Amino acid transport by resealed ghosts from pigeon erythrocytes

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Resealed ghosts from pigeon erythrocytes were shown to haemolyse during incubation in isotonic media with pH values greater than about 7, and high concentrations of Na⁺ inside the ghosts seemed to enhance this effect. At lower pH values the ghosts were stable but still highly permeable to Na⁺ and K⁺, and moderately permeable to sucrose. Under the latter conditions the ghosts transported amino acids in a way qualitatively but not quantitatively similar to intact erythrocytes. The Na⁺-dependent transport of serine and alanine by the ghosts consisted essentially of an exchange of extracellular for intracellular amino acids, with no significant net flux. In contrast, net fluxes of glycine in the direction of the Na⁺-concentration gradient across the ghost membrane were demonstrated. However, under one condition a small net influx of glycine occurred against the prevailing Na⁺-concentration gradient. Unlike Na⁺-dependent glycine uptake, the uptake of six other amino acids by intact pigeon erythrocytes was not influenced by the nature of the anion present. The significance of these findings in relation to previous work on the Na⁺-gradient hypothesis of membrane transport is discussed.

Na⁺-dependent uptake of amino acids and sugars by various cells and tissues has given rise to the 'sodium-gradient' theory of membrane transport (reviewed by Eddy, 1975; Crane, 1977). Some of the most convincing evidence supporting this concept derives from the work of Vidaver and his colleagues on the transport of glycine by both intact pigeon erythrocytes and resealed ghosts from these cells (Vidaver, 1964*a,b,c,d*, 1971; Vidaver *et al.*, 1964, 1976; Vidaver & Shepherd, 1968; Imler & Vidaver, 1972; Terry & Vidaver, 1972, 1973). This transport system, however, is almost certainly peculiar to glycine, and several other amino acids are transported in the pigeon erythrocyte via Na⁺-dependent processes that have different characteristics from the glycine system (Wheeler & Christensen, 1967; Eavenson & Christensen, 1967; Christensen, 1975). Indeed, work with intact cells led to the suggestion that the Na⁺-dependent transport of most amino acids other than glycine by pigeon erythrocytes occurs via obligatory exchanges of extracellular for intracellular amino acids, the actual Na⁺-concentration gradient being unimportant (Wheeler & Christensen, 1967). Although that proposal seemed to be testable with the use of the resealed-erythrocyte-ghost system, the advantages of the latter had

been applied only to the study of the glycine system, and no general description of the properties of resealed ghosts, in comparison with those of intact erythrocytes, has been reported. The results presented here attempt to do this, with the use of a few amino acids selected as representative of different classes as far as transport is concerned (Christensen, 1975).

Materials and methods

Incubation media

Two standard incubation media were used, an Na⁺ medium and a K⁺ medium. Both contained 2 mM-MgSO₄ and 20 mM-imidazole and their pH values were adjusted by titration with HCl at about 20°C, as indicated in the legends to Figures and Tables. The Na⁺ medium also contained 140 mM-NaCl plus 5 mM-KCl, whereas the K⁺ medium contained 145 mM-KCl. In experiments concerning the effects of anions, similar media were prepared with the use of Na⁺ and K⁺ salts of other acids, as indicated in the legend, and the pH of these media was adjusted with acetic acid instead of HCl.

Intact erythrocytes

Pigeon erythrocytes were collected as described previously (Wheeler & Christensen, 1967) in the appropriate isotonic medium containing heparin as

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anticoagulant and washed two or three times, with removal of white cells, by sedimentation (about 3000 *g* for 5 min at 4°C) and resuspension. The final suspension was made with 1 vol. of packed cells plus 0.5 vol. of medium, and this mixture was kept on ice until used.

Resealed ghosts

Washed and packed erythrocytes (1 vol.) were suspended in 1 vol. of 165 mM-KCl or -NaCl, as required, and then 20 vol. of lysing solution was added with gentle mixing. All solutions were maintained at 0–4°C. The lysing solution contained 4 mM-MgSO₄ plus 10 mM-imidazole/acetic acid (pH 6 at 20°C). The mixture was stirred gently on ice for 5 min and then 1 vol. of either 3 M-KCl or 3 M-NaCl was added. The suspension was transferred to a water bath at 38°C, where it was gently shaken for 45–60 min. The resealed ghosts were sedimented by centrifugation at about 3000 *g* and 4°C for 15 min, and then washed twice by resuspension and sedimentation in either Na⁺ medium, K⁺ medium or 165 mM-choline chloride. When ghosts containing an amino acid were required the latter was added at the appropriate concentration to the lysing solution. (The required concentration had to be found by trial and error, because the final concentration in the resealed ghosts was invariably slightly lower than that in the lysing solution.) The resealed ghosts were kept on ice and were always used for experiments immediately after they were prepared. (For clarity, ghosts containing K⁺ as the main cation will be termed K⁺-ghosts, and those containing mainly Na⁺, Na⁺-ghosts.)

Incubations

Amino acid fluxes were measured by incubating the erythrocytes or ghosts in the appropriate media containing ¹⁴C-labelled amino acids at 38°C, as described by Wheeler & Christensen (1967) except that (a) 0.3 ml of cell suspension was added to 3 ml of incubation medium, (b) for the final sedimentations intact cells were centrifuged at 3000 *g* for 5 min and ghosts for 15 min and (c) the proportion of extracellular fluid associated with the sedimented cells or ghosts was determined with the use of [¹⁴C]sucrose. The use of special racks to hold syringes, flasks and tubes enabled five simultaneous incubations to be made (Eavenson & Christensen, 1967). Where necessary trichloroacetic acid was added to all samples analysed by scintillation spectroscopy so that quenching was constant throughout. Na⁺ and K⁺ concentrations in the cell extracts were measured by flame photometry with the use of a Unicam SP-90 flame photometer operated in the emission mode.

Chemicals

[U-¹⁴C]-labelled L-amino acids and [U-¹⁴C]-sucrose were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were supplied by the Sigma (London) Chemical Co., or by BDH, Poole, Dorset, U.K., and were of AnalaR grade when possible. All solutions were prepared with water that had been distilled twice from a glass still. Only the L-isomers of amino acids were used.

Results

Properties of resealed ghosts

Initial attempts to make ghosts by the method described by Vidaver (1964*b*) gave unsatisfactory results and the procedure finally adopted was arrived

Fig. 1. Effects of pH and cations on the integrity of resealed ghosts

Resealed Na⁺-ghosts and K⁺-ghosts were prepared as described in the text and then incubated for 2 h at 38°C in both Na⁺ media and K⁺ media at different pH values. The indicated pH values were those measured at 20°C, to conform to the standard preparative procedure. The extent of lysis of the ghosts was then quantified by measurement of the concentration of haemoglobin in the clear supernatant fluids (Bull, 1964) obtained by centrifugation of the suspension at 3000 *g* for 30 min. The concentration of haemoglobin found in the supernatant from Na⁺-ghosts incubated in Na⁺-medium at pH 7.8 was set at a value of 1, and all other values were expressed relative to that. ●, Na⁺-ghosts incubated in Na⁺ medium; ○, Na⁺-ghosts incubated in K⁺ medium; ▲, K⁺-ghosts incubated in Na⁺ medium; △, K⁺-ghosts incubated in K⁺ medium.

at by trial and error after taking into account the extensive work that has been done with human erythrocyte ghosts (Bodemann & Passow, 1972; Lepke & Passow, 1972; Schwoch & Passow, 1973; Romero, 1974). We found that both vigorous mechanical stirring and the inclusion of Ca^{2+} at the lysing step (Vidaver, 1964b) tended to produce fragile and leaky ghosts. Also, the resealed ghosts were easily sedimented at about 3000g, in contrast with those prepared from human erythrocytes, presumably because of the nucleus in the pigeon cells. However, the main peculiar property of the pigeon erythrocyte ghosts was their susceptibility to lysis as a function of both the pH and the cation compositions of the solutions used. Fig. 1 shows the effects of the pH and the cation composition of the incubation media on the extent of haemolysis of

Fig. 2. *Cation concentrations in erythrocytes and resealed K⁺-ghosts during incubation in Na⁺ medium* Washed pigeon erythrocytes and resealed K⁺-ghosts prepared from them were incubated at 38°C in Na⁺ medium that had been adjusted to pH6.8 at 20°C. In some experiments an amino acid was present during the incubation, but no effects of the amino acids on the cation contents of the cells or ghosts were detected. The concentrations of Na⁺ and K⁺ in acid extracts of the sedimented cells and ghosts were measured as described in the text. Mean values ($\pm$ S.E.M.) from four to ten experiments are given. ■, Concentration of Na⁺ in intact erythrocytes; □, concentration of K⁺ in intact erythrocytes; ▲, concentration of Na⁺ in K⁺-ghosts; △, concentration of K⁺ in K⁺-ghosts.

resealed ghosts. At pH values above about 7 the ghosts haemolysed rapidly when incubated in Na⁺-medium and significantly, but more slowly, in K⁺-medium. Moreover, ghosts prepared with Na⁺ inside were consistently more fragile than those prepared with K⁺ inside. To overcome this effect all further experiments with resealed ghosts were carried out with incubation media buffered to pH6.8.

Although the ghosts usually did not haemolyse during incubations at pH6.8, they were much more permeable to Na⁺ and K⁺ than were intact erythrocytes. Fig. 2 shows how the intracellular concentrations of Na⁺ and K⁺ in both intact cells and K⁺-containing ghosts varied during incubation in Na⁺ medium, and Fig. 3 shows the corresponding values for Na⁺-containing ghosts incubated in K⁺ medium. Clearly both kinds of ghosts were relatively permeable to both Na⁺ and K⁺, but the Na⁺-ghosts seemed to be slightly more permeable than the K⁺-ghosts.

The resealed ghosts were also significantly permeable to sucrose. For example, when intact cells and K⁺-ghosts were incubated at 38°C for 60 min in

Fig. 3. *Concentrations in resealed Na⁺-ghosts during incubation in K⁺ medium*

Details are as described in the legend to Fig. 2, except that only Na⁺-ghosts were incubated in K⁺ medium. Mean values ($\pm$ S.E.M.) from four experiments are given. ●, Na⁺ concentration in Na⁺-ghosts; ○, K⁺ concentration in Na⁺-ghosts.

media buffered at pH 6.8 and containing 1.7 mM-sucrose, the distribution ratios (intracellular/extracellular concentration) were 0.24 and 0.28 for K^+ -ghosts in Na^+ medium and K^+ medium respectively, but only 0.006 for intact cells in both media. This meant that when measurements of extracellular fluid were made, it was essential to sediment the ghosts immediately after adding them to the medium containing $[^{14}C]$ sucrose. The average values for extracellular fluid were about 0.1 and 0.2 (ml per g wet wt. of cells) for intact cells and ghosts respectively.

Uptake of amino acids by resealed ghosts and intact cells

The uptakes of glycine, serine and leucine, from both Na^+ medium and K^+ medium, were measured with intact erythrocytes, K^+ -ghosts and K^+ -ghosts containing 5 mM unlabelled amino acid. The Na^+ -dependent influx of glycine was about 3–4 times faster in intact cells than in K^+ -ghosts, whereas the

Fig. 4. Uptake of glycine by intact erythrocytes and resealed K^+ -ghosts

Either intact erythrocytes or resealed K^+ -ghosts were incubated at $38^\circ C$ in Na^+ - and K^+ -media (pH 6.8) containing labelled 0.5 mM-glycine and the uptake of glycine was measured as described in the text. ■, Intact cells in Na^+ medium; □, intact cells in K^+ medium; ▲, K^+ -ghosts in Na^+ medium; △, K^+ -ghosts in K^+ medium. The values for the K^+ -ghosts are the means ($\pm$ S.E.M.) from four different sets of ghosts, one with no internal amino acid initially, and the other three with 5 mM-glycine, serine or leucine. Abbreviation used: ICF, intracellular fluid.

Fig. 5. Uptake of serine by intact erythrocytes and resealed K^+ -ghosts

Intact erythrocytes and resealed K^+ -ghosts were incubated as described in the legend to Fig. 4, but labelled serine replaced the glycine. Only Na^+ -dependent influx of serine is shown, i.e. the difference between influx from Na^+ and K^+ media. ■, Intact cells; △, K^+ -ghosts; ▲, K^+ -ghosts containing 5 mM unlabelled serine. Abbreviation used: ICF, intracellular fluid.

Fig. 6. Uptake of leucine by intact erythrocytes and resealed K^+ -ghosts

The experiments were carried out as described in Fig. 4, with leucine in place of glycine. ■, Intact cells in Na^+ medium; □, intact cells in K^+ medium; ▲, K^+ -ghosts in Na^+ medium; △, K^+ -ghosts in K^+ medium. Abbreviation used: ICF, intracellular fluid.

small Na^+ -independent influx was somewhat higher in the ghosts than in the intact cells (Fig. 4). The inclusion of glycine, serine or leucine inside the ghosts had no significant effect on glycine influx (Fig. 4). With serine the difference between its Na^+ -dependent influx into intact cells and ghosts was much larger, there being only a very low uptake by ghosts, about 7% of that by the intact cells (Fig. 5). Again, the very small Na^+ -independent influx was higher in the ghosts than in the intact cells (results not shown). However, when 5 mM-serine was present inside the ghosts, Na^+ -dependent serine influx increased markedly and reached more than 80% of that measured in intact cells during the first 15 min incubation (Fig. 5). The inclusion of glycine inside the ghosts had no effect, whereas leucine induced a small increase in serine influx (results not shown). In absolute terms the uptake of leucine by intact cells was considerably lower than the uptakes of glycine and serine, and leucine influx into K^+ -ghosts was only about 50% lower than into intact cells (Fig. 6). Some small increases in leucine influx were observed when the ghosts contained

either serine or leucine, but all of these values were too close to be considered significant without many more experiments, and these results have been omitted for the sake of clarity.

Effluxes from resealed ghosts

To see if Na^+ -dependent effluxes could be detected, both Na^+ -ghosts and K^+ -ghosts were prepared with labelled glycine or serine inside and incubated in K^+ medium. Fig. 7 shows that the loss of glycine from Na^+ -ghosts was faster than from K^+ -ghosts, whereas serine efflux was the same from both types of ghost preparation. However, just as serine influx from Na^+ medium into K^+ -ghosts was accelerated by the presence of internal unlabelled serine (Fig. 5), so alanine efflux from K^+ -ghosts into Na^+ medium (but not into K^+ medium) was accelerated in the presence of extracellular unlabelled alanine (Fig. 8). Although these K^+ -ghosts contained only about 9 mM- Na^+ inside when prepared, their relatively high permeability to Na^+ frustrated attempts to see if internal Na^+ was necessary for the exchange process.

Net fluxes

To examine net fluxes of the amino acids, ghosts were prepared containing labelled amino acid and then incubated in media containing the same

Fig. 7. Effluxes of glycine and serine from resealed ghosts

Na^+ -ghosts and K^+ -ghosts were prepared by lysis in solutions containing 1 mM- ^{14}C glycine or 1 mM- ^{14}C serine, giving final concentrations of 0.63 mM and 0.61 mM respectively inside the ghosts. The concentrations of the amino acids inside the ghosts were then monitored during incubations at 38°C in K^+ medium, pH 6.8. ●, Glycine concentration in Na^+ -ghosts; ○, glycine concentration in K^+ -ghosts; ▼, serine concentration in Na^+ -ghosts; ▽, serine concentration in K^+ -ghosts. Abbreviation used: ICF, intracellular fluid.

Fig. 8. Stimulation of alanine efflux from K^+ -ghosts by external alanine

Resealed K^+ -ghosts containing labelled alanine were prepared as described in the text and then incubated at 38°C in both Na^+ medium and K^+ medium, in the presence and absence of unlabelled 10 mM-alanine. ●, Na^+ medium; ○, K^+ medium; ■, Na^+ medium plus alanine; □, K^+ medium plus alanine. Abbreviation used: ICF, intracellular fluid.

Fig. 9. Net fluxes of glycine and serine in K^+ -ghosts. K^+ -ghosts containing labelled 0.2 mM-glycine or labelled 0.2 mM-serine were prepared as described in the text and then incubated in Na^+ and K^+ media containing the same amino acid at the same concentration and specific activity. The incubations were at $38^\circ C$ and pH 6.8. ∇ , Serine concentration in ghosts incubated in Na^+ medium; $\bullet$, glycine concentration in ghosts incubated in Na^+ medium; $\triangle$, serine concentration in ghosts incubated in K^+ medium; $\circ$, glycine concentration in ghosts incubated in K^+ medium. Abbreviation used: ICF, intracellular fluid.

Fig. 10. Net fluxes of glycine in Na^+ -ghosts. Na^+ -ghosts containing labelled 0.2 mM-glycine were prepared as described in the text and then incubated as described in the legend to Fig. 9. $\bullet$, Na^+ medium; $\circ$, K^+ medium. Abbreviation used: ICF, intracellular fluid.

concentration of the same amino acid, at the same specific radioactivity. Thus any change in the amino acid concentration within the ghosts would show that a net flux had occurred, with the establishment of an amino acid concentration gradient across the membrane. Fig. 9 shows that when K^+ -ghosts containing 0.2 mM-glycine were incubated in Na^+ medium containing 0.2 mM-glycine, there was a clear net flux of the amino acid into the ghosts, which continued even though the Na^+ concentration gradient diminished throughout the incubation (Fig. 2). In contrast, Fig. 9 also shows that, under similar conditions, only a very small apparent net influx of serine was detected and that there was no net flux of either amino acid in the absence of extracellular Na^+ . Under similar conditions leucine behaved in the same way as serine (results not shown).

Repetition of these experiments with glycine and Na^+ -ghosts, instead of K^+ -ghosts, gave the results shown in Fig. 10. There was a small net influx of glycine from Na^+ medium, despite the fact that the measured intracellular concentration of Na^+ was about 180 mM throughout the incubation, whereas the extracellular concentration was only 140 mM- Na^+ . In K^+ medium, however, there was a small net

efflux of glycine from the Na^+ -ghosts. No consistent net fluxes were recorded under similar conditions with either serine or leucine.

Effects of anions on amino acid uptake

The effects of anions on the uptake of a number of amino acids by intact pigeon erythrocytes was examined. Preliminary experiments showed that when the usual chloride in the incubation media was replaced by acetate, Na^+ -dependent glycine uptake was markedly decreased, as shown previously by Imler & Vidaver (1972). Hence all the media used in these experiments contained imidazole adjusted to pH 7.5 with acetic acid instead of HCl, although the rest of the chloride normally present was replaced by different anions. The details of the experiments are given in the legend to Table 1, which shows that although Na^+ -dependent glycine influx was clearly dependent of the nature of the anion present, neither Na^+ -dependent nor Na^+ -independent uptake of any other amino acid tested was significantly affected by replacement of chloride.

As a further check, the uptakes of glycine and alanine from both chloride-containing and acetate-containing media into ghosts containing potassium acetate were measured. Table 2 shows again that only Na^+ -dependent glycine uptake was affected significantly, and under these conditions no Na^+ -dependent uptake of glycine could be detected in the acetate medium. This suggested that the

Table 1. *Effect of anions on the influxes of amino acids into intact erythrocytes*

Na⁺ and K⁺ media containing the indicated anions were prepared and their pH values adjusted to pH 7.5 at 20°C as described in the text. Samples of washed pigeon erythrocytes were incubated for two successive periods of 15 min at 38°C in fresh batches of each medium and then incubated separately in the appropriate media containing 0.5 mM of the indicated amino acid, for 15 min at 38°C. The uptake of each amino acid by cells incubated in NaCl medium has been set at 100% and the other uptakes are expressed relative to that value. The values given are from one to four experiments and means (±S.E.M.) are indicated where possible.

Extracellular		Relative uptake of:				
Cation	Anion	Glycine	Alanine	Serine	Leucine	Phenylalanine
Na ⁺	Cl ⁻	100	100	100	100	100
Na ⁺	Br ⁻	108	111	—	—	100
Na ⁺	SCN ⁻	84	111	95	—	108
Na ⁺	I ⁻	71	107	95	—	100
Na ⁺	F ⁻	51	95	94	—	100
Na ⁺	CH ₃ CO ₂ ⁻	29 ± 1	93, 96	89, 91	86, 92	84, 100
Na ⁺	SO ₄ ²⁻	20 ± 1	92	90, 94	88	115
K ⁺	Cl ⁻	10 ± 1	3, 4	3, 4	64, 67	84, 123
K ⁺	I ⁻	6	3	4	—	100
K ⁺	CH ₃ CO ₂ ⁻	7 ± 1	4, 4	5, 6	59, 71	84
K ⁺	SO ₄ ²⁻	9 ± 1	1	3, 4	75	115

Table 2. *Effects of anions on amino acid influxes into ghosts*

Washed pigeon erythrocytes were incubated for 15 min at 38°C in K⁺ medium containing acetate in place of chloride. K⁺-ghosts were then prepared from the cells as described in the text, except that acetate replaced chloride throughout. The resealed ghosts were incubated in Na⁺ and K⁺ media, with either chloride or acetate as the anion, and containing 0.5 mM amino acid, as indicated. The uptakes of the amino acids during 10 min incubation at 38°C were measured. Abbreviation used: ICF, intracellular fluid.

Medium	Influx of amino acid (μmol/ml of ICF per 10 min)		
	Glycine	Alanine	Phenylalanine
NaCl	0.28	0.13	0.15
KCl	0.11	0.08	0.16
Sodium acetate	0.13	0.13	0.14
Potassium acetate	0.13	0.10	0.16

small Na⁺-dependent uptake of glycine from acetate medium observed in intact cells could arise from influx via the system that normally transports amino acids such as alanine, serine and cysteine, i.e. the so-called 'ASC-system' (see Christensen, 1975, for detailed definition). To test this, the effect of adding unlabelled 10 mM-alanine as an inhibitor of glycine uptake from acetate medium was measured. As shown in Fig. 11, alanine did effectively inhibit the small Na⁺-dependent component of glycine uptake under these conditions.

Fig. 11. *Inhibition of Na⁺-dependent glycine uptake from acetate medium by alanine*

Pigeon erythrocytes were incubated for 15 min at 38°C in K⁺ medium containing acetate as the anion. The cells were separated by centrifugation at 3000 g for 5 min and the incubation was repeated. The uptake of glycine (0.2 mM) by the cells was then measured, as described in the text, during incubations in both sodium acetate medium and potassium acetate medium, in the presence and absence of unlabelled 10 mM-alanine. ●, Sodium acetate medium; ○, potassium acetate medium; ■, sodium acetate medium plus 10 mM-alanine; □, potassium acetate medium plus 10 mM-alanine. Abbreviation used: ICF, intracellular fluid.

Discussion

Properties of resealed ghosts

The marked effect of pH on the susceptibility of the resealed ghosts to haemolysis, coupled with the relatively high permeability of the ghosts to Na^+ and K^+ , raises some questions about previous work on the glycine transport system in similar preparations. Vidaver (1964*b*) did not state the pH of the incubation media he used for his original experiments with pigeon erythrocyte ghosts, but the composition of his buffer indicates that the pH probably was about 6.8, which we found to be satisfactory. However, more recently a detailed analysis of the glycine system was made with the use of results derived partly from the effects of pH on glycine uptake by pigeon erythrocyte ghosts (Vidaver *et al.*, 1976). The findings reported here suggest that the conclusions drawn from that study should be reviewed, although the properties of the ghosts used then might well have differed from those described here because the preparative methods were different. As a check on our methods we did prepare resealed ghosts from human erythrocytes in the same way, and tested the effect of pH during prolonged incubations, but no significant effects were detected over the range tested, which was as in Fig. 1. Hence the effects described above indicate that some property specific to the pigeon cell membranes is involved. Since there is a differential effect of pH on the dissociation of spectrin from human and pigeon erythrocyte membranes (Watts & Wheeler, 1980), it is quite feasible that other similar differences exist. The apparently differential effects of internal Na^+ and K^+ on ghost fragility at the higher pH values (Fig. 1) is particularly intriguing and further study of these effects might be worthwhile.

Transport of amino acids by resealed ghosts

In spite of the obvious drawbacks of these preparations, they do mimic the intact cells in a qualitative fashion as far as amino acid transport is concerned. Exact quantitative comparisons between ghosts and intact cells are difficult to make, because their relative volumes might differ. To check this we measured the wet weights, dry weights and extracellular fluid of samples of intact cells and of both Na^+ -ghosts and K^+ -ghosts prepared from exactly the same volume of intact cells. The ghosts were resealed but not washed, to avoid any losses during washing. Four replicate measurements gave the following mean ($\pm$ S.E.M.) values for the intracellular fluid: intact cells, 0.136 ± 0.0001 ml; K^+ -ghosts, 0.128 ± 0.004 ml; Na^+ -ghosts, 0.124 ± 0.002 ml. Hence there was not a large difference in the volumes, so that it is reasonable to conclude that the results described above that show quantitative

differences between intact cells and ghosts are valid. Accepting this quantitative difference, the results described above for serine and alanine transport, as representative of the ASC-system, support the notion that their Na^+ -dependent movements through the cell membrane consist essentially of exchange fluxes. If only exchange is involved, the very small Na^+ -dependent influxes of serine into ghosts noted in Fig. 5 and Table 2 must be attributed to exchange with residual endogenous amino acids in the ghosts. It is not clear if the small apparent net influx of serine shown in Fig. 9 was significant. Exact replication of such experiments was difficult because no two preparations of ghosts were identical, and statistical verification, or contradiction, of such small effects would therefore have required more experiments than seemed justified. Also, of course, residual and irreproducible amounts of endogenous amino acids in the ghosts could again have contributed to such findings. Hence it seems reasonable to conclude that for this transport system exchange fluxes (Figs. 5 and 7) would be overwhelmingly important under more physiological conditions, and the actual difference in Na^+ concentration across the membrane is not important. In fact, it is clear only that extracellular Na^+ is required for the operation of this system, but the possible requirement of intracellular Na^+ as well could not be excluded because the K^+ -ghosts took up Na^+ so quickly (Fig. 8). The fact that both influx and efflux of Na^+ accompanies amino acid transport via the ASC-system in intact cells (Wheeler & Christensen, 1967) suggests that intracellular Na^+ is required.

As far as glycine transport is concerned, the results described here are in keeping with those originally described by Vidaver (1964*b*) in so far that net movements of glycine both into (Fig. 9) and out of (Fig. 10) the ghosts were demonstrated when the initial Na^+ -concentration gradient was in the appropriate direction. However, the constantly changing internal concentrations of Na^+ and K^+ in the ghosts seem to exclude any sound theoretical calculations concerning the magnitudes of the gradients involved. Also, it is important to note that a small net influx of glycine occurred against the prevailing Na^+ -concentration gradient under one set of conditions (Fig. 10). Since similar experiments, but with different concentrations of glycine, gave the same pattern of results, this observation seemed to be significant. Also, although the net fluxes recorded with Na^+ -ghosts were always small, the net effluxes of glycine into K^+ -medium were similar in magnitude to those originally reported by Vidaver (1964*b*). The asymmetry of this transport system is well known (Vidaver & Shepherd, 1968) and it has been shown previously that these cells tend to accumulate more, and to expel less, glycine than predicted from theoretical calculations (Vidaver, 1971), but

such apparently contradictory movement under similar conditions has not been reported. To account for this finding in terms of the Na^+ -gradient hypothesis, it seems necessary to assume that there was a sufficient electrical potential difference across the ghost membrane.

Effects of anions

The results in Table 1 confirm previous observations concerning the anion-dependency of Na^+ -dependent glycine uptake (Imler & Vidaver, 1972) and extend them by showing the anion requirement to be an apparently unique property of the glycine system. The results in Fig. 11 parallel those found recently for glycine uptake by human erythrocytes (Ellory *et al.*, 1981; Al-Saleh & Wheeler, 1982) and indicate that normally part of Na^+ -dependent glycine influx occurs via the ASC-system. Since only the glycine system seems to be capable of bringing about net Na^+ -dependent transport in these cells, it would be interesting to examine the anion dependency of Na^+ -dependent amino acid transport via different systems in other cells, to see if the anion effect is unique to glycine transport, or to net transport.

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References

- Al-Saleh, E. A. & Wheeler, K. P. (1982) *Biochim. Biophys. Acta* in the press
- Bodemmann, H. & Passow, H. (1972) *J. Membr. Biol.* **8**, 1–26
- Bull, H. B. (1964) *An Introduction to Physical Biochemistry*, p. 195, F. A. Davis Co., Philadelphia
- Christensen, H. N. (1975) *Curr. Top. Membr. Transp.* **6**, 227–258
- Crane, R. K. (1977) *Rev. Physiol. Biochem. Pharmacol.* **78**, 99–159
- Eavenson, E. & Christensen, H. N. (1967) *J. Biol. Chem.* **242**, 5386–5396
- Eddy, A. A. (1975) *Excerpta Medica International Congress Series* no. 391, 332–349
- Ellory, J. C., Jones, S. E. M. & Young, J. D. (1981) *J. Physiol. (London)* **310**, 22P
- Imler, J. R. & Vidaver, G. A. (1972) *Biochim. Biophys. Acta* **288**, 153–165
- Lepke, S. & Passow, H. (1972) *Biochim. Biophys. Acta* **255**, 696–702
- Romero, P. J. (1974) *Biochim. Biophys. Acta* **339**, 116–125
- Schwoch, S. & Passow, H. (1973) *Mol. Cell. Biochem.* **2**, 197–218
- Terry, P. M. & Vidaver, G. A. (1972) *Biochem. Biophys. Res. Commun.* **47**, 539–543
- Terry, P. M. & Vidaver, G. A. (1973) *Biochim. Biophys. Acta* **323**, 441–455
- Vidaver, G. A. (1964a) *Biochemistry* **3**, 662–667
- Vidaver, G. A. (1964b) *Biochemistry* **3**, 795–799
- Vidaver, G. A. (1964c) *Biochemistry* **3**, 799–803
- Vidaver, G. A. (1964d) *Biochemistry* **3**, 803–808
- Vidaver, G. A. (1971) *Biochim. Biophys. Acta* **233**, 231–234
- Vidaver, G. A. & Shepherd, S. L. (1968) *J. Biol. Chem.* **243**, 6140–6150
- Vidaver, G. A., Romain, L. F. & Haurowitz, F. (1964) *Arch. Biochem. Biophys.* **107**, 82–87
- Vidaver, G. A., Shepherd, S. L., Lagow, J. B. & Wiechelman, K. J. (1976) *Biochim. Biophys. Acta* **443**, 494–514
- Watts, C. & Wheeler, K. P. (1980) *Biochim. Biophys. Acta* **602**, 460–466
- Wheeler, K. P. & Christensen, H. N. (1967) *Biochim. Biophys. Acta* **602**, 460–466