine transport across the rat jejunum the present paper presents further information on these aspects of transepithelial permeability.

METHODS

Materials. Male albino rats (125-150 g) were used for all the experiments. Before use the rats were kept in the laboratory for at least 18 hr with free access to food and water. The rats were anaesthetized by I.P. injections of sodium pentobarbitone and the total small intestine was removed, whereupon the rats were killed. The mid 15–20 cm of the total small intestine were used for the measurement of tissue uptake, transmural fluxes, and influx across the brush-border membrane.

[^{3}H]methoxy-inulin, [^{3}H]mannitol, and [^{14}C]lysine were purchased from New England Nuclear Co.

All chemicals were of the highest purity commercially available.

Methods. In all experiments the tissue was incubated in a Krebs phosphate buffer whose composition was: Na, 140 mM; K, 8 mM; Ca, 2·6 mM; Mg, 1 mM; Cl, 140 mM; P, 8 mM; SO_4, 1 mM; pH 7·4. All experiments were made at 37 °C during aeration with pure oxygen.

Unidirectional transmural fluxes were measured by the Ussing-Zerahn technique used as described by Munck (1972). In all experiments four segments of tissue were used from each rat using the middle approximately 10 cm of the total small intestine. These four segments were studied simultaneously and in most of the experimental series were used to provide two sets of control and test preparations. J_{ms} and J_{sm} are used as symbols for mucosa to serosa and serosa to mucosa fluxes respectively.

Steady-state epithelial uptake was measured using the isolated mucosal tissues prepared according to the description of Dickens & Weil-Malherbe (1941). The same part of the intestine was used as for measurements of transmural fluxes. The details were as previously described (Munck, 1972).

Influx across the brush border membrane (J_{mc}) was measured by the method (Schultz, Curran, Chez & Fuisz, 1967) described for rabbit ileum, with the modifications previously described (Munck & Rasmussen, 1975). J_{mc} was also measured under voltage-clamp conditions using the technique described by Frizzell & Schultz (1972) as modified to allow for oxygenation and circulation of the solution bathing the serosal side of the intestinal segment (Munck & Schultz, 1974).

Symbols and conventions. In addition to flux symbols defined above, J_{cm} and J_{cs} stand for the flux from inside the epithelial cell to its mucosal and serosal sides respectively. [S] with subscript m, c or s indicates the concentration (mM) of substance S in the mucosal, cellular or serosal compartment, respectively.

Unless otherwise stated, the transepithelial electrical potential difference (p.d.) is defined as $\psi_{ms} = \psi_s - \psi_m$.

The radioactivity of the different samples was measured using the scintillation fluid described by Bray (1960).

Evaluation of experimental data. Statistical evaluation of the data is based on the t test (Kemp & Nielsen, 1961). All data are given as means ± 1 S.E. with the number of observations in brackets. P values by the t test below 0·05 are taken as evidence of statistical significance.

RESULTS

Lysine transport across the brush-border membrane. Influx of lysine (lys) across the brush-border membrane was measured in the presence of 5·5 mM-D-glucose and 0·1, 1·0, 5·0, 20·0, 40·0, and 60·0 mM-lysine, using all six concentrations on preparations from each rat. These experiments were performed in the open-circuit state. Any diffusional component of J^{lys}_{mc} may therefore be assumed to have been opposed by a p.d. of approximately 5 mV and thus to amount to about 90% of its value in the short-circuit state. The results of the measurements are shown in Fig. 1 which

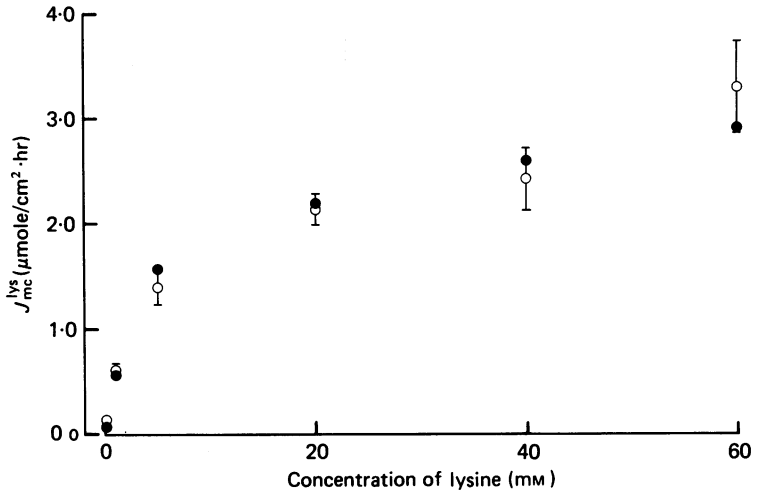

Fig. 1. Lysine influx across the brush-border membrane measured at the concentrations stated, under open-circuit conditions, in the presence of 5·5 mM-D-glucose. ○, average ± S.E. of five to seven observations. ●, calculated as described by eqn. (1).

demonstrates that as a function of the concentration of lysine, J^{lys}_{mc} is quite well described by the equation:

$$J^{lys}_{mc} = 2{\cdot}25\,[lys]_m/(3+[lys]_m)+[lys]_m\,.\,0{\cdot}9\,.\,0{\cdot}014\ \mu mole/cm^2\,.\,hr \qquad (1)$$

that is as the sum of a saturable transport with a K_t of 3 mM and a J_{max} of 2·25 μmole/cm².hr and a diffusional transport which in the short-circuit state would be related to the lysine concentration similarly to the apparently linear fraction of J^{lys}_{ms} (Table 1). The data from these experiments agree with those previously reported (Munck & Rasmussen, 1975).

J^{lys}_{mc} was measured at 60 mM-lysine + 17 mM-glucose under voltage-clamp conditions. The p.d. was clamped at + 50, 0, and − 50 mV. The concentration of 60 mM-lysine was chosen to obtain conditions where a proposed paracellular fraction should become a relatively easily detectable contribution to the total J^{lys}_{mc}. The results are shown in Fig. 2 which demonstrates that, indeed, a fraction of J^{lys}_{mc}, not far from the expected magnitude, is sensitive to the transmural p.d.

Table 1. The influence of the concentrations of lysine and glucose on the unidirectional transmural fluxes of lysine

mM-lysine		mM-glucose 0	5·5	17	28
1	ms	0·09 ± 0·02 (5)	0·20 ± 0·021 (6)	0·14 ± 0·014 (5)	0·14 ± 0·013 (5)
	sm	0·01 ± 0·002 (5)	0·02 ± 0·001 (6)	0·03 ± 0·005 (9)	0·03 ± 0·004 (5)
5	ms			0·32 ± 0·02 (9)	
	sm			0·29 ± 0·04 (5)	
10	ms	0·45 ± 0·5 (5)	0·45 ± 0·02 (7)	0·35 ± 0·07 (9)	
	sm	0·23 ± 0·03 (5)	0·30 ± 0·03 (7)	0·36 ± 0·13 (5)	
20	ms		0·61 ± 0·15 (4)	0·53 ± 0·05 (12)	
	sm		0·66 ± 0·09 (4)	0·73 ± 0·06 (12)	
30	ms	0·60 ± 0·04 (3)	0·78 ± 0·06 (4)	0·64 ± 0·03 (5)	0·52 ± 0·02 (3)
	sm	0·61 ± 0·04 (3)	0·90 ± 0·06 (4)	1·09 ± 0·05 (5)	0·99 ± 0·17 (3)
40	ms	0·83 ± 0·08 (4)	1·02 ± 0·08 (4)	0·69 ± 0·09 (7)	
	sm	0·80 ± 0·07 (4)	1·40 ± 0·05 (4)	1·22 ± 0·12 (7)	
40*	ms		1·41 ± 0·07 (4)		
	sm		1·32 ± 0·03 (4)		
60	ms			1·29 ± 0·22 (4)	
	sm			2·36 ± 0·18 (4)	

Errors are S.E. The number of experiments are given in the parentheses.
* Choline substituted for sodium.

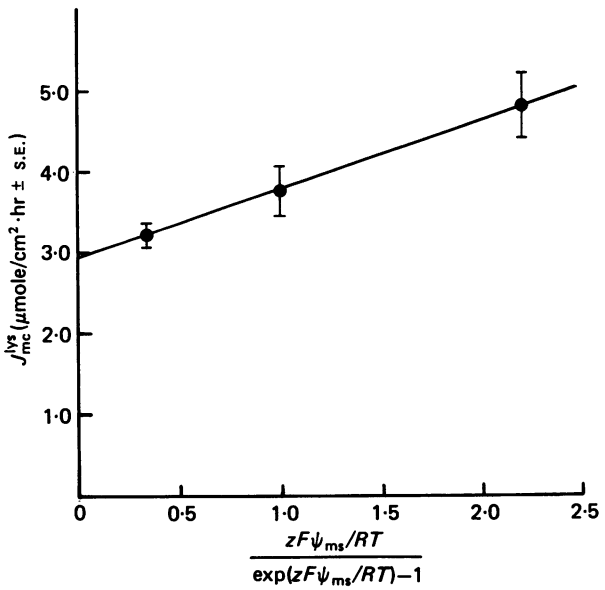

Fig. 2. Effect of transmural p.d. on J^{lys}_{mc} measured at 60 mM-lysine in the presence of 17 mM-D-glucose. Each symbol indicates the mean ± 1 S.E. of nine to fourteen observations. The straight line is described by the eqn.:

$$J^{lys}_{mc} = 2{\cdot}93 + (0{\cdot}88 \pm (\text{S.D.} = 0{\cdot}24)) \frac{a\psi_{ms}}{(\exp(a\psi_{ms}) - 1)} \mu\text{mole/cm}^2\text{.hr}$$

which is derived by the least square method. Its slope is equal to 0·0146. 60; $a = zF/RT$.

Unidirectional transmural fluxes of lysine. Effects of increasing concentrations of lysine and glucose, and of changing the transmural electrical potential difference

The unidirectional transmural fluxes of lysine were measured at several concentrations of lysine at both 5·5 and 17 mM-D-glucose (Table 1). The data on lysine transport at 5·5 mM-glucose represent experiments performed within a period of a few weeks on preparations which were comparable with respect to p.d., I_{sc}, and time course of these parameters. They therefore constitute the basis (Table 1) on which the transcellular transport of lysine will be evaluated. At 28 mM-D-glucose J^{lys}_{ms} and J^{lys}_{sm} were measured (Table 1) using lysine concentrations, which were also used in

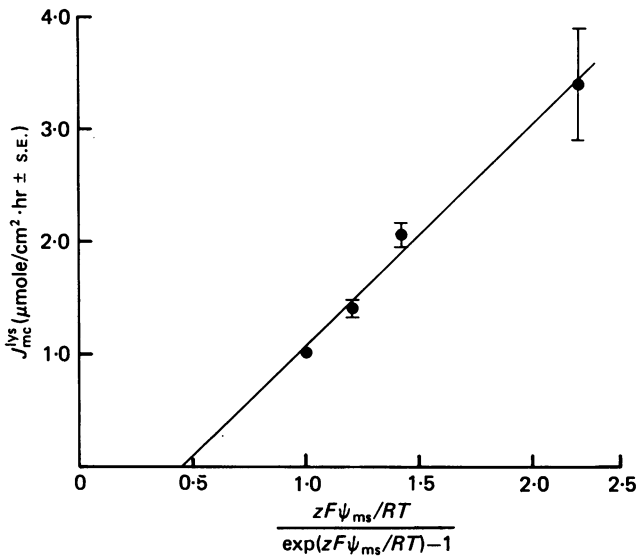

Fig. 3. Effect of transmural p.d. on J^{lys}_{ms} measured at 60 mM-lysine in the presence of 17 mM-D-glucose. Each symbol indicates the mean ± 1 S.E. of four observations. The line is described by $J_{ms} = (1{\cdot}95 \pm (\text{S.D.} = 0{\cdot}14)).a\psi_{ms}/(\exp(a\psi_{ms}) - 1) - 0{\cdot}87$; $a = zF/RT$, which, derived from the experimental data by the method of the least squares, shows the correlation between J^{lys}_{ms} and the transmural p.d.

the first two series of experiments. At several concentrations of lysine paired flux measurements were performed at 0 and 5·5, 17, or 28 mM-D-glucose. In another series of experiments paired measurements of J^{lys}_{ms} and J^{lys}_{sm} were performed at 40 mM-lysine in choline-substituted, sodium-free media, with 5·5 mM-glucose (Table 1). The ability of glucose to induce a net secretion of lysine can be seen to be sodium-dependent. It also appears that glucose can induce a net secretion of lysine only when an apparently diffusion fraction of the transmural flux becomes comparable in magnitude to the J_{max} of a saturable fraction.

In one series of experiments the effects of the transmural p.d. were examined at the rather high concentration of 60 mM-lysine + 17 mM-D-glucose (Fig. 3). In these experiments all four preparations from each rat were used to measure J^{lys}_{ms}, clamping the preparations at 0, −10, −20, and −50 mV respectively. The effect on J^{lys}_{ms} of a p.d. of −50 mV was examined in paired experiments performed at 10 mM-lysine or

10 mM-lysine + 2 mM-leucine in the presence of 11 mM-D-glucose. In the absence of leucine the fluxes (μmole/cm^2.hr) were $0{\cdot}35 \pm 0{\cdot}04$ ($n = 8$) at 0 mV and $0{\cdot}51 \pm 0{\cdot}04$ ($n = 8$) at -50 mV. In the presence of 2 mM-leucine the fluxes were $0{\cdot}79 \pm 0{\cdot}08$ ($n = 8$) at 0 mV and $0{\cdot}84 \pm 0{\cdot}02$ ($n = 8$) at -50 mV. Within the range used in this study the transcellular fraction of J^{lys}_{ms} is thus not affected by the transmural p.d.

The procedure of examining for p.d. effects on J^{lys}_{ms} and J^{lys}_{sm} was to measure the fluxes in the short-circuited state for the first 60–80 min and then at the desired p.d.

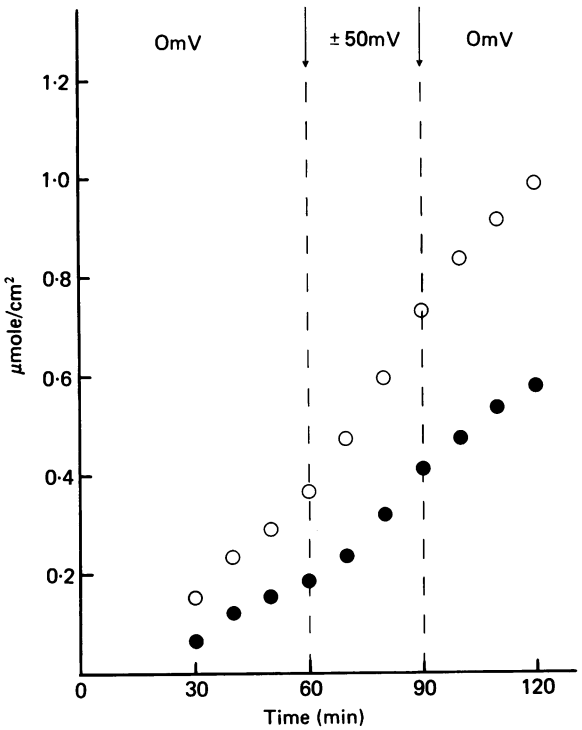

Fig. 4. The effect of changing transmural p.d. on J^{lys}_{ms} (●) and J^{lys}_{ms} (○) measured at 10 mM-lysine in the presence of 5·5 mM-D-glucose. J^{lys}_{ms} was measured at -50 mV and J_{sm} at $+50$ mV.

for the next 40–60 min. In some experiments fluxes were measured, first in the short-circuited state for 60 min, then at 50 respectively -50 mV for the next 30 min, and finally in the short-circuited state for the last 30 min of incubation.

As shown by Fig. 4 the effects of clamping at ± 50 mV are reversible. The data of Table 1 show that except at 1 mM-lysine J^{lys}_{ms} is not significantly affected by the presence of glucose. It is also seen that in the absence of glucose, but with increasing lysine concentration, J^{lys}_{sm} approaches, but never significantly exceeds, J^{lys}_{ms}. However, with increasing concentration of glucose, J^{lys}_{sm} is increasingly stimulated. This is most clearly seen from the data of Table 1 which demonstrates that whereas at 5·5 mM-glucose the lysine concentration must be raised to 40 mM before J^{lys}_{sm} significantly exceeds J^{lys}_{ms}, at 17 mM-glucose this has happened already at 20 mM-lysine. The mechanism by which glucose stimulates J^{lys}_{sm} also appears to be saturable with respect to glucose.

The changes in unidirectional transmural fluxes of lysine produced by applying a 50 mV p.d. across the preparations are shown in Table 2. According to eqn. (2) (see below) this p.d. should increase the fluxes measured in the short-circuited state by 1·2.0·014.[lys] μmole/cm^2.hr. It is seen that at 1 and 10 mM-lysine the effects of p.d. on J_{ms} are compatible with the passive permeability deduced from Table 1.

TABLE 2. Effect of lysine concentration on the responses of J^{lys}_{ms} and J^{lys}_{sm} to changes in transmural p.d.

	J_{ms} (μmole/cm^2.hr)		J_{sm} (μmole/cm^2.hr)	
mM-lysine	0 mV	− 50 mV	0 mV	+ 50 mV
1	0·138 ± 0·013 (5)	0·165 ± 0·006 (5)	0·027 ± 0·004 (5)	0·032 ± 0·002 (5)
10	0·35 ± 0·04 (8)	0·51 ± 0·04 (8)	0·31 ± 0·03 (8)	0·41 ± 0·03 (8)
20	0·42 ± 0·03 (7)	0·76 ± 0·12 (7)		
30	0·61 ± 0·03 (7)	1·35 ± 0·12 (7)	0·99 ± 0·12 (3)	1·29 ± 0·14 (8)
60	1·03 ± 0·03 (4)	3·39 ± 0·48 (4)		

All experiments were performed at 17 mM-D-glucose.
Errors are S.E. The number of experiments are given in the parentheses.

However, at higher concentrations the data would indicate that the total J^{lys}_{ms} becomes sensitive to the transmural p.d. to the same degree as expected for a paracellular, diffusional flux. And finally at 40, and particularly at 60 mM-lysine (Fig. 3), the effects on J^{lys}_{ms} of a serosa negative p.d. of 50 mV cannot be accounted for in terms of the transmural p.d. on the unidirectional fluxes alone.

Finally the data of Table 2 show that generally J^{lys}_{sm} is less sensitive to the transmural p.d. than J^{lys}_{ms}.

Effects of an electric current on the transmural electrical resistance

Electric currents have been passed across the rat jejunum in both directions. At the different concentrations of glucose the electrical resistance was always unaffected (Fig. 5*A*) when the current was passed from the mucosal to the serosal side, while it was increased when the current was passed in the opposite direction (Fig. 5*B*). In one such series (10 mM-lysine + 5·5 mM-glucose) the resistance increased from 20 ± 2 (n = 11) to 58 ± 2 (n = 11) Ω cm^2 with a half-time of 7 ± 0·5 min (n = 6).

In the absence of glucose the current has been passed only from mucosa to serosa. In one such series of experiments (1 mM-mannitol) the passage of current reduced the electrical resistance from 59 ± 3 (n = 10) to 24 ± 0·3 (n = 10) Ω cm^2 with a half-time of 7 ± 0·5 min (n = 10) (Fig. 5*C*).

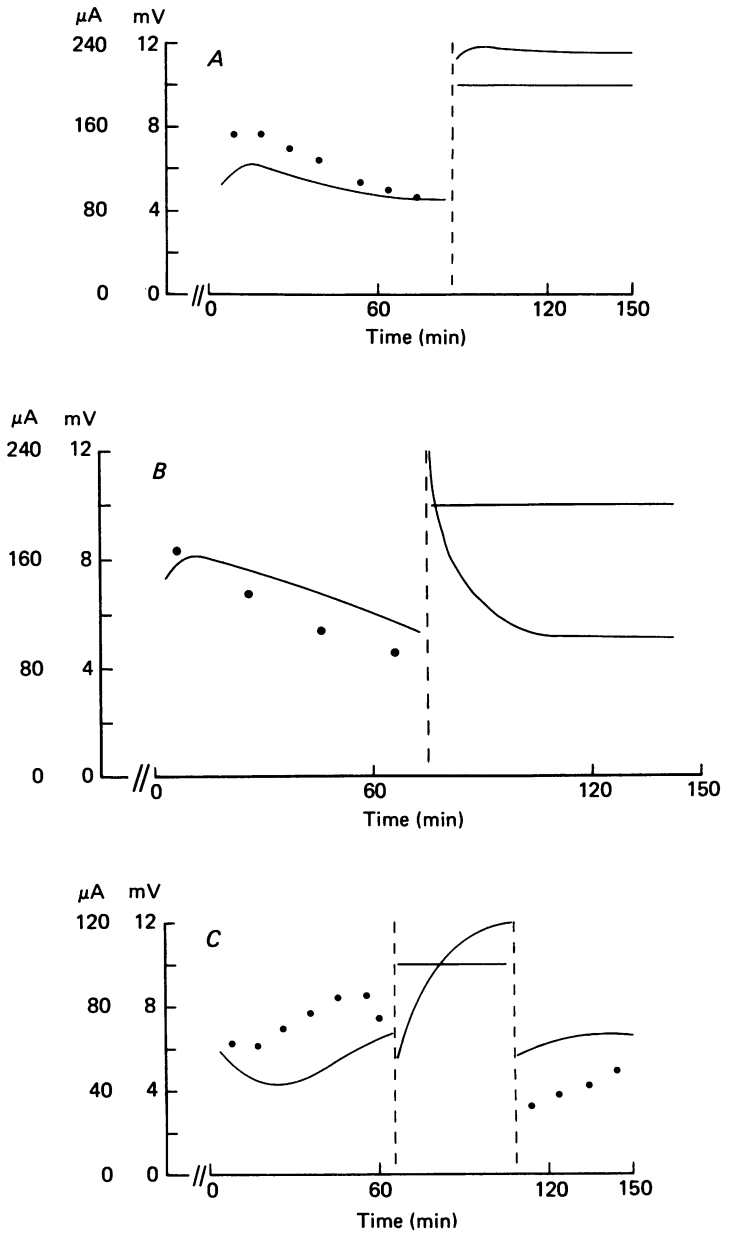

Fig. 5. Current–p.d. relationship of rat jejunum during incubation. *A*, at 10 mM-lysine + 11 mM-glucose; during the first 80 min of incubation the preparation was short-circuited; during the last 70 min it was clamped at −50 mV and the full range of the ordinate corresponds to 1200 μA or 60 mV. *B*, at 10 mM-lysine + 11 mM-glucose; during the first 75 min of incubation the preparation was short-circuited; during the last 70 min it was clamped at +50 mV and the full range of the ordinate corresponds to 1200 μA and 60 mV respectively. *C*, at 1 mM-mannitol without glucose present; during the first 65 and the last 40 min of incubation the preparation was short-circuited. From 66 to 110 min it was clamped at −50 mV, and the full range of the ordinate corresponds to 1200 μA and 60 mV respectively.

The effect of glucose on the ability of an electric current to change the unidirectional transmural fluxes of mannitol (man)

J^{man}_{ms} and J^{man}_{sm} were measured in paired experiments at 1 mM-mannitol and at 1 mM-mannitol + 17 mM-glucose. Generally the preparations were kept short-circuited for the first 80 min of incubation, whereupon they were clamped at − 50 mV for the next 60 min. In some experiments one of the four preparations was kept short-circuited throughout the 140 min of incubation. In these the steady-state

TABLE 3. Transmural fluxes of mannitol. Effects of glucose and transmural p.d.

	J_{ms} (μmole/cm².hr)	
	0 mV	− 50 mV
1 mM-mannitol + 0 mM-glucose	0·022 ± 0·001 (6)	0·057 ± 0·002 (5)
J^{50}/J^{0}	2·56 ± 0·10 (5)	
1 mM-mannitol + 17 mM-glucose	0·018 ± 0·002 (8)	0·035 ± 0·003 (8)
J^{50}/J^{0}	1·92 ± 0·12 (8)	
	J_{sm} (μmole/cm².hr)	
	0 mV	− 50 mV
1 mM-mannitol + 0 mM-glucose	0·017 ± 0·0016 (5)	0·044 ± 0·002 (5)
J^{50}/J^{0}	3·01 ± 0·35 (5)	
1 mM-mannitol + 17 mM-glucose	0·047 ± 0·005 (7)	0·048 ± 0·006 (6)
J^{50}/J^{0}	1·00 ± 0·001 (5)	

Errors are S.E. The number of experiments are given in parentheses.

fluxes were maintained unchanged during the last 60 min of incubation. The results of passing the electrical current necessary to maintain a p.d. of − 50 mV are shown in Table 3. It is seen that in the absence of glucose both unidirectional fluxes were increased, whereas in the presence of glucose, when in the short-circuit state J^{man}_{sm} was approximately twice J^{man}_{ms}, J^{man}_{sm} was unchanged and J^{man}_{ms} was doubled.

Steady-state epithelial accumulation of lysine

Steady-state uptake of lysine by the isolated mucosa was measured at a number of lysine concentrations in the presence of 17 mM-glucose. The results of these experiments are shown in Table 4. It is apparent that with increasing concentration

TABLE 4. Intracellular accumulation of lysine

Lysine concentration in medium (mM)	Tissue/medium concentration ratio
1	7·1 ± 0·3 (12)
5	3·1 ± 0·2 (10)
10	2·67 ± 0·14 (9)
15	2·50 ± 0·19 (10)
20	2·48 ± 0·16 (10)
30	2·77 ± 0·17 (12)
40	2·58 ± 0·22 (10)

Errors are S.E. The number of experiments are given in parentheses.

of lysine the tissue/medium concentration ratio is decreasing, but that this decline stops at 10 mM-lysine, beyond which a steady level of 2·5–3·0 is maintained.

DISCUSSION

The results presented in Table 1 and Fig. 1 show that, in addition to being saturable functions of the lysine concentration of the incubation medium, both J^{lys}_{ms} and J^{lys}_{mc} include a component which is linearly related to this concentration. Furthermore both J^{lys}_{ms} and J^{lys}_{sm} are affected by the transmural p.d. and the data of Fig. 2 demonstrate that a fraction of J^{lys}_{mc} is sensitive to the transmural p.d. The magnitude of this fraction (0·015 μmole/cm^2.hr.mM) is comparable to the contribution expected from the apparently non-saturable fraction of J^{lys}_{mc} or J^{lys}_{ms}. We propose that this unsaturable, p.d.-sensitive component represents a passage of lysine through a paracellular pathway, probably the tight junctions, of the epithelium. Other interpretations may be entertained: (1) a carrier-mediated transcellular passage with a J_{max} above 2·5 μmole/cm^2.hr and a K_t above 100 mM; (2) transcellular diffusion; or (3) edge damage. J_{ms} is a function of J_{mc}, J_{cm}, and J_{cs} (Chez, Palmer, Schultz & Curran, 1967). The first alternative is made unlikely by the equal magnitude of the linear components of J_{ms} and J_{mc}. The second alternative can be ruled out on the grounds that at 1 mM-lysine Munck & Schultz (1974) could not significantly affect J^{lys}_{mc} by changing the p.d., and that the leucine-stimulated J^{lys}_{ms} was relatively less sensitive to the p.d. than the unstimulated J^{lys}_{ms}. The third possibility that the linear flux contributions represent the effect of edge damage can be ruled out by the effects of glucose, the low permeability 0·02 cm/hr for chloride (Munck & Schultz, 1974), and by the observation (B. G. Munck, unpublished observation) that the electrical conductance (m-mho/cm^2) of the similar preparation of the rabbit ileum was unchanged when the exposed area was increased from 0·6 to 4·8 cm corresponding to a perimeter/area ratio (cm^{-1}) (Dobson & Kidder, 1968) of 5·0 and 1·6 respectively. Finally our interpretation that the fractions of J^{lys}_{ms} and J^{lys}_{mc} which are linearly related to the ambient lysine concentration should be ascribed to paracellular diffusion is supported by the details of the effects of p.d. on the transepithelial fluxes of lysine and mannitol (Tables 2 and 3). In addition, the estimated paracellular lysine permeability of 0·014 cm/hr is reasonable compared with the permeabilities of 0·08 cm/hr for sodium and 0·001 cm/hr for polyethyleneglycol (mol. wt. 4000).

According to this interpretation the total transepithelial flux of lysine should be described by the equation (Schultz & Zalusky, 1964):

$$J^{lys}_{ms} = {}_cJ_{ms} + {}_dJ^0_{ms} . zF\psi_{ms}/RT/(\exp(zF\psi_{ms}/RT) - 1) \quad (2)$$

in which ${}_cJ_{ms}$ indicates the transcellular fraction of J^{lys}_{ms} and ${}_dJ^0_{ms}$ its paracellular fraction in the short-circuited state. When estimated on the basis of this relationship, ${}_dJ^0_{ms}$ should be linearly related to the concentration of lysine and equal to ${}_dJ^0_{sm}$. Furthermore the transcellular fraction of the total transepithelial flux should be defined by the ordinate intercept of the line described by eqn. (2), and the paracellular permeability by its slope. However, these expectations were not fulfilled. From the data of Table 2 and Fig. 3 it appears that when evaluated on the basis of p.d. effects on J^{lys}_{ms}, the paracellular permeability of lysine increases with increasing

lysine concentration, that at 30, 40 and 60 mM-lysine the intercepts with the ordinate would correspond to negative values for J^{lys}_{ms}, and that generally the lysine permeability is higher when estimated from data on J^{lys}_{ms} than from data on J^{lys}_{sm}. Of these deviations, the low estimates of the paracellular permeability which follows from the p.d. effects on J^{lys}_{sm} can be explained by the observation (Fig. 5*B*) that applying a serosa-positive p.d. of 50 mV leads to a substantial increase in transmural electrical resistance. According to Bindlsev *et al.* (1974) this increase may be caused by a narrowing of the paracellular shunt. It must therefore be assumed to have as a corollary a reduction of the partial conductance of lysine which must, however, be p.d.-independent in order for eqn. (2) to apply (Schultz & Zalusky, 1964).

The effect of -50 mV on J^{lys}_{ms} could approach a factor of 2·2, only if the transcellular fraction of J^{lys}_{ms} were negligible. However, at and above 30 mM-lysine this factor is exceeded. Therefore the model described by eqn. (2) is inadequate.

Neglect of the compartment of the lateral intercellular spaces is one obvious oversimplification of the model described by eqn. (2). As its most serious error this simplification disregards that in the steady state the backflux through a paracellular pathway is decreased by serosa negative p.d. values. However, an analysis of lysine movements through this compartment based on the results of the present study showed that even thus improved the model could not account for the observations of Table 2 and Fig. 4.

Another possibility was that passing an electrical current across the epithelium induced volume flows, which by convective effects on lysine movements and concentration gradients would in turn affect the transepithelial passage of lysine. Like the frog gall-bladder (Bindslev *et al.* 1974) the rat jejunum (Wright, 1966; Munck & Schultz, 1974) is cation-selective. Therefore, according to the analysis by Bindslev *et al.* (1974), passing a current in the direction mucosa to serosa will generate a volume flow in the same direction, through the lateral intercellular spaces, which are thereby expanded. Passing a current in the direction serosa to mucosa will create a volume flow towards the mucosal side leading to a collapse of the lateral intercellular spaces. These latter effects were confirmed for the rat jejunum by the data on the effects of $+50$ mV on J^{lys}_{sm} and by the observation of resistance changes exemplified by Fig. 5*B*. The effects of a current passed from mucosa to serosa were confirmed by Fig. 5*C*; while Fig. 6*A*, in demonstrating the lack of a resistance effect in addition to that of glucose, makes it unlikely that currents or p.d.s of the magnitude used here have effects directly on tight junctions or cell membrane (Bindslev, Tormey, Pietras & Wright, 1974). Whether the volume flows indicated by these observations would suffice to account for the excess in p.d./current effects on J^{lys}_{ms} was examined using mannitol as a probe. That a mucosa to serosa electrical current doubled J^{man}_{ms} both in the absence and presence of 17 mM-glucose indicates first that the effect is convective and, secondly, since a convective effect depends on the presence of a cation-selective barrier, it points to the tight junctions as the effective route. From Table 3 it is seen that at 17 mM-glucose the current needed to maintain the -50 mV increases the J_{ms} of an uncharged molecule by a factor of 1·9. The charge-related effect of this p.d. on J^{lys}_{ms} would be a factor of 2·2 on the paracellular fraction. The total effect will then be a 4·2-fold increase of the paracellular flux. This is enough to account for the observations shown in Fig. 3. Therefore the observed deviations from the predictions

of eqn. (2) are well accounted for by known effects of electrical currents on the paracellular, lateral intercellular space pathway of leaky epithelia.

Independent support for the notion that the paracellular route plays a role in epithelial transport of sugars and amino acids is found in the applicability of the Patlak & Rappoport (1971) fluid-circuit model on the transport of lysine by the glucose-transporting rat jejunum. From this application one would, in accordance with the data of Table 1 on J_{sm}^{lys} at 0 and 17 mM-glucose, expect an effect of glucose on lysine transport similar to its effect on mannitol transport (Table 3 and Munck & Rasmussen, 1977).

Because of the relatively substantial paracellular contributions to the unidirectional transmural fluxes a three compartment analysis of the transcellular lysine transport is not fruitful. Nevertheless, together with the data on intracellular accumulation of lysine (Table 4) the concentration dependence of the transcellular fraction of J_{ms}^{lys} (Table 1) suggests that the kinetics of J_{cs}^{lys} are similar to those deduced for J_{cs}^{lys} of the rabbit ileum (Munck & Schultz, 1969).

In the context of the present study it is of interest to note that changes in the transmural conductance were not seen when approximately 0·5 min exposures were used to measure voltage-clamped influx across the brush-border membrane of the rat jejunum (Munck & Schultz, 1974). The time course for the conductance changes reported here suffices to explain this difference and to demonstrate that the current-induced conductance changes do not inadvertently interfere with the use of voltage-clamped influx to characterize the permeability of the paracellular pathway of this epithelium.

This work was supported by grants from the Danish Medical Research Council and the Novo Foundation.

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