Characteristics of the Transport of Alanine, Serine and Glutamine across the Plasma Membrane of Isolated Rat Liver Cells

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1. Alanine, glutamine and serine were actively accumulated in liver cells isolated from starved rats. 2. This accumulation was inhibited when either Na^+ or HCO_3^- ions were omitted from the incubation medium. In general the degree of dependence on Na^+ was quantitatively similar to that on HCO_3^- . 3. The apparent K_m values for the transport of all three amino acids were in the range 3–5 mM with $V_{\max}$ values in the range 15–25 nmol/min per mg of cell protein at 37°C. 4. Alanine and serine transport were mutually competitive; glutamine inhibited the transport of alanine and serine non-competitively. 5. The initial rate of transport of these amino acids was inhibited when the intracellular content of ATP was decreased. 6. Ouabain inhibited the rate of alanine transport without inhibiting the rate of alanine metabolism. 7. It is concluded that a minimum of three transport systems must be postulated to exist in the liver cell plasma membrane to account for the transport of alanine, serine and glutamine. The rate of transport of these amino acids in isolated hepatocytes is unlikely to limit the rate at which they are metabolized.

Amino acids produced from the hydrolysis of dietary proteins pass through the liver before entering the general circulation. It may be expected therefore that the liver exercises an important influence on the concentrations of these amino acids in the blood and hence on the availability of amino acids for extra-hepatic tissues. Amino acid transport, amino acid metabolism or both may be influenced in the intact liver by regulatory factors such as glucagon (Mallette *et al.*, 1969), somatotropin (growth hormone; Jefferson *et al.*, 1975) and changes in the dietary state (Bloxham, 1972).

Previous studies on amino acid transport into liver have been largely confined to the use of non-metabolizable analogues of naturally occurring amino acids such as α -aminobutyrate in liver slices (Crawhall & Segal, 1968; Tews & Harper, 1969; Tews *et al.*, 1970) and more recently in isolated hepatocytes (Le Cam & Freychet, 1977). Such studies provide valuable information about the mechanism of transport of these analogues, but do not necessarily reflect the properties of the transport of the natural amino acid substrates.

In the present investigation, alanine, serine and glutamine transport has been studied. These three amino acids occur at relatively high concentrations in rat plasma and are readily metabolized to glucose and urea in perfused liver (Ross *et al.*, 1967) and isolated liver cells (Krebs *et al.*, 1976). The transport of these amino acids into isolated liver cells has been studied under conditions where no net metabolism of these compounds occurs or where metabolism has

been specifically inhibited. The purpose of this investigation was to obtain information on the transport of these amino acids with particular regard to overall kinetic parameters, ion requirements, involvement of ATP and competition between different amino acids. Such experiments have provided quantitative data about the rates of transport of these amino acids and have permitted a preliminary resolution of the transport systems involved. Since amino acid analogues were not used in the present study, no rigorous kinetic analysis has been attempted.

Materials and Methods

Incubation conditions

Hepatocytes were isolated from 24 h-starved male Wistar rats of about 300 g weight by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974). Unless otherwise stated, the cells were incubated in Krebs–Henseleit bicarbonate buffer (pH 7.4) (Krebs & Henseleit, 1932) containing 2% (w/v) dialysed bovine albumin (fraction V) at 37°C under a gas phase of O_2/CO_2 (19:1). For the measurement of alanine and serine transport the cells were pre-incubated in the above medium together with 0.5 mM-amino-oxyacetate and 10 mM-DL-cycloserine for 15 min before the initiation of transport studies. Cells were preincubated without any inhibitors when glutamine transport was to be measured.

Measurement of transport

The transport of amino acids was initiated by the

addition of cells (5–10mg of cell protein/ml final concentration) to the incubation medium containing ^{14}C -labelled amino acid (0.05 μCi), together with ^3H -labelled inulin (0.1 μCi), which acted as a marker of the extracellular space. Samples (0.2ml) were removed at appropriate intervals and the transport was terminated by centrifuging the cells through a layer of silicone oil into HClO_4 as described previously (McGivan *et al.*, 1977a). The uptake of amino acid was taken as the amount of labelled compound appearing in the inulin-impermeable space.

When amino acid uptake was to be measured by enzymic methods, the cells were preincubated as described above and transport was initiated by adding cells (10–15mg of protein/ml final concentration) to a medium containing the amino acid and also including $^3\text{H}_2\text{O}$ and ^{14}C -labelled inulin. Samples (1ml) were removed and the cells were separated from the medium by centrifugation at 10000g for 2s in an Eppendorf centrifuge (model 3200). The supernatant was decanted carefully and acidified with HClO_4 (3%, w/v, final concentration). The pellet was resuspended in 0.5 ml of water and similarly acidified. Samples of both pellet and supernatant were then measured for radioactivity as described previously (McGivan *et al.*, 1977a) and the neutralized extracts were used for enzymic assays of amino acids. By this method, approx. 60% of the total pellet volume was found to be extracellular, and the internal volume of the cells was $2.67 \pm 0.23 \mu\text{l}/\text{mg}$ of cell protein (mean $\pm$ S.E.M. for ten cell preparations).

Determination of metabolites

Standard enzymic methods were used for the measurement of alanine (Williamson *et al.*, 1967), glutamate (Bernt & Bergmeyer, 1965) and ATP (Lamprecht & Trautschold, 1965). Glutamine was assayed as glutamate after treatment of the sample with glutaminase (Lund, 1974). Cell protein was measured by a biuret method (Gornall *et al.*, 1949).

Estimation of the extent of metabolism of amino acids during the course of the transport studies

In order that the uptake of labelled amino acids should provide an accurate estimate of the rate of transport it is necessary to ensure that the metabolism of the transported amino acids is inhibited as far as possible. It has been shown previously that preincubation of liver cells with amino-oxyacetate and cycloserine completely prevents the metabolism of low concentrations of alanine in transport studies (Edmondson *et al.*, 1977), and these results have been confirmed in our laboratory.

The first enzyme reaction in the major pathway of serine metabolism in liver is catalysed by serine dehydratase, a pyridoxal phosphate-dependent enzyme that should also be inhibited by amino-

oxyacetate. It was found that the rate of gluconeogenesis from serine at concentrations of up to 20mM was totally inhibited by the addition of 0.5mM-amino-oxyacetate (results not shown). In further experiments, hepatocytes were incubated with ^{14}C -labelled serine (0.5mM) for periods of up to 20min in the presence and absence of 0.5mM-amino-oxyacetate plus 10mM-DL-cycloserine. The incubations were terminated by acidification and the extracts were neutralized. Serine, which has no net charge at neutral pH, was separated from its negatively charged metabolic products by the use of ion-exchange resins (Dowex AG-1X8; formate form) as described by McGivan *et al.* (1977b). In the presence of the inhibitors all the radioactivity was recovered as serine, but in the absence of inhibitors 25% of the added serine was metabolized to negatively charged products in the 20min incubation period.

By using the same technique, no significant loss of label from 0.5mM-glutamine was observed over 20min even in the absence of these inhibitors. This is consistent with the observation that low concentrations of glutamine are not metabolized by hepatocytes (Lund & Watford, 1976). It is concluded that the preincubation conditions used were adequate to inhibit completely the metabolism of these amino acids at low concentrations. This is not necessarily the case when high concentrations of amino acids were used (i.e. concentrations > 2mM).

Materials

Collagenase (type I), bovine albumin (fraction V) and glutaminase (grade V) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All other enzymes and nucleotides were purchased from the Boehringer Corp., Mannheim, Germany.

Results

Ion-dependence of amino acid transport in liver cells

Fig. 1 shows the time course of the transport of alanine, serine and glutamine when these amino acids were added at a concentration of 0.5mM. The uptake of these amino acids was linear with time over a period of 2min, and the initial rates of transport for all three were similar. The internal and external concentrations of all three amino acids became equal within 1min of initiation of transport and the subsequent uptake represented entry of the amino acid against a concentration gradient. Fig. 1 also shows the time course of the uptake of these amino acids in a medium in which the NaCl and NaHCO_3 of the standard incubation medium had been replaced with equivalent concentrations of choline chloride and choline bicarbonate. Cells incubated in this medium did not show any marked decrease in cell ATP contents compared with the controls. In this Na^+ -free medium, the transport of all three amino acids

Fig. 1. Time course of the transport of alanine, serine and glutamine, and the effect of the omission of Na⁺. Cells were washed three times and resuspended in either Krebs–Henseleit bicarbonate buffer (○) or in a modification of this buffer in which NaCl and NaHCO₃ were replaced by corresponding concentrations of choline chloride and choline bicarbonate respectively (●). Amino acid transport was initiated by the addition of alanine (a), serine (b) or glutamine (c), all at 0.5 mM final concentration. The results obtained in a typical experiment with a single batch of cells are shown.

was impaired, but the inhibition of glutamine transport was consistently found to be much less than that of either alanine or serine. In the absence of Na⁺, equilibration of alanine and serine occurred within 5 min, but no concentrative uptake was observed. A similar dependence on Na⁺ has previously been shown for the transport of alanine (Edmondson *et al.*, 1977) and α -aminoisobutyrate (Le Cam & Freychet, 1977) in isolated liver cells. A small apparent accumulation (<2-fold) of glutamine was observed even in the absence of Na⁺ (Fig. 1).

Fig. 2 shows the effect of varying the NaCl concentration in the range 0–120 mM on the initial rate of transport of alanine, serine and glutamine. The ionic strength was kept constant by replacing the NaCl with choline chloride. The dependencies of the transport of alanine and serine on Na⁺ concentration were similar. For glutamine, 50% of the initial rate was found to be Na⁺-independent and maximal rates of glutamine transport were attained at a concentration of 50 mM-Na⁺. Considerably higher concentrations of Na⁺ were necessary to obtain maximal rates of alanine and serine transport.

Maximal rates of amino acid uptake were obtained only when bicarbonate was present in the medium. Table 1 shows the effect of replacing the bicarbonate in the medium with an equal concentration of the buffer Na⁺/Mops (4-morpholinepropanesulphonate). The transport of all three amino acids was inhibited, but glutamine transport was inhibited to a lesser extent than was the transport of

serine or alanine. In control experiments, the addition of 25 mM-Na⁺/Mops to the standard Krebs–Henseleit medium did not affect the rate of amino acid transport. For all three amino acids, the inhibition resulting from the omission of Na⁺ was similar in magnitude to that caused by the omission of bicarbonate from the medium.

Table 1. Ion-dependence of amino acid transport into liver cells

Liver cells were washed, resuspended and incubated in three different media: (a) Krebs–Henseleit bicarbonate buffer (control); (b) the same medium except that NaCl and NaHCO₃ were replaced with corresponding concentrations of choline chloride and choline bicarbonate (Na⁺-free); (c) the same medium as in (a) except that the NaHCO₃ was replaced with an equal concentration of Na⁺/Mops (bicarbonate-free). All media were at 37°C and pH 7.4. The initial rate of transport of amino acids (0.5 mM final concentration) was measured after 2 min. The results are the means of paired observations on two separate batches of cells. These agreed within a range of $\pm 15\%$.

Medium	Initial rate of transport (nmol/min per mg)		
	Alanine	Serine	Glutamine
Control	2.89	3.93	2.25
Na ⁺ -free	0.58	0.55	1.12
Bicarbonate-free	0.40	0.54	0.97

Kinetic constants for the transport of alanine, serine and glutamine

The dependence of the rate of serine transport on the concentration of serine is shown in Fig. 3. Similar curves were obtained for alanine and glutamine. Such saturation kinetics have not been observed for the transport of cycloleucine (Le Cam & Freychet, 1977) or of leucine (McGivan *et al.*, 1977a) in hepatocytes where the non-saturable component has been attributed to diffusion. Lineweaver-Burk plots of

the present data yield straight lines from which the kinetic parameters of the uptake of all three amino acids have been evaluated (Table 2). The apparent K_m values for transport of alanine, serine and glutamine are similar and are an order of magnitude higher than the concentrations at which these compounds are found in the portal blood (Fernstrom *et al.*, 1971). The maximum rate of transport of all these amino acids is similar at 37°C.

Specificity of the amino acid-transporting systems

The transport of amino acids across the plasma membrane of ascites-tumour cells has been shown to be mediated by several transporting systems which show a broad specificity with respect to their substrates (Christensen, 1969). Evidence based on

Fig. 2. Dependence of the initial rate of amino acid transport on the external concentration of Na^+

Cells were washed three times and suspended in a modification of Krebs-Henseleit bicarbonate buffer in which the NaHCO_3 was replaced with choline bicarbonate throughout and the concentration of NaCl varied from 0 to 120 mM. The osmolarity of the solutions was kept constant by the addition of an appropriate concentration of choline chloride. Amino acids were added at a final concentration of 0.5 mM and uptake was measured after 2 min. The results are expressed as a percentage of the rate obtained in a medium containing 120 mM Na^+ . $\circ$, Alanine; $\bullet$, serine; $\blacksquare$, glutamine.

Fig. 3. Initial rate of serine transport as a function of serine concentration

Serine transport was measured after 1 and 2 min. The symbols $\bullet$ and $\circ$ represent values obtained on two separate batches of cells. Each point is the mean of duplicate determinations. The rates measured are the sum of Na^+ -dependent and -independent transport processes.

analogue studies has also been presented for the existence of multiple amino acid-transporting systems in hepatocytes (Le Cam & Freychet, 1977). Further evidence for such systems is provided by the data in Table 3, which show the effect of adding various amino acids (5mM) on the initial rates of transport of alanine, serine and glutamine (present at 0.5mM).

The following points may be noted: asparagine, methionine, proline, glycine and threonine inhibited the transport of alanine to the same extent as that of serine. Glutamine inhibited the transport of both alanine and serine. Glutamine transport was also inhibited by methionine, glycine and proline. Asparagine and threonine inhibited glutamine transport to a lesser extent than that of alanine or serine. Leucine inhibited the transport of glutamine, but had little effect on that of alanine or serine. The observation that the influx of one amino acid is inhibited by the addition of another amino acid does not necessarily imply that the transport of both

amino acids is mediated by a common carrier. With this in mind, the interaction between the transport of alanine, serine and glutamine was further investigated.

Fig. 4(a) is a Dixon plot of the reciprocal of the initial rate of alanine transport as a function of the added concentration of an inhibitor, in this case serine. Inhibition of alanine transport by serine was competitive, with a K_i for serine of approx. 3mM. The inhibition of serine uptake by alanine was also competitive (K_i 2.8mM) (results not shown). In contrast, the inhibition of alanine transport by glutamine was non-competitive (Fig. 4b), with an apparent K_i for glutamine of 10.5mM. Similarly, the inhibition of serine transport by glutamine was non-competitive (K_i 7mM) (results not shown).

Dependence of amino acid transport on the intracellular ATP concentration

The distribution ratios of alanine and glutamine determined enzymically after an incubation period of

Table 2. *Kinetic parameters of amino acid transport*

The initial rates of transport of alanine, serine and glutamine were measured after 1 and 2 min. Amino acids were added at ten different concentrations between 0.1 and 25mM. For each experiment, values of K_m and V_{max} were evaluated from Lineweaver-Burk plots. Considerable variations in the values of V_{max} were observed between different batches of cells. Far less variation occurred in the values of apparent K_m . The values shown give the mean values and the range for several cell preparations. Both Na^+ -dependent and Na^+ -independent transporting systems are involved in the transport of all three amino acids; the values shown are those obtained for the overall transport process.

Amino acid	Apparent K_m (mM)		V_{max} (nmol/min per mg)		No. of preparations
		Range		Range	
Alanine	4.5	4.1–4.9	22.5	17–28	3
Serine	3.7	3.3–4.1	18.8	17.8–19.8	2
Glutamine	4.5	4.0–5.2	23.9	22.6–25.3	3

Table 3. *Inhibition of the transport of alanine, serine and glutamine by various amino acids*

The initial rate of transport of alanine, serine or glutamine (each added at 0.5mM final concentration) was measured after 2min in the presence and absence of other amino acids added at a concentration of 5mM. The results are expressed as percentage inhibition of the control rates, and the numbers of cell preparations used are shown in parentheses. Values given are the means $\pm$ s.e.m.; statistical significance is represented as follows: * $P < 0.05$ versus the uninhibited rate in the same column; † $P < 0.05$ versus inhibition by the same inhibitor with alanine as substrate; ‡ $P < 0.05$ versus inhibition by the same inhibitor with serine as substrate.

Inhibitor	Substrate	Percentage inhibition of initial rate of transport		
		Alanine	Serine	Glutamine
Alanine	...	—	61.5 $\pm$ 2.2 (6)*	27.0 $\pm$ 3.2 (5)*†
Asparagine		45.2 $\pm$ 4.8 (5)*	50.2 $\pm$ 2.2 (4)*	28.3 $\pm$ 3.5 (3)*†‡
Glutamine		40.8 $\pm$ 2.4 (5)*	37.1 $\pm$ 3.8 (4)*	—
Glycine		34.9 $\pm$ 2.4 (5)*	34.3 $\pm$ 2.0 (6)*	21.9 $\pm$ 8.7 (4)
Leucine		7.73 $\pm$ 2.5 (3)	6.1 $\pm$ 2.9 (3)	26.2 $\pm$ 2.3 (5)*†‡
Methionine		39.0 $\pm$ 5.8 (4)*	47.7 $\pm$ 3.0 (3)*	31.8 $\pm$ 5.1 (5)*
Proline		49.2 $\pm$ 9.6 (3)*	26.8 $\pm$ 4.4 (4)*	37.9 $\pm$ 7.0 (3)*
Serine		41.2 $\pm$ 5.0 (5)*	—	7.7 $\pm$ 1.9 (5)*†
Threonine		16.2 $\pm$ 8.4 (3)	20.4 $\pm$ 4.6 (4)*	7.7 $\pm$ 1.9 (5)*†

Fig. 4. Dixon plots of the inhibition of alanine transport by serine and glutamine

In these experiments, the initial rate of transport of labelled substrate was measured by taking the value for the uptake at 2 min. Three different concentrations of substrate were used in the presence of various concentrations of inhibitor. The experiments shown are (a) inhibition of alanine transport by serine and (b) inhibition of alanine transport by glutamine. Each experiment was performed on a separate batch of cells; each point is the mean of duplicate determinations. The Na^+ -independent contribution to alanine transport at this concentration is very small (Fig. 1).

Fig. 5. Distribution of alanine and glutamine across the plasma membrane as a function of external concentration

Liver cells were incubated with various concentrations of alanine or glutamine, and the internal concentration of these compounds was measured after 20 min as described in the Materials and Methods section. The results shown for each amino acid are means of paired results obtained on two separate batches of cells. ○, Alanine; ●, glutamine.

20 min are shown in Fig. 5. At an external concentration of 0.5 mM, liver cells can maintain a concentration gradient of 5.7-fold for alanine and 7.0-fold for glutamine. These results are consistent with previous reports that liver cells can accumulate 1 mM-alanine 6-fold (Edmondson *et al.*, 1977) and 2 mM- α -amino-isobutyrate 6.5-fold (Le Cam & Freychet, 1977). Extrapolation of the data suggests that concentration gradients in excess of 7 would exist across the plasma membrane at the low concentrations of amino acids found in portal blood, provided that transport was not rate-limiting for the metabolism of these amino acids. As the concentration of added amino acid increased, the accumulation ratio decreased so that at an external concentration of 4 mM an accumulation of only 2.5–3-fold was observed. Interpretation of these results at high concentrations is complicated by the fact that at such concentrations over a period of 20 min a significant degree of metabolism occurred (20% of both amino acids were metabolized at an added concentration of 5 mM).

The possible involvement of cellular ATP was assessed by observing the effect on the initial rate of amino acid transport of varying the ATP content by the addition of limiting amounts of the uncoupling agent carbonyl cyanide *m*-chlorophenylhydrazone. It was found that the initial uptake of the amino acids which occurred over the first minute was relatively insensitive to inhibition by uncoupler (results not shown). The major proportion of the influx of amino acids occurring over the first minute represents

Fig. 6. Effect of the uncoupling agent carbonyl cyanide *m*-chlorophenylhydrazone on the initial rate of amino acid transport and on the ATP content in liver cells

Liver cells were preincubated for 10 min as described in the Materials and Methods section with the addition of various concentrations of carbonyl cyanide *m*-chlorophenylhydrazone as shown. A sample of the cell suspension was then deproteinized for the determination of ATP; to another sample was added (a) alanine or (b) glutamine at 0.5 mM final concentration in each case. Uptake of amino acids was measured at 1 and 2 min. The results shown represent the uptake at 2 min minus that at 1 min (see the text). ●, Cell ATP (nmol/mg of cell protein); ○, uptake at 2 min minus that at 1 min (nmol of amino acid/mg).

Fig. 7. Inhibition of alanine transport by ouabain
Liver cells were preincubated with transaminase inhibitors as described in the Materials and Methods section and were then preincubated for a further 10 min in the presence of various concentrations of ouabain. The initial rate of transport of 0.5 mM-alanine was measured over a 2 min time interval.

simple equilibration, which would not be expected to be energy-dependent. A much larger inhibition was observed between 1 and 2 min of the incubation period. Fig. 6 shows that the rate of transport of serine and of glutamine decreased as the cell ATP content decreased. When the intracellular ATP content decreased to zero, serine transport was completely inhibited; however, glutamine was still transported at a significant rate under these conditions. On increasing the uncoupler concentration from 50 to 100 μM the rate of glutamine transport was further decreased, although no ATP was detectable in the cells at either concentration. This further inhibition of glutamine transport is probably due to a non-specific cytotoxic effect of the uncoupler.

Effect of ouabain

The energy for the active accumulation of amino acids is thought to be derived in part from the asymmetric distribution of Na^+ across the cell membrane. This Na^+ distribution is maintained by a membrane-bound Na^+/K^+ -dependent adenosine triphosphatase that is inhibited by ouabain. The effect of ouabain on alanine transport into liver cells is shown in Fig. 7. At a concentration of 0.5 mM-amino acid, the addition of 0.5 mM-ouabain caused a $66.1 \pm 1.5\%$ inhibition of the initial rate of alanine transport and a $51.9 \pm 3.1\%$ inhibition of glutamine transport (mean $\pm$ s.e.m. of observations for four separate batches of cells). In a typical experiment

Table 4. *Effect of ouabain on the transport and metabolism of alanine*

A suspension of liver cells was preincubated with transaminase inhibitors plus 0.5mM-ouabain for 10min. The rate of transport of alanine (0.5mM) in this suspension and also in a control experiment in the absence of ouabain was measured over a 2min time interval. A second suspension of cells was preincubated with or without ouabain in the absence of transaminase inhibitors: 0.5mM-alanine was then added and the disappearance of alanine from the medium by metabolism was measured enzymically after an incubation period of 10min. The results shown are the mean $\pm$ S.E.M. of the numbers of observations in parentheses.

Additions	Rate of alanine transport (nmol/min per mg)	Rate of metabolism (nmol/min per mg)
0.5mM-Alanine	3.17 $\pm$ 0.21 (11)	1.80 $\pm$ 0.31 (9)
0.5mM-Alanine plus 0.5mM- ouabain	1.62 $\pm$ 0.21 (9)	1.66 $\pm$ 0.11 (5)

ouabain caused a fall in the intracellular ATP content from 11.4 to 8.4nmol/mg of cell protein. It is not known from these experiments whether the effect of ouabain was exerted primarily through changes in ion gradients, as is the case in other cell systems.

The effect of ouabain on the transport and metabolism of 0.5mM-alanine is illustrated in Table 4. At this concentration the rate of alanine transport was considerably faster than its metabolism. The addition of ouabain decreased the initial rate of alanine transport without affecting the rate of alanine metabolism. No effect of ouabain on the rate of alanine metabolism was observed at concentrations of alanine up to 10mM. At a concentration of 10mM, alanine was metabolized at a rate of 6.5nmol/min per mg of cell protein, and the initial rate of transport at this concentration was 18.7nmol/min per mg in a representative experiment. These results suggest that alanine transport is not rate-limiting for alanine metabolism at alanine concentrations between 0.5 and 10mM.

Discussion

In isolated hepatocytes alanine, serine and glutamine are accumulated by an Na^+ -dependent process. Although the kinetic constants of transport are similar for each amino acid, it appears that they are not all transported by a common carrier system.

It is probable that alanine and serine are transported by the same carrier. This conclusion is supported by the following observations, which fulfil the criteria necessary for the existence of a common carrier for two or more amino acids according to Heinz (1972). These are as follows.

(a) The transport of alanine and serine is mutually competitive.

(b) The K_m for the uptake of alanine is similar, within experimental error, to the K_i for the inhibition of serine uptake by alanine. Similarly, the K_m for serine transport is the same as the K_i for the inhibition of alanine transport by serine.

(c) Amino acids that inhibit the uptake of alanine inhibit the uptake of serine to the same extent (Table 3).

The transport of glutamine is catalysed by a transport system distinct from that which catalyses the transport of serine and alanine. This conclusion is supported by the following evidence.

(a) The inhibition of the uptake of alanine and serine by glutamine is non-competitive.

(b) The K_i for glutamine as an inhibitor of alanine and serine transport is not equal to the K_m for glutamine transport itself.

(c) Amino acids, such as asparagine and threonine, that inhibit alanine and serine transport do not inhibit glutamine transport to the same extent. Further, serine inhibits the transport of alanine, but not that of glutamine, whereas leucine inhibits glutamine transport but has little or no effect on that of alanine or serine.

(d) Glutamine transport was less dependent on the presence of Na^+ , HCO_3^- or of cellular ATP than was the transport of alanine or serine.

The results presented in this paper are consistent with the existence of a minimum of three separate systems catalysing the transport of these three amino acids (Scheme 1). Transport system I catalyses the accumulation of alanine and serine. System II catalyses the accumulation of glutamine. Both systems I and II are dependent on Na^+ , HCO_3^- and ATP for their activity. System III catalyses the equilibration of alanine, serine and glutamine and is independent of Na^+ , HCO_3^- and ATP. It must be emphasized that the Na^+ -independent influx of all three amino acids represented by system III may be catalysed by more than one carrier. Identification of a single carrier system would require the demonstration of saturation kinetics and competitive interactions in the Na^+ -independent transport of the amino acids. At low concentrations alanine and serine are predominantly transported by system I, whereas the net transport of glutamine is shared equally between systems II and III. The precise number of transporting systems involved cannot be determined without the use of structural analogues, and the three systems shown in Scheme 1 represent the minimum number consistent with the data. It is probable that system I may be identical with the A and/or ASC systems that have been characterized in Ehrlich ascites cells (Oxender & Christensen, 1963). The limitation of analogue studies in predicting the actual rates of transport of naturally

Scheme 1. Minimum number of carrier systems required to account for alanine, serine and glutamine transport. For details see the text. Systems I and II are dependent on Na^+ , HCO_3^- and ATP. The symbol $\sim$ is intended to represent energy-linked accumulation. System III may in turn be composed of more than one transport system.

occurring amino acids is exemplified by the finding that the Na^+ -dependent accumulation of α -aminoisobutyrate in hepatocytes follows a much slower time-course and has a much higher apparent K_m than does that of alanine in the present studies (Le Cam & Freychet, 1977).

A new observation reported here is that the maximum rate of transport of alanine, serine and glutamine is dependent on the presence of HCO_3^- as well as Na^+ . This is not the case for the transport of the branched-chain amino acids, which are not accumulated by hepatocytes (McGivan *et al.*, 1977a). The rates of transport were also very sensitive to changes in the intracellular content of ATP (Fig. 6). In view of these findings, it would seem to be of importance to ensure that cell ATP contents are adequate and to avoid the use of organic buffers to replace bicarbonate when studying amino acid transport and metabolism in liver cells.

Alanine, serine and glutamine at a concentration of 10 mM are readily metabolized to glucose and urea in perfused liver (Ross *et al.*, 1967) and in isolated hepatocytes (Krebs *et al.*, 1976). The transport of alanine into perfused liver has been shown to be stimulated by glucagon (Mallette *et al.*, 1969), and the transport of α -aminoisobutyrate is stimulated by somatotropin (Jefferson *et al.*, 1975). In the present experiments the rate of transport of alanine into isolated hepatocytes was faster than its metabolism at all concentrations tested. Furthermore, the inhibition of alanine transport by ouabain did not lead to a corresponding inhibition of alanine metabolism. It therefore appears that the transport of alanine is not rate-limiting for its metabolism. It is

likely that similar considerations apply also to serine, which is metabolized more slowly than alanine but is transported at a comparable rate. Glutamine is not metabolized by isolated liver cells when added at a concentration of less than 2 mM in the absence of activators such as NH_4Cl and glucagon (Lund & Watford, 1976; Joseph & McGivan, 1978). Under these conditions glutamine is nevertheless transported into the hepatocyte, and the addition of NH_4Cl and glucagon, which stimulate glutamine metabolism, does not increase the rate of transport (Joseph & McGivan, 1978).

It has been observed previously in isolated cells (Joseph & McGivan, 1978) and in perfused liver (Chamalaun & Tager, 1970) that after the addition of glutamine, at both 2 mM and 10 mM, a lag period of 15–20 min ensues before a steady maximal rate of glutamine metabolism occurs. It is conceivable that this lag period represents the time required for the intracellular content of glutamine to rise to a value high enough to permit a rapid rate of glutamine hydrolysis by glutaminase I (EC 3.5.1.2).

It has been pointed out (see Krebs, 1972) that in general the K_m values of the enzymes that initiate amino acid degradation are much higher than the concentrations of amino acids present in plasma. One factor that regulates the rate of degradation of an amino acid in liver is the activity of the appropriate aminotransferase in the tissue. It appears that a second factor of importance may be the presence or absence of an active transport system for the amino acid in the plasma membrane. Thus alanine, serine and glutamine are actively accumulated in hepatocytes and these three amino acids are rapidly metabolized by liver.

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