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# INTERACTIONS BETWEEN AMINO ACIDS DURING TRANSPORT AND EXCHANGE DIFFUSION IN NOVIKOFF AND EHRLICH ASCITES TUMOR CELLS

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SUMMARY

(1) Novikoff hepatoma ascites tumor cells accumulate amino acids by transport and exchange diffusion. The general characteristics of these phenomena are almost identical to those relating to the Ehrlich ascites tumor cells.

(2) Studies of the interaction between amino acids during transport and exchange diffusion suggest that the "A" type of site binds methionine, ethionine and α-aminoisobutyric acid with the interaction involving sodium and potassium ions. The "L" or exchange type of site which does not interact with cations seems to have no affinity for α-aminoisobutyric acid but methionine and ethionine attach to this site for exchange diffusion purposes, the attachment being partially additive at low concentrations and competitive at high concentrations.

(3) The importance of the concentration of amino acid within the membrane is emphasized, particularly in studies involving "trans-inhibition" phenomena.

## INTRODUCTION

It is now commonly assumed that during the process of translocation of amino acids from the extracellular fluid to the intracellular fluid there is a reaction between the amino acids and a large molecule termed a carrier (as reviewed in refs. 1–4). However, much evidence has accumulated to indicate that several carriers, or alternatively several sites on one carrier, must be involved in the active transport of the amino acids (see, for example, refs. 1–7). This conclusion has been reached principally on the basis of studies of interactions between amino acids during the course of their transport into various types of cells. Most of these interactions are of such a nature as to indicate that amino acids compete for the sites on the carrier which are involved in transport[5,8–12]. Kinetic analysis of the mutual inhibitory effects of amino acids on each others transport by the method of LINEWEAVER AND BURK[13] has usually yielded simple linear relationships from which characteristic values for the affinity constants may be obtained[14]. Analysis of these kinetic constants to identify specific sites or specific carrier systems, though sometimes yielding positive results[15], has more often led to confusion. The demonstration by CHRISTENSEN[16] that the operation of two

Abbreviation: ACPC, 1-aminocyclopentane carboxylic acid.

separate transport systems, during the translocation of one amino acid, may often be closely approximated by a single linear Lineweaver and Burk relationship has shed some light on this situation. Further, the proposal by OXENDER AND CHRISTENSEN[7] that two types of sites (termed the A and the L sites) may be involved in the transport of neutral amino acids, has facilitated a clearer understanding of the possible nature of the interaction between amino acids during transport.

Some of the results cited by OXENDER AND CHRISTENSEN[7] suggest that the affinity of an amino acid for the transport system remains the same even when the cell has been prepacked with the amino acid under consideration. During incubation under such conditions, amino acids enter the cell as the result of exchange diffusion in addition to the continuing operation of transport and simple diffusion phenomena. The contribution of the process of exchange diffusion to the total movement of amino acid under these circumstances may be estimated when care is taken to minimize the transport process *e.g.* by lowering the temperature or by incubating in the absence of cations. In this way it has been shown that the affinity of the carrier(s) for amino acids as determined by the $K_m$ value, is the same in pancreas for the process of transport as for the process of exchange diffusion[17]. Preliminary investigations of the interaction occurring between amino acids during exchange diffusion have been carried out[18] and we now report in detail on these interactions as they occur in Ehrlich and Novikoff ascites tumor cells. The results obtained with these two ascitic tumors are compared with those previously obtained using slices of mouse pancreas[17,18].

## MATERIALS AND METHODS

The $^{14}C$-labelled amino acids were purchased from the Radiochemical Centre, Amersham (Great Britain) and were usually employed at a specific activity of approx. 20 counts/min per m$\mu$mole. Non-radioactive amino acids were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio (U.S.A.) and were of the purest grade available. All amino acids used were the L isomer unless otherwise indicated.

The Ehrlich ascites tumor cells were grown in Swiss white mice and harvested as previously described[19]. The Novikoff hepatoma was grown in ascitic form in male Sprague–Dawley rats weighing 160–180 g which were obtained locally. The tumor was maintained by serial transplantation of 1.5 ml ascitic fluid and cells were routinely harvested after 5 days. Harvesting and washing were carried out as described for the Ehrlich ascites tumor cells.

Measurements of amino acid uptake were made as described by JOHNSTONE AND SCHOLEFIELD[19]. In studies of exchange diffusion the tumor cells were prepacked by incubating with amino acid for a suitable time at 37°. Subsequently the cells were separated from the medium by centrifugation, washed once in ice-cold Krebs–Ringer medium (calcium free) and suspended in 0.25 M sucrose to yield a preparation containing 0.3 ml packed cells per ml. The exchange diffusion process was initiated by addition of 1 ml of such a suspension to 8 ml medium. The incubation was carried out at 20° and 2-ml samples were removed as required (generally after 2, 5, 9 and 15 min). The aliquot was added to 8 ml ice-cold 0.9% saline, centrifuged, the supernatant discarded and the labelled amino acid content of the cells estimated as cited above[19].

Estimation of intracellular fluid by subtraction of the weight of intercellular

fluid (obtained from $^{35}SO_4^{2-}$ space determinations) and the dry weight from the total weight (obtained by assuming a density of 1.00 and from a knowledge of packed cell volume) gave a value of 0.65 ml per ml packed cells. All results are presented as mM concentrations calculated assuming the specific activity of the added amino acid remains unchanged throughout the various incubation periods.

## RESULTS

### *The uptake of amino acids by Novikoff ascites tumor cells*

Accumulation of amino acids by the ascitic form of the Ehrlich tumor[8], sarcoma 37 (ref. 19), leukemia L1210 (ref. 20) and a plasma cell tumor[21] has been demonstrated but no reports are available concerning the ability of the ascitic form of the Novikoff hepatoma to do so. This became of some interest since in previous unpublished experiments we have been unable to demonstrate extensive amino acid accumulation by normal liver. The abilities of the Novikoff ascites and Ehrlich ascites cells to concentrate various amino acids were estimated and the results obtained are compared in Table I. They indicate a striking quantitative as well as qualitative similarity between the results of the operation of the active transport processes as they occur in these two ascitic forms of tumors. In both tumors, proline and α-aminoisobutyric acid were the most extensively concentrated among the amino acids tested and in both tumors the aromatic amino acids tryptophan and phenylalanine were concentrated to the least extent. Even the two D-amino acids examined (methionine and tryptophan) attained similar concentration ratios in the two tumors.

### *Exchange diffusion in Novikoff ascites tumor cells*

In view of the close similarity seen between the transport systems operating in these two forms of tumors and the known ability of the Ehrlich tumor to bring about exchange diffusion between amino acids (see ref. 4), an attempt was made to detect exchange diffusion in Novikoff hepatoma ascites tumor cells. The cells were

TABLE I

THE UPTAKE OF AMINO ACIDS BY EHRLICH AND NOVIKOFF ASCITES TUMOR CELLS

Cells were incubated in Krebs–Ringer phosphate medium at 37° for 45 min in the presence of 2 mM labelled amino acid.

| *Amino acid added* | *Intracellular concentration (mM)* | |
|---|---|---|
| | *Ehrlich carcinoma* | *Novikoff hepatoma* |
| L-Proline | 19.0 | 20.2 |
| α-Aminoisobutyric acid | 18.2 | 17.4 |
| ACPC | 13.0 | 14.2 |
| Glycine | 13.2 | 12.8 |
| L-Methionine | 8.0 | 11.4 |
| L-Alanine | 13.0 | 10.0 |
| L-Tryptophan | 6.0 | 3.4 |
| L-Phenylalanine | 5.4 | 3.4 |
| D-Tryptophan | 8.4 | 8.6 |
| D-Methionine | 5.0 | 5.2 |

first incubated in a calcium-free Krebs–Ringer phosphate medium in the presence of 2 mM [$^{14}$C]ACPC for 45 min at 37°. They were then washed as described in MATERIALS AND METHODS, separated into four aliquots and incubated at 20° in fresh media containing (a) no amino acid, (b) 5 mM proline, (c) 5 mM methionine or (d) 5 mM ACPC. The amount of labelled amino acid remaining in the cells was then determined on sequential aliquots and the results obtained are presented in Table II. They indicate that there is a slow leak of ACPC from the Novikoff ascites tumor cells when incubated at 20°. The efflux is not greatly altered when proline is present in the incubation medium but when either 5 mM methionine or 5 mM ACPC are present the rate of loss of labelled amino acid is increased to three times its original value, a result analogous to that previously observed for the Ehrlich tumor. To demonstrate that the process is, in fact, one of exchange diffusion, advantage was taken of the previous observation that exchange diffusion is independent of the presence of ions in the incubation medium[22]. Cells were prepacked with ACPC from a medium containing 2 mM labelled ACPC and then incubated in the presence and absence of 4 mM methionine at 20° in media containing either the normal level of $K^+$ or media in

TABLE II

EXCHANGE DIFFUSION OF ACPC IN NOVIKOFF HEPATOMA ASCITES TUMOR CELLS

Cells were prepacked by incubating with 2 mM [*carboxy*-$^{14}$C]ACPC for 45 min at 37°. Efflux was measured by incubating at 20° as described under MATERIALS AND METHODS. Figures in parentheses refer to the increased efflux due to the presence of amino acid in the incubation medium.

| *Time* (*min*) | *Additions* | *ACPC remaining* (*μmoles per g tissue water*) |
|---|---|---|
| 0 | Nil | 14.6 |
| 15 | Nil | 11.6 |
| 15 | 5 mM Proline | 10.9 (0.7) |
| 15 | 5 mM ACPC | 3.9 (7.7) |
| 15 | 5 mM Methionine | 4.4 (7.2) |

TABLE III

THE EFFECTS OF POTASSIUM IONS ON EFFLUX AND INFLUX OF AMINO ACIDS FROM NOVIKOFF HEPATOMA ASCITES TUMOR CELLS BY EXCHANGE DIFFUSION

Prepacking at 37°; experiment at 20°.

| *Amino acid prepacked* (*concentration in medium*) | *Time* (*min*) | $K^+$ *concentration in medium* (*mM*) | *Amino acid in medium* | *Concentration labelled amino acid in cells* (*mM*) |
|---|---|---|---|---|
| 2 mM [$^{14}$C]ACPC in each | 0 | — | — | 8.7 |
| | 15 | 7.5 | Nil | 7.2 |
| | 15 | 0 | Nil | 6.4 |
| | 15 | 7.5 | 4 mM L-Methionine | 3.4 |
| | 15 | 0 | 4 mM L-Methionine | 2.9 |
| Nil | 15 | 7.5 | 4 mM [$^{14}$C]ACPC in each | 5.1 |
| Nil | 15 | 0 | | 5.0 |
| 2 mM L-Methionine | 15 | 7.5 | | 10.6 |
| 2 mM L-Methionine | 15 | 0 | | 10.5 |

which all the KCl had been replaced by choline chloride. The results obtained (Table III) indicate that the increment in flux of amino acid is independent of the concentration of $K^+$. For purposes of comparison, results are cited from a parallel experiment in which the effect of $K^+$ on the exchange diffusion of prepacked methionine with labelled ACPC into Novikoff ascites cells was examined. In neither case was the increased flux of amino acid sensitive to the presence of $K^+$. Similar experiments were conducted in which all the NaCl in the Krebs–Ringer phosphate medium was replaced by choline chloride and no decrease in the rate of exchange diffusion of amino acids became apparent. The results obtained with Ehrlich ascites carcinoma cells were quantitatively similar and the increased fluxes observed in the Novikoff hepatoma ascites cells are therefore concluded to be due to exchange diffusion.

*Interactions between amino acids during exchange diffusion into Novikoff ascites cells*

To determine whether the exchange diffusion process measured in the Novikoff ascites cells is saturable, the effects of various amino acids at several concentrations were examined. In Table IV the effects of ethionine on the efflux of prepacked labelled ACPC are reported. With increase in the concentration of ethionine there was increased efflux of the ACPC and a saturation phenomenon. Half maximal stimulation of efflux appears to be achieved in this experiment at a concentration of ethionine of 0.2 mM. In another group of experiments ethionine was replaced by methionine and the concentration of external methionine at which half maximal increased efflux of ACPC took place was also approx. 0.2 mM. These values will hereafter be referred to as "$K_m$ values for exchange diffusion". The corresponding values characterizing amino acid transport into the Ehrlich and Novikoff ascites tumor cells have been measured and found to be approx. 1 mM for ethionine and methionine in both types of cells*. Such values are in good agreement with previous observations made in this and other laboratories. Hence, there is a 5-fold difference between the $K_m$ values for transport and for exchange diffusion of methionine or ethionine. However, if only one site is involved in both processes there can be only one affinity constant for this site for any given amino acid.

TABLE IV

THE EFFECTS OF ETHIONINE AT VARIOUS CONCENTRATIONS ON THE EFFLUX OF PREPACKED ACPC FROM NOVIKOFF HEPATOMA ASCITES TUMOR CELLS

Figures in parentheses refer to changes in the intracellular concentration of labelled ACPC due to the presence of ethionine in the incubation medium. Incubations were conducted as described in MATERIALS AND METHODS.

| *Time (min) at 20°* | *Ethionine present in medium (mM)* | *ACPC remaining in cells (μmoles per g tissue water)* |
|---|---|---|
| 0 | Nil | 12.7 |
| 15 | Nil | 9.0 (0.0) |
| 15 | 0.2 | 6.7 (2.3) |
| 15 | 0.5 | 5.5 (3.5) |
| 15 | 1.0 | 5.3 (3.7) |
| 15 | 4.0 | 4.7 (4.3) |

* Previous values reported for Ehrlich cells include ~1 mM (ref. 7), 1.0 ± 0.1 mM (ref. 27), 1.24 mM (ref. 12) and 1.5 mM (ref. 23). Present experiments (not reported) yielded values of 1.0 mM for Ehrlich and Novikoff ascites cells.

The effects of several other amino acids on the exchange diffusion of external methionine or ethionine with internal ACPC were investigated. Except where otherwise stated the internal amino acid has always been ACPC and in the first experiments to be described in this section the external amino acids consisted of methionine, ethionine or a mixture of the two. If these two amino acids when present extracellularly exchange with intracellular ACPC as the result of combination with the identical site then, at low concentrations, they should give effects on ACPC efflux which are partially but not completely additive. The increased efflux of ACPC due to the presence of 0.1 mM ethionine and 0.1 mM methionine separately and together was estimated since this concentration represents approximately one-half the $K_m$ value of exchange diffusion in each case and should permit additive effects to be observed. From the results presented in Table V it is apparent that partially additive results are obtained *i.e.* the values obtained when methionine and ethionine are added

TABLE V

THE EFFECTS OF METHIONINE *plus* ETHIONINE ON THE EFFLUX OF ACPC FROM NOVIKOFF ASCITES TUMOR CELLS

Tumor cells were prepacked with labelled ACPC by incubating for 45 min with 2 mM ACPC at 37° under aerobic conditions. The second incubation was carried out at 20° in media containing amino acid as indicated. Four experiments were carried out and in all four the increased flux due to added methionine *plus* ethionine was greater than either of the individual increments but less than the sum of the two.

| *Time* (*min*) | *Additions* | *ACPC remaining* (*μmoles per g tissue water*) |
|---|---|---|
| 0 | Nil | 10.1 |
| 15 | Nil | 7.7 (0.0) |
| 15 | 0.1 mM Ethionine | 6.3 (1.4) |
| 15 | 0.1 mM Methionine | 6.0 (1.7) |
| 15 | 0.1 mM Ethionine *plus* 0.1 mM Methionine | 5.2 (2.5) |

TABLE VI

THE EFFECTS OF ETHIONINE ON METHIONINE INFLUX INTO ASCITES TUMOR CELLS BEFORE AND AFTER PREPACKING WITH ACPC

All cells were preincubated for 45 min at 37° in the absence (control cells) or presence (prepacked cells) of 2 mM ACPC. The second incubation was carried out for 15 min at 20° in the presence of 0.1 mM [*Me*-$^{14}$C]methionine and the uptake of labelled amino acid determined.

| *Cells* | *Ethionine added* (*mM*) | *μmoles methionine taken up per g tissue water in 15 min* | | |
|---|---|---|---|---|
| | | *Control cells* | *Prepacked cells* | *Increment* |
| Ehrlich | Nil | 0.48 | 1.37 | 0.89 |
| | 0.1 | 0.42 | 1.00 | 0.58 |
| | 0.4 | 0.32 | 0.60 | 0.28 |
| Novikoff | Nil | 0.82 | 1.63 | 0.81 |
| | 0.1 | 0.70 | 1.21 | 0.51 |
| | 0.4 | 0.42 | 0.74 | 0.32 |

simultaneously are greater than those for methionine or ethionine individually but less than their sum. This partially additive effect of methionine and ethionine at low concentrations during exchange diffusion suggests a further criterion for determining whether the amino acids interact by competing for a single site. If this is the case, there should be competition between the two amino acids for the intracellular ACPC *e.g.* the presence of non-radioactive ethionine in the medium should decrease the influx by exchange of radioactive methionine. Novikoff ascites tumor cells were therefore prepacked by previous incubation with 2 mM ACPC, subsequently incubated for 15 min at 20° in fresh medium in the presence of 0.1 mM labelled methionine *plus* increasing amounts of ethionine and the flux of methionine measured. The results obtained are presented in Table VI. They confirm that ethionine inhibits the uptake of methionine in cells which had been preincubated in the absence of added amino acid. However the increased influx of methionine due to the presence of prepacked ACPC was also diminished by the presence of extracellular ethionine. A similar result was obtained in a parallel experiment in which Ehrlich ascites cells were used and the inhibition of uptake by exchange diffusion in the prepacked cells was even more striking than the inhibition of uptake by transport in the control cells. These results amply confirm previous indications that methionine and ethionine compete for at least one site during their transport into Ehrlich ascites tumor cells and suggest that a similar situation prevails during exchange diffusion. However, they do not explain the five-fold difference in the $K_m$ values for uptake and exchange diffusion.

*Studies with α-aminoisobutyric acid, proline and other amino acids*

The abilities of many amino acids to inhibit methionine uptake and methionine exchange were studied. Two of the amino acids (α-aminoisobutyric acid and proline) gave rise to results which warranted further investigation. Both amino acids are able to bring about significant inhibition of methionine uptake (Table VII (a)) but neither was able to cause any significant alteration in the increased flux of ACPC due to the presence of extracellular methionine (Table VII (b)). In addition they did not give rise to a significant extent of exchange diffusion on their own. In some experiments, the concentration of α-aminoisobutyric acid was increased to 2, 4 and 8 mM with the concentration of labelled methionine maintained at 0.1 mM and in others the concentration of labelled methionine was increased to 1.0 mM with α-aminoisobutyric acid being added at 0.8 mM and 4.0 mM. In no case was there any evidence that α-aminoisobutyric acid (or proline) could exchange diffuse with ACPC or alter the exchange diffusion caused by the presence of extracellular methionine.

The foregoing results establish that α-aminoisobutyric acid and proline do not undergo exchange diffusion with intracellular ACPC, that they have no effect on the exchange diffusion of extracellular methionine with intracellular ACPC and that they have a positive effect on the transport of methionine by the two ascitic tumors under consideration. The effect of methionine on the transport and accumulation of labelled α-aminoisobutyric acid and proline by these cells was therefore investigated and the relevant results are recorded in Table VIII. Methionine effectively inhibits the transport of both α-aminoisobutyric acid and proline, the concentration gradient being reduced almost to zero when 8 mM methionine is added to 2 mM α-aminoisobutyric acid or 2 mM proline. Similar results were obtained with the Novikoff ascites tumor cells. It is concluded that methionine and proline or methionine and α-aminoisobutyric

TABLE VII

INTERACTION OF α-AMINOISOBUTYRIC ACID OR PROLINE WITH METHIONINE DURING ITS INFLUX INTO NOVIKOFF ASCITES TUMOR CELLS BY TRANSPORT AND BY EXCHANGE DIFFUSION

In (a) cells were incubated for 15 min at 37° in the presence of 2 mM [*Me*-$^{14}$C]methionine and the uptake of radioactive amino acid estimated as described in MATERIALS AND METHODS. In (b) exchange diffusion of prepacked ACPC was measured as described in Table V. Figures in parentheses refer to increment in efflux due to extracellular amino acid.

(a) DURING TRANSPORT

| *Concentration of inhibitor added (mM)* | *μmoles methionine taken up per g tissue water* | |
|---|---|---|
| | *α-Aminoisobutyric acid added* | *Proline added* |
| 0 | 9.0 | 11.6 |
| 2 | 7.2 | 8.7 |
| 4 | 6.1 | 7.5 |
| 8 | 5.2 | 6.0 |

(b) DURING EXCHANGE

| *Time (min)* | *Expt. 1* | | *Expt. 2* | |
|---|---|---|---|---|
| | *Additions* | *ACPC remaining (μmoles per g tissue water)* | *Additions* | *ACPC remaining (μmoles per g tissue water)* |
| 0 | Nil | 12.2 | Nil | 10.0 |
| 15 | Nil | 8.7 (0.0) | Nil | 7.8 (0.0) |
| 15 | 0.8 mM α-Amino-isobutyric acid | 8.6 (0.1) | 0.8 mM Proline | 7.5 (0.3) |
| 15 | 0.1 mM Methionine | 6.8 (1.9) | 0.1 mM Methionine | 5.6 (2.2) |
| 15 | 0.1 mM Methionine *plus* 0.8 mM α-Amino-isobutyric acid | 7.0 (1.7) | 0.1 mM Methionine *plus* 0.8 mM Proline | 5.4 (2.4) |

acid compete with each other during transport but do not interact during the exchange diffusion of methionine.

When attempts were made to measure exchange diffusion of proline, the technique was to prepack cells with labelled ACPC or methionine and to measure the ability of proline (or other amino acids) to increase the efflux. The negative results obtained with proline and α-aminoisobutyric acid mean only that neither proline nor α-aminoisobutyric acid increased this efflux. To be certain that there was no exchange diffusion the converse experiment was performed in which the effects of prepacked ACPC on the uptake of labelled proline were measured. The results obtained from such a series of experiments (Table IX) show that the presence of prepacked ACPC, rather than causing an increased influx of labelled proline, gave rise to a significant inhibition in both Novikoff ascites and Ehrlich ascites tumor cells. It is important to note that this inhibition was just as extensive after 2 min incubation at 20° as it was after 15 min incubation at this temperature. When parallel experiments were conducted with Ehrlich and Novikoff ascites tumor cells which had been prepacked

TABLE VIII

THE EFFECTS OF METHIONINE ON THE UPTAKE OF LABELLED α-AMINOISOBUTYRIC ACID AND PROLINE BY EHRLICH ASCITES TUMOR CELLS

Labelled α-aminoisobutyric acid and proline were added to the medium to yield a final concentration of 2 mM. Incubations were carried out at 37° in air for 60 min (α-aminoisobutyric acid) or 30 min (proline).

| *Methionine added (mM)* | *Intracellular concentration of labelled amino acid (mM)* | |
|---|---|---|
| | *α-Amino-isobutyric acid* | *Proline* |
| 0 | 7.8 | 11.4 |
| 2 | 3.4 | 5.2 |
| 4 | 2.9 | 3.5 |
| 8 | 2.1 | 2.2 |

TABLE IX

THE INFLUENCE OF PREPACKED ACPC AND/OR EXTRACELLULAR METHIONINE ON THE UPTAKE OF PROLINE BY ASCITES TUMOR CELLS

| *Amino acid present during preincubation* | *Time (min)* | *Proline uptake (μmoles per g tissue water)* | | | |
|---|---|---|---|---|---|
| | | *Ehrlich cells* | | *Novikoff cells* | |
| | | *No methionine present* | *0.8 mM methionine present* | *No methionine present* | *0.8 mM methionine present* |
| Nil | 2 | 0.62* | 0.45 | 0.52 | 0.32 |
| | 9 | 1.44 | 0.95 | 1.35 | 0.76 |
| | 15 | 1.96 | 1.18 | 1.93 | 1.05 |
| 2 mM ACPC | 2 | 0.44 | 0.37 | 0.31 | 0.29 |
| | 9 | 1.00 | 0.69 | 0.83 | 0.58 |
| | 15 | 1.25 | 0.96 | 1.04 | 0.73 |

* All values for each type of tumor cell are taken from a typical experiment.

with non-radioactive methionine in place of ACPC a similar result was obtained *i.e.* there was an inhibition of proline uptake from the onset of incubation which did not increase during an incubation period of 15 min at 20°. These results also show that the effect of intracellular ACPC prepacked from a medium containing 2 mM ACPC was approximately the same as the effect obtained with 0.8 mM extracellular methionine added to Novikoff or Ehrlich ascites cells not prepacked with any amino acid.

## DISCUSSION

In most of the present studies of transport and exchange diffusion of amino acids, the results obtained with the widely studied Ehrlich ascites carcinoma cells were compared with those obtained using the Novikoff hepatoma ascites cells. Although these two tumors grow in different species and although the Ehrlich tumor was originally a mammary gland tumor while the Novikoff was derived from liver, their ascitic forms concentrate each amino acid studied to almost exactly the same

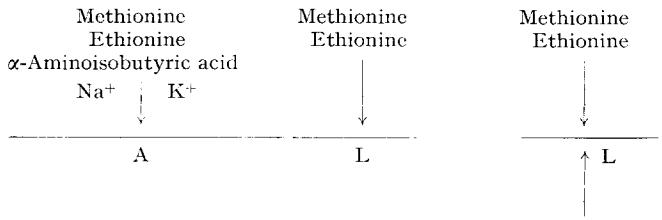
extent. Further the Novikoff tumor, like the Ehrlich tumor, is now shown to be able to bring about exchange diffusion between amino acids located on opposite sides of the membrane. In all cases, the specificities and characteristic kinetic constants were very similar and the general conclusions to be reached are taken to apply equally well to both types of tumor cells.

It should be noted that if exchange diffusion between ACPC and methionine occurs at the site designated as the "L" site by OXENDER AND CHRISTENSEN[7] (where $Na^+$ and $K^+$ play a minor role in the uptake of amino acids by transport) it is not surprising that these ions are not involved in uptake of the amino acids through exchange diffusion at the same site. What is of interest is that there is a saturation effect of methionine and ethionine for this exchange process and that the concentration at which half maximum saturation occurs (approx. 0.2 mM, Table IV) is considerably less than the concentration at which half maximum saturation of the transport process occurs (1 mM for the Ehrlich and the Novikoff ascites tumor cells). A simple explanation of these results is available if methionine enters the ascites tumor cells *via* the "A" and the "L" sites during transport but enters primarily by the "L" site during exchange diffusion. It was shown by INUI AND CHRISTENSEN[23] that transport *via* the "L" site may be studied by conducting the experiments in the absence of $Na^+$ since these ions are essential for the activity of the "A" site but are without effect on the activity of the "L" site. If the "L" site is the only type involved in exchange diffusion of methionine and the only type involved in transport in the absence of $Na^+$, then the corresponding $K_m$ values for methionine should be the same. Unpublished experiments (R. H. MATTHEWS AND P. G. SCHOLEFIELD) suggest that this is indeed the case.

INUI AND CHRISTENSEN[23] pointed out that the sodium-independent transport of methionine was insensitive to the presence of α-aminoisobutyric acid and on this basis it may be predicted that the exchange diffusion of methionine with intracellular amino acids should also be insensitive to the presence of α-aminoisobutyric acid. This prediction was confirmed by the demonstration that neither α-aminoisobutyric acid or proline (at concentrations up to eight times that of methionine) had any significant effect on the exchange diffusion of extracellular methionine with intracellular ACPC (Table VII (b)). The concentration of 0.1 mM was chosen because it represented approximately one-half of the $K_m$ value of methionine for exchange and would correspond to approximately one-third saturation of a carrier system thus permitting detection of either additive or inhibitory effects of other amino acids.

It is now predicted that if exchange diffusion involves common site(s) then the use of methionine and ethionine at low extracellular concentrations should give additive effects when the efflux of an intracellular exchangeable amino acid is examined. The validity of this prediction is evident from the results presented in Table V. Under the same conditions, however, a competition between methionine and ethionine during influx should be apparent and this is seen from the results presented in Table VI. It is concluded that methionine and ethionine react with common site(s) during exchange diffusion and that this site may be characterized as the "L" site as defined by INUI AND CHRISTENSEN[23] on the basis of its insensitivity to $Na^+$, $K^-$ and α-aminoisobutyric acid. This situation is described in Scheme I.

Amino acid entering or leaving a cell passes through the membrane region and it is likely that at some stage it becomes associated with a molecular site within that




Scheme I.

region. The same site may presumably be reached from the extracellular fluid or from the internal spaces within the cell. If two amino acids have an affinity for this site and both are present in the extracellular fluid then they will compete with each other and the movement of both into the cell will decrease. If the two amino acids are initially present on opposite sides of the membrane, the site may participate in the interchange of the two amino acids by the process of exchange diffusion. If the site does not cause exchange diffusion to occur a characteristic concentration of each amino acid will still be achieved within the membrane irrespective of the direction of movement or its cause. It is then possible to envisage that the intracellular amino acid may compete with the extracellular amino acid for association with this specific molecular site within the membrane. As a result there will be an inhibition of uptake of the extracellular amino acid by the process of transport without the intracellular amino acid having leaked out into the extracellular medium. The data presented in Table IX indicate that this situation actually occurs. In the experiments cited, prepacked intracellular ACPC proved to be an effective inhibitor of the uptake of proline in both the Ehrlich and the Novikoff ascites tumor cells. If the concentration of ACPC during the preincubation period was 2 mM the accumulated amino acid proved to be as effective an inhibitor of proline uptake as 0.8 mM extracellular methionine. For reasons discussed in the text, ACPC could not have leaked out from the cell and inhibited the uptake of proline simply by competing for the system by proline transport into the cell. It should therefore be borne in mind during studies of interaction between amino acids that the concentration of amino acids in the membrane region is the determining factor rather than the concentration of amino acid in the external medium. The phenomenon of "trans-inhibition"[25,26] may well be another example of this phenomenon and the results obtained by PAINE AND HEINZ[24] concerning the interaction between prepacked methionine with extracellular glycine are easily explicable on this basis without invoking the requirement for efflux followed by inhibition of uptake as suggested by these authors.

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