Biochimica et Biophysica Acta, 367 (1974) 88–101

BBA 76734

THE QUESTION OF COUNTER-TRANSPORT IN THE INTESTINE

J. W. L. ROBINSON

Département de Chirurgie Expérimentale, Hôpital Cantonal Universitaire, Lausanne (Switzerland)

(Received April 11th, 1974)

SUMMARY

The mechanism of counter-transport in the small intestine was investigated and an explanation for the asymmetry of the phenomenon was sought. The experiments were performed with everted sacs of guinea-pig intestine, and the outward or inward fluxes of labelled amino acids or sugars across the brush border membrane were measured after a preliminary preloading of the tissue.

Efflux of preloaded amino acid can be stimulated by the presence of the same amino acid or homologue in the external medium. This increase is a saturable function of the external amino acid concentration and can also be observed in the absence of Na^+ in the external medium. Efflux of preloaded hexoses can also be increased by the presence of the same hexose or phloridzin in the external medium. However, the stimulation of sugar efflux is dependent on the presence of Na^+. These effects cannot be seen at 0 °C, and are independent of the volume of the external medium. They do not occur if the Na^+ gradient across the luminal membrane is destroyed by preloading the tissue with Na^+ as well as the non-electrolyte.

No stimulation of influx can be observed by preloading the tissue with unlabelled amino acid. Experiments attempting to increase influx by reversing the sodium gradient were not consistently successful.

These results are most compatible with a "competitive exchange diffusion" model, provided a considerable unstirred layer at the surface of the cell prevents thorough mixing with the external medium. The asymmetry of the phenomenon can be attributed to the different affinities of the substrate for its carrier at the two faces of the luminal membrane.

INTRODUCTION

Counter-transport can be defined as the acceleration of the movement of a substance across a membrane by the presence of the substance or its homologue on the opposite side of the membrane. In many cells, the influx of a substrate can be stimulated by preloading the tissue with the substrate or a homologue, just as its efflux can be increased by the presence of the substrate or its homologue in the incubation medium. However, in absorptive epithelia, the latter process has been observed on many occasions, but with one exception, attempts to demonstrate the former have

failed. This lack of symmetry has led to some confusion in the literature: Hajjar et al. [1], for instance, recently wrote of the rabbit ileum: "The increase in efflux shown (in the presence of external amino acid) could also be due to a "trans-concentration effect" mediated by the transport system. However, such an effect is unlikely in the present case because there are no trans-concentration effects on alanine influx; influx is not affected by alanine in the cell". Whereas nobody has ever demonstrated an increased influx across intact intestinal brush border by preloading with homologous substrates, there is ample evidence for stimulation of efflux in the presence of a homologue in the external medium apart from that of Hajjar et al. [1]. Although many experiments were performed with intestinal rings [2–4], and are therefore open to the possible criticism that movements could be occurring across membranes other than the brush border (though this is rather unlikely owing to diffusion barriers), a more rigorous demonstration of counter-transport in the outward direction across the brush border of rabbit ileum was presented by Alvarado et al. [5]. In these experiments, the inward flux of amino acid across the brush border of an everted sac of rabbit ileum was reversed on addition of a second amino acid to the incubation medium. It is worth recording that analogous observations have been made in the renal cortex slice: the efflux of *p*-aminohippuric acid across the basal membrane of the proximal cell can be stimulated by the presence of the substrate in the external medium, but uptake is not influenced by preloading the tissue with the substrate [6]. Similarly, the efflux of neutral amino acids across the luminal membrane of renal proximal cells may be increased by the presence of homologues in the external medium [7], but the influx of these compounds remains unchanged by preloading [8]. On the other hand, preloading this tissue with dibasic amino acids does lead to an acceleration of uptake (this is the exception mentioned above [8]). Interestingly, the same situation is found in brain slices where efflux can always be stimulated in the presence of an elicitor, but uptake can only be accelerated by preloading in the case of lysine [9].

Counter-transport is a term that has been used for two different processes at the cellular level, which have been designated by Stein [10], as "competitive exchange diffusion" and "accelerative exchange diffusion", respectively. The aims of the present study are to decide which model is more appropriate to explain the findings in the small intestine, and to provide a possible rationale for the asymmetry of the phenomenon.

THEORY

A model for reversible membrane transport is proposed in Fig. 1, in which both loaded and unloaded forms of the carrier are capable of crossing the membrane. In the condition depicted, there is an excess of labelled substrate (A*) within the cell, and an excess of unlabelled substrate (A) in the external medium. The models are required to explain the fact that the presence of unlabelled substrate at the external face of the membrane accelerates the flow of labelled substrate from the cell.

According to the "accelerative exchange diffusion model" (which will henceforth be designated as the "accelerative model"), the labelled substrate at the internal face combines with the carrier (C) to form a complex C–A* which crosses the membrane at a certain velocity. The complex dissociates at the external face, A* being liberated into the medium. If there is no other substrate in the external medium, then

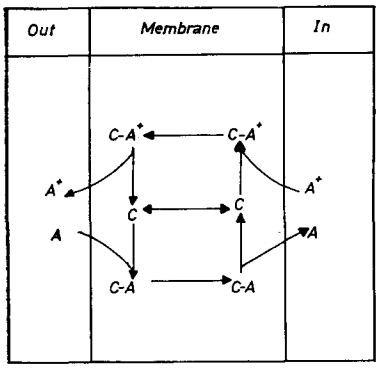

Fig. 1. General model for the stimulated efflux of a labelled amino acid (A*) in the presence of unlabelled amino acid (A) at the external face of the cell. C, carrier.

the carrier is bound to return unloaded. The essence of this model is that the rate of translocation of the unloaded carrier is slower than that of the loaded form. In this case, the presence of unlabelled substrate, A, in the medium enables the latter to return in the loaded form. Hence the cycle proceeds at a more rapid rate when a substrate is present at the trans face of the membrane. This model is evidently applicable to both influx and efflux.

According to the "competitive exchange diffusion model" (henceforth to be called the "competitive model"), the complex C–A* crosses the membrane and dissociates at its external face. There exists, however, a tendency for the liberated A* to recombine with the carrier and re-enter the cell. This tendency will be reduced in the presence of unlabelled substrate at the external face, due to competition for the binding site. This model may be applied both to influx and efflux, provided the affinity of the substrate for the carrier is the same at both faces of the membrane.

METHODS

The experiments were performed with everted sacs of guinea-pig intestine. Various criticisms have been levelled against this preparation in rats because of morphological deterioration [11, 12], but it is nevertheless uniquely suitable for the measurement of fluxes across the brush border [5], since the influence of other membranes can be unequivocally eliminated. The relative stability of the guinea-pig intestine during long incubations led to the choice of this species.

Everted ileal sacs, of wet weight 500–800 mg, were prepared and filled to slight distension with Krebs bicarbonate buffer. They were then subjected to two successive incubations in Krebs bicarbonate buffer with or without substrate or in corresponding buffers in which all Na^+ were replaced by equimolar quantities of K^+ or choline. When changes in the radioactivity of the medium were being monitored, the solution (generally 10 ml) was contained in a double-walled chamber which was thermostatically controlled and gassed (with O_2–CO_2 (95 : 5, by vol.)) from below, and open at the top so that 50-μl samples could be removed every 5 min.

The experimental design differed from one experiment to another. In most series, the sacs were preincubated for 30 min in the presence of the labelled substrate,

and then the release of the isotope into a medium was monitored during a second 25-min incubation. Alternatively, the tissue was preloaded with unlabelled substrate, and then incubated in radioactive solutions, the rate of loss of isotope from the medium being followed and equated with uptake. In yet another series, modelled on a previously published design [5, 13], the sacs were incubated in radioactive substrate, then, at a given moment, a second substrate was added, and the flux changes, as noted by alterations in the radioactivity of the medium, were recorded. In all cases, the radioactivity was determined in a liquid scintillation counter.

At the end of the final incubation, the sac was removed from the incubation flask, opened longitudinally, rinsed in cold buffer, carefully blotted on filter paper and weighed. Samples of the tissue (approx. 30 mg) were then cut out for analysis: they were weighed on a torsion balance, dissolved in 0.1 ml 30 % KOH and counted in a toluene–ethanol medium in a liquid-scintillation counter [13]. In addition, if the sacs had first been incubated in a radioactive solution, small samples of the tissue were excised at the end of this incubation after making a new ligature in the sac, and analysed in the same manner.

Auxiliary experiments were performed to study the inhibition of uptake by intestinal rings during short incubations. Tissues were processed in the same manner as the segments of the sacs. These methods have been described in detail elsewhere [13].

Expression of results

In each series of experiments, the rate of loss or gain of radioactivity by the tissue was recorded by plotting a time-course of the changes in activity of the incubation medium. In addition, the radioactivity in the tissue of the sac at the end of the experiment was also measured. In cases where the tissue at the end of the first incubation contained the isotope, the level at the end of the second incubation could be expressed either in absolute terms or with reference to the amount existing in the tissue when the incubation medium was changed. In every case, there was close agreement between the different methods of calculating the results, whether in terms of tissue levels or medium concentrations. In view of this identity, only one series of results concerning tissue levels will be presented in this article, all the rest being expressed solely by their time-course curves. Each experiment was repeated with six or eight different animals; in fact, a considerable variation between animals was encountered, as well as a fairly large variation between sacs. However, owing to the large number of replicates, there was never a significant difference between the tissues of different series of sacs at the end of the first incubation.

Statistical evaluation

Analysis of differences between curves was performed by a two-way analysis of variance with replicates [14] considering the times and the conditions as the two entries and the different animals as replicates. Analysis of differences between tissues was performed by a global two-way analysis of variance, in which the conditions and the animals were the two entries. In this case, a value of "*D*", the least significant difference between two means at a given significance level, was extracted [15].

Materials

L-[U-^{14}C]Phenylalanine was purchased from New England Nuclear Corp.,

β-methyl-D-[U-^{14}C]glucoside from Calbiochem, and L-[U-^{14}C]methionine from the Radiochemical Centre, Amersham. The corresponding unlabelled products came from Merck, Darmstadt, or (in the case β-methylglucoside) from Fluka, Buchs.

RESULTS AND DISCUSSION

The first experiment was performed using a model developed earlier [5, 13], in which the reversal of net flow of the substrate across the brush border membrane of the intestine is evoked by addition of a homologous elicitor (Fig. 2). When the final concentration of the elicitor was greater than that of the substrate, a reversal of the flux of the latter occurred, the labelled amino acid returning to the medium; the magnitude of this effect was dependent on the concentration of the elicitor. Apart from the evident counter-flow observed in the figure, analysis of the tissues disclosed a very large and significant difference in the amount of labelled methionine in the tissue under the different conditions. Analogous results were obtained using labelled β-methylglucoside as substrate, and adding different concentrations of the unlabelled substance as elicitor. This result is clearly explicable in terms of both the models presented in Theory.

The next experiment was performed using a new design. The sacs were preloaded with labelled phenylalanine, and then incubated in different solutions, namely Krebs bicarbonate buffer without any addition, the same buffer containing 1 mM unlabelled phenylalanine, the same containing 10 mM elicitor, a Na^+-free, choline-substituted

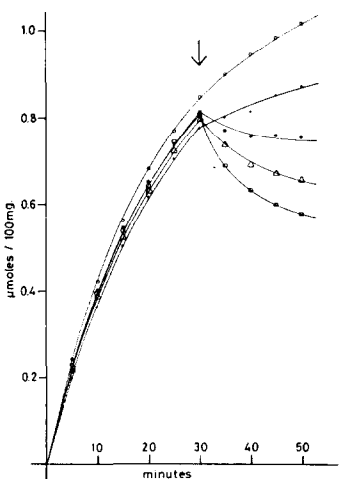

Fig. 2. Everted sacs of guinea-pig intestine were incubated in a solution of 1 mM L-methionine in Krebs bicarbonate buffer. The uptake of the substrate was monitored by taking 50-μl samples of the incubation medium every 5 min. At Minute 30, mixtures of mannitol (to maintain osmolarity) and unlabelled methionine were added to give the following final concentrations: (1) 20 mM methionine +10 mM mannitol (□–□); (2) 10 mM methionine+20 mM mannitol (△–△); (3) 4 mM methionine +26 mM mannitol (●–●); (4) 1 mM methionine +29 mM mannitol (+–+); and (5) 30 mM mannitol (○–○). Then sampling was continued until the 50th min. The tissue was analysed after the final sample, and the following values for the amino acid in the tissue were obtained (in μmoles/g fresh tissue): (1) 1.48; (2) 1.71; (3) 2.23; (4) 2.80; (5) 3.45 ($D_{0.05} = 0.27$; $D_{0.001} = 0.50$). The results are the means of six experiments.

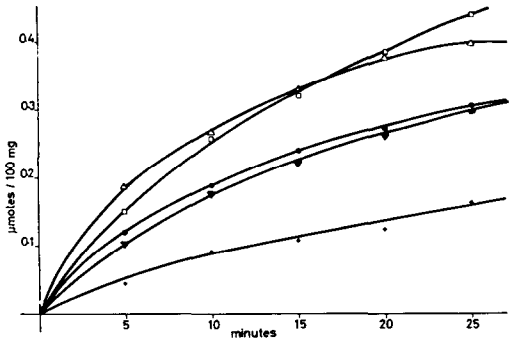

Fig. 3. Everted sacs of guinea-pig intestine were preloaded by incubating for 30 min in 1 mM [^{14}C] phenylalanine. The curves show the efflux of labelled phenylalanine into (1) Krebs bicarbonate buffer (+–+), (2) buffer + 1 mM unlabelled phenylalanine (▼–▼), (3) buffer + 10 mM unlabelled phenylalanine (□–□), (4) Na^+-free, choline-substituted buffer (●–●) or (5) choline buffer + 1 mM unlabelled phenylalanine (△–△). The following statistical differences between the curves were calculated: between 1 and 2: $F_{1,50} = 47.8$; between 2 and 3: $F_{1,50} = 19.8$; between 1 and 4: $F_{1,50} = 147.3$; between 4 and 5: $F_{1,50} = 24.3$; and between 2 and 5: $F_{1,50} = 22.4$. The results are the means of six guinea-pigs. The values pertaining to the tissue levels at the end of the second incubation are presented in Table I.

buffer, and finally the choline buffer containing 1 mM unlabelled phenylalanine. The efflux of labelled phenylalanine from the tissue into these different media was monitored, and the results are illustrated in Fig. 3. The tissues were analysed at the end of each incubation, and the findings are presented in Table I. It is clear that the efflux

TABLE I

TISSUE LEVELS OF PHENYLALANINE DURING THE EFFLUX EXPERIMENT ILLUSTRATED IN FIG. 3

The results are of the means of six experiments. The results in the first column indicate the levels of phenylalanine in the tissue of the sacs at the end of the first incubation (30 min); this series is homogenous ($F_{4,20} = 2.18$). The results in the second column demonstrate the level of the amino acid remaining in the tissue at the end of the second incubation (25 min). In the final column is shown the percentage loss of phenylalanine during the second incubation. This value is calculated for each individual sac in each experiment from the values before and after the second incubation. Statistical evaluation is performed by a two-way analysis of variance, from which values of "D", the least significant difference at various probability levels, can be extracted.

Medium of the second incubation	Tissue level of phenylalanine (μmoles/g fresh tissue)		% of tissue phenylalanine liberated
	Beginning	End	
Krebs buffer alone	3.89	3.22	20.2
Krebs buffer + 1 mM phenylalanine	4.21	2.70	34.6
Krebs buffer + 10 mM phenylalanine	4.89	2.07	57.5
Choline buffer alone	4.60	2.85	38.9
Choline buffer + 1 mM phenylalanine	4.27	2.47	42.5
$D_{0.05}$	0.76	0.52	10.2
$D_{0.01}$	1.04	0.70	13.9
$D_{0.001}$	1.41	0.95	18.8

of labelled substrate is stimulated substantially when unlabelled phenylalanine is added to the incubation medium. Furthermore, the acceleration is greater at higher elicitor concentrations. In addition, the efflux is more rapid into the Na^+-free medium than into the Na^+-containing solution both in the presence and absence of phenylalanine; and finally, the stimulation by external phenylalanine is observed both in the presence and absence of Na^+.

These results are readily explained in terms of the "competitive model" provided labelled and unlabelled phenylalanine can compete for transport in the virtual absence of Na^+, a fact that will be discussed below, and provided the reversal of the Na^+ gradient across the mucosal membrane can itself accelerate phenylalanine efflux; experimental evidence for this assumption is available both in the intestine [1] and in brain slices [16].

Interpretation of this experiment in terms of the "accelerative model" is possible, but is complicated by the fact that the scheme presented in Fig. 1 is rather oversimplified, since at the intestinal brush border, amino acid transfer occurs apparently in the form of a ternary complex between the carrier, the amino acid, and Na^+ [17]. Thus various forms of the complex can be envisaged, namely

Na^+–C–Φ; Na^+–C–; –C–Φ; and –C–

where C denotes the carrier, and Φ a phenylalanine molecule. In order to interpret the results in Fig. 3 in terms of the "accelerative model", it can easily be demonstrated that the following order of mobilities of the different forms of the carrier would be required:

$$-C-\Phi > Na^+-C-\Phi > -C- > Na^+-C-$$

Unfortunately one of the cornerstones of the widely accepted model for amino acid transport developed by Curran et al. [17] is precisely that the mobilities of the different forms of the carrier should be equal. Furthermore, it is rather unlikely that the mobility of –C–Φ is greater than that of the ternary complex, in view of the great reduction in entry of phenylalanine when Na^+ is removed from the bathing medium

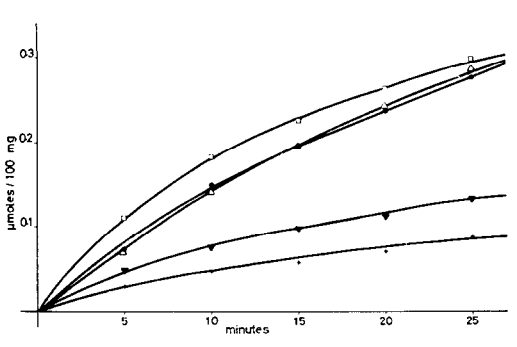

Fig. 4. Efflux of labelled β-methylglucoside (following preloading with 1 mM substrate) into (1) Krebs bicarbonate buffer (+–+), (2) buffer containing 1 mM unlabelled β-methylglucoside (▼–▼), (3) buffer containing 10 mM unlabelled β-methylglucoside (□–□), (4) Na^+-free, choline-substituted buffer (●–●) or (5) choline-buffer containing 1 mM unlabelled β-methylglucoside (△–△). The following differences are significant at $P < 0.01$: 1 and 2, 2 and 3, 1 and 4, and 2 and 5. The tissue analyses show the same tendencies.

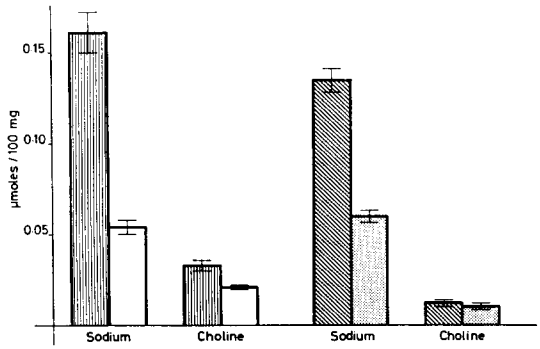

Fig. 5. Inhibition of initial velocity of uptake of sugars and amino acids in presence and absence of Na^+. Rings of guinea-pig intestine were incubated for 5 min in Krebs bicarbonate buffer or Na^+-free, choline-substituted buffer containing various substrates and mixtures: Vertical bars, 1 mM phenylalanine alone; open columns, 1 mM phenylalanine + 5 mM methionine; diagonally hatched columns, 1 mM β-methylglucoside; dotted columns, 1 mM β-methylglucoside + 5 mM glucose. The results are the means of six experiments and demonstrate that methionine significantly inhibits the initial rate of entry of phenylalanine in both sodium and choline buffers, whereas glucose inhibits the entry of β-methylglucoside only in Na^+-buffer.

(see Fig. 5 below). However, until these mobilities can be determined experimentally, the above discussion must remain within the realm of conjecture.

When this experiment was repeated using β-methylglucoside instead of phenylalanine, slightly different results were obtained (Fig. 4). In this case, qualitatively similar stimulations were observed in the Na^+-containing media, and the efflux was more rapid in the absence of Na^+ than in its presence, but there was no additional acceleration of efflux by external sugar in the choline medium. Analogous results were found when K^+-substituted buffers were used, confirming earlier observations of Alvarado [2]. In terms of the "accelerative model", this behaviour could be explained by suggesting that the ratios of the mobilities of the different forms of the sugar carrier are not identical to the corresponding ratios pertaining to the different forms of the amino acid carrier. On the other hand, this difference can be most easily explained in terms of the "competitive model", simply by implying that no competition between sugars occurs in the absence of Na^+. Such an explanation has in fact been suggested by earlier research [18, 19], and is confirmed by the results shown in Fig. 5. In this experiment, methionine is seen to inhibit the initial velocity of uptake of phenylalanine in both Na^+ and choline buffers, but glucose can only inhibit the initial velocity of uptake of β-methylglucoside in the Na^+-containing buffer. There is no interaction in choline buffer where all entry of sugars is assumed to occur by simple diffusion. Thus the results of the experiments in Figs 3 and 4 are more compatible with the "competitive model".

The classical method of distinguishing between the "accelerative model" and the "competitive model" is to change the volume of the external medium and investigate whether the stimulation of efflux is altered [10]. If the volume is increased, the labelled amino acid leaving the tissue will be subjected to greater dilution, and therefore competitive effects between the labelled and unlabelled amino acid will be modified. On the other hand, stimulation according to the "accelerative model"

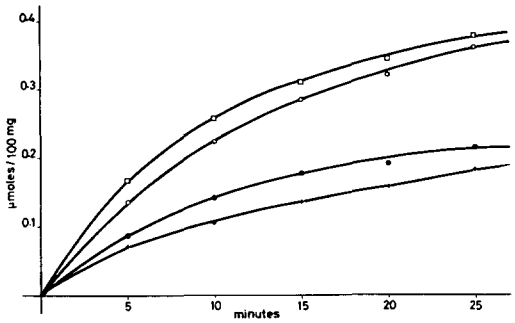

Fig. 6. Influence of the external volume on the acceleration of efflux. Efflux of labelled phenylalanine (following preloading with 1 mM substrate) into (1) Krebs bicarbonate buffer (+–+), (2) Krebs buffer+10 mM unlabelled phenylalanine (□–□), (3) Na^+-free, choline-substituted buffer (●–●), and (4) choline buffer+10 mM unlabelled phenylalanine (○–○). The differences between curves 1 and 2 and between 3 and 4 are significant at the 1 % level, and the difference between 1 and 3 at the 5 % level. The results are the means of six experiments. The external volume was 100 ml in this experiment, instead of the 10 ml used in the experiments illustrated in Figs 3 and 4.

should be unaffected by the volume of the external medium. The effect of employing a 10-fold greater external medium without changing the concentration of the elicitor is shown in Fig. 6. Comparing this figure quantitatively with Fig. 3, it can readily be seen that in the Krebs buffer, the influence of 10 mM phenylalanine is almost identical in the two experiments. Exactly analogous results were obtained in the case of β-methylglucoside. This result is more compatible with the "accelerative model", though it could be explained in terms of the "competitive model", provided a considerable unstirred layer at the surface of the sac is assumed to prevent adequate mixing of the labelled amino acid with the large external medium. The presence of notable unstirred layers in the intestine, due partly to the topography of the organ, has been evoked to explain various transport phenomena in this tissue [20, 21], and their importance should not be underestimated.

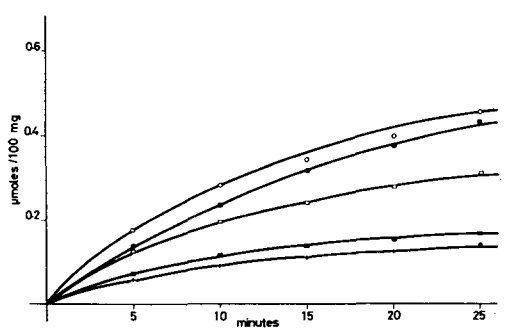

Fig. 7. Influence of the external phenylalanine concentration on the level of stimulation of the efflux from preloaded tissue. Tissues loaded with 1 mM labelled phenylalanine, then incubated in (1) Krebs bicarbonate buffer alone (+–+), (2) buffer+1 mM unlabelled phenylalanine (■–■), (3) buffer+4 mM unlabelled phenylalanine (□–□), (4) buffer+10 mM unlabelled phenylalanine (●–●) or (5) buffer+25 mM unlabelled phenylalanine (○–○). Results are means of seven experiments.

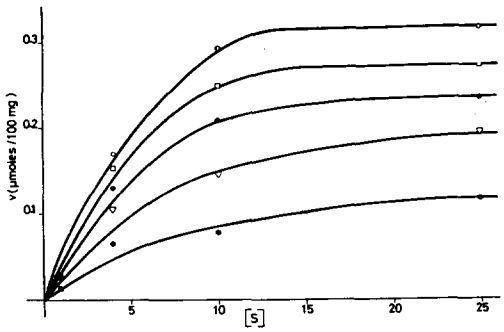

Fig. 8. Saturability of the stimulation of efflux of phenylalanine from preloaded sacs as a function of the concentration of phenylalanine added to the second incubation medium. Results taken from Fig. 7. The values represent the difference in efflux in the presence and absence of phenylalanine. Lowest curve: 5-min data; second curve: 10-min data, etc.

A kinetic study of the stimulation of efflux, using the same experimental design, has been undertaken, and the results are illustrated in Figs 7–9. The first figure shows that there is a gradual increase in the acceleration provoked on augmenting the concentration of the elicitor, and Fig. 8 (where the base-line efflux has been subtracted from each value) reveals that this is a saturable function of the external concentration. The curves in Fig. 8 can be transformed into straight lines by classical methods, as revealed in Fig. 9.

Mathematical analysis of this phenomenon according to the "accelerative model" is complex, in view of the various forms of the carrier mentioned above; the lack of knowledge of the mobilities of these different forms and of events at the internal face of the membrane renders such an analysis highly speculative. Analysis according to the "competitive model" is much simpler, and it can be shown that the straight lines obtained in Fig. 9 are compatible with this model.

According to the model, the stimulation produced (i.e. the difference in efflux in the presence and absence of external amino acid) is numerically equivalent to the inhibition of re-entry caused by the external amino acid (i.e. the difference in re-entry

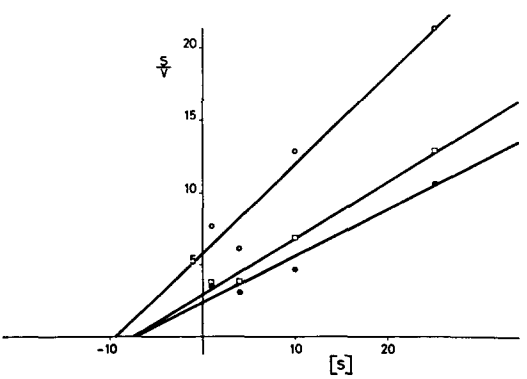

Fig. 9. Linearisation of the data in Fig. 8. ○–○, 5-min data; □–□, 10-min data; ●–●, 15-min data. Lines constructed by the method of least squares.

in the presence and absence of the external amino acid). We can therefore write two kinetic equations concerning the re-entry in the absence and presence of the inhibitor:

$$v = \frac{V}{1 + \frac{K_m}{[S]}} \quad \text{and} \quad v' = \frac{V}{1 + \frac{K_m}{[S]}\left(1 + \frac{[I]}{K_i}\right)}$$

These two equations can be combined to give the following form:

$$\frac{[I]}{v - v'} = [I] \cdot \left(\frac{1 + \frac{K_m}{[S]}}{V}\right) + \frac{[S] \cdot K_i \left(1 + \frac{K_m}{[S]}\right)^2}{V \cdot K_m}$$

This is clearly the well-known form of a linear transformation of the Michaelis equation of the type S/v against S. The concentration, [I], represents the concentration of the elicitor added, since it is supposed to be acting as an inhibitor of the re-entry, and the velocity, $(v - v')$, represents the difference in re-entry in the presence and absence of the elicitor, which, as has been pointed out, is numerically equivalent to the stimulation of efflux that has been measured. Thus the linearity revealed in Fig. 9 would be compatible with the "competitive model".

Counter-transport occurring in other systems according to the "accelerative model" generally takes place at 0 °C as well as at 37 °C [22]. This possibility was explored in the present system by preloading tissue sacs at 37 °C with labelled phenylalanine and then incubating them at 0 °C to study the efflux. Under these circumstances, the efflux was extremely slow, and was not affected significantly by the presence of the amino acid. This result would, of course, be expected in terms of the "competitive model", since neither transport nor competitive inhibition may be observed at 0 °C.

The most compelling evidence in favour of the "competitive model" is presented

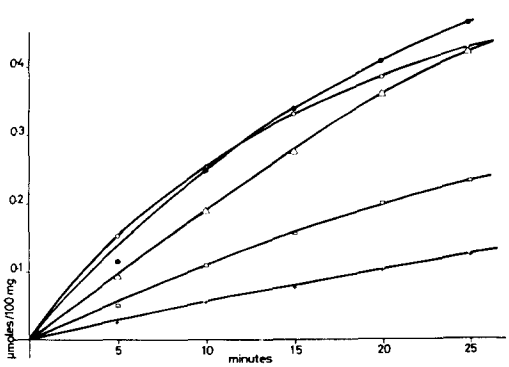

Fig. 10. Effect of phloridzin on efflux of β-methylglucoside from preloaded sacs. Tissues preloaded with 1 mM β-methylglucoside, then incubated in (1) Krebs bicarbonate buffer (+–+), (2) buffer + 1 mM phloridzin (□–□), (3) Na^+-free, choline-substituted buffer (●–●), (4) choline-buffer + 1 mM phloridzin (△–△), or (5) choline-buffer + 10 mM unlabelled β-methylglucoside (○–○). The results are the means of nine experiments. The difference between curves 1 and 2 is highly significant ($F_{1,80} = 47.4$), as is the difference between curves 1 and 3. There is no difference between the three curves in choline buffer ($F_{2,120} = 1.41$).

in Fig. 10, where it is seen that phloridzin is capable of stimulating β-methylglucoside efflux. Note that neither phloridzin nor unlabelled β-methylglucoside exerts any significant effect in choline buffer, in agreement with the findings in Fig. 4. The observation that phloridzin elicits β-methylglucoside counterflow is not original: it has been demonstrated in hamster intestinal rings by Alvarado [2], and in an elegant manner in toad intestine using electrophysiological techniques by Hoshi and Komatsu [23]. Phloridzin is a competitive inhibitor of sugar transport in the intestine, but it appears to be unable to enter the epithelial cells [24]. Nevertheless, recent work with isolated rat kidney tubules [25] has questioned the premise that phloridzin is unable to enter the proximal cells of the kidney, and by analogy casts doubt on the intestinal results cited. However, if we do assume that phloridzin is retained extracellularly, the finding that it elicits counter-transport is incompatible with the "accelerative model", since the elicitor must, according to that hypothesis, also enter the cell. It is, nevertheless, entirely feasible in terms of the "competitive model". Thus the majority of the evidence obtained indicates that the "competitive model" is the more likely explanation for the phenomenon in the intestine, as also suggested by Alvarado [26].

One last test was performed using this experimental design: the tissue was preloaded with both amino acid and Na^+ by means of a preliminary incubation in labelled phenylalanine plus 1 mM ouabain, and then incubated in the presence or absence of unlabelled phenylalanine. Under these conditions, external phenylalanine was unable to stimulate the amino acid efflux. This indicates that the Na^+ gradient must be maintained for the stimulation of efflux to be elicited, and suggests that the difference between internal and external Na^+ concentrations plays an important role in the counter-transport effect.

Finally, experiments were designed in an attempt to demonstrate acceleration

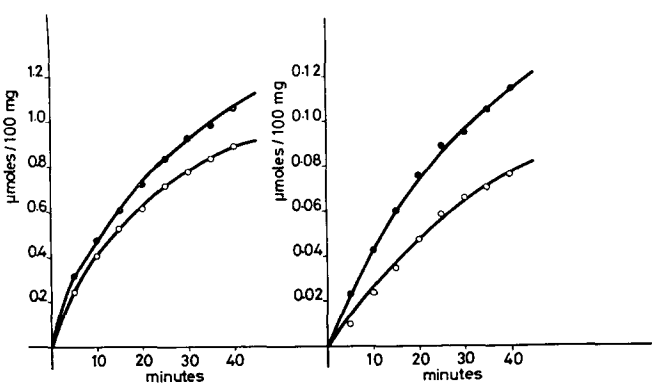

Fig. 11. Effect of preloading of guinea-pig sacs on influx of phenylalanine. Tissues were preincubated in unlabelled phenylalanine (○–○) or simply in Krebs bicarbonate buffer (●–●), then incubated a second time in [^{14}C]phenylalanine. The disappearance of the labelled substrate is illustrated by the curves. At left, the concentration of phenylalanine in both incubations was 1 mM, and at right, the concentration in the first incubation was 20 mM, and in the second 0.1 mM. The two curves are significantly different (at left $F_{1,80} = 18.7$ and at right $F_{1,80} = 139$). Analysis of the tissues at the end of the incubation revealed that preincubation did not significantly alter the level of labelled amino acid in the experiment shown at the left ($F_{1,5} = 0.18$), but had a very significant effect in the experiment at the right (uptakes of 0.374 and 0.457 µmole/g fresh tissue, respectively, $F_{1,5} = 17.3$). The results are the means of six experiments.

of uptake of amino acid following preloading of the tissue. Sacs were preloaded with unlabelled phenylalanine, and were then incubated in [^{14}C]phenylalanine, the rate of disappearance of the label from the incubation medium being monitored. Two series were carried out: in the first, both incubations were performed in 1 mM phenylalanine, and in a second series, the tissue was preincubated in 20 mM unlabelled phenylalanine, and then incubated in 0.1 mM labelled amino acid. The results are shown in Fig. 11. Instead of stimulating the influx, the preloading has diminished it, a larger effect being observed under the extreme conditions. This finding is presumably due to an unstirred-layer effect, the unlabelled amino acid being trapped in the vicinity of the carriers at the end of the first incubation, thus inhibiting the uptake during the second incubation. All attempts to prevent this phenomenon by vigorous washing between the two incubations (even to the extent of performing an intervening incubation in ice-cold mannitol for 15 min) were unsuccessful.

Whether this interpretation for the inhibition is correct or not, an explanation must be sought for the lack of acceleration, which would be expected in terms of a symmetrical carrier model (Fig. 1). The answer must surely be found in the different rates of efflux and influx. A direct comparison of the curves in Figs 3 and 11 in the absence of any trans-concentration effect reveals that about seven times as much phenylalanine has been taken up by the mucosa than has been liberated into the medium after 25 min, though the internal concentration of phenylalanine in Fig. 3 was at least 5 times as large as the external concentration in Fig. 11. Thus efflux is a relatively sluggish process, a fact that is presumably caused by the differences in affinity of the carrier for its substrate on the two sides of the membrane due to the different ionic content of its surroundings [2, 26]. If the K_m for transport varies in accordance with the local ionic concentrations, then the K_i for interactions between two homologues will be similarly dependent. Hence, according to the "competitive model", inhibition of re-exit will not occur, because of the virtual absence of interaction between the two homologues at the internal face of the membrane. On the other hand, it should theoretically be possible to induce interactions at the internal face of the membrane by changing the local Na^+ concentration. Furthermore, it has been shown above that counter-transport can only occur in the presence of an intact Na^+ gradient. Thus in theory, one should be able to provoke an acceleration of influx by reversing the Na^+ gradient. Accordingly, sacs were loaded with unlabelled phenylalanine and Na^+ by means of an incubation in the presence of ouabain, and they were then incubated in labelled phenylalanine in a low-Na^+ medium. Unfortunately all attempts at consistently demonstrating a stimulation of influx by these means were unsuccessful, possibly since inhibition by unlabelled amino acid trapped in the unstirred layer (as shown in Fig. 11) masks any small effects that occur. Various Na^+ concentrations in the second incubation medium and various phenylalanine levels were tried, but although occasionally, an acceleration was observed, it was never reproducible; the experiment was therefore abandoned.

ACKNOWLEDGEMENTS

I am grateful to Zyma S. A. for financial support for this project, to Mlles H. Asper, C. Brandt, D. Mettraux and U. Pässler for skilful technical assistance, and to Mlle J. Taylor-Medhurst for secretarial aid and for drawing the graphs.

REFERENCES

1 Hajjar, J. J., Lamont, A. S. and Curran, P. F. (1970) J. Gen. Physiol. 55, 277–296
2 Alvarado, F. (1965) Biochim. Biophys. Acta 109, 478–494
3 Jéquier, J.-Cl., Robinson, J. W. L. and Felber, J.-P. (1965) Biochem. Biophys. Res. Commun. 18, 507–511
4 Robinson, J. W. L. (1966) Biochim. Biophys. Acta 126, 61–72
5 Alvarado, F., Torres-Pinedo, R., Mateu, L. and Robinson, J. W. L. (1970) FEBS Lett. 8, 153–156
6 Ross, C. R. and Farah, A. (1966) J. Pharmacol. Exp. Ther. 151, 159–167
7 Williams, W. M. and Huang, K. C. (1970) Am. J. Physiol. 219, 1468–1474
8 Schwartzman, L., Blair, A. and Segal, S. (1967) Biochim. Biophys. Acta 135, 120–126
9 Blasberg, R., Levi, G. and Lajtha, A. (1970) Biochim. Biophys. Acta 203, 464–483
10 Stein, W. D. (1967) The Movement of Molecules across Cell Membranes, pp. 129–157, Academic Press, New York
11 Levine, R. R., McNary, W. F., Kornguth, P. J. and LeBlanc, R. (1970) Eur. J. Pharm. 9, 211–219
12 Plattner, H., Klima, J., Mehnert, A. and Berger, H. (1970) Virchows Arch. Zellpathol. 6, 337–349
13 Robinson, J. W. L. and Alvarado, F. (1971) Pflügers Arch. Eur. J. Physiol. 326, 48–75
14 Lison, L. (1968) Statistique Appliquée à la Biologie Expérimentale, pp. 171–176, Gauthier-Villars, Paris
15 Pearce, S. C. (1965) Biological Statistics: An Introduction, pp. 34–39, McGraw-Hill, New York
16 Joanny, P., Barbosa, E., Hillman, H. and Corriol, J. (1971) Biochem. J. 125, 255–260
17 Curran, P. F., Schultz, S. G., Chez, R. A. and Fuisz, R. E. (1967) J. Gen. Physiol. 50, 1261–1286
18 Goldner, A. M., Schultz, S. G. and Curran, P. F. (1969) J. Gen. Physiol. 53, 362–383
19 Robinson, J. W. L. (1970) Comp. Biochem. Physiol. 34, 641–655
20 Saltzman, D. A., Rector, F. C. and Fordtran, J. S. (1972) J. Clin. Invest. 51, 876–885
21 Winne, D. (1973) Biochim. Biophys. Acta 298, 27–31
22 Lacko, L., Wittke, B. and Kromphardt, H. (1972) Eur. J. Biochem. 25, 447–454
23 Hoshi, T. and Komatsu, Y. (1970) Tohoku J. Exp. Med. 100, 47–59
24 Stirling, C. E. (1967) J. Cell Biol. 35, 605–618
25 Heath, D. A. and Aurbach, G. D. (1973) J. Biol. Chem. 248, 1577–1581
26 Alvarado, F. (1970) Bol. Soc. Esp. Hist. Nat. Biol. 68, 33–68
27 Crane, R. K., Forstner, G. and Eichholz, A. (1965) Biochim. Biophys. Acta 109, 467–477