Interactions between Potassium Ions and Glycine Transport in the Yeast *Saccharomyces carlsbergensis*

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(Received 3 August 1970)

A study has been made of the effects of both varying the pH and extracellular $[K^+]$ on the initial rate of uptake of glycine (v) by a strain of *Saccharomyces carlsbergensis* that concentrated the amino acid, with respect to the extracellular phase, by up to 1400 times. When no other substrate than glycine was provided and $[glycine]$ was relatively small (≤ 0.2 mM) (1) v increased fivefold when the pH was lowered from 7 to 4.5; (2) v fell by up to about 80% as $[K^+]$ rose, K^+ behaving as a non-competitive inhibitor of the system, with K_i 0.33 mequiv./l at pH 7; (3) the absorption of glycine caused up to about 2 or 3 equiv. of K^+ to leave the yeast cells. These three phenomena were each less evident when glucose was present. An analogy is drawn between the respective interactions of H^+ and K^+ with the yeast system and the well recognized effects of Na^+ and K^+ on amino acid transport in certain mammalian systems.

Na^+ ions and K^+ ions are known to be intimately involved in the active transport of various amino acids and carbohydrates in certain mammalian tissues (Heinz, 1967). The spontaneous movements of Na^+ across the cell membrane of the pigeon erythrocyte (Vidaver, 1964*a,b*) and the analogous movements of both Na^+ and K^+ in mouse ascites-tumour cells (Eddy & Mulcahy, 1965; Eddy, 1968*a,b*) may set up substantial gradients of glycine concentration across the cell membrane in circumstances where ATP is virtually absent. Likewise the spontaneous movement of glycine into the tumour cells may lead to a Na^+ -concentration gradient (Eddy, 1969). These phenomena, which appear to involve physical rather than chemical events, can be understood quantitatively in terms of equations (cf. Eddy & Hogg, 1969) that are extensions of the classical model of carrier kinetics (Wilbrandt & Rosenberg, 1961).

The possibility that ionic gradients of K^+ or Na^+ might also be implicated in microbial transport systems has received little attention in the literature. The occasional reports that K^+ or Na^+ may interact with the transport of amino acids in yeast (Conway & Duggan, 1958; Eddy & Indge, 1962; Surdin, Sly, Sire, Bordes & De Robichon-Szulmajster, 1965) and in certain bacteria (Wong,

Thompson & MacLeod, 1969; Mora & Snell, 1963; Harold & Baarda, 1968*a*; Gale, 1953) are difficult to interpret, especially when the mechanisms by which these cells transport Na^+ and K^+ themselves are poorly understood. The present work involves a strain of *Saccharomyces carlsbergensis* that exhibited some of the properties that Surdin *et al.* (1965) and Gits & Grenson (1967) found in an earlier study of the so-called amino acid permeases of yeast. The new observations show that there are circumstances where glycine transport in this yeast is markedly affected by the values of $[K^+]$, $[Na^+]$ and $[H^+]$ in the extracellular phase and to an extent depending on the availability of glucose. The observations lead to the idea that, in certain circumstances, H^+ and K^+ may play a part in the transport of amino acids by yeasts that is analogous to the roles of Na^+ and K^+ in the mammalian systems (Eddy, 1966). The possibility that H^+ ions are involved in certain bacterial systems has been advocated on the grounds that these are readily inhibited by various reagents that increase the permeability of natural membranes to protons (Mitchell, 1963; Harold & Baarda, 1968*b*).

MATERIALS AND METHODS

Organism and culture conditions. Strain N.C.Y.C. 74 (British National Collection of Yeast Cultures, Nutfield, Surrey, U.K.) of *Sac. carlsbergensis* was maintained on nutrient agar slopes at 4°C and subcultured every 6 months. The slopes contained yeast extract (Difco

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Laboratories, Detroit, Mich., U.S.A.; 3g), malt extract (Difco; 3g), mycological peptone (Oxo Ltd., London S.E.1, U.K.; 5g), glucose (10g), agar (Oxo Ltd.; 20g) and water to 1 litre. Secondary stock cultures (10ml) were prepared in McCartney bottles (28ml capacity) in a similar solution from which the agar was omitted. They were maintained at 25°C for up to ten transfers. Portions (1ml of a culture 24h old) were transferred to larger volumes (500ml) of a mineral-salts-nutrient medium containing glucose (Eddy & Rudin, 1958). The cultures were kept for 17–20h in Roux flasks (1 litre capacity) that were shaken at 25°C with a 50cm stroke at 80 oscillations/min. The yeast cells (about 500mg dry wt./l of culture medium), in the exponential phase of growth, were harvested by centrifugation (MSE Minor centrifuge, unrefrigerated), washed with water (3 × 50ml) and stored at 4°C for up to 3h.

Assay of glycine transport. The assay mixture (3–10ml) typically contained yeast cells (1–10mg dry wt./ml of suspension), 16mM-tris solution, from pH9 to pH3, as adjusted by the addition of citric acid, and a selected concentration (0.01–14mM) of glycine containing 0.1–0.3 μ Ci of [14 C]glycine/ml of suspension. The uptake of glycine was usually initiated by mixing a suspension of the yeast cells in 1ml of water at 30°C with the other reagents in an open conical flask (capacity 25ml) at 30°C. The mixture was shaken through 4cm at 80 oscillations/min. Samples (1ml) were withdrawn at selected intervals and mixed with 16mM-tris-HCl buffer solution (7ml, pH7.2) at 0°C. The yeast cells were separated by centrifugation at 1000g for 2min, the supernatant solution (A) was removed and the cells were resuspended in the buffer solution at 0°C. The latter procedure displaced <5% of the accumulated [14 C]glycine from the yeast cells. Each yeast sample was washed twice more similarly, mixed with water (2ml) and kept at 100°C for 10min. Cellular debris (B) was separated at 1000g for 3min, the supernatant solution (C) being retained. A different extraction procedure, involving cold 5% (w/v) trichloroacetic acid (Gale & Folkes, 1953), was used in preliminary work. The 14 C contents, or in certain instances the 42 K contents, of fractions A, B and C were assayed as described by Eddy, Mulcahy & Thomson (1967). To determine cellular Na⁺ and K⁺ the yeast cells (50mg) were boiled with water (5ml) for 10min. The suspension was cooled, acidified with 2M-HNO₃ (0.2ml) and centrifuged at 1000g for 5min. The Na⁺ and K⁺ contents of the supernatant solution was determined by using an EEL flame photometer (Evans Electroselenium Ltd., Halstead, Essex, U.K.).

Radioactive compounds were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Other compounds were of A.R. grade where these were available.

RESULTS

Preliminary work

The initial rate (v) of accumulation of 14 C in the hot-water extract was studied as a function of the extracellular glycine concentration ([glycine]) at pH6 when 100mM-glucose was present. Omission of glucose lowered v by 50–90%. By plotting v

Fig. 1. Variation of v at 30°C with the pH of the 16mM-tris solution containing graded amounts of citric acid. ○, 100mM-glucose, 0.6mg of yeast/ml; ●, no glucose, 2.5mg of yeast/ml. [Glycine] was 0.2mM.

against v /[glycine], two kinetic components were revealed with K_m values of 64 μ M and 2.8mM. Analogous components have been found with L-methionine (Gits & Grenson, 1967) and with *p*-fluorophenylalanine (Kempner & Cowie, 1960).

Merely starving the yeast cells at 20–30°C for 90min, after they were harvested, lowered the glycine uptake rate from a 0.2mM solution containing 100mM-glucose by up to 70%. The fall was much less marked at 4°C. It is noteworthy that when the cells that had been aged at 30°C for 2h were kept with glucose for 45min before being tested with glycine v was progressively restored to the relatively large values characteristic of the fresh cells. The presence of 5mM-potassium chloride accelerated the recovery (cf. Eddy & Indge, 1962; Stachiewicz & Quastel, 1963). These aging phenomena appear to involve principally the transport system operating at small values of [glycine].

Variation of v with pH. Omission of glucose from the system inhibited both the metabolism of glycine and its transport into the cells. The results in Fig. 1 show that v exhibited a more marked pH-dependence when glucose was absent than when it was present. The uptake of L-lysine and L-aspartic acid also then showed a relatively sharp pH optimum. The results in Fig. 1 appear to represent the effect on the system of varying [H⁺] rather than the other components of the buffers. Previous work (cf. Surdin *et al.* 1965; Magaña-Schwenke & Schwenke, 1969) is open to the objection that complex solutions were employed in which [Na⁺] and [K⁺] also varied and might be expected to have influenced the result (see below).

Relation of v to amino acid metabolism. The yeast cells were put at 30°C with glucose and either a 0.2mM solution of [¹⁴C]glycine for 5min or a 10mM solution for 60min. In the latter instance the yeast contained an amount of [¹⁴C]glycine representing 8–10% of the cellular dry wt., in an approx. 200mM solution in the cellular water, whereas in the former instance a 10mM solution was obtained. Chromatographic analysis on paper indicated that at least 90% of the ¹⁴C extracted from the cells by hot water was associated with glycine. Some serine was formed and the cellular fraction insoluble in hot water (15% of the ¹⁴C absorbed) contained both seryl and glycyll peptides. About 5% of the ¹⁴C absorbed was converted into ¹⁴CO₂ that was assayed after it had been trapped in 40% (w/v) potassium hydroxide placed in the centre well of a Warburg flask. These and other observations indicated that the ¹⁴C content of the hot-water extract was probably a fair measure of the amount of [¹⁴C]glycine absorbed during a typical measurement of v , when the yeast was usually exposed for 1 or 2min to the labelled compound. On the other hand, metabolism of a significant fraction of the absorbed glycine appeared to occur during the 30 or 40min it took for the cellular glycine content to approach a steady value under a given set of conditions.

Inhibition by K⁺

The value of v fell when extracellular [K⁺] was raised at a constant pH value of 7.4 in the absence of glucose. The observations in the range up to

Fig. 2. Non-competitive inhibition of glycine uptake by K⁺, at pH 7.4 in the absence of glucose (2.8mg of yeast/ml). v was lowered to v' at a given value of [K⁺], in accordance with type IIb kinetics of Dixon & Webb (1964). In these circumstances, $v/v-v'$ varies linearly with [K⁺]⁻¹, K_i being the values of [K⁺] when $v/v-v' = 0$. Glycine (10μM) was present.

10mequiv. of K⁺/l are plotted in Fig. 2. They show that as [K⁺] became very large the inhibition would be expected to approach a maximum value of about 80%, K_i being 0.33mequiv./l. Independent observations showed that increasing [K⁺] from 10 to 200mequiv./l in fact lowered v only by about a further 10%. The principal effects of K⁺ at pH 7.4 can be explained therefore in terms of one binding site for K⁺.

Other work showed that the glycine-transport system responded immediately to a change either of [K⁺] or [H⁺], as though both ionic species acted directly at the cell surface. At pH 7.4 with [K⁺] zero, the variation of v with [glycine] in the range up to 0.2mM corresponded to K_m 58±12μM (4). When [K⁺] was 100mequiv./l K_m was not significantly different, at 80±12μM (4). As V_{max} was 0.34±0.01nmol/min per mg in the former instance and 0.09±0.01 in the latter ($P < 0.001$), K⁺ behaved, in relation to glycine itself, as a non-competitive inhibitor of glycine transport.

Role of anions. Observations on the system lacking glucose showed that 100mM-potassium chloride, 100mM-potassium methanesulphonate or 50mM-potassium sulphate each lowered v by the same amount, whereas the action of sodium chloride (see below) differed from that of potassium chloride. Hence the cation rather than the anion seemed to be the inhibitor.

Effect of pH. Table 1 shows that whereas K⁺ inhibited the uptake of glycine at both pH 4.5 and pH 7.4, Na⁺ did so only at pH 7.4 and to a smaller extent than K⁺ at the lower concentration studied. A given concentration of K⁺ exerted a somewhat smaller effect at the lower pH. Such behaviour loosely parallels the differential effects of these variables on the transport of Na⁺ and K⁺ themselves (see Armstrong & Rothstein, 1964).

Table 1. *Effect of pH on the percentage inhibition of glycine accumulation by K⁺ and Na⁺*

The initial rate of accumulation was determined at pH 4.5 and 7.4 in the absence of glucose, at 30°C, with [glycine] 0.2mM and [K⁺] and [Na⁺] as indicated. The mean extent to which v was lowered by the alkali cations, in a series of assays, is expressed as a percentage of the rate, at the same pH, in their absence.

pH	[K ⁺] (mequiv./l)	[Na ⁺] (mequiv./l)	Inhibition (%, ±s.e.m.)
4.5	2	0	39.8±5.4 (6)
	20	0	57.8±2.7 (6)
	0	2	-6.7±1.5 (6)
	0	20	6.6±3.9 (9)
7.4	2	0	67.3±2.7 (6)
	20	0	72.4±3.4 (6)
	0	2	18.4±3.1 (6)
	0	20	52.3±4.6 (6)

Glycine uptake during 30 min. K^+ also lowered the net amount of [^{14}C]glycine the yeast cells absorbed from a $10\mu M$ solution in 30 min, during which time the glycine concentration approached a steady state. The net uptake and v were proportionately affected (Fig. 3). Fig. 4 shows how the cellular ^{14}C content changed when extracellular [glycine] varied below 0.1 mM , in the absence of glucose, (1) at pH 7.4, (2) at pH 7.4 with 100 mequiv. of K^+/l and (3) at pH 4.5 with 30 mequiv. of K^+/l . At a given value of [glycine], the amount of glycine absorbed in 30 min increased in parallel with the initial rate v , being greater at pH 4.5 than at pH 7.4 and greater when extracellular $[K^+]$ was relatively small (Fig. 4). The amount of glycine absorbed also increased with [glycine] and extrapolation of the results in Fig. 4 suggests that as $[glycine]^{-1}$ approached zero the glycine content would approach a definite limit in each of the three situations studied. The limits defined in that way, i.e. on the basis of the observations at $[glycine] < 0.1\text{ mM}$, appeared to be reproducible within a factor of 2, as between different cell preparations. For instance, the results in Fig. 4 indicate that at pH 4.5 with 30 mM-potassium chloride present the maximum amount of glycine absorbed in 30 min represented approximately a 3 mM solution in the cellular water, or about 13 nmol of glycine/mg dry wt. The presence of glucose increased the limit about 50-fold. A substantial increase in the amount of glycine absorbed was also brought about by incubating the yeast with 100 mM-glucose at pH 4.5 for 20 min before washing the cells and exposing them to the amino acid.

The observations illustrated in Fig. 4, when taken in conjunction with the intracellular water content of $3.62\mu l/\text{mg}$ dry wt. of cells, also define the cellular [glycine]/extracellular [glycine] ratio in each situation. The above ratio was 1320 when extracellular [glycine] was $0.5\mu M$ at pH 4.5 with 30 mequiv. of K^+/l . It was 138 and 43 respectively when [glycine] was $10\mu M$ and $90\mu M$, whereas at pH 7.4 with 100 mequiv. of K^+/l it was 2.5 when [glycine] was $10\mu M$ and 0.8 when [glycine] was $100\mu M$. It is emphasized that with $[glycine] > 100\mu M$ the yeast in fact absorbed considerably more glycine than was predicted on the above basis. This behaviour was presumably associated with the transport system exhibiting the larger of the two K_m values for glycine.

Glucose addition. K^+ had a smaller inhibitory effect, at both pH 7.4 and pH 4.5, in the presence than in the absence of glucose (Table 2). Potassium chloride (100 mM) caused a marked inhibition at pH 9 in the system with glucose.

Putting the yeast with glucose for 20 min, before the addition of glycine without glucose, virtually abolished the dependence of v on $[K^+]$, in

Fig. 3. Comparison of the effect of varying $[K^+]$ from 0.1 to 200 mequiv./l on the initial rate of glycine uptake (v), from a $10\mu M$ solution at pH 7.4, and its effect on the amount of glycine the yeast accumulated in 30 min at $30^\circ C$. Both v and the net uptake were expressed as percentages of the control values observed in the absence of K^+ . Glucose was absent.

Fig. 4. Effect of varying [glycine] in the range below $100\mu M$ on the uptake of glycine in 30 min at $30^\circ C$. Glucose was absent. $\square$, pH 7.4, 100 mequiv. of K^+/l ; $\circ$, pH 7.4 and $\triangle$, pH 4.5, with 30 mequiv. of K^+/l .

the range up to 5 mequiv./l, that is illustrated in Fig. 2. It seems possible that an increase in energy metabolism is the common factor modifying the response to extracellular $[K^+]$ after either the preliminary treatment with glucose or the addition of glucose to the assay system.

Osmotic effects. The question arose whether the

Table 2. *Effect of glucose on the percentage inhibition of glycine accumulation by K⁺ at pH 4.5 and 7.4*

The glycine concentration was 0.2mM. The mean extent to which 100mM-KCl lowered *v* is expressed as a percentage of the rate, at the same pH and glucose concentration, when [K⁺] was zero.

pH	Glucose concn. (mM)	Inhibition (%; $\pm$ s.e.m.)
4.5	0	70.7 $\pm$ 1.7 (11)
	100	24.0 $\pm$ 6.3 (6)
7.4	0	76.9 $\pm$ 4.1 (5)
	100	42.5 $\pm$ 3.8 (4)
9.0	100	95.4 $\pm$ 1.1 (9)

Table 3. *Effects of cations on glycine accumulation, in the absence of glucose, at pH 7.4*

The glycine concentration was 0.2mM. The extent to which *v* was lowered in the presence of a given compound is expressed as a percentage of the rate in the absence of that compound. Similar results were obtained in a second series of experiments.

Compound added	Inhibition (%)
5mM-KCl	77
2mM-MgCl ₂	49
5mM-KCl+2mM-MgCl ₂	79
0.1mM-CaCl ₂	55
1.0mM-CaCl ₂	85
3.0mM-CaCl ₂	89
10mM-Tris chloride	6
25mM-Tris chloride	15
50mM-Tris chloride	35
100mM-Tris chloride	72
25mM-Imidazole	80
100mM-Choline chloride	30
100mM-LiCl	52
100mM-KCl	76

action of 100mM-potassium chloride was a function of the raised osmotic pressure. The addition of 200mM-mannitol, to which yeast cells are relatively impermeable, lowered *v* by <10% (three experiments) both when glucose was absent at pH 4.5 or at pH 7.4 and when glucose was present at pH 8 or pH 9. Hence, in these instances, potassium chloride acted (Tables 1 and 2) in some other way. On the other hand, at pH 4.5 or pH 7.4, with glucose present, the addition of mannitol depressed *v* by up to 30% (three experiments). As potassium chloride had a comparable effect (Table 2) it may have affected *v*, under these conditions, largely by raising the osmotic pressure.

Respiration rate. Manometric measurements showed that the presence of 100mM-potassium chloride increased the rate of endogenous res-

Fig. 5. Efflux of K⁺ during the absorption of the glycine zwitterion in the absence of glucose: comparison with the behaviour during the absorption of the L-lysine cation and the L-aspartate anion in the presence of glucose, with 3–6mg of yeast/ml of suspension, at pH 4.5, 30°C. Aspartic acid uptake (▲) occurred from solutions containing 5mM-⁴²KCl (0.01 μ Ci/ml), 100mM-glucose and, respectively, zero (the control), 0.25mM-, 0.7mM- or 2.5mM-L-[G-¹⁴C]aspartic acid (0.1 μ Ci/ml). Three samples were collected from each solution during 15min. The extra ⁴²K⁺ retained by the yeast in the presence of aspartic acid, as compared with the control, was computed at each stage. To study the efflux of K⁺ the yeast was put for 15min in ⁴²K⁺ (1 μ Ci/ μ equiv. of K⁺) with 100mM-glucose. The cells were washed and transferred to either (1) 100mM-glucose–30mM-KCl, with or without 4.5mM-L-[G-¹⁴C]lysine (0.1 μ Ci/ml) (●); or (2) 5mM-KCl and, respectively, zero (the control), 0.3mM- (□), 1.3mM- (○) or 2.6mM-[L-¹⁴C]glycine (0.1 μ Ci/ml) (△). The yeast and the extracellular phase were sampled at intervals during 20min. The specific radioactivity of the cellular ⁴²K⁺ was approximately constant between 5 and 20min. The extra ⁴²K⁺ appearing in the extracellular phase at a given time is compared with the corresponding amount of the amino acid the yeast had absorbed.

piration (approx. 1 μ g-atom of O/h per mg of dried yeast) by up to 20%.

Other cations. The value of *v* determined in the absence of glucose was lowered by calcium chloride, magnesium chloride and certain other compounds (Table 3). These effects were smaller at pH 4.5. Table 3 also shows that the actions of tris chloride and potassium chloride were quite distinct at relatively low concentrations.

Interactions with K⁺ transport

Aspartic acid and lysine. When the yeast accumulated L-aspartic acid in the presence of glucose, approximately 1 extra equivalent of K⁺ entered the cells (Fig. 5). The influx of ⁴²K⁺ was stimulated

by aspartic acid whereas its efflux appeared to be unchanged. In contrast, the uptake of L-lysine from a 4.5 mM solution resulted in the loss of about 1 equivalent of K^+ from the cells (Fig. 5). Up to 40% of the initial cellular content of the K^+ was replaced by lysine under these conditions. The results shown in Fig. 5 also show that a small amount of lysine (about 8 nmol/mg of yeast) was absorbed without displacing an equivalent of K^+ . Other studies with $^{42}K^+$ indicated that the factors governing the stoichiometry were complex. (1) The influx of $^{42}K^+$ was completely inhibited by lysine after a delay of 5 min. (2) The efflux of $^{42}K^+$ was stimulated in the presence of lysine. (3) When the lysine concentration was lowered to 0.5 mM, less than 1 equivalent of the K^+ was displaced and the cell suspension tended to become acidified more rapidly than when lysine was absent. Titration of the acid with tris solution indicated that the influx of the lysine cation was now balanced by the secretion both of H^+ and K^+ . It seems possible that the two modes of ion exchange represent two distinct modes of lysine entry, exhibiting a dependence on [lysine] like that found for glycine.

Glycine. Both the influx (see Fig. 6) and the efflux of $^{42}K^+$ in the presence of glucose occurred at the same rate in the presence and absence of 0.2 mM-glycine. On the other hand, when glucose was omitted glycine strikingly stimulated the efflux of $^{42}K^+$ (Fig. 5). Between 2 and 3 equivalents of $^{42}K^+$ were displaced/mol of glycine absorbed, in the range up to about 30 nmol/mg of yeast, and relatively smaller amounts of $^{42}K^+$ when larger amounts of glycine were absorbed. Now the observations in Fig. 4 suggest that the yeast would be expected to absorb no more than about 20–30 nmol of glycine/mg of yeast by the mechanism with K_m about 60 μM for glycine. We tentatively conclude that the largest efflux of $^{42}K^+$ was associated with that mechanism.

In six experiments 1 mM-glycine caused no consistent change in the influx of $^{42}K^+$ from a 100 mequiv./l solution, either at pH 7.4 or at pH 4.5 in the absence of glucose. Nevertheless K^+ inhibited glycine influx under these conditions by 65% at pH 7.4 and about 30% at pH 4.5. An effect of glycine on the influx of K^+ would be most easily detected at pH 4.5 when the two species entered the cells at similar rates. We infer that, in the absence of glucose, K^+ retarded the entry of glycine by a mechanism that did not involve the majority of the sites through which K^+ ions themselves were absorbed, or simple competition for a limited source of metabolic energy, or some other factor common to the two processes (cf. Eddy, 1966).

Studies with 'sodium yeast'. Conway & Duggan (1958) reported that the presence of 5 mM-glycine, in the absence of glucose, accelerated the flow of

Fig. 6. Effect of 0.2 mM-glycine on the influx of $^{42}K^+$ in the presence of 100 mM-glucose at pH 4.5, 30°C. Yeast concentration was 2.7 mg/ml. The yeast suspension (30 ml) was kept with glucose and 5 mM-KCl at pH 4.5 for 15 min, the cells were collected by centrifugation and put in a similar solution containing ^{42}KCl (0.01 $\mu Ci/ml$). [^{14}C]-Glycine (0.2 mM; 0.1 $\mu Ci/ml$) was added 10 min later ($\uparrow$) to one portion of the suspension (●), the remainder serving as a control (○). The increase in the cellular [^{14}C]glycine content is also shown (▲).

cellular Na^+ to the extracellular phase. For this purpose they used preparations of baker's yeast in which cellular K^+ had been largely replaced by Na^+ during a preliminary treatment with 0.2 M-sodium citrate solution containing 5% glucose. We were unable to reproduce their observations with our system when [glycine] was either 5 mM (three experiments) or 0.2 mM (two experiments). Our results show that it was the efflux of K^+ (Fig. 5) rather than Na^+ that was associated with the influx of glycine.

DISCUSSION

We were interested in the question whether the ideas developed to explain the actions of Na^+ and K^+ on substrate transport in other eukaryotic systems (Vidaver, 1964a,b; Crane, 1964; Eddy, 1968a,b; Curran, Schultz, Chez & Fuisz, 1967), of which the mouse ascites system is an important example, might be relevant to the transport of amino acids in yeast. Amino acid transport in the mouse tumour cells appears not to be driven directly by ATP, though that compound is the indirect energy source. The direct source appears to be the energy inherent in the gradients of Na^+ and K^+ concentrations that normally exist across the cell membrane. These form under the influence of the

sodium pump in a process that utilizes ATP (Skou, 1965). The ionic gradients thus serve, as it were, as an intermediate energy reservoir on which amino acid transport depends.

The yeast functioning in the absence of glucose is especially interesting in this connexion and a number of intriguing parallels can be drawn with the behaviour of the mouse cells. (1) Glycine uptake was stimulated by H⁺ (Fig. 1) and inhibited by K⁺ (Fig. 2). (2) It was also associated with an accelerated efflux of K⁺ (Fig. 5). The effect was largest when [glycine] was small. Hence it may be principally a function of the transport system exhibiting K_m about 60 μ M. The above two circumstances are consistent with the working hypothesis (Eddy, 1966) that, under these specific conditions, the H⁺/K⁺ ion pair serve as co-substrates of a glycine carrier system functioning without the direct intervention of ATP. Na⁺ stimulates glycine uptake into the mouse cells and K⁺ competitively inhibits the role of Na⁺. In the yeast system the inhibition of v by K⁺ was smaller at pH 4.5 than at pH 7.4 (Table 1) but whether the actions of H⁺ and K⁺ were competitively related was not established. As Na⁺, Mg²⁺, Ca²⁺ and, indeed, even a large concentration of the tris cation also lowered the glycine entry rate (Tables 1 and 3), the special significance attached to the functions of K⁺, bearing in mind that Na⁺ ions were not displaced by glycine, stems from the observations in Fig. 5.

The movements of K⁺ ions, shown in Fig. 5, accompanying the absorption of the lysine cation and the aspartic acid anion respectively, can be explained in general terms of the need to preserve electroneutrality during the influx of these species (Davies, Folkes, Gale & Bigger, 1953). Such an explanation can hardly be relevant to the absorption of the glycine zwitterion, unless other ions necessarily participate in the process. We suggest that the displacement of K⁺ from the yeast by glycine, like its displacement from the mouse ascites cells (Eddy, 1968a) means that K⁺ ions play an essential role, under these conditions, in the functioning of the glycine carrier system. In terms of the ion-gradient hypothesis, the efflux of K⁺ from the yeast would be expected to be accompanied by the absorption of protons, just as in the mouse system the influx of Na⁺ induced by glycine was accompanied by an efflux of K⁺ (Eddy, 1968a). The predicted influx of H⁺ with glycine has been described in a preliminary report (Eddy, Backen & Nowacki, 1970).

These intriguing parallels are associated with certain striking differences between the yeast and the mammalian systems. (1) The yeast concentrated glycine in the cellular water by a factor of at least 10³ when [glycine] was 10 μ M, whereas ratios of <30 are found in the mammalian systems. (2)

The yeast, in contrast with the mouse system, appears to be regulated in a complex fashion by its intracellular amino acid content (Grenson, Crabeel, Wiame & Béchet, 1968; Ring & Heinz, 1966). Both the biosynthesis and the degradation of a component of the transport system are possibly involved (Schwenke & Magaña-Schwenke, 1969; Gale & Folkes, 1967). (3) The effects of Na⁺ and K⁺ on the absorption of glycine by the mouse ascites cells were almost independent of energy metabolism (Eddy *et al.* 1967; Eddy & Hogg, 1969). In contrast, the addition of glucose to the yeast system, which would increase the supply of ATP, perhaps for that reason also (a) greatly modified the effects on v of both H⁺ (Fig. 1) and K⁺ (Table 2) and (b) appeared to remove the connexion shown in Fig. 5 with the efflux of K⁺, which was no longer stimulated by the presence of glycine. The influxes of K⁺ and of glycine also appeared to be independent processes (Fig. 6). Such behaviour suggests that the glycine carrier system functioned in a different way in the presence of glucose than in its absence, a distinction that further work has tended to support.

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