

Alanine Efflux across the Serosal Border of Turtle Intestine

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ABSTRACT The exit of alanine across the serosal border of the epithelial cells of turtle intestine was measured by direct and indirect techniques. A decrease or an increase in cell Na did not affect the amino acid flux from cell to serosal solution. Cells loaded with Na and alanine did not exhibit any extrusion of alanine when their serosal membranes were exposed to an Na-free medium containing alanine. However, substantial amino acid extrusion was observed across the mucosal cell border under similar conditions. Although alanine flux across the serosal membrane appeared to be Na-independent, it showed a tendency toward saturation as cellular alanine concentration was elevated. The results are consistent with the postulate that the serosal and mucosal membranes of intestinal cells are asymmetrical with respect to amino acid transport mechanisms. The serosal membrane appears to have an Na-independent carrier-mediated mechanism responsible for alanine transport while transport across the mucosal border involves an Na-dependent process.

An interaction between neutral amino acids and Na at the brush border of intestinal cells has been observed either directly or indirectly in many experimental animals (1-3). Detailed study of alanine transport across the brush border membrane in rabbit ileum by Curran et al. (3), led to a kinetic model that characterizes this interaction, and seems consistent with the "Na gradient hypothesis" suggested by Crane (4) for sugar transport. There is, however, little information concerning the exit of amino acids across the serosal or lateral borders of intestinal epithelial cells primarily because of the inaccessibility of this cellular surface to direct measurements. Since intestinal cells ordinarily concentrate amino acids to levels in excess of those in their bathing media (5, 6), exit at the serosal side usually occurs down a concentration gradient. However, this exit process is probably not entirely one of simple diffusion since most amino acids are relatively large molecules to which biological membranes would be fairly impermeable. The present study involves an attempt to obtain direct information on this process by

examining alanine transport across the serosal border of the Greek tortoise intestine. This tissue, as reported by Baillien and Schoffeniels (7), can be easily stripped of its muscular coats and most of its submucosa and muscularis mucosa leaving a sheet of mucosal cells that can be mounted in chambers without difficulties.

METHODS

Before measuring the unidirectional efflux of alanine from the cell to the serosal solution, we felt it necessary to examine some general aspects of alanine transport in turtle intestine since the present study might be of more general interest if the process were similar to that observed in mammalian intestine. Certain features of the transport process, particularly with respect to Na dependence, required further study in view of observations of Gilles-Baillien and Schoffeniels (8-11) suggesting differences between turtle and mammalian intestine. Thus several types of experiments were carried out as described below. In all studies, the upper or mid-intestine of the freshwater turtle (*Testudo graeca*) was resected after pithing the animals and removing their ventral shells. The tissue was washed free of intestinal contents, placed in Ringer solution, and bubbled with compressed air. In all experiments, the muscle coats and most of the submucosa were removed, utilizing the method described by Parsons and Paterson (12). The mucosal sheets were subsequently treated in various ways depending on the nature of the experiment. The normal Ringer solution contained 120 mM NaCl, 1.9 mM KCl, 0.45 mM CaCl₂, 1 mM K₂HPO₄, 0.65 mM KH₂PO₄ (pH = 7.2). Na-free Ringer was prepared by substituting all the NaCl by choline chloride. In some experiments, ouabain was added at a concentration of 10⁻⁴ M. L-alanine, uniformly labeled L-alanine-³H, and inulin-¹⁴C (The Radiochemical Centre, Amersham/Searle Corp., Arlington Heights, Ill.) were added from stock solutions. All experiments were done at room temperature (24°-27°C).

Transmural Fluxes

Adjacent segments of intestinal mucosa were mounted as flat sheets between lucite chambers, and mucosal-to-serosal and serosal-to-mucosal fluxes were determined in an apparatus similar to that described by Schultz and Zalusky (13). The tissues were bathed on both sides with identical solutions containing the same concentration of alanine (5 mM in all experiments). Labeled alanine was added to one bathing solution and, after an equilibration period of 90 min, the steady-state rate of appearance of isotope in the opposite solution was determined. In each experiment, four pieces of intestine from the same animal were examined, two in the presence of sodium and two in its absence.

Cellular Alanine Accumulation and Electrolyte Composition

To examine the ability of the cells to accumulate amino acid, strips of mucosa were incubated in different media containing 5 mM unlabeled L-alanine, L-alanine-³H, and inulin-¹⁴C. After 90 min, the intracellular concentration of alanine was determined essentially as described by Schultz, Fuisz, and Curran (6). The strips of mucosa were blotted, weighed, and extracted for 3-4 hr in 0.1 N HNO₃. Extracts of tissues

and portions of incubation media were counted in a three-channel liquid scintillation spectrometer using Bray's solution (14). The extracted tissues were dried at 90°C for 12 hr to determine dry weights. The cell water content and cell concentration were determined using inulin to estimate extracellular space. Similar experiments were carried out to estimate cell Na and K concentrations under different conditions. Na and K in tissue extracts and bathing solutions were measured with a Baird-Atomic flamephotometer (Baird-Atomic, Inc., Bedford, Mass.).

Direct Measurement of Efflux from Cell to Serosal Solution

The method employed was similar to the one described previously (15) for measurement of efflux toward the mucosal solution in rabbit ileum. Sheets of mucosa were incubated for 90 min in solutions containing alanine-³H and different concentrations of nonlabeled alanine. They were then dipped in isotonic mannitol, blotted gently with filter paper, and mounted between lucite half chambers. Solution with the same concentration of L-alanine as the incubation media, but without isotope, was placed in the serosal chamber only and replaced every minute for 8 min in order to follow washout of alanine. The washout samples were transferred directly to counting vials by suction. At the end of an experiment, the exposed mucosa was cut out, extracted in 0.1 N HNO₃, and its dry weight determined. The cellular alanine concentration $[A]_c$ was estimated as previously described (15) from the expression

$$[A]_c = \frac{S_c \times 10^3}{V_c^0 + 4.0 S_c}, \quad (1)$$

in which S_c is the alanine content (micromoles per milligram dry weight), determined from the counts in the tissue extract, and the specific activity of the incubation medium. V_c^0 is cell volume in the absence of alanine, taken as 4.9 μ l/mg dry weight for intestinal pieces incubated in normal Ringer solution; 5.4 μ l/mg for the ouabain-treated tissues; and 4.3 μ l/mg for those in choline medium (see Table III). The factor 4.0 is the change in cell volume per micromole of solute required to maintain isotonicity.

Indirect Measurements of Alanine Efflux

These experiments utilized the method described by Curran et al. (16) and used by Hajjar et al. (15) to study alanine transport in rabbit ileum. The tissue was mounted in chambers similar to those used for transmural fluxes, and labeled and nonlabeled alanine were added to the mucosal solution. 10 samples were taken from the serosal solution at timed intervals (ranging between 3 and 7 min depending on the nature of the experiment). After the 10th sample, three additional samples were taken at 20-min intervals. This procedure provides an estimate of the time-course of approach to a steady-state unidirectional tracer flux. The steady-state concentration of alanine in mucosal cells was estimated by nitric acid extraction of the exposed area of mucosa. From these data, the exit of alanine from cell to serosal solution (J_{cs}) was calculated as previously described (15).

Measurement of Net Transfer of Alanine

The method used was essentially the one described above for cell alanine accumulation. The mucosal pieces, however, were transferred from one incubation medium to another to study the effect of a Na concentration gradient on net alanine transfer. All tissues were first incubated for 45 min in Ringer's solution containing 10^{-4} M ouabain in order to elevate cell Na concentration to about that of the medium. Labeled and unlabeled alanine, and inulin- 14 C were next added to the solution, and incubation continued for another 45 min to allow entry of the amino acid into the cell. In some experiments, tissues were next transferred to Na-free choline medium containing labeled alanine and inulin, at the same specific activities as the original solution. As described in a previous study (15), the sequence of change in cell alanine was examined after transfer to the new solution by removing pieces of tissue for analysis at various times. In other experiments, incubated tissues were mounted (within 1-3 min) as sheets between lucite half chambers,¹ and only one of their surfaces was exposed to the Na-free choline solution (containing labeled alanine and inulin). The exposed tissues were subsequently cut out at various time intervals and examined.

RESULTS

Transmural Fluxes of L-Alanine

Mucosal-to-serosal (J_{ms}), and serosal-to-mucosal (J_{sm}) fluxes, in the presence and absence of sodium, are summarized in Table I. The turtle intestine, like rabbit ileum (17), or other mammalian intestine, is capable of net transfer of alanine from mucosal to serosal solution in the absence of a concentration difference. This net transport is completely inhibited by removal of sodium from the bathing media mainly as a result of a marked decrease in mucosal-to-serosal flux. This observation is also in agreement with the results of similar experiments on rabbit ileum (17).

TABLE I
TRANSMURAL FLUXES OF ALANINE ACROSS TURTLE INTESTINE

Incubation medium	n	J_{ms}	J_{sm}	J_{net}
			$\mu\text{moles/hr, cm}^2$	
120 mM Na + 5 mM alanine	4	0.60 ± 0.06	0.12 ± 0.93	0.48 ± 0.04
Choline + 5 mM alanine	4	0.10 ± 0.01	0.07 ± 0.02	0.03 ± 0.01

J_{ms} and J_{sm} were determined on adjacent segments of tissue from the same animal. All errors are standard errors of the mean. n, number of experiments, each with four sampling periods.

¹ These chambers consist of two open lucite cylinders in between which the tissue is mounted and one surface bathed with at least 3 ml of solution. The solution is stirred by bubbling with air.

Intracellular Alanine Accumulation

Mucosal strips of intestine were incubated in three different media, containing 5 mM alanine. In an experiment in which the time-course of accumulation was assayed, a steady-state cellular alanine concentration was found after 70–80 min, and in all subsequent studies tissues were examined after 90 min of incubation. The results of these experiments are presented in Table II. In normal Ringer, the mucosal cells accumulate the amino acid to a concentration 6 times that of the bathing medium. In Na-free medium, and in Ringer's solution containing ouabain, cellular accumulation to concentrations above that of the bathing solution is almost completely abolished. These results are also comparable to those reported by Schultz et al. (6) for rabbit ileum.

To determine whether measurements of tracer in this system can be used to predict the behavior of bulk material and whether the alanine molecule is

TABLE II
STEADY-STATE ALANINE ACCUMULATION BY
MUCOSAL STRIPS

Incubation medium	n	Cell alanine concentration
		mm
120 mM Na	10	32.5±3.3
120 mM Na + ouabain	12	7.0±0.7
Choline	12	6.7±0.9

Initial alanine concentration of the media in all experiments was 5 mM. Mucosal strips from the same animal were incubated in the above three media. Three animals were used. n, number of determinations.

altered during these experiments, paper-chromatography studies were carried out simultaneously on tissue extract, incubation medium, and a standard L-alanine solution. A single radioactive ninhydrin positive spot was found in each of the two tested solutions. The positions of the spots corresponded closely to that for the alanine standard. Thus the nonlabeled alanine appears to follow its isotopic marker closely within the time limits of the present experiments and metabolism of alanine seems minimal.

Intracellular Na and K in the Absence of Alanine

As shown in Table III, mucosal strips, incubated in the same media as above but in the absence of alanine, have cell Na and K concentrations that are qualitatively similar to rabbit mucosal cells incubated under similar conditions (6). In normal Ringer's solution, the cells maintain a relatively low Na concentration and a high K concentration. In the presence of ouabain, cell Na increases to a level nearly equal to that in the external solution; there is a

TABLE III
STEADY-STATE Na AND K INTRACELLULAR CONCENTRATION

Incubation medium	n	[Na] _c	[K] _c	Cell volume
		mM	mM	μl/mg dry wt
120 mM Na	14	50.3±4.1	102.6±7.3	4.9±0.2
120 mM Na + ouabain	12	112.7±4.3	55.0±6.8	5.4±0.1
Choline	13	6.2±5.2	110.3±8.3	4.3±0.3

Mucosal strips from the same animal were incubated in the above three media. Four animals were used. n, number of determinations.

decrease in cell K and an increase in cell volume. Removal of Na from the bathing solution causes a marked loss of cell Na and a decrease in cell water.

Direct Measurements of Efflux

The analysis of washout experiments was similar to that described previously (15). As shown in Fig. 1, plotting the fraction of activity remaining in the mucosa against the time of exposure to unlabeled solution yields a curve that can be described by two exponentials with half-times of 1.2 and 23.4 min. The slower component was fitted by a method of least squares and its intercept was taken as an estimate of the cellular pool of labeled alanine. In previous studies (15) in which the efflux of alanine from the rabbit intestinal cell to mucosal solution was examined, this method was shown to provide a reasonable estimate of the pool. There are however, certain differences between the present studies and those on rabbit ileum that merit comment. In rabbit, the half-time of the fast component for efflux toward the mucosal solution was approximately 0.5 min compared to 1.2 min for efflux toward the serosal solution in the present study. This difference is apparently due to a larger (thicker) extracellular space between the cells and the serosal solution than between the cells and the mucosal solution. These spaces were estimated in turtle mucosa by exposing separately the two surfaces of the epithelium to inulin-¹⁴C. The mucosal space measured in 10 pieces of tissue was 0.07 ± 0.02 ml/g wet weight while the serosal space was 0.18 ± 0.04 ml. In spite of the slower initial washout, the difference between the half-times for the two components of the curve is still sufficient to permit use of the intercept of the slow component to estimate the cellular pool of alanine. In the studies on rabbit ileum, there was a significant "recapture" of radioactive amino acid during the washout toward the mucosal solution. This effect did not occur in the present studies on the serosal side of the tissue since elevation of the alanine concentration in the washout solution to 40 mM did not cause an increased efflux of alanine-³H in any of the three media used. Thus the corrections used previously to account for "recapture" were not necessary and alanine efflux was calculated directly

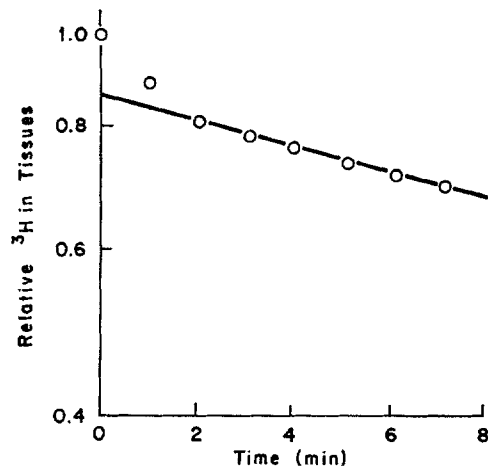


FIGURE 1. Washout of L-alanine-³H across the serosal border of turtle intestine. 5 mM alanine in Ringer solution was used as the bathing medium.

as the product of pool size and rate coefficient obtained from the slow component of washout.

Results of experiments carried out under three conditions, normal tissue, Na-loaded tissue, and low Na tissue, are presented in Fig. 2 in which efflux of alanine from the cells to the serosal solution is shown as a function of cellular alanine concentration. Since the cell alanine concentration could not be accurately predicted in these experiments, several determinations were made, and tissues having almost equal concentrations (within ± 1 mM) were grouped. At a given cell alanine concentration, the efflux from a cell with normal Na concentration is about the same as that from a cell with high or low Na concentration.

In normal Ringer, alanine efflux, J_A^e , shows a fairly clear tendency toward saturation as concentration increases, and can be described by the expression

$$J_A^e = \frac{J_A^{e \max} [A]_c}{K_t + [A]_c}, \quad (2)$$

in which $[A]_c$ is cell alanine concentration, $J_A^{e \max}$ is the maximal flux, and K_t is the "apparent Michaelis constant." In the Na-loaded and low Na cells, the points can be fit by straight lines, but within the errors, they can also be described by the above equation. The values of $J_A^{e \max}$ and K_t were determined as shown in Fig. 3. The lines in Fig. 2 were drawn according to equation 2 using these values.

Indirect Measurement of Efflux

Results of these experiments are presented in Fig. 4. The fluxes again do not differ markedly at the same cell alanine concentration for the three conditions,

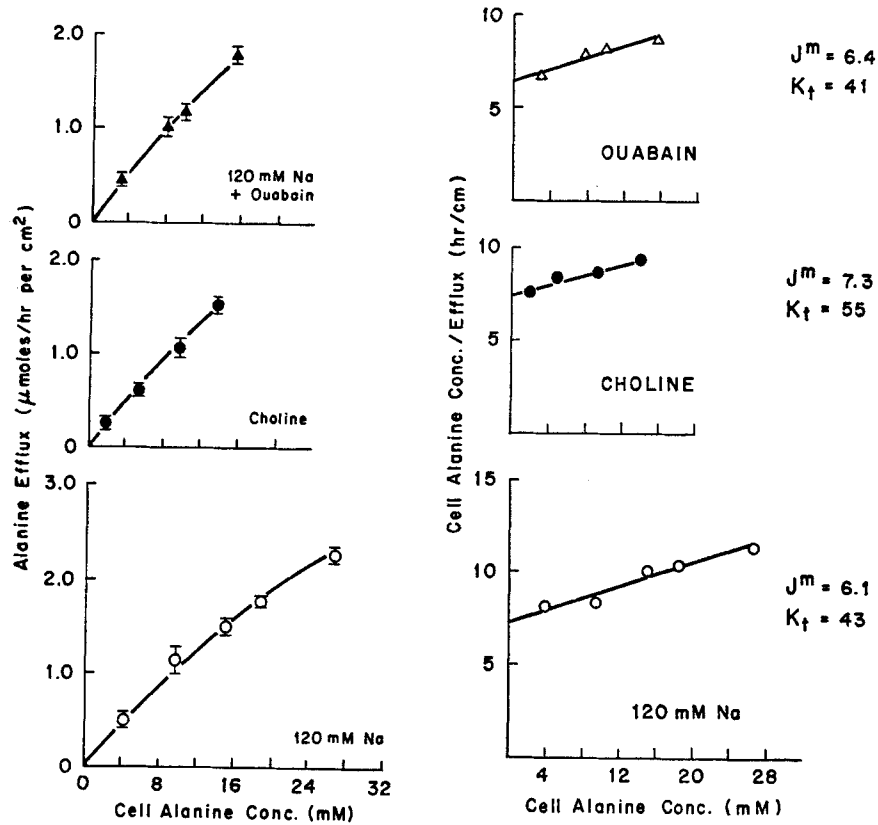


FIGURE 2

FIGURE 3

FIGURE 2. Relation between cellular alanine concentration and efflux from the cell to the serosal solution. Each point is the average of at least six determinations. The bars represent ± 1 SEM. The lines drawn from equation 2 using values of $J_A^{e\max}$ and K_t given in Fig. 3.

FIGURE 3. The relations between alanine concentration and concentration divided by efflux using data from Fig. 2. The tissues are preincubated in 120 mM Na (\circ), 120 mM Na + ouabain (Δ), and choline (\bullet). $J_A^{e\max}$ is in micromoles/hour square centimeter and K_t in millimoles/liter.

normal Ringer, Na-free Ringer, and normal Ringer plus 10^{-4} M ouabain. The lines in Fig. 4 are the same as those in Fig. 2 which were determined from direct efflux data as indicated above. The individual indirect efflux measurements show a reasonable scatter around these lines indicating good agreement between the two methods used to estimate this flux.

Net Transfer of Alanine

In these experiments, the influence of an Na-concentration gradient on net alanine transfer across the two faces of intestinal cells was studied. Tissues

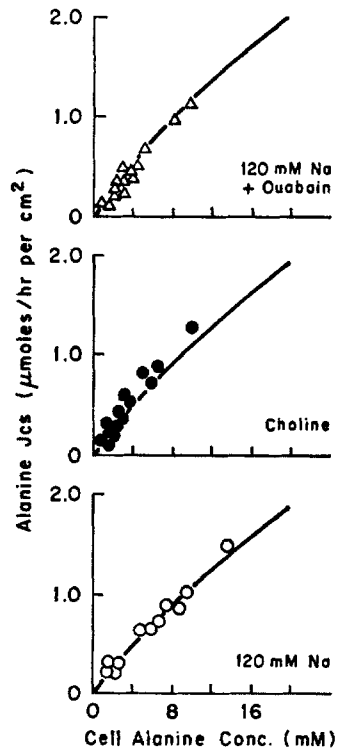


FIGURE 4. Relation between cellular alanine concentration and indirect efflux measurements from tissues preincubated in 120 mM Na (○), 120 mM Na + ouabain (Δ), and choline (●). The lines are drawn from the direct efflux data in Fig. 2.

were preincubated in Ringer's solution containing 10 mM alanine and ouabain. As indicated in Tables II and III, cellular concentrations of alanine and Na became approximately equal to the external concentrations under these conditions. When these tissues were transferred to an Na-free medium containing 10 mM alanine, alanine was extruded from the cells as shown in Fig. 5 and cellular alanine concentration decreased to a level significantly below the level observed in tissues not exposed to Na-free medium (Fig. 6). Although the initial cellular alanine concentration was approximately 14 mM, somewhat higher than that in the bathing solution, sufficient alanine was extruded to reduce cellular concentration to 8.8 mM at 10 min. Thus extrusion apparently occurred against a concentration difference. To determine the extent of extrusion of alanine from each of the two faces of the epithelium, one face of the tissue only was exposed to the Na-free medium after the initial incubation. As shown in Figs. 5 and 6, tissues with their mucosal surface bathed with Na-free medium extruded alanine to levels comparable to those in which both surfaces were exposed. This similarity is particularly apparent when alanine con-

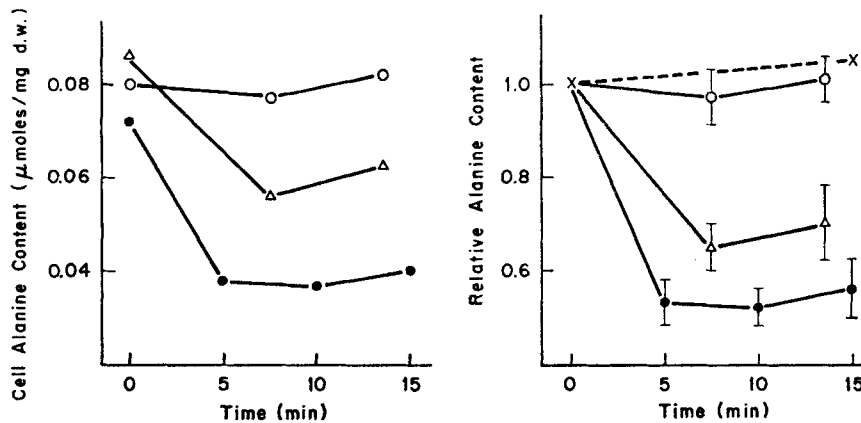


FIGURE 5. Extrusion of alanine from mucosal cells in the presence of an Na concentration difference. Tissues were preincubated in Na Ringer plus ouabain and 10 mM alanine. At zero time strips of tissue were transferred to Na-free solution (containing 10 mM alanine) (●) or only the mucosal (Δ) or serosal (○) surface was exposed to this medium. Left, cellular alanine content per milligram dry weight as a function of time. Right, alanine content relative to the value at zero time. The dashed line shows behavior of control tissue kept in the initial incubation medium.

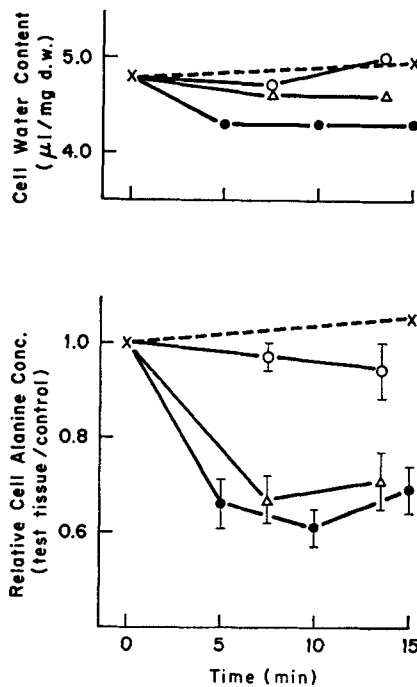


FIGURE 6. Cell water content and cellular alanine content relative to initial values as functions of time after transfer of tissues to Na-free medium (see Fig. 5). Both surfaces exposed (●), mucosal surface only (Δ), and serosal surface only (○). The dashed line shows the behavior for tissues kept in the initial incubation solution. Initial concentrations were 13.6 mM for tissues denoted ●, 16.2 mM for ○, and 18.2 mM for Δ.

tents and concentrations are expressed relative to their initial values for the different types of experiment. On the other hand, practically no extrusion of alanine occurred when the serosal surface alone was perfused with Na-free solution. These findings suggest that the exit of alanine from intestinal cells across their serosal border is independent of an Na concentration difference between the cells and the serosal solution while exit across the mucosal border is clearly influenced by such a concentration difference.

DISCUSSION

In order to obtain a complete understanding of amino acid transport across the intestinal epithelium, properties of both the mucosal and serosal borders of the cells must be examined since the polarized nature of the process indicates that these two barriers must have different properties. The movements of neutral amino acids across the brush border membrane have been studied in some detail (3, 18), but information regarding the serosal membrane is based largely on indirect evidence and inference. The main reason for this shortcoming is that the serosal aspect of the cells is usually much less accessible for direct measurement than the brush border. The turtle intestine thus appears to offer a unique opportunity to study the serosal membrane directly because it can be stripped almost completely of muscular and submucosal layers.

The present results suggest that the turtle intestine, like rabbit and other mammalian species, has an Na-dependent amino acid transport system in the brush border membrane. As shown in Tables I and II, the mucosal cells accumulate relatively high concentrations of alanine while actively transporting the amino acid from mucosa to serosa suggesting that the "active" step of transport must be located at the mucosal membrane. When Na in the bathing solutions is replaced by choline, cellular accumulation and active transmural transport are almost completely abolished. These observations, together with the finding that addition of amino acids to the mucosal solution causes a rapid increase in transmural electrical potential difference (19), suggest an interrelationship between Na and amino acid transport at the brush border similar to that in rabbit intestine.

These results are, however, considerably at variance in several respects with those reported by Gilles-Baillien and Schoffeniels for alanine transport by the intestine of the terrestrial tortoise *Testudo hermanni hermanni* Gmelin. They were unable to demonstrate cellular accumulation of alanine in the presence of Na. After 3 hr of incubating tissue with 20 mM alanine in the mucosal solution (but not the serosal solution), cellular alanine concentration was approximately 20 mM. This behavior was unaffected by a 10-fold reduction of Na concentration in the mucosal solution (8). Gilles-Baillien (11) also notes that fragments of mucosa incubated in solution containing 20 mM alanine do not

show cellular accumulation. The mucosal-to-serosal flux of alanine was reduced when 90% of the NaCl in the mucosal solution was replaced by sucrose (11). However, if Na was replaced by tris(hydroxymethyl)aminomethane (Tris) (constant Cl) or if Cl was replaced by SO_4 (constant Na), no effect on alanine flux was observed. Finally, Gilles-Baillien and Schoffeniels (9) found that alanine added to the mucosal solution had no clear effect on net Na flux but markedly altered net Cl flux. On the basis of these results, they argue for a completely different mechanism of amino acid transport in turtle intestine than that proposed for rabbit intestine (3, 18). They suggest that the major transport site may be at the serosal side of the cell, that intracellular metabolism of alanine may be involved in some of the observed effects, and that the role of Na may be an indirect one.

We have at present no explanation for the rather major differences between those results and the present ones. They could be due in part to differences in the animals used or in their condition at the time of the experiment. Differences in experimental techniques could also be involved. It is perhaps of interest to note that Gilles-Baillien (10) gives values for cellular Na of 60–90 mM and for cellular K of 50–70 mM which are quite different from the values we have observed (Table III). These differences might well influence the transport of amino acids, particularly in view of the well established ionic dependence of these processes. At any rate, our results are consistent with an amino acid transport system in the brush border membrane of turtle intestine that is Na-dependent and at least qualitatively similar to the one in rabbit ileum. Thus, the present results on alanine movement should be representative of relatively "normal" intestinal amino acid transport.

The absence of an Na-dependent transport process in the serosal membrane has usually been suggested in overall explanations of net absorption of amino acids (20). This speculation is now confirmed by two observations involving direct study of the serosal barrier. First, equal alanine efflux rates were observed across the serosal membrane of cells containing identical concentrations of the amino acid but high and low Na concentrations. Second, the cells did not extrude alanine in the presence of an Na concentration difference when the serosal solution was Na free (Figs. 5 and 6). In this regard, it is important to note that substantial extrusion of alanine did occur across the *mucosal* border of the cells when an outwardly directed Na concentration difference was imposed. Thus these results indicate that in turtle intestine alanine movement across the mucosal membrane is Na-dependent while movement across the serosal membrane is not.

The exact mechanism of amino acid transfer across the serosal membrane remains unclear, but it is probably not simple diffusion. Munck and Schultz (21) have suggested a carrier-mediated exit process for lysine at the serosal side of rabbit intestine. Newey and Smyth (22) studying glycine and glycygly-

cine transfer and intracellular peptidase activity in rat intestine, concluded that the exit process required metabolic energy. The present results, particularly those in the lower portions of Fig. 2, provide direct evidence against a simple diffusion process. The rather clear indication of saturation of efflux as cellular concentration increases suggests a mediated mechanism such as facilitated transfer. The question of whether or not the serosal transfer mechanism is symmetrical with respect to transfer in the two directions remains unclear. Some of the data presented could be interpreted as suggesting an asymmetry. Thus with 5 mM alanine in the bathing solutions, net flux was $0.5 \mu\text{moles/hr cm}^2$ (Table I) and cellular alanine concentration was 32 mM (Table II). Fig. 2 indicates that flux from cell to serosa would be approximately $2.5 \mu\text{moles/hr cm}^2$ under these conditions and the steady state condition would then require a flux from serosa to cell of $2 \mu\text{moles/hr cm}^2$. This ratio of unidirectional fluxes at the serosal membrane does not seem consistent with a symmetrical facilitated transfer system given a 6-fold concentration ratio and the characteristics proposed for the efflux process. In fact, it would appear that either the maximal flux must be greater for movement into the cell than out or that the K_t for influx must be smaller than for efflux. Alternatively, there could be a significant component of exchange diffusion in movement of alanine across the serosal membrane. This possibility is made somewhat unlikely, but is not ruled out entirely, by the observation that efflux of alanine- ^3H is not altered by raising alanine concentration in the serosal solution from 5 to 40 mM. Finally, there may be an effective asymmetry in the serosal system due to effects of other amino acids present in the cytoplasm. Hajjar et al. (15) found, in studies on rabbit ileum, a substantial cellular pool of several amino acids even after fairly long incubations in solution containing only alanine. These amino acids could compete with alanine for exit via the serosal transfer mechanism and would elevate the effective K_t of the process above the intrinsic value.

The above considerations may, however, be somewhat misleading since in the experiments in which efflux was measured directly (Fig. 2), cellular alanine concentration reached a level of only 15 mM with an external concentration of 5 mM. In this case, efflux at the serosal side would be $1.5 \mu\text{moles/hr cm}^2$ and influx $1.0 \mu\text{moles/hr cm}^2$, a situation more nearly compatible with a symmetrical system. A final answer to this question will have to await attempts to characterize by direct measurement the flux from serosal solution to cell. However, the data in Figs. 5 and 6 seem to indicate that if the exit step is asymmetrical (which would make it capable of transport against a concentration difference) the asymmetry must also be inhibited by ouabain since alanine is not extruded to any significant extent at the serosal side under the conditions of these experiments. At present, there is no real reason to postulate an active exit step; a "downhill" mechanism seems sufficient since the cells are able to accumulate amino acids via the mucosal transport system to concentrations in excess of those in the bathing media.

The existence of an Na-independent mechanism for amino acid transfer in intestinal cells does not represent an entirely anomalous situation. Although active amino acid transport mechanisms in a wide variety of cells and tissues display strong Na dependence (see Schultz and Curran [20] for a summary), there are also reports of Na-independent systems. Thus, the so-called "L" or "leucine preferring" system observed by Christensen and his coworkers (23-25) in Ehrlich ascites cells, rabbit reticulocytes, and pigeon erythrocytes displays minimal Na sensitivity and Na-independent mechanisms for cellular accumulation of lysine have been reported (21, 26). As discussed by Schultz and Curran (20), the extent to which these Na-independent mechanisms are capable of uphill transport is not entirely clear. Finally, it is perhaps of interest to note that the neutral amino acid transport system in the mucosal membrane of rabbit ileum continues to function in the absence of Na but is unable to cause active transport and shows characteristics of facilitated transfer. Thus in the absence of Na, this system displays characteristics that are somewhat similar to the system in the serosal membrane of turtle intestine.

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