The Concentration of Amino Acids by Yeast Cells Depleted of Adenosine Triphosphate

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1. The ATP content of preparations of a strain of *Saccharomyces carlsbergensis* was lowered below 0.3 nmol/mg of yeast by starving the yeast cells in the presence of both antimycin and 5 mm-deoxyglucose. 2. When the depleted cells were put at pH 4.5 with glycine up to about 20 nmol of the amino acid/mg of yeast was absorbed without being chemically modified. The mechanism did not depend on an exchange with endogenous amino acids. 3. The concentration of the absorbed glycine could apparently reach 100–200 times that outside the cells. 4. Replacement of the cellular K^+ by Na^+ almost stopped amino acid absorption in the presence of antimycin and deoxyglucose, but not in their absence. 5. It is suggested that, when energy metabolism itself had stopped, a purely physical process, namely the movements of H^+ and K^+ into and out of the yeast respectively, served to concentrate the amino acids in the cells. Both ionic species appear to be co-substrates of the system transporting amino acids.

Eddy, Indge, Backen & Nowacki (1970b) found that whereas the H^+ present in the extracellular phase increased the rate of uptake of glycine by a certain strain of *Saccharomyces carlsbergensis*, the presence of K^+ lowered that rate provided glucose was absent from the system. Moreover, there were circumstances in which the uptake of glycine resulted in the K^+ being expelled from the yeast cells. These effects are consistent with the working hypothesis (Eddy, 1966) that H^+ played a role in the yeast system like that of Na^+ in certain mammalian transport systems (Heinz, 1967). The analogy would include the interactions with K^+ . The present work with starved yeast cells, depleted of ATP by treatment with antimycin and 2-deoxyglucose, was designed to test the working hypothesis further. Does the spontaneous flow of H^+ that takes place into the starved yeast at pH 4.5, in exchange for cellular K^+ , lead to the influx of a given amino acid against its own concentration gradient? An analogous process in a mammalian system would be the forced accumulation of glycine that may accompany the flow of Na^+ into mouse ascites cells when their energy metabolism is blocked (Eddy, 1968).

MATERIALS AND METHODS

These were mainly as described by Eddy *et al.* (1970b), except that, before a given experiment, the yeast cells (strain N.C.Y.C. no. 74; British National Collection of Yeast Cultures, Nutfield, Surrey, U.K.) were fed at 30°C, for 20–40 min, with 100 mm-glucose in 20 mm-tris solution (about 5 mg dry wt. of yeast/ml) adjusted to pH 4.5 with citric acid. The yeast was then separated by centrifugation and washed with a large volume of ice-cold water.

Recovery of the absorbed amino acids. The washed yeast (50 mg), prepared as described above, was suspended in 5 mm-tris (4 ml), adjusted to pH 4.5 with citric acid and containing 5 mm-2-deoxyglucose and 25 μ g of antimycin. The suspension was shaken gently at 30°C for 5 min in a small conical flask (25 ml capacity). The ^{14}C -labelled amino acid (0.5 μ mol, 0.25 μ Ci) was then added and after a further 3.5 min the suspension was cooled to 0°C. The yeast was quickly separated by centrifugation and the supernatant solution (A) removed. The yeast pellet, containing both cellular and free water (0.28 ml), was mixed with water (0.5 ml) and kept at 100°C for 10 min. The product was cooled and centrifuged, the supernatant solution (B) being retained. The supernatant solution (A) was assayed (1) for ^{14}C and (2), by using the method of Rosen (1957), for ninhydrin-reactive materials. Portions (5 μ l) of the cellular extract B were assayed chromatographically for [^{14}C]glycine and [^{14}C]serine, in parallel with known amounts of authentic samples of these compounds. Resolution was accomplished (1) by paper chromatography (Whatman no. 1), for 18 h, with *n*-butanol–acetone–water–diethylamine (10:10:5:2, by

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vol.), by the ascending technique (three experiments); (2) by paper chromatography for 66h with a mixture of *n*-butanol-ethyl methyl ketone-water-17M-NH₃ (5:3:1:1, by vol.), by the descending technique (one experiment); (3) for 18h, by t.l.c. on silica gel film (Eastman Kodak type 6061, without fluorescent indicator; Kodak Ltd., Liverpool, U.K.), in the above solvent containing diethylamine by the ascending technique (one experiment). In one experiment L-[G-¹⁴C]phenylalanine was assayed after resolution, in 18h, by the ascending technique of t.l.c. on the above films. The solvent mixture was *n*-butanol-water-acetic acid (12:5:3, by vol.). The papers and films were subsequently dried and cut into strips (3 cm × 1.5 cm). Each strip was transferred to a vial with 10 ml of scintillation fluid and its ¹⁴C content determined in a scintillation spectrometer (Packard Tri-Carb model 3310; Packard Instruments Ltd., Wembley, Middlesex, U.K.). The scintillation fluid contained toluene (2.1 litres) and ethanol (0.9 litre) and 2,5-diphenyloxazole (PPO) (12g) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (POPOP) (300mg) (see Bernlohr & Novelli, 1963). The gain was set at 8% and the discriminator gates at 45 and 1000 in the first two channels and at 45 and 85 in the third channel of the spectrometer. Sample quenching was assessed by the channels-ratio method.

Preparation of 'sodium yeast'. This was based on the observations of Conway & Moore (1954). After being harvested from the standard growth medium, the yeast (250mg) was suspended in 0.2M-sodium citrate (100ml) adjusted to pH 6 with citric acid and containing 5% (w/v) glucose. The suspension was shaken at 30°C for 2h, after which the cells were recovered and washed with a large volume of water. Such preparations typically contained about 0.1 μmol of K⁺ and about 0.65 μmol of Na⁺/mg of yeast.

Other assays. (1) The cellular contents of 3-phosphoglycerate, 2-phosphoglycerate and phosphoenolpyruvate were assayed as described by Czok & Eckert (1963) after extraction as follows. A portion (5ml) of the cell suspension (20mg of yeast/ml) at pH 4.5, containing the other compounds specified in the text, was centrifuged at 30°C. The yeast was quickly resuspended for 2min in 3.3% (w/v) HClO₄ at 0°C. The mixture was frozen in a bath containing solid CO₂ and methylated spirits. It was then thawed at 5°C and refrozen, three times in all. The cellular debris was separated by centrifugation, washed twice with 5% (w/v) HClO₄ (2 × 0.5ml) and the washings were combined with the original extract. The mixture (4ml) was adjusted to pH 7 by the addition of solid K₂CO₃. The precipitated KClO₄ was separated by centrifugation and the supernatant collected and stored at -20°C for the assays.

(2) Cellular ATP was assayed as described by Eddy & Hogg (1969). The appropriate yeast suspension (0.5ml, containing about 6mg of yeast) was mixed with 7.5% (w/v) HClO₄ (2.0ml) at 0°C. After 10min the mixture was neutralized with 0.7M-KOH solution (2.2ml) containing 0.1M-triethanolamine hydrochloride. It was kept at 0°C for a further 30min and clarified by centrifugation. The supernatant solution was assayed for ATP.

Antimycin A and 2-deoxyglucose were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. ATP, 3-phosphoglyceric acid, 2-phosphoglyceric acid and phosphoenolpyruvate, together with the other compounds and

enzymes used in their spectrophotometric assay, were obtained from the Boehringer Corp. (London) Ltd., London W.5, U.K.

RESULTS

Depletion of the cellular ATP content. Studies with a Clark-type oxygen electrode showed that within 1min of the addition of antimycin A (0.1 μg/mg of yeast) the endogenous respiration rate fell by 90%. 2-Deoxyglucose (10mm) stimulated respiration in the absence of antimycin by about 50%, as others have observed (Maitra & Eastabrook, 1962). The cellular contents of ATP, 3-phosphoglyceric acid, 2-phosphoglyceric acid and phosphoenolpyruvic acid were lowered within 1.5min of the addition of both compounds together (Table 1). From that time up to 12min the yeast contained no more than about 0.3nmol of either ATP or phosphoenolpyruvate/mg dry wt. of yeast. The addition of 10nmol of glycine/mg of yeast to the system, 5min after the metabolic inhibitors, appeared not to affect these metabolite contents. When 14mm-deoxyglucose was used together with 0.2, 0.6 or 2 μg of antimycin/mg of yeast both the cellular ATP content and the absorption of glycine by the yeast (see below) were independent of the amount of antimycin present.

In other work the yeast (10mg/ml) was suspended at 30°C in 5mm-tris solution adjusted to pH 4.5 with citric acid and containing 5mm-deoxyglucose and 0.1 μg of antimycin/mg of yeast. The pH in the extracellular phase was recorded with a glass electrode and samples were withdrawn for the assay of both intracellular and extracellular [K⁺] by flame photometry. A series of observations showed that the pH increased steadily by 0.1–0.2 unit during 10min and, simultaneously, the concentration of K⁺ in the extracellular phase increased steadily from about 0.5mequiv./l to 1mequiv./l at the expense of cellular K⁺ (150–200mequiv./l of cellular water). The ejection of H⁺ from the yeast, a process actuated by metabolism (Conway & Brady, 1950), appeared to have stopped and the ion movements were presumably controlled by their diffusion gradients. Nevertheless the yeast absorbed selected amino acids in the rather striking fashion described below.

Amino acid absorption in the presence of antimycin and deoxyglucose at pH 4.5. Fig. 1 illustrates two important aspects of the transport behaviour under these conditions. (1) Glycine appeared to be concentrated in the cells, relative to the solution phase, though to a smaller extent than when the two inhibitors were omitted. (2) These lowered the net uptake of glycine. For instance, in the assay illustrated in Fig. 1(a), a relatively small amount of yeast was used (0.7mg/ml). The uptake of glycine

Table 1. *Effects of 2-deoxyglucose and antimycin on the cellular contents of ATP and glycolytic intermediates in yeast*

The concentrations of 2-deoxyglucose and antimycin used were 5mM and 0.5 μ g/mg of yeast respectively. The yeast was kept for the stated period at pH 4.5, either with or without the two metabolic inhibitors, and then assayed as described in the Materials and Methods section. The results of three independent determinations were averaged and are followed by the s.e.m.

Control		Antimycin + deoxyglucose	
Time (min)	ATP content (nmol/mg of yeast)	Time	ATP content (nmol/mg of yeast)
1	2.34 $\pm$ 0.61	0.5	0.48 $\pm$ 0.20
2	4.32 $\pm$ 1.93	1.5	0.30 $\pm$ 0.09
4	3.10 $\pm$ 1.36	3.5	0.27 $\pm$ 0.08
		6.0	0.22 $\pm$ 0.04
7.5	3.71 $\pm$ 2.09	7.0	0.28 $\pm$ 0.09
10.5	2.86 $\pm$ 0.97	10.0	0.24 $\pm$ 0.06
		15.0	0.17 $\pm$ 0.03
Time	Phosphoenolpyruvate content (nmol/mg of yeast)	Time	Phosphoenolpyruvate content (nmol/mg of yeast)
1	0.33 $\pm$ 0.01	1	0.15 $\pm$ 0.03
5	0.27 $\pm$ 0.04	5	0.10 $\pm$ 0.01
8	0.23 $\pm$ 0.04	8	0.10 $\pm$ 0.02
10	0.33	10	0.10
12	0.35 $\pm$ 0.04	12	0.10 $\pm$ 0.04
Time	3-Phosphoglycerate content (nmol/mg of yeast)	Time	3-Phosphoglycerate content (nmol/mg of yeast)
1	0.98 $\pm$ 0.07	1	0.53 $\pm$ 0.08
5	0.87 $\pm$ 0.05	5	0.36 $\pm$ 0.03
8	0.66 $\pm$ 0.03	8	0.21 $\pm$ 0.01
12	0.65 $\pm$ 0.06	12	0.23 $\pm$ 0.03
Time	2-Phosphoglycerate content (nmol/mg of yeast)	Time	2-Phosphoglycerate content (nmol/mg of yeast)
1	0.16 $\pm$ 0.06	1	0.09 $\pm$ 0.03
5	0.08 $\pm$ 0.03	5	0.03 $\pm$ 0.01
8	0.04 $\pm$ 0.02	8	0.01 $\pm$ 0.01
12	0.10 $\pm$ 0.04	12	0.02 $\pm$ 0.02

from a 0.2mM solution during 25min reached 37nmol/mg of yeast in the presence of the metabolic inhibitors and about 180nmol/mg in their absence. The accumulation ratio (cellular [glycine])/(extracellular [glycine]) was 45 in the former instance and about 730 in the latter. About 13% and 65% respectively of the available glycine was absorbed. In Fig. 1(b) the yeast concentration was 5.7mg/ml, about 50% of the glycine was absorbed and the concentration ratio reached about 50, both in the presence and in the absence of the metabolic inhibitors. Raising the concentration of yeast to 12mg/ml increased by up to about 90% the proportion of the available glycine (0.12mM) that was absorbed in 5min. The mean accumulation ratio ($\pm$ s.e.m.) in the presence of deoxyglucose and antimycin was then 174 ± 8 (4). These observations show that in the presence of the two metabolic inhibitors, complex circumstances govern the

accumulation ratio exhibited by the standard yeast, containing K^+ . Two factors contribute to the variation with the yeast concentration. (1) A marked depletion of the glycine content of the extracellular phase may occur when a large amount of yeast is used. (2) Only about 10–20nmol of glycine/mg of yeast is absorbed rapidly in the presence of deoxyglucose and antimycin.

The absorption of glycine under these conditions was not affected by the removal of O_2 from the system in a stream of N_2 (two experiments).

Effect of dinitrophenol. In two experiments with 10mg of yeast/ml, the presence of 0.1mM-2,4-dinitrophenol at pH 4.5 (1) lowered by $55 \pm 2\%$ the amount of glycine accumulated in 1min from a 0.05mM solution containing the other two metabolic inhibitors, but (2) the amount of glycine accumulated in the period after 5min was scarcely affected by dinitrophenol, the ratio (cellular

Fig. 1. Time-course of the uptake of glycine at two yeast concentrations and the effects on that process of (1) replacing cellular K^+ by Na^+ and (2) the addition of both 5 mM-2-deoxyglucose and 0.5 μ g of antimycin/mg of yeast. [Glycine] was 0.2 mM; the pH was 4.5; (a), 0.7 mg of yeast/ml; (b) 5.7 mg of yeast/ml. Neither inhibitor present: Δ , K^+ ; $\circ$, Na^+ as major cellular cations. Deoxyglucose and antimycin present: $\blacktriangle$, K^+ ; $\bullet$, Na^+ as major cellular cations.

[glycine]]/(extracellular [glycine]) at 10 min being 127 ± 1 without, and 90.5 ± 0.4 with, dinitrophenol present.

Dependence on cellular K^+ as opposed to cellular Na^+ . The absorption of glycine in the presence of deoxyglucose and antimycin was greatly restricted when cellular K^+ was replaced by Na^+ (Fig. 1). The 'sodium yeast' functioning in the absence of the metabolic inhibitors, however, concentrated glycine almost as well as did the yeast containing K^+ as principal cation (Fig. 1).

Phenylalanine. The effects on the absorption of L-[U- ^{14}C]phenylalanine of (1) both 2-deoxyglucose and antimycin and (2) the replacement of most of the cellular K^+ by Na^+ were similar to those observed with glycine (two experiments).

Fate of the absorbed amino acid. A yeast preparation (50 mg) in 4 ml of tris-citrate buffer, pH 4.5, containing 5 mM-2-deoxyglucose and 0.5 μ g of antimycin/mg of yeast (see the Materials and Methods section), was exposed to 0.48 μ mol of [1- ^{14}C]glycine for 3.5 min. In three experiments (1) the ^{14}C content and (2) the ninhydrin-reactive material remaining in the extracellular phase were each assayed. The former was equivalent to 42 ± 3 (S.E.M.) nmol of [1- ^{14}C]glycine and the latter to 55 ± 5 nmol of glycine. Thus the glycine was absorbed without exchanging with the endogenous cellular amino acids. The ^{14}C content of the cells was extracted with hot water (0.5 ml), as described

in the Materials and Methods section, and portions of the extract were assayed for [^{14}C]glycine. In this way $89 \pm 3\%$ (S.E.M.) of the [^{14}C]glycine taken into the yeast was recovered in a fraction exhibiting the chromatographic behaviour of glycine. Less than 1% of the cellular ^{14}C was associated with serine. In one similar experiment 88% of the L-phenylalanine absorbed was recovered apparently unchanged. We conclude that these two amino acids were absorbed into the yeast, under these conditions of severely restricted energy metabolism, without undergoing chemical modification. In three assays $78 \pm 4\%$ of the glycine absorbed by the 'sodium yeast' in the absence of the metabolic inhibitors was likewise recovered as glycine and $9.9 \pm 1.5\%$ as serine.

Dependence on extracellular $[H^+]$ and $[K^+]$. Eddy *et al.* (1970b) found that the glycine absorption taking place when glucose was omitted from the yeast suspension was strikingly stimulated by lowering both the pH and extracellular $[K^+]$. The object of the new measurements was to see (1) whether similar factors were involved in the presence of deoxyglucose and antimycin and (2) whether they applied to amino acids other than glycine. Fig. 2 compares the initial rates of uptake (v) of five amino acids in the presence of deoxyglucose and antimycin, at pH 4.5 and 7.4, with and without the addition of 5 mM- or 100 mM-potassium chloride. In each case the value of v with a given amino acid

Fig. 2. Histograms showing the initial rate of uptake (v) of each of five amino acids (Leu, Lys, Phe, Gly, Aib = 2-aminoisobutyric acid) from a 0.2mM solution at either (a) pH 4.5 or (b) pH 7.4, containing 0, 5 or 100mM-KCl and 5mM-2-deoxyglucose with 0.5 μ g of antimycin/mg of yeast. The yeast concentration was 5mg/ml.

was largest at pH 4.5, in the absence of added K^+ . The results with glycine are rather similar to the behaviour found by Eddy *et al.* (1970b) with yeast cells respiring their endogenous energy reserves. We conclude that these interactions do not necessarily involve ATP.

Eddy *et al.* (1970b) obtained a value of 0.33mequiv./l for the inhibition constant for K^+ on glycine uptake at pH 7.4, a result which appears consistent with the further data in Fig. 2. The value of v for glycine, measured in the presence of the two metabolic inhibitors, was lowered by about 10% by 200mM-mannitol at pH 7.4 and by 35% at pH 4.5. The effect shown in Fig. 2 of increasing $[K^+]$ from 5mequiv./l to 100mequiv./l at pH 4.5 is thus only partly the result of raising the osmotic pressure. It seems rather that a larger concentration of K^+ was needed at the lower pH to produce a given effect. This suggests that the actions of H^+ and K^+ were perhaps competitively related.

Effect of Na^+ and Ca^{2+} . In the presence of deoxyglucose and antimycin 10mM-sodium chloride lowered v by 36% at pH 7.4 and by -2% at pH 4.5 (two experiments). [Glycine] was 0.2mM in both cases. In two other experiments 1mM-calcium chloride lowered v by 10% at pH 4.5 and by 33% at pH 7.4. Similar effects occurred in the absence of antimycin and deoxyglucose (Eddy *et al.* 1970b).

Observations with the 'sodium yeast'. In one of two similar sets of observations, when [glycine] was 0.2mM, v was 4.0, 1.5 and 0.7nmol/min per mg, respectively, at pH 4.5, 7.4 and 8.5. The two

metabolic inhibitors were absent. The addition of 200mM-mannitol lowered v by 10-28% over the pH range. Glycine uptake at pH 8.5 was lowered by 38% in the presence of 5mM-potassium chloride and by 94% with 100mM-potassium chloride. The corresponding values at pH 7.4 were 23% and 83% respectively. At pH 4.5, on the other hand, 5mM-potassium chloride increased v by about 35% and 100mM-potassium chloride lowered it by about 5%. It is clear that only a minor part of the effects of potassium chloride on the system containing the 'sodium yeast' is due to the change in osmotic pressure.

DISCUSSION

The present results establish that yeast preparations in which the energy metabolism associated with phosphoenolpyruvate and ATP was severely restricted nevertheless rapidly absorbed small amounts, of the order of 10-30nmol/mg of yeast, of glycine and L-phenylalanine. Most of the glycine and L-phenylalanine taken up was recovered from the cells unchanged. Further, the use of large concentrations of yeast resulted, after about 5 min, in the amino acid concentration in the extracellular phase falling well below that in the cells. The cells would initially contain a pool of unlabelled amino acids. The concentration gradient across the cell membrane, for a given amino acid, would be even larger, therefore, than was indicated by the distribution of ^{14}C . Typically a glycine or phenylalanine concentration of at least 3mM was found in the yeast when the concentration outside the cells was of the order of 0.02mM. Further work is needed to show whether larger factors of concentration can be achieved in the presence of the metabolic inhibitors by, for instance, varying the conditions in the extracellular phase. The evidence presented here is sufficient to show clearly that the amino acids were concentrated to a striking degree in the yeast. This was despite the marked inhibition of respiration and ATP metabolism.

Possible mechanisms. (1) The observations with glycine indicate that the accumulation process did not in general depend on a counter-flow of selected endogenous amino acids. (2) The maximum amount of amino acid absorbed, up to about 30nmol/mg of yeast (Fig. 1), corresponds roughly to an 8mM solution in the cellular water. This is rather more than is likely to be retained by adsorption on macromolecular binding sites. For if the molecular weight associated with each binding site is 5000, an 8mM solution of the hypothetical agent would then represent an unacceptably large fraction, namely 14%, of the cellular dry weight. Thus an explanation of the degree of concentration achieved that postulates simple binding of most of the absorbed

amino acid in the cells seems implausible. (3) Alternatively the glycine carrier system might be supposed to be linked to an unspecified exergonic chemical reaction. The hypothetical reaction would involve neither ATP nor phosphoenolpyruvate, two compounds that have been implicated, however, in other transport processes (Albers, 1967; Rothfield & Finkelstein, 1968).

(4) *A preferred mechanism.* In view of the various phenomena involving H^+ and K^+ , we favour a different interpretation in terms of the ion-gradient hypothesis (Eddy, 1966; Eddy *et al.* 1970b). Similar concepts were originally applied to the possible roles of Na^+ and K^+ in the accumulation of amino acids by mouse ascites cells (Christensen, Riggs, Fischer & Palatine, 1952; Riggs, Walker & Christensen, 1958). The hypothesis envisages that the flows of H^+ and K^+ taking place across the cell membrane of the energy-depleted cells are coupled, by means of an appropriate carrier system, to the amino acid influx. On that view, amino acid influx in the presence of the metabolic inhibitors is regarded as a complex physical process in which chemical changes are not directly involved. The notion that a gradient of H^+ , across the plasma membrane, may actuate substrate transport in certain bacterial systems has been argued notably by Mitchell (1963) and by other workers (Harold, Pavlasová & Baarda, 1970). The systems in question are especially prone to inhibition by various compounds, such as dinitrophenol, that uncouple oxidative phosphorylation, allegedly by accelerating the passage of protons across the functional membranes. Mitchell (1963) suggests that the uncouplers may accordingly affect substrate transport by a similar mechanism, namely by short-circuiting a proton gradient that drives the accumulation of the substrate. The argument is obviously indirect and requires experimental support in two essential aspects on which the present work also bears. (1) Proof is required that the movements either of H^+ or of other ions necessarily take place in these systems, during the absorption of substrates bearing no net charge. (2) The effect of stopping energy metabolism on the postulated ionic interactions needs to be elucidated. The present observations may be considered in both contexts.

Because dinitrophenol slowed glycine influx without greatly affecting the extent to which the yeast concentrated the amino acid, in the presence of deoxyglucose and antimycin, its effect on the yeast system, at any rate, cannot be interpreted simply in terms of the short-circuiting of a proton gradient. Again, to return to aspects (3) and (4) above, the latter hypothesis rather than the former would account for (a) the circumstance illustrated in Fig. 2 that, when energy metabolism is largely

blocked, H^+ and K^+ in the extracellular phase both interact with the influx of each of five amino acids. It would also account for (b) the requirement for cellular K^+ , as opposed to cellular Na^+ , if the rapid absorption either of glycine (Fig. 1) or of phenylalanine is to take place in the presence of the two inhibitors; and for (c) the intimate connexion, described in a preliminary report (Eddy, Backen & Nowacki, 1970a), that is then found between, on the one hand, the movements of H^+ and K^+ and, on the other hand, the influx of various amino acids.

Dual modes of amino acid absorption distinguished by their relations to cellular K^+ and to energy metabolism. Replacing most of the normal complement of cellular K^+ by Na^+ almost stopped the absorption of both glycine and phenylalanine in the presence but not in the absence of deoxyglucose and antimycin. Whereas amino acid transport in the 'sodium yeast' therefore appears to depend rather directly on energy metabolism being maintained (mode 1), the preparations containing K^+ could apparently function independently of energy metabolism (mode 2). It remains to be seen how these two modes are related to the complex effects of glucose on the characteristics of the glycine transport system found in yeast preparations containing K^+ and functioning in the absence of the metabolic inhibitors (Eddy *et al.* 1970b).

REFERENCES

- Albers, R. W. (1967). *A. Rev. Biochem.* **36**, 727.
- Bernlohr, R. W. & Novelli, G. D. (1963). *Archs Biochem. Biophys.* **103**, 94.
- Christensen, H. N., Riggs, T. R., Fischer, H. & Palatine, I. M. (1952). *J. biol. Chem.* **198**, 1.
- Conway, E. J. & Brady, T. G. (1950). *Biochem. J.* **47**, 360.
- Conway, E. J. & Moore, P. T. (1954). *Biochem. J.* **57**, 523.
- Czok, R. & Eckert, L. (1963). In *Methods of Enzymatic Analysis*, p. 229. Ed. by Bergmeyer, H. U. New York and London: Academic Press.
- Eddy, A. A. (1966). *Proc. 2nd Symp. Yeast*, p. 457. Bratislava: Slovak Academy of Sciences.
- Eddy, A. A. (1968). *Biochem. J.* **108**, 489.
- Eddy, A. A., Backen, K. & Nowacki, J. A. (1970a). *Biochem. J.* **116**, 34p.
- Eddy, A. A. & Hogg, M. C. (1969). *Biochem. J.* **114**, 807.
- Eddy, A. A., Indge, K. J., Backen, K. & Nowacki, J. A. (1970b). *Biochem. J.* **120**, 845.
- Harold, F. M., Pavlasová, E. & Baarda, J. R. (1970). *Biochim. biophys. Acta*, **196**, 235.
- Heinz, E. (1967). *A. Rev. Physiol.* **29**, 21.
- Maitra, P. K. & Eastabrook, R. W. (1962). *Biochem. biophys. Res. Commun.* **9**, 1.
- Mitchell, P. (1963). *Biochem. Soc. Symp.* no. 22, p. 142.
- Riggs, T. R., Walker, L. M. & Christensen, H. N. (1958). *J. biol. Chem.* **233**, 1479.
- Rosen, H. (1957). *Archs Biochem. Biophys.* **67**, 10.
- Rothfield, L. & Finkelstein, A. (1968). *A. Rev. Biochem.* **37**, 463.