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THE UPTAKE OF AMINO ACIDS BY MOUSE PANCREAS *IN VITRO*

IV. THE ROLE OF EXCHANGE DIFFUSION*

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SUMMARY

1. The phenomenon of exchange diffusion occurs in mouse pancreas and may lead to a threefold increment in the flux of certain amino acids.

2. The ability of various amino acids to exchange is similar to that observed in other tissues and appears to be independent of whether the amino acid is intra- or extracellular.

3. The exchange diffusion process is independent of the presence of cations, is characterized by a molar influx to efflux ratio which approaches 1:1 and has a half maximum value at a concentration which is equal to the K_m value for transport.

4. Interaction between amino acids can occur during exchange diffusion in mouse pancreas.

INTRODUCTION

The term exchange diffusion was originally introduced to designate certain types of ion movement across the membrane barrier[1,2] and it is now established that similar phenomena occur during entry of amino acids into various tissues[3,4]. Exchange diffusion of amino acids is independent of the presence of external ions[5] and is believed to utilize the same carrier system(s) as the active transport process[6,7]. OXENDER AND CHRISTENSEN[8,9] have concluded that, in the Ehrlich cell, neutral amino acids may be divided into at least two separate groups on the basis of their modes of entry into the cell, one criterion being the ability of the amino acid to undergo exchange diffusion. For example, valine, ACPC and methionine are excellent exchangers, while α-aminoisobutyric acid, glycine and alanine are very poor at exchanging. Exchange diffusion has also been studied in a number of other systems. Experiments *in vivo* with brain[10] showed that [^{14}C]lysine administered intravenously or intraperitoneally entered the circulating blood and exchanged rapidly with the free lysine of the brain. Exchange diffusion has been demonstrated *in vitro* in rat brain slices (between histidine and methionine) by NAKAMURA[11] and the factors controlling exchange diffusion in the intestine have been investigated by MOCHIDA, SAKURAI AND SUDA[12] and MUNCK[13,14].

Abbreviation: ACPC, 1-aminocyclopentane carboxylic acid.

* A preliminary communication of some of the present data was made at a symposium and the proceedings are contained in *Brain Barrier Systems*[18].

The general characteristics of the active transport of amino acids into mouse pancreas have been reported previously[15–17] and the purpose of the present communication is (a) to provide evidence for the occurrence of exchange diffusion in the pancreas, (b) to examine the factors affecting and controlling exchange diffusion, and (c) to indicate the possible relation of this process to carrier-mediated active transport. A preliminary account of some of these studies has already appeared[18].

MATERIALS AND METHODS

Amino acids

[1-^{14}C]Glycine, uniformly ^{14}C-labelled L-proline, uniformly ^{14}C-labelled L-lysine and L-[*Me*-^{14}C]methionine were purchased from the Radiochemical Centre, Amersham (England). L-[3-^{14}C]Tryptophan, α-[1-^{14}C]aminoisobutyric acid and 1-aminocyclopentane [*carboxy*-1-^{14}C]carboxylic acid were obtained from the New England Nuclear Corporation, Boston, Mass. (U.S.A.). The unlabelled amino acids were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio (U.S.A.) and were used without further purification.

Animals

Male Swiss white mice weighing 20–25 g were used throughout.

Preparation of the tissue

The mice were killed by cervical dislocation and an incision was made to the left of the mid-line on the ventral side. The pancreas and spleen were removed as a single unit, the spleen discarded and the pancreas placed on a Petrie dish filled with crushed ice in order to keep the tissue at a temperature of 0–4° prior to incubation. The pancreas was spread out and cut into several thin strips by means of small scissors as previously described[15–17]. The amount of tissue used in each vessel consisted of several strips having a total weight of 50–70 mg.

Incubation conditions for transport

The strips of pancreas were incubated in a calcium-free Krebs–Ringer solution containing 145 mM NaCl, 5.8 mM KCl, 1.4 mM KH_2PO_4 and 1.4 mM $MgSO_4$ buffered with 10 mM sodium phosphate to a final pH of 7.4. The final volume in all cases was 3 ml. The vessels were gassed with oxygen for 3 min and the incubation was carried out at 37° in stoppered 25-ml erlenmeyer flasks in a water-bath shaker.

Determination of amino acid uptake

At the end of the incubation period, the flasks were removed from the water-bath shaker and placed in a tray containing crushed ice. The tissue was carefully removed, washed with ice-cold saline and the free amino acids extracted with 3 ml 5 % trichloroacetic acid as previously described[15]. 0.2-ml aliquots of the trichloroacetic acid extract were counted in 10 ml scintillant using a Series 3000 Packard Liquid Scintillation Spectrometer.

The counts/min thus obtained were corrected for background, multiplied by 15 (0.2-ml sample taken from 3 ml), multiplied by 1000/the weight of the tissue in mg (to express the results per g wet weight), divided by 0.75 (to correct for the

amount of water in the tissue) and finally divided by the specific activity of the amino acid (counts/min per μmole) to obtain μmoles amino acid taken up per g tissue water.

Measurement of influx by exchange diffusion

The strips of pancreas were incubated as described above in the absence of amino acid and in the presence of sufficient non-radioactive compound to obtain the desired level of amino acid in the tissue. At the end of the incubation period, the pancreatic tissue was removed with forceps, washed in ice-cold saline and freed from excess moisture by passing the strips along "Parafilm". The tissue was transferred to 3 ml fresh medium containing radioactive amino acid and the second incubation was then carried out at 15°. The uptake of radioactive amino acid by the "loaded" and "non-loaded" tissues was determined as previously described.

Measurement of efflux by exchange diffusion

A preincubation was carried out in which all flasks contained labelled amino acid. The tissue was subsequently removed, washed, freed from excess moisture as above and transferred to new oxygenated media with and without unlabelled amino acids being present. This second incubation was carried out at 15° using a large volume of external medium (9 ml) in order to minimize the possibility of reconcentration. In experiments where the initial concentration of radioactive amino acid in the tissue was greater than 10 mM, the volume of the external medium was increased accordingly in order to maintain the same dilution factor for the exiting amino acid. The efflux of the labelled amino acid was followed by taking aliquots of the external medium at definite time intervals and counting as previously described.

The amount of amino acid exiting from the pancreas was calculated as follows: the radioactivity of the first 0.2-ml aliquot was multiplied by 45 to obtain the total counts/min in the 9 ml of external medium. Subtraction of the amount of radioactivity in this aliquot from the total efflux gave the radioactivity (counts/min) remaining in the incubation medium. Subsequently, another 0.2-ml sample of the medium was removed and the radioactivity determined. The total radioactivity was then calculated by multiplying the counts/min of the aliquot by 44 (since now the volume of external medium was 8.8 ml). The radioactivity which had exited in the second interval of time was obtained by subtraction of the counts/min of the medium left after the first aliquot from the total medium counts/min as determined by the second aliquot. Similarly, the remaining medium radioactivity was again obtained by subtracting the counts/min removed in the second aliquot from the calculated total counts/min of the medium. Then a third aliquot was removed at a definite time to yield a volume of external medium of 8.6 ml. In this way it was possible to calculate the total counts per min leaving the tissue at specific time intervals. This figure was then multiplied by 1000/weight of the tissue in mg, and divided by 0.75 (to correct for the amount of water in the tissue) and by the specific activity of the amino acid in order to determine the decrease in the concentration of this substrate in the tissue, the amount being expressed as μmoles amino acid per g tissue water.

After the last aliquot had been taken, the tissue was removed, washed, homogenized in 3 ml 5% trichloroacetic acid, and the concentration of amino acid in the trichloroacetic acid soluble portion determined. This value was then added to the

calculated decrease in amino acid content of the tissue in order to determine the concentration of amino acid present prior to the start of the second incubation. Only when these totals were within 5 % of each other for the individual flasks was it felt that a valid comparison could be made between the effects of the presence of different external amino acids. This led to rejection of about one flask in 15, estimated from more than 200 determinations.

RESULTS

Evidence for exchange diffusion

In previous studies[16,17] kinetic aspects of interactions between amino acids during transport into preparations *in vitro* of mouse pancreas have been investigated to obtain information relevant to the specificity of the carrier system(s) which are thought to be involved. Results obtained with tryptophan as substrate, particularly when methionine was used as inhibitor, did not fit into the linear forms predicted by the application of the LINEWEAVER AND BURK[19] relationship to transport problems[20]. It was felt that the disparity might be a consequence of the involvement of exchange diffusion phenomena. In preliminary experiments designed to demonstrate the operation of exchange diffusion, methionine and tryptophan were therefore chosen as the amino acid pair. The tissue was preincubated alone or with sufficient methionine (0.7 mM) to achieve a tissue content of approx. 5 μmoles per g water (actually 5.4). Tryptophan, when added to the second incubation medium, was present at a concentration of 1 mM. The results obtained are presented in Table I.

The top half of the table shows the movement of radioactive tryptophan into methionine "loaded" and "non-loaded" cells and the bottom portion shows methionine exit in the presence and absence of external tryptophan. The presence of methionine inside the tissue led to an increased tryptophan influx (in this case amounting to more than 30 % after 15 min), and the presence of tryptophan in the medium led to a

TABLE I

THE EXCHANGE DIFFUSION REACTION BETWEEN L-TRYPTOPHAN AND L-METHIONINE IN MOUSE PANCREAS AT 15°

The concentration of L-methionine in the tissue was 5.4 μmoles per g tissue water and the concentration of L-tryptophan in the medium was 1.0 mM. All values cited were the means of duplicates from a typical experiment. Movement of radioactivity: (a) Inward. The uptake of 1 mM radioactive L-tryptophan at 15° into L-methionine "loaded" and "non-loaded" tissue was followed as described in MATERIALS AND METHODS. (b) Outward. Pancreas was preincubated in the presence of 0.7 mM radioactive L-methionine for 60 min at 37° and efflux at 15° into new media was followed as described in MATERIALS AND METHODS.

Amino acid prepacked	*Amino acid in medium*	*Direction of flow of radioactivity*	*Flux (μmoles per g tissue water) at*		
			5 min	*10 min*	*15 min*
Nil	L-Tryptophan*	In	1.45	1.95	2.40
L-Methionine	L-Tryptophan*	In	1.85	2.60	3.27
L-Methionine*	Nil	Out	0.82	1.13	1.36
L-Methionine*	L-Tryptophan	Out	1.57	2.30	2.80

* Indicates radioactive amino acid.

marked increase in the efflux of prepacked methionine from the pancreas (approximately double after 15 min). The increased fluxes which might be attributable to exchange diffusion are of the same order of magnitude (*i.e.* the ratio is between 1 and 2) and the total fluxes in and out are approximately equal.

Apparent stimulation of efflux could also arise from inhibition of reconcentration of the amino acid lost from the cell. This possibility seems to be disproved by the demonstration that on incubation of pancreas, prepacked with methionine, the efflux is stimulated when tryptophan is present in the incubation medium but the presence of intracellular methionine increases the flux of tryptophan into the tissue *i.e.* that the increased flux can occur in both directions. More conclusive proof of exchange diffusion is apparent from the results presented in Table II in which the effects of 1 mM methionine and 5 mM α-aminoisobutyric acid on the transport and exchange diffusion of ACPC are compared. The concentrations of α-aminoisobutyric acid and methionine employed were chosen so that the two amino acids inhibited the uptake of ACPC by transport to about the same extent. If the effects indicated in Table I were obtained through inhibition of reconcentration, then these two amino acids at these concentrations should have exactly the same effect on the loss of ACPC from prepacked tissue. However, 5 mM α-aminoisobutyric acid had almost no effect on the efflux of prepacked ACPC, but 1 mM methionine caused a 75 % increase in efflux. The increased flux of amino acid must therefore be due to an exchange diffusion process which is called into play by ACPC but not by α-aminoisobutyric acid.

TABLE II

THE EFFECTS OF 5 mM α-AMINOISOBUTYRIC ACID AND 1 mM L-METHIONINE ON TRANSPORT AND EXCHANGE DIFFUSION OF ACPC

Uptake: The uptake of 1 mM radioactive ACPC in the presence and absence of 5 mM α-aminoisobutyric acid and 1 mM L-methionine was measured at 37° for 60 min as described in MATERIALS AND METHODS. Exchange: The pancreas was preincubated in the presence of 0.5 mM radioactive ACPC at 37° for 60 min to obtain a tissue concentration of 5.4 μmoles per g tissue water. Efflux of ACPC at 15° into the various media was followed as described in MATERIALS AND METHODS.

Movement of ACPC	*Addition to external medium*		
	Nil	*5 mM α-amino-isobutyric acid*	*1 mM* L-*methionine*
Uptake	9.35*	6.35	6.02
Exchange			
5 min at 15°	0.73	0.73	1.28
15 min at 15°	1.35	1.51	2.41

* Cellular content of amino acid (μmoles per g tissue water). All values cited are the means from four determinations. Exchange values refer to decrease in content.

Effects of external Na^+

It was shown by JOHNSTONE AND SCHOLEFIELD[5] that the homo-exchange of methionine in Ehrlich ascites cells is independent of the presence of either Na^+ or K^+. In contrast, WHEELER AND CHRISTENSEN[21] have reported that the counter transport process for L-alanine in rabbit red cells is dependent upon the presence of Na^+ in the external medium. The results of a similar study undertaken with mouse pancreas strips are shown in Table III. The tissue was preloaded with radioactive ACPC in all

cases and efflux followed into normal media in the presence and absence of external methionine (first portion of the table) and into low Na^+ media in the presence and absence of external L-methionine (last two columns of the table). At each of the time intervals indicated, reduction in the external Na^+ content had no significant effect on efflux of ACPC, either in the presence or absence of external amino acid. Similar experiments (not quoted) involving the addition of 1 mM ouabain to the external medium of the exchange system produced no significant changes. These results indicate that interference with the Na^+–K^+ pump has no effect on the exchange diffusion process in mouse pancreas.

TABLE III

THE EFFECT OF A DECREASED EXTERNAL Na^+ CONCENTRATION ON THE EXCHANGE DIFFUSION PROCESS IN MOUSE PANCREAS

Pancreas was preincubated with 0.2 mM radioactive ACPC for 60 min at 37° in order to obtain a tissue concentration of 2.3 μmoles of ACPC per g tissue water. The efflux of ACPC at 15° into the various media was followed as described in MATERIALS AND METHODS. The low Na^+ medium contained 17 mM Na^+ and the decreased NaCl content was replaced by an equimolar concentration of choline chloride.

Time (min)	*ACPC efflux into a normal Na^+ medium containing*		*ACPC efflux into low Na^+ (17 mM) medium containing*	
	nil	*1 mM L-methionine*	*nil*	*1 mM L-methionine*
2	0.19*	0.30	0.20	0.27
8	0.43	0.83	0.41	0.79
15	0.64	1.24	0.63	1.21

* Efflux is expressed as μmoles of ACPC per g tissue water. All values cited are the means of duplicates from a typical experiment.

Specificity of the exchange diffusion process

The results presented in Table II suggested that the exchange process had its own specificity. This apparent specificity is further exemplified by the results presented in Table IV which concern the ability of various extracellular amino acids to stimulate the efflux of intracellular amino acids from mouse pancreas. The effect of the extracellular compound is expressed as percent in excess of the control efflux.

(*a*) *Efflux of intracellular glycine.* Taurine is a poor inhibitor of glycine transport and caused little change in the efflux of intracellular glycine but α-aminoisobutyric acid and L-proline which are excellent inhibitors of glycine transport, also had little effect on the efflux. γ-Aminobutyric acid which is the physiological isomer for α-aminoisobutyric acid is poorly concentrated by the pancreas, but appeared to have a significant affinity for the system involved in the exchange process since it led to a stimulated efflux of glycine. Homo-exchange was quite significant but L-tryptophan and L-valine caused the greatest efflux, the increase amounting to 74% over the control.

(*b*) *Efflux of intracellular α-aminoisobutyric acid.* At the concentrations examined homo-exchange of α-aminoisobutyric acid was significant (efflux was 21% more than in the control) and several other amino acids caused slightly greater effluxes. Amino acids such as glycine and L-ethionine, which were excellent inhibitors of α-amino-

TABLE IV

THE EFFECT OF SEVERAL AMINO ACIDS ON THE EFFLUX OF RADIOACTIVE AMINO ACIDS FROM MOUSE PANCREAS

Efflux (μmoles amino acid per g tissue water) measured at the end of 15 min incubation. Numbers in parentheses show the efflux as a percent in excess of the control efflux in the absence of external amino acid. All values cited are the means of 3–5 determinations and in no case did an individual value differ from the mean by more than 10%. No significance has been attached to mean stimulations amounting to less than 20%.

Pancreas was preincubated with:

(1) 1.7 mM uniformly ^{14}C-labelled L-lysine for 60 min, resulting in an initial tissue concentration of 6.4 μmoles per g tissue water.
(2) 0.6 mM uniformly ^{14}C-labelled L-proline at 37° for 90 min, resulting in an initial tissue concentration of 5.8 μmoles per g tissue water.
(3) 0.5 mM α-amino[1-^{14}C]isobutyric acid for 90 min, resulting in an initial tissue concentration of 5.4 μmoles per g tissue water.
(4) 0.5 mM [1-^{14}C]glycine at 37° for 60 min resulting in an initial tissue concentration of 6.6 μmoles per g tissue water.
(5) 0.8 mM L-[3-^{14}C]tryptophan for 90 min, resulting in an initial tissue concentration of 6.5 μmoles per g tissue water.
(6) 0.5 mM [^{14}C]ACPC for 60 min, resulting in an initial tissue concentration of 5.4 μmoles per g tissue water.
(7) 0.7 mM L-[*Me*-^{14}C]methionine for 60 min, resulting in an initial tissue concentration of 5.4 μmoles per g tissue water.

In all cases efflux into media containing various amino acids was then followed as described in MATERIALS AND METHODS.

Amino acid added (5 mM)	*Intracellular amino acid*						
	Lysine	*Proline*	*α-Amino-isobutyric acid*	*Glycine*	*Tryptophan*	*ACPC*	*Methionine*
Nil	1.73 (100)	1.63 (100)	1.21 (100)	1.26 (100)	1.95 (100)	1.35 (100)	1.41 (100)
Taurine	2.00 (114)	—	1.50 (124)	1.25 (100)	1.99 (102)	1.35 (100)	1.41 (100)
α-Aminoisobutyric acid	—	1.92 (118)	1.47 (121)	1.34 (106	—	1.51 (112)	1.57 (111)
L-Proline	2.06 (120)	1.95 (120)	1.49 (123)	1.72 (136)	1.97 (100)	1.81 (134)	1.62 (115)
Glycine	2.03 (117)	1.81 (111)	1.29 (106)	2.01 (160)	—	1.88 (139)	1.82 (129)
γ-Aminobutyric acid	—	—	—	1.89 (150)	2.88 (148)	1.92 (142)	2.34 (166)
L-Threonine	—	—	1.53 (126)	1.92 (152)	4.02 (206)	2.50 (185)	3.08 (219)
L-Tryptophan	2.07 (120)	1.84 (113)	1.62 (134)	2.20 (174)	3.75 (192)	2.43 (180)	3.56 (252)
L-Leucine	2.00 (114)	2.13 (114)	1.68 (139)	2.08 (165)	4.43 (227)	2.45 (182)	3.81 (270)
L-Cysteine	—	—	—	—	—	3.31 (245)	3.77 (267)
ACPC	1.93 (111)	1.84 (113)	1.41 (116)	2.04 (162)	3.53 (181)	3.51 (260)	3.53 (251)
L-Valine	2.07 (120)	—	1.57 (130)	2.19 (174)	4.03 (206)	3.48 (258)	3.91 (277)
L-Methionine	2.00 (114)	—	—	2.04 (162)	4.26 (218)	3.69 (274)	4.19 (297)

isobutyric acid transport, were quite poor as exchangers in this system. Although L-proline was a much more effective inhibitor of α-aminoisobutyric acid transport than taurine, these two amino acids stimulated α-aminoisobutyric acid efflux to approximately the same extent. The maximum stimulations of efflux were observed with L-tryptophan and L-leucine in the external medium and were only 34 and 39% respectively, above the control level.

(*c*) *Efflux of* L-*proline.* BÉGIN AND SCHOLEFIELD[17] have suggested that proline is transported into mouse pancreas only after two molecules of the amino acid have combined with similar sites on the surface of the carrier and in this respect L-proline differs from α-aminoisobutyric acid and glycine. Although each of the amino acids examined appeared to have some affinity for the system involved in the efflux of L-proline, their effects were quite small (maximum stimulation of efflux occurred with L-leucine and was only 24% above the control). The results clearly indicate that intracellular L-proline is a very poor exchanger.

(*d*) *Efflux of intracellular* L-*methionine.* Amino acids such as α-aminoisobutyric acid, L-proline and glycine, although good inhibitors of L-methionine uptake, were extremely poor participants in the exchange reaction. γ-Aminobutyric acid, though poorly concentrated itself, caused a significant stimulation of efflux. The amino acids ACPC and L-valine, previously shown to migrate by a system distinct from that of L-methionine, had an affinity for the system involved in the efflux of methionine, as indicated by stimulated effluxes of 151 and 177%, respectively, above the control. In addition, the homo-exchange situation showed a greater efflux than any of the twelve hetero-exchange possibilities. The maximum stimulation of efflux amounted to 197% over the control, and was greater than any of the homo- or hetero-exchange effects of amino acids discussed in the preceding sections.

(*e*) *Efflux of intracellular ACPC.* Just as in the case of L-methionine, the amino acids α-aminoisobutyric acid, L-proline and glycine had the least ability to exchange with intracellular ACPC under the present experimental conditions. L-Threonine and L-leucine, good inhibitors of ACPC transport, gave rise to marked increases in efflux. Here too γ-aminobutyric acid exhibited an affinity for the exchange system, although the value of 42% in excess of the control was slightly less than that seen with L-methionine. Methionine, which apparently enters by a transport system distinct from that serving for ACPC, caused an increase of 174% over the control. Homo-exchange in this situation was also quite large and the extent compared favourably with that obtained with L-methionine.

(*f*) *Efflux of intracellular* L-*tryptophan.* As seen with other intracellular amino acids, L-proline and taurine were extremely poor choices for exchange, while γ-aminobutyric acid appeared to have a small but significant affinity for the exchange system. Homo-exchange was quite good but several other amino acids were most effective in increasing the efflux. When present intracellularly, tryptophan had a much greater capacity for exchange than glycine, α-aminoisobutyric acid or L-proline and had less capacity than L-methionine or ACPC.

(*g*) *Efflux of intracellular lysine.* Although all amino acids examined produced some stimulation of efflux, the maximum effect was only 20% in excess of the control and it is concluded that L-lysine, when present intracellularly, behaves similarly to α-aminoisobutyric acid and L-proline.

Concentration effects on the exchange diffusion reaction

It was of interest to determine whether the concentration effects controlling exchange differed in any way from those controlling transport. The system investigated first was the exchange of prepacked ACPC with L-methionine in the incubation medium. The results obtained are presented in Fig. 1. In the first set of experiments, shown in Fig. 1A, pancreas was preloaded with ACPC to different levels and efflux was followed in the presence and absence of 1 mM L-methionine in the external media. That portion due to exchange (difference between efflux in the presence and absence of external L-methionine over the first 5–15 min) at each of the ACPC concentrations is plotted against the concentration of ACPC in the tissue at the start of the incubation.

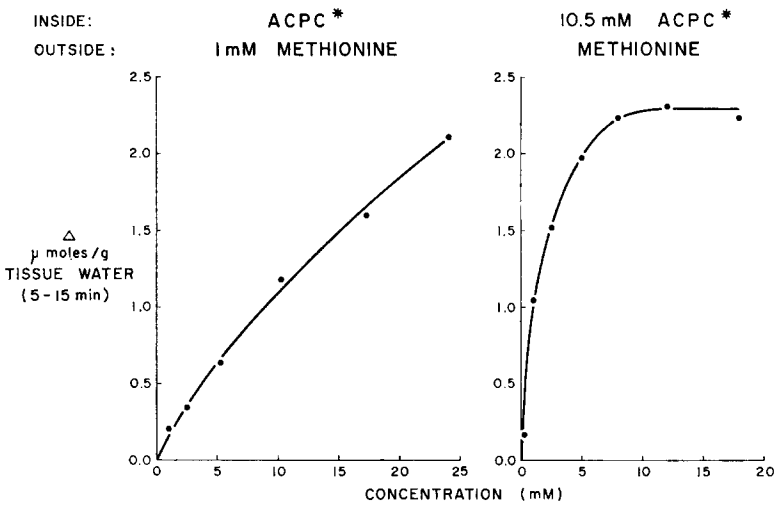

Fig. 1. The influence of 1 mM extracellular methionine on the efflux of intracellular labelled ACPC prepacked to various concentrations and the influence of various levels of extracellular methionine on the efflux of labelled ACPC prepacked to a level of 10.5 mM.

It should be noted that these are only approximate values since they involve data in which large blank values have been subtracted from the overall flux *i.e.* the rate of movement of ACPC into an amino acid free medium has been subtracted from the total flux observed in the presence of methionine. It is apparent that the system would only be termed saturated at extremely high levels of ACPC inside the cell. On the other hand, when the concentration of ACPC inside the cell was maintained at 10.5 mM and the concentration of methionine in the outside medium was varied the results indicated that this amino acid when present extracellularly is able to saturate the exchange system at quite low concentrations (Fig. 1B). Estimates of affinity for each amino acid were obtained by plotting the data according to the method of LINEWEAVER AND BURK[19]. For the other systems, the experimental set-up was similar to that shown in Fig. 1B and a summary of some of the affinity constants obtained is presented in Table V. When the concentration of methionine present in the outside medium was varied, the affinity constant was approx. 2 mM and it is worth noting that the affinity constant characterizing the transport of methionine into mouse pancreas has previously been shown[16] to be 2.9 mM. Similarly, the uptake

of ACPC is characterized by an affinity constant of 5.8 mM (ref. 16) which is of the same order as the constant characterizing the affinity of ACPC present in the medium for the system responsible for exchanging it with the prepacked methionine. The affinity constant for methionine influx by exchange seemed to be relatively independent of the intracellular amino acid (compare lines 2 and 4 of Table V) and of its concentration (compare lines 2 and 3 of Table V).

TABLE V

THE AFFINITY CONSTANTS CHARACTERIZING THE EXCHANGE DIFFUSION PROCESS IN MOUSE PANCREAS

The incubation conditions were as described in the text and in MATERIALS AND METHODS. ACPC in and ACPC out refer to ACPC inside the cell and ACPC in the medium, respectively. L-Methionine out refers to L-methionine in the external medium. In all cases the efflux of radioactive amino acid was followed. The values cited were obtained from a LINEWEAVER AND BURK[19] plot of data which represented the mean values of at least three determinations at 5 or 6 concentrations of the variable amino acid.

Amino acid inside the pancreas	*Amino acid in the external medium*	*Amino acid whose concentration is varied*	K_{ex} (*mM*) *Affinity constant for exchange*
ACPC	L-Methionine	ACPC	30
ACPC (5.4 mM)	L-Methionine	L-Methionine	1.5
ACPC (10.5 mM)	L-Methionine	L-Methionine	2.2
L-Methionine (5.5 mM)	L-Methionine	L-Methionine (outside)	3.0
L-Methionine (6.0 mM)	ACPC	ACPC	7.1
ACPC (5.4 mM)	ACPC	ACPC (outside)	5.0

Effects of extracellular amino acid mixtures on the exchange reactions

The similarity of the affinity constants characterizing transport and exchange systems suggests that they have much in common and that competition or interaction between amino acids might occur for the exchange system as for the transport system. In Table VI are cited the fluxes of prepacked L-methionine into media containing non-radioactive L-threonine, L-methionine or a mixture of these two amino acids. The concentrations employed were below the saturation level to permit the expression of additive effects. If competition for one exchange site occurred through the presence of two amino acids at less than saturation levels, then there should be a significant increase in the total flux of L-methionine. As seen in Table VI the presence of both amino acids in the incubation medium produced a greater effect than the presence of either alone. This additive effect clearly indicates that an interaction between amino acids can occur in the exchange system.

Since inhibitory effects might also occur, a similar experiment (Table VII) was carried out in which 1 mM ACPC, 5 mM α-aminoisobutyric acid or a mixture of the two amino acids at these concentrations, was present in the incubation medium and the pancreatic tissue was again prepacked with labelled methionine. In this case the concentration of ACPC in the medium was made 1 mM in order to have the minimum concentration of substrate that would show a significant exchange diffusion effect. At a concentration of 5 mM, α-aminoisobutyric acid caused a marked inhibition of accumulation from a medium containing 1 mM ACPC (see Table II). As seen from the

results presented in Table VII there was no significant effect of α-aminoisobutyric acid on the homo-exchange of ACPC under these conditions, although it is an effective inhibitor of ACPC transport.

TABLE VI

THE EFFECTS OF MIXTURES OF L-THREONINE AND L-METHIONINE IN THE EXTERNAL MEDIUM OF THE EFFLUX OF L-[*Me*-^{14}C]METHIONINE FROM MOUSE PANCREAS

Pancreas was preincubated with 0.75 mM labelled L-methionine for 60 min at 37° in order to obtain a tissue concentration of 5.3 μmoles per g tissue water. Efflux at 15° into the various media was followed as described in MATERIALS AND METHODS.

Time (*min*)	*Additions to external medium*		
	5 mM L-*trheonine*	*1.4 mM* L-*methionine and 5 mM* L-*trheonine*	*1.4 mM* L-*methionine*
2	1.15*	1.58	1.23
8	2.38	3.11	2.35
15	3.15	3.79	3.10

* Efflux expressed as μmoles/g tissue water. All values cited are the means of duplicate determinations from a typical experiment. Individual values did not differ from the mean by more than 6%.

TABLE VII

THE EFFECTS OF MIXTURES OF ACPC AND α-AMINOISOBUTYRIC ACID IN THE EXTERNAL MEDIUM ON THE EFFLUX OF [^{14}C]ACPC FROM MOUSE PANCREAS

Pancreas was preloaded with an initial concentration of 5.5 μmoles of ACPC per g tissue water as described in MATERIALS AND METHODS. Efflux at 15° into the various media was followed as described in the text.

Time (*min*)	*Additions to external medium*			
	Nil	*1 mM ACPC*	*1 mM ACPC and 5 mM α-amino-isobutyric acid*	*5 mM α-amino-isobutyric acid*
2	0.43*	0.63	0.71	0.42
8	0.93	1.64	1.74	0.93
15	1.35	2.52	2.48	1.32

* Efflux is expressed as μmoles per g tissue water. All values cited are the means of duplicate determinations from a typical experiment.

DISCUSSION

It is apparent from the results presented above that movement of amino acids across the cell membrane of mouse pancreas may occur *via* an exchange diffusion reaction as well as by transport and simple diffusion. This statement is based on the demonstration that an amino acid located on one side of the membrane may stimulate the movement of an amino acid initially located on the other side of the membrane. In the case of efflux of a prepacked amino acid, the stimulation could be due to a prevention of a reconcentration of the effluxed amino acid as suggested by WINKLER

AND WILSON[22] to explain their results in studies of galactoside transport in *E. coli*. This suggestion may be discounted in the present studies on the basis of the demonstration that α-aminoisobutyric acid has an appreciable inhibitory effect on the uptake of ACPC by mouse pancreas but has no effect on the efflux of ACPC, while methionine, when used at a concentration which has an equal effect on the uptake of ACPC, has a very marked effect on the efflux of ACPC under the conditions which have been interpreted as leading to exchange diffusion. JOHNSTONE AND QUASTEL[6,7] have concluded that the same amino acid carriers are involved in both transport and exchange diffusion in the Ehrlich cell. The present demonstration that the apparent K_m values for transport and for exchange diffusion are similar, lends some weight to this suggestion. It is possible, however, that the "A" type of site and the "L" type of site, as envisaged by OXENDER AND CHRISTENSEN[8,9] for the Ehrlich ascites tumor cells, may also operate in mouse pancreas. If this is the case, then one is led to assume that either the two sites have approximately equal K_m values or that the exchanging or "L" site dominates the movement of amino acid into the cell even when transport is being considered.

The method of presentation of results in Table IV merits comment. The percentage increase in efflux due to the presence of extracellular amino acid was greatest in the case of methionine and ACPC. The amino acids added are therefore listed in order of the averages of their abilities to stimulate the efflux of methionine and of ACPC. The order of the columns was arrived at by calculating the average value of all the numbers in parentheses listed under a given amino acid to obtain a measure of the "exchangeability" of each. The two orders in which the amino acids fall are almost exactly the same *i.e.* the "exchangeability" of a given amino acid is the same whether one is considering extracellular amino acid or intracellular amino acid. It is of interest to note that OXENDER AND CHRISTENSEN[8] have concluded that "Essentially identical relationships as to flux stimulation among these amino acids were observed when the experiments were performed in the reverse fashion . . .". Among the several possible simple explanations of this fact is the suggestion that there is only one site at which exchange takes place and hence that exchangeability should be exactly the same whether one considers intra- or extracellular amino acid. Another alternative would suggest that exchange is dominated by the rate of movement of the more sluggish of the pair of amino acids and that a sluggish amino acid will move slowly irrespective of the other amino acid or of its location relative to the membrane. All explanations, however, seem to hinge upon some measure of uniformity being involved in exchange diffusion and lend weight to the argument proposed by OXENDER AND CHRISTENSEN[8,9] that the "L" site for transport is the primary site for exchange, possibly the only site. If this is indeed the case, then attempts to study relative affinities, numbers of sites involved, relative velocities *etc* . . . would probably be better undertaken by first determining the relative parameters involved in interaction with the exchange site only and then seeking to disentangle this factor from the more complicated situation which will obtain under conditions where net transport *via* all sites is being considered.

An attempt was made in designing these experiments to secure a relatively constant concentration of intracellular amino acid and the values obtained ranged from 5.4 to 6.6 μmoles amino acid per g tissue water. Under these conditions, the unstimulated efflux of amino acid from the pancreas varied from 1.21 to 1.95 μmoles

amino acid per g tissue water per 15-min period, *i.e.* it was relatively constant and independent of the amino acid.

The availability of non-exchangeable amino acids was made use of in the experiment cited in Table VII. If an amino acid is non-exchangeable because of sluggish movement rather than because of a low affinity then it should be able to displace an exchangeable amino acid and cause a decrease in the rate of exchange diffusion. As may be seen from this table, α-aminoisobutyric acid at a concentration five times that of ACPC had no effect on the stimulated efflux brought about by the presence of ACPC in the external medium. Hence failure to undergo exchange diffusion probably arises from a low affinity of the amino acid for the site at which exchange diffusion occurs. The nature of these interactions and the relation of the transport site to the exchange site in two ascitic tumors are discussed in the following paper[23].

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