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CATIONS, TRANSPORT AND EXCHANGE DIFFUSION OF METHIONINE IN EHRLICH ASCITES CELLS

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SUMMARY

The rate of methionine uptake (a) against the methionine concentration gradient as well as (b) along the methionine concentration gradient (c) in Na^+-replete and (d) in Na^+-free media is dependent on the ATP level of the cell. Exchange diffusion of methionine is completely independent of the cellular ATP level as well as of the magnitude and direction of ion gradients. The evidence obtained indicates that the exchange carrier is incapable of catalyzing unidirectional movement of methionine into the cell. Under the experimental conditions net accumulation of methionine against its concentration gradient required extracellular sodium, but not a sodium gradient.

INTRODUCTION

It is well established that not only net accumulation but also exchange diffusion of amino acids can occur in Ehrlich ascites cells[1-5]. Although it has been demonstrated that exchange diffusion of amino acids, unlike net accumulation, can occur in absence of continuous ATP production[1,3,6-8] it has not been established whether the exchange carrier is in an "activated state" and protected against inactivation by amino acids as proposed by HEINZ AND WALSH[9]. In Ehrlich ascites cells the rate of net amino acid uptake does not cease for several minutes after the addition of dinitrophenol[10]. These data indicate that the "active" carrier is not inactivated immediately. The process of exchange diffusion, however, is complete within 5–10 min at temperatures above 20°. Thus far no attempts have been made to deplete cells of ATP in absence of exogenous amino acid before measuring exchange diffusion. Therefore no definitive data is available to assess whether exchange diffusion in Ehrlich ascites cells is dependent on a "activated" carrier. GARRAHAN AND GLYNN[11] have shown that for Na^+ exchange in red blood cells, ATP is required although there is no net breakdown of ATP.

Moreover, although it is generally agreed that Na^+ plays a role in net accumulation of organic compounds in a variety of cellular preparations, there is less agreement on the role of Na^+ in exchange diffusion processes in general. Thus, JOHNSTONE AND SCHOLEFIELD[12] demonstrated that in Ehrlich ascites cells, methionine exchange occurred at the same rate with or without extracellular Na^+. BIHLER AND CRANE[13], on the other hand, concluded that carbohydrate exchange in the small intestine depended on the Na^+ concentration. The work of WINTER AND CHRISTENSEN[14] and

INUI AND CHRISTENSEN[15] appeared to resolve some of these problems by proposing that there are two types of transport systems; a Na^+-dependent type and a Na^+-independent type. In reticulocytes, the Na^+-dependent system required Na^+ for exchange as well as net transport and the Na^+-independent system did not require Na^+ for either type of movement[14]. It was concluded that in Ehrlich ascites cells, net methionine uptake is accomplished by both a Na^+-dependent and a Na^+-independent system (to approximately equal extents)[15].

To the best of our knowledge, no attempt has been made to assess the degree of independence of the exchange system in Ehrlich ascites cells for monovalent cations and the cation distribution across the cell membrane. Most measurements of exchange have been made over brief periods (1–5 min) of incubation[12,15,16] by introducing cells with normal intracellular cationic composition into Na^+-free media or Na^+-complete media. Since exchange is complete within 5 min at temperatures of 20° or higher, there is little opportunity for detecting the effect of altered intracellular Na^+ and/or K^+ distributions.

Finally, although several authors have concluded that the Na^+ gradient determines the direction of amino acid movement and is the actual energy source for amino acid uptake[17–22] other data suggest that the Na^+ concentration in the extracellular medium, not the Na^+ gradient, is the critical factor[23,24]. Since it is possible to alter the magnitude and direction of the ionic gradients without metabolic inhibitors and/or depleting cellular ATP, we examined the role of ion gradients in net accumulation of amino acids.

Our objective in the present work was to answer the following questions: (1) Can large changes in the monovalent cation composition of the cell and/or the medium change the rate constant for exchange diffusion? (2) Does exchange diffusion of amino acids depend on a preactivated carrier? (3) Does the Na^+ gradient, rather than the concentration of Na^+ in the medium, control the accumulation and net movement of an amino acid? (4) Is the exchange carrier capable of catalyzing net methionine uptake?

MATERIALS AND METHODS

Seven day old Ehrlich ascites carcinoma cells were isolated from male Swiss white mice and prepared as previously described[3]. 100 mg wet weight of cells per 3 ml of incubation medium were used.

Unless otherwise specified, cells which were incubated in Krebs–Ringer medium (145 mM NaCl, 5.8 mM KCl, 1.5 mM KH_2PO_4, 1.5 mM $MgSO_4$) at 37° for 40–45 min contained 20–30 mM Na^+ and 140–160 mM K^+. To obtain cells containing 120–160 mM Na^+, incubations were performed in Krebs–Ringer medium at 10° for 40–45 min. Krebs–Ringer solutions in which the NaCl was replaced by choline chloride or KCl were used to obtain cells containing 0 to 5 mM Na^+. Cellular K^+ levels of 0 to 5 mM were achieved by incubation in a modified Krebs–Ringer solution in which all K^+ salts were replaced by their Na^+ equivalents. The buffer used in these experiments was 4 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonate, (Hepes) pH 7.4.

Extracellular water, determined with [*carboxy*-^{14}C]inulin, did not vary with the media changes introduced in these experiments, and was estimated to be 30 ± 5 % of the total wet weight of tissue. The latter was always determined by weighing the

cell pellet after the incubation and prior to extraction of the intracellular contents. The dry weight of the cell pellet was found to be 15 % of its wet weight and intracellular water, by difference, was calculated to be 55 % of the wet weight.

To determine the rate constant for exchange diffusion the following procedure was applied. A batch of cells was prepared and divided into three portions; the first was preincubated with L-[*Me*-^{14}C]methionine, the second with L-[^{12}C]methionine, and the third without any methionine. After the 40–45 min preincubation period, the cells were spun down and resuspended in the second incubation medium in which they would undergo exchange diffusion. That portion of cells preincubated with L-[*Me*-^{14}C]-methionine was used to measure the exchangeable intracellular methionine at time zero, thus serving the function of a "prepack" control. The remaining portions (those preincubated with and without L-[^{12}C]methionine) were incubated with L-[*Me*-^{14}C]-methionine and uptake of radioactivity was estimated with time. Exchange was presumed to be complete when the uptake of L-[*Me*-^{14}C]methionine in the second incubation period, (after correction for net uptake) attained at least 90 % of the level of uptake observed with the "prepack" control. The net uptake was determined by measuring uptake of methionine in the non-prepacked cells. 30 min at 10° was usually more than adequate to achieve completion of exchange diffusion.

The rate constant, k, was calculated from the equation

$$\log_{10} \frac{U_0}{U_0 - U_e} = \frac{kt}{2.3}$$

where U_0 is the exchangeable cellular methionine concentration at time zero, and U_e is the uptake due to exchange at any time, t. It has previously been shown that a 1:1 relationship exists between uptake and efflux of methionine during exchange diffusion[3].

[^{14}C]Amino acid uptake, was measured as described before[3].

Amino acid incorporation into proteins was estimated by a modification of the SCHNEIDER procedure[25,26]. Na^+ and K^+ analyses were performed with a Zeiss PMQ II flame photometer. Cellular ATP was estimated using the method of SLEIN *et al.*[27] as modified by NIGAM[28] in an acid extract of the tissue.

Amino acid analyses were carried out with the aid of a Technicon Amino Acid Analyser.

[^{14}C]Inulin and [^{14}C]amino acids were purchased from New England Nuclear Corp. Glucose 6-phosphate, $NADP^+$, hexokinase (Sigma, Type III), and glucose-6-phosphate dehydrogenase (Sigma, Type XI) were purchased from Sigma Chemical Co. ATP (disodium) was the product of Nutritional Biochemical Co., Cleveland, Ohio. All other chemicals used were of reagent grade.

Except where otherwise stated (Fig. 1 and Table I) the variation from day to day for similar experiments was within 15 %. In most experiments where single time values are reported, time-course analyses were performed at 4–5 separate time intervals.

RESULTS

It is known that exchange diffusion of amino acids in Ehrlich ascites cells occurs rapidly even at room temperature[3]. Since we were interested in obtaining samples over

a period of 30 min, we sought the lowest working temperature at which to measure exchange and to maintain unusual cation distributions. It is also known that re-establishment of normal cation distributions in Ehrlich ascites cells occurs extremely rapidly at 37° unless metabolic poisons are added[29,30]. The results in Fig. 1 show that at 15° the exchange reaction is almost complete in 5 min but that 10° offers a suitable working temperature. It may be noted that exchange occurs, albeit slowly, at 0°. The inset in Fig. 1 shows that at 10° the intracellular Na^+ concentration can be maintained at approximately 40–60 mM in a medium containing 145 mM Na^+.

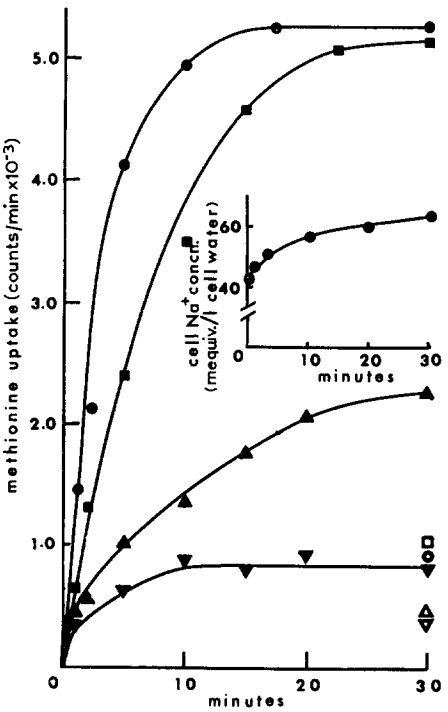

Fig. 1. Effect of temperature on the rate of methionine exchange. Cells were preincubated for 45 min at 37° with 2 mM L-methionine. Then the cells were transferred to fresh medium containing L-[*Me*-^{14}C]methionine. The exchange medium was isotonic mannitol containing 4 mM *N*-2-hydroxyethylpiperazine-*N*-2 ethane sulfonic acid (Hepes). Exchange temperature: ●—●, 15°; ■—■, 10°; ▲—▲, 5°; ▼—▼, 0°. The open symbols correspond to the control uptakes in cells preincubated without methionine. The effect of temperature on exchange was estimated at least three times for each of the temperatures. 10–20 % variation occurred in the rate of any one temperature from day to day. The results presented are typical.

Exchange diffusion and the direction of Na^+ and K^+ movements

In our earlier work on ions and exchange diffusion[3,12] we examined the effects of Na^+ replacement without attempting to reverse the ionic gradients or to measure the changes in the intracellular ionic composition. The possibility exists that in experiments of short duration we did not alter the intracellular ionic composition sufficiently to cause a measurable change in the exchange rate. In the present experiments we examined the effects of varying the ionic compositions on both sides of the membrane on exchange diffusion of methionine.

In Table I are shown values for the rate constant for exchange diffusion of methionine determined in cells of various intracellular and extracellular Na^+ and K^+ concentrations. It is apparent that the rate constant fluctuated somewhat from day to day. However, it is evident that in any experiment the rate constant is unaffected by the presence or absence of monovalent cations or ionic gradients. It may be pointed out that the methionine concentration in the incubation medium is well below the saturation level for exchange diffusion under these conditions.

TABLE I

IONS AND EXCHANGE DIFFUSION OF METHIONINE

Cells were preincubated as described in MATERIALS AND METHODS. They were then transferred to fresh Krebs–Ringer medium or to modified Ringer solutions in which isoosmotic amounts of mannitol or K^+ had replaced the Na^+. All flasks contained 2 mM L-[*Me*-^{14}C]methionine, specific activity $70 \cdot 10^3$ counts/min per μmole. Uptake was measured for 30 min at 10°.

	Medium		*Cellular*				k^* (min^{-1})
	Na^+ (mM)	K^+ (mM)	Na^+ (mM)		K^+ (mM)		
			t_0	t_{30}	t_0	t_{30}	
(1)	145	5	41	61	86	66	0.067
			121	107	30	27	0.068
(2)	145	0	41	—	90	—	0.043
			20	—	160	—	0.041
			160	—	10	—	0.045
(3)	0	5	26	19	129	100	0.077
			12	8	104	85	0.073
			0	0	117	80	0.081
(4)	0	5	26	—	130	—	0.060
			0	—	202	—	0.061
			190	—	42	—	0.063
(5)	0	145	10	0	248	248	0.055
			0	0	235	258	0.055
			69	47	100	100	0.054

* In a further experiment, cells preincubated at 37° for 40–45 min in Krebs–Ringer containing L-methionine were allowed to exchange in all media listed above. The rate constants for exchange averaged 0.067 ± 0.003 min^{-1} (mean $\pm$ S.D.). It was therefore concluded that the differences in exchange rate constant quoted in the table were due to day to day variations in the cellular activity.

Turning from the factors governing the exchange system to total amino acid uptake, INUI AND CHRISTENSEN[15] concluded that about 50 % of the total methionine uptake, at concentrations below 4 mM, is independent of Na^+ and mediated by the L system which also catalyzes exchange[2,16]. The present data (Fig. 2) show that at the steady state there is at least 70 % reduction of uptake in Na^+-free media. This result is not a consequence of examining the uptake over a range of concentrations which would primarily estimate the Na^+-dependent transport system as classified dy OXENDER AND CHRISTENSEN[2] and INUI AND CHRISTENSEN[15]. The lack of identity of response of all amino acid transport systems to the medium Na^+ concentration is also shown in Fig. 2. Methionine uptake is relatively insensitive to diminishing Na^+ ion concentrations whereas glycine uptake is very sensitive. But in Na^+-free media, relatively little uptake of either amino acid is observed (Fig. 2). In contrast with

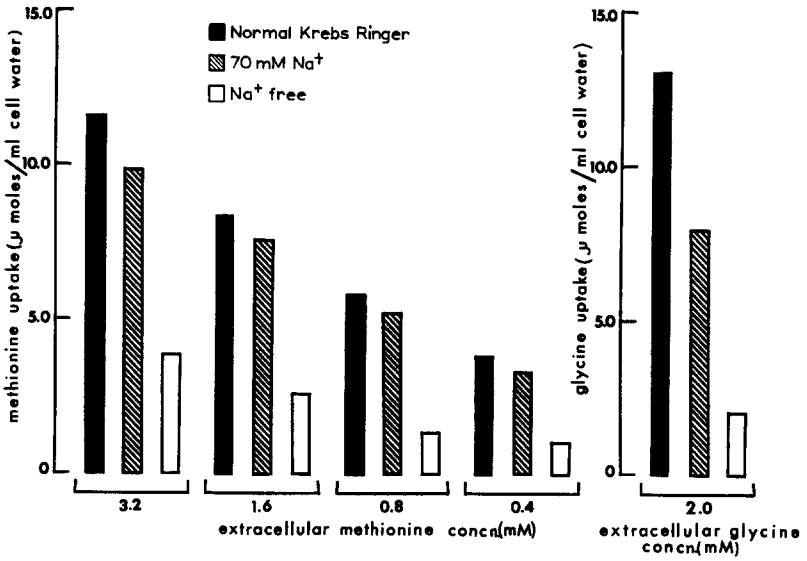

Fig. 2. Extracellular Na^+ and methionine uptake. Uptake of methionine from solutions of 3.2–0.4 mM. Choline chloride replaced NaCl. [1-^{14}C]Glycine, 2 mM; incubation time, 25 min, at 37°.

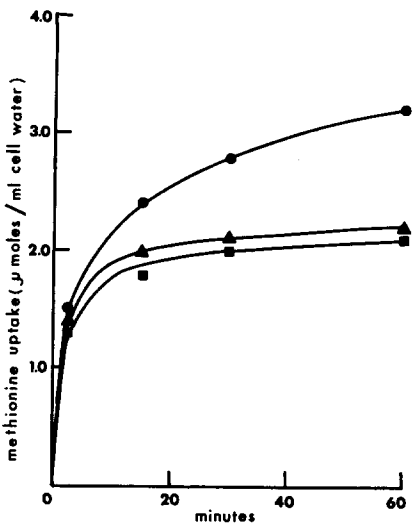

Fig. 3. Uptake of methionine at 25° in Na^+-free media. Cells were incubated in Na^+-free isotonic choline chloride and uptake was measured at 25° from 2 mM L-[*Me*-^{14}C]methionine, specific activity $140 \cdot 10^3$ counts/min per μmole. ●—●, control; ▲—▲, *plus* 10 mM α-aminoisobutyric acid; ■—■, *plus* 10 mM L-alanine. In three separate experiments the uptake of methionine at 60 min at 25° in a choline chloride medium was estimated at 3.5 and 4.0 μmoles/ml cell water. In presence of α-aminoisobutyric acid or alanine the uptake of methionine varied between 2.3 and 2.5 μmoles.

the data obtained by INUI AND CHRISTENSEN[15], α-aminoisobutyric acid and alanine were found to suppress methionine uptake in Na^+-free media by about 35 % at 60 min (Fig. 3). However, in agreement with INUI AND CHRISTENSEN[15], if one examines methionine uptake at 1 or 2 min of incubation, α-aminoisobutyric acid and alanine

TABLE II

ENDOGENOUS POOL OF "EXCHANGEABLE" AMINO ACIDS IN PRESENCE AND ABSENCE OF ADDED METHIONINE

The incubation was carried out at 25° in a medium in which isotonic choline chloride replaced the Krebs–Ringer solution as noted in Fig. 3. Half of the cells were incubated in a medium free from amino acids and the other half with 2 mM L-[*Me*-^{14}C]methionine. The amino acids whose concentration decreased in presence of methionine were: valine, isoleucine, leucine, tyrosine and phenylalanine. This group of amino acids is called the "exchangeable" pool and its concentration is given in the table. No difference in content in presence and absence of methionine at either 2 or 60 min was detected for the group of amino acids consisting of aspartic acid, threonine, serine, glutamic acid, glycine and alanine. Practically identical differences (within 15%) in the size of the endogenous pool with and without methionine were obtained in a duplicate experiment.

Time (min)	*Exchangeable amino acid pool (μmoles/ml cell water)*		*Δ due to methionine columns 2 minus 3 (μmoles/ml cell water)*	[^{14}C]*Methionine uptake (μmoles/ml cell water)*
	Control	*With 2 mM methionine*		
2	2.0	0.65	1.35	1.5
60	1.2	0.68	0.52	3.8

have little effect. The difference in the behaviour of α-aminoisobutyric acid on methionine uptake at 2 min and at 60 min is due to the fact that at 2 min, uptake is largely a measure of exchange diffusion with endogenous amino acids whereas the increment between 2 and 60 min is primarily net methionine uptake (Table II).

Carrier activation and exchange diffusion

The data in Table II suggest that the α-aminoisobutyric acid-insensitive carrier catalyzes exchange but not net accumulation. Further evidence that exchange diffusion and net movement of methionine in Na^+-free media occur *via* independent

TABLE III

Na^+ CONCENTRATION, CELLULAR ATP LEVELS AND METHIONINE UPTAKE

To obtain cells with high and low ATP levels all the cells were first preincubated with 0.1 mM dinitrophenol for 60 min at 37°. At the end of this time the ATP level in all cases was 0.1 mM or less. Then the cells were reintroduced into fresh medium (dinitrophenol-free) with and without 10 mM glucose and incubated for 90 min at 25° in air. The ATP level was restored within 5 min to the values given in the table. Samples were analyzed at 5, 15, 50 and 90 min. Data presented was taken from the 90-min incubation period. The same relationship between uptake and ATP level was found at all periods of incubation. All flasks contained 2 mM L-[Me-^{14}C]methionine of specific activity $150 \cdot 10^3$ counts/min per μmole. The incubation medium was isotonic Krebs–Ringer with 10 mM *N*-2-hydroxyethylpiperazine-*N*-2 ethane sulfonic acid (Hepes). The results presented are typical of those obtained in 3 experiments.

Medium Na^+ concn. (mM)	*Glucose*	*Intracellular ATP (mM)*	*Methionine uptake (μmoles/ml cell water)*
150	+	1.6	7.2
150	−	0.6	4.5
25	+	1.8	5.0
25	−	0.4	2.5
0–5	+	1.8	2.0
0–5	−	0.4	0.9

mechanisms comes from studies on the relationship between the cellular ATP level and net uptake. The data in Table III show that net accumulation in normal Na^+, low Na^+, and Na^+-free media is closely tied to the ATP level in the cell. A 60–75 % drop in the cellular ATP level causes about 50 % decrease in methionine uptake in normal as well as low Na^+ media. In Fig. 4 are shown data indicating that cellular

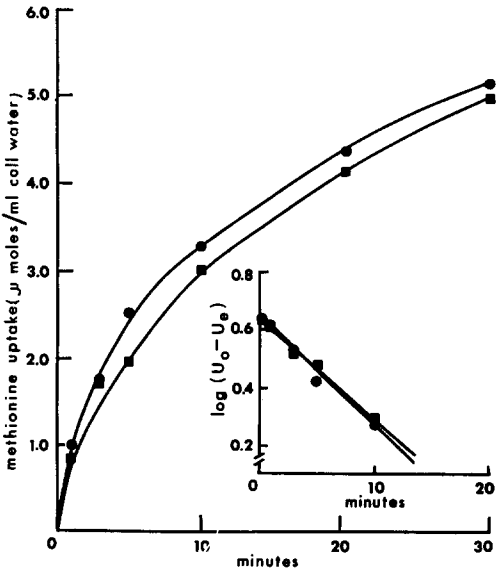

Fig. 4. Cellular ATP levels and exchange diffusion of methionine. To obtain cells with low levels of ATP, incubations were carried out for 60 min at 37° with and without 0.1 mM dinitrophenol. At the end of this time the cellular ATP levels were 0.1 and 1.6 mM, respectively. Then the cells with low ATP levels were incubated for 45 min with 15 mM L-[^{12}C]methionine and the cells with high ATP levels incubated with 2 mM L-[^{12}C]methionine. These preparations had practically identical intracellular methionine concentrations at the end of 45 min. The cells were transferred to media of identical composition in which mannitol replaced the NaCl and containing 2 mM L-[*Me*-^{14}C]methionine specific activity $70 \cdot 10^3$ counts/min per μmole. Exchange was measured at 10°. ●—●, cells containing 1.6 mM ATP; ■—■, cells containing 0.1 mM ATP.

TABLE IV

RECOVERY OF AMINO ACID TRANSPORT AFTER PREINCUBATION WITH DINITROPHENOL

All cells were preincubated with 0.1 mM dinitrophenol for 60 min at 37°. Then the cells were transferred to fresh medium and uptake measured in a normal Krebs–Ringer medium for 60 min at the temperatures indicated. The amino acids were 2 mM [^{14}C]glycine, specific activity, $130 \cdot 10^3$ counts/min per μmole, and 2 mM L-[*Me*-^{14}C]methionine, specific activity $150 \cdot 10^3$ counts/min per μmole.

Additions	*Temp.*	*Uptake (μmoles/ml cell water)*
Glycine + dinitrophenol	37°	4.5
Glycine	37°	6.3
Glycine + glucose (10 mM)	37°	24.0
Methionine + dinitrophenol	25°	2.6
Methionine	25°	3.8
Methionine + glucose (10 mM)	25°	7 0

ATP does not influence methionine exchange. The results in Fig. 4 also show that exchange diffusion does not require activation of the carrier by ATP since these cells were prepared for exchange studies after ATP was depleted and the cells were incapable of appreciable net accumulation. Such cells should not mediate exchange diffusion if a "preactivated" carrier is required. It cannot be claimed that extensive pretreatment with dinotrophenol results in irreparable destruction of the accumulating system. The results in Table IV show that accumulation can be restored by reincubation in dinitrophenol-free media particularly if glucose is also present. Protein synthesis is not involved in the restoration of activity since the presence of puromycin alters neither the rate nor extent of reappearance of net transport activity. Similar results are obtained for glycine and methionine transport. Thus it is evident that unidirectional movement (with or without Na^+) is stimulated by ATP (Table III) whereas exchange diffusion is not (Fig. 4).

Net accumulation and the Na^+ gradient

In recent years several investigators have argued that the Na^+ gradient across the cell membrane rather than the presence of extracellular Na^+ is required for net accumulation of organic compounds[17–22,32]. At 10°, although net accumulation of methionine is small, it is measurable and consistent. Since it is possible to maintain abnormal ionic distributions at this temperature without resorting to the use of extraneous inhibitors, we were in a position to estimate the dependence of net accumulation on the Na^+ gradient.

TABLE V

THE SODIUM GRADIENT AND NET UPTAKE OF L-METHIONINE

All cells were preincubated to obtain the appropriate intracellular ion concentrations as described in MATERIALS AND METHODS. They were then transferred to fresh Krebs–Ringer medium or to modified Ringer solutions in which the Na^+ was replaced with isoosmotic amounts of mannitol (Expt. 1) or choline$^+$ (Expt. 2). All flasks contained 2 mM L-[*Me*-^{14}C]methionine, specific activity $70 \cdot 10^3$ counts min per μmole (Expt. 1), $280 \cdot 10^3$ counts/min per μmole (Expt. 2), or $150 \cdot 10^3$ counts/min per μmole (Expt. 3). The data presented are averages obtained from at least two or three separate experiments. The magnitude in variation from one experiment to the next for this series can be seen by comparing the uptakes in Expts. 1 and 2 for 0–2 mM extracellular Na^+.

Expt. No.	*Temp.*	*Additions*	*Medium Na^+ (mM)*	*Cellular Na^+ (mM)*		*Methionine uptake (μmoles/ml cell water per 30 min)*
				t_0	t_{30}	
1	10°	2 mM L-methionine	145	41	61	2.1
		2 mM L-methionine	145	121	107	2.9
		2 mM L-methionine	0–2	26	19	1.3
		2 mM L-methionine	0–2	135	80–100	1.4
		2 mM L-methionine	0–2	0–5	0–5	1.5
2	10°	2 mM L-methionine	0–2	25–30	0–5	1.2
		+ 5 mM L-ethionine	0–2	25–30	0–5	0.7
		+10 mM L-ethionine	0–2	25–30	0–5	0.5
		+10 mM L-valine	0–2	25–30	0–5	0.6
		+10 mM L-alanine	0–2	25–30	0–5	0 9
3	25°	2 mM L-methionine	145	170	148	3.5

The results in Table V show that methionine uptake is not decreased when the Na^+ gradient is abolished, provided that a normal level of extracellular Na^+ is present. Indeed, the reverse is true at 10°; there is small but consistent enhancement of methionine uptake when there is nearly an equivalent Na^+ concentration of 150–180 mM on both sides of the membrane. At 25° when the Na^+ gradient is abolished, but medium Na^+ is maintained at 145 mM, significant accumulation of methionine against its concentration gradient is obtained. It should be noted, (Table V) that we are examining a carrier-mediated process at 10° the activity of which is reduced without Na^+ or in presence of other amino acids. It is also clear from Table V that cells exposed to a reversed Na^+ gradient take up as much methionine as do those containing little Na^+ inside and/or outside. If a reversed Na^+ gradient were to cause a change in the direction of net amino acid movement, methionine uptake would be expected to be appreciably lower in cells with a reversed Na^+ gradient than with any other type of gradient.

DISCUSSION

The data presented in Fig. 3 show that even in Na^+-free media, methionine uptake may be divided into two components, an initial rapid component and a slower component. The initial component is unaffected by the presence of α-aminoisobutyric acid or alanine and can be associated with an additional loss of endogenous amino acids known to be capable of exchanging with methionine. These data are therefore consistent with the conclusion that the uptake in the first few min of incubation represents exchange diffusion[2,16,33] and agrees with the known rates for exchange diffusion (see Fig. 1). The slower component of uptake is markedly inhibited by α-aminoisobutyric acid and alanine, occurs too slowly to represent exchange and cannot be associated with a corresponding loss of intracellular amino acids. It is possible that the slower component of uptake is the residue of the Na^+-stimulated transport system. If, as suggested[15], the exchange carrier catalyzed 50 % of the total uptake of methionine, removal of Na^+ should not decrease the uptake of methionine by more than 50 %, and α-aminoisobutyric acid would not inhibit the residual uptake in Na^+-free media. Clearly, both the latter deductions are incorrect; the steady state level is reduced by at least 70 % in absence of Na^+ and α-aminoisobutyric acid and alanine inhibit the uptake in Na^+-free media. These data indicate that the α-aminoisobutyric acid-insensitive system (the exchange system) does not catalyze net uptake of methionine.

Additional evidence that the exchange carrier does not contribute to net uptake comes from our data relating cellular ATP levels to net uptake of methionine. If the exchange mechanism contributed to net uptake, two consequences would ensue: (a) As the $[Na^+]$ in the medium were decreased, a higher percentage of the residual uptake would be mediated by the exchange carrier. (b) Since the exchange carrier is insensitive to the level of cellular ATP, a decrease in cellular ATP should affect methionine uptake to a lesser extent at low extracellular Na^+ levels. This is clearly not the case, (Table III) and indicates that net movement, even with limiting Na^+ in the medium, is not mediated by the exchange carrier. This evidence points to a fundamental difference in the mechanism of uptake catalyzed by the "exchange" carrier and by the carrier(s) capable of mediating net movement.

In contrast with previous reports, the maintenance of a Na^+ gradient does not appear to be an absolute requirement for net accumulation of methionine against its concentration gradient. At 10°, and especially at 25°, significant accumulation of methionine against its concentration gradient occurred in virtual absence of a Na^+ gradient. Although EDDY[22] concluded that the Na^+ gradient controlled the rate and extent of glycine uptake, it is in fact apparent from EDDY's[22] data (Figs. 1a and 1c) that a decrease in external Na^+ from 150 to 61 mM resulted in a marked decrease in uptake despite a constant $[Na_o]/[Na_i]$ ratio of about 2.0.

EDDY *et al.*[21] further concluded that if the effects of internal Na^+ on glycine efflux were considered, the presence or absence of ATP would not contribute to the uptake of glycine provided Na^+ gradients of equal magnitude were considered. This is in contrast with our observation on methionine transport where the cellular level of ATP appeared to control the rate of methionine uptake even in absence of Na^+ gradients. The present data are also at variance with those presented by VIDAVER[17,18,34] on pigeon red cells and are more consistent with the conclusions made by KIPNIS AND PARISH[23], KIPNIS[35] and SHAFER AND JACQUEZ[24], that ATP and not the Na^+ gradient is required as an energy source for accumulation of organic compounds.

Although we have not attempted to determine whether the Na^+-stimulated transport of methionine is catalyzed by more than one system, we have attempted to determine how much methionine uptake occurs by simple physical diffusion. At 10°, in a Na^+-free medium, a 1:1 distribution ratio is barely obtained in 30–60 min of incubation with 2 mM methionine or higher. Yet we find that this residual uptake may be suppressed by alanine, valine, leucine and ethionine. In view of the known effects of ethionine on methionine uptake[3] it is not surprising that ethionine is the most effective inhibitor. 50–75% of the "no Na^+" uptake at 10° can be suppressed. Considering that (a) the diffusion component would be least affected by a decrease in temperature and (b) that the Na^+-stimulated uptake is at least twice the "Na^+-independent" uptake at 10°, and (c) that the net uptake at 37° is 4–5 times that at 10°, the present data show that simple diffusion accounts for no more than 5% of the net uptake at 37° from a 2 mM methionine solution during the 30-min incubation period.

ACKNOWLEDGEMENTS

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