Lysine Transport across Isolated Rabbit Ileum

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ABSTRACT Lysine transport by in vitro distal rabbit ileum has been investigated by determining (*a*) transmural fluxes across short-circuited segments of the tissue; (*b*) accumulation by mucosal strips; and (*c*) influx from the mucosal solution across the brush border into the epithelium. Net transmural flux of lysine is considerably smaller than that of alanine. However, lysine influx across the brush border and lysine accumulation by mucosal strips are quantitatively comparable to alanine influx and accumulation. Evidence is presented that the "low transport capacity" of rabbit ileum for lysine is due to: (*a*) a carrier-mediated process responsible for efflux of lysine out of the cell across the serosal and/or lateral membranes that is characterized by a low maximal velocity; and (*b*) a high "backflux" of lysine out of the cell across the mucosal membrane. A possible explanation for the latter observation is discussed with reference to the relatively low Na dependence of lysine transport across the intestinal brush border.

The ability of small intestine to transport neutral amino acids from a lower concentration in the mucosal solution to a higher concentration in the serosal solution was first demonstrated by Wiseman in 1951 (1). Since then the transport of neutral amino acids by in vitro preparations of mammalian small intestine has been the subject of numerous investigations and has been described in considerable detail. In 1961, Hagihira et al. (2) demonstrated transport of the basic amino acids across everted sacs of hamster small intestine against a concentration difference using low initial concentrations in the mucosal and serosal fluids. These investigators noted that the maximal rates of transport of the basic amino acids were only 5–10% of the rates of transport of neutral amino acids and concluded that the earlier failure to demonstrate net transport in the presence of high initial mucosal and serosal concentrations (3) could be attributed to this small transport "capacity." Since 1961, there have been relatively few studies dealing with the characteristics of basic amino acid transport across in vitro small intestine, but nevertheless the initial observations and conclusions of Hagihira et al. have been substantially confirmed (4–8).

This "small transport capacity" of in vitro small intestine for basic amino acids raises several questions.

First, is the net transmural transport of these cationic amino acids influenced by the transmural electrical potential difference across small intestine? It is well-established that the serosal surface of the small intestine is electrically positive with respect to the mucosal surface by approximately 5–15 mv in the presence of actively transported sugars or amino acids (9). Although this potential difference is not large, the possibility that it influences the net movements of cationic amino acids cannot be dismissed. Thus, if the tissue is highly permeable to these cationic amino acids an adverse electrical potential difference, though small, could significantly reduce net transport from mucosa to serosa.

Second, evidence has been presented that the rate of neutral amino acid transport across small intestine is influenced by an interaction between the amino acid carrier mechanism and Na at the mucosal border of the epithelial cell. Further, preliminary studies have suggested that the influxes of acidic, neutral, and basic amino acids across the brush border of rabbit ileum may differ significantly with respect to their dependence upon the presence of Na in the mucosal solution; the influx of lysine appeared to be significantly less dependent upon Na than were the influxes of glutamic acid and alanine (10). These results were obtained using a single lysine concentration, and, though suggestive, they could not be considered conclusive. The question that arises is: Do the influxes of lysine and alanine differ with respect to their dependence upon Na over a wide concentration range and, if so, could the difference between the rates of cationic and neutral amino acid transport across small intestine be related to this difference?

The present communication is concerned with a detailed investigation of lysine transport by in vitro rabbit ileum which was undertaken in order to gain further insight into the apparent differences between the transport of neutral and basic amino acids across mammalian small intestine.

METHODS

Male and female white rabbits (2–4 kg), that had been maintained on normal food intake, were sacrificed by intravenous injection of pentobarbital. A section of distal ileum was excised, opened along the mesenteric border, and rinsed free of intestinal contents with normal buffer.

Transmural fluxes across segments of rabbit ileum were determined under voltage-clamped conditions using the methods and apparatus that have been described in detail previously (11, 12). Briefly, a segment of tissue was clamped as a flat sheet between two identical Lucite half-chambers and both surfaces of the tissue were perfused and oxygenated using water-jacketed gas-lift circulating systems that maintained the bathing solutions at 37°C. In all experiments the mucosal and serosal bathing solutions had identical compositions and unidirectional fluxes from mucosa to serosa (J_{ms}) and from serosa to mucosa (J_{sm}) were determined using lysine-^{14}C (New England Nuclear Corp., Boston, Mass.). Sampling of the initially unlabeled bathing solution was begun

60 min after the addition of lysine-^{14}C to the opposite bathing solution, and three to six samples were then withdrawn at intervals of 10–20 min for the subsequent 60 min. The delay in the initiation of sampling was sufficient to ensure a steady-state transmural flux of lysine-^{14}C, and in all instances the flux remained constant, within experimental error, for at least 1 hr after sampling commenced. In all experiments, flux measurements were made on two adjacent pieces of ileum from the same animal thus permitting either (*a*) determination of the two oppositely directed transmural unidirectional fluxes under the same condition, or (*b*) comparison of the unidirectional flux in one direction under two different experimental conditions. Whenever possible paired analyses of these data are presented.

The methods employed for the determination of the unidirectional influx of lysine from the mucosal solution across the brush border into the intestinal epithelium have also been described in detail (13). Briefly, a defined area of the mucosal surface of the tissue is exposed to a solution containing lysine-^{14}C and inulin-^{3}H for a period of time up to 1 min. The exposed tissue is then rinsed for approximately 5 sec with ice-cold isotonic mannitol solution, and extracted in 0.1 N HNO_3 for 12–24 hr. Control studies, described previously (13), indicated that more than 98% of the label is recovered by the extraction prodecure. The tissue extract and an aliquot of the mucosal solution are assayed for ^{14}C and ^{3}H simultaneously. The ^{3}H content of the tissue extract is taken as a measure of adherent radioactive solution that was not removed by the mannitol wash. The ^{14}C content of the extract, corrected for this volume of adherent mucosal medium, represents the lysine-^{14}C uptake by the tissue across the brush border. The apparatus employed permits determination of eight influxes on tissue from the same animal. Thus, in the studies of influx vs. concentration, lysine influxes in the presence of four different lysine concentrations were determined in duplicate on tissue from the same animal.

Tissue accumulation of L-lysine by mucosal strips[1] of rabbit ileum was determined as described previously (14). All tissues were preincubated for 30 min at 37°C in a lysine-free buffer prior to the addition of lysine-^{14}C. Intracellular concentrations of L-lysine were calculated after correction for the lysine-^{14}C content of the extracellular space. The size of the extracellular space was taken as 0.30 ml/g wet weight. Intracellular concentrations determined after 40 min of incubation and after 60 min of incubation did not differ significantly indicating that a steady-state distribution ratio was achieved prior to 40 min.

Unless otherwise indicated, the composition of the buffer used for washing, perfusion, and incubation in each of the procedures outlined above was: NaCl, 140 mM; $KHCO_3$, 10 mM; K_2HPO_4, 1.2 mM; KH_2PO_4, 0.2 mM; $CaCl_2$, 1.2 mM; and $MgCl_2$, 1.2 mM. The gas mixture employed was 95% O_2–5% CO_2 and the pH of the buffer at 37°C was between 7.0 and 7.2. Na-free medium was obtained by replacing NaCl with choline chloride.

The determination of transmural fluxes and tissue accumulation of lysine according to the procedures outlined above assumes that lysine-^{14}C is not metabolized by the

[1] The mucosal strip consists of the epithelial cell layer, the underlying lamina propria, and a portion of the muscularis mucosa. The submucosal connective tissue and muscular layers are removed as described previously (14).

tissue.[2] This assumption was shown to be valid in two ways. First, tissue was incubated in a solution containing 0.1 mM L-lysine-^{14}C for 1 hr. The tissue was extracted in 0.1 N HNO_3 and both the tissue extract and the incubation medium were subjected to ascending paper chromatography using a sec-butanol-formic acid–water (65-15-20 v/v) solvent system (15). More than 95% of the ^{14}C initially in the incubation solution was recovered and radiochromatographic scanning revealed a single peak (accounting for more than 95% of the recovered ^{14}C) that migrated with L-lysine. Second, in several experiments, whole rabbit ileum was incubated in a Krebs-phosphate buffer containing lysine-^{14}C at high specific activity. The system was oxygenated by bubbling with 100% O_2 and the CO_2 evolved by oxidative metabolism was collected by bubbling the effluent gas through 2 M NaOH. Assay of aliquots of the NaOH after 1 and 2 hr of incubation did not reveal the presence of any $^{14}CO_2$. These results indicate that under the present conditions L-lysine is not metabolized to any significant extent by rabbit ileum and they are in agreement with the findings of other investigators using rat (5), hamster (8), and rabbit (6) small intestine.

TABLE I

TRANSMURAL FLUXES OF LYSINE ACROSS SHORT-CIRCUITED RABBIT ILEUM*

Lysine concentration	Na concentration = 140 mM				Na concentration = 0			
	n	J_{ms}	J_{sm}	J_{net}	n	J_{ms}	J_{sm}	J_{net}
mM								
0.1	4	0.078±0.013	0.001±0.001	0.08±0.01	4	0.005±0.000	0.001±0.000	0.004±0.001
1.0	3	0.27 ±0.04	0.023±0.001	0.25±0.04	3	0.035±0.007	0.020+0.004	0.015±0.008
10.0	7	0.30 ±0.04	0.21 ±0.03	0.09±0.05	6	0.29 ±0.02	0.26 ±0.03	0.03 ±0.04

J_{ms} and J_{sm} were determined on adjacent segments of tissue from the same animal.
Fluxes are in units of μmoles/hr, cm² and all errors are standard errors of the mean.
* n = number of experiments; each experiment involved three–five sampling periods.

RESULTS AND DISCUSSION

Transmural Fluxes of L-Lysine

The unidirectional transmural fluxes of lysine from mucosa to serosa (J_{ms}) and serosa to mucosa (J_{sm}) across short-circuited segments of rabbit ileum in the presence of 140 mM Na are given in Table I. In each experiment, both fluxes were determined on tissue from the same animal. When the concentration of lysine in both bathing solutions is increased from 0.1 mM to 1.0 mM there is a 3.5-fold increase in J_{ms}. However, increasing the lysine concentration in both bathing solutions from 1.0 mM to 10 mM does not result in a significant further increase in J_{ms}. In contrast, J_{sm} increases almost linearly over the 100-fold range of concentrations from 0.1 mM to 10 mM. These data are

[2] The determination of unidirectional influx is based on the assumption that all the radioactive label that crosses the brush border is retained within the total tissue. Thus, it is not influenced by metabolic modification of the original compound providing that none of the products is volatile.

plotted in Fig. 1. It is clear that the net flux of lysine from mucosa to serosa (J_{net}) reaches a maximal value at a concentration somewhere between 0.1 and 1.0 mM and then declines with increasing concentrations above 1.0 mM. It is of interest to compare these data with previously published data on the transmural fluxes of L-alanine across the identical preparation of rabbit ileum (12). In the latter study it was found that in the presence of 5 mM L-alanine, J_{ms}, J_{sm}, and J_{net} averaged 1.3, 0.13, and 1.2 μmoles/hr, cm² respectively. From Fig. 1 we can deduce that the values of J_{ms}, J_{sm}, and J_{net} in the presence of 5 mM lysine would be approximately 0.28, 0.11, and 0.17 μmole/hr, cm² respectively. Thus, the serosa-to-mucosa fluxes are approximately the same in the presence of 5 mM L-alanine and 5 mM L-lysine. In contrast, the

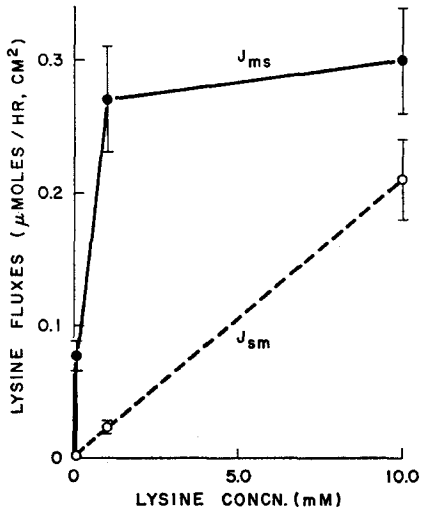

FIGURE 1. Transmural fluxes of lysine across short-circuited segments of rabbit ileum.

mucosa-to-serosa flux of lysine is significantly lower than that of alanine, and, as a result, the net flux of lysine is only 14% that of alanine.

Also given in Table I are the transmural fluxes of L-lysine across short-circuited segments of rabbit ileum when both bathing solutions were rendered Na-free by replacement of NaCl with choline chloride. In all instances, J_{net} was markedly reduced as a result of a decline in J_{ms} alone; J_{sm} in the absence of Na does not differ significantly from that observed in the presence of Na over the 100-fold range of lysine concentrations studied. These findings resemble those reported for L-alanine transport across the same preparation of rabbit ileum (12). It is important to note that although J_{net} is markedly reduced in the absence of Na, it does not appear to be entirely abolished. In every sampling period in the presence of 0.1 mM lysine (20 sampling periods) and 1.0 mM lysine (15 sampling periods) J_{ms} exceeded J_{sm}, and J_{net}, though small, is statistically different from zero. In the presence of 10 mM

lysine the unidirectional transmural fluxes in the absence of Na are equal, within experimental error, and J_{net} does not differ significantly from zero.

The Effect of a Transmural Electrical Potential Difference on Transmural Lysine Fluxes

Previous studies on the effect of transmural electrical potential differences on unidirectional and net Na fluxes across rabbit ileum have suggested that the serosa-to-mucosa flux of Na is attributable to simple ionic diffusion (11). Clarkson has reported a similar finding for the case of in vitro rat ileum (16). These observations suggest that a large fraction of the bidirectional Na fluxes across isolated rat and rabbit ileum may be the result of ionic diffusion through intercellular shunt pathways (13, 16). It was therefore of interest to examine the effect of a transmural electrical potential difference on lysine fluxes. This was accomplished in the following manner.

Unidirectional fluxes in the same direction (i.e. either J_{ms} *or* J_{sm}) were determined at the same time on adjacent segments of ileum from the same animal. One segment of tissue was maintained under short-circuit conditions and the other segment of tissue was voltage-clamped at a transmural electrical potential difference (PD) of 35 mv, mucosa negative with respect to serosa. In five experiments (lysine concentration, 10 mM) J_{ms} averaged 0.35 ± 0.03 μmole/hr, cm² under short-circuit conditions, and 0.35 ± 0.07 μmole/hr, cm² in the presence of the 35 mv electrical potential difference. The ratio of J_{ms} at PD = 0 to J_{ms} at PD = 35 mv in paired tissues is 1.07 ± 0.16 and does not differ significantly from unity. In nine experiments (lysine concentration, 10 mM) J_{sm} averaged 0.23 ± 0.06 μmole/hr, cm² under short-circuit conditions, and 0.32 ± 0.03 μmole/hr, cm² when PD was 35 mv. The ratio of J_{sm} at PD = 0 to J_{sm} at PD = 35 mv in paired tissues exceeded unity in each experiment and averaged 1.34 ± 0.11, a value that is significantly different from 1.0 ($p < 0.001$).

Although the increase in J_{sm} when the mucosa is clamped electrically negative with respect to the serosa is what one would expect if part of the J_{sm} were due to ionic diffusion through a transmural shunt, the observation that J_{ms} is not affected by the transmural electrical potential difference essentially rules out this possibility. If a shunt pathway were present, an increase in J_{sm} in response to an electrical potential difference should be accompanied by a decrease in J_{ms} ; that is, the electrical potential difference should affect both unidirectional fluxes. Although the lack of an effect on J_{ms} could be due to two events that fortuitously cancel, the close agreement between the average fluxes in the presence and absence of the PD makes this possibility unlikely and we are forced to conclude that lysine diffusion through a transmural (intercellular) shunt pathway does not contribute significantly to the bidirectional movements of lysine across in vitro rabbit ileum. Possible explanations for the observed increase in J_{sm} will be discussed below.

These findings together with the observations of Schultz and Zalusky (11) and Clarkson (16) on Na diffusion across isolated ileum raise interesting implications regarding the properties of the proposed shunt pathways. For, if such shunts permit transmural diffusion of Na, K, and Cl, but do not provide a significant diffusion pathway for lysine, they must be highly selective. If this selectivity is based on ionic size, then the pathway must be capable of distinguishing between ionic radii of 2–3 A (Na, K) and an ionic radius of perhaps 4–6 A (lysine). Clarkson has suggested that the passive channel for Na diffusion might be holes in the epithelial layer caused by the exfoliation of epithelial cells. He has calculated that these holes would have a diameter of 4 μ and a negative surface charge density of 3000 esu/cm^2. It seems highly unlikely that such holes could possess the high degree of selectivity indicated by the present data and by previous observations that suggest that in vitro rabbit ileum is impermeant to the organic cation, Tris[2-amino-2(hydroxymethyl)-1,3 propanediol] (17).[3] The most obvious structure that could provide a transmural shunt pathway across intact epithelium is the lateral intercellular space. This space is bounded at the luminal pole by the tight junction (zona occludente) and at the basal pole by the basement membrane. Although the properties of the basement membrane are poorly defined it is generally believed to be freely permeable to the many solutes transported by the small intestine. If this were not the case, special transport mechanisms would be necessary to provide for movement across the basement membrane into the underlying capillary bed after exit from the epithelial cells. The permeability properties of the tight junctions are also incompletely defined. However, Lowenstein (18) has reported that the pathway between the intercellular space and the lumen is characterized by a high electrical resistance in a variety of epithelial structures. Thus, the tight junctions could possess

[3] If J_{sm} is attributable to ionic diffusion and if the tissue conductance is independent of the transmural electrical potential difference, J_{sm} under short-circuit conditions ($_oJ_{sm}$) is related to J_{sm} in the presence of a PD by (11).

$$J_{sm}/{}_oJ_{sm} = \frac{F(\psi_m - \psi_s)/RT}{\exp\left[F(\psi_m - \psi_s)/RT\right] - 1}$$

where ψ is the electrical potential and the subscripts m and s denote the mucosal and serosal surfaces of the tissue (equation 6 of reference 11). When $\psi_m - \psi_s = -35$ mv, $J_{sm}/{}_oJ_{sm} \approx 2$; that is, if the J_{sm} of lysine were due to diffusion, the imposition of a 35 mv transmural electrical potential difference would have doubled the flux. If the observed increase by a factor of 1.3 is due to the fact that only a fraction of the J_{sm} of lysine is due to diffusion, it can be readily shown that under short-circuit conditions this portion could not exceed 0.08 μmole/hr, cm^2 or approximately one-third of the observed flux. The partial Na conductance of rabbit ileum in the presence of 140 mM Na is 6.1 mmhos/cm^2. If the shunt pathway could not discriminate between Na and lysine, J_{sm} of lysine under short-circuit conditions due to diffusion would be 0.4 μmole/hr, cm^2, a value that is approximately twice that of the actually observed flux. Further, if 0.08 μmole/hr, cm^2 was due to lysine diffusion through a shunt pathway, the resistance of this pathway to lysine diffusion would be five times the resistance to Na diffusion. Compared to Na diffusion a hole with a diameter of 4μ could hardly be expected to impose such a restriction on lysine diffusion.

the high degree of selectivity consistent with our observations and could control the admission of solutes into the shunt pathway. Additional studies are indicated in order to define further the properties of transmural diffusion pathways and the possible role of the tight junctions in determining the selectivity of these extracellular shunts.

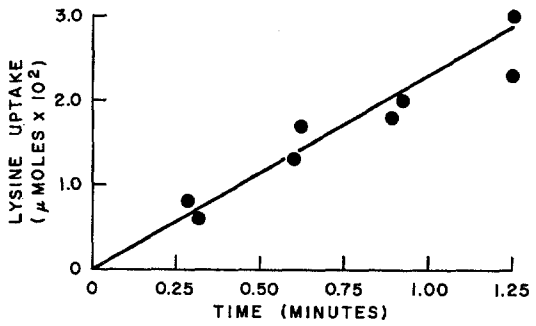

FIGURE 2. Time course of lysine uptake across mucosal border of rabbit ileum.

TABLE II

UNIDIRECTIONAL LYSINE INFLUX*

Lysine concentration	$[Na]_m$ = 140 mM		$[Na]_m$ = 0	
	n	J_{mc}	n	J_{mc}
mM		μmoles/hr, cm²		μmoles/hr, cm²
0.10	18	0.17±0.01	7	0.13±0.01
0.17	7	0.27±0.03		—
0.20		—	5	0.20±0.02
0.50	7	0.43±0.03	4	0.31±0.01
1.0	11	0.74±0.04	9	0.50±0.05
5.0	6	1.70±0.17	10	1.00±0.09
10.0	9	2.40±0.17	15	1.34±0.11
20.0	8	3.63±0.29	9	1.95±0.08

$[Na]_m$ denotes the Na concentration in the mucosal solution.
* n = number of determinations; all errors are standard errors of the mean.

Lysine Influx

The unidirectional influx of lysine from the mucosal solution across the brush border into the epithelium was determined as a function of lysine concentration in the mucosal medium. A typical time course of lysine uptake as a function of duration of exposure of the mucosal surface of the tissue to a solution containing 10 mM lysine and 140 mM Na is shown in Fig. 2. These data were obtained on tissue from the same animal using each of the eight available ports for an exposure of different duration. Lysine uptake is a linear function of time for at least 1.25 min and the line extrapolates through the origin. Thus, the 1 min uptake accurately reflects the unidirectional influx of lysine across the mucosal border into the epithelium. Further, the observation that the

line extrapolates to the origin indicates that the data are not influenced by surface binding, or by contamination of the tissue by adherent radioactive medium that was not accounted for by the inulin-^{3}H assay. One would expect that surface binding would be a rapid process, and that the degree of contamination would be time-independent so that if either of these were present the line should be shifted from the origin toward a positive intercept on the ordinate.

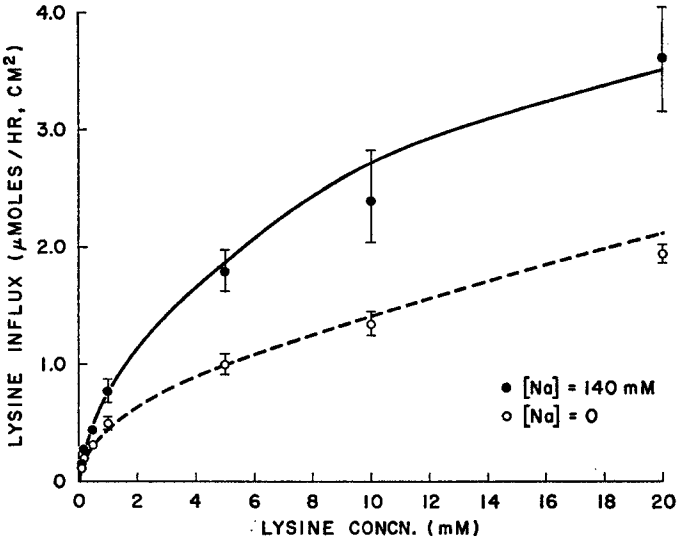

FIGURE 3. Unidirectional lysine influx across brush border of rabbit ileum as a function of mucosal lysine concentration in the presence of 140 mM Na (●) and in the presence of a Na-free, choline medium (○).

The unidirectional influxes of lysine from mucosal solutions containing 0.1–20 mM lysine and 140 mM Na are given in Table II and are plotted as a function of lysine concentration in Fig. 3 (solid circles). In all these experiments, the tissue was preincubated with a mucosal solution consisting of normal buffer containing neither lysine nor glucose. The radioactive test solution consisted of the same buffer to which inulin-^{3}H and the desired concentration of lysine-^{14}C were added. In each experiment influxes in the presence of four different concentrations of lysine were determined in duplicate using tissue from the same animal. The data shown in Fig. 3 cannot be described by a rectangular hyperbola typical of a single saturable process, nor can they be described by the combination of a single saturable process and an influx that is linear with concentration (i.e. the so-called "nonsaturable uptake" described by Christensen and Liang for amino acid influx into ascites tumor cells [19]). Furthermore, the data do not fit the typical kinetics of a carrier model that involves the combination of two lysine molecules with a

single carrier molecule. However, the data can be adequately described by two saturable processes operating in parallel. Under these conditions, J_{mc} at any lysine concentration represents the sum of two influx processes and is given by:

$$J_{mc} = J'_{mc} + J''_{mc} = [L]_m \left[\frac{J^{m'}_{mc}}{K' + [L]_m} + \frac{J^{m''}_{mc}}{K'' + [L]_m} \right]$$

J^m_{mc} is the maximal influx, K is the mucosal lysine concentration at which influx is half-maximal, and $[L]_m$ is the lysine concentration in the mucosal medium. If K' and K'' are sufficiently different, $J^{m'}_{mc}$, $J^{m''}_{mc}$, K', and K'' can be evaluated by a method of successive approximations. In this manner the following equation was obtained relating J_{mc} to $[L]_m$ when $[\mathrm{Na}]_m = 140$ mM.

$$J_{mc} = [L]_m \left[\frac{0.4}{0.3 + [L]_m} + \frac{4.6}{10.0 + [L]_m} \right]$$

This equation is represented by the solid line in Fig. 3 and it appears to provide an adequate description of the experimental data.

Experiments were performed to evaluate the effect of intracellular lysine on lysine influx into the epithelial layer. Tissues were preincubated for 30 min with normal buffer containing 4 mM unlabeled lysine, and paired tissues from the same animal were preincubated under identical conditions except that the buffer did not contain lysine. Lysine influx was then determined using normal buffer containing 10 mM lysine-^{14}C. Lysine influx into tissue preexposed to lysine for 30 min averaged 2.7 ± 0.3 μmoles/hr, cm^2 and lysine influx in the paired control tissues averaged 2.7 ± 0.5 μmoles/hr, cm^2. These data indicate that lysine influx is not influenced by exchange diffusion or transconcentration effects at the concentrations studied.

The unidirectional influxes of lysine from a medium rendered Na-free by replacement with choline are also given in Table II and plotted in Fig. 3 (open circles). In all these experiments the tissue was preincubated for 30 min with a mucosal solution consisting of the Na-free choline buffer. Inspection of the data in Table II indicates that lysine influx is inhibited by Na replacement at all concentrations but that the per cent inhibition is greater at higher concentrations than at lower concentrations. This suggested that J'_{mc}, the influx process that predominates at low $[L]_m$, might be Na-independent and that the entire effect of Na on J_{mc} might be attributable to a change in J''_{mc} (the process that predominates at high $[L]_m$). Accordingly, J'_{mc} ($K' = 0.3$ mM, $J^{m'}_{mc} = 0.4$ μmole/hr, cm^2) was subtracted from the values of J_{mc} obtained in the absence of Na and the remainder was plotted according to the method of Lineweaver and Burk. Double reciprocal plots of J''_{mc} (i.e. $J_{mc} - J'_{mc}$) in the presence of 140 mM Na (closed circles) and in the absence of Na (open circles) for $[L]_m = 5$, 10, and 20 mM are shown in Fig. 4. The points can be

described by two lines that have a common intercept on the ordinate corresponding to a common $J_{mc}^{m''}$ equal to 4.6 μmoles/hr, cm^2. The slopes of the two lines differ and correspond to a K'' of 10 mM when [Na] = 140 mM and a K'' of 35 mM when $[Na]_m = 0$. The dashed line in Fig. 3 represents the equation

$$J_{mc} = [L]_m \left[\frac{0.4}{0.3 + [L]_m} + \frac{4.6}{35 + [L]_m} \right]$$

and adequately describes the experimental values of J_{mc} in the absence of Na.

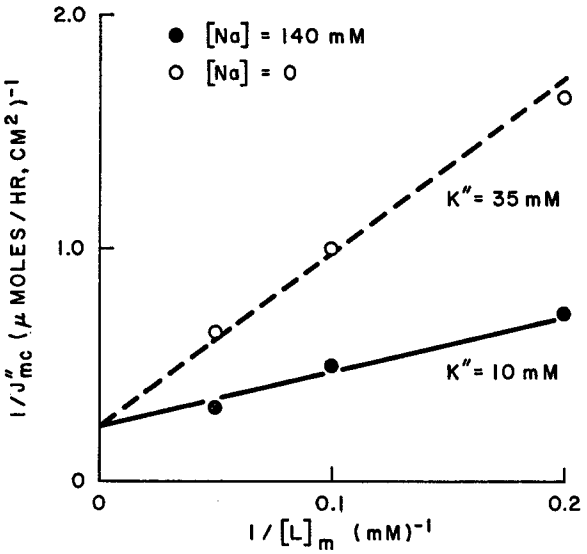

FIGURE 4. Lineweaver-Burk plot of J''_{mc} vs. $[L]_m$. Closed circles denote J''_{mc} in the presence of 140 mM Na, and open circles denote J''_{mc} in the presence of a Na-free, choline medium.

Thus, unidirectional influx of lysine across the brush border of isolated rabbit ileum appears to be mediated by two saturable processes that operate in parallel. One predominates at low mucosal lysine concentrations and appears to be Na-independent. The second predominates at high lysine concentrations and is Na-dependent. The Na dependence of the latter process is reflected in the lysine concentration at which influx is half-maximal (K''); K'' increases from 10 mM to 35 mM when the mucosal medium is changed from one that contains 140 mM Na to one that is Na-free. On the other hand, the maximal influx of the Na-dependent process is unaffected by total replacement of the Na in the mucosal medium with choline. These characteristics of the Na-dependent lysine influx process are qualitatively similar to those described previously for the Na dependence of neutral amino acid influx across the brush border of rabbit ileum (20). Christensen and Liang have

demonstrated that lysine influx into Ehrlich ascites tumor cells may also be described by two saturable processes, one that predominates at low lysine concentrations and one that predominates at higher lysine concentrations. However, neither of these processes appears to be dependent upon extracellular Na (21).

Before discussing the implications of these findings a brief digression on the kinetic analysis of lysine influx is warranted. We have demonstrated that J_{mc} can be adequately described by two parallel saturable influx processes each of which displays classic Michaelis-Menten kinetics. Several alternative possibilities have been eliminated; however, other kinetic treatments could be devised that would equally satisfy the experimental observations. The present treatment is justified on several grounds. First, it is almost certainly the simplest alternative that will describe the data. Second, and more important, it serves to distinguish between a Na-dependent and a Na-independent portion of lysine influx. The Na-dependent portion has all the kinetic characteristics of the many Na-dependent carrier processes described for sugar and amino acid transport in small intestine as well as other cells and tissue (20, 22), and the Na-independent portion displays the kinetic characteristics of many Na-independent carrier mechanisms. It is, of course, tempting to speculate that two distinct carrier mechanisms are involved in lysine influx, one associated with J'_{mc} and the other with J''_{mc}. Although this may be the simplest interpretation of our findings, it should be stressed that our kinetic analysis is strictly operational and that any extrapolation from this analysis to molecular mechanism is, at present, unwarranted.

Another possibility that should be considered is that J'_{mc} represents a Na-independent binding or adsorption to the brush border rather than influx across this barrier. The observation that high concentrations of leucine inhibit both J'_{mc} and J''_{mc} (B. G. Munck and S. G. Schultz, unpublished observations) suggests that J'_{mc} cannot simply represent adsorption of a cationic solute by fixed anionic groups on the brush border. Further, it is clear that under any circumstances $J_{mc} > J_{ms}$ (see equation 1 *a* below). If only J''_{mc} represented true influx, then when $[L]_m = 0.1$ mM, $J_{mc} = J''_{mc} = 0.05$ μmole/hr, cm². However, when $[L]_m = 0.1$ mM, $J_{ms} = 0.078$ μmole/hr, cm². Thus, at low lysine concentrations J''_{mc} alone cannot account for J_{ms}. This analysis supports the interpretation of J'_{mc} as a true influx rather than adsorption.

It is of interest to compare the Na-dependent portion of lysine influx with the kinetic characteristics of L-alanine influx determined using the identical methods and preparation (13, 20). The unidirectional influx of alanine was shown to display classic Michaelis-Menten kinetics and, in the presence of 140 mM Na, the maximal influx was 6 μmoles/hr, cm² and the alanine concentration required to elicit a half-maximal influx was 9 mM. For the Na-dependent portion of lysine influx $J^{m''}_{mc} = 4.6$ μmoles/hr, cm² and $K'' = 10$ mM, and the

maximal total lysine influx ($J_{mc}^{m'} + J_{mc}^{m''}$) is 5 μmoles/hr, cm². Thus, in spite of the fact that the J_{ms} and J_{net} of lysine are much lower than the corresponding transmural alanine fluxes, the characteristics of lysine and alanine influxes into the cell in the presence of Na are quantitatively similar. Therefore the low transport capacity for lysine, and, in particular, the difference between the rates of transmural transport of lysine and alanine cannot be attributed to differences in the unidirectional influxes of these amino acids across the brush border into the epithelium.

Although the Na-dependent component of lysine influx, and alanine influx are kinetically very similar in the presence of 140 mM Na, they differ signifi-

TABLE III

LYSINE ACCUMULATION BY MUCOSAL STRIPS*

	Medium Na concentration = 140 mM			Medium Na concentration = 0		
Initial lysine concentration	*n*	Final lysine concentration	Intracellular lysine concentration	*n*	Final lysine concentration	Intracellular lysine concentration
mM		*mM*	*mM*		*mM*	*mM*
0.1	6	0.05	1.2±0.2	6	0.07	0.6±0.01
1.0	8	0.7	20±0.8	8	0.95	4.8±0.4
10.0	16	9.0	57±1	12	9.5	27±1

In most experiments, accumulation in the presence and absence of Na was determined on tissue from the same animal.

* *n* = number of determinations; all errors are standard errors of the mean.

cantly in the absence of Na in one important respect. In a Na-free, choline buffer the alanine concentration required to elicit a half-maximal influx (K_t) is 70 mM, a sevenfold increase over the value observed in the presence of 140 mM Na (20). In contrast, K'' for lysine influx in the absence of Na is 35 mM, a value that is only 3.5 times that observed in the presence of 140 mM Na. Thus, alanine influx is much more markedly influenced by Na concentration than is the Na-dependent component of lysine influx. This observation confirms preliminary studies reported previously (10).

Intracellular Accumulation of L-Lysine

Mucosal strips of rabbit ileum were incubated in either normal buffer or Na-free choline buffer that initially contained either 0.1, 1.0, or 10 mM lysine-^{14}C. Since the medium-to-tissue ratio was not infinite, the extracellular lysine concentration decreased during the course of the 40–60 min incubation. Assays of the initial and final incubation media for lysine-^{14}C were employed to calculate final steady-state extracellular lysine concentrations. The intracellular lysine concentrations calculated after correction for the lysine content of the extracellular space (0.30 ml/g wet weight) are given in Table III and

Fig. 5. In the presence of 140 mM Na, intracellular lysine concentrations greatly exceeded the extracellular lysine concentrations at all concentrations studied. The ratio of intracellular to extracellular lysine ranged from approximately 25 at final extracellular concentrations of 0.05 and 0.7 mM to approximately 6 at a final extracellular concentration of 9 mM. These values are considerably higher than those reported for in vitro preparations of whole intestine of rat (7) and hamster (8) and also exceed the values reported by McCarthy et al. for lysine uptake by human intestinal mucosa (23). However,

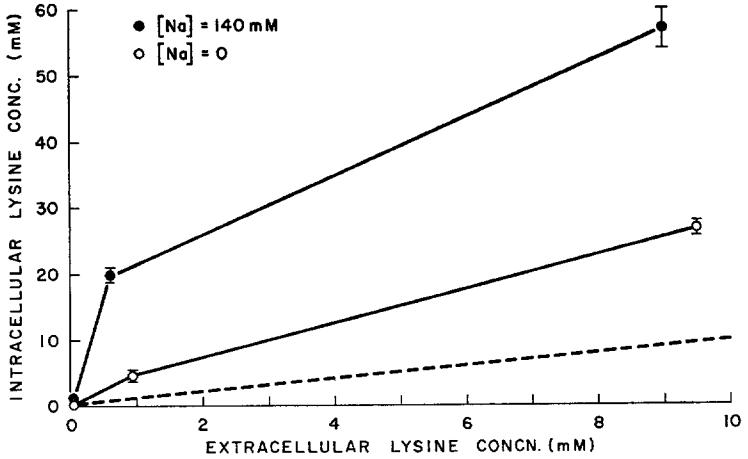

FIGURE 5. Intracellular lysine concentration as a function of extracellular lysine concentration in the presence (●) and absence (○) of Na. The dashed line represents a distribution ratio of unity.

Rosenberg et al. (6) have reported intracellular to extracellular distribution ratios for lysine in whole segments of rabbit jejunum of more than 50 following incubation in the presence of 0.025 mM lysine and 144 mM Na.

It is of interest to compare, once more, alanine transport and lysine transport as reflected by accumulation within mucosal strips. We have previously demonstrated that when mucosal strips of rabbit ileum are incubated in a medium containing 5 mM alanine and 140 mM Na a steady-state intracellular alanine concentration of 40–50 mM is achieved within 30 min. From the data shown in Fig. 5 we may deduce that the steady-state intracellular lysine concentration in the presence of 5 mM lysine and 140 mM Na would be approximately 40 mM (and, if the points were joined by means of a curve rather than by a line a somewhat higher value would be obtained). Thus, as was the case for influx, the low transmural fluxes of lysine compared with those of alanine are not paralleled by differences in the ability of mucosal strips to accumulate these two amino acids.

Tissue accumulation of lysine was markedly inhibited when the mucosal

strips were incubated in the Na-free, choline buffer. However, as shown in Table III and Fig. 5, steady-state distribution ratios that significantly exceeded unity were obtained at all lysine concentrations studied; these ratios ranged from a value of approximately 9 at a final extracellular lysine concentration of 0.07 mM to approximately 3 at a final extracellular lysine concentration of 9.5 mM.

The interpretation of these data is complicated by several factors. First, there are always uncertainties regarding the adequacy of the correction for the lysine content of the extracellular space. An overestimate of this space would lead to an overestimate of the intracellular lysine concentration. However, even if our results were expressed in terms of the lysine concentration in total tissue water, the distribution ratios would significantly exceed unity in the presence as well as in the absence of Na. Second, even if the intracellular lysine contents are correct, to a first approximation, there is no justification for assuming that the intracellular distribution of the amino acid is homogeneous. Finally, and perhaps most important, the extent to which the electrical potential differences across the mucosal and serosal membranes influence the steady-state distribution ratio of lysine is, at present, unknown. The only reported measurements of transmembrane electrical potential differences across the mucosal and serosal membranes of mammalian small intestine are those of Wright (24). He found that the potential differences across the mucosal and serosal membranes were 8.4 and 21 mv, respectively, in the presence of 28 mM glucose and 115 mM Na, with the cell interior electrically negative with respect to the mucosal and serosal bathing solutions. These values cannot be applied directly to the present studies, because, apart from the fact that they were determined using hamster jejunum, the mucosal strips of rabbit ileum are largely "short-circuited" due to the low electrical resistance of the surrounding incubation medium. However, using Wright's data as a rough index, it is probably safe to assume that in the presence of Na the cell interior of mucosal strips of rabbit ileum is no more than 10–20 mv negative with respect to the surrounding fluid. Assuming that the activity coefficients of intracellular and extracellular lysine are equal, this could account for distribution ratios of 1.5 to 2.1 if the steady-state lysine distribution were determined solely by the transmembrane electrical potential difference. These ratios are considerably lower than the range of distribution ratios of 6 to 25 observed in the presence of 140 mM Na, so that it appears safe to conclude that under these conditions lysine accumulation by the tissue represents net transport against an electrochemical potential difference.[4]

[4] In view of these arguments, the finding of a lysine distribution ratio less than unity (7) implies either (*a*) that a steady-state distribution was not achieved, or (*b*) the presence of a transport mechanism that brings about the extrusion of lysine from the cell against an electrochemical potential difference.

The situation in the absence of Na is far more uncertain. There is no direct information on steady-state transmembrane electrical potential differences across mammalian small intestine in the presence of a Na-free buffer containing 140 mM choline. Previous studies have shown that incubation of mucosal strips of rabbit ileum in a Na-free choline buffer results in a marked reduction in the intracellular Na concentration but that the intracellular K concentration is not significantly affected and is greater than the K concentration in the incubation medium by a factor of 10 (14). Thus, although the transmural electrical potential difference is abolished when both surfaces of the tissue are perfused with a Na-free choline buffer (11), there is every reason to believe that an electrical potential difference between the cell interior and the surrounding medium persists and, indeed, the relative electrical negativity of the cell interior may be greatly increased over that in the normal buffer. The recent studies of Gilles-Baillien and Schoffeniels on the electrical potential profile across the small intestine of the Greek tortoise indicate that reduction of the sodium concentration in the mucosal or serosal perfusion media leads to marked increases in the transmucosal and transserosal electrical potential differences, respectively, and values as high as 45 mv, cell interior negative, were observed (25). The steady-state lysine distribution ratios observed in the absence of Na ranged from 3 to 9. If these distribution ratios were determined entirely by the electrical potential difference, the cell interior would have to be 30–56 mv negative with respect to the incubation medium. These values, especially the former, are not unreasonable. Therefore, without direct measurements of the transmembrane electrical potential differences, we cannot conclude that lysine uptake in the absence of Na involves net movement against an electrochemical potential difference.

The purpose of this somewhat lengthy discussion is to stress the difficulties inherent in interpreting data on intracellular lysine accumulation. Previous studies on lysine accumulation by intestinal and renal tissue have not explicitly considered the fact that lysine is a monovalent cation. Although it is possible that lysine movements and the steady-state intracellular lysine concentrations are not significantly influenced by transmembrane electrical potential differences, we have no direct information on this point. In the absence of this information, interpretation of data on lysine accumulation by intestinal and renal tissue, and on the effects of changes in the ionic environment on these distribution ratios, is hazardous.

CONCLUSIONS

At present there are three experimental approaches that can be applied to the study of amino acid transport by in vitro preparations of mammalian small intestine; namely, the determination of (*a*) transmural fluxes, (*b*) ac-

cumulation by segments of epithelium, and (*c*) unidirectional influx from the mucosal bathing solution into the epithelium. All these approaches involve the use of somewhat different preparations of the tissue. Although there is no apparent reason why the resulting data should not be quantitatively compatible, there is no direct evidence on this point. In the following discussion we will assume that the data obtained in the present investigation can be combined in a qualitative fashion to give a composite picture of lysine transport across in vitro rabbit ileum.

One of the conclusions that emerges from an examination of all the data is that there may be a Na-independent mechanism that is capable of bringing about net lysine transport across the tissue in the absence of an electrochemical pontential difference of lysine. In the absence of Na, net lysine flux across short-circuited segments of rabbit ileum is only 5–10% of that observed in the presence of Na, but the differences between J_{ms} and J_{sm} in the presence of 0.1 and 1.0 mM lysine appear to be significant. Furthermore, in the absence of Na the calculated intracellular lysine concentration in the presence of 0.1 mM lysine is nine times that in the surrounding medium. As discussed previously, we cannot conclude that this represents transport against an electrochemical potential difference; however, the electrical potential difference of 56 mv that would be required for a passive distribution of lysine is somewhat high though not inconceivable. Finally, our analysis of lysine influx kinetics is consistent with the presence of a saturable Na-independent influx process. None of these findings was observed in previous studies of alanine transport by rabbit ileum (12, 14, 20); alanine transport against a concentration difference is entirely abolished in the absence of Na. Taken individually, each of these pieces of evidence does not provide a compelling argument for a Na-independent mechanism for net transmural lysine transport; however, when *all* these data are considered it is difficult to reject this possibility. Further studies are necessary to establish this point conclusively. It should be stressed that if such a mechanism is present, its contribution to net lysine transport across the tissue in the presence of physiological Na concentrations is minute. It is of interest to note that lysine influx into Ehrlich ascites tumor cells appears to be Na-independent (21) and that Fox et al. (26) have concluded that lysine transport into slices of rat kidney cortex is mediated by two mechanisms, one Na-dependent and the other Na-independent.

The low transport capacity of small intestine for transmural lysine transport, first noted by Hagihira et al. (2) using hamster intestine and subsequently demonstrated using everted sacs of rat intestine (4, 5, 7) has been confirmed for the case of isolated rabbit ileum. Further, by employing the short-circuit technique we have demonstrated that the reported low transport capacity for lysine cannot be entirely attributed to the fact that previous studies of lysine transport were performed in the presence of an adverse trans-

mural electrical potential difference. We have demonstrated that the net transport of lysine from mucosa to serosa is much smaller than that of alanine even when the electrical potential difference across the tissue is abolished. Since the carrier mechanisms primarily responsible for the transport of amino acids from a lower concentration in the mucosal solution to a higher concentration in the cell appear to be located on or near the brush border of the cells (14, 27), one might have presumed that the low transport capacity for lysine might be the result of a low influx across the mucosal border and a

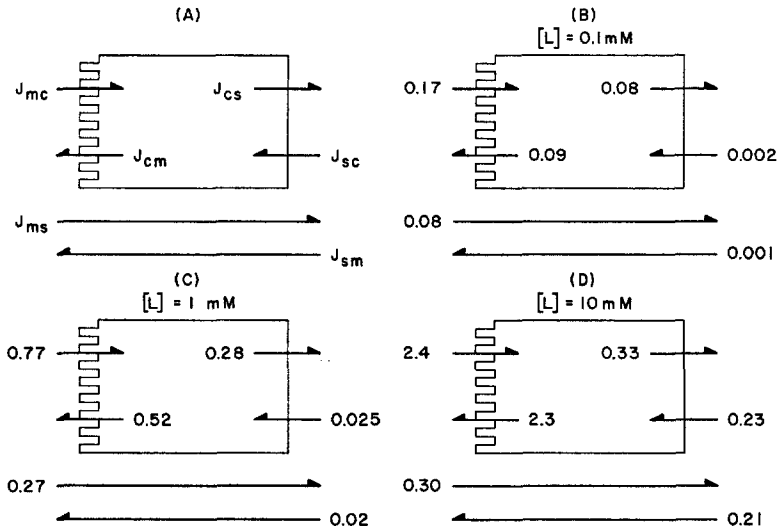

FIGURE 6. Unidirectional lysine fluxes across the mucosal and serosal membranes. All values are in units of μmoles/hr, cm^2.

low level of lysine accumulation within the epithelial cells. Our results exclude this possible explanation. We have demonstrated that lysine influx and intracellular accumulation are quantitatively comparable to alanine influx and accumulation that were determined on the same preparations using identical methods. Why then is the net transmural flux of lysine less than one-fifth the net transmural flux of alanine at comparable concentrations?

A possible answer to this question emerges from an analysis of the steady-state bidirectional fluxes of lysine across the mucosal and serosal borders of the epithelium. These fluxes are illustrated in Fig. 6 A and are related by the following equations:

$$J_{ms} = J_{mc}J_{cs}/(J_{cm} + J_{cs}) \qquad (1a)$$

$$J_{sm} = J_{sc}J_{cm}/(J_{cm} + J_{cs}) \qquad (1b)$$

$$J_{net} = J_{ms} - J_{sm} = J_{mc} - J_{cm} = J_{cs} - J_{sc} \qquad (1c)$$

As discussed previously (13) these equations are based on the assumption that the mucosal and serosal solutions and the intervening epithelium may be represented by a three compartment system. This representation ignores the fact that the tissues underlying the epithelial cells constitute a diffusion barrier for solute transport and cannot be described by a homogeneous compartment. However, in a composite system consisting of a series-array of membranes, the steady-state net flux across the total system must be equal to the net flux

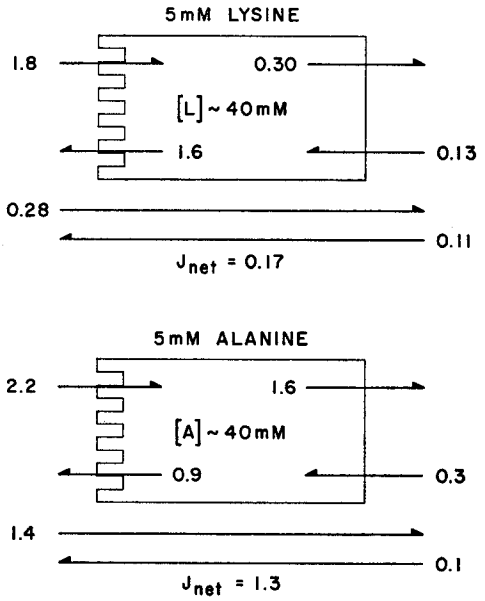

FIGURE 7. Comparison of unidirectional fluxes of lysine and alanine across the mucosal and serosal membranes in the presence of 5 mM amino acid and 140 mM Na. All values are in units of μmoles/hr, cm^2.

across each of the membranes arranged in series. Therefore, J_{cm} can be derived directly as the difference between J_{mc} and J_{net} and only J_{cs} and J_{sc} are subject to the shortcomings of this representation. The unidirectional fluxes of lysine across the mucosal and serosal membranes obtained using equations 1, are illustrated in Fig. 6. It is apparent from Fig. 6 D that one reason why the net flux of lysine is extremely low in the presence of 10 mM lysine in spite of a high unidirectional influx is that the efflux of lysine out of the cell across the mucosal border is almost equal to the influx; all but approximately 5% of the lysine influx across the mucosal border is counterbalanced by this high efflux. The difference between lysine and alanine fluxes is illustrated in Fig. 7. The alanine data represent typical fluxes in the presence of 5 mM alanine and were published previously (13). The lysine fluxes in the presence of 5 mM lysine were obtained from Table II and Fig. 1; the intracellular lysine concentration was interpolated from the data shown in Fig. 5. Of interest is the fact that in the case of alanine, J_{cm} is only 40% of J_{mc} giving a net flux of 1.3 μmoles/hr, cm^2, whereas in the case of lysine J_{cm} is 90% of J_{mc} resulting in a small net

flux of 0.17 μmole/hr, cm². Thus, in spite of the fact that the calculated intracellular concentrations of lysine and alanine are equal, lysine efflux out of the cell across the brush border is approximately twice that of alanine.

The relatively low Na dependence of lysine influx compared to that of alanine influx may provide a possible explanation for this difference in J_{cm}. We have demonstrated that the Na-dependent portion of lysine influx has a K'' of 10 mM in the presence of 140 mM Na and a K'' of only 35 mM in the absence of Na. The K_t for alanine influx in the presence of 140 mM Na is 9

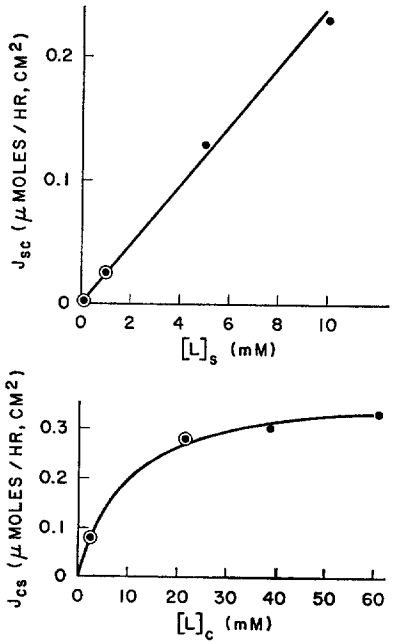

FIGURE 8. (Upper) J_{sc} as a function of $[L]_s$; (lower) J_{cs} as a function of $[L]_c$. As discussed in the text, the values calculated for 0.1 mM and 1 mM lysine are subject to error and are designated by the double circles.

mM but increases to 70 mM in the absence of Na. The maximal values of alanine influx and the Na-dependent portion of lysine influx do not differ significantly. Thus, at low Na concentrations alanine influx is more markedly inhibited than is the Na-dependent portion of lysine influx. Indirect evidence has been presented that the process that mediates amino acid efflux out of the cell across the brush border may be influenced by the local intracellular Na concentration in much the same manner as is the influx process (28). If so, at a given intracellular amino acid concentration, J_{cm} would increase with increasing intracellular Na and vice versa. The net flux across the brush border ($J_{mc} - J_{cm}$) would be determined by the difference between the Na concentrations in the mucosal medium and the cell interior, and by the degree to which the transport constant, K_t (i.e. the concentration at which the unidirectional flux is half-maximal), is affected by Na. If this model of the

efflux process is correct, then at a given low intracellular Na concentration lysine efflux out of the cell across the brush border would be expected to exceed that of alanine when the intracellular concentrations of these two amino acids are equal. To illustrate this point, if the influx and efflux processes were identical and if the local intracellular Na concentration were close to zero, lysine efflux would be half-saturated at an intracellular lysine concentration of 40 mM but at the same intracellular concentration alanine efflux would be well below the half-saturation point.

As discussed above, the calculation of J_{sc} and J_{cs} is based on the assumption that the serosal tissues do not impose a significant restriction on the movement of lysine across the tissue; that is, it assumes that the serosal tissues may be considered a homogeneous extension of the serosal bathing medium. This assumption is clearly incorrect. However, the errors introduced in the calculation of J_{sc} and J_{cs} depend upon the permeability of the serosal tissues to lysine and increase as diffusion through this barrier becomes rate-limiting. In order to estimate the validity of the calculated values of J_{cs} and J_{sc} we have measured the diffusion of lysine across segments of rabbit ileum after removing the epithelial cell layer by gentle scraping with a glass slide. In the presence of 10 mM lysine, the bidirectional fluxes across this *serosal strip* were equal and averaged 1.1 $\pm$ 0.1 μmoles/hr, cm^2, a value that is 10 times greater than the net flux of lysine across the total tissue. Thus, when the net flux across the tissue is 0.1 μmole/hr, cm^2, the concentration of lysine at the serosal surface of the epithelial cell layer need be only 11 mM compared to 10 mM in the serosal bathing medium. Under these conditions, transport across the cell layer is rate-limiting compared to diffusion across the serosal tissues, and the error introduced by considering the serosal tissues an extension of the serosal medium is minimal. Using a permeability coefficient of 0.11 cm/hr for lysine diffusion across the serosal tissues the lysine concentrations at the serosal border of the epithelial cells needed to produce the net fluxes observed in the presence of 5 mM, 1 mM, and 0.1 mM lysine are 6.5 mM, 3.2 mM, and 0.9 mM, respectively. Clearly, in the presence of 1 mM and 0.1 mM lysine there are steep concentration gradients across the serosal tissues and the assumption that this region can be treated as an extension of the serosal medium is invalid. At present we are unable to provide an estimate of the errors involved in the calculations of J_{sc} and J_{cs} in the presence of these low lysine concentrations.

With the above reservations in mind, it is of interest to examine the behavior of J_{cs} and J_{sc}, particularly in the presence of high lysine concentrations where the errors involved are minimal. The values of J_{cs} are plotted as a function of the intracellular lysine concentration in Fig. 8 (lower). It is clear that J_{cs} does not increase appreciably when the intracellular lysine concentration increases from 40 to 60 mM. Further, all the data shown in Fig. 8

(lower) can be described by the equation

$$J_{cs} = \frac{0.38[L]_c}{9.5 + [L]_c} \tag{2}$$

that has the typical Michaelis-Menten form (solid curve). Although the uncertainty of the lower two points introduces an uncertainty in the value of the intracellular lysine concentration at which J_{cs} is half-maximal (9.5 mM), it seems safe to conclude that with increasing intracellular lysine concentration J_{cs} approaches a maximal rate of approximately 0.35–0.4 μmole/hr, cm². These data are consistent with the view that lysine efflux out of the cell across the serosal and/or lateral membranes is a carrier-mediated process[5] and provide important insight into the low transport capacity of lysine. It is clear that under *any* condition the maximal rate of net transmural lysine transport must be less than J_{cs} since J_{sc} must always have a finite positive value. It should be noted (Fig. 7) that in the presence of equal intracellular concentrations J_{cs} for lysine is only one-fifth the value of J_{cs} for alanine.

On the basis of these findings the mechanism of the low transport capacity for lysine can be visualized as follows. Lysine enters the cell across the brush border by means of an influx mechanism that has a relatively high maximal velocity, one that compares favorably with that of alanine. Efflux of lysine out of the cell across the serosal or lateral membranes is mediated by a mechanism that is characterized by a low maximal velocity and that fixes the upper limit of net transmural lysine flux. Prior to the achievement of a steady state, influx into the cell will exceed efflux out of the cell leading to an accumulation of lysine within the cell. A steady-state intracellular lysine concentration is reached when the net flux across the brush border equals the net flux across the serosal border. At this point, unidirectional efflux out of the cell across the brush border counterbalances all but a few per cent of the influx across that boundary due to the high intracellular lysine concentration possibly combined with a low Na-dependence of the efflux process. According to this model, the *primary reason for the low rate of transmural lysine transport is the presence of an efflux process at the serosal or lateral boundaries of the cell that is characterized by a low maximal rate*. It is quite likely that this rate-limiting process is mediated by a carrier mechanism but more direct evidence is required to establish this point conclusively.

As shown in Fig. 8 (upper), J_{sc} is a linear function of the lysine concentra-

[5] Previous investigators have suggested the presence of a carrier mechanism for amino acids at the serosal or lateral membranes that is responsible for exit from the absorptive cell (29). Indeed the observation that most cell membranes are essentially impermeable to molecules having five or more carbon atoms has long implied that the exits of sugars and amino acids from intestinal cells are carrier-mediated processes in spite of the fact that net transport is directed down an electrochemical potential gradient.

tion in the serosal bathing medium over the range studied.[6] This relation can provide a possible explanation for the observation that J_{sm} increases whereas J_{ms} is unchanged when the mucosal surface is clamped at 35 mv negative to the serosal surface. Examination of equations 1 *a* and 1 *b* reveals that J_{sm} is directly proportional to J_{sc} but that J_{ms} is unrelated to J_{sc}. Thus, an increase in J_{sc} by a factor of 1.3 in the presence of a 35 mv PD would produce the observed increase in J_{sm} without affecting J_{ms}. When the tissue is voltage-clamped so that the mucosal surface of the tissue is 35 mv electrically negative with respect to the serosal surface, the region immediately adjacent to the serosal borders of the epithelial cells must be electrically negative with respect to the serosal solution because the serosal tissues must offer some resistance to the flow of current. Thus, the steady-state lysine concentration in the region adjacent to the serosal borders of the cells will be greater when the tissue is clamped at 35 mv than under short-circuit conditions. In the presence of 10 mM lysine the increase in J_{sm} produced by imposing a 35 mv PD across the tissue (mucosa negative) is sufficient to abolish net transport; that is, under these conditions J_{ms} and J_{sm} do not differ significantly. In the absence of net transmural transport, the region adjacent to the serosal surface of the epithelial cell layer need be only 8 mv negative with respect to the serosal fluid in order to increase the lysine concentration in this region by a factor of 1.3 (i.e. from 10 mM to 13 mM). According to the relation shown in Fig. 7 (upper) this increase in local lysine concentration would account for the observed increases in J_{sc} and J_{sm}. Although other explanations for the observed increase in J_{sm} in the presence of an imposed PD are possible (i.e. that J_{sc} is affected by the PD across the serosal or lateral membranes), the linear relations between J_{sm} and $[L]_s$ (Fig. 1) and between J_{sc} and $[L]_s$ (Fig. 8) suggest that a simple increase in the "effective" serosal lysine concentration is sufficient and that more complex mechanisms need not be invoked at present.

Finally, it has been generally assumed that the ability of the intestine to transport a given solute may be evaluated by investigating *either* net transport across the tissue or accumulation of the solute within the tissue; the implicit assumption being that these two phenomena parallel each other. The present results demonstrate that this assumption is invalid. Obviously, if exit of a solute from the cell is mediated by a transport mechanism having a low maximum velocity, high levels of accumulation within the cell may be associated with

[6] [Note added in proof] At first glance it might seem that the relation shown in Fig. 8 (upper) is inconsistent with the saturable process that describes J_{cs}. It should be noted that J_{sc} appears to be a linear function of $[L]_s$ over a concentration range that is relatively low compared to that of $[L]_c$ and significant deviations from linearity might become apparent only at higher values of $[L]_s$. Equation 2 predicts values of 0.13 and 0.19 μmole/hr, cm² for J_{cs} when $[L]_c$ is 5 mM and 10 mM, respectively. These values compare favorably with the values of 0.13 and 0.23 μmole/hr, cm² for J_{sc} when $[L]_s$ is 5 mM and 10 mM, respectively. Thus, in view of the uncertainties in evaluating J_{sc}, J_{cs}, and $[L]_c$, these data are not inconsistent with the suggestion that the same saturable process mediates J_{cs} and J_{sc}.

very low rates of net transmural transport. Further, there is a widespread tendency to associate rates of net transport or levels of intracellular accumulation with properties of carrier mechanisms at the brush border. The present results indicate that this approach can be misleading. For example, Lineweaver-Burk plots of the rate of net transmural transport vs. concentration have been used to estimate the "affinity" of the "carrier mechanism" for a given amino acid. We have shown that the rate of net lysine transport passes through a maximum and then declines, and Matthews and Laster (30) have demonstrated a similar phenomenon for glycine, alanine, leucine, and α-aminoisobutyric acid transport across everted sacs of hamster small intestine. This is to be expected whenever the mucosa-to-serosa and serosa-to-mucosa unidirectional fluxes conform to the patterns shown in Fig. 1, and need not reflect any specific inhibitory effect at high amino acid concentrations. Clearly the analysis of these net transport processes in terms of Michaels-Menten kinetics is not valid and kinetic parameters derived from such an analysis are neither descriptive of the entire process nor do they have meaningful physical counterparts. Furthermore, it is apparent from the present results that J_{ms} need not reflect the properties of the carrier mechanism at the brush border. Indeed, if as for the case of lysine, $J_{mc} \cong J_{cm} + J_{cs}$, or $J_{mc} \cong J_{cm} > J_{cs}$, equation 1 *a* predicts that $J_{ms} \cong J_{cs}$; that is, the unidirectional flux from mucosa to serosa closely parallels the rate-limiting efflux process. Indeed, from the data given in Table I and Fig. 1, one may estimate that the maximum value of J_{ms} is approximately 0.33 μmole/hr, cm^2 and that a half-maximal J_{ms} is observed in the presence of 0.3 mM lysine. From Fig. 5 we find that when the extracellular lysine concentration is 0.3 mM the intracellular lysine concentration is 9–10 mM. Thus the kinetic parameters that characterize J_{ms} appear to be in excellent agreement with those that characterize J_{cs} (equation 2), indicating that J_{ms} is primarily influenced by this rate-limiting process rather than by events at the mucosal border. Clearly, an accurate picture of intestinal transport demands that efforts be directed toward defining the separate events at the mucosal and serosal boundaries of the absorptive cells.

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