Kinetic Relations of the Na-Amino Acid Interaction at the Mucosal Border of Intestine

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ABSTRACT The relation between unidirectional influxes of Na and amino acids across the mucosal border of rabbit ileum was studied under a variety of conditions. At constant Na concentration in the mucosal bathing solution, amino acid influx followed Michaelis-Menten kinetics permitting determination of maximal influx and the apparent Michaelis constant, K_t. Reduction in Na concentration, using choline as substitute cation, caused an increase in K_t for alanine but had no effect on maximal alanine influx. The reciprocal of K_t was a linear function of Na concentration. Similar results were obtained for valine and leucine and these amino acids competitively inhibited alanine influx both in the presence and in the absence of Na. These results lead to a model for the transport system which involves combination of Na and amino acid with a single carrier or site leading to penetration of both solutes. The model predicts that alanine should cause an increase in Na influx and the ratio of this extra Na flux to alanine flux should vary with Na concentration. The observed relation agreed closely with predicted values for Na concentrations from 5 to 140 mM. These results support the hypothesis that interactions between Na and amino acid transport depend in part on a common entry mechanism at the mucosal border of the intestine.

In the preceding paper (1), a method for measuring unidirectional solute influx across the mucosal border of intestinal epithelial cells was described and some characteristics of L-alanine and Na influxes were presented. These studies provided the first unequivocal evidence that the flux of an actively transported amino acid from the mucosal solution into the cell is influenced by the Na concentration in the mucosal solution and is independent of the bulk cellular Na concentration. Further study of the influx processes is necessary in order to define more completely the transport mechanisms for non-

electrolytes and the nature of the interaction with Na. Data on cell accumulation or transmural transport of amino acids or sugars do not provide this information because these phenomena depend in a rather complex way on events at both the mucosal and serosal borders of the cell. The present experiments are concerned primarily with the kinetics of L-alanine influx and the interaction between L-alanine and Na. In addition, measurements have been made of valine and leucine fluxes, and the interaction between these amino acids and alanine influx has been examined.

METHODS

The apparatus and methods used to measure amino acid and Na influxes have been described in detail in the preceding paper (1). The basic Ringer solution used contained 140 mM NaCl, 10 mM $KHCO_3$, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM K_2HPO_4, and 0.2 mM KH_2PO_4. In most experiments, the tissue segments were bathed with solutions having the same Na concentration, but, from one experiment to another, Na concentration of the Ringer solution was altered by replacing NaCl with choline chloride. The tissue was preincubated for 30 min in Ringer's solution having the same Na concentration as that used in the influx measurements. The preincubation solution did not contain amino acid. In many of the experiments, fluxes were determined at four different amino acid concentrations on tissue from the same animal. Since the experimental arrangement permitted eight separate influx measurements, duplicate determinations at each concentration were possible. The solution for flux measurement (test solution) was prepared by adding ^{14}C-labeled L-alanine, L-valine, or L-leucine to the appropriate Ringer solution to give concentrations varying from 1.7 to 20 mM; this solution also contained inulin-^{3}H and ^{22}Na. In experiments testing the effect of valine or leucine on alanine influx, the alanine concentration was 3.3 mM and the concentration of the other amino acid was 20 mM. Methods of tissue extraction, of assaying the three isotopes, and of flux calculations have been described (1).

All influx values reported were obtained by measuring tracer uptake over a 60 sec interval. Since the present experiments involved higher alanine concentrations and higher fluxes than previously observed, a single experiment was performed to determine the time course of alanine-^{14}C uptake at 20 mM alanine and 140 mM Na. Tracer uptake was linear for more than 60 sec in agreement with observations at 5 mM alanine (1).

RESULTS

Kinetics of Alanine Influx

Alanine influx was determined as a function of alanine concentration at four Na concentrations ranging from zero (nominally Na-free medium) to 140 mM. The results of two individual experiments at the extreme Na concentrations are shown in Fig. 1. As expected from previous data (1), alanine influx is considerably lower in the absence of Na than in its presence. The influx increases

with alanine concentration but shows a tendency toward saturation. The apparent hyperbolic relation between flux and concentration was confirmed by plotting the reciprocal of flux against the reciprocal of concentration[1] (Fig. 2). The resulting straight lines indicate that the alanine influx, J_A^i, can be described by a relation of the form

$$J_A^i = \frac{J_A^{im}\,[A]_m}{K_t + [A]_m} \qquad (1)$$

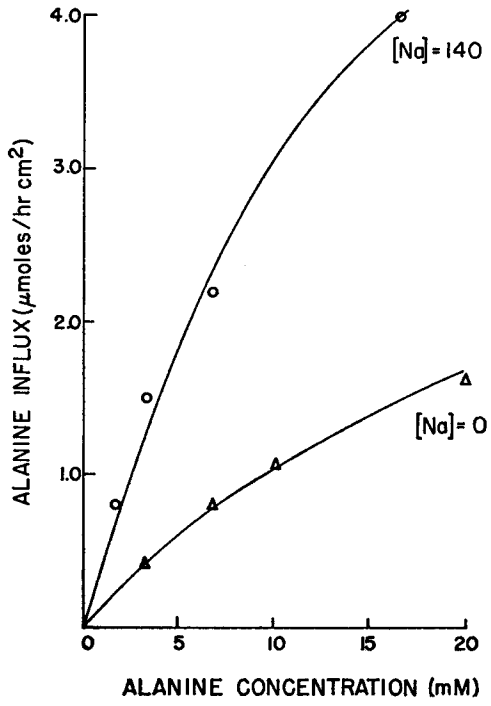

FIGURE 1. Alanine influx as a function of alanine concentration. Data at each Na concentration were obtained using tissue from a single animal and each point is the average of duplicate flux determinations.

in which $[A]_m$ is alanine concentration in mucosal solution and J_A^{im} is the maximal influx. K_t is the "apparent Michaelis constant" (the value of $[A]_m$ for which $J_A^i = J_A^{im}/2$). The fact that the intercepts of the lines shown in Fig. 2 are identical indicates that J_A^{im} is the same at the two Na concentrations while the difference in slopes shows that K_t is different.[2] This behavior was found at all Na concentrations tested and the average values of J_A^{im} and K_t

[1] Though it would have been desirable to examine alanine influx at higher alanine concentrations in order to obtain values closer to saturation, concentrations of amino acid greater than 20 mM were not employed because of the inhibitory effects reported by other investigators (21) and because it was desirable to maintain the test solution at approximately constant osmolarity and ionic strength.

[2] Alternative methods of plotting the data which weigh the points differently were also tried (36). Least squares analysis of the various lines showed no significant differences for J_A^{im} and K_t among the various methods.

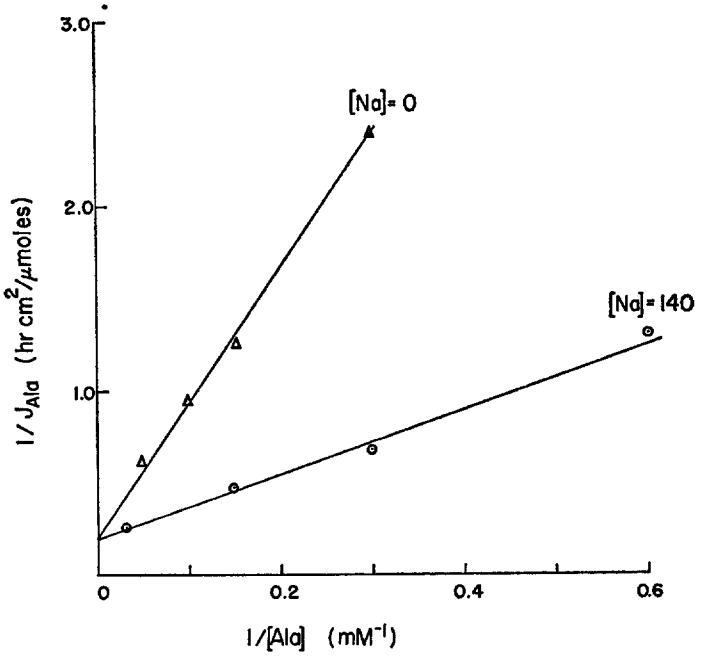

FIGURE 2. Double reciprocal plot of data shown in Fig. 1.

for alanine are summarized in Table I. Three experiments, yielding 24 determinations of alanine influx, were carried out at each Na concentration and J_A^{im} and K_t were determined by least squares analyses of lines such as those shown in Fig. 2. The relatively high value of J_A^{im} at 70 mM Na appears to be due to animal variation rather than to Na concentration. The data in Table I show that there is no consistent variation of J_A^{im} with Na concentration, and fluxes consistent with a value for J_A^{im} as high as 13 μmoles/hr cm² were ob-

TABLE I

PROPERTIES OF THE AMINO ACID TRANSPORT SYSTEM

Amino acid	[Na]	K_t	J_A^{im}
	mM	*mM*	*μmoles/hr cm²*
Alanine	140	9.1 (7.2–11.0)*	6.1±0.9
	70	16.3 (11.5–21.2)	13.7±1.9
	22	31.2 (25.0–34.5)	7.8±1.5
	0	70.0 (60.0–79.6)	6.8±0.7
Valine	140	5.0 (3.3–6.4)	4.3±3.1
	0	31.5 (23.1–36.0)	6.5±0.8
Leucine	140	4.2 (3.0–5.7)	6.3±1.2
	0	29.0 (24.0–34.0)	4.5±1.3

* Range.

served in different experiments at 140 mM Na. In addition, one experiment was carried out in which J_A^{im} was estimated at 140 and 70 mM Na on tissue from a single animal; no significant effect of Na concentration on J_A^{im} was observed. We conclude, therefore, that variation in mucosal Na concentration does not significantly affect the maximum alanine influx. On the other hand, K_t increases markedly with reduction in Na concentration. Examination of these data indicates that the reciprocal of K_t is a linear function of Na con-

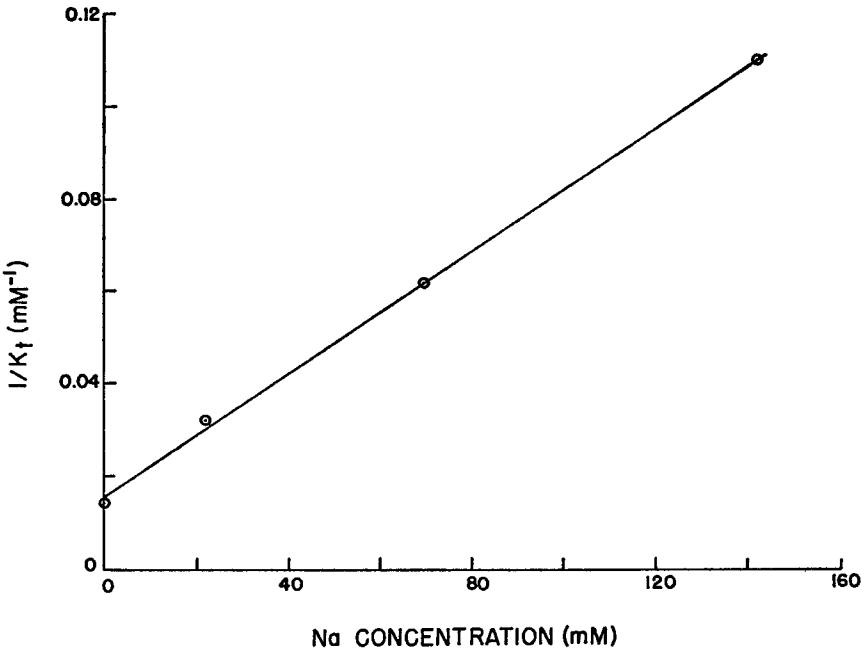

FIGURE 3. Reciprocal of the apparent Michaelis constant (K_t) for alanine as a function of Na concentration in the mucosal bathing solution.

centration, as shown in Fig. 3. Thus the relation between K_t and Na concentration can be described by an expression of the form

$$K_t = \frac{\alpha}{\beta + [\mathrm{Na}]_m} \quad \text{or} \quad \frac{1}{K_t} = \frac{1}{\alpha}[\mathrm{Na}]_m + \frac{\beta}{\alpha} \qquad (2)$$

in which α and β are constants, and $[\mathrm{Na}]_m$ is Na concentration in the mucosal solution. The values of α and β can be obtained from the slope and intercept of the line in Fig. 3.

Valine and Leucine Fluxes

The kinetics of valine and leucine influxes were investigated at 140 mM Na and in the absence of Na and were found to resemble the kinetics of alanine

influx. Values of maximal flux and K_t were determined as described above; the results are summarized in Table I. Within experimental error, the maximum fluxes were independent of Na concentration and did not differ significantly from those obtained for alanine. The values of K_t for both valine and leucine were smaller than those observed for alanine but also showed a marked increase when Na was absent from the bathing medium.

Neutral amino acids are known to compete with each other in transport across the intestine (2, 3), and the competition appears to occur, at least in part, at the mucosal border (4). An examination of these competitive properties can provide evidence regarding modes of alanine entry. Experiments were carried out to test the effects of 20 mM valine or leucine on alanine influx at 140 mM Na and zero Na; the results are summarized in Table II. Valine and

TABLE II

INHIBITION OF ALANINE INFLUX BY LEUCINE AND VALINE

		Alanine influx*			
Competitor	[Na]	Control	+ Competitor	K_I	K_t
	mM	μmoles/hr cm²		mM	mM
Leucine	140	1.20	0.27	3.9	4.2
(20 mM)	0	0.44	0.25	30.7	29.0
Valine	140	1.72	0.38	3.9	5.0
(20 mM)	0	0.26	0.16	31.0	31.5

* Alanine concentration was 3.3 mM in all experiments. Influx values are the average of six or more determinations under each condition.

leucine decreased alanine influx below control levels under both conditions, but the effect was greater at 140 mM Na. If the effect of these amino acids is assumed to be due entirely to competitive inhibition, the "K_I" for valine and leucine can be calculated (6) from the relation

$$\frac{J_A^{i'}}{J_A^i} = \frac{K_t + [A]_m}{K_t + [A]_m + \frac{K_t [I]_m}{K_I}}$$

in which J_A^i and J_A^i are alanine influxes in the presence and absence of competitor respectively and $[I]_m$ is the concentration of competitor in the mucosal solution. The values of K_t for alanine were taken as those in Table I. The resulting K_I's of valine and leucine, given in Table II, are nearly identical with the K_t's of these amino acids. Thus, the effects of valine and leucine are consistent with "classical" competition with alanine for the influx mechanism at both 140 mM and zero Na. These observations have two important implica-

tions. First, alanine influx does not take place by a mechanism not shared, at least in part, by valine and leucine; if it did, agreement between K_I and K_t would not be obtained. Second, amino acid entry in the absence of Na cannot be attributed to simple diffusion since, in addition to displaying saturation kinetics, it is subject to competitive inhibition. Indeed, the good agreement between K_I and K_t suggests that simple diffusion does not contribute significantly to alanine entry at 140 mM Na or at zero Na.

Oxender and Christensen (5) have suggested that neutral amino acid transport in ascites tumor cells is mediated by multiple entry routes having overlapping affinities. Their data indicate that satisfactory agreement between K_t and K_I may not be a sufficiently critical criterion for the presence of a single transport site. However, there is at present no compelling evidence for multiple systems in intestine so for simplicity the agreement between K_I and K_t will be interpreted as evidence for a single site. If subsequent information indicates that a single site is insufficient, the model presented below will have to be modified.

A Transport Model

The data reported above seem sufficient to warrant consideration of possible models which could describe the Na-alanine interaction at the mucosal border of the cell. A number of observations (7, 8) suggest that such a model should include a provision for the specific entry of Na into the cell together with alanine, and a variety of models involving combination of alanine and Na with a carrier or site located in the mucosal membrane were, therefore, examined. In addition, the model should provide for the following characteristics of alanine influx: (i) alanine influx is independent of cellular Na concentration (1); (ii) at constant Na concentration, alanine influx is given by equation 1; (iii) J_A^{im} is independent of $[Na]_m$; (iv) alanine influx can occur in the absence of Na (Fig. 1) by a mechanism similar to that involved in the presence of Na; (v) K_t is dependent on $[Na]_m$ as shown in equation 2.

Of the possibilities considered, a model of the type shown in Fig. 4 seemed particularly promising. A detailed analysis of the kinetic behavior of this system is given in Appendix A. As a first step, a complete solution for the initial alanine influx was derived assuming that the concentration of alanine in the cell was zero. This solution is extremely unwieldy and is not presented. It predicts that alanine influx should depend on cell Na concentration and, in view of point (i) above, cannot describe the present data. If P_1, P_2, and P_3 are assumed to be small relative to the rates of reaction with the carrier so that translocation is the rate-limiting step, the expression is simplified and J_A^i no longer depends on cell Na. However, the resulting solution (equation A4) predicts that J_A^{im} should be dependent on external Na concentration, and does not agree with point (iii). If we further assume that the permeabilities of all

forms of the carrier are equal ($P_1 = P_2 = P_3 = P$), we obtain the following expression for J_A^i

$$J_A^i = \frac{C_t P [A]_m}{\frac{K_1 K_2}{[\mathrm{Na}]_m + K_2} + [A]_m} = \frac{J_A^{im} [A]_m}{K_t + [A]_m} \quad (3)$$

in which C_t is total carrier concentration; $K_1 = k_{-1}/k_1$ and $K_2 = k_{-2}/k_2$ where k_1 and k_{-1} are the forward and reverse rate coefficients of the reaction $A + X \rightleftharpoons XA$ and k_2 and k_{-2} are similar coefficients for $XA + \mathrm{Na} \rightleftharpoons XA\mathrm{Na}$. The coefficient K_t is given by

$$K_t = \frac{K_1 K_2}{[\mathrm{Na}]_m + K_2} \quad (4)$$

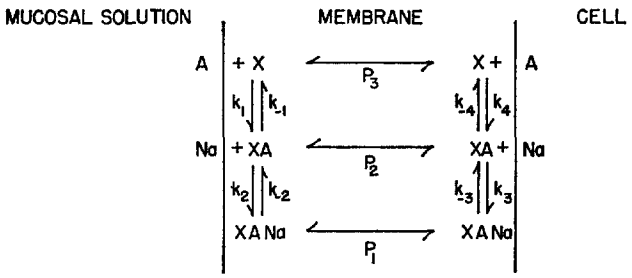

FIGURE 4. Model system for alanine and Na transport across the mucosal border of intestine. A represents amino acid and X is a carrier molecule. The $k_{\pm i}$ are rate coefficients for the chemical reactions and the P_i are permeability coefficients.

which is the form required by equation 2. The values of the dissociation constants for alanine and Na, determined from the slope and intercept of the line in Fig. 3, are $K_1 = 70$ mM and $K_2 = 17$ mM.

Equation 3 fulfills the five requirements outlined above so that this model satisfies all the data so far considered on alanine influx. This agreement is not, however, particularly compelling evidence in favor of the model because the requirements are rather simple and could, no doubt, be satisfied by a number of other approaches. The proposed model would gain further support if it could predict other characteristics of the system which are independent of the observations employed in its construction. One such prediction, the linear relation between $1/K_t$ and $[\mathrm{Na}]_m$, has already been noted (equation 4). Two additional predictions can be made. First, the model predicts that the unidirectional influx of amino acid will be unaffected by intracellular amino acid concentration. That is, there should be no significant transconcentration effect on influx. Data presented in the preceding paper (1) have shown the absence of a transconcentration effect on alanine influx. Second, a portion of the Na

influx should be directly related to alanine influx and the coupling between these two movements can be predicted (equation A6). If we designate J'_{Na} as that portion of Na influx which is independent of alanine influx, the total Na influx, J^i_{Na}, should be given by

$$J^i_{Na} = \left[\frac{[Na]_m}{K_2 + [Na]_m}\right] J^i_A + J'_{Na} \quad (5)$$

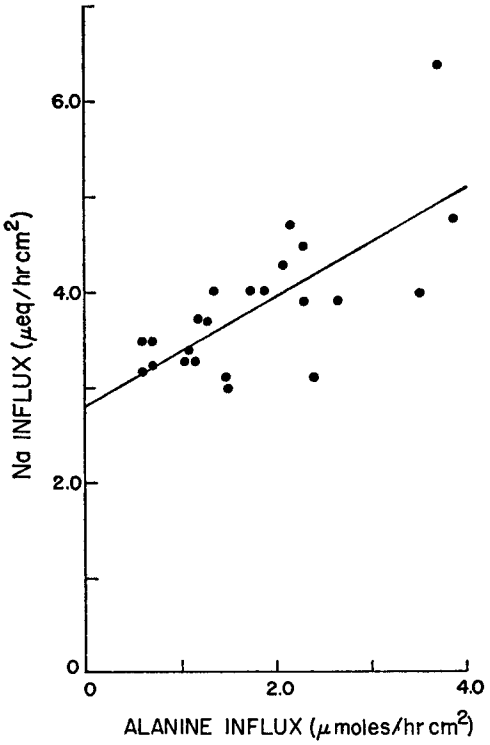

FIGURE 5. Relation between Na and alanine influxes for experiments at a Na concentration of 22 mM in the mucosal solution. Each point represents a single tissue sample in which both fluxes were determined simultaneously. The line was determined by least squares analysis.

Thus, the model predicts that J^i_{Na} will vary linearly with J^i_A at constant Na concentration and that the slope of this relation ($\Delta J^i_{Na}/\Delta J^i_A$) will vary with Na concentration. Further, since the value of K_2 has been determined from the relation between K_t and $[Na]_m$, the slope can be predicted quantitatively.

Relation between Alanine and Na Fluxes

Since both alanine and Na influxes were measured simultaneously in many experiments, an evaluation of equation 5 is possible. Experiments such as those shown in Fig. 1 provide the necessary data since variation in alanine flux at constant Na concentration was obtained by changing alanine concentration. In each experiment there was a direct relation between Na influx and alanine influx which suggested a coupling between these processes. In order

to evaluate this relation Na influx was plotted against the simultaneously measured alanine influx for all experiments at a given Na concentration. An example of the results is shown in Fig. 5 for experiments at 22 mM Na. At all four Na concentrations tested, (140, 70, 22, and 5 mM), there appeared to be a linear relation between the two fluxes and correlation coefficients for linear regression were statistically significant ($p < 0.01$) in all cases. The slopes and intercepts of the lines were determined by least squares analyses. Fig. 6 shows a comparison between experimentally determined slopes and those predicted

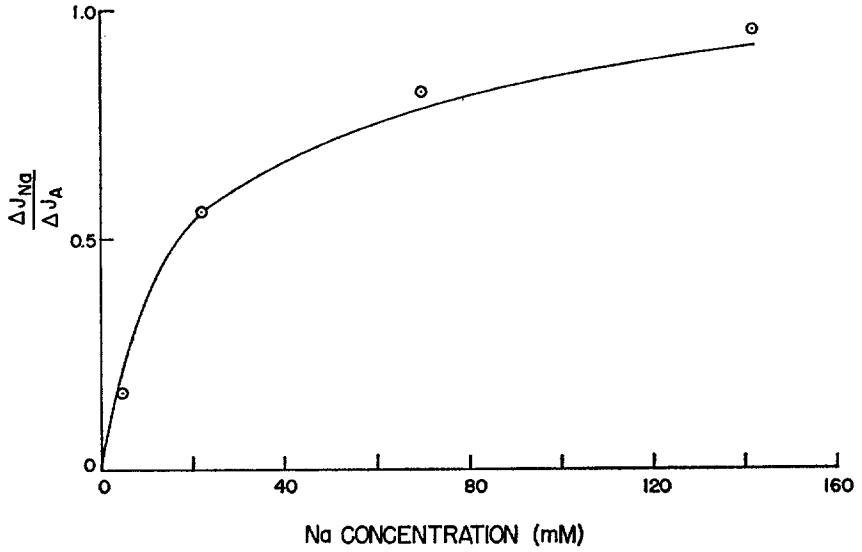

FIGURE 6. Ratio of alanine-dependent Na influx to alanine influx as a function of Na concentration in the mucosal solution. Points are experimental values given by the slopes of lines such as that shown in Fig. 5. The solid curve was calculated from equation 5 as described in the text.

by equation 5; the points are experimental values and the solid curve was calculated from $[Na]_m/(K_2 + [Na]_m)$ using $K_2 = 17$ mM as determined from the line in Fig. 3. The agreement is satisfactory and lends strong support to the proposed model.

The intercept of the line relating alanine and Na influxes provides information on the Na influx in the absence of alanine, J'_{Na} in equation 5. Fig. 7 shows J'_{Na} as a function of Na concentration; this portion of Na influx increases linearly with concentration at least up to 140 mM. The rate of active Na transport across isolated rabbit ileum is also a linear function of Na concentration over this range (9). In the presence of 5 mM alanine, the alanine-dependent Na influx makes up less than 15% of the total Na influx at all Na concentrations. Thus, unless alanine concentration is quite high, the major

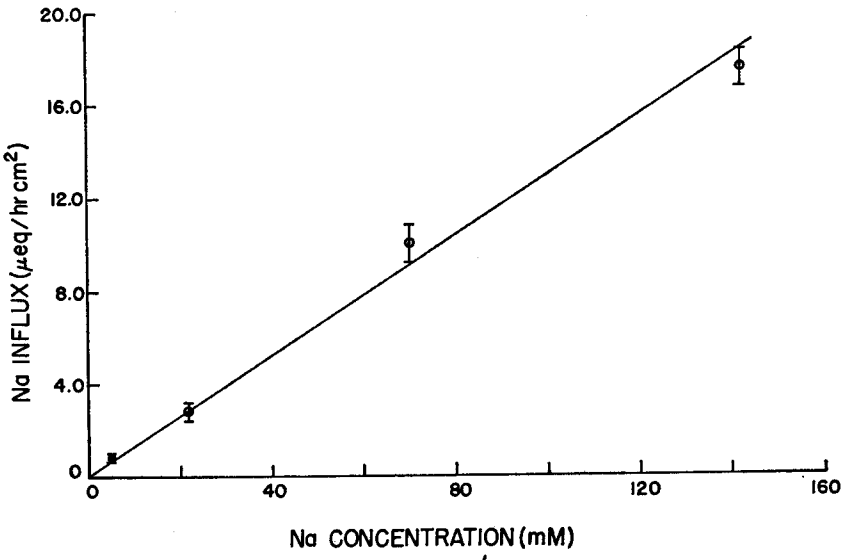

FIGURE 7. Alanine-independent Na influx, J'_{Na}, as a function of Na concentration in the mucosal solution.

portion of Na influx across the mucosal border of the cells takes place via a mechanism other than that proposed in Fig. 4.

Effects of Temperature

The influence of temperature on alanine and Na influxes was examined in several experiments in which one piece of tissue was kept at 37°C and the other at room temperature (23°C). The results are summarized in Table III.

TABLE III
EFFECT OF TEMPERATURE ON Na AND ALANINE INFLUXES

	Influx			
	Alanine*		Sodium‡	
	[Na] = 140	[Na] = 0	Ala-dependent	Ala-independent
	μmoles/hr cm²		μmoles/hr cm²	
37°	2.6	0.5	2.5	18.0
23°	0.3	0.1	0.3	11.4
Q_{10}	4.5	3.2	4.5	1.4
n§	8	6	6	6

* Alanine concentration was 5 mM in all experiments.
‡ Alanine-dependent and alanine-independent Na fluxes are defined by equation 5.
§ No. of observations.

The effect of temperature on alanine flux in the presence of Na was quite marked; a Q_{10} of 4.5 was calculated from the data. Since in the presence of 140 mM Na, approximately 1 Na enters with 1 alanine (Fig. 5), the Q_{10} of the alanine-dependent Na influx is also approximately 4.5. The effect of temperature on total Na flux was, however, much smaller, and if the total Na fluxes were corrected for alanine-dependent flux, a Q_{10} of 1.4 was calculated for the alanine-independent Na influx, J'_{Na}. At zero Na, alanine influx had a Q_{10} of 3.2. This value is not known accurately, however, because at room temperature alanine influx in the absence of Na was quite small so that experimental errors could have a rather marked effect on the calculated Q_{10}.

These observations on the effects of temperature on influx at the mucosal border may be compared with the findings of Schultz and Zalusky (9) on active Na transport across rabbit ileum. They found a Q_{10} of 4.8 for the sugar-stimulated increase in net Na transport (measured by short-circuit current) and a Q_{10} of only 1.6 for net Na flux in the absence of actively transported nonelectrolyte. These values are in good agreement with those observed here for the alanine and Na influx processes. The marked differences in response of Na fluxes to temperature in the presence and absence of alanine lend further support to the concept of two independent paths for Na influx as expressed in equation 5. The explanation of these rather high temperature coefficients is unclear at present but the phenomenon certainly merits further study since it may offer clues to the molecular mechanism of the transfer process.

DISCUSSION

Model for Amino Acid Transport

The model shown in Fig. 4 provides a satisfactory explanation for all the data presented in this and the preceding paper on amino acid and Na influxes across the mucosal border of the intestine. The available data rule out several other possible models involving a ternary complex between amino acid, Na, and a carrier (or site). The presence of two independent and parallel pathways for alanine influx, one Na-dependent and the other Na-independent, is rendered unlikely by the observation that J_4^{im} is the same in the presence and absence of Na; if there were two independent pathways, the maximum influxes should be additive. For this reason the first reaction in the proposed model cannot involve association with Na rather than amino acid since, under these conditions, there would be no flux of amino acid in the absence of Na. The same point would apply to a system involving an obligatory simultaneous reaction among Na, amino acid, and carrier. A model in which there is no preferred order for association of Na and amino acid with the carrier is extremely complex and has not been fully evaluated. Preliminary calculations

suggest, however, that amino acid influx would depend on intracellular Na concentration in such a system. Thus the model presented seems the simplest one which can offer a reasonable explanation of the experimental observations.

The quantitative considerations of this model involve a number of assumptions which merit comment. The assumption that the steps involving transfer of carrier across the membrane are rate-limiting is based primarily on the fact that the general expression for alanine influx obtained without this assumption predicts dependence on cell Na concentration, a prediction which is contrary to our observations (1). However, the influence of cell Na on alanine influx would depend strongly on relative values of the rate coefficients for the chemical reactions. In particular, if k_3 were small, dependence of amino acid influx on cell Na concentration would be minimized. The general solution also has the form of Michaelis-Menten kinetics although the meanings of the quantities J_A^{im} and K_t are much more complex than for the simplified case. In this general case, J_A^{im} would depend on Na concentration in the mucosal solution, $[Na]_m$, and the relation between K_t and $[Na]_m$ would be different from that observed. Thus these points, together with failure to observe an effect of cell Na on amino acid influx, seem to offer reasonable support for the assumption that the carrier transfer processes are rate-limiting.

The assumption that the three permeability coefficients are equal is perhaps more difficult to justify on experimental grounds although it is reasonable in terms of the concept of translocation of a relatively large carrier molecule. The data are, however, adequate to demonstrate that if the model is correct, the coefficients cannot differ markedly. First, the prediction that there will be no transconcentration effect on influx arises directly from the assumption of equal permeability coefficients (see for example the discussion of Heinz and Durbin, 10). Appreciable differences in permeabilities would lead to either a decrease or an increase of influx in tissues preloaded with alanine, depending on the relative values of the permeability coefficients. Since no significant effect of preloading was observed (1), the coefficients must be similar. Second, the assumption that $P_1 = P_2$ leads to the prediction that J_A^{im} will be independent of $[Na]_m$ and is justified by the observation that there is no consistent variation of J_A^{im} with $[Na]_m$. However, if P_1 and P_2 do not differ markedly, the dependence of J_A^{im} on $[Na]_m$ might be sufficiently small to escape detection. Thus, the available evidence lends support to these assumptions but does not completely prove their validity.

In developing expressions for behavior of the model system, we have tacitly assumed that all forms of the carrier are uncharged or, alternatively, that there is no electrical potential difference across the mucosal border of the cell. The latter is clearly incorrect (11, 12, footnote 3) and since an ion (Na^+) is assumed to associate with the carrier, at least one of the forms might be expected to carry

[3] Field, M., and P. F. Curran, unpublished observations.

a net charge. As discussed by Britton (13), inclusion of a potential difference in consideration of carrier models is not entirely straightforward. In the present case, the simplest approach would be to assume that the complex XANa is positively charged and that the effect of the electrical potential difference is confined to its influence on the translocation of this complex. If the flux of XANa is assumed to be described by the Goldman equation (14), the quantity $P_1([XA\text{Na}]_m - [XA\text{Na}]_c)$ in equations A2 must be replaced by $P_1\xi([XA\text{Na}]_m - [XA\text{Na}]_c e^{-\xi})/(1 - e^{-\xi})$ where $\xi = z_i\mathfrak{F}\Delta\psi/RT$, and $\Delta\psi$ is the electrical potential difference across the mucosal membrane. The resulting expression for amino acid influx contains terms dependent on the electrical potential difference. Even with the assumption that $P_1 = P_2 = P_3$, J_A^{im} should depend on both Na concentration and potential difference. The failure to observe a dependence of J_A^{im} on Na thus suggests that the potential difference may not influence the transport system unless the effects of changes in Na concentration and in potential difference cancel in a rather fortuitous manner. On the other hand, if the association of Na with the carrier involved an exchange with another cation such as hydrogen ion, none of the carrier forms would involve a net charge and a potential difference would have no effect. Further studies are clearly necessary to examine these factors in detail and there is no point at present in speculating on their possible implications in the transport mechanism.

Finally, the proposed model has been discussed mainly in terms of the mobile carrier hypothesis, but this concept need not be invoked to explain the experimental observations. The steps involved in transfer of materials across the membrane phase could involve chemical reactions or molecular rearrangements such as the "gate" mechanism suggested by Patlak (15). In this case, the P's would represent rate coefficients for the translocation process. If the steps involving transfer across the membrane were slow relative to the association-dissociation reactions at the surfaces, the kinetic description of the system would be unaltered and all considerations presented above would remain valid. Thus, the ability of the model to describe the kinetics of the Na-alanine interaction should not be taken as evidence for the existence of a mobile carrier.

Alanine-Dependent Na Influx

The addition of actively transported sugars or amino acids to the solution bathing the mucosal surface of rabbit ileum results in an immediate increase in the short-circuit current due to an increased rate of active Na transport from mucosa to serosa (7, 16). The increase in the short-circuit current is a saturable function of the mucosal alanine (or sugar) concentration (7). These observations supported the concept that a ternary complex involving Na is responsible for the influx of the nonelectrolyte (17). The present experiments

provide the first clear demonstration of a coupled entry of amino acid and Na from the mucosal solution into the cell and the alanine-dependent Na influx is of sufficient magnitude to account for the increased rate of transmural Na transport in the steady state. Thus at 5 mM alanine, the steady-state influx of alanine across the mucosal border averages 2.2 μmoles/hr cm². Since the coupling ratio between alanine and Na is 0.9 at 140 mM Na (Fig. 6), the increased Na influx will be 2.0 μmoles/hr cm². The rate of net transmural alanine transport under similar conditions averages 1.3 μmoles/hr cm² (footnote 3), so that alanine efflux out of the cell across the mucosal border must be 0.9 μmole/hr cm². Assuming that the transport mechanism is symmetrical,[4] as shown in Fig. 4, the coupling ratio between alanine efflux and Na efflux in the presence of a cell Na concentration of 50 mM (18) is 0.75. The efflux of Na coupled to alanine would be (0.75) (0.9) = 0.7 μmole/hr cm², and the net alanine-dependent flux of Na across the mucosal border becomes 1.3 μmoles/hr cm². This prediction for the increased rate of Na transport in the presence of 5 mM alanine is in good agreement with the value of 1.5 μmoles/hr cm² observed by Schultz and Zalusky (Fig. 2, reference 7).

Alanine-Independent Na Influx

The conclusions drawn from the model of Na and amino acid interaction permit a more detailed examination of the data presented in the preceding paper on the effects of K and Li on Na and alanine influxes. Since Na influx can be described adequately by equation 5 and since the coefficient multiplying J_A' in the expression has been evaluated, the effects of K and Li on the alanine-independent Na influx, J'_{Na}, can be estimated. Table IV gives values of J'_{Na} at 22 mM Na under control conditions (118 mM choline) and in the presence of various other replacement solutes. As expected from the data given previously, replacement of choline by Tris or of choline chloride by mannitol does not alter J'_{Na} significantly. K causes a depression of the alanine-independent Na influx under all conditions tested. When K is used in the test solution only, so that the tissue is exposed to high K for approximately 60 sec, the effect on Na influx is small. Preincubation of the tissue in high K for 30 min causes a significant further inhibition of Na influx and, as shown in the last row of the table, the preincubation in high K has an effect even if the K concentration in the test solution is normal (12 mM). The effects of K on the alanine-independent Na influx are approximately the same as those observed on alanine influx (Fig. 5, reference 1). Since these two processes appear to be independent, this observation suggests that the decrease in fluxes caused by K is not specific but is the result of a general effect on the tissue. The effect of Li is of particular interest. The substitution of Li for

[4] The model shown in Fig. 4 is considered symmetrical if $K_1 = K_4$ and $K_2 = K_3$.

choline caused only a 18% inhibition of alanine influx (Fig. 4, reference 1) but resulted in a 60% inhibition of the alanine-independent Na influx. As discussed previously, the observation suggests that this portion of the Na influx may not be the result of a simple diffusion process; it may involve a carrier-mediated process or some other specific interaction with the membrane which is subject to inhibition by Li.

TABLE IV

Treatment	Na influx		n*	p
	Control‡	Test		
	μmoles/hr cm²			
118 mM Tris	4.6	4.7	6	>0.1
236 mM mannitol	4.4	5.0	4	>0.1
118 mM Li	4.8	2.0	8	<0.01
130 mM K	4.1	3.4	10	>0.05
130 mM K (preincubation) 130 mM K (test)	4.7	2.4	6	<0.01
130 mM K (preincubation) 12 mM K (test)	4.1	2.8	4	<0.01

* No. of observations.

‡ Control tissue preincubated in solution containing 118 mM choline, 22 mM Na and uptake measured from same solution. Test tissue was preincubated in this same solution unless otherwise noted and uptake measured from solution shown in column 1. All solutions contained 22 mM Na. Control and test data for each condition were obtained on tissues from the same animals.

Net Transport and Tissue Accumulation of Amino Acids

The model presented in Fig. 4 makes no provision for the direct utilization of metabolic energy in the processes of amino acid entry. Since amino acids are accumulated by the mucosal cells to concentrations considerably above those in the bathing medium (5, 18, 19) and are transported across the intestine against substantial concentration differences (19–21), a consideration of energetics is necessary. Both cell accumulation and net transmural transport of amino acids are inhibited by metabolic poisons (22) but this observation does not necessarily demonstrate a *direct* coupling of amino acid transport to metabolism. The same results would be obtained if amino acid influx were coupled to the movement of another solute whose transport is, in turn, directly coupled to metabolic energy (see references 23 and 24 for a phenomenological description of such coupled processes). That amino acid transport "could be wholly driven by the energy inherent in the asymmetry of cellular alkali metal distribution" was suggested by Christensen and his coworkers (25), and the possibility of a ternary complex between Na, glycine,

and carrier was entertained by these investigators. More recently Crane (26) has proposed that accumulation and, hence, net transport of sugars can be explained by the concentration differences of Na and K between the cell and its environment and does not require a direct coupling to metabolic energy. Vidaver (27) has suggested that glycine accumulation by pigeon erythrocytes is due to a Na concentration difference. The models involved are rather similar to that proposed here. However, the data available do not provide conclusive evidence that alanine transport by rabbit ileum can be accounted for by the Na concentration difference alone. This point may be illustrated by examining some simple thermodynamic implications of the system shown in Fig. 4. The most straightforward approach is the evaluation of the dissipation function Φ (or the entropy production)[5] of the system which must be positive if the process under consideration is to occur spontaneously (24). As shown in Appendix B, the dissipation function for the model depicted in Fig. 4 can be written simply

$$\Phi = J_A \Delta\mu_A + J_{Na} \Delta\mu_{Na} \tag{6}$$

in which the J's are the *coupled* net fluxes of alanine and Na across the mucosal membrane and are positive in the direction mucosal solution to cell. The $\Delta\mu$'s are differences in chemical potentials across the membrane. As indicated above, the coupled net fluxes of Na and alanine across the membrane are approximately equal at 140 mM Na. If we assume that cellular concentrations determined on mucosal strips (18) apply to the condition of steady-state net transfer, equation 6 can be evaluated. With mucosal solution concentrations of 140 mM for Na and 5 mM for alanine, cellular concentrations were found to be approximately 50 mM Na and 40 mM alanine. Assuming that cellular and extracellular activity coefficients are equal, the dissipation function then becomes

$$\Phi = RTJ_A\{\ln 0.125 + \ln 2.80\} = -1.05RTJ_A$$

Since the dissipation function is negative, the proposed process cannot occur spontaneously. As indicated in Appendix B, if one of the carrier complexes is charged, $\Delta\mu_{Na}$ should be replaced by $\Delta\bar{\mu}_{Na}$. Assuming a potential difference of 15 mv, cell-negative (footnote 3), Φ becomes -0.49 RT J_A. Since Φ is still negative, a spontaneous process of net alanine transport under the conditions assumed seems impossible in terms of this simple model. There is, however, a net alanine flux across the mucosal border so that the correct dissipation function must involve terms in addition to the two indicated, or the evaluation of Φ must be incorrect. For example, the association-dissociation

[5] The dissipation function is given by the absolute temperature times the rate of entropy production due to irreversible processes occurring within a system.

reactions were considered to be at equilibrium and, thus, to make no contribution to the dissipation function. Since net flows of the reactions must occur, this assumption cannot be entirely correct but the contribution to Φ cannot be evaluated. Further, cell Na may be compartmentalized in such a way that the concentration at the membrane itself is considerably lower than the average value determined for total cell water. In order to give a positive dissipation function, the Na concentration at the cytoplasmic side of the mucosal border would have to be less than 17 mM (or less than 30 mM if the transport is influenced by the potential difference[6]). Finally, the intracellular Na and alanine concentrations pertaining under conditions of steady-state net transport may not be the same as those observed in mucosal strips even though the two conditions seem comparable.

The above considerations are all based on the assumption that the transport system is symmetrical, having the same properties at both sides of the membrane. Alternatively, the system may not be symmetrical. The proposal of Crane (26) (see also Kipnis and Parrish (28)) that the high cellular K influences the affinity of substrate for the carrier is a possible source of asymmetry. However, we have been unable to obtain any evidence for a major effect of extracellular K on alanine influx and there are indications that even the observed 20% inhibition may be a relatively nonspecific effect. Thus, a marked influence of the K concentration difference seems unlikely in the present case. Nonetheless, the possibility remains that the influence of K on the cellular side of the membrane is different from that on the outer side. In addition, other properties of the cytoplasm could contribute to an asymmetry. Finally, there is the clear possibility that a direct participation of metabolic energy is involved in providing the asymmetry necessary to account for tissue accumulation and net transport. For example, a metabolic alteration of the carrier at the inner surface which reduced its affinity for substrate would increase the accumulation ratio. In this case a new, detailed formulation of the model system would be required and the dissipation function of the system would take a different form. A more detailed understanding of the cellular compartment is clearly necessary for an explicit thermodynamic description. In addition, the influence of metabolic inhibitors on the influx process must be examined and information on outflux from the cell must be obtained before the process of accumulation can be fully understood.

Although the present model may not account quantitatively for the observed levels of amino acid accumulation by mucosal cells, the inhibitory effects of ouabain or a Na-free medium are readily explicable in terms of a symmetrical transport system and the Na concentration difference. When

[6] The cell Na concentration of 50 mM was calculated after correction for the Na content of the inulin space of mucosal strips. Since, as noted previously (18), inulin may underestimate the extracellular space, it is likely that the true intracellular Na concentration is less than 50 mM.

mucosal strips are incubated in the presence of ouabain or in a Na-free medium, the difference between intracellular and extracellular Na concentrations is essentially abolished within 30 min (18). Examination of Fig. 4 or equation A7 shows that if $K_1 = K_4$ and $K_2 = K_3$, and if $[Na]_m = [Na]_c$ the system will reach a steady state when $[A]_c = [A]_m$ (that is, net flux across the mucosal border will be zero when the concentration ratio for alanine reaches unity). Thus the inhibition of amino acid accumulation and transmural transport against a concentration difference by Na-free medium, ouabain, or metabolic inhibitors may be attributed to the fact that under all these conditions $[Na]_c \cong [Na]_m$.

This explanation for the inhibitory effect of ouabain is consistent with a number of experimental observations. Ouabain inhibits transmural transport of sugars (29) and amino acids (30) only when it is present in the serosal bathing solution even though the mechanisms responsible for transmural transport of sugars and amino acids against concentration differences are located in the brush border (4). While it is possible that some ouabain crosses the serosal membrane and gains access to the nonelectrolyte transport mechanisms, two observations mitigate against this possibility. First, when ouabain is placed in the serosal solution, very little enters the mucosal cells and virtually none appears in the mucosal solution (29). Second, in squid axon (31) and erythrocytes (32) there is evidence suggesting that the site of ouabain action on cation transport is at the extracellular surface of the membrane. While these observations do not rule out an intracellular action of ouabain on nonelectrolyte transport in intestine, a much simpler explanation is suggested by the finding that ouabain inhibits active Na transport across rabbit ileum only when it is present in the serosal solution (9). Thus, inhibition of the mechanism by which Na is extruded from the cell could abolish the difference between $[Na]_c$ and $[Na]_m$ and thereby inhibit sugar and amino acid transport. The effect of ouabain may be restricted only to its well known role as an inhibitor of cation transport and no direct action on nonelectrolyte transport need be postulated.

Other Studies

It is somewhat difficult to compare these studies quantitatively with other investigations of interrelations between Na and nonelectrolyte transport by intestine. The present experiments are unique in that they provide data on unidirectional influx across the mucosal border. Other studies involving measurement of tissue accumulation or transmural transport do not provide unequivocal information on this influx process. In fact, extrapolation of data on accumulation or transport to conclusions regarding the transport mechanism may be misleading. Thus, the careful studies of Mathews and Laster (21) on transmural transport indicated that the maximal transport rates for

alanine, valine, and leucine were in the ratio 1.0:0.56:0.29 while the data shown in Table I indicate that, for the mucosal entry step, the maximal rate is the same, within experimental error, for all three compounds. While this difference could be due in part to species variation, it probably also reflects the fundamental difference between mucosal influx and transmural flux. Even measurements of tissue uptake over relatively short time intervals (5–10 min) do not provide data which are directly comparable to the present measurements for two reasons. First, the tissue is exposed to fluid on both sides and there is no clear way of separating uptake across the serosal side from that at the mucosal side. Second, in the presence of Na, sufficient amino acid enters the cells in 5–10 min to make estimation of a unidirectional influx impossible. In 10 min, alanine has reached approximately 75% of its steady-state concentration in mucosal strips (18). Thus, such experiments actually measure a complex relation between influx and outflux at the mucosal and serosal borders. Coefficients such as K_t or maximal rates calculated from these data are extremely difficult to interpret as Mathews and Laster (21) have pointed out. The studies most similar to the present ones are those of Rosenberg et al. (19) examining the effect of Na on the steady-state influx and efflux of the model amino acid 1-aminocyclopentane-5-carboxylic acid (ACPC) in segments of rabbit jejunum. A decrease in Na concentration of the bathing medium caused a marked depression of ACPC influx into the tissue. On the assumption that the major route of ACPC entry into the tissue was via the mucosal border, their results are similar to those reported here. However, quantitative comparisons cannot be made until information on the relative contributions of the mucosal and serosal borders to total influx into tissue segments has been evaluated.

Studies on several nonepithelial systems have yielded data on amino acid transport which are similar to the present observations but in no case do precisely the same factors seem to be involved. Vidaver (27) has suggested that his observations on the influence of Na on glycine transport by pigeon erythrocytes could be explained by a system involving a carrier or site which combines with two Na ions and one amino acid molecule. His data appear to be fitted best by a model in which the site must first combine with the Na ions. The studies of Kipnis and Parrish (28) on the influence of Na on amino acid transport by rat diaphragm and isolated lymph node cells also seem qualitatively similar to our observations with respect to effects of changes in Na concentration. They have suggested that amino acid accumulation is the result of Na and K concentration differences as proposed by Crane (26) for sugars in intestine. Finally, the transport of amino acids by Ehrlich ascites cells is strongly dependent on Na (33). In contrast to the present observations, Wheeler et al. (34) have recently reported that both the K_t and the maximal velocity for influx of aminoisobutyric acid are reduced by lowered Na concentration. Interpretation of observations on amino acid transport by ascites

cells may be complicated by the possibility that there are multiple modes of transport (5) but this conclusion has been questioned by Jacquez and Sherman (35). In intestine, we have been unable to obtain any evidence that more than one mechanism is involved in the transport of alanine, valine, and leucine across the mucosal membrane.

It is not clear whether the differences between these other tissues and intestine indicate the presence of different transport mechanisms or whether they represent different expressions of the same basic mechanism. Certainly the Na dependence of amino acid influx is present in all cases and many observations seem compatible with variants of the model shown in Fig. 4. For example, as indicated by equation A4, the present model predicts, under certain conditions, that both K_t and J_A^{im} would vary with Na concentration. It would be of interest to have information on the influence of amino acids on Na influx in these tissues since such data have proved particularly useful in suggesting and supporting the present model for the intestinal transport system.

APPENDIX A

We wish to examine the kinetic behavior of the model shown in Fig. 4 under steady-state conditions. Under these conditions, the concentrations of all components in the membrane phase are constant at all points of the phase so that, for example,

$$\frac{d\,[X]}{dt} = 0$$

and at the mucosal boundary

$$\frac{d\,[X]_m}{dt} = -k_1\,[A]_m\,[X]_m + k_{-1}\,[XA]_m + P_3\,[X]_c - P_3\,[X]_m = 0 \qquad \text{(A1)}$$

Similar expressions may be written for the other components of the membrane phase at both the "m" (mucosal solution) and "c" (cell) boundaries. The net steady-state velocity of the system, J_A, is given by

$$J_A = P_1([XA]_m - [XA]_c) + P_2([XA\text{Na}]_m - [XA\text{Na}]_c)$$

Following the approach discussed by Hearon (37), the set of equations of which equation A1 is an example can be used to form the set

$$\begin{aligned} J_A &= w_1[X]_m - w_{-1}[XA]_m \\ &= w_2[XA]_m - w_{-2}[XA\text{Na}]_m + P_2([XA]_m - [XA]_c) \\ &= P_1([XA\text{Na}]_m - [XA\text{Na}]_c) + P_2([XA]_m - [XA]_c) \\ &= w_3[XA\text{Na}]_c - w_{-3}[XA]_c + P_2([XA]_m - [XA]_c) \\ &= w_4[XA]_c - w_{-4}[X]_c \\ &= P_3([X]_c - [X]_m) \end{aligned} \qquad \text{(A2)}$$

in which

$$w_1 = k_1[A]_m \qquad w_2 = k_2[\mathrm{Na}]_m \qquad w_3 = k_{-3} \qquad w_4 = k_{-4}$$

$$w_{-1} = k_{-1} \qquad w_{-2} = k_{-2} \qquad w_{-3} = k_3[\mathrm{Na}]_c \qquad w_{-4} = k_4[A]_c$$

The $k_{\pm i}$ are forward and backward rate coefficients for the various reactions and the P_i are permeability coefficients. In addition, the total amount of carrier is assumed to be constant so that

$$2C_t = [X]_m + [X]_c + [XA]_m + [XA]_c + [XA\mathrm{Na}]_m + [XA\mathrm{Na}]_c \qquad (A3)$$

in which C_t is total carrier concentration. The factor 2 enters because the mean concentration of each form has been defined by relations of the type $[X]_{\mathrm{mean}} = ([X]_m + [X]_c)/2$. The set of equations A2 together with equation A3 may be solved simultaneously for J_A. Since we are, at present, interested in the initial alanine influx into cells which have not been preloaded, the condition $[A]_c = 0$ has been imposed. The resulting expression for J_A will then represent the unidirectional influx from mucosal solution to the cell, J_A^i. The general solution of this set is straightforward but tedious; the resulting expression for J_A^i contains 32 terms in the denominator involving products of the w's and P's. An expression of this complexity is of little use in quantitative considerations of alanine flux. In addition, the general expression for J_A^i contains terms in $[\mathrm{Na}]_c$ in the denominator while our observations have indicated that J_A^i is independent of $[\mathrm{Na}]_c$. While it is impossible to evaluate the relative importance of these terms in $[\mathrm{Na}]_c$, their presence suggests that the general expression for J_A^i might not fit the experimental data and we have, therefore, examined the influence of some simplifying assumptions.

The diffusion of carrier may be assumed to be much slower than the postulated association-dissociation reactions; that is, $P_1, P_2, P_3 < w_i, w_{-i}$ for all i. This assumption leads to considerable simplification and the resulting expression for J_A^i is

$$J_A^i = \frac{2C_t P_3 k_1\{P_1 k_2 [\mathrm{Na}]_m + P_2 k_{-2}\} [A]_m}{2P_3 k_{-1} k_{-2} + k_1 [A]_m\{(P_1 + P_3)k_2 [\mathrm{Na}]_m + (P_2 + P_3)k_{-2}\}} \qquad (A4)$$

This expression could satisfy much of the present data but, depending on the relative values of P_1, P_2, and P_3, the maximal influx rate, J_A^i, could depend on $[\mathrm{Na}]_m$. Since the data suggest that such a dependence does not occur, the additional assumption that the three permeability coefficients are equal has been made. This assumption is also necessary to insure that there be no transconcentration effect (see the analysis of Heinz and Durbin, 10). Equation A4 then reduces to

$$J_A^i = \frac{C_t P [A]_m}{\frac{K_1 K_2}{[\mathrm{Na}]_m + K_2} + [A]_m} \qquad (A5)$$

in which $P = P_1 = P_2 = P_3$, $K_1 = k_{-1}/k_1$, and $K_2 = k_{-2}/k_2$. The use of equation A5 in analysis of the present data is discussed in the text.

An expression of the form of A5 can be obtained without assuming that all three permeability coefficients are equal. If $P_1 = P_2$ and $P_1 \neq P_3$, the term C_tP becomes $2C_tP_1P_3/(P_1 + P_3)$ and K_1 becomes $2K_1P_3/(P_1 + P_3)$. For the present, we shall retain the simpler assumption of uniform permeabilities since none of the basic considerations presented here is affected by choice of these two possibilities.

The unidirectional Na influx arising from the system shown in Fig. 4 should be given by $P_2[XA\ \mathrm{Na}]_m$. In solving the set A2 for J_A^i using the simplifying assumptions, the following expression is obtained:

$$[XA\mathrm{Na}]_m = \frac{k_2\,[\mathrm{Na}]_m\,J_A^i}{P(k_2\,[\mathrm{Na}]_m + k_{-2})}$$

The total measured Na influx, J_{Na}^i, is given by the flux via the carrier system plus a flux J_{Na}' through other paths so that

$$J_{\mathrm{Na}}^i = \left[\frac{[\mathrm{Na}]_m}{[\mathrm{Na}]_m + K_2}\right] J_A^i + J_{\mathrm{Na}}' \qquad \text{(A6)}$$

As in equation A5, $K_2 = k_{-2}/k_2$. An experimental test of equation A6 is described in the text.

If the restriction that $[A]_c = 0$ is removed, the set of equations A2 may be solved for the rate of *net* transfer, J_A. Using the assumptions that the permeation steps are rate-limiting and that $P_1 = P_2 = P_3 = P$, the resulting expression is

$$J_A = \frac{C_t\,P\{K_3\,K_4([\mathrm{Na}]_m + K_2)\,[A]_m - K_1\,K_2([\mathrm{Na}]_c + K_3)[A]_c\}}{K_1\,K_2\,K_3\,K_4 + K_3\,K_4([\mathrm{Na}]_m + K_2)[A]_m + K_1\,K_2([\mathrm{Na}]_c + K_3)[A]_c + ([\mathrm{Na}]_m + K_2)([\mathrm{Na}]_c + K_3)[A]_m\,[A]_c} \qquad \text{(A7)}$$

in which $K_3 = k_3/k_{-3}$ and $K_4 = k_4/k_{-4}$.

APPENDIX B

The entropy production, d_iS/dt, or dissipation function, $\Phi = T\,d_iS/dt$, in the system illustrated in Fig. 4, may be evaluated easily under steady-state conditions (24). Since the steps involving transfer across the membrane are rate-limiting, the chemical reactions at the boundaries may be assumed to be approximately in equilibrium and their contribution to entropy production within the membrane may be neglected. Thus, to a first approximation

$$\Phi = J_{XA}\Delta\mu_{XA} + J_{XA\mathrm{Na}}\Delta\mu_{XA\mathrm{Na}} + J_X\Delta\mu_X \qquad \text{(B1)}$$

The J's are positive in the direction mucosal solution to cell and Δ indicates mucosal solution minus cell. At the mucosal solution side of the membrane, the condition of equilibrium for the chemical reactions requires that

$$\mu_A' + \mu_X' = \mu_{XA}'$$

$$\mu_{\mathrm{Na}}' + \mu_{XA}' = \mu_{XA\mathrm{Na}}'$$

and at the opposite side

$$\mu''_A + \mu''_X = \mu''_{XA}$$

$$\mu''_{Na} + \mu''_{XA} = \mu''_{XANa}$$

Thus,

$$\Delta\mu_{XA} - \Delta\mu_X = \Delta\mu_A \tag{B2}$$

$$\Delta\mu_{XANa} - \Delta\mu_X = \Delta\mu_{Na} + \Delta\mu_A \tag{B3}$$

Further, since the carrier is confined to the membrane phase,

$$J_{XA} + J_{XANa} + J_X = 0 \tag{B4}$$

Introducing equations B2, B3, and B4 into equation B1 and rearranging yields

$$\Phi = (J_{XA} + J_{XANa})\Delta\mu_A + J_{XANa}\Delta\mu_{Na} \tag{B5}$$

Assuming that there are boundary equilibria between the solutions and the membrane phases (24), $\Delta\mu_A$ and $\Delta\mu_{Na}$ may be taken as the differences between the two solution phases and equation B5 may be written

$$\Phi = J_A\Delta\mu_A + J_{Na}\Delta\mu_{Na} \tag{B6}$$

in which J_A and J_{Na} are the coupled net fluxes of amino acid and Na. If XANa is assumed to be positively charged, similar arguments including electrical potentials show that

$$\Phi = J_A\Delta\mu_A + J_{Na}\Delta\tilde{\mu}_{Na} \tag{B7}$$

in which $\tilde{\mu}_{Na}$ is the electrochemical potential of Na. Thus under steady-state conditions the dissipation function for the proposed model can be expressed simply in terms of flows and forces external to the membrane.

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