CATION TRANSPORT IN YEAST*

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INTRODUCTION

The maintenance of the internal environment of living cells is well known to demand the expenditure of metabolic energy. This energy requirement may arise in part out of the need to overcome a permeability barrier or because of the existence of gradients across the cell membrane. There is no agreement yet on the site and mechanism of the active processes involved. In the special case of electrolytes the problem of active transport may be complicated by the existence of electrochemical gradients across the cell membrane. The potential, if any, across the yeast cell membrane is not known.

It has been established that work must be done to enable anions to penetrate yeast (1). Similarly cation accumulation requires the supply of energy. The mechanism of cation transport in yeast has been studied in considerable detail by Conway, Ryan, and Carton (2). These workers found that an azide-sensitive carrier system transports potassium into the cells. They further postulate that a separate carrier is responsible for the extrusion of sodium. They conclude that their evidence "appears strongly in favor of respiratory ferment carriers and the direct utilization of electron energy as in the redox pump." (2) The concept of such redox carriers, transporting ions by virtue of cyclic changes in their state of oxidation, has previously been discussed by several workers (*e.g.*, reference 3).

The experiments reported in the present paper further explore the mechanisms of cation transport in yeast. The existence of the postulated Na carrier is reconsidered in the light of new experiments. Experimental proof is also presented further supporting the assumption that an azide-sensitive carrier in the cell membrane is responsible for K transport into the cell.

A preliminary statement of part of these results has already been presented (4).

EXPERIMENTAL

As in previous reports (1, 5), fresh Anheuser-Busch commercial bakers' yeast was used for all experiments. It was exhaustively washed immediately before each experi-

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ment. The preparation of K-deficient sodium-rich yeast was carried out following the instructions of Conway, Ryan, and Carton (2). The fresh yeast contained on the average 145 μM K and 21 μM Na/gm. By allowing the yeast to ferment in the presence of sodium citrate at a temperature of 35°C., the K content can be reduced in 2 hours to as low as 35 μM/gm. At the same time, the Na content rises to about 140 μM/gm. The sodium yeast, after thorough washing, contains little fermentable substrate. Ammonium yeast was obtained by substituting NH_4 citrate for Na citrate.

In studies on cation transport, the final concentration of yeast was 1 in 40 (*w/v*). No appreciable changes in extracellular concentrations were thus caused by the addition of yeast. Experiments on K transport were begun by the addition of one-ninth volume of KCl of suitable concentration at time zero. Controls were run in the absence of inhibitor (control A) and in the presence of 0.02 M Na azide or NaCN (control B). Incubation was carried out in a Dubnoff shaker at the desired temperature. The active K transport was stopped by inhibiting O_2 uptake with excess NaN_3 or NaCN (final concentration 0.02 M). The yeast was then thoroughly washed with ice cold, distilled H_2O. The washed cells were extracted with 10 volumes of 10 per cent (*w/v*) trichloracetic acid, and the Na and K levels determined on a Barclay flame photometer. Unless otherwise stated the results reported are the mean of determinations on at least three separate flasks. Equating the difference between controls A and B to 100 per cent activity, the percentage inhibition by low concentrations of inhibitor could be calculated. For reasons discussed below, control B could not be run above pH 6, even in experiments in which all other flasks were at a higher pH.

Azide was estimated spectrophotometrically at 500 mμ as the ferric azide complex formed in slightly acid solution (6). The pH of the solution to be assayed was adjusted when necessary with HCl to pH 5–6, and $FeCl_3 \cdot 6H_2O$ was added to a final concentration of 0.1 per cent (*w/v*). The method is very sensitive and gives satisfactory results over a range of 0.01–0.5 μM azide/ml. Because many substances interfere with color formation, standards and $FeCl_3$ blanks were always made up in the supernatant fluid from the yeast suspension studied. In the presence of buffers, it was necessary to carry out a preliminary distillation of azide from acid solution in Conway microdiffusion units. Full recoveries were invariably obtained.

In order to avoid as far as possible changes in the ionic composition of the suspension, buffers were not employed except when stated. The pH of a washed suspension of yeast varies from 5.6 to 5.8. The pH was raised when desired by the addition of small amounts of NaOH. The final pH value was determined electrometrically on the supernatant from the suspension after incubation. The pH readings were taken at room temperature and corrected for the temperature of incubation.

The theoretical extracellular space and the ion-permeable outer region of the cell were calculated as in previous experiments (1) by the method of Conway and Downey (7). This total space, referred to as the anion space of the suspension, was in good agreement with the chloride space determined under experimental conditions. From the volume of the anion space and the azide concentration in the supernatant after incubation, azide recovery could be estimated. The difference between added azide and azide recovered in the anion space was equated with azide taken up by cells. Reported azide concentrations represent the sum of the concentration of azide ions and of undissociated hydrazoic acid (HN_3).

Conventional Warburg manometers were used for respiratory measurements. Manometers were shaken at 120 cycles/minute.

RESULTS

1. *The Uptake of Azide by Cells.*—The following experiments were carried out at 0°C. because of the possible existence in yeast of an active anion transport mechanism (1). It was further desired to exclude if necessary the oxida-

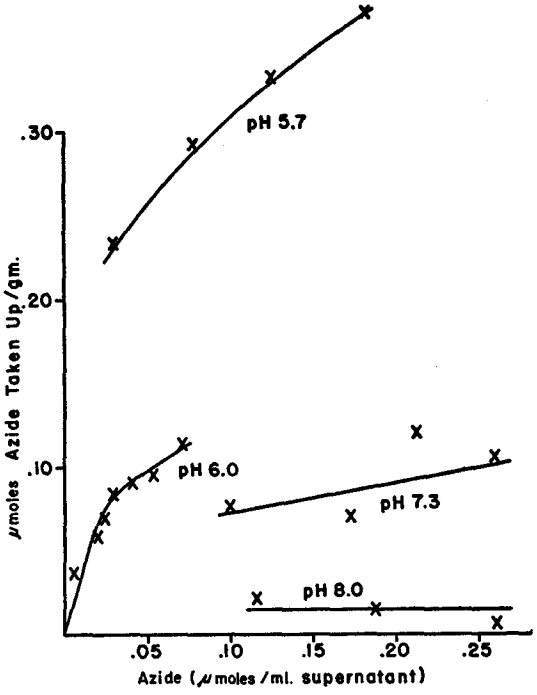

FIG. 1. Uptake of azide by yeast. 60 per cent (*w/v*) suspension of washed yeast in H_2O shaken 30 minutes at 0°C. pH adjusted with NaOH. Azide concentration and pH were determined after incubation. Each point represents the mean of determinations on triplicate flasks.

tion of azide. This may occur even at 0°C. in the presence of hemin enzymes (6, 8). Despite the widespread occurrence of an azide-oxidizing system in animal and plant tissues (9), no evidence was found in the present work of azide oxidation by yeast or leucocytes.

The uptake of azide by cells was calculated as discussed above from the difference between total azide added and azide recovered in the anion space. Representative values for the micromoles azide taken up per gram cells are shown in Fig. 1, for fresh yeast at various final hydrogen ion and azide concentrations.

The pH dependence of azide uptake shown in Fig. 1 suggests that, as frequently observed in other systems, it is undissociated hydrazoic acid rather than azide ion which is taken up by the cells.

Fig. 1 also shows that at pH 5.7 azide accumulates in the cell to a level higher than its concentration in the medium. As pH 5.7 closely approaches the over-all intracellular pH of yeast (10) azide accumulation cannot be explained by the buffering action of the cell contents. Nor is there sufficient metabolic energy available at 0°C. to establish concentration gradients. The accumulation of azide under these conditions must therefore be attributed to its combination with cell constitutents. This is a reversible combination, because all azide can invariably be recovered in the extracellular fluid (anion space) when either the azide is added to the yeast suspension at alkaline pH or when the pH is raised above 7.5 after preliminary incubation at pH 5.7.

Similar experiments were carried out with fresh rabbit leucocytes suspended in physiological saline at pH 6.5 and incubated at 22°C. The volume of the extracellular phase (anion space) in this case was equated with the volume of distribution of added KCNS after 10 minutes' incubation. As in the case of yeast, the volume of distribution of azide exceeded the volume of the anion space. At 22°C., pH 6.5, and an extracellular azide concentration of 0.6 μM/ml., calculated azide uptake showed approximately twofold accumulation of azide by the cell.

2. *K Uptake by Yeast and Its Inhibition by Azide.*—In the presence of fermentable substrates yeast will rapidly take up K under both aerobic and anaerobic conditions. If an azide- and cyanide-sensitive system is involved in all K transport, these inhibitors should depress anaerobic K uptake. That this is not the case is shown in Table I for the case of cyanide. Similar results have been obtained with azide. It will be seen that, in the absence of glucose and PO_4 from the medium, suppression of respiration causes practically complete cessation of K gain. When glucose and PO_4 are added, an increase in K uptake occurs which is now no longer coupled with respiration. This effect is produced by glucose and fructose only, and cannot be duplicated with galactose and oxidizable substrates such as acetate and ethanol.

The Na yeast prepared as described showed little anaerobic CO_2 production and was thus presumably relatively free of fermentable substrates. The experiments reported below were all carried out without the addition of such substrates and thus are concerned exclusively with the azide- and cyanide-sensitive portion of K transport.

The addition to Na yeast of a non-fermentable oxidizable substrate such as acetate considerably stimulated respiration but did not accelerate K uptake. Nor did K cause a significant or consistent increase in the respiration of Na yeast. As an excess of K was always added to the medium, the rates of K uptake were therefore maximal for the conditions used. Assuming that

the Michaelis-Menten equation is applicable to whole yeast cells (*cf.* Rothstein (11)), a K_s of about 6×10^{-3} M K was obtained for K-H exchange in Na yeast (*cf.* below).

Attempts were made to analyze the azide inhibition data by the graphic method of Lineweaver and Burk (12). It was not possible to obtain sufficient accuracy for an unequivocal conclusion on the competitive nature of the inhibition. Usually the maximum rate of K uptake was depressed by the inhibitor suggesting the presence of non-competitive factors in the inhibition.

In Fig. 2 are shown the simultaneous changes in electrolyte pattern when

TABLE 1

Glucose and Potassium Transport

Additions	Atmosphere	Micromoles K taken up/gm. yeast
None	Air	42
Cyanide	Air	3
None	N_2	5
PO_4	Air	42
Glucose	Air	41
Glucose + PO_4	Air	81
Glucose + PO_4 + cyanide	Air	45
Glucose + PO_4 + cyanide	N_2	42
Glucose + PO_4	N_2	44

Each flask contained Na yeast in 40 volumes of M/10 KCl. Na phosphate pH 5.8 M/100, glucose M/50, cyanide M/1000. Similar results were obtained with M/2000 Na azide. Incubation 10 minutes at 21°C. The results presented are typical of many similar experiments although the additive rates of K uptake in air alone and in the presence of glucose and PO_4 when respiration was suppressed were not always identical.

a Na-rich yeast is suspended in aerated KCl solution in the absence of fermentable substrate.

It will be seen that in the later stages of incubation, potassium gain is balanced by sodium loss. This confirms the findings of Conway *et al.* (2). During the first few minutes after the addition of KCl, the rate of K uptake, however, greatly exceeds the rate of sodium loss. In an unbuffered suspension of sodium yeast, the addition of KCl (but not of NaCl) causes a rapid fall in pH. Thus, in a typical experiment, there occurred under these conditions a fall in pH from 5.5 to 3.4 in 5 minutes at 31°C. It is concluded that the initial rapid K uptake in excess over Na loss represents at least partially an exchange of K for hydrogen ions. As Na yeast secretes organic anions (2) the extracellular fluid acquires considerable buffering capacity. No attempt was made to account quantitatively for the K uptake in excess over Na out-

put by the secretion of hydrogen ions, although such 1 to 1 exchange has previously been reported (*cf.* reference 16).

Both potassium-hydrogen and potassium-sodium exchanges are inhibited by azide, as illustrated in Fig. 2. Detailed studies of the inhibition of K trans-

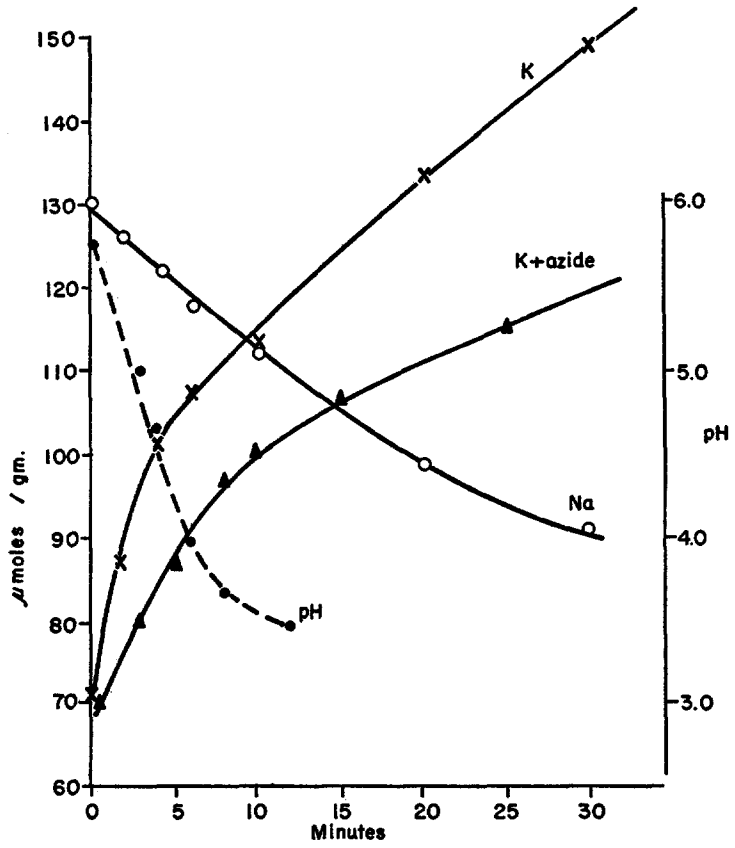

FIG. 2. Cation exchange. Yeast shaken at 31°C. in air. Potassium 0.1 M. To show that both phases of K uptake are azide-sensitive, the uptake of K in the presence of 1.2×10^{-4} M azide is also illustrated. The values reported are typical of several similar experiments and in each case represent the mean of determinations on duplicate flasks.

port by azide could thus be carried out at a time when both Na and H exchanged for K. The experiments reported below were mostly restricted to this initial period.

The duration of the initial exchange is not affected by strong buffering of the suspension. It follows that H extrusion does not stop because of the establishment of an excessive concentration gradient but more likely because of a limited intracellular concentration of succinic and similar acids.

In agreement with this is the finding that many variables such as temperature and oxygen pressure greatly affect the K uptake in the first 5 minutes of incubation. Depending on the length of preincubation, the total K taken up in the first 5 minutes at 31°C. may not exceed the total uptake at 21°C. Similarly, preincubation with inhibitors may alter the intracellular concentration of metabolites. This certainly is the case with azide which increases the rate of carbohydrate utilization by yeast (13).

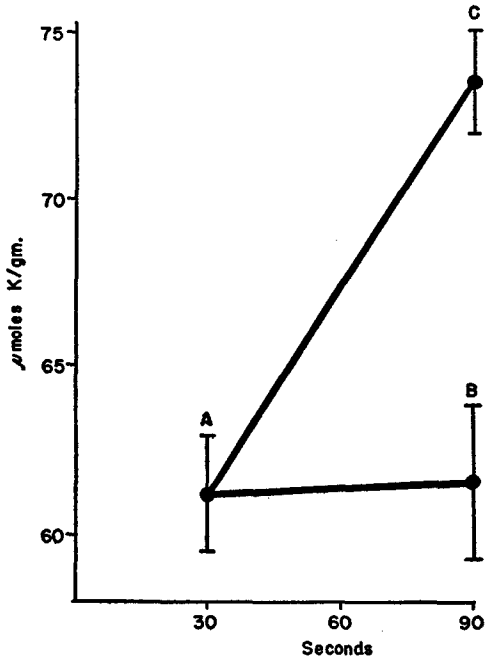

FIG. 3. Time course of azide inhibition of potassium uptake. For details, see text. Temperature 31°C. Suspension continuously stirred with O_2. Na phosphate pH 5.8, 1×10^{-3} M. Potassium 0.1 M. The mean values of 5 experiments for A, B, and C are shown with their respective standard deviation.

The study of the inhibition of K transport therefore requires not only the simultaneous determination of the control values as outlined in the experimental section. It is further necessary to avoid preincubation in the presence of inhibitor. In practice, this was achieved by adding at time zero one-ninth volume of M/1 KCl containing the appropriate amount of inhibitor. The justification for this procedure is based on the results illustrated in Fig. 3, where it is seen that azide exerts instantaneous inhibition of K transport.

In these experiments KCl was added at time zero. After 30 seconds, the reaction was stopped in one tube (A) by the addition of a large excess of sodium cyanide. At the same time, sodium azide to a final concentration of

7.5×10^{-4}M was added to a second tube (B), which was incubated for a further 60 seconds before the addition of excess cyanide. K uptake was allowed to proceed in a third tube for 90 seconds before being stopped by cyanide (C). If azide inhibition in (B) only sets in after a lag phase, the final K level in (B) would be intermediate between (A) and (C), depending on the length of the lag. The fact that K levels in (A) and (B) do not differ significantly, shows that there is no appreciable lag phase in the azide inhibition of K transport. Identical results were obtained when using 10^{-3}M NaCN to inhibit K transport in (B).

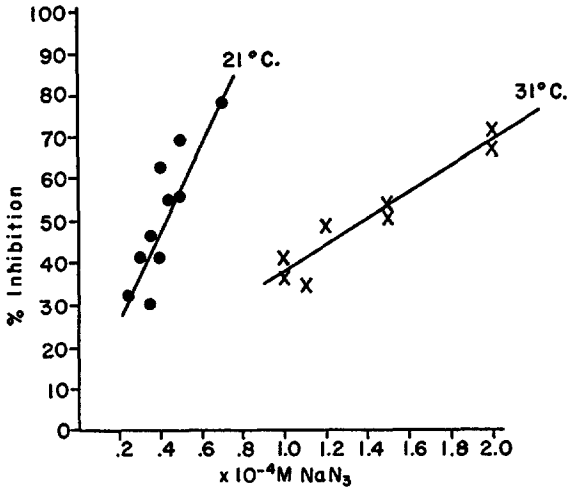

FIG. 4. Azide inhibition of potassium uptake. Na phosphate pH 5.8, 1.5×10^{-2} M. Incubation, 4 minutes in air.

The inhibition of K transport in yeast by varying concentrations of azide in the absence of fermentable substrate is illustrated in Fig. 4. These experiments were carried out with K-deficient yeast at pH 5.8. It is seen that the degree of inhibition varies with temperature. 50 per cent inhibition of K uptake in exchange for hydrogen ions was observed at 1.4×10^{-4}M NaN_3 at 31°C., and 0.4×10^{-4}M NaN_3 at 21°C. These figures are somewhat lower than those previously reported (4) and represent the results of a careful reinvestigation at accurately controlled pH values.

Typical experiments on the pH dependence of the azide inhibition of K transport are reported in Table II. No significant effect is noted at pH 8 of concentrations of azide which are strongly inhibitory at pH 6.

Experiments on leucocytes rendered potassium-deficient by preliminary incubation in ammonium-Ringer solution (14) show that these resemble yeast in the sensitivity of their K transport system to azide.

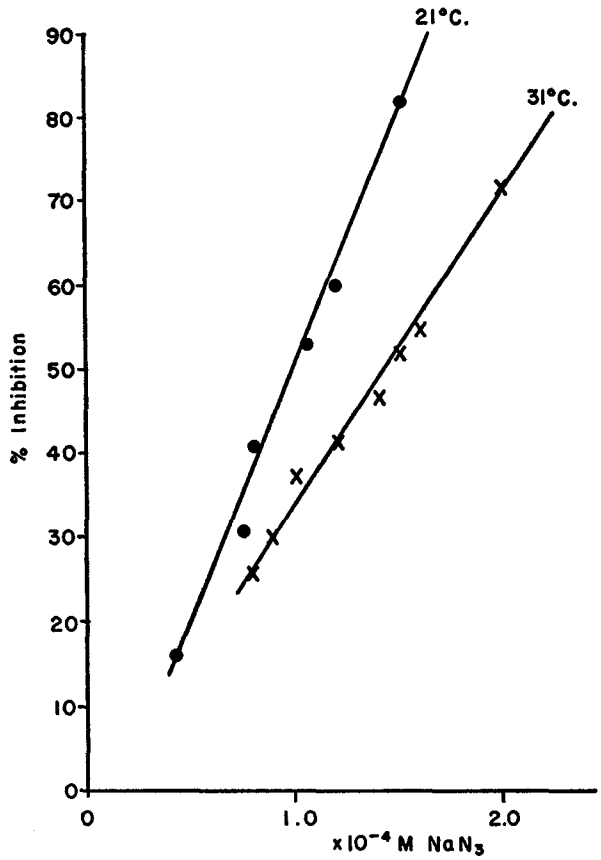

FIG. 5. Azide inhibition of O_2 uptake. Each flask contained 40 mg. Na yeast in 1 per cent ethanol and 0.02 M Na phosphate pH 5.8. Final volume 2.5 ml. 0.2 ml. 2 N KOH in center well. Inhibition measured over 20 minutes. Each point represents mean of duplicate determinations.

TABLE II

pH Dependence of Azide Inhibition of Potassium Transport

Experiment	pH	Azide concentration	Inhibition
		M	*per cent*
1	6	3.3×10^{-4}	82
	8	3.3×10^{-4}	0
2	6	5×10^{-4}	87
	8	5×10^{-4}	8

The yeast was suspended in 40 volumes 0.01 M Na phosphate buffer. Na levels were kept constant by the addition of NaCl. Incubation, 5 minutes at 31°C.

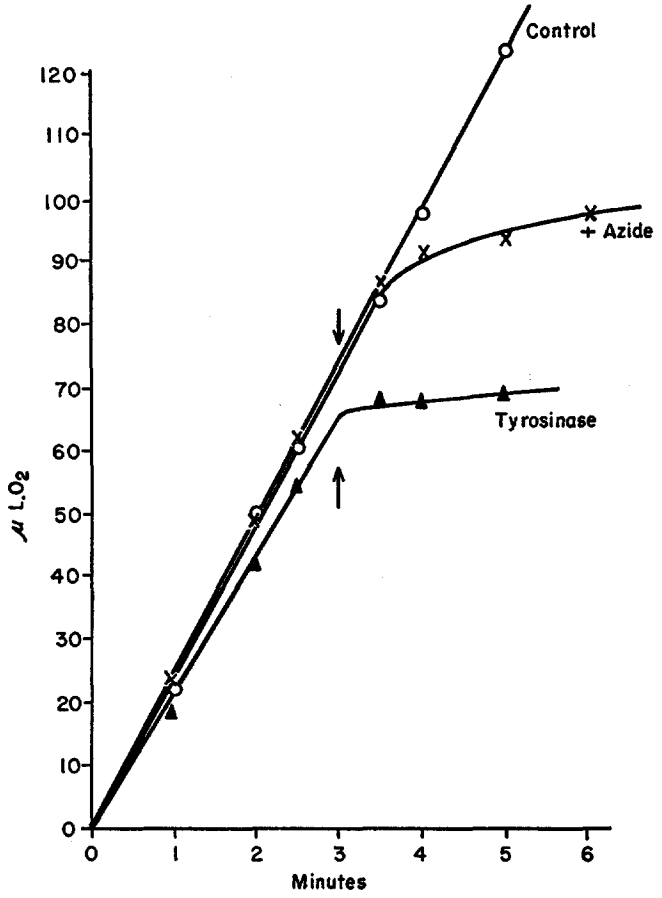

FIG. 6. Time course of inhibition of O_2 uptake. Flasks contained 40 mg. yeast in 2 ml. 1 per cent ethanol, 0.2 ml. M/15 phosphate pH 5.8, and 0.3 ml. H_2O. Alkali in center well. Inhibitor solution or water in side arm (0.5 ml.) tipped in at arrow. Final azide concentration 10^{-3} M. Plotted points represent the mean of three experiments with a standard deviation of ± 1.5 μl. Control experiment was carried out with purified mushroom tyrosinase in 6×10^{-3} M phosphate pH 7. Substrate 2.5 mg. *p*-cresol. Final cyanide concentration 2×10^{-3} M. Curve shown is representative of three experiments run at different enzyme and inhibitor concentrations.

3. *Azide Inhibition of O_2 Uptake.*—These experiments were carried out under conditions strictly comparable to those described for Fig. 4. The sodium yeast was suspended in 1 per cent alcohol to assure the presence of excess of an easily oxidizable substrate to which the cells are freely permeable. Fig. 5 illustrates the degree of inhibition of O_2 uptake by various concentrations of azide. 50 per cent inhibition was obtained at 31°C. at 1.4×10^{-4}M azide,

corresponding to 1.4×10^{-5} hydrazoic acid. The same hydrazoic acid concentration caused a 50 per cent inhibition at pH 5.0. At 21°C., 50 per cent inhibition of O_2 uptake was caused by 1.0×10^{-4}M azide corresponding to 1.2×10^{-5}M HN_3. Greater saturation of the cytochrome oxidase with O_2 (*i.e.* use of pure oxygen in gas phase) led to somewhat greater inhibition by azide. For instance, 50 per cent inhibition was caused at 31°C. by 8×10^{-6}M HN_3. The respiration of intact rabbit leucocytes was considerably more resistant to azide.

The kinetics of the azide inhibition of O_2 uptake are shown in Fig. 6.

In these experiments, a dilute inhibitor solution was tipped from the side arm into the reaction mixture at the indicated time. Water or phosphate buffer pH 7.0 was similarly tipped into the control vessel. The suspension

TABLE III

Loss of Cation from Yeast

	Loss of Na from Na yeast	Loss of K from K yeast
	per cent	*per cent*
Control	9	9
0°C	1	3
Azide (M/1000)	17	10
N_2	13	—
Cyanide (M/1000)	14	—

Each flask contained yeast in 40 volumes of distilled H_2O. Incubation, 40 minutes at 31°C. in air unless otherwise stated. Na levels were kept constant by addition of M/1000 NaCl. Losses are expressed in per cent of original concentration, and were calculated from the mean of determinations on triplicate flasks.

was buffered at pH 6.0 for the azide experiments, and at pH 8.0 for cyanide. That the manometric method is capable of revealing an instant inhibition was demonstrated with an homogeneous enzyme system (tyrosine oxidase). Oxygen uptake by this enzyme was stopped instantaneously on the addition of inhibitor. When azide or cyanide is added to a yeast suspension, a significant lag of about 30 seconds elapses before the O_2 uptake comes to a complete standstill. Under the same conditions, both azide and cyanide exert an immediate inhibition of K transport. (See Fig. 3.)

4. *The Transport of Na.*—Washed yeast was suspended in distilled water and the loss of sodium and potassium was measured after incubation under varying conditions. Typical results are summarized in Table III.

The results show that metabolism is linked to the extrusion both of Na and of K. Indeed, little cation is lost at 0°C. The apparent specificity of the extrusion system appears to be a reflection of the intracellular cation levels. Na is lost from Na yeast, but K is extruded from fresh yeast. Similarly am-

monium is lost from a K-deficient, NH_4-enriched yeast. Conway *et al.* (2) observed that azide, at a concentration sufficient to cause complete inhibition of O_2 uptake in the present experiments, had no effect on Na transport into a K-free medium. Anoxia and cyanide on the other hand are reported strongly to inhibit Na extrusion. These observations are not confirmed by the above experiments. Indeed, Na loss under all conditions where O_2 uptake was abolished consistently exceeded the control values. When Na was exchanged for K, it was observed in agreement with Conway *et al.* that azide strongly depresses Na loss (*cf.* Fig. 2).

DISCUSSION

The results presented in this paper and their significance are discussed under several headings. An attempt is made to interpret them in the light of a carrier hypothesis of cation transport in yeast.

1. *Aerobic and Anaerobic K Uptake.*—The data shown in Table I are best explained on the basis of two separate mechanisms of K transport. One is inhibited by anoxia but does not require any addition to resting cells of Na yeast. The second mechanism appears to be connected with the uptake of sugars and is not affected by anoxia, azide, or cyanide. It neither involves the azide-sensitive carrier, nor does it represent primarily a mechanism which will respond to the physiological stimulus of K deficiency. Indeed, when normal yeast cells are suspended in KCl, no further net K uptake occurs; *i.e.*, the first mechanism will not raise the K level above that of normal cells. The further addition of glucose and PO_4 causes a K influx leading to final K levels of more than 150 per cent of normal. A relationship between K and carbohydrate metabolism in yeast has been frequently observed in the past (15, 16).

The studies of Conway and Kernan (17) on the influence of oxidation-reduction potential on electrolyte transport were carried out under conditions where only the azide-insensitive mechanism of K transport is operative. These studies can shed no light therefore on the properties of an azide-sensitive redox carrier in the membrane.

All further discussion deals only with the azide-sensitive aerobic pathway of potassium uptake.

2. *Respiration and K Uptake.*—Winzler (18) reported a value of 2.2×10^{-6} for the dissociation constant of the azide complex of yeast cytochrome oxidase at 20°C. Recalculation on the basis of undissociated hydrazoic acid concentration reduces this figure to less than 1×10^{-6}. The corresponding value for rat heart cytochrome oxidase at 25°C. is 7×10^{-7} (19). In the present experiments 50 per cent inhibition of respiration, even in an atmosphere of O_2, was observed at somewhat higher levels of inhibitor.

In kidney cortex slices Mudge (20) observed a greater azide inhibition of K transport than of O_2 uptake. Similar results have been obtained in this

laboratory with rabbit leucocytes (unpublished). One explanation for this phenomenon may be the well known action of azide in inhibiting oxidative phosphorylation (21.) In yeast there is a much smaller difference in the azide sensitivity of K transport and of respiration. A system of oxidative phosphorylation less sensitive to azide or a greater relative cytochrome oxidase activity could account for this observation.

Another explanation for the greater azide sensitivity of K transport than of respiration may be a specific interaction of azide with active sites on the cell membrane not protected by permeability barriers. The fact that azide inhibition of K transport sets in without appreciable lag, compared to a significant lag before the inhibitor reaches cytochrome oxidase in the mitochondria strongly supports the view of such a specific interaction. The localization of enzymes on yeast cell walls has previously been described as for instance in the studies of Rothstein and Larrabee (22) on the inhibition of glucose transport by the binding of uranyl ions.

Cyanide also appears to react specifically with the carrier system, as judged by the time course of the inhibition of O_2 uptake and K transport. This observation makes it unlikely that the action of azide and cyanide on K transport is caused either by inhibition of cytochrome oxidase or by interference with oxidative phosphorylation. It is therefore concluded that an azide- and cyanide-sensitive component of the transport system is directly affected by the inhibitors. This agrees with the view of Conway *et al.* (2) on the presence of cytochrome-like redox carriers.

As K transport is inhibited before respiration it is necessary to assume either that the immediate electron acceptor leading to oxidation of reduced carrier differs from O_2, or that the O_2 involved in this reaction is negligible when compared to the over-all oxidative metabolism of the cell. Either of these assumptions is compatible with the observation that respiration but not K transport is greatly accelerated by the addition of an oxidizable substrate to Na yeast.

3. The Reaction of Azide with K Carrier.—Keilin (23) reported that the azide inhibition of yeast respiration varies with pH. Armstrong and Fisher (24) explained the pH dependence of the azide inhibition of biological systems by the fact that only undissociated hydrazoic acid can penetrate the cell. Keilin and Hartree (25) subsequently confirmed this with the erythrocyte. The internal pH of the yeast cell, and therefore the degree of dissociation of intracellular hydrazoic acid, is little affected by changes in pH of the extracellular compartment between 6 and 8 (26). The effect of such pH changes on the azide inhibition of yeast respiration is thus attributed to the impermeability of the cell membrane to azide ion rather than to the changes in the intracellular dissociation of hydrazoic acid. The K carrier must be assumed to be localized in the cell membrane, not protected by a permea-

bility barrier. If azide inhibits K transport by direct interaction with the carrier system, the changes in the azide inhibition of K transport with pH are therefore best explained by the assumption that it is undissociated hydrazoic acid rather than azide ion which combines with the carrier. It must be emphasized that even if this interpretation is not correct, the concentration of K carrier as calculated below would not be appreciably affected. Stannard and Horecker (19) described the analogous case of mammalian cytochrome oxidase. Provided their preparation contained no mitochondrial permeability barriers, their results show that only the undissociated hydrazoic acid combines with the oxidase.

4. The Concentration of K Carrier.—The reaction of hydrazoic acid with the postulated carrier can be represented by Equation 1.

$$C + nHN_3 \rightleftharpoons C(HN_3)_n \tag{1}$$

If, as in the case of yeast and rat heart cytochrome oxidase (18, 19), $n = 1$, the dissociation constant k is given by

$$k = \frac{(C)(HN_3)}{(C \cdot HN_3)} \tag{2}$$

By using the values for the dissociation constant of hydrazoic acid at various temperatures (27), values for the concentration of free hydrazoic acid can be calculated for the experiments illustrated in Fig. 4. At 31°C., 50 per cent inhibition of K transport is obtained at a concentration of 1.4×10^{-5} M undissociated hydrazoic acid. The corresponding value for 21°C. is 0.5×10^{-5} M. From these values, the change of pk with temperature $\left(-\frac{\Delta pk}{\Delta t}\right)$ is calculated at 0.045/°C. over the range studied. The corresponding value determined by Scheler and Jung (28) for methemoglobin-azide is 0.033. The dissociation constant of the K carrier–HN_3 complex at 0°C. is derived from the above values by van't Hoff's equation in which H is the heat content, R the gas constant, and T the absolute temperature.

$$\frac{d \ln k}{dt} = \frac{\Delta H}{RT^2} \tag{3}$$

A value of 2×10^{-6} is obtained. No attempt has been made to account for possible changes in pk with ionic composition of the medium (29).

From the dissociation constant of hydrazoic acid at 0°C. (0.8×10^{-5}) the concentration of undissociated hydrazoic acid is calculated for the experiments illustrated in Fig. 1. The total concentration of K carrier (Pc) in the cell wall can then be calculated as follows:—

$$Pc = C + C(HN_3) \tag{4}$$

$C(HN_3)$ is experimentally determined as combined azide as in Fig. 1. No

correction has been applied for uncombined azide within the cell. Indeed the concentration of the latter is a function of the extracellular concentration of undissociated hydrazoic acid. Azide taken up per gram cells at a pH at which azide is mainly dissociated therefore closely approximates azide bound by cell constituents.

From equations (2) and (4) is obtained equation (5):

$$Pc = C(HN_3) \times \left(1 + \frac{2 \times 10^{-6}}{(HN_3)}\right) \tag{5}$$

The values of Pc obtained in three separate experiments in which the combined azide was determined in the concentration range of 1.7 to 4.4 $\times$ 10^{-6} M HN_3 varied from 0.08 to 0.10 μM/gm. yeast. If the azide-sensitive carrier contains 1 mole Fe/mole, this corresponds to roughly one-seventh of the total Fe content of the cells as determined by the thiocyanate method after digestion with sulfuric acid.

The value calculated for the concentration of K carrier is of course a maximum value as other cell constituents may significantly contribute to the binding of azide in the concentration range studied. That this does indeed occur is shown by a consideration of the change of azide uptake with temperature.

Equation (5) can be rearranged to equation (6):

$$\text{Bound azide} = \frac{Pc \times (HN_3)}{k + (HN_3)} \tag{6}$$

If temperature is represented by subscripts, and if $Pc_0 = Pc_t$

$$\frac{(\text{Bound azide})_0}{(\text{Bound azide})_t} = \frac{(HN_3)_0}{(HN_3)_t} \times \frac{k_t + (HN_3)_t}{k_0 + (HN_3)_0} \tag{7}$$

At HN_3 concentrations of 3 to 4 $\times$ 10^{-6} M the experimentally observed value for the ratio of equation (7) at t = 21°C. and t = 31°C., was considerably smaller than the theoretical ratio calculated for the values of k of the potassium carrier–inhibitor complex. Other cell constituents whose azide complex possesses a smaller value of H in equation (3) than the potassium carrier–inhibitor complex therefore appear to react significantly with azide at the concentration studied. One such compound is cytochrome oxidase whose azide inhibition is less sensitive to temperature changes than is the K carrier (*cf.* Fig. 5).

A concentration of 0.1 μM carrier/gm. cells would approximately correspond to 6 $\times$ 10^6 molecules per cell. This compares to a figure of 4.6 $\times$ 10^7 calculated by Rothstein (11) for the active centers in the yeast cell wall involved in glucose uptake.

5. The Na Carrier.—Conway *et al.* (2) believe that a Na carrier is responsi-

ble for the extrusion of Na from yeast. As under present conditions we could not fully confirm all the findings of Conway *et al.* (2), the question of the existence of a separate carrier for Na was reconsidered.

The experimental results presented above do not support the view that in the yeast studied Na extrusion into distilled water is coupled to oxidative metabolism. The fact that energy is required (*cf.* Table III) may be explained by the need to produce anions like succinate or bicarbonate to accompany the extruded Na. The movement of Na may indeed be consequent on the excretion of anion. A higher anaerobic organic anion production could then account for the increased Na extrusion when O_2 uptake is inhibited. The apparent specificity of the azide-resistant Na pump observed by Conway *et al.* appears to be a reflection of intracellular Na and K concentrations (*cf.* Table III). It is concluded therefore that Na extrusion by Anheuser-Busch yeast in the absence of extracellular K does not necessitate the assumption of an active Na carrier at work.

The exchange of Na for extracellular K is inhibited by azide. We further find, in contrast to Conway *et al.* (2), that Na extrusion by Na yeast is as much inhibited by 2:4 dinitrophenol (DNP) as is K uptake. If in the results of Conway *et al.* (2) the DNP-resistant Na extrustion into a K-free medium may be subtracted from the DNP-resistant Na extrusion into KCl, it is seen that the action of DNP on K transport does not greatly differ from its effect on simultaneous Na extrusion. Since Na and K transport are equally sensitive to the action of all inhibitors tested, the need for assuming a separate Na carrier in yeast rests on the specificity of Na exchange for K observed by Conway *et al.* (2).

There are two considerations germane to this argument. First, the postulated Na carrier is apparently able to transport NH_4^+ as easily as Na. This can be deduced from the experimental observation that K uptake by NH_4 yeast is identical in all respects to K uptake by Na yeast. In both cases, moreover, there occurs a K-H exchange. It would be necessary to assume therefore that the outward carrier will also carry H ions. The second consideration refers to the possibility that the apparent preference of the Na carrier for Na over K is a reflection of differences in the intracellular state of Na and K ions. Such differences have previously been described, for instance in the work of Mudge (30) on K and Na exchange in kidney cortex slices, and by Cowie, Roberts, and Roberts (31) in *E. coli*.

In view of the above considerations it is felt that further work would be required to establish the existence of a separate Na carrier in yeast.

SUMMARY

1. The distribution of azide added to suspensions of bakers' yeast was studied under various conditions. The recovery of azide was estimated in the

volume of water into which low concentrations of electrolytes can readily diffuse (anion space). Considerable azide disappeared from this anion space.

2. The incomplete recovery of azide in the anion space is due to its uptake by the cells. This uptake occurs against a concentration gradient at 0°C., and is attributed to binding of azide by cell constituents.

3. Confirmatory evidence is presented that one such constituent is the K carrier in the cell membrane. The azide inhibition of K transport is not mediated by inhibition of cytochrome oxidase in the mitochondria.

4. From the amount of combined azide and the experimentally determined dissociation constant of the K carrier–inhibitor complex, the maximum value for the concentration of this carrier is calculated as 0.1 μM/gm. yeast.

5. The addition of glucose and PO_4 causes a secondary K uptake which is not azide-sensitive and is clearly distinct from the primary, azide-sensitive mechanism.

6. The existence of a separate carrier responsible for Na extrusion is reconsidered. It is concluded that present evidence does not necessitate the assumption that such a carrier is active in yeast.

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