BBA 75078

HOMO- AND HETERO-EXCHANGE DIFFUSION OF AMINO ACIDS IN EHRLICH ASCITES CARCINOMA CELLS*

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(Received June 1st, 1967)

SUMMARY

1. The specificity and multiplicity of mechanisms for the exchange diffusion of amino acids in Ehrlich ascites cells have been studied by comparing rates of homo- and hetero-exchange diffusion of pairs of amino acids. It was found that amino acid concentrations could be chosen such that homo-exchange rates were greater than the corresponding hetero-exchange rates for the following pairs of amino acids: glycine:L-alanine, L-alanine:DL-α-amino-*n*-butyric acid, DL-α-amino-*n*-butyric acid:L-methionine, L-methionine:L-phenylalanine and L-methionine:L-lysine. These results are interpreted to indicate that a minimum of three mechanisms exists for the exchange diffusion of these amino acids.

2. A large number of amino acids were found to exchange with previously accumulated glycine, DL-α-amino-*n*-butyric acid, L-methionine, L-phenylalanine and/or L-lysine. In general, the smaller neutral amino acids, the amide amino acids and the imino acids exchanged relatively well with glycine while the larger neutral amino acids and those with branched or cyclic side-chains exchanged well with methionine and phenylalanine. Diamino acids and larger neutral amino acids underwent exchange diffusion with intracellular lysine.

3. The results of the present study are consistent with the idea that the transport and exchange diffusion of amino acids occur through the operation of the same carrier systems and that the different carrier systems can all operate to bring about exchange diffusion.

INTRODUCTION

The accumulation of amino acids in Ehrlich ascites cells by a process of 'active' transport is well documented (see refs. 1, 2). In recent years several investigators

* This work is taken in part from a thesis submitted to McGill University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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have concluded that more than one mechanism exists for the net transport of amino acids into these cells[3–6]. In each case this view is based on studies of the inhibition of the uptake of various amino acids by others.

In contrast with transport, the possible multiplicity of the mechanisms involved in exchange diffusion has been the subject of considerably less study. The term 'exchange diffusion', introduced by USSING[7,8], describes the one-for-one equilibration of ions or molecules across membranes by a mechanism considered to involve a complex between substrate and carrier. With amino acids hetero-exchange diffusion, *i.e.* the exchange of two different amino acids, as well as homo-exchange diffusion has been observed[9–12]. The occurrence of hetero-exchange diffusion always has been taken to indicate that the amino acids in question have affinity for a common carrier. HEINZ[13] and HEINZ AND WALSH[9] early demonstrated that several amino acids, when present inside the cell, increase influx of glycine or exchange with this amino acid. These authors postulated that the same carrier mechanism is involved in the transport and exchange diffusion of glycine. On the other hand, OXENDER AND CHRISTENSEN[5,12] have suggested that only one of two principal systems proposed for the transport of neutral amino acids is capable of effecting exchange diffusion. More recently, JACQUEZ AND SHERMAN[14] have demonstrated that a large group of amino acids exchange with tryptophan and have questioned whether the assumption of more than one transport mechanism for neutral amino acids is necessary.

The purpose of the present work was to explore in detail the exchange relationships between various amino acids. The experimental design represents a new approach to the study of the specificity of carrier mechanisms, an approach based on exchange diffusion rather than on the inhibition of uptake. Using hetero-exchange diffusion as an indication that two amino acids have affinity for a common carrier, comparisons have been made of the rates of homo- and hetero-exchange of selected pairs of amino acids. This approach permits the determination of the minimum number of exchange mechanisms operating for the amino acids studied. A preliminary account of a portion of this work has appeared[15].

MATERIALS AND METHODS

The Ehrlich ascites cells were of the Lettré strain. They were carried in male CF1 Swiss white mice by the intraperitoneal injection of ascitic fluid derived from the solid form of the tumor.

Preparation of the cells

Mice that had been injected with cells 6–9 days previously were killed by cervical dislocation. An incision was made in the abdominal wall and the cell suspension transferred to centrifuge tubes kept in an ice–water mixture. The cells were washed 3 or 4 times with ice-cold saline to remove ascitic fluid and any blood cells present. By centrifuging the cell suspension for 20 sec at 1600 × *g* the tumor cells can be sedimented and blood cells left in suspension. After washing, the cells were packed by centrifuging for 2 min at 1600 × *g*. Cells from 3 or 4 mice were pooled for each experiment.

Incubation medium

Cells were incubated under air in a calcium-free Krebs–Ringer medium containing 145 mM NaCl, 5.8 mM KCl, 1.4 mM KH_2PO_4, and 1.4 mM $MgSO_4$ buffered with 20 mM sodium phosphate to a final pH of 7.4.

Calculation of intracellular concentrations

The intracellular amino acid concentrations were calculated using a factor of 0.65 ml/ml packed cells to represent the intracellular space. This figure was arrived at by subtracting the dry weight and the extracellular space from the total packed cell volume. The dry weight of 1 ml of packed cells was 12–13 mg and the extracellular space determined with [^{35}S]sulphate (see ref. 16) averaged 0.22 ml/ml packed cells. When results are expressed as mM, it is assumed that the intracellular amino acid is evenly distributed throughout the intracellular water.

Sources of amino acids

[1-^{14}C]Glycine, L-[*Me*-^{14}C]methionine, and uniformly labelled L-[^{14}C]lysine were purchased from The Radiochemical Centre, Amersham, Great Britain; L-[1-^{14}C]alanine from the Volk Radiochemical Co., Chicago, Ill.; and L-[1-^{14}C]phenylalanine and DL-α-amino-*n*-[1-^{14}C] butyric acid from the New England Nuclear Corp., Boston, Mass. The radiochemical purity of the amino acids was confirmed by two-dimensional paper chromatography. Unlabelled amino acids of the best grade available were purchased from the Nutritional Biochemical Corp., Cleveland, Ohio and used without further purification.

Measurement of exchange diffusion

Exchange diffusion was followed by measuring the rate of appearance of previously accumulated radioactive amino acid in the medium. Cells were first incubated at 37° in the presence of a suitable concentration of radioactive amino acid. These incubations were carried out in 250-ml erlenmeyer flasks with the cells in a total volume of 30 ml at a dilution of approx. 1:30 (1 ml packed cells per 30 ml medium). The time of incubation varied with the amino acid but was usually under 60 min. At the end of the incubation period the cells were centrifuged for 1 min at 1600 × *g*, suspended in ice-cold saline, and recentrifuged, again for 1 min. They were then suspended in ice-cold calcium-free Krebs–Ringer solution and several 5.0-ml portions transferred to a series of test tubes. To start the exchange reactions the cells were poured from the test tubes into 50-ml beakers containing unlabelled amino acids in 5.0 ml of medium. The contents of these beakers were at room temperature and all subsequent manipulations were carried out at this temperature. The temperature of this incubation was initially 14° and increased to 16° within the next 4 min. The final cell dilution was between 1:50 and 1:100.

With practice it was possible to pour the contents of 4 or 5 test tubes simultaneously to start this second incubation. Samples were then taken by pouring portions of the cell suspensions into centrifuge tubes. Cells and supernatants were separated by centrifuging for 1 min at 1600 × *g* at the desired time intervals. Supernatants were immediately decanted. The radioactivity in aliquots of each supernatant was determined in a liquid-scintillation counter using accepted techniques. A minimum count of 10 000 was obtained for each sample. The radioactive amino acid remaining in the

final cell sample was measured by extracting the cells with 95% ethanol and counting a portion of the extract. With this experimental procedure it was possible to measure accurately and simultaneously the efflux of labelled amino acid from cells in the presence of a variety of different extracellular amino acids.

EXPERIMENTAL DESIGN

Many different factors govern the rate of exchange diffusion. Using the terminology of the currently favoured carrier concept, these include (1) the affinity of the amino acid for the carrier at the outer surface of the membrane; (2) the rate of movement or reorientation of the carrier–substrate complex inward; (3) the affinity of the amino acid for the carrier at the inner surface of the membrane; (4) the rate of movement or reorientation of the carrier–substrate complex outward. If more than one carrier mechanism is involved in the exchange diffusion of any given amino acid then these four kinetic constants for both mechanisms will govern the observed rate. If the exchange under consideration is between two different amino acids one final condition must be met. The two amino acids both must have some affinity for at least one common carrier and must be able to move across the membrane with that carrier.

In order to determine if only one carrier is responsible for the exchange diffusion of two different amino acids, experiments were designed so that the individual kinetic constants cancel out leaving as the important factor whether or not more than one mechanism is responsible for the exchange diffusion. Portions of cells preloaded with radioactive amino acids (A or B) were added to exchange media containing no amino acid or either A or B in unlabelled form. In other words, the rates of exchange diffusion of four different combinations were compared. These four combinations were (1) x concentration of A inside the cells and m concentration of A outside; (2) x concentration of A inside and n concentration of B outside; (3) y concentration of B inside and m concentration of A outside; and (4) y concentration of B inside and n concentration of B outside. With this experimental design the relative saturation of sites by amino acid A at the inner surface of the membrane and the rate of movement of carrier–A complex outward will be the same whether amino acid A or B is present in the external medium (Cases 1 and 2). Similarly the relative saturation of sites by B at the inner surface of the membrane and the rate of movement of carrier–B complex outward will be the same in Cases 3 and 4. In addition the relative saturation of sites by A at the outer surface of the membrane and the rate of movement of carrier–A complex inward will be the same regardless of whether A or B is inside the cell (Cases 1 and 3) and the same statement can be made for B (Cases 2 and 4). It follows that if all the exchange diffusion of the two amino acids proceeds through only one carrier, then either amino acid A or B in the extracellular medium should have the greater effect on the efflux of both intracellular A and B. If, however, two or more carriers are responsible for the observed exchange diffusion and the two amino acids do not have the same relative affinities for these, it should be possible to chose amino acid concentrations such that extracellular A will have the greater effect on the efflux of intracellular A and extracellular B on the efflux of intracellular B. This reasoning assumes that amino acids do not have inverse relative affinities for different carriers at the inner and outer surfaces of the membrane. It does not require, however, that affinities be the same at both membrane surfaces.

Each of the experiments of this design was repeated four times. The same qualitative results were obtained in each case.

RESULTS

Comparison of homo- and hetero-exchange diffusion of glycine and L-*alanine*

Since glycine, alanine, α-amino-*n*-butyric acid and methionine bear simple structural relationships to each other their exchange diffusion was studied in detail. The results of a typical experiment comparing the homo- and hetero-exchange diffusion of glycine and L-alanine are presented in Fig. 1. Extracellular glycine has a greater

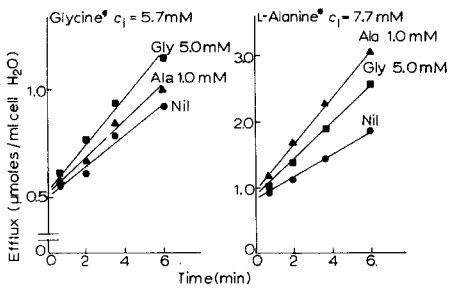

Fig. 1. Comparison of homo- and hetero-exchange diffusion of glycine and L-alanine. Cells were first incubated with the labelled amino acid indicated at the top of each graph. c_1 is the intracellular amino acid concentration at the start of the exchange incubation. The amino acid and concentration indicated opposite each curve refer to the extracellular amino acid. The curves labelled nil represent efflux into a medium containing no amino acid. The exchange incubations were carried out at an average temperature of 15° at a cell dilution of 1:60 in Ca^{2+}-free Krebs–Ringer solution buffered with phosphate to pH 7.4.

effect on the efflux of intracellular glycine than on the efflux of alanine and extracellular alanine has a greater effect on the efflux of intracellular alanine indicating that more than one system is responsible for the exchange diffusion of these two amino acids. This result and those that follow can be understood as being due to a 'better matching' of affinities for different carriers at the inner and outer surfaces of the membrane in the case of homo-exchange diffusion.

A numerical example might help to clarify this idea. Suppose only two carriers are involved in exchange diffusion and that the relative affinities of glycine for these are such that Site 1 is 50% satd. and Site 2 is 25% satd. both inside and outside the membrane at the concentrations of glycine used in this experiment. Suppose also that with alanine the situation is exactly reversed and Site 2 is 50% satd. and Site 1, 25% satd. both inside and outside the membrane at the alanine concentrations chosen. As additional simplifications suppose that both carriers are present in equal concentration and that the rates of movement of both amino acids with both carriers are the same. The observed rates of exchange diffusion would then be the result of the combined operation of the two carriers. For glycine the rate of exchange *via* Site 1 would be 50% times 50% or 1/4 the maximum possible rate and *via* Site 2 would be 25% times 25% or 1/16 the maximum possible. The total exchange, therefore, would be 5/32 the maximum. Since the relative saturation of the two sites by alanine was chosen to be numerically the same, the rate of exchange diffusion of alanine would also be 5/32 the

maximum possible rate. In the case of hetero-exchange, Site 1 is assumed to be 50% satd. with glycine on one side of the membrane and 25% satd. with alanine on the other side. The relative rate of exchange diffusion would therefore be 50% times 25% or 1/8 the maximum possible rate. Similarly for Site 2 the rate of exchange would also be 1/8 the maximum. The combined operation of the two carriers therefore would lead to rates of hetero-exchange diffusion that are 4/32 the maximum possible rate and only 80% of either homo-exchange rate. In actual fact, of course, the various affinity constants could have any numerical value and the rates of movement of the carrier–substrate complexes need not be the same for the different carriers or for the different amino acids. If, however, exchange diffusion is taking place through the operation of more than one carrier and the relative affinities of the two amino acids being compared are different for these two carriers then, at suitable concentrations of the two amino acids, the rates of homo-exchange diffusion should be greater than the rates of hetero-exchange diffusion.

The rates in these and the following graphs do not extrapolate back to zero time. There are two reasons for this. Firstly, there is a certain amount of radioactive amino acid unavoidably lost during the preparation of the cells for the exchange incubation and secondly, the times on the graphs represent the times that centrifugation to separate cells and medium was started and not the times that exchange diffusion was stopped. The various rates extrapolate back to a common point at about *minus* 30 sec. This is approximately the time necessary to separate cells and medium and thereby stop exchange diffusion.

Comparison of homo- and hetero-exchange diffusion of L-*alanine and* DL-*α-amino-n-butyric acid*

As with glycine and alanine the rates of homo-exchange are greater than the corresponding rates of hetero-exchange with alanine and DL-α-amino-*n*-butyric acid (Fig. 2) although the differences are not large. Throughout the present work intracellular amino acid levels were always kept below 10 mM to minimize the possibility of saturating sites at the inner surface of the membrane. The choice of extracellular concentrations was largely a matter of trial and error. It is obvious that if the concentration of either amino acid in the pairs studied is altered markedly then homo-exchange rates will not both be greater than hetero-exchange rates. The relative rates of exchange with the two intracellular amino acids do remain different however.

The use of α-amino-*n*-butyric acid in its DL form requires consideration of the

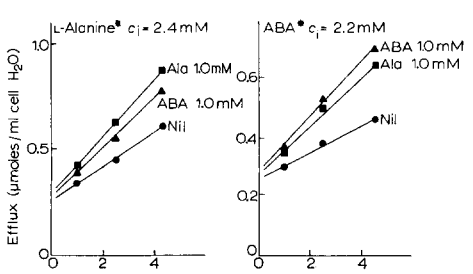

Fig. 2. Comparison of homo- and hetero-exchange diffusion of L-alanine and DL-α-amino-*n*-butyric acid (ABA). Experimental details were as described for Fig. 1.

possibility that the two isomers use different exchange mechanisms. The transport of the D and L forms of alanine and valine has been studied in detail and the conclusion reached that the two forms use the same carriers[17]. With respect to exchange diffusion a comparison of the homo- and hetero-exchange rates of D- and L-methionine gave no indication that more than one mechanism was involved. It appears unlikely, therefore, that the D and L forms of α-amino-*n*-butyric acid are exchanging by different mechanisms.

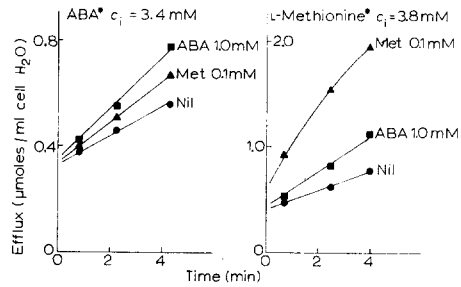

Fig. 3. Comparison of homo- and hetero-exchange diffusion of DL-α-amino-*n*-butyric acid (ABA) and L-methionine. Experimental details were as described for Fig. 1.

Comparison of homo- and hetero-exchange diffusion of DL-α-amino-n-butyric acid and L-*methionine*

There is a large difference in the rates of homo- and hetero-exchange diffusion of DL-α-amino-*n*-butyric acid and methionine (Fig. 3). The difference in the structures of these two amino acids is, of course, greater than the difference in the structure of glycine and alanine or alanine and DL-α-amino-*n*-butyric acid. These results, along with those above, can be explained by assuming that there are two mechanisms operating for the exchange diffusion of the four amino acids studied and that, as the chain lengths of the amino acids increase, the relative affinities for the two sites shift. Glycine could be seen as having a higher affinity for one carrier than the other, alanine and DL-α-amino-*n*-butyric acid could occupy intermediate positions, and methionine could have a higher affinity for the other carrier. Alternatively, the existence of three, four, or any number of carrier mechanisms can also explain the results obtained. In

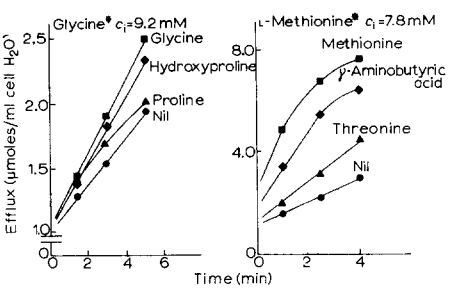

Fig. 4. The effects of various amino acids on the efflux of glycine or L-methionine. Cells were first incubated with radioactive glycine or L-methionine to contain the intracellular concentrations (c_i) indicated. They were then washed and transferred to media containing the amino acids indicated opposite each curve at a concentration of 5 mM. The exchange incubations were carried out at an average temperature of 15° and at a cell dilution of 1:100.

this case each amino acid could be seen as having the highest affinity for one carrier and lower affinities for those primarily responsible for the exchange diffusion of structurally similar amino acids.

The exchange diffusion of L-*methionine and* L-*phenylalanine*

OXENDER AND CHRISTENSEN[5] have concluded that methionine is transported into the Ehrlich ascites cell by two different transport systems. Alanine and glycine are considered to be transported predominantly by one system and phenylalanine and leucine predominantly by another. The exchange relationships between methionine, glycine and alanine have been indirectly established above and detailed studies on the exchange of methionine and glycine are reported in the following section. It was also pertinent to compare the rates of homo- and hetero-exchange diffusion of methionine and either phenylalanine or leucine. Phenylalanine was chosen for this study (Table I). When the two amino acids were both present at an extracellular concentration of 0.02 mM, phenylalanine had a greater effect than methionine on the efflux of both the intracellular amino acids while at the two higher concentrations methionine had the

TABLE I

COMPARISON OF HOMO- AND HETERO-EXCHANGE DIFFUSION OF L-METHIONINE AND L-PHENYLALANINE

Cells were incubated with radioactive L-methionine or L-phenylalanine, washed and transferred to media containing either no amino acid or L-methionine or L-phenylalanine at the concentrations indicated. The exchange incubations were carried out at an average temperature of 15° and at a cell dilution of 1:100. In each case a time course consisting of three samples taken within 4 min was determined. Initial efflux of the radioactive intracellular amino acid is reported. Efflux in the absence of extracellular amino acid has been subtracted from other values.

Radioactive intracellular amino acid (mM)*	*Extracellular amino acid (mM)*	*Efflux (mμmoles/ml cell H_2O per min)*		
		No extracellular amino acid	*Extracellular methionine*	*Extracellular phenylalanine*
Methionine (4.8)	0.02		108	208
	0.1	162	377	323
	0.5		770	377
	5.0		1015	446
Phenylalanine (2.6)	0.02		109	294
	0.1	57	331	395
	0.5		770	528
	5.0		800	654

greater effect. At a concentration of 0.1 mM, however, methionine had the greater effect on the efflux of intracellular methionine while phenylalanine had the greater effect on the efflux of intracellular phenylalanine indicating that more than one carrier mechanism is involved in the exchange diffusion of these two compounds.

The effects of L-*methionine on the efflux of glycine*

If methionine does exchange by more than one mechanism and only two mechanisms exist for the exchange diffusion of neutral amino acids then methionine

should exchange with glycine. This possibility has been explored by PAINE AND HEINZ[18] and OXENDER AND CHRISTENSEN[12] who found that preloading cells with methionine decreased rather than increased the subsequent influx of glycine. The first authors suggested that sufficient methionine was leaking from the cells to inhibit the influx of glycine and concluded that it was impossible to say whether or not glycine and methionine underwent exchange diffusion.

The results of studies with these two amino acids under the present experimental conditions are summarized in Table II. Several different combinations of intracellular

TABLE II

THE EFFECTS OF EXTRACELLULAR METHIONINE ON THE EFFLUX OF GLYCINE

Cells were incubated with radioactive glycine, washed and transferred to media containing either no amino acid or L-methionine. The exchange incubations were carried out at an average temperature of 15° and at a cell dilution of 1:60.

Radioactive glycine (mM)*	*L-Methionine (mM)*	*Efflux (mμmoles/ml cell H_2O)*		
		Time (min:sec)	*No extracellular amino acid*	*Extracellular methionine*
0.65	1.0	1:30	75	80
		3:15	94	88
		5:30	114	114
3.2	1.0	1:35	348	366
		3:35	430	430
		5:15	550	505
4.6	0.1	1:00	500	498
		2:30	560	608
		4:20	673	660
7.9	5.0	1:30	1420	1520
		3:30	1970	1820
		5:30	2260	2150
9.5	0.3	1:00	1030	1070
		3:00	1260	1290
		5:15	1660	1680
15.9	1.0	1:30	1680	1800
		3:00	2100	2060
		5:20	2620	2520

glycine and extracellular methionine were used in an attempt to demonstrate unequivocal exchange between these two compounds. In five of six experiments efflux of intracellular glycine was increased by a few per cent at the earliest time interval only. After 5 min efflux had been significantly decreased in three cases. These results suggest the possibility that glycine and methionine do exchange with each other but that methionine enters the cells rapidly and in sufficient amount to inhibit the efflux of glycine.

The exchange diffusion of L-*methionine and* L-*lysine*

It has been suggested that the dibasic amino acids are transported by a system or systems different from those serving for the neutral amino acids in a variety of

TABLE III

COMPARISON OF HOMO- AND HETERO-EXCHANGE DIFFUSION OF L-METHIONINE AND L-LYSINE

Cells were incubated with radioactive L-methionine or L-lysine, washed and transferred to media containing either no amino acid or L-methionine or L-lysine at the concentrations indicated. Other experimental details were as described in Table I.

Radioactive intracellular amino acid (mM)	*Extracellular amino acid (mM)*	*Efflux (mμmoles/ml cell H_2O per min)*		
		No extracellular amino acid	*Extracellular methionine*	*Extracellular lysine*
Methionine (4.9)	0.1		377	0
	0.3		685	0
	1.0	162	893	0
	3.0		963	15
Lysine (2.6)	0.1		6	34
	0.3		17	77
	1.0	66	29	114
	3.0		59	129

tissues including kidney[19–22], intestine[23,24], brain[25] and Ehrlich ascites cells[6,26]. In each tissue neutral amino acids have been shown to inhibit the uptake of dibasic ones and it has been proposed that these two groups of compounds have overlapped specificities. In the intestine exchange diffusion between lysine and leucine has been noted[27]. In the Ehrlich ascites cell it has been observed that, at concentrations of 20 mM, several neutral amino acids increase the efflux of previously accumulated lysine or diaminobutyric acid[6]. It was therefore of interest to compare the rates of homo- and hetero-exchange diffusion of representative neutral and dibasic compounds. Methionine and lysine were chosen for detailed study (Table III). Lysine, up to an extracellular concentration of 3 mM, has little or no effect on the efflux of intracellular methionine. Extracellular methionine, on the other hand, increases the efflux of intracellular lysine though not to the same extent as does extracellular lysine. This result indicates that a separate mechanism exists for the exchange diffusion of lysine for which methionine has some affinity. This mechanism must be different from the one serving for the exchange diffusion of glycine since lysine has no effect on the efflux of intracellular glycine (see Table IV).

The effects of other amino acids on the efflux of glycine, DL-*α-amino-n-butyric acid,* L-*methionine,* L-*phenylalanine and* L-*lysine*

The effects of a large number of amino acids on the efflux of glycine, DL-*α*-amino-*n*-butyric acid, methionine, phenylalanine and lysine are summarized in Table IV. Representative experiments from this series are presented in detail in Fig. 4. Efflux in the absence of extracellular amino acid and the rate of homo-exchange diffusion were determined in each experiment. Before calculating percentages, rates were corrected for efflux in the absence of extracellular amino acid as measured in the individual experiments. With the exception of the efflux of glycine in the presence of extracellular alanine, proline and methionine, all rates appeared linear.

Almost all the amino acids studied exchange with one or more of the intracellular amino acids. Among the neutral amino acids, the smaller straight chain and

TABLE IV

THE EFFECTS OF OTHER AMINO ACIDS ON THE EFFLUX OF GLYCINE, DL-α-AMINO-*n*-BUTYRIC ACID, L-METHIONINE, L-PHENYLALANINE AND L-LYSINE

Cells were first incubated with 1 mM radioactive glycine, α-amino-*n*-butyric acid, methionine, phenylalanine or lysine, as indicated at the top of the five columns. They were then washed and transferred to media containing the amino acids listed in the left hand column at a concentration of 5 mM. The exchange incubations were carried out at an average temperature of 15° and at cell dilutions of from 1:60 to 1:100. Unless otherwise noted amino acids were of the L form. The initial intracellular amino acid concentrations were (mM): glycine, 7.7–10.7; α-amino-*n*-butyric acid, 4.6–7.1; methionine, 4.4–9.8; phenylalanine, 1.8–3.2; lysine, 1.7–2.6. Efflux in the absence of extracellular amino acid was (mμmoles/ml cell H_2O per min): glycine, 80–200; α-amino-*n*-butyric acid, 115–230; methionine, 138–245; phenylalanine, 60–90; lysine, 42–70. Initial rates of homo-exchange diffusion were (mμmoles/ml cell H_2O per min): glycine, 77–123; α-amino-*n*-butyric acid, 165–212; methionine, 875–1070; phenylalanine, 570–890; lysine, 155–180.

Amino acid in medium (5.0 mM)	*Hetero-exchange as percent of homo-exchange*				
	Glycine	*α-Amino-n-butyric acid*	*Methionine*	*Phenyl-alanine*	*Lysine*
Glycine	100	17	2	0	6
Alanine	54*	51	10	10	15
α-Amino-*n*-butyric acid (DL)	100	100	27	41	12
Methionine	0*	50	100	164	42
Ethionine	0	32	86	154	—
Serine	71	80	15	12	7
Threonine	27	66	35	31	—
Cysteine	52	116	58	87	14
α-Aminoisobutyric acid	13	2	5	18	0
Proline	31*	12	3	0	0
Hydroxyproline	77	30	0	0	0
Asparagine	77	28	12	16	10
Glutamine	60	50	19	26	32
Valine	16	100	75	115	8
Leucine	0	23	68	144	47
1-Amino-cyclopentanecarboxylic acid	19	114	65	135	5
Histidine	0	37	69	154	67
Tryptophan	0	25	37	100	2
Phenylalanine	0	10	35	100	15
β-Alanine	33	9	0	0	8
γ-Aminobutyric acid	50	54	40	103	22
ε-Aminocaproic acid	—	—	0	—	0
Taurine	0	0	0	0	0
D-Methionine	0	41	83	145	—
Glutamic acid	0	0	1	0	0
Lysine	0	0	1	2	100
α,β-Diaminopropionic acid (DL)	25	—	—	—	10
α,γ-Diaminobutyric acid (DL)	0	0	0	—	80
Ornithine	—	—	0	—	92
Arginine	—	—	0	—	93
Cystine (0.4 mM)	—	—	0	—	25

* Rates not linear.

the amide amino acids as well as the imino acids have a relatively large effect on the efflux of glycine and only a small effect on the efflux of methionine and phenylalanine. The larger neutral amino acids and those with branched or cyclic side-chains exchange best with methionine and phenylalanine. It is of interest to compare the relative effects of structurally similar amino acids. Serine and threonine provide such a comparison. Serine is seen to have a much greater effect than threonine on the efflux of glycine

(71% *vs.* 27% of the homo-exchange rate) and only a slightly greater effect on the efflux of DL-α-amino-*n*-butyric acid (80% *vs.* 66%) while threonine has the greater effect on the efflux of methionine (35% *vs.* 15%) and phenylalanine (31% *vs.* 12%). The large effect of γ-aminobutyric acid on the efflux of all four of the neutral amino acids is noteworthy. When the results obtained with lysine are considered the basic amino acids as well as cystine and several other neutral amino acids are seen to exchange.

Amino acid transport at 15°

In previous sections it has been assumed that when efflux is increased in the presence of an extracellular amino acid then exchange diffusion is occurring. It is also possible that what is being measured is not true exchange diffusion but rather the inhibition of the retransport of the radioactive amino acid. It was therefore of importance to determine the rates of transport of glycine, alanine, DL-α-amino-*n*-butyric acid, methionine, phenylalanine and lysine under the experimental conditions used for the measurement of exchange diffusion (Table V). To duplicate these conditions exactly, cells were first preincubated for 30 min in a medium containing no amino acid. The amino acid concentrations chosen for study correspond to the highest and lowest concentrations present in the medium at the end of the exchange incubations in previous sections. Compared with exchange diffusion, transport at 15° is a slow process. The rates of exchange reported in previous sections are, in almost every case, considerably faster than the rates of transport of the radioactive amino acid partner. Comparing the exchange (Table IV) and transport rates for methionine it is seen that amino acids which have an effect on efflux that is greater than 20% of the homo-exchange rate must be increasing efflux by exchange diffusion rather than by inhibiting retransport. Taking another example, all the amino acids indicated in Table

TABLE V

THE TRANSPORT OF VARIOUS AMINO ACIDS AT 15°

Incubations were carried out at an average temperature of 15° as described in the text. The amino acid concentrations chosen correspond to the concentrations present in the medium at the end of the exchange incubations in previous sections.

Amino acid	*Amount (mM)*	*Uptake (mμmoles/ml cell H_2O) after*	
		1 min	*5 min*
Glycine	0.015	10, 15	48, 63
L-Alanine	0.008	71	128
	0.025	180	370
DL-α-Amino-*n*-butyric acid	0.008	15	47
	0.013	32	75
L-Methionine	0.013	146	266
	0.023	212	403
L-Phenylalanine	0.010	235	267
	0.050	485	550
L-Lysine	0.007	10	32
	0.015	26	43

IV as having an effect on the efflux of glycine have an effect that is greater than 12 mμmoles/ml cell H_2O per min indicating that all these amino acids do undergo exchange diffusion with glycine.

DISCUSSION

The first conclusion that can be reached on the basis of the present study is that a multiplicity of mechanisms exist for the exchange diffusion of amino acids in the Ehrlich ascites carcinoma cell. A minimum of two exchange mechanisms must be postulated to explain the results obtained with the straight chain neutral amino acids (Figs. 1, 2, and 3). The existence of a third mechanism is necessary to explain the behaviour of lysine since this amino acid, when present as the extracellular partner, does not exchange with any of the neutral amino acids but does exchange with itself. When the results obtained with the neutral amino acids alone are considered a second conclusion can be reached. Individual amino acids do not necessarily exchange by only one mechanism; considerable overlapping of affinities exists. The relative affinities of glycine, alanine, DL-α-amino-*n*-butyric acid and methionine for the minimum two exchange mechanisms appear to shift in an orderly fashion, glycine having the higher relative affinity for one site and methionine for the other while alanine and DL-α-amino-*n*-butyric acid occupy intermediate positions. This same feature of broad and overlapping specificities has been emphasized by OXENDER AND CHRISTENSEN[5] in their studies of amino acid transport systems present in the Ehrlich ascites cell. These authors have suggested that two systems, termed the alanine-preferring and leucine-preferring mediations, are primarily responsible for the transport of neutral amino acids. They have assigned different amino acids to one or both mediations on the basis of whether they inhibit the uptake of glycine and alanine (alanine-preferring) or leucine and phenylalanine (leucine-preferring). When those amino acids that exchange relatively well with glycine (Table IV) are compared with those that markedly inhibit the uptake of glycine and alanine[5], the correspondence is very good indeed. Similarly those amino acids that exchange well with methionine and phenylalanine are largely the same group that inhibit the uptake of leucine and phenylalanine. This correspondence supports the view that exchange diffusion and net transport take place through the operation of the same carrier systems and that the different systems are both (or all) capable of bringing about exchange diffusion.

The good correlation between the present exchange studies and the transport studies of OXENDER AND CHRISTENSEN[5] is somewhat surprising when the observations of JACQUEZ[28] and GUROFF, FANNING AND CHIRIGOS[29] are considered. These investigators have found that the extracellular presence of various amino acids increases, rather than decreases, the 1-min uptake of tryptophan. Similarly the steady-state level of intracellular leucine is increased in the presence of several other amino acids[30]. Whatever the detailed sequence of events that leads to this stimulated uptake might be, it is reasonable to suppose that the same sequence of events could alter levels of inhibition from what they should be on the basis of true affinity constants. Indeed the evidence supporting the view that more than one transport system exists for the neutral amino acids has recently been questioned[14].

In considering what controls the rates of exchange diffusion of different amino acids four factors suggest themselves. These are: (1) an inherent difference in different

carrier mechanisms; (2) different concentrations of different carrier mechanisms in the cell membrane; (3) differences in affinities of various amino acids for the carriers; (4) differences in the rates of movement or reorientation of the different carrier–amino acid complexes. It is simpler to assume that some combination of Factors 2, 3 and 4 is responsible for the widely different exchange rates observed rather than to adopt Factor 1 as the likely explanation. The present study is consistent with this view.

ACKNOWLEDGEMENTS

I wish to thank Dr. P. G. SCHOLEFIELD and Dr. R. M. JOHNSTONE for helpful advice during the course of this work. The financial support of the National Cancer Institute of Canada is gratefully acknowledged.

REFERENCES

1 H. N. CHRISTENSEN, *Advan. Protein Chem.*, 15 (1960) 239.
2 R. M. JOHNSTONE AND P. G. SCHOLEFIELD, *Advan. Cancer Res.*, 9 (1965) 143.
3 A. TENENHOUSE AND J. H. QUASTEL, *Can. J. Biochem. Physiol.*, 38 (1960) 1311.
4 K. AHMED AND P. G. SCHOLEFIELD, *Can. J. Biochem. Physiol.*, 40 (1962) 1101.
5 D. L. OXENDER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 238 (1963) 3686.
6 H. N. CHRISTENSEN, *Proc. Natl. Acad. Sci. U.S.*, 51 (1964) 337.
7 H. H. USSING, *Nature*, 160 (1947) 262.
8 H. H. USSING, *Physiol. Rev.*, 29 (1949) 127.
9 E. HEINZ AND P. M. WALSH, *J. Biol. Chem.*, 233 (1958) 1488.
10 R. M. JOHNSTONE AND P. G. SCHOLEFIELD, *Cancer Res.*, 19 (1959) 1140.
11 J. A. JACQUEZ, *Am. J. Physiol.*, 200 (1961) 1063.
12 D. L. OXENDER AND H. N. CHRISTENSEN, *Nature*, 197 (1963) 765.
13 E. HEINZ, *J. Biol. Chem.*, 211 (1954) 781.
14 J. A. JACQUEZ AND J. H. SHERMAN, *Biochim. Biophys. Acta*, 109 (1965) 128.
15 E. GILLESPIE AND P. G. SCHOLEFIELD, *Federation Proc.*, 25 (1966) 592.
16 E. HEINZ AND A. A. MARIANI, *J. Biol. Chem.*, 228 (1957) 97.
17 D. L. OXENDER, *J. Biol. Chem.*, 240 (1965) 2976.
18 C. M. PAINE AND E. HEINZ, *J. Biol. Chem.*, 235 (1960) 1080.
19 C. E. DENT AND G. A. ROSE, *Quart. J. Med.*, 20 (1951) 205.
20 E. B. ROBSON AND G. A. ROSE, *Clin. Sci.*, 16 (1957) 75.
21 W. A. WEBBER, J. L. BROWN AND R. F. PITTS, *Am. J. Physiol.*, 200 (1961) 380.
22 W. A. WEBBER, *Am. J. Physiol.*, 202 (1962) 577.
23 H. HAGIHIRA, E. C. C. LIN, A. H. SAMITY AND T. H. WILSON, *Biochem. Biophys. Res. Commun.*, 4 (1961) 578.
24 P. R. LARSEN, J. E. ROSE AND D. F. TAPLEY, *Biochim. Biophys. Acta*, 88 (1964) 361.
25 R. BLASBERG AND A. LAJTHA, *Arch. Biochem. Biophys.*, 112 (1965) 361.
26 H. N. CHRISTENSEN AND M. LIANG, *J. Biol. Chem.*, 241 (1966) 5542.
27 B. G. MUNCK, *Biochim. Biophys. Acta*, 120 (1966) 282.
28 J. A. JACQUEZ, *Biochim. Biophys. Acta*, 71 (1963) 15.
29 G. GUROFF, G. R. FANNING AND M. A. CHIRIGOS, *J. Cellular Comp. Physiol.*, 63 (1964) 323.
30 P. G. SCHOLEFIELD, *Can. J. Biochem. Physiol.*, 39 (1961) 1717.