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Mechanism of the Stimulation of Serine and Alanine Transport into Isolated Rat Liver Cells by Bicarbonate Ions

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1. Bicarbonate ions stimulate the transport of serine and alanine into isolated hepatocytes. 2. The effect of bicarbonate is to increase the $V_{max.}$ of the transport process without changing the apparent K_m. 3. The intracellular pH was estimated from the distribution of the weak base methylamine and the weak acid 5,5'-dimethyloxazolidine-2,4-dione (DMO) across the plasma membrane. 4. The addition of bicarbonate to a cell suspension caused the internal pH to become more acid. 5. The initial rate of serine, alanine and glycine transport was a linear function of the initial difference in pH across the membrane. 6. It is concluded that bicarbonate activates the transport of these amino acids primarily by increasing the pH difference across the plasma membrane. 7. It is suggested that the uptake of serine together with Na^+ ions occurs in exchange for H^+ ions, which are translocated outwards on the same carrier system. Some preliminary evidence consistent with this model is presented.

Neutral amino acids are transported across the plasma membrane of isolated liver cells by a number of specific carrier systems, which in general appear to have characteristics similar to those originally identified in Ehrlich ascites cells (Oxender & Christensen, 1963). The uptake of the amino acid analogue α-aminoisobutyrate is largely Na^+-dependent, and this compound is accumulated by liver cells, whereas the transport of cycloleucine occurs mainly via a Na^+-insensitive system (Le Cam & Freychet, 1977). Other aspects of the transport of amino acid analogues in isolated hepatocytes in suspension or in culture have been characterized (see Guidotti *et al.*, 1978).

It is of importance to determine also the characteristics of transport of naturally occurring amino acids, since these may be of significance in the regulation of amino acid metabolism in liver. It has been shown that branched-chain amino acids are transported into liver cells via a Na^+-independent system which does not lead to amino acid accumulation and is not energy-dependent (McGivan *et al.*, 1977; Seglen & Solheim, 1978), whereas alanine, serine and glutamine are accumulated by Na^+-dependent carrier systems which are also very dependent on the intracellular content of ATP (Joseph *et al.*, 1978). It was reported that the rate of transport of alanine and serine into isolated liver cells was much decreased if the bicarbonate in the incubation medium was replaced with an equal concentration of Mops (Joseph *et al.*, 1978).

Abbreviations used: Mops, 4-morpholinepropanesulphonic acid; DMO, 5,5'-dimethyloxazolidine-2,4-dione.

The transport of branched-chain amino acids was not bicarbonate-dependent. The mechanism by which bicarbonate ions stimulate the transport of certain amino acids into isolated liver cells is the subject of the present report.

Materials and Methods

Hepatocytes were isolated from the livers of normally fed male Wistar rats of about 300g weight by collagenase digestion as described by Berry & Friend (1969), with the modifications of Krebs *et al.* (1974). The cells were washed and resuspended either in Krebs–Henseleit bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), or in a modification of this medium in which the $NaHCO_3$ was replaced by an equal concentration of Mops (sodium salt) at pH 7.4. In each case, the medium also contained 2% (w/v) dialysed bovine albumin (fraction V). When the bicarbonate-buffered medium was used, this was equilibrated with a gas phase of O_2/CO_2 (19:1). The cells at a concentration of 30mg of cell protein/ml were stored on ice for up to 1 h before use. It is known that storage of cells on ice leads to changes in the distribution of ions across the cell membrane. Baur *et al.* (1975) have established that the ionic steady state of hepatocytes (as judged by measurement of membrane potential, pH gradient, intracellular volume and ATP concentration) is re-established after storage on ice by incubation at 37°C for 10min.

The cell suspension was preincubated at 37°C for 15min with 1mM-amino-oxyacetate. This procedure

has been shown to inhibit completely the metabolism of subsequently added alanine (Edmondson *et al.*, 1977) and serine (Joseph *et al.*, 1978) in this system. The inclusion of cycloserine as originally suggested by these authors was found to be unnecessary. Glutamine is not metabolized by hepatocytes over a period of 20min when added at a concentration of 0.5mM (Lund & Watford, 1976; Joseph *et al.*, 1978). Glycine is a very poor substrate for gluconeogenesis in isolated hepatocytes (results not shown) and it was assumed therefore that glycine metabolism was negligible over the 2min period used for the determination of initial rates. The transport of amino acids was initiated by the addition of a sample of the concentrated cell suspension to a small volume of the appropriate suspension medium containing ^{14}C-labelled amino acid at the required concentration together with ^{3}H-labelled inulin, which acted as a marker of the extracellular space. The transport reaction was terminated after the appropriate time by centrifuging a sample of the cell suspension through a layer of silicone oil into $HClO_4$ as previously described (McGivan *et al.*, 1977). The ^{14}C and ^{3}H radioactivities in the pellet and supernatant were assayed as described by Joseph *et al.* (1978). The amount of protein used in each determination was 2–3mg. Since no metabolism of the added amino acids occurs under these conditions, the uptake of amino acid was taken as the amount of labelled compound appearing in the inulin-impermeable space. The internal volume of the cells was determined in parallel experiments as the difference in volume occupied by [^{14}C]inulin and $^{3}H_2O$.

Cell protein was determined by a biuret method (Gornall *et al.*, 1949).

Estimation of intracellular pH in hepatocytes

In closed vesicles which are too small to permit the introduction of a pH electrode to measure intravesicular pH directly, the internal pH is usually estimated by measuring the equilibrium distribution of a weak acid or a weak base across the membrane. Intramitochondrial pH has been measured by using the weak acid (Addanki *et al.*, 1968), and also more recently acetate and methylamine (Nicholls, 1974) or D-lactate (Halestrap, 1978). The pH inside lysosomes has been estimated from the distribution of DMO and of various amines (Reijngoud *et al.*, 1976). The assumptions made in the use of this method for the determination of intravesicular pH have been discussed (Addanki *et al.*, 1968; Waddell & Bates, 1969). DMO distribution has been used to estimate the intracellular pH of perfused liver (Sies *et al.*, 1973) and Ehrlich ascites cells (Geck *et al.*, 1978). However, in a heterogeneous system containing several intracellular compartments, each of which may be of a different size and have a different internal pH, the interpretation of the results obtained by measuring the distribution of a weak acid or base is not straightforward.

In the present investigation, the distribution of [^{14}C]methylamine and [^{14}C]DMO across the inulin-impermeable liver cell plasma membrane was measured. The amount of un-ionized methylamine inside the cell may be assumed to be negligible, and for methylamine the following estimation of internal pH was made:

$$\text{Apparent } pH_i = pH_o - \log\left(\frac{[[^{14}C]\text{methylamine}]_i}{[[^{14}C]\text{methylamine}]_o}\right)$$

where the subscripts o and i represent the extracellular and intracellular media respectively.

For DMO, the internal and external concentrations of un-ionized DMO are not negligible and the apparent internal pH was taken as:

$$\text{Apparent } pH_i = pK + \log\left[\frac{[[^{14}C]DMO]_i}{[[^{14}C]DMO]_o} \cdot [1 + 10^{(pH_o - pK)}] - 1\right]$$

where $[DMO]_o$ and $[DMO]_i$ represent the sum of the concentrations of the charged and uncharged forms of DMO in the extracellular and intracellular compartments respectively (Addanki *et al.*, 1968), and pK represents the negative logarithm of the dissociation constant of DMO. This latter value was taken to be 6.13 at the ionic strength used and at 37°C (Waddell & Butler, 1959). The relation between the apparent pH values derived from the above equations and the cytoplasmic pH in the liver cells is examined in the Discussion section.

Materials

Collagenase was purchased from Boehringer Corp., Mannheim, Germany, and bovine albumin (fraction V) was from Sigma Chemical Co., St. Louis, MO, U.S.A. ^{14}C-labelled inulin, DMO, alanine, serine, glycine, glutamine, ^{3}H-labelled inulin and H_2O, and $^{22}NaCl$ were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

Results

Stimulation by bicarbonate of amino acid transport in isolated liver cells

Fig. 1(*a*) shows the time course of the uptake of 1mM-serine into liver cells suspended either in Krebs–Henseleit bicarbonate buffer (bicarbonate-containing medium) or in a modification of this medium where the bicarbonate was replaced by

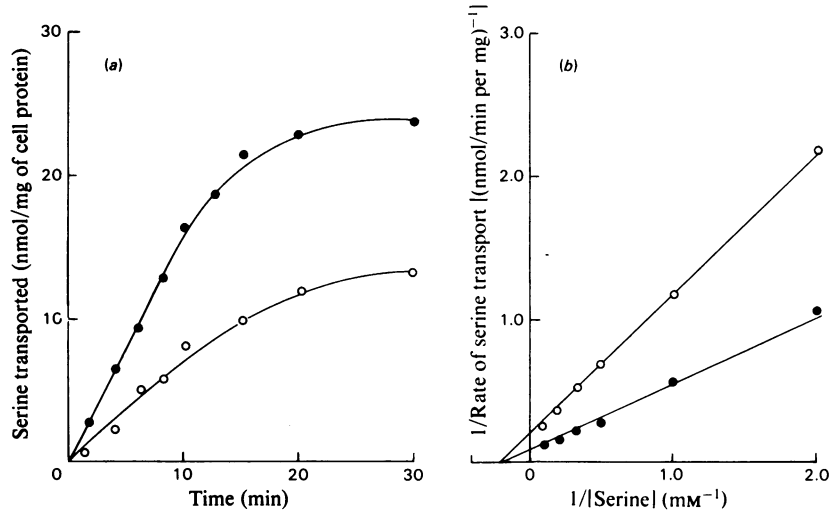

Fig. 1. *Effect of bicarbonate ions on serine transport in liver cells*

Hepatocytes were preincubated as described in the Materials and Methods section. [^{3}H]Inulin (50μg/ml) was added, and in (*a*) transport was initiated by the addition of 1mM-serine and the uptake of serine was measured at the times shown. In (*b*), serine was added at concentrations between 0.5 and 15mM. The initial rate of transport was calculated from the uptake measured 1 and 2min after the addition of serine. The external pH was 7.4 throughout. ●, Bicarbonate medium; ○, bicarbonate-free medium.

Table 1. *Activation of serine transport by bicarbonate ions*

Cells were preincubated in Krebs–Henseleit buffer containing 25mM-Mops (sodium salt) instead of 25mM-$NaHCO_3$. [^{3}H]Inulin was then added, followed by small volumes of 1M-$NaHCO_3$ to give final bicarbonate concentrations between 0 and 20mM as shown. Then 1mM-[^{14}C]serine was added, and the initial rate of transport was measured by taking samples after 1 and 2min. The initial pH of the medium was 7.40. The addition of bicarbonate caused a further alkaline pH change, which, in the case of the addition of 20mM-bicarbonate, increased the pH to 7.65.

[Bicarbonate] (mM)	Initial rate of serine transport (nmol/min per mg of cell protein)
0	0.57
1	0.69
2	0.80
5	0.96
10	1.18
15	1.45
20	1.61

25mM-Mops (sodium salt) (bicarbonate-free medium). The pH of the medium in each case was 7.4. The initial rate of transport was considerably faster in the bicarbonate-containing medium, in agreement with previous results (Joseph *et al.*, 1978). The concentration-dependence of the initial rate of transport on serine concentration is shown in Fig. 1(*b*). The maximum rate of transport in the presence of bicarbonate was approx. 13nmol/min per mg of cell protein, and the corresponding value in the bicarbonate-free medium was 5.1nmol/min per mg; the apparent K_m for serine was approx. 5mM in each case. The effect of bicarbonate is therefore to increase the maximum velocity of serine transport without affecting the apparent K_m. Similar results were obtained for the transport of alanine (results not shown).

The replacement of bicarbonate ions by Mops in the incubation medium decreased the intracellular content of ATP by less than 10% (results not shown), indicating that the decreased rate of serine transport in the absence of bicarbonate was not due to non-specific cell damage by the Mops buffer used. This conclusion was substantiated by the experiment shown in Table 1. Here, cells were suspended in a medium containing 25mM-Mops (sodium salt) and bicarbonate was then added. The rate of transport increased on the addition of bicarbonate to a value comparable with that obtained in the normal bicarbonate-buffered medium, indicating that Mops is not an inhibitor of amino acid transport.

In this type of experiment, the Na^+ concentration was increased from 145mM to a maximum of 165mM by the addition of $NaHCO_3$. The increase in rate observed was not due to an increase in Na^+ concentration, since in similar experiments (results not

shown) it was found that serine transport was not activated by the weak acids formate, acetate, butyrate, propionate or DMO when these were added separately as Na^+ salts at a concentration of 20mM. These experiments indicate also that the stimulation of amino acid transport is relatively specific for bicarbonate ions.

Several possibilities exist for the mechanism of stimulation of alanine and serine transport by bicarbonate ions. The transport system itself or the ATP-dependent outwardly directed Na^+ pump may be directly activated by this compound. Alternatively, the effect of bicarbonate ions may be secondary to a change in intracellular pH brought about by the transport of HCO_3^- as H_2CO_3 or as H_2O plus CO_2 if the transport of amino acids were linked in some way to the movement of H^+ ions. It was therefore necessary to obtain an estimate of the intracellular pH.

Table 2. *Concentration-dependence of the distribution of DMO and methylamine across the liver-cell plasma membrane*
After preincubation in bicarbonate medium, [^{3}H]inulin (50μg/ml) was added to the cell suspension followed by the appropriate concentration of [^{14}C]DMO or [^{14}C]methylamine. The distribution of DMO and methylamine was measured after a further period of 10min. The pH of the cell suspension was 7.45. The apparent internal pH was calculated as described in the Materials and Methods section. The subscripts 'i' and 'o' represent internal and external concentrations respectively.

Concn. of DMO or methylamine (mM)	Ratio $\frac{[DMO]_i}{[DMO]_o}$	Apparent pH_i	$\frac{[Methylamine]_i}{[methylamine]_o}$	Apparent pH_i
0.01	0.47	7.10	11.63	6.38
0.025	0.39	7.00	11.60	6.39
0.05	0.43	7.05	11.64	6.38
0.10	0.43	7.05	11.78	6.38
0.20	0.41	7.03	10.54	6.43
0.50	0.46	7.09	8.49	6.52
1.00	0.43	7.05	6.94	6.61

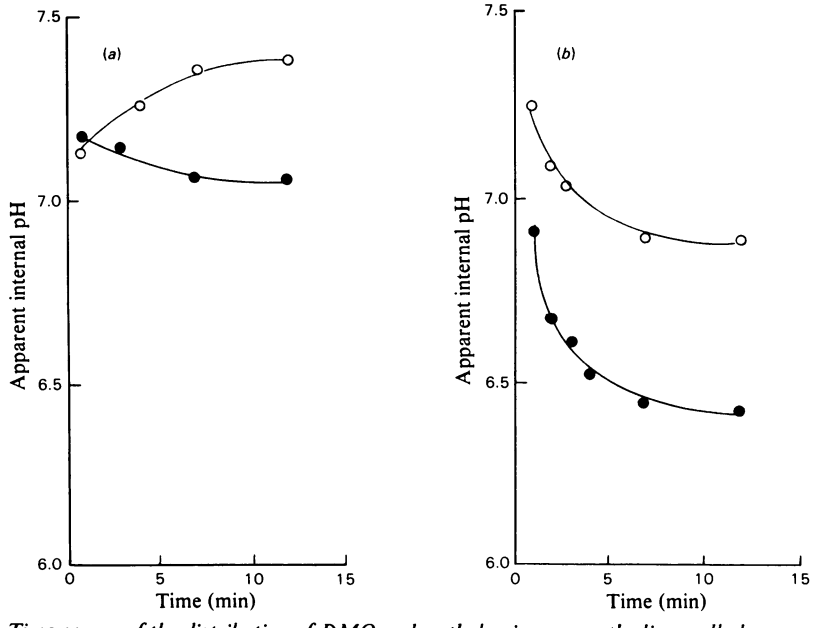

Fig. 2. *Time course of the distribution of DMO and methylamine across the liver-cell plasma membrane*
After preincubation of the cells, [^{3}H]inulin (50μg/ml) was added together with in (*a*) 0.5mM-[^{14}C]DMO and in (*b*) 0.01mM-[^{14}C]methylamine. The distribution of the labelled compounds across the membrane was measured after the times shown, and the apparent internal pH was calculated as described in the Materials and Methods section. ●, Bicarbonate medium; ○, bicarbonate-free medium.

Estimation of intracellular pH in liver cells

The distribution of the base methylamine and the weak acid DMO across the liver-cell plasma membrane was measured and the apparent intracellular pH was calculated as described in the Materials and Methods section. Table 2 shows that the apparent internal pH measured by DMO distribution was independent of the DMO concentration in the range 0–1 mM-DMO. With methylamine, a constant value was obtained only in the range 0–0.1 mM-methylamine. The apparent pH measured by using methylamine was considerably more acid than that calculated from the distribution of DMO. This is to be expected, since, as discussed below, methylamine will accumulate in compartments of the cell which are more acid than the cytosol (e.g. lysosomes). The time courses of the distribution of methylamine and DMO across the plasma membrane at pH 7.4 in media containing either 25 mM-bicarbonate or 25 mM-Mops as buffer are shown in Fig. 2. A constant value in each case was obtained after an interval of 5–10 min. With DMO as indicator, it was observed that the apparent internal pH was lower by 0.3–0.4 pH unit when the medium contained bicarbonate than when bicarbonate was absent. A similar result was obtained with methylamine, although the absolute values of apparent internal pH calculated from methylamine distribution were lower than those calculated from the distribution of DMO, in agreement with Table 2.

These experiments were extended to determine the apparent internal pH of the liver cell at various values of external pH in the presence and absence of bicarbonate. Fig. 3 shows the values of internal and external pH observed in a series of experiments with 12 different batches of cells. In the absence of bicarbonate, the internal pH measured by DMO distribution increased from 7.25 to 7.70 as the external pH was raised from 7.0 to 8.0. In the presence of an added concentration of 25 mM-bicarbonate, the internal pH was lower throughout this range, the values at external pH values of 7.0 and 8.0 being 6.75 and 7.5 respectively. Again, the calculation of internal pH from methylamine distribution gave values that were lower than those calculated from the distribution of DMO. However, in this case also, the apparent internal pH was lower in the presence of bicarbonate than in its absence.

It was concluded from these results that methylamine is unlikely to give a true estimation of cytosolic pH because of the fact that it will accumulate in acidic compartments in the cell. DMO distribution will give an approximate estimate of the pH in the cytosol, as discussed below. However, both DMO and methylamine can be used to indicate changes in the pH of the cytosol. There appears to be no doubt that the omission of bicarbonate from the incubation medium causes the internal pH to become relatively more alkaline at all values of external pH between 7 and 8. Sies *et al.* (1973) have noted a similar effect of bicarbonate on the intracellular pH of perfused liver as measured by DMO distribution.

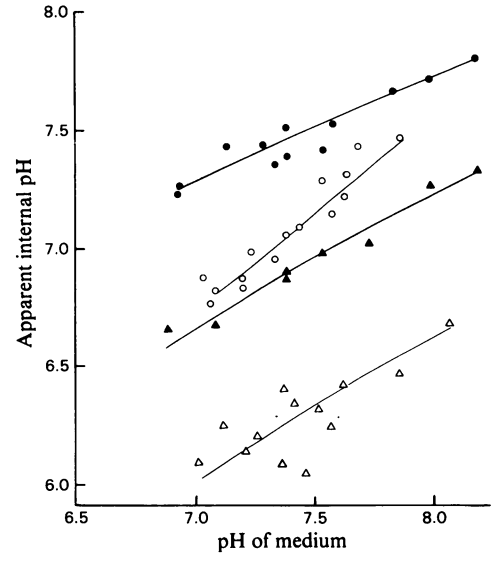

Fig. 3. *Apparent internal pH as a function of external pH as measured by the distribution of DMO and methylamine in the presence and absence of bicarbonate*

Cells were preincubated in either a bicarbonate medium or the bicarbonate-free medium. The pH of the cell suspension was adjusted by the addition of a small volume of 3 M-NaOH or 3 M-HCl. [^{3}H]Inulin was added together with 0.5 mM-[^{14}C]DMO or 0.01 mM-[^{14}C]methylamine. After a further 10 min, the pH of the suspension was measured and samples were withdrawn for the determination of the apparent internal pH. The data were obtained from experiments on 12 different batches of cells. ●, Internal pH calculated from distribution of DMO (bicarbonate-free medium); ○, internal pH calculated from distribution of DMO (bicarbonate medium); ▲, internal pH calculated from distribution of methylamine (bicarbonate-free medium); △, internal pH calculated from distribution of methylamine (bicarbonate medium).

Relationship between amino acid transport and intracellular and extracellular pH

In a series of experiments, hepatocytes were incubated at different values of external pH between 7 and 8 in the presence or absence of bicarbonate. The initial rate of transport of 1 mM-serine was determined and the distribution of [^{14}C]DMO was simultaneously measured in a parallel incubation. The intracellular ATP content was unaltered by this procedure (results not shown). Values of apparent

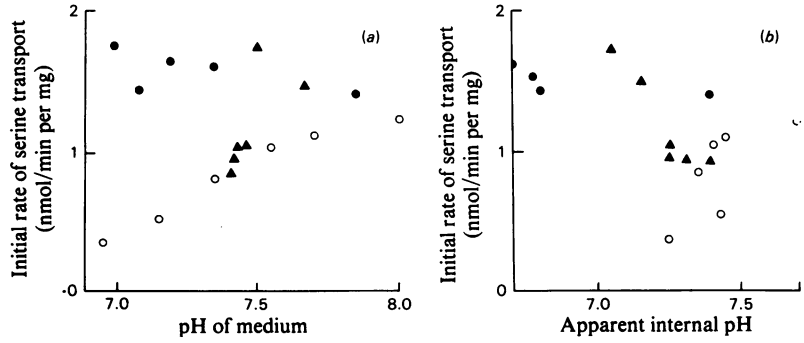

Fig. 4. *Effect of varying the external pH on the initial rate of serine transport in the presence and absence of bicarbonate*
Hepatocytes were preincubated, [^{3}H]inulin was added and the pH of the suspension was adjusted by adding small volumes of 3M-NaOH or 3M-HCl. In some experiments where cells were preincubated in a bicarbonate-free medium, small volumes of 1M-$NaHCO_3$ were added to produce final bicarbonate concentrations between 1 and 20mM. The cell suspensions were divided into two equal portions. To one half was added [^{14}C]DMO (0.5mM), and after a further 10min, DMO distribution was measured in these samples. The other half of the suspension was incubated with unlabelled DMO (0.5mM) for 10min; 1mM-[^{14}C]serine was then added and the uptake was measured after 1 and 2min to obtain the initial rate. The pH of the suspension was measured at the same time. The rate is plotted against the external pH in (*a*) and against the calculated values of internal pH in (*b*). The data were obtained from six separate batches of cells. ○, Bicarbonate-free medium; ●, Krebs–Henseleit bicarbonate buffer; ▲, cells preincubated in bicarbonate-free medium and various concentrations of bicarbonate then added. The concentrations of bicarbonate used in this type of experiment were 1, 2, 5, 10, 15 and 20mM; the initial rate of serine transport increased with increasing bicarbonate concentration (see Table 1).

internal pH were calculated as described in the Materials and Methods section.

Fig. 4(*a*) shows the dependence of the initial rate of serine transport on the pH of the incubation medium. In the absence of bicarbonate, the rate of serine transport increased from 0.35nmol/min per mg at pH6.95 to 1.30nmol/min per mg at pH8.0. The rate was higher at all values of external pH if bicarbonate (25mM) was also present. At intermediate concentrations of bicarbonate added to a medium already containing 25mM-Mops, the rates obtained were intermediate between those in the presence and absence of 25mM-bicarbonate. In this case, the addition of bicarbonate to the Mops medium increased the rate of transport markedly, but had little effect on the external pH. These results indicate that the rate of serine transport is not a simple function of extracellular pH.

Fig. 4(*b*) shows data from the same experiments with the rate of transport now plotted against calculated values of internal pH. The rate of transport increases with increasing internal pH in the absence of bicarbonate, but at any value of internal pH the rate is faster in the presence of bicarbonate, indicating that the effect of bicarbonate is not simply due to a change in the intracellular pH.

In Fig. 5 the same data are plotted with the calculated difference in pH ($pH_o - pH_i$) as the abscissa. A linear relation is obtained between the initial rate of transport and the apparent pH difference across the

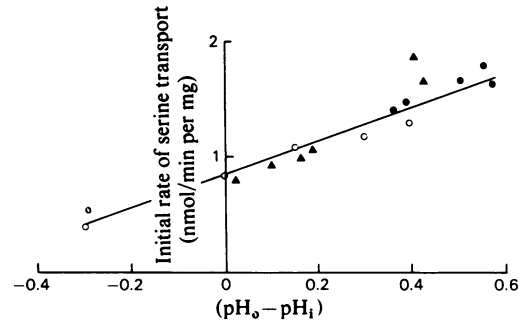

Fig. 5. *Relationship between the rate of serine transport and the pH difference across the plasma membrane*
The data of the experiments shown in Fig. 4 are replotted. The abscissa is the calculated difference in apparent internal pH derived from the distribution of DMO across the plasma membrane. The symbols have the same significance as in Fig. 4.

membrane. This relationship is independent of the presence of bicarbonate, since the values obtained at all concentrations of bicarbonate at all tested values of external pH lie on the same line. It follows that the stimulation of serine transport by bicarbonate can be correlated with the increase in the pH difference that occurs on addition of bicarbonate to a cell suspension. It may be noted that a similar linear relationship

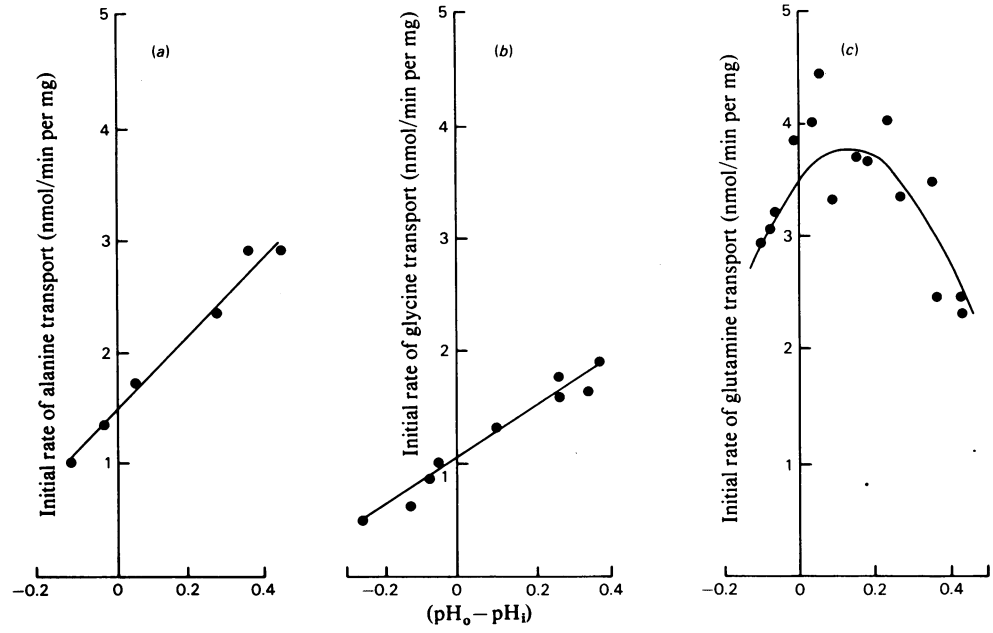

Fig. 6. *Initial rate of alanine, glycine and glutamine transport as a function of the pH difference across the plasma membrane*
Hepatocytes were preincubated in bicarbonate-free medium. [^{3}H]Inulin was added together with the appropriate volumes of 1 M-$NaHCO_3$ to give final bicarbonate concentrations between 0 and 25 mM. The distribution of DMO and the initial rates of uptake of 1 mM-alanine (*a*), 1 mM-glycine (*b*) and 1 mM-glutamine (*c*) were measured as described in the legend to Fig. 4.

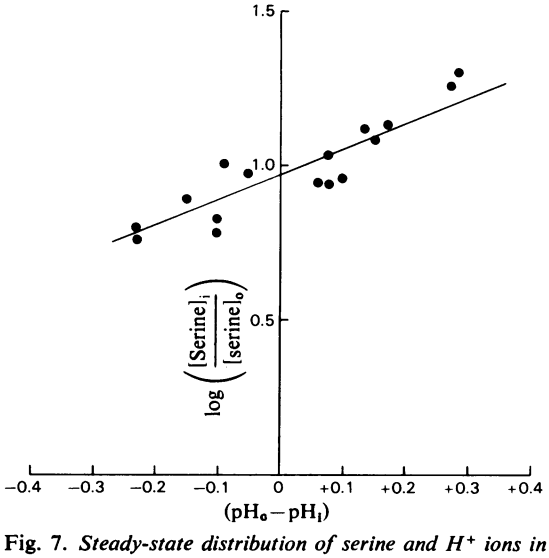

Fig. 7. *Steady-state distribution of serine and H^+ ions in isolated hepatocytes*
After preincubation in bicarbonate-free medium, the pH of the cell suspension was adjusted and bicarbonate at various concentrations was added as described in the legend to Fig. 4. [^{3}H]Inulin was added and the cell suspension was divided into two portions. [^{14}C]Serine (0.5 mM) and unlabelled DMO (0.5 mM) were added to one sample. [^{14}C]DMO (0.5 mM) and unlabelled serine (0.5 mM) were added to the other. After a further 20 min, the distribution of serine and DMO were measured in these parallel incubations. It has been shown previously that after preincubation with amino-oxyacetate, no metabolism of serine occurs during a 20 min incubation period when serine is present at a concentration of 0.5 mM (Joseph *et al.*, 1978).

between the rate of serine transport and the pH difference across the plasma membrane is observed when the internal pH is calculated from the distribution of methylamine (results not shown).

Fig. 6 shows that a similar relationship between the rate of transport and the pH difference obtains also for the transport of alanine and glycine, but not for that of glutamine, which is relatively insensitive to the pH difference. Evidence has been presented previously that alanine and serine are transported on the same carrier system, whereas glutamine is transported mainly on a separate system. Glutamine transport is also much less stimulated by bicarbonate (Joseph *et al.*, 1978).

One interpretation of the dependence of the rate of serine transport on the pH gradient across the membrane is that the transport of serine requires the simultaneous outward transport of H^+. If this is the

case, a relationship should exist between the distribution of serine across the membrane in the steady state and the pH gradient under these conditions. In a series of experiments, cells were incubated with 1mM-serine at 37°C, and the distributions of serine, DMO and $^{22}Na^+$ were measured after 20min when the intracellular concentration of serine had reached a maximum value. The results obtained from experiments with six separate batches of cells are shown in Fig. 7. A linear relation between the logarithm of the serine distribution and the apparent pH difference was obtained (correlation coefficient = 0.892). The slope of the line was 0.87, and the intercept on the ordinate was 0.973. The mean value of $[Na^+]_o/[Na^+]_i$ in these experiments was 7.50 ± 0.62 (mean ± S.E.M. for 20 observations), and this value was independent of external pH in the range 7.0–7.6 and was also independent of the presence or absence of bicarbonate ions. The possible significance of these results is considered below.

Discussion

The results presented above show that the initial rate of transport of alanine, serine and glycine into isolated liver cells is a linear function of the pH difference across the plasma membrane as measured by the distribution of methylamine or DMO (Figs. 5 and 6). The use of this approach for the measurement of pH gradients assumes that no metabolism of these compounds occurs, that the charged form of the indicator molecule does not penetrate the membrane, and that the dissociation constants of the weak acid or base are the same inside and outside the cell (see Addanki *et al.*, 1968). These assumptions appear to be valid for DMO and amine distribution in mitochondria (Addanki *et al.*, 1968) and lysosomes (Reijngoud *et al.*, 1976). In liver cells it is not easy to obtain an independent measurement of the cytosolic pH, which alone could validate these assumptions. However, the fact that DMO distribution is independent of DMO concentration (Table 2) indicates that no significant metabolism of this compound occurs, and a similar argument is applicable to the distribution of methylamine at the concentration used.

Geck *et al.* (1978) have discussed the problems associated with the use of weak acids and bases to estimate intracellular pH in cells containing more than one intracellular compartment. In a hypothetical cell containing two compartments of equal volume but differing pH, the distribution of DMO across the cell membrane will reflect mainly the pH of the more-alkaline compartment, whereas that of methylamine reflects the pH of the more-acidic compartment where it accumulates. For the liver cell, it may be expected that the distribution of methylamine across the plasma membrane will indicate a pH value intermediate between that of the cytosol (which is the largest compartment) and the lysosomes, which represent the most acidic compartment where methylamine will accumulate. The internal pH of isolated lysosomes is approx. 6.0 at an external pH of 7.4 (Reijngoud *et al.*, 1976). DMO will accumulate in relatively alkaline compartments and will be excluded from lysosomes. There is some uncertainty about the intramitochondrial pH in the liver cell. This value has, however, been estimated to be 0.3 pH unit higher than that of the cytoplasm (Tischler *et al.*, 1977). DMO distribution should therefore indicate a value intermediate between that of the cytosol and that of the mitochondrial matrix. Since the pH difference between these compartments is relatively small and the volume of the cytosol is much larger than the intramitochondrial volume, it may be concluded that DMO distribution indicates a pH value which approximates closely to that of the cytosol. Methylamine distribution, however, should indicate a pH value considerably more acid than that of the cytosol. The fact that the apparent internal pH measured by methylamine distribution changes in parallel with that measured by DMO distribution (Fig. 3) when the external pH is varied suggests that changes in the distribution of both DMO and methylamine can be taken as a measure of changes in cytoplasmic pH.

With the reservations discussed above, it can be concluded that the addition of bicarbonate ions to isolated cells suspended in a bicarbonate-free medium causes the cytoplasmic pH to become more acid. Since in both the presence and the absence of bicarbonate the rate of transport of alanine, serine and glycine is a function of the pH difference across the cell membrane as measured by the distribution of either DMO or methylamine (Fig. 5), it follows that the stimulatory effect of bicarbonate on the transport of these amino acids is due primarily to an increase in the transmembrane pH difference caused by bicarbonate addition. The acidification of the cytosol by the addition of bicarbonate ions can be readily understood if this compound penetrates the cell membrane primarily in the form of CO_2 or H_2CO_3, since this process leads to the net influx of H^+ ions. Although there appears to be no direct evidence that HCO_3^- ions cross the plasma membrane in this way, the HCO_3^- anion is known to be unable to cross the mitochondrial membrane (Chappell & Crofts, 1966), whereas CO_2 is a small neutral molecule which readily crosses biological membranes.

The possibility that bicarbonate ions stimulate either the amino acid-transporting systems or the Na^+ pump itself is not directly eliminated by the present evidence. However, the postulation of such a direct activating effect of bicarbonate is unnecessary to explain the present results.

On the basis of the results in the present paper, a preliminary model for the mechanism of alanine,

serine and glycine transport into rat liver cells may be proposed. It is assumed that these amino acids are transported together with Na^+ ions according to the gradient theory (Crane, 1962), and that the energy for active transport is derived from the Na^+ gradient, which is maintained by an outwardly directed ATP-dependent Na^+ pump. It is proposed here that the transfer of charge across the membrane as a result of the movement of amino acid and Na^+ is partially compensated by the simultaneous outward transport of H^+ ions. It is proposed also that, at the initial external concentrations of serine and Na^+, the initial rate of transport is limited by the difference in H^+ concentration across the cell membrane.

In the steady state, the rates of inward and outward transport are equal. The above model predicts that:

$$[Na^+]_o^x \cdot [\text{serine}]_o \cdot [H^+]_i^y = [Na^+]_i^x \cdot [\text{serine}]_i \cdot [H^+]_o^y$$

and:

$$\log\left(\frac{[\text{serine}]_i}{[\text{serine}]_o}\right) = x \log\left(\frac{[Na^+]_o}{[Na^+]_i}\right) + y\,(pH_o - pH_i)$$

where the subscripts i and o represent intracellular and extracellular concentrations respectively.

In Fig. 7, it is shown that there is a linear relationship between $\log([\text{serine}]_i/[\text{serine}]_o)$ and the pH difference as predicted by this equation if the value of $[Na^+]_o/[Na^+]_i$ is independent of the pH difference. This was found to be the case within experimental error over the pH range used. Taking the values for the slope and intercept derived from Fig. 7 together with the measured ratio $[Na^+]_o/[Na^+]_i = 7.5$, values of 0.87 and 1.10 for y and x respectively are obtained.

These data are compatible with a mechanism in which the inward transport of serine is accompanied by the inward transport of approx. 1 Na^+ ion and the simultaneous outward transport of approx. 1 H^+ ion. Assessment of this preliminary model and data on its general applicability will require simultaneous measurements of Na^+ and H^+ movements during amino acid transport.

Note Added in Proof (Received 6 July 1979)

It has been shown (van Amelsvoort *et al.*, 1978) that isolated plasma-membrane vesicles from rat liver take up alanine in a Na^+-dependent manner. This alanine uptake is stimulated by CNS^- ions, suggesting that Na^+–alanine symport in liver is an electrogenic process. These findings are not inconsistent with those in the present paper if a system exists in the liver plasma membrane for the electrogenic uptake of HCO_3^-. The entry of one alanine molecule together with 1 Na^+ and 1 HCO_3^- ion is equivalent to the uptake of alanine with the overall net exchange of 1 Na^+ for 1 H^+, owing to the subsequent exit of bicarbonate with a proton in the form of H_2CO_3 or CO_2 plus H_2O.

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