Relationship of Cation Influxes and Effluxes in Yeast

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ABSTRACT  The Na⁺ efflux from Na⁺-rich yeast cells into a cation-free medium is largely balanced by the excretion of organic anions. In the presence of Rb⁺, K⁺, or high levels of H⁺ (pH 3–4), the Na⁺ efflux is increased and the organic anion excretion is suppressed so that stoichiometric cation exchanges occur. H⁺ participates in the exchanges, moving into or out of the cells depending on the external pH and on the concentration of external Rb⁺(K⁺). The total cation efflux is dependent on the external Rb⁺ concentration in a “saturation” relationship, but the individual cations in the efflux stream are not. The discrimination factor in the efflux pathway between H⁺ and Na⁺ is very large (of the order of 10,000), and between Na⁺ and K⁺ considerable (of the order of 50). For the latter pair, the recycling of K⁺ from the cell wall space is an important factor in the discrimination. In addition, the Na⁺ efflux as a function of Na⁺ content follows a sigmoidal curve so that the discrimination factor is increased at high levels of cellular Na⁺. Although the influx and efflux pathways behave as a tightly coupled system, the mechanism of coupling is not entirely clear. A single system with different cation specificities and kinetic behaviors on the inside and outside faces of the membrane could account for the data.

Yeast cells can, in the presence of substrate, take up potassium with great rapidity. The characteristics of the uptake have been determined in detail in terms of kinetics, specificity, and relationship to metabolism (1, 6, 21, 22). The system is saturable by monovalent cations with the specificity sequence, K⁺ > Rb⁺ > Cs⁺ > Na⁺ > Li⁺ (2, 10). Under certain conditions bivalent cations such as Mg²⁺ can also be transported by the same system (7), although a separate and distinct transport system for bivalent cations is also present (16, 26). The potassium transport site also has a high affinity for H⁺, which competes for transport. The relative complexity of the transport system is illustrated by findings that H⁺ and certain other cations, especially Ca²⁺ and Cs⁺, can inhibit uptake by interaction with a nontransporting “modifier”
site (3), and that very low concentrations of K+ and Rb+ can activate transport by interaction with an "activator" site (4, 5).

In contrast to the uptake of cations, the characteristics of efflux pathways for cations are not well defined. Yeast cells do not require Na+ for growth. Consequently, commercial yeast used in most experiments is grown in low Na+ media and contains much K+ and very little Na+. In such K+-rich cells, in the presence of impermeant, nontransported anions such as Cl−, K+ uptake is balanced by a stoichiometric efflux of H+ (12, 23, 25) derived ultimately from metabolism of substrate (6). The efflux of K+ on the other hand, is normally slow and it is not much increased by raising the external K+ concentration, indicating that K+ for K+ exchange accounts for only a small component of the K+ influx in the presence of glucose. In view of the high internal K+ concentration, of the order of 150–200 mM, and the low H+ concentration (pH over 5 [9, 27]), the apparent discrimination of the efflux pathway for H+ over K+ might be as high as 10,000 to 1. The large discrimination factor is also evident from the studies of Ryan and Ryan (27) who examined the K+ and H+ efflux as a function of intracellular pH. The optimum intracellular pH for K+ efflux was 6.4 and for H+ efflux, 5.7 with the rates of efflux about equal at pH 5.9. The concentration ratio K+/H+ at the latter pH, is of the order of 5,000 to 1.

Yeast cells can be loaded with Na by exposure to high concentrations of the cation in the absence of K+(11). Such Na+-loaded cells suspended in water lose Na+ slowly accompanied by an almost equivalent loss of organic anions and bicarbonate, but with very little efflux of K+. Addition of K+ to the medium results in a large enhancement of Na+ efflux and concomitant gain of K+ (13). Na+ efflux is further enhanced by the presence of sugars (20). The Na+ efflux is influenced appreciably by two internal factors, the Na+ content and the intracellular pH. The relationship to Na+ content roughly follows a sigmoid function (14), whereas that to pH shows an optimum at a value of 6.5 (27).

The relationships between the efflux and influx pathways have not yet been clearly established. Conway (6, 13), based on inhibitor studies, suggested that separate carriers are involved in the influx of K+ and in the efflux of Na+, a conclusion disputed by Foulkes (15) who found that Na+ extrusion and K+ uptake by Na+-loaded cells were equally sensitive to various inhibitors. More recently, on the basis of the relationship of H+, Na+, and K+ efflux to the intracellular pH, Ryan and Ryan (27) have supported the concept of two separate cation transport systems in yeast, one involved in K+-Na+ or K+-K+ exchanges and the other in K+-H+ exchange. In another fungus, *Neurospora*, the kinetics of K+ influx, H+ efflux, and Na+ efflux can be accounted for either by a single system or by two distinct systems (29, 30).

In part the difficulties of interpretation arise from deficiencies in the
existing data. Much of the information on cation efflux in yeast is qualitative in nature. In the two studies in which quantitative relationships were determined (14, 27), only certain of the parameters were varied. For example, the movements of $H^+$ have been measured exclusively in cells virtually free of $Na^+$ so that the effect of internal $Na^+$ concentrations on $H^+$ efflux is not known. Nor has the influence of intracellular $Na^+$ concentrations on $K^+$ efflux been evaluated. Although it is clear that extracellular $pH$ and $K^+$ markedly influence $Na^+$ efflux, the quantitative relationship between extracellular $H^+$ and $K^+$ concentrations and ion fluxes in $Na^+$-loaded yeast have not been determined. In the present paper, a series of studies were undertaken in which the effluxes of $Na^+$, $H^+$, and $K^+$ were determined simultaneously in cells in which intracellular $K^+$ was replaced to varying degrees by $Na^+$. The effects of extracellular cations and $pH$ were systematically determined and in many cases the influx of $H^+$ and $K^+$ (or $Rb^+$) was also measured. From the observations, the factors that contribute to discrimination between cations in the efflux and influx pathways could be evaluated.

METHODS

Fresh commercial baker’s yeast (Standard Brands Inc.) was washed three times with 10 vol of water. In the washing procedure, centrifugation was at slow speeds for 5 min sufficient to spin out only 85–90% of the cells on the first wash so that cell debris, colloidal material, and lighter cells were discarded.

The cells were loaded with $Na^+$ by exposure to sodium citrate plus 0.1 M glucose for 4 h at 22°C (11). In the loading process the total cation ($Na^+ + K^+$) remained relatively constant, but varying degrees of substitution of $Na^+$ for $K^+$ were achieved by using different sodium citrate concentrations in the range of 0.2–200 mM (Table I). The maximum substitution by the procedure is almost 80%.

| Initial Na citrate (mM) | Final cellular $Na^+$ concentration (nM) | Final cellular $K^+$ concentration (nM) | Total cations $Na^+ + K^+$ (nM) |
|------------------------|-----------------|----------------|------------------|
| 0                      | 0               | 140            | 140              |
| 0.2                    | 5               | 137            | 142              |
| 1.0                    | 16              | 126            | 142              |
| 5.0                    | 30              | 109            | 139              |
| 20                     | 46              | 88             | 134              |
| 50                     | 51              | 84             | 135              |
| 100                    | 87              | 56             | 143              |
| 150                    | 108             | 29             | 137              |
| 200                    | 108             | 29             | 137              |

Cellular concentrations are millimoles per kilogram of cells (wet weight).
The Na⁺-loaded cells were washed three times with 100 vol of cold water (4°C) and suspended in medium at 22°C containing specified concentrations of alkali cations added as chlorides. The pH was maintained by a pH stat using HCl or triethylamine chloride. At specified times samples of suspension were separated by centrifugation. The supernatants were saved for various determinations. The cells were washed twice with 100 vol of cold water (4°C) and an aliquot was counted for radioactivity. A second aliquot was boiled to release cellular Na⁺ and K⁺ for analysis.

Na⁺ and K⁺ were estimated in the supernatants and in the boiled cell extracts by flame photometry. Rb⁺ was estimated by isotopic techniques using ⁸⁶Rb. Net fluxes of H⁺ were estimated by the amount of acid or alkali used in the pH stat to maintain constant pH. Organic acid anion release to the medium was determined by acid-base titration between the limits pH 3.0–8.0. Packed cell volumes were determined for each suspension by centrifugation in calibrated tubes. The interspace between cells in the packed pellet was assumed to be 26% and the cell wall space 12% (8).

In some experiments, cation effluxes were measured using the cell column technique (24). A pad of yeast (300 mg) was packed on a medium porosity scintered glass disk (diameter 30 mm) by suction. The chosen medium was passed through the pad of yeast, under pressure (about 3.5 psi) at a rate of 3 ml/min. The effluent was collected over a period of time in tubes in a fraction collector and analyzed for Na⁺ and K⁺ by flame photometry.

RESULTS

The reported (13) stimulation of Na⁺ loss from Na⁺-rich cells by K⁺ was readily confirmed (Fig. 1). Of the other alkali metal cations only Rb⁺ was effective with Mg++, Ca⁺, and Li⁺ giving little or no enhancement. It has also been reported (13) that the Na⁺ loss into salt-free solutions is balanced by an equivalent loss of organic anions, but that the loss in the presence of extracellular K⁺ involves a stoichiometric exchange of K⁺ for Na⁺. In our experiments, on the other hand, H⁺ was also found to play a significant role in the cation exchanges. In Na⁺-loaded cells suspended in salt-free solutions, a large fraction of the Na⁺ loss was balanced by H⁺ gain, about 70% in the experiment of Fig. 2 (lower section), with the remainder balanced against excreted titrable organic anions. In the presence of Rb⁺ (upper section of Fig. 2), the organic anion secretion was repressed, so that stoichiometric cation exchanges were observed. In this particular experiment organic acid determinations were carried out only at 30 min. The loss into salt-free solution was 9.1 mmol/kg, approximately the difference between the Na⁺ loss and H⁺ gain. The value in the presence of Rb⁺ was <1.0 mmol/kg, the limit of detection. In three other similar experiments the organic acid values were determined at each of three time points. On the average, organic acid excretion accounted for 86% of the surplus of Na⁺ loss over H⁺ gain with yeast suspended in salt-free medium. In the presence of Rb⁺ or K⁺ (concentrations from 3 to 100 mM) no significant excretion of organic acid was detected by the titration method. The Na⁺ loss was stimulated about threefold by Rb⁺.
Nevertheless, Na⁺ loss was appreciably exceeded by the Rb⁺ gain, with the difference accounted for by H⁺ loss, the direction of H⁺ movement being reversed in the presence of Rb⁺. Virtually no loss of K⁺ was detectable in cells suspended in salt-free solutions or in the presence of Rb⁺.

The initial cation influx (Rb⁺) or efflux (Na⁺ + H⁺) as a function of external Rb⁺ concentration, followed an asymptotic relationship (Fig. 3), that gave a reasonably good fit on a reciprocal plot (Fig. 4). The calculated values of $K_m$ and $V_m$ were 0.8 mM and 4.8 mmol/kg yeast/min. These data compare reasonably well with previously determined kinetic characteristics of Rb⁺ transport in K⁺-rich baker’s yeast (2, 3, 10).

Although the total cation efflux followed simple kinetic behavior with respect to external Rb⁺, the fluxes of the individual cations, H⁺ and Na⁺, did not. As already pointed out, a substantial efflux of Na⁺ and influx of H⁺ proceeds in the absence of Rb⁺. Furthermore, the proportions of H⁺ and Na⁺ in the total cation efflux depended on the Rb⁺ concentration. At low concentrations (below 0.7 mM), the efflux of Na⁺ exceeded the influx of Rb⁺ with the difference largely balanced by an influx of H⁺ (Fig. 3). At 0.7 mM Rb⁺,
the efflux of Na\(^+\) was equal to the influx of Rb\(^+\), with no net flow of H\(^+\). At high concentrations (above 0.7 mM), the efflux of Na\(^+\) was less than the influx of Rb\(^+\) with the difference balanced by efflux of H\(^+\). At concentrations that gave a maximal effect (above 3 mM) the ratio of Na\(^+\) to H\(^+\) efflux was constant, approximately 2 to 1.

To specify the role of H\(^+\) in the cation exchanges more precisely, Na\(^+\)-loaded cells were maintained at different levels of external pH with a pH stat and the movements of cations were determined. For cells suspended in salt-free medium, the Na\(^+\) efflux decreased somewhat as the pH was increased (Fig. 5a), and the efflux of K\(^+\) was exceedingly low except at pH 2.0. At low pH, the efflux of Na\(^+\) was balanced by an influx of H\(^+\), but as the pH was increased the influx of H\(^+\) was not only decreased, but at pH 7 and 8, a net efflux occurred. At low pH (2–4) where the influx and efflux of cations were approximately equal, no detectable excretion of organic acid anions was found. At higher values of pH where a net efflux of cations occurs, organic acid anion excretion was significant. No fluxes are reported, because estimates were made only at one time point (15 min). The values correlated well with the net loss of cations, accounting for over 83% in all cases. Thus at low pH, the cells behaved predominantly as a Na\(^+\)-H\(^+\) exchange system, at intermediate pH, they lost Na\(^+\)-organic anion, and at high pH, Na\(^+\)-organic anion plus H\(^+\)-organic anion.

In the presence of 3 mM RbCl, the system was considerably modified especially at higher values of pH (Fig. 5b). At pH 2 or 3, little Rb\(^+\) uptake

**FIGURE 3**

The effect of external Rb\(^+\) concentration on the cation influxes and effluxes. The initial Na\(^+\) content was 60 mmol/kg (wet weight), the pH was 5.0, and the concentration of cells, 11 mg (wet weight)/ml of suspension. The rates are taken in the initial 5 min when they are relatively linear.

**FIGURE 4**

Relationship of the reciprocals of Rb\(^+\) concentration and reciprocal of cation influx and efflux. Data taken from Fig. 3.
occurred, the Na⁺ efflux being the same as in the salt-free medium (Fig. 5 a), balanced by an influx of H⁺. Above pH 4, a net H⁺ efflux occurred and an enhanced Na⁺ efflux, both balanced by an uptake of Rb⁺. Virtually no efflux of K⁺ was observed (not shown on the graph to avoid confusion). Except at pH 8, the excretion of organic anions was completely suppressed.

In the experiments on Na⁺-loaded cells reported above, as in the previous experiments of Conway et al. (13), the measured efflux of K⁺ was very low compared to that of Na⁺. They reported that the Na⁺ efflux was about 20 times as high as the K efflux in yeast in which the Na⁺ content was 65 mmol/kg and the K⁺ content 85 mmol/kg. The discrimination factor was, therefore, of the order of 25 to 1 in favor of Na⁺. In the experiment of Fig. 5 a, the amount of K⁺ that escaped from the cells was also very low, and the discrimination factor was at least of the same order. The net K⁺ efflux measured in cell suspensions may, however, be subject to large errors. The inward transporting system has a high relative affinity for K⁺ ($K_0 0.5$ mM [2, 10]), so that K⁺ released from the cells would tend to be taken up again. The amount of recycling of K⁺ would undoubtedly be increased by the presence of a cell wall that encloses a poorly mixed water space adjacent to the membrane, equal to about 12% of the total cell water (8). The amount of K⁺ that recycles was estimated by use of a cell column technique in which K⁺-free solution rapidly flows through a layer of packed cells. The rate of flow can be maintained at a sufficiently high level that the K⁺ concentration in the effluent remains very low (24) with rapid and continuous washout of K⁺ from the cell wall space. A typical experiment is illustrated in Fig. 6. The pattern of Na⁺ loss in the presence and absence of K⁺ was very similar to that measured by the batch sampling procedure (Fig. 1) except that many more...
data points were obtained. One characteristic of the Na⁺ loss that became clearly evident from this and many similar experiments was that the stimulating effect of K⁺ only lasted for about 50 min. Thereafter the loss was not influenced by the external K⁺. Furthermore, the loss as a function of time was not first order, nor did it follow any simple compartmental model.

When K⁺ loss was compared using the batch technique and the cell column technique, it was clear that recycling of K⁺ must be very high. With the latter technique, K⁺ loss was appreciable (Fig. 7), increasing with time as the rate of Na⁺ loss diminished. Indeed after 30 min, the rate of K⁺ loss exceeded the rate of Na⁺ loss the total of Na⁺ plus K⁺ being relatively linear. These data indicate that the discrimination between Na⁺ and K⁺ in the outflow pathway is considerably less than has been previously assumed.

The discrimination between Na⁺ and K⁺ can be quantitatively defined as follows:

\[
\text{Discrimination} = \frac{\text{Na}^+ \text{ efflux}}{\text{K}^+ \text{ efflux}} \times \frac{\text{K}^+ \text{ content}}{\text{Na}^+ \text{ content}}.
\]

The data calculated on this basis for Fig. 7, are presented in Fig. 8. The discrimination was not constant with time. It was initially about 5 but continued to decrease until it reached a value of 1 after about 40 min and was, thereafter, constant.

In order to determine whether the loss of discrimination was due to some time-dependent factor, or to changes in intracellular cation levels, a series of yeast samples were prepared with different initial levels of Na⁺ using the technique described in Table I. By this procedure, the total Na⁺ + K⁺ content remained constant, but K⁺ was replaced to varying degrees by Na⁺. The initial efflux of K⁺ was determined by the cell column technique and those of Na by either batch or column technique. The fluxes were plotted against the initial Na⁺ content in Fig. 9. It is evident that Na⁺ and K⁺ effuxes
are related to the cellular content of Na⁺ and K⁺, but in a complex manner. The discrimination factor calculated as in Fig. 8 and plotted against the Na⁺ content (Fig. 10) showed a sharp inflection point at 40 mM of Na⁺/kg of yeast. Below that level discrimination was minimal (value of 1.0), but above

![Figure 7](image)

**Figure 7.** A comparison of Na⁺ and K⁺ loss from yeast measured by the cell column technique and by the suspension technique. The initial Na⁺ content was 59 and K⁺, 80 mmol/kg (wet weight) and pH 6.0.

**Figure 8.** The change in Na⁺/K⁺ discrimination in the efflux pathway with time. Calculations from data of Fig. 7.

![Figure 9](image)

**Figure 9.** K⁺ and Na⁺ effluxes as a function of the cellular content of K⁺ and Na⁺. Data are from various experiments.

**Figure 10.** Na⁺/K⁺ discrimination in the efflux pathway as a function of Na⁺ content. Data are from various experiments in which Na⁺ and K⁺ effluxes were simultaneously measured in Figs. 8 and 9.
that level, the discrimination increased markedly. Similar calculations are also plotted for the data of Fig. 8. In this case, the Na⁺ and K⁺ contents were changing with time as a result of the continuous loss of the cations. Again the discrimination showed a sharp break at about 40 mM of Na⁺/kg of yeast, with little discrimination below that level (solid dots of Fig. 10). The parallel behavior of the two sets of data suggested that the discrimination in each case depends on the cation content per se and not on some other time-dependent factor.

**DISCUSSION**

The pathway for uptake of cations in yeast, although complex, is relatively well defined in terms of its kinetics and specificity. In general, it follows simple Michaelis-Menten kinetics with specificity in the order H⁺ > K⁺ > Rb⁺ > Cs⁺ > Na⁺ > Li⁺ (2, 10), but under certain conditions the pattern is more complicated. At relatively low cation concentrations a cooperative effect is observed, reflected in a sigmoidal curve for Rb⁺ uptake with increasing concentrations, and in a stimulation of Rb⁺ uptake by K⁺ or Na⁺ (4, 5). At higher concentrations of cations, especially H⁺, Cs⁺, and Ca++, a non-competitive inhibition occurs (2, 3). Both effects have been explained by assuming two-site models.

The pathway for outflow of cations cannot be defined with the same degree of precision because the concentrations of cations cannot be easily controlled. K⁺ can only be partially replaced and H⁺ is particularly troublesome because it has a high affinity for the transport systems and can move rapidly, either into or out of the cell. Only net movements of H⁺ can be measured, so its contribution to outflow cannot be determined unless the inflow is minimized by increasing the external pH to high values. Nevertheless, certain properties of the outflow pathways can be stated: (a) In the absence of external cations and with low external H⁺ (high pH), Na⁺ loss is balanced by the appearance of organic anions in the medium. The efflux is about 50% of that in the presence of higher H⁺ concentrations (pH below 4.0, Fig. 5a) and 30% of that in the presence of external cations such as Rb⁺ (compare Na⁺ efflux in Fig. 5a and b at high pH). The limiting factor, at low external cation concentrations, would seem to be the rate of anion outflow.

(b) In the presence of external cations (including H⁺), organic anion secretion is almost completely repressed (also noted previously [13]), so that the cation efflux is stoichiometric with the total cation uptake. Thus the in and out pathways behave as though they were tightly coupled.

(c) The total cation efflux seems to be limited by the cation influx. For example, in Fig. 4, the efflux (H⁺ + Na⁺) follows a Michaelis-Menten relationship with respect to the external concentrations of Rb⁺ with constants the same as those previously observed for Rb⁺ uptake (2, 3). It is difficult to
account for this finding except to suggest that the limiting factor that determines the efflux is the rate of entry of Rb⁺.

(d) The contribution of H⁺ to the cation outflow depends on several factors. The proportion of H⁺ changes with time. For example, in Fig. 2 (upper section), H⁺ loss is 40% of the total cation loss for the first 5 min, but almost ceases after 20 min. A similar sequence of a relatively high H⁺ loss followed by a relatively high Na⁺ loss has been reported in bacteria (28). The proportion of H⁺ in the total cation outflow is also dependent on the external concentration of Rb⁺ (Fig. 3) and on the external pH (Fig. 5). As noted previously, however, the data on H⁺ represent net fluxes, so that the reported effects may represent changes in influx, efflux, or both. They certainly, however, represent the minimal efflux. On this basis, the selectivity for H⁺ in the pathway for cation outflow must be very high. The average internal pH, although dependent on many factors, especially the external pH and the metabolic state (9, 27), is unlikely to be much lower than 6.0 under the conditions of the present experiments. In the experiment of Fig. 2, the cellular Na⁺ concentration is 60 mM or 60,000 times as great as the H⁺ concentration. Yet the H⁺ efflux is about 60% of that of Na⁺. Granted that the local pH at the site of transport may be considerably lower than the average cellular pH, the data indicate that the apparent discrimination for H⁺ over Na⁺ must be of the order 10,000-fold.

(e) The large apparent discrimination between Na⁺ and K⁺ in the pathway is due to a large degree to a recycling of K⁺ that is enhanced by the presence of a relatively unmixed water space within the cell wall structure amounting to about 12% of the total water space of the cell (8). In addition, the total pool of cations in the cell wall space is increased because of the presence of fixed anionic groups capable of binding cations (22). A similar cation binding component in the cell wall space has been demonstrated in Neurospora (30). As K⁺ and Na⁺ pass through the membrane into the wall space, they would be exposed to an inward directed transport system that is highly specific for K⁺ (2, 10). The K⁺ would, therefore, be recycled, whereas the Na⁺ would diffuse into the medium. Such recycling would amplify the inherent selectivity of the efflux pathway. In cells subjected to a continuous washout in the cell column technique (Fig. 8), the recycling is minimized. The observed K⁺ loss is considerably increased, whereas the Na⁺ loss is not. Under conditions of washout, the largest observed discrimination between Na⁺ and K⁺ is 8 to 1, compared to an apparent value of as much as 50 to 1 in cells in suspension.

(f) The relationship between Na⁺ efflux and cellular Na⁺ is not asymptotic, but exponential or perhaps sigmoidal (Fig. 9), confirming previous observations (14). Such a relationship cannot result from competition of cations for a transport site on a one-to-one basis (Michaelis-Menten kinetics). It has
been suggested that a cooperative phenomenon might be involved with as many as three Na⁺ ions being cotransported (14). Sigmoidal characteristics have also been observed for Na⁺ influx (4), but at lower concentrations (below 10 mM if Rb⁺ is present). In *Neurospora* at high pH, K⁺ uptake also follows sigmoidal pattern (30). In both of the latter cases models have been proposed involving two-site systems. Whatever the explanation may turn out to be, the end result is a marked increase in the Na/K discrimination in the efflux pathway from 1/1 to over 8/1 as the Na⁺ content is increased (Fig. 10).

The relationship of the pathway for uptake to the pathway for outflow of cations is of interest. The system behaves as a tightly coupled one-for-one exchange, except in the absence of external cations (H⁺ concentrations also low). In the latter situation, a net loss of cations occurs, balanced by the appearance of organic anions in the medium. The outflow may, however, still involve a one-to-one exchange of cellular Na⁺ for H⁺ with the undisassociated organic acid diffusing out of the cell, dissociating in the high pH medium into H⁺ and A⁻, thereby providing H⁺ that can be recycled into the cell in exchange for Na⁺.

The “tight” coupling of uptake and outflow may have two explanations. The transport system may be nonelectrogenic, requiring an equivalent loading of cations on either side before transfer can occur, or it may involve an electrical coupling that results in a one-for-one cation exchange, with anions unable to participate to any substantial degree because of low permeability. A choice between the two possibilities could be made if measurements of membrane potentials and ion conductances could be made, but the small size and tough cell wall of yeast have prevented direct measurements of potential. Measurements have been made, however, in another fungus, *Neurospora*. The potential is not influenced by internal concentrations of Na⁺ or K⁺, nor by the magnitudes of the fluxes, suggesting that electrical coupling is not involved (29). On the other hand, in the bacterium *Streptococcus faecalis*, indirect measurements using passively distributed lipid soluble anions and cations suggest that the transport of Na⁺ and H⁺ out of the cell creates a potential which acts as the driving force for the uptake of K⁺ (17, 18, 19). The question remains open at this time in the case of yeast cell, although on a priori grounds, it seems more likely to behave like *Neurospora* than a bacterium.

The experimental data do not allow a sorting out of the number of transport systems in yeast that contribute to the uptake and outflow of cations. A single tightly coupled system relatively specific for K⁺ in the inward direction and for Na⁺ and H⁺ in the outward direction could account for the present observations, provided that the various complexities were taken into account, such as the noncompetitive inhibitions by cations on influx (2, 3), the cooperative effects on uptake (4), the sigmoid relationship of Na⁺ loss
to Na⁺ content (Fig. 9 and reference 14), and the recycling of K⁺ and probably H⁺ from the cell wall space, as demonstrated in this paper.

On the basis of differences in the pH optima (cellular pH) for K⁺-H⁺ exchange, as compared to K⁺-K⁺ exchange and K⁺-Na⁺ exchange, Ryan and Ryan (27) suggested that two systems were involved. It is possible that the internal pH could alter cation outflow through a variety of mechanisms, but perhaps the simplest explanation would be a single transport system with affinities in the order of H⁺ > Na⁺ > K⁺. Based on this assumption, the relative affinities of H⁺/Na⁺ for outward transport would be about 50,000 to 1 calculated from their data showing equal rates of H⁺ and Na⁺ at a cellular pH of about 5.9 and a Na⁺ content of 60 mmol/liter of cells. The calculation gives the same order of discrimination as that presented earlier in the discussion based on competition for a common site (calculated from Fig. 3). Until specific inhibitors are found that can differentiate the efflux pathway into more than one component, or until mutants are identified in which only certain fluxes are altered, it seems unnecessary to postulate more than one cation transport system.

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