Biochimica et Biophysica Acta, 455 (1976) 200–213

BBA 77473

MUCOSAL AND SEROSAL FLUXES OF ALANINE IN RABBIT ILEUM

GIUSTINA DANISI*, YUAN-HENG TAI** and PETER F. CURRAN†

Department of Physiology, Yale University, School of Medicine, New Haven, Conn. 06510 (U.S.A.)

(Received March 22nd, 1976)

SUMMARY

The four unidirectional fluxes of alanine across the mucosal and serosal borders of rabbit ileum were evaluated as functions of the alanine concentration on a single piece of tissue using a method previously described (Naftalin, R. J. and Curran, P. F. (1974) J. Membrane Biol. 16, 257–278). The effects of Na^+ removal and of ouabain on these fluxes were investigated. Alanine was actively transported across the mucosal membrane under control conditions; Na^+ removal or ouabain inhibited this process as a result of a decrease in flux from the mucosal solution to the cell and an increase in the flux in the opposite direction. The results concerning mucosal efflux of alanine are apparently inconsistent with the carrier model for alanine transport at this border. Alanine transfer across the serosal membrane appeared to involve a facilitated transfer mechanism. Alanine movement at the serosal side of the cell was not influenced by Na^+.

INTRODUCTION

The knowledge of the fluxes of a solute across the mucosal and the serosal membranes of an epithelium is fundamental for the understanding of the net transport of the solute by the epithelium. As far as amino acid transport is concerned considerable information has been collected on alanine transport by mammalian small intestine [1–4]. These studies used separate measurements of bidirectional transmural fluxes across the epithelium and influx across the mucosal border, measurements made on three separate pieces of tissue, for calculating the remaining unidirectional fluxes [3, 4]. In these calculations it is assumed that the tissue behaves as a single compartment for the amino acid involved. Recently, Naftalin and Curran [5], assuming as in earlier studies that the tissue is operationally a single compartment for the solute involved, were able to calculate all four unidirectional fluxes of galactose in rabbit

* Present address: Département de Chirurgie Expérimentale, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Suisse.

** Present address: Department of Gastroenterology Walter Reed Army Institute of Research, Washington, D.C. 20012, U.S.A.

† Deceased October 16th, 1974.

ileum from direct measurements made on a single piece of tissue. Their results were similar to those obtained by other investigators from direct measurements and were consistent with conclusions regarding sugar transport drawn from a variety of other studies [6]. Such information is not available for alanine transport in rabbit ileum. Mucosal influx and efflux have been measured directly [4, 7, 8], but direct information regarding the serosal membrane is available only for turtle small intestine [9].

In the present studies the unidirectional fluxes of alanine across the mucosal and serosal borders of rabbit ileum were evaluated according to the approach of Naftalin and Curran under a variety of experimental conditions.

METHODS

Male New Zealand white rabbits were killed by intravenous injection of sodium pentobarbital. A segment of ileum was removed rapidly, washed free of intestinal contents with ice-cold Ringer's solution and stripped of serosal and muscle layers as previously described [10]. The stripped tissue consists of mucosa, lamina propria, muscularis mucosae and occasional adherent portions of the inner muscular layer [10]. The mucosal tissue was then mounted as a flat sheet between Lucite half-chambers with an area of 3.14 cm^2 in the apparatus described by Schultz and Zalusky [11]. The tissues were then exposed to Ringer's solution (15 ml at each side) containing varying concentrations of L-alanine labelled with L-[^{3}H]alanine (mucosal solution) and L-[^{14}C]alanine (serosal solution). The Ringer's solution contained, in mM, 140 NaCl, 10 $KHCO_3$, 0.2 KH_2PO_4, 1.2 K_2HPO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$ and was continuously bubbled with 95 % O_2/5 % CO_2. All experiments were carried out at 37 °C. In some experiments all NaCl in the Ringer's solution was replaced by choline chloride.

30 min after the tissues were exposed to the bathing solutions and to the isotopes, one-ml samples were removed from both mucosal and serosal solutions for counting at 20- or 25-min intervals for 100 min. At the end of the experiment, the "hot" solutions were removed, and both sides of the chambers were rapidly washed with ice-cold Ringer's solution with the membrane still in place. The chambers were then opened; the exposed circle of tissue was cut out and blotted carefully on both sides to remove the excess moisture. The mucosa was scraped off using glass microscope slides to remove any muscle left from the stripping procedure. The resultant tissue was then weighed wet in a small tared flask and extracted by shaking for 2 h in 3–5 ml of 0.1 M HNO_3. Samples from the extract were taken for counting. Tissue samples were dessicated overnight at 80 °C to determine the dry weight. Tissue water was calculated as the difference between wet and dry weights. Cellular water was calculated taking an extracellular space of 30 % of tissue wet weight (inulin space determined in this laboratory).

Radioactive samples were counted in a liquid scintillation spectrometer set to provide adequate discrimination between ^{3}H and ^{14}C. Appropriate standards were used to separate counts of the two isotopes in the various samples. Transmural fluxes were calculated from the rate of tracer appearance on the "cold" side and the specific activity of the "hot" side. As the flux values calculated between the first and the last 20-min intervals did not differ significantly, they were averaged over the 100 min for each tissue. In most experiments, four pieces of tissue were studied simultaneously,

each with a different concentration of alanine in the bathing solutions. An experiment was repeated 9 or more times for each condition.

Assuming that rabbit ileum can be described in terms of a three compartment system, mucosal solution (compartment m), cell (compartment c) and serosal solution (compartment s), the unidirectional fluxes of a solute across individual barriers have been derived using the following relationships [5]:

$$J_{mc} = J_{ms} + J_{sm}R \quad (1)$$

$$J_{cm} = J_{sm}(1+R) \quad (2)$$

$$J_{cs} = J_{ms}(1+1/R) \quad (3)$$

$$J_{sc} = J_{sm} + J_{ms}/R \quad (4)$$

If solute concentrations are identical in compartment m and s,

$$R = \frac{J_{mc}}{J_{sc}} = \frac{(\text{cpm})_c^T/(\text{cpm/ml})_m^T}{(\text{cpm}_c^C)/(\text{cpm/ml})_s^C} \quad (5)$$

Superscripts T and C refer to ^{3}H- and ^{14}C-labelled solute.

RESULTS

The measured transmural fluxes of alanine as a function of external alanine concentration are shown in Fig. 1 for control conditions. The mucosa to serosa flux, J_{ms}, bears a convex relationship to concentration. The flux in the opposite direction, from serosa to mucosa, J_{sm}, appears to bear a concave relationship with external alanine concentration, although the points could be fitted also by a straight line. The net active flux of alanine across the tissue is also shown. Fig. 2 presents the change of the ratio R and of the intracellular alanine concentration with external alanine concentration. R is the ratio of the two isotopes in the tissue, normalized to the specific activity of each isotope in the external solutions. It reflects the relative

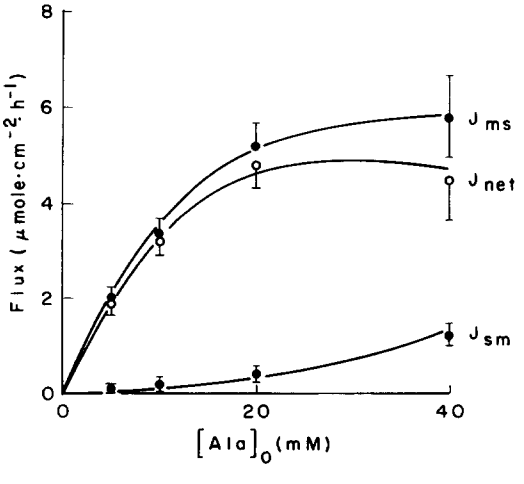

Fig. 1. Measured transmural fluxes of alanine under control conditions as functions of external alanine concentration. Points are means ±S.E. of 10 or more experiments at each concentration.

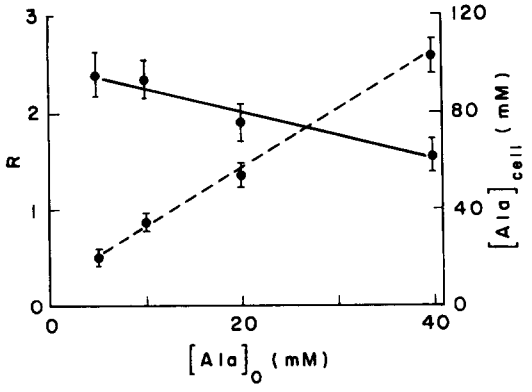

Fig. 2. The measured ratio R and cellular alanine concentration as functions of external alanine concentration in control experiments. —, R; – – –, intracellular alanine concentration. Points are mean ±S.E. of at least 10 experiments at each concentration.

quantities of solute which enter the cell from the mucosal and serosal solutions. This ratio decreases with an increase in external alanine concentration, from 2.4 at 5 mM to 1.6 at 40 mM external alanine. This means that at the lower concentration about 2.5 times more alanine enters the cells from the mucosal side than from the serosal medium. As the alanine concentration in the medium increases the serosal contribution to the cellular alanine is increased with respect to that of the mucosal solution, and at 40 mM external alanine, approximately equal quantities of alanine enter from both sides. The total intracellular alanine concentration is a linear function of the alanine in the bathing solutions in the range of concentrations employed in these experiments. However, the accumulation ratio, Ala_{cell}/Ala_{medium}, decreases from 4.3 at 5 mM to 2.6 at 40 mM external alanine. The accumulation ratio of 4.3 at 5 mM external alanine is about half that previously reported in the literature at the same external alanine concentration [1]. This apparent discrepancy was not due to too short a time of extraction in our experiments (2 h) as compared to 24 h in earlier data. There was no significant difference between the cellular alanine concentration estimated after 2 h and after 24 h of extraction for all concentrations of alanine employed in these experiments. This suggests that the difference between our values for cellular alanine and those reported by other investigators at 5 mM external alanine may be due to animal variation.

From the results presented in Figs. 1 and 2 the unidirectional fluxes across the mucosal and serosal borders were calculated. They are shown in Fig. 3. On the left side are shown the influxes of alanine from mucosal and serosal solutions into the cells as a function of the external alanine concentration. On the right side we can see the effluxes of alanine from cell to mucosal and to serosal solutions as functions of the cellular alanine concentration. The mucosal influx shows a clear tendency towards saturation, in agreement with data from direct measurements [7, 12]. The hyperbolic shape of the mucosal influx with concentration indicates that the effective permeability coefficient for unidirectional alanine movement from mucosal solution to cell, $P_{mc} = J_{mc}/Ala_{medium}$, decreases with increase of external alanine concentration. The mucosal efflux, J_{cm}, increases as the cellular alanine rises, but the increase in the flux

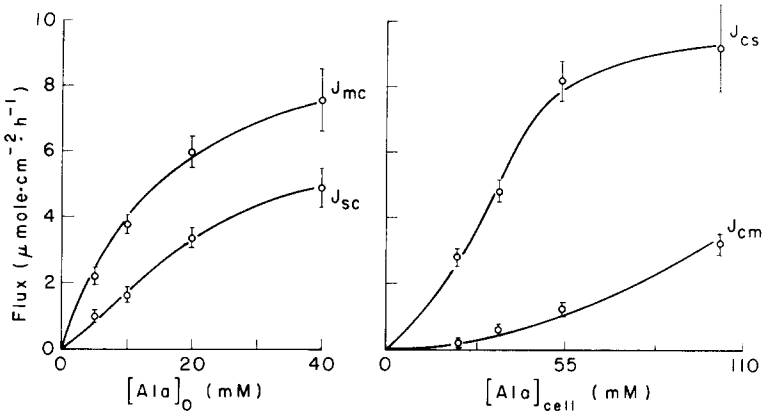

Fig. 3. Calculated unidirectional fluxes across the mucosal and serosal boundaries. Points are mean ±S.E. The points for J_{cm} and J_{cs} are average values obtained at each of the 4 external alanine concentrations and are plotted against the corresponding average cellular concentration.

is more pronounced than the increase in concentration, suggesting that there is an increase in the effective permeability coefficient for the exit of alanine at this border, $P_{cm} = J_{cm}/\text{Ala}_{cell}$. However, the uncertainties in the estimated values and the physical meaning of cellular alanine concentration make it difficult to establish clearly the behaviour of J_{cm} as a function of intracellular alanine concentration. There is net absorption of alanine across the mucosal border at all concentrations of external alanine. The data presented in Fig. 3 provide a clear evidence against a simple diffusion process for the movement of alanine at the serosal border, as the ratio of the fluxes is consistently lower than the ratio of concentrations. This difference may be even greater, because of an underestimation of the cellular alanine, due to the washing procedure (see Methods). J_{cs} increases quite linearly with concentration, up to approx-

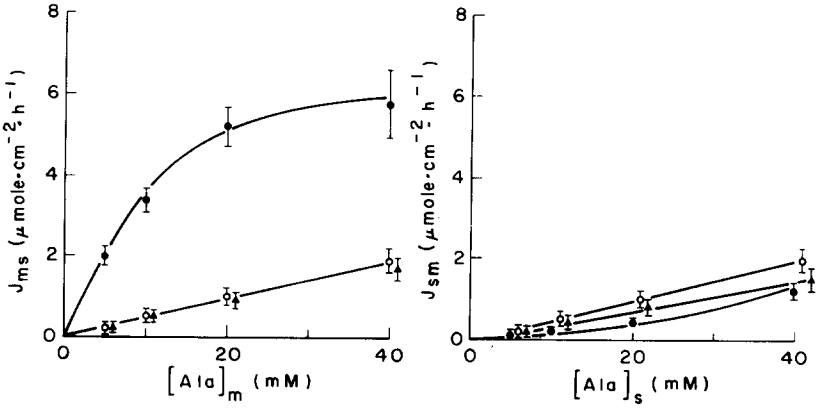

Fig. 4. Measured transmural fluxes of alanine as function of external alanine concentration in Na^+-free and ouabain-treated tissues. ●, control; ○, Na^+-free; ▲, ouabain. Some points are displaced on the X-axis for clarity. Each point in the experimental conditions is mean +S.E. of 9 experiments or more.

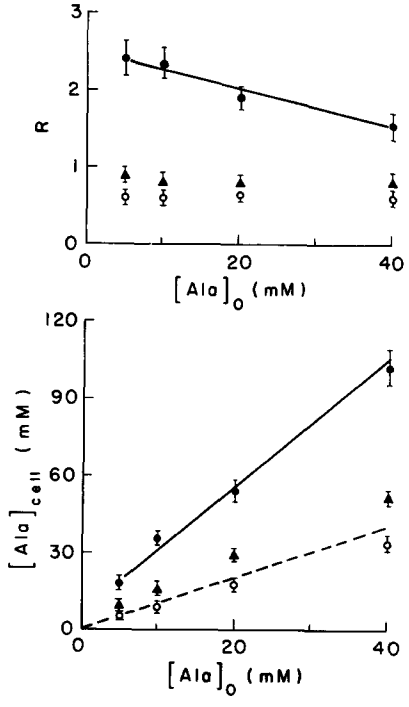

Fig. 5. The effect of Na^+-free and ouabain-treatment on the ratio R and on cellular alanine concentration. Symbols as in Fig. 4. Each point in the experimental conditions is the mean $\pm$S.E. of at least 9 experiments.

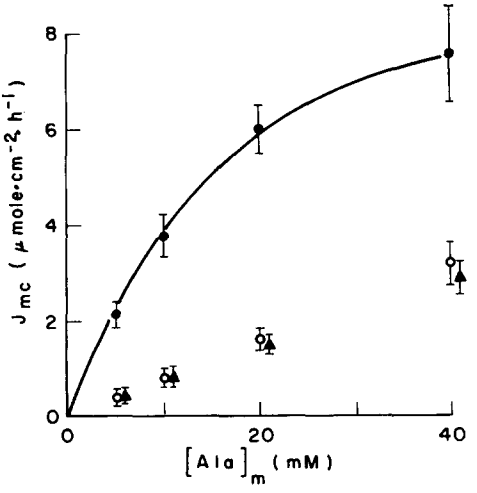

Fig. 6. J_{mc} (mean $\pm$S.E.) as a function of external alanine concentration in Na^+-free and in ouabain-treated tissues. Symbols as in Fig. 4. The ouabain points are displaced on the X-axis for clarity.

imately 50 mM cellular alanine. However at the highest cellular alanine this increase in J_{cs} is not proportional to concentration, suggesting a saturation of the transport mechanism. The serosal influx, J_{sc}, also suggests some tendency towards saturation, but within the limits of experimental error these results may be fitted also by a straight line.

The experiments that will be presented below were carried out in Na^+-free Ringer and in Na^+-Ringer plus 10^{-4} M ouabain. Mucosal strips of rabbit ileum, incubated in Na^+-Ringer and 5 mM alanine, have a cellular Na^+ concentration of about 50 mM [1]. Incubation in Na^+-free Ringer results in a rapid depletion of intracellular Na^+, to only 7–10 mM [1]. Ouabain leads to an increase in cellular Na^+, resulting in a Na^+ concentration in the cell not different from that of the medium [1]. In our experiments these experimental manipulations were intended to change the external and/or the intracellular Na^+ concentrations, so that the importance of the Na^+ gradient for the movement of alanine at the mucosal border could be verified and the dependence of the serosal fluxes of alanine on Na^+ could be ascertained.

Figs. 4 and 5 show effects of these treatments on the measured quantities. Control values are also shown for the sake of comparison. As shown in Fig. 4, the net flux of alanine across the intestine is abolished in Na^+-free Ringer and following pretreatment with ouabain. This is mainly due to a sharp reduction in J_{ms}, significantly lower than control levels. J_{sm} is slightly increased compared to control conditions, and the increase is significant except at 40 mM external alanine for ouabain-treated tissues, where J_{sm}, in these tissues, is not significantly different from the control values. There appeared to be a linear relationship between the fluxes and concentration. Na^+-free conditions and Na^+-Ringer plus ouabain reduced the ratio R to values below unit (Fig. 5), indicating that under these conditions more than 50 % of the cellular alanine comes from the serosal solution. This ratio does not change with concentration, which implies that the same relative quantities of the amino acid enter the cell from the mucosal and serosal sides at all concentrations. These findings suggest that, at least for the Na^+-free condition, the serosal membrane is more permeable to alanine than the mucosal membrane. Fig. 5 also shows that cellular alanine accumulation is abolished in the Na^+-free condition. Except at 5 mM external alanine, the intracellular alanine concentration is significantly lower than its concentration in the bathing solutions. However because of the washing procedure the cellular alanine concentration may be somewhat underestimated. Treatment with ouabain decreases the cellular accumulation of alanine, but its concentration in the presence of ouabain is significantly higher than that of the external solution. Intracellular alanine concentrations above the concentration in the external medium were also reported by Schultz et al. [1] in some experiments where the time course of alanine uptake from normal buffer (5 mM alanine) containing $5 \cdot 10^{-5}$ M ouabain was followed up to 50 min.

The unidirectional fluxes across the individual borders, calculated from the data presented in Figs. 4 and 5, are shown from Figs. 6 to 9. Fig. 6 illustrates the flux from the mucosal solution into the cell, J_{mc}, as a function of external alanine concentration. After both treatments, J_{mc} was markedly reduced and became a linear function of concentration with no evidence of saturation. The flux values for each concentration in Na^+-free Ringer and after ouabain are not significantly different. The decrease in J_{mc} upon withdrawal of external Na^+ is predicted by the carrier model

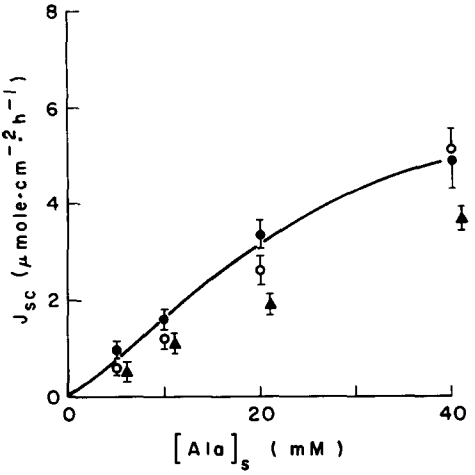

Fig. 7. J_{cs} (mean $\pm$S.E.) as a function of external alanine concentration in Na^+-free and ouabain-treated tissues. Symbols as in Fig. 4.

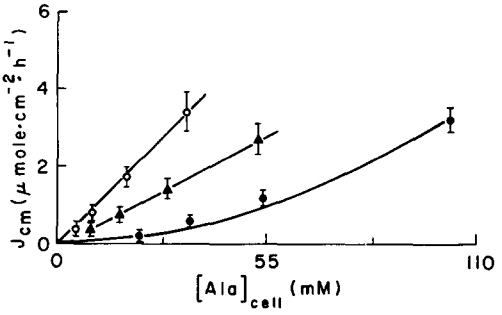

Fig. 8. J_{cm} (mean $\pm$S.E.) in Na^+-free and ouabain conditions as a function of intracellular alanine concentration. Symbols as in Fig. 4.

for the movement of alanine at this border [7], whereas the decrease in J_{mc} after ouabain is not. Fig. 7 presents the relationship between the serosal influx and the serosal alanine concentration in Na^+-free Ringer and ouabain-treated tissues. As in the case of the mucosal influx, the serosal influxes are linearly related to the serosal alanine concentration under both conditions. The values of J_{sc} in the absence of external Na^+ are significantly lower than the controls, except at 40 mM external alanine, but the difference is rather small. This suggests that the movement of alanine from the serosal solution into the cells does not depend strongly on external Na^+. However ouabain treatment significantly reduces J_{sc} below control levels at all external alanine concentrations.

The change in J_{cm} with intracellular alanine concentration for Na^+-free and ouabain-treated tissues can be seen in Fig. 8. Control values are also shown for the sake of comparison. The flux of alanine per unit cellular concentration is increased after ouabain treatment with respect to control conditions. This result is consistent with the carrier model proposed for the movement of alanine at this border. However the results in Na^+-free Ringer do not seem consistent with this carrier model, because

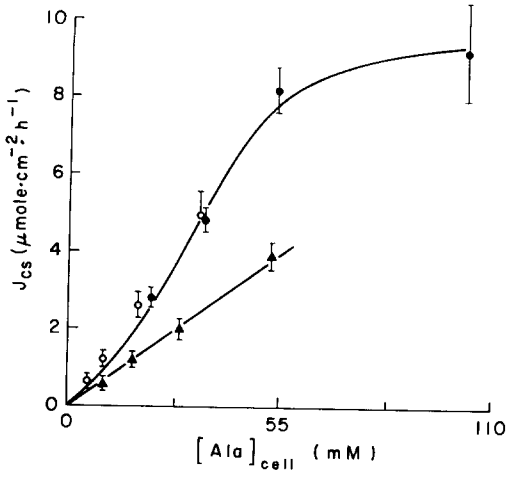

Fig. 9. J_{cs} (mean $\pm$S.E.) as a function of intracellular alanine concentration in Na^+-free and ouabain-treated tissues. Symbols as in Fig. 4.

in these conditions the mucosal efflux per unit concentration of alanine is higher than the control and ouabain-treated tissue levels. The carrier model would predict a lower efflux at a given cellular alanine concentration when cellular Na^+ is low (Na^+-free) than when cellular Na^+ is higher (control and ouabain).

The movement of alanine from the cell to the serosal solution J_{cs}, for Na^+-free and ouabain-treated tissues is presented in Fig. 9. Control values are also presented for comparison. In the absence of external Na^+, the intestine loses its capacity to accumulate alanine, however, J_{cs} per unit cellular alanine concentration is the same as in control conditions. This observation strongly suggests that the exit of alanine at the serosal border does not depend on cellular Na^+, which in the situation above is decreased with respect to control conditions. However our values for J_{cs} in ouabain treated tissues are slightly but significantly lower than the values of J_{cs} in control and Na^+-free conditions at similar cellular alanine concentrations.

DISCUSSION

In the present analysis, it is assumed that all radioactivity measured in the solutions and tissue represents alanine. Schultz et al. [1] have reported that at least 95 % of the radioactivity in the incubation media and that extracted from rabbit ileum migrated with cold alanine on ascending paper chromatography. It is also assumed that the transmural movement of alanine follows a transcellular route. Recent studies of Frizzell and Schultz [13] have demonstrated a significant paracellular pathway for ion movement in rabbit ileum. However this shunt does not appear to play a major role in the transepithelial movement of amino acids, as mucosal influx of amino acids, under control and Na^+-free conditions, is subjected to competitive inhibition by other amino acids [7, 14]. The recent studies of Rinaldo et al. [15] suggesting that the transepithelial movement of sugars in Na^+-free media occurs through the shunt pathway may not be extended to amino acids, as the movements of

sugars in the absence of external Na appear to be diffusional. The basic assumption inherent to this approach for estimating unidirectional fluxes, i.e., that the tissue acts as a single compartment with respect to alanine, is rather difficult to verify. The small intestine is composed of at least three kinds of cells which may not be involved to the same extent in transmural transport of alanine. Some investigators [16–18] have suggested an anatomical separation between Na^+ and fluid absorption and Na^+ and fluid secretion, absorption occurring in the villa and secretion in the crypts. However, recently De Jonge [19] has presented experimental evidence that both villa and crypts contribute to the secretion of water and electrolytes during cholera, suggesting that absorptive and secretory processes occur within the same epithelial compartment. On the other hand, Kinter and Wilson [20] have suggested that the crypts of Lieberkühn do not communicate with the mucosal fluid and may constitute a compartment that is not involved in the transmural transport of alanine. However, both villous and crypt epithelium are able to bind the cholera toxin present in the lumen to their surface [21]. The fairly good agreement between some of our calculated fluxes and data from direct measurements (see below) would suggest that the assumption made in our calculation is reasonable.

Using a different approach for obtaining unidirectional fluxes of alanine across individual cell borders, our results of J_{mc}, under control conditions, are consistent with those reported earlier by direct measurements of influx across the brush border [3, 4, 7]. Under control conditions, J_{mc} is a saturating function of the external alanine concentration, in agreement with results from Curran et al. [7] and Schultz et al. [12]. Our experiments yield values for K_t and J_{mc}^m, 24.5 and 12.5 $\mu mol \cdot cm^{-2} \cdot h^{-1}$ respectively which are higher than those reported by Curran et al. We do not have a clear explanation for this discrepancy. Differences in unstirred layer under different experimental conditions may be responsible, at least in part, for differences in K_t values [22].

The behaviour of J_{mc} in Na^+-free Ringer, sharply decreased as compared to control conditions, is consistent with the carrier model proposed for the movement of alanine across the mucosal barrier [7]. In the absence of external Na^+, there is no net flux of alanine across the mucosal membrane and no alanine accumulation by the tissue, both results being consistent with the hypothesis that the Na^+-gradient across the mucosal border determines the asymmetry of alanine fluxes at this barrier under control conditions. Despite the linearity of J_{mc} with external alanine concentration, this flux is probably a carrier mediated transport because the mucosal influx in the absence of external Na^+ is subjected to inhibition by other amino acids [7]. The sharp decrease in J_{mc} in the presence of ouabain, similar to the results of Luisier and Robinson [23], may suggest a direct effect of ouabain on J_{mc}. Direct measurement of influx after ouabain treatment is decreased by 30 % with respect to control conditions, and this effect of ouabain was attributed to cell swelling that occurs when the tissue is treated by ouabain [3]. Swelling, with no ouabain present, causes a decrease of 30 % of mucosal influx respective to control levels. A direct effect of ouabain on initial rates of uptake of amino isobutyrate (AIB) has been shown in membrane vesicles from Ehrlich ascites cells [24]. Such an effect is not likely in our experiments. Inspection of Figs. 6–9, shows that, after ouabain, the four fluxes per unit concentration are decreased with respect to control conditions. Such observation would suggest a rather unspecific effect of ouabain. Charalampous [25] has shown that the effect of ouabain on AIB

influx in KB cells is of an indirect nature, preventing an adequate intracellular concentration of K^+. The 80 % decrease of influx in ouabain-treated tissues in our experiments may be due to the K^+ gradient effect on the carrier mediated transport process and to changes in electrical potential difference across the mucosal membrane in addition to the cell swelling.

The relationship between J_{cm} and concentration of alanine in the three conditions, control, Na^+-free and ouabain, is puzzling and difficult to interpret in terms of the carrier model for alanine movement at this membrane. As seen in Fig. 8, at similar cellular alanine, J_{cm}, in ouabain experiments, where cellular Na is increased respective to control conditions, is higher than in control tissues. This finding alone supports the carrier model proposed for the exit of alanine at this border. However, in Na^+-free Ringer, the value of J_{cm} at similar cellular alanine levels as in control and ouabain-treated tissues, is higher than the values of J_{cm} in the two conditions above. This finding is not predicted by the model, where, a lower efflux for the Na^+-free condition than in control and ouabain-treated tissues is expected. Similar behaviour of galactose efflux at the mucosal membrane was found by Naftalin and Curran [5] in rabbit ileum. This finding was interpreted by these investigators as a recapture of galactose leaving the cell at this border back to cell, in control and ouabain tissues, which was absent when Na^+ was substituted by choline in the external solutions. In rabbit ileum, Hajjar et al. [8] have shown that labelled alanine leaving from cell to mucosal solution was recaptured back to the cell. The recapture was virtually absent when external alanine was raised to 40 mM or in the absence of external Na. However we do not think this phenomenon alone can account for the higher efflux of alanine per unit concentration in Na^+-free Ringer than in ouabain and control experiments. At 40 mM external alanine, recapture should be virtually absent in the control and ouabain experiments; however, at this external alanine concentration, the effective permeability coefficient for alanine exit at the mucosal border, $P_{cm} = J_{cm}/Ala_{cell}$, in the Na^+-free conditions is significantly higher than its values in control and ouabain-treated tissues at such concentration. No conclusive answer can be reached from our experiments, however they suggest that the adequacy of the carrier model for the exit of alanine at the mucosal border should be reevaluated. For example, if in the equation for the net flux of alanine at the mucosal border (see appendix) we employ the accepted values for cellular Na^+ and alanine concentration, a net secretion of alanine at this border is forecast. Influx is predicted according to experimental data, but the efflux is not, thus resulting in a net secretion, which is contrary to what is observed experimentally, i.e., net absorption. Net absorption will be predicted by Eqn. A3 only if $K_1K_2 \neq K_3K_4$ or if $K_1K_2 = K_3K_4$ and the "effective" alanine and/or Na^+ concentrations are less than those experimentally measured. Pietrzyk and Heinz [26] have shown that in Ehrlich cells cellular Na is sequestrated by the necleus, thus the cytoplasmic Na^+ is only 15 % of the intracellular Na^+. No similar evidence is available for rabbit ileum, but Lee and Armstrong [27] have shown that in isolated bullfrog intestine the activity of cellular Na^+ is lower than the concentration of this ion. Besides the potential difference probably plays some role on the exit of alanine at this border inasmuch as the entry appears to be rheogenic [28]. Some investigators have reported values of mucosal alanine efflux that do not quite agree with what is predicted by the carrier model for the exit of alanine at this border. For example, Hajjar et al. [8] have shown that alanine effluxes in ouabain-treated tissues, having similar cellular alanine

concentrations as the controls, are about equal to the effluxes under control conditions. On the other side, Naftalin and Holman [29] have shown that the effective permeability for the exit of galactose from cell to mucosal solution $P_{cm} = J_{cm}/Gal_{cell}$, decreases when external Na^+ is increased, condition in which the cellular Na^+ would be rising too, thus leading to an increase in P_{cm}.

J_{cs} in control experiments saturates when cellular alanine increases. This finding is consistent with the results of Hajjar et al. [9] who measured directly the serosal efflux of alanine in turtle intestine. These investigators observed also that alanine movement across the serosal membrane was a Na^+-independent process. This fact is confirmed in our experiments, i.e. at equal cellular alanine concentrations in control tissues, where cellular Na^+ is about 50 mM, J_{cs} is not different from its values in tissues where the external Na is replaced by choline, and cellular Na^+ is only 7–10 mM. The movement of alanine in the opposite direction also does not seem to depend strongly on external Na^+, as J_{cs} in control conditions (external Na^+ 140 mM) is only slightly higher than J_{sc} in Na^+-free experiments. Naftalin and Curran [5], Bihler and Cybulsky [30], and Murer et al. [31] also reported that the movement of sugars at the serosal side of intestine was Na^+-independent. The decrease in flux per unit concentration gradient for the movement of alanine at this border in ouabain-treated tissues would not be expected if these movements were Na^+-independent. However, the cell swelling and/or the decrease in cellular K^+, that occurs in the presence of ouabain, may account, at least in part, for this effect.

In summary, under control conditions, our studies have provided results, in a single set of experiments, that agree reasonably well with conclusions regarding alanine transport drawn from a variety of other studies. Our results for mucosal influx in control conditions are consistent with data for influx at the mucosal border obtained from direct measurements. Some of our results in Na^+-free and ouabain conditions may not be consistent with the carrier model for alanine transport at the mucosal border. At the serosal border, we have provided, with these experiments, the first specific information about alanine transport across this barrier in rabbit ileum.

APPENDIX

Curran et al. [7] have derived an expression for alanine influx across the mucosal membrane in rabbit ileum according to the carrier model of Fig. 10.

$$J_A^i = \frac{C_t P [A]_m}{\frac{K_1 K_2}{[Na^+]_m + K_2} + [A]_m}, \text{ as } [A]_c = 0 \tag{A1}$$

where J_A^i is the influx, C_t is the total carrier concentration, $K_1 = k_{-1}/k_1$, $K_2 = k_{-2}/k_2$. The fundamental assumptions involved are: the translocation of the free carrier or complexes is the rate limiting step and the system is in the steady state. Schultz et al. [4] have demonstrated experimentally that, within the limits of experimental error, alanine influx is not subjected to a transconcentration effect.

Following the same model and fundamental assumptions, we can show that the alanine efflux has exactly the same form as Eqn. A1 when $[A]_c \neq 0$ and the efflux can be expressed as:

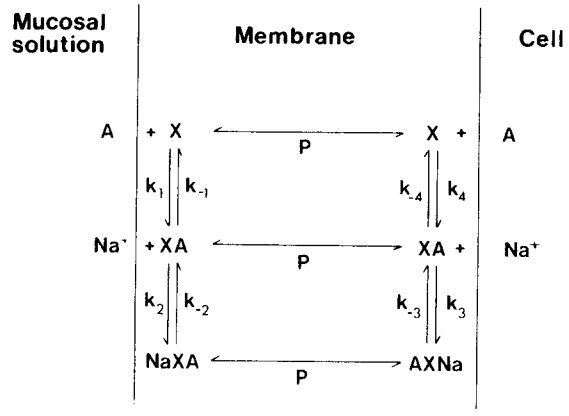

Fig. 10. Carrier model for alanine transport at the mucosal border of rabbit ileum.

$$J_A^e = \frac{C_t P[A]_c}{\frac{K_3 K_4}{[Na^+]_c + K_3} + [A]_c} \tag{A2}$$

where $K_3 = k_{-3}/k_3$ and $K_4 = k_{-4}/k_4$. The formula for the net alanine flux across the mucosal membrane in the steady state obtained by taking the difference between J_A^i and J_A^e is exactly the same as that derived by Curran et al. [7] for the net flux, i.e.

$$J_A = \frac{C_t P\{K_3K_4([Na^+]_m + K_2)[A]_m - K_1K_2([Na^+]_c + K_3)[A]_c\}}{K_1K_2K_3K_4 + K_3K_4([Na^+]_m + K_2)[A]_m + K_1K_2([Na^+]_c + K_3)[A]_c + ([Na^+]_m + K_2)([Na^+]_c + K_3)[A]_m[A]_c} \tag{A3}$$

There have been numerous pieces of evidence from direct influx measurements and some from direct efflux measurements for the validity of this carrier model. Since the extracellular Na^+ and alanine concentrations are well known, and the intracellular Na^+ and alanine concentrations have been estimated, we would like to examine how the net alanine flux across the mucosal membrane can be predicted by Eqn. A3. It is known that when $[A]_m = 5$ mM and $[Na^+]_m = 140$ mM, then $[A]_c$ is approximately 40 mM and $[Na^+]_c$ about 50 mM [1]. Substituting these values into the numerator of Eqn. (A3), surprisingly $J_A < 0$, if $K_1K_2 = K_3K_4$. The predicted result is contradictory to what actually occurs, i.e., $J_A > 0$. $J_A > 0$ can be predicted by Eqn. A3 only if (1) $K_1K_2 \neq K_3K_4$ or (2) $K_1K_2 = K_3K_4$ and the "effective" $[A]_c$ and/or $[Na^+]_c$ are less than those experimentally measured.

ACKNOWLEDGEMENTS

This work was supported by the United States-Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases (Grant AI-09277) and by United States Public Health Service Grants (AM-12028 and AM-17433) from the National Institute of Arthritis, Metabolism, and Digestive Diseases. Dr Danisi was supported by a grant from Fundação de Amparo a Pesquisa do Estado de São Paulo, Brazil (Processo Biologicas 72/574). We should like to thank Miss Winefred Happee for skilful secretarial aid.

REFERENCES

1 Schultz, S. G., Fuisz, R. E. and Curran, P. F. (1966) J. Gen. Physiol. 49, 849–866
2 Schultz, S. G. and Curran, P. F. (1970) Physiol. Rev. 50, 637–718
3 Chez, R. A., Palmer, R. R., Schultz, S. G. and Curran, P. F. (1967) J. Gen. Physiol. 50, 2357–2375
4 Schultz, S. G., Curran, P. F., Chez, R. A. and Fuisz, R. E. (1967) J. Gen. Physiol. 50, 1241–1260
5 Naftalin, R. J. and Curran, P. F. (1974) J. Membrane Biol. 16, 257–278
6 Goldner, A. M., Schultz, S. G. and Curran, P. F. (1969) J. Gen. Physiol. 53, 362–383
7 Curran, P. F., Schultz, S. G., Chez, R. A. and Fuisz, R. E. (1967) J. Gen. Physiol. 50, 1261–1286
8 Hajjar, J. J., Lamont, A. S. and Curran, P. F. (1970) J. Gen. Physiol. 55, 277–296
9 Hajjar, J. J., Khuri, R. N. and Curran, P. F. (1972) J. Gen. Physiol. 60, 720–734
10 Powell, C. W., Binder, H. J. and Curran, P. F. (1972) Am. J. Physiol. 223, 531–537
11 Schultz, S. G. and Zalusky, R. (1964) J. Gen. Physiol. 47, 567–584
12 Schultz, S. G., Yu-Tu, L. and Strecker, C. K. (1972) Biochim. Biophys. Acta 288, 367–379
13 Frizzell, R. A. and Schultz, S. G. (1972) J. Gen. Physiol. 59, 318–345
14 Peterson, S. C., Goldner, A. M. and Curran, P. F. (1970) Am. J. Physiol. 219, 1027–1032
15 Rinaldo, J. E., Jennings, B. L., Frizzell, R. A. and Schultz, S. G. (1975) Am. J. Physiol. 228, 854–860
16 Yardley, J. H., Bayless, T. M., Luebbers, E. H., Halsted, C. H. and Hendrix, T. R. (1972) Hopkins Med. J. 131, 1–10
17 Banwell, J. G. and Sherr, H. (1973) Gastroenterology 65, 467–497
18 Hendrix, T. R. and Bayless, T. M. (1970) Ann. Rev. Physiol. 32, 139–164
19 De Jonge, H. R. (1975) Biochim. Biophys. Acta 381, 128–143
20 Kinter, W. B. and Wilson, T. H. (1965) J. Cell. Biol. 25, 19–39
21 Peterson, J. W., LoSpalluto, J. J. and Finkelstein, R. A. (1972) J. Infect. Dis. 126, 617–628
22 Wilson, F. A. and Dietschy, J. M. (1974) Biochim. Biophys. Acta 363, 112–126
23 Luisier, A. -L. and Robinson, J. W. L. (1973) in: Comparative Physiology (Bolis, L., Schmidt-Nielsen, K. and Maddrell, S. H. P., eds.), pp. 465–475, North-Holland, Amsterdam
24 Colombini, M. and Johnstone, R. M. (1974) J. Membrane Biol. 18, 315–334
25 Charalampous, F. C. (1971) J. Biol. Chem. 246, 461–465
26 Pietrzyk, C. and Heinz, E. (1974) Biochim. Biophys. Acta 352, 397–411
27 Lee, C. O. and Armstrong, W. McD. (1972) Science 175, 1261–1264
28 Rose, R. C. and Schultz, S. G. (1971) J. Gen. Physiol. 57, 639–663
29 Naftalin, R. J. and Holman, G. D. (1974) Biochim. Biophys. Acta 373, 453–470
30 Bihler, I. and Cybulsky, R. (1973) Biochim. Biophys. Acta 298, 429–437
31 Murer, H., Hopfer, U., Kinne-Saffran, E. and Kinne, R. (1974) Biochim. Biophys. Acta 345, 170–179