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A Cation Carrier in the Yeast Cell Wall

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The active transport of K^+ ions in exchange for H^+ ions during fermentation has been described in various publications (e.g. Conway & O'Malley, 1946; Rothstein & Enns, 1946; Conway, Brady & Carton, 1950). Without any added buffering, the pH of the external fluid when the volume of this is restricted as far as possible can fall as low as 1·5.

When 1 ml. of external fluid, containing 5% glucose (w/v) but no potassium chloride or buffer, is present/g. of yeast the pH external to the cells is in the region of 3, owing to the excretion of succinic acid. When under such conditions the external fluid contains sodium chloride, lithium chloride or other inorganic chloride, even in high concentration, no appreciable uptake of the inorganic cation species occurs, with the exception of rubidium. When, however, the external fluid is buffered to pH 6–7, not only is the uptake of potassium markedly increased, but also that of sodium, lithium, magnesium and other metal cations. There is then marked competition between cation species.

In this paper such uptake and competition are studied at pH 6–7 and the evidence leads to the conclusion that only one carrier system is involved (Conway & Duggan, 1956).

Since K^+ ions are taken up much more readily than is any other metal cation, the carrier may be regarded as the physiological K^+ ion carrier, but is not the only mechanism involved in the uptake of cations. Magnesium, for example, can be actively transported also, by a different system operating at pH 3–5, and the transport is not then inhibited by small concentrations of K^+ ions, the magnesium being taken up in association with phosphate (Rothstein, 1955).

This physiological K^+ ion carrier is to be distinguished from that which transports sodium from within outwards, when, for example, a considerable quantity of Na^+ ions has been introduced (Conway, Ryan & Carton, 1954). It also appears to be operative when external cations exchange for Na^+ ions within the cells of sodium-rich yeast.

An account is given of the determination of the concentration of the carrier in terms of milli-equivalents of combining power/kg. of washed and centrifuged yeast.

EXPERIMENTAL

Chemical analysis and materials

Sodium, potassium, caesium, rubidium and lithium. The concentration of these in yeast was determined by means of the Beckman flame photometer, with wavelengths of 589, 769, 852, 795 and 670·8 mμ for Na^+, K^+, Cs^+, Rb^+ and Li^+ ions respectively. The yeast for analysis was first centrifuged, washed twice and then 1 g. (moist wt.) was suspended in 20 ml. of water. This suspension was then brought to 100° for 1–2 min., cooled, centrifuged and suitably diluted. The readings were compared with those obtained with standard solutions.

pH *determinations.* These were carried out by means of the Beckman model G meter.

Magnesium determinations. (i) Titan-yellow method: Determinations of Mg were carried out mainly by the method of Young & Gill (1951). (ii) Flame-photometer method: This method was also used in the earlier determinations. Small quantities of yeast (0·8–1·0 g.) were treated with a few drops of 4 N-H_2SO_4 and ashed in platinum crucibles. The ash was dissolved in 2 ml. of warm N-HCl, the solution then neutralized with dilute aq. NH_3 soln. and Mg was precipitated by the addition of 1 ml. of 8-hydroxyquinoline reagent (Cruess-Callaghan, 1935). The separation of the Mg in this way from Na and K was found necessary because of interference by Na^+ and K^+ ions with the photometer readings for Mg. The precipitate was then dissolved in very dilute HCl and water was added so that the final concentration of Mg in the solution was between 25 and

50 mg./l. The emission was then determined at 371 mμ with the Beckman flame photometer. The concentration was evaluated from a curve for solutions of $MgSO_4$ containing 10–50 mg. of Mg./l.

Amino acids. These were determined by the colorimetric ninhydrin method of Troll & Cannon (1953).

Volatile amines and ammonia. These were determined by microdiffusion procedures (Conway, 1957).

Experiments with isotopes. ^{42}K, ^{24}Na, ^{86}Rb and ^{131}Cs were used. They were obtained from the Atomic Energy Research Establishment, Harwell. The counts from 0·35 ml. of the medium were recorded under similar conditions.

Baker's yeast. In all the experiments fresh baker's yeast, as supplied by the Cork Yeast Co., was used.

Uptake of cation at different external concentrations

Suspensions were made of 1 g. of washed centrifuged yeast (centrifuged at approx. 2500 rev./min.) in 5–200 ml. or more depending on the cation being examined. The suspending fluid contained 5 % (w/v) of glucose and varying concentrations of the cation. Mixing was effected by shaking or bubbling with air or oxygen. The fermentation was allowed to proceed at room temperature (average of 18°) for a fixed time, samples being then taken and immediately centrifuged for 5 min. at about 2500 rev./min., the holders for the centrifuge tubes containing ice-cold water. The supernatant fluid was removed and the pellet of yeast washed twice with ice-cold water, and centrifuged in a similar manner each time. Alternatively, as with potassium uptake, the supernatant fluid was analysed for change in concentration. In this case the suspension was usually 1:5 (w/v).

The mixture was maintained at about pH 6 either by using the citrate or the acetate of the cation, or, as with $MgCl_2$, by adding small amounts of lithium carbonate throughout the fermentation. With citrate or acetate, either the volume of the suspending fluid was relatively large, or the time of fermentation was comparatively short, so that the buffering, even with the acetate, was such as to prevent much change of pH. In general, with the neutral salt the pH very quickly changed to near 6·0 and did not drop below about 5·9. In experiments where a greater constancy of pH was desirable, as in examining effects of competition at about pH 7·2, the suspending fluid contained 2-amino-2-hydroxymethylpropane-1:2-diol (tris) buffer (50 mM) (Gomori, 1946). This buffer has pK 8·1 at 23° and was shown to have no inhibitory effect on the respiration of rat-kidney slices by Barron (1946), or on enzymes such as alkaline phosphatase, and is not appreciably absorbed by fermenting yeast. The buffer mixture was prepared by incorporating 45 ml. of N-HCl in 1 l. of the 0·05 M pure base. The time allowed for the fermentation varied from 10 min. with K to 30–120 min. with Mg.

From the data obtained the K_m values corresponding to the Michaelis–Menten constants under the experimental conditions could be determined either directly from the graphs of uptake against external concentration, the concentration corresponding to half the maximum uptake being taken as the K_m value, or by applying equation 4 in the Theoretical section.

Competition in uptake of ions

Fermenting yeast. The experimental conditions were as described above except that a second competing cation species was introduced into the suspending fluid.

The competition of the two cations, or the inhibiting effect on uptake of the one by the other, was then examined by maintaining the concentration of one constant and varying the other, and applying the equations 4 and 5 in the Theoretical section based on Michaelis–Menten kinetics.

It was also examined by determining the concentration of one ion required to depress the uptake of the other by 50 % with the external concentrations maintained approximately constant. In the latter case the results could be expressed as relative transport affinities, with K taken as 100. The figure could be calculated as

$$A = [K]/[X]_{0\cdot5} \times 100,$$

where $[X]_{0\cdot5}$ is the external concentration of the second inorganic cation species depressing the uptake of K by 50 %. This relative affinity could also be expressed as $A = 100 \times K_k/K_m$, where K_k and K_m are the Michaelis–Menten constants (see Theoretical section) for the K^+ ion and the competing ion respectively.

For examination of the effect of another cation on the uptake of K in this way, the latter was present usually as 2 m-equiv. of potassium citrate/l., concentration of the other cation being varied. Ten minutes was usually allowed for the fermentation. Alternatively the second cation species was maintained constant and the concentration of K varied. The pH was maintained at approx. 6–7 as described above.

Sodium-rich yeast. The sodium-rich yeast was prepared by suspending washed and centrifuged yeast at room temperature (18°), 1:20 (w/v) of 5 % glucose containing 0·2 M-sodium citrate for 2 hr. After two washes with 20 vol. of tap water, the Na content of the centrifuged yeast was usually 50–80 m-moles of Na/kg. (Conway & Moore, 1954; Conway *et al.* 1954).

Samples (1 g.) of the sodium-rich yeast were then suspended in 20–200 ml. of a solution containing a constant concentration of a given ion species and varying concentrations of KCl, and the concentration of the latter required to reduce the ion uptake by 50 % was determined.

Stoppered flasks of about 500 ml. capacity were half-filled with the yeast suspension and shaken for 90 min., samples for analysis being removed immediately before the shaking and at the end. These samples were immediately centrifuged and washed twice with about 20 vol. of tap water; the concentrations of K and of the competing cation were then determined.

The relative affinity of the cation with respect to K could be expressed as before by $A = 100 \times [K]/[X]_{0\cdot5}$, where [K] and $[X]_{0\cdot5}$ are the concentrations of both cations at one-half the full rate of uptake of K.

Effects of amino acids on excretion of sodium from sodium-rich yeast

Twenty-four amino acids were investigated. A portion (1 g.) of washed and centrifuged sodium-rich yeast was suspended in 20 ml. of 5 mM-solution of each amino acid at approx. pH 6, small amounts of NaOH being added if necessary to bring the pH to 6. The suspensions were treated as described above, and the excretion of Na after 90 min. was compared with the excretion into water at the same pH.

Procedure used for the determination of concentration of the cation carrier

The principle of this determination is described later in the Results section. A sample (5 g.) of freshly washed and centrifuged yeast was suspended in 2·5 ml. of a solution containing 5% (w/v) of glucose and 20 mM-magnesium acetate, and cooled to 4–5°. Potassium chloride was then added as 0·5 ml. of a solution containing 7–10 mM-KCl, including sufficient ^{42}K for satisfactory counting.

After adding the labelled KCl, 30 sec. was allowed, with shaking, for the uptake of K by the carrier. During this time there would occur an active transport of only about 0·02 m-equiv. of K/kg. of washed and centrifuged yeast (this will appear from Fig. 7). A sample (3 ml.) was removed and centrifuged immediately for 2 min. at 2000 rev./min. in cooled centrifuged tubes (jacketed in water at 0°). The upper layer of the supernatant fluid was at once removed.

To a suspension similarly treated 0·1 ml. of 0·5 M-RbCl was added 30 sec. after the addition of the labelled KCl solution, and the suspension was shaken for 30 sec.; a sample (3 ml.) was then withdrawn and centrifuged as before, the supernatant fluid being removed at once. Counts were then carried out on the supernatant fluids, and the increase in external K caused by introducing the RbCl was expressed as m-equiv. of K/kg. of the centrifuged yeast.

THEORETICAL

Relation of rate of uptake to external cation concentration. The presence of only one species of metal cation in the external fluid is considered, and the external H^+ ion concentration is assumed to be negligible. It is also assumed, for illustrative purposes, that the cyclically re-formed carrier is the reduced form (M^-) of a metal–redox system (this having the merit of being the simplest hypothesis interpreting the facts, and for which there is now much evidence, e.g. Conway, 1951, 1953, 1955; Foulkes, 1956). The metal–redox system receives electrons through the passage of H atoms, as from a flavine.

The carrier molecule at the external surface of the membrane forms a complex with a cation B^+ in the external solution. This complex, interpreted as of a physical kind, may be written $M_1^- \cdot B^+$, and the formation expressed by the equation

$$M_1^- + B^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} M_1^- \cdot B^+, \qquad (1)$$

where k_1 and k_2 have high values compared with those of other kinetic constants of the system. The complex then moves in the membrane and transfers its electrons on the inner side to another acceptor, releasing B^+ into the cells. If the rate at which the oxidized form M_1 is again reduced is much faster than the rate of the passage of the complex through the membrane and that of the passage of its electrons to another system, then the total concentration of the carrier molecules, $[M_1^-]+[M_1^- \cdot B^+]$, may be regarded as constant with respect to changes of $[B^+]$.

From equation 1 it may be deduced that

$$\frac{[M_1^- \cdot B^+]}{[M_1^-]+[M_1^- \cdot B^+]} = \frac{[B^+]}{K_m+[B^+]}, \qquad (2)$$

where $K_m = k_1/k_2$. If the rate of transport of B^+ into the cell, written v, is proportional to $[M_1^- \cdot B^+]$ then the maximum rate is proportional in a similar way to

$$[M_1^-]+[M_1^- \cdot B^+],$$

and one obtains the relation

$$v = \frac{V[B^+]}{K_m+[B^+]}, \qquad (3)$$

which is in the usual form of the Michaelis–Menten equation. [It may be pointed out here that the derivation of equation (3) would also follow, whatever the nature of the carrier system, provided that its total concentration was constant independent of changes in the external concentration of the transported cation.]

When the substrate concentration is such that the rate of uptake is one-half of the maximum, and written $[B^+]_{0\cdot5}$, then $K_m = [B^+]_{0\cdot5}$. For this estimate it is necessary to know the full curve of rate of uptake with varying external $[B^+]$. Also, if a second cation species is added so that a maximum rate of uptake (V) already in being is reduced to $0\cdot5V$, then it may be assumed, as a first approximation, that the carrier is being equally shared by the two ions and the ratio of the concentration outside the yeast cell varies inversely with these K_m values.

It is often convenient to arrange equation (3) in the form

$$\frac{1}{v} = \frac{1}{[B^+]} \times \frac{K_m}{V} + \frac{1}{V} \qquad (4)$$

(after Woolf, as noted by Haldane & Stern, 1932; Haldane, 1957; also used by Lineweaver & Burk, 1934, and subsequently by many others). On plotting $1/v$ against $1/[B^+]$, the slope of the line gives the value of K_m/V and the intercept on the ordinate that of $1/V$.

Where an inhibitor, which is here a second cation species, competes for the same active site as the first, equation (4) may be written

$$\frac{I}{v} = \frac{1}{[B^+]} \times \frac{K_m}{V}\left[1+\frac{[I]}{K_i}\right] + \frac{1}{V}, \qquad (5)$$

where $[I]$ is the concentration of the second cation species, and K_i the constant similar to K_m. Here the effect of the inhibitor acting on the same active group is to cause an increase of the slope of the line $1/v$ against $1/[B^+]$, but the intercept on the ordinate remains the same.

When the inhibitor acts on some group other than that to which the first cation species is attached (non-competitive inhibition), the slope of the line is increased, but the intercept on the ordinate is also increased by the same factor.

An effect similar to non-competitive inhibition could be produced if the second and inhibiting cation species slowed the movement of the carrier through the membrane.

Lastly, there may be mentioned the case where the inhibitor acts not on the free carrier, but only on the compound of carrier and cation (and at a different site from the first species). The slope is then unchanged but the intercept is increased.

The application of equation (4) to yeast metabolism was shown by Hopkins & Roberts (1935) and by Gottschalk (1944). Recently these equations were used by Rothstein (1955) in his investigations on various inhibitions of fermentation and of respiration in yeast.

Epstein and co-workers (Epstein & Hagen, 1952; Epstein & Leggett, 1954; Epstein, 1955) have also used them in an extensive series of investigations in connexion with the

absorption of alkali and alkaline-earth cations, as well as sulphate, in barley roots. Their findings, in so far as they are relevant to the present investigations with yeast, are considered in the Discussion. Here the inhibition of uptake of Na by K^+ ions is studied in a similar fashion.

RESULTS

Rate of uptake of K^+ ions with varying external concentrations of K^+ ion

It has been shown that with unbuffered suspensions (1 g. of yeast/0·6 ml. of suspending fluid) the uptake of K increases with the external K^+ ion concentration (Conway & O'Malley, 1946), the K^+ ions exchanging for H^+ ions, and the pH falling to low levels (about 1·6–2·0) depending on the previous conditioning of the yeast. When the pH is maintained in the region 5–7 with relatively large volumes of the suspending fluid, and potassium acetate or citrate, the maximum uptake per unit time increases markedly and a maximum is reached at a low level of external K^+ ion concentration, about 1·6 mM (Fig. 1). This may be explained by relative competition of K^+ and H^+ ions for the carrier, the H^+ ion competition rapidly declining with increasing pH.

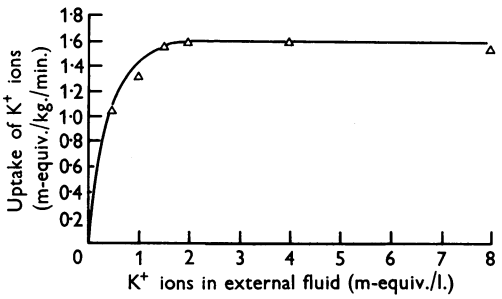

Fig. 1. Rate of uptake of potassium by fermenting yeast at pH 5–6. Medium contained 1 g. of yeast/20 ml. of 5% glucose and potassium as citrate; 10 min. was allowed for the fermentation and the potassium decrease in the external solution was measured.

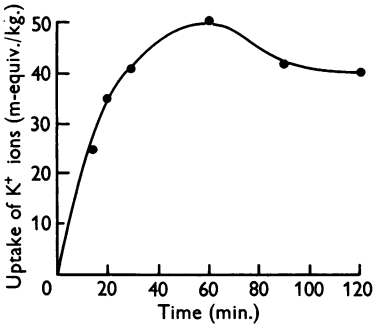

Fig. 2. Time curve of uptake of potassium by fermenting yeast (1:20, w/v, suspension in 5% glucose and 0·2 M-potassium acetate); approx. pH 6.

The net uptake of K^+ ions at pH 5–7 increases almost linearly with the time up to about 60 min. at room temperature and then comes somewhat abruptly to an end (Fig. 2), after which there may even be some loss of K from the yeast cells.

Rate of uptake of other inorganic cations

Fig. 3 shows the rate of uptake of K, Rb, Na and Mg as m-equiv./kg. of centrifuged yeast, plotted against the external concentrations. The pH values were at or near to 6. Each cation species was present alone (apart from the very small concentration of H^+ ions), except for Rb (rubidium chloride), with which the pH was maintained by 10 mM-magnesium acetate, Mg at this concentration interfering very little with the uptake of Rb. Fig. 4 shows the application of equation (4) to the uptake of K^+ ions in one series of experiments under the above conditions, and Fig. 5 illustrates a series on uptake of Rb^+ ions.

Competitions between inorganic cations for the carrier in uptake of ions

Table 1 gives a list of the results obtained, both for fermenting yeast and non-fermenting sodium-rich yeast, with the method by which the concentration of one ion which causes a 50% inhibition of the uptake of another is determined, with K^+ ion as the standard species.

The values give the transport affinities relative to that of K^+ ion, taken as 100. The similar values obtained under the two conditions suggest that a single mechanism is at work. It may be inferred from Fig. 3, or by applying equation (4) to the lines in Figs. 4 and 5 and the lower line in Fig. 6, and

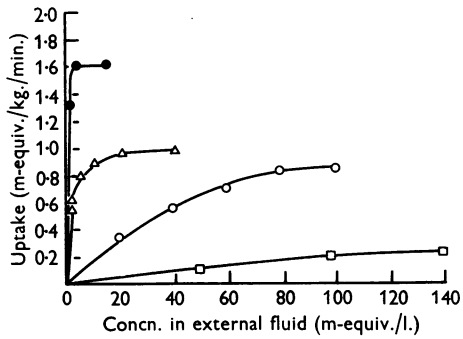

Fig. 3. Comparison of rates of uptake of K, Rb, Na and Mg by fermenting yeast with varying concentrations of cation in the external fluid; the yeast was suspended (1 g./20 ml.) in fluid which contained 5% of glucose (w/v) and was buffered to approx. pH 6. ●, Potassium; △, rubidium; ○, sodium; □, magnesium.

from the results given for magnesium in the next paper (Conway & Beary, 1958), that while the relative transport affinities vary greatly from K^+ to Mg^{2+} and over some hundreds of times, the maximum uptake rates vary comparatively little, though these rates are reached at widely different external concentrations.

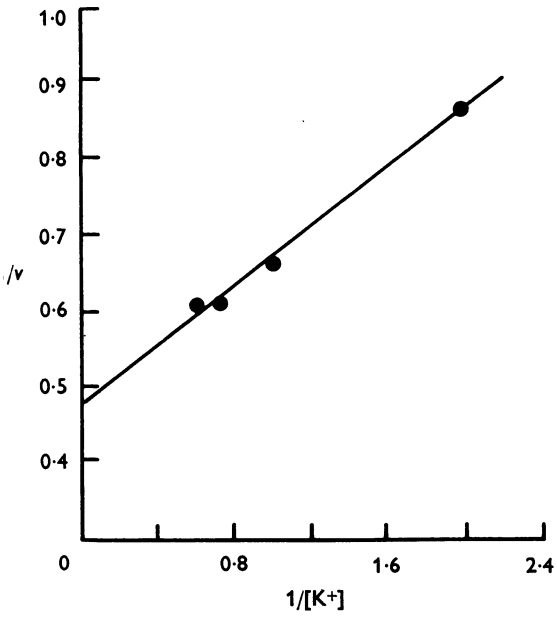

Fig. 4. Double reciprocal graph of $1/v$ against $1/[K^+]$, where v is the rate of uptake of K^+ ions (m-equiv./kg./min.) by the fermenting yeast, and $[K^+]$ is the external potassium concentration, the conditions being the same as for Fig. 1.

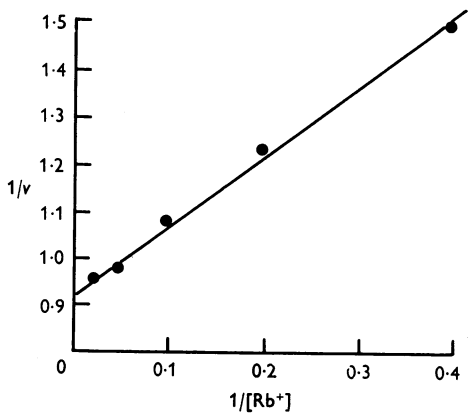

Fig. 5. Double reciprocal graph $1/v$ against $1/[Rb^+]$, where v is the rate of uptake of Rb^+ ions (m-equiv./kg./min.) by fermenting yeast, and $[Rb^+]$ the external rubidium concentration, the conditions being the same as for Fig. 3.

Do inorganic cations compete for the same active group on the carrier?

This was studied by using the inhibiting effect of K^+ ions on the rate of uptake of Na^+ ions and the double reciprocal graph of $1/v$ against $1/[B^+]$. To ensure a constancy of pH tris buffer was used. The washed and centrifuged yeast was suspended (1 g./20 mol.) in fluid containing 5% of glucose, 5 mM-potassium acetate, varying concentrations of sodium acetate and 50 mM-tris buffer. The fermentation was allowed to proceed for 30 min. at room temperature, air being bubbled through the suspensions. After 30 min. the suspension was centrifuged, washed twice with water and centrifuged, the Na content being determined as described in Methods.

Fig. 6 shows that the results correspond with the requirement for competition for the same active group.

The regression lines:

without K^+ ions in the suspending fluid

$$1/v = 26{\cdot}1 \times \frac{1}{[Na^+]} + 0{\cdot}35,$$

with 10 mM-K^+ ions

$$1/v = 45{\cdot}9 \times \frac{1}{[Na^+]} + 0{\cdot}35,$$

show that the intercepts on the ordinate are the same. Here the uptake rate v for the Na^+ ion is given as m-moles/kg. of centrifuged yeast/min. at room temperature and $[B^+]$ is given as m-moles/l. of external fluid.

It will be shown in the next paper that a similar picture is presented for the inhibition of uptake of Mg^{2+} ions by Rb^+ ions.

Transport affinities of organic cations during fermentation

A group of substituted ammonium ions (ethylamine, ethanolamine, taurine, acetamide, glycine and alanine) was examined as described in Methods.

Table 1. *Relative affinities for cation carrier*

The relative transport affinity of a cation was determined from the relation $[K]/[X]_{0{\cdot}5} \times 100$, where [K] and [X] are the concentrations of potassium and the cation investigated, when the uptake of potassium is depressed by 50%. Figures in parentheses give the number of experiments.

Cation species	Relative transport affinities during fermentation	Relative transport affinities in sodium-rich yeast, not fermenting
K	100	100
Rb	42 (2)	37 (2)
Cs	7 (3)	8 (2)
Na	3·8 (4)	—
Li	0·5 (2)	0·9 (2)
Mg	0·5 (3)	0·5 (2)

The uptake of the NH_4^+ ion was also examined, and the results are expressed in terms of transport affinities, taking that of the K^+ ion as 100. The results are summarized in Table 2.

The relative transport affinity for ethylamine is quite appreciable, about one-tenth of that of K; those for glycine and alanine were not measurable under the conditions. The values for ethanolamine, acetamide and taurine ranged from 1·4 to 0·2 % of the value for K.

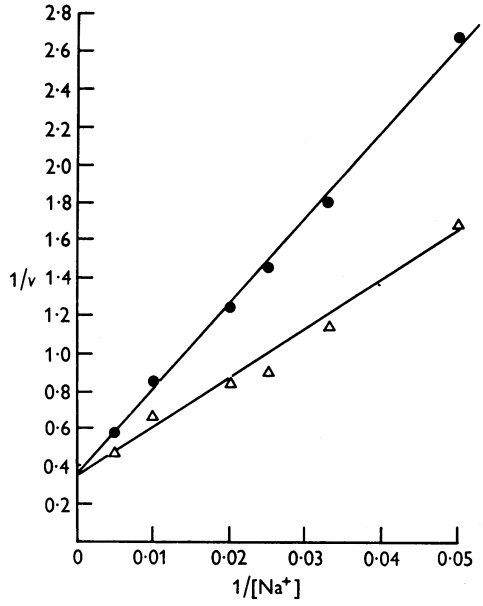

Fig. 6. Demonstration of competitive inhibition of uptake of sodium by potassium during fermentation: △, sodium acetate alone; ●, sodium acetate with 5 mM-potassium acetate. Yeast was suspended (1:20, w/v) in a solution containing 5% of glucose and 50 mM-tris buffer; 30 min. was allowed for uptake. Abscissa is the reciprocal of the sodium concentration (mM) and the ordinate the reciprocal of the rate of uptake expressed as m-equiv. of sodium/kg. of yeast/min. While the slope was changed by the presence of K, the intercept of $1/v$ on the ordinate remained the same, indicating competition for the same active site (see equation 5 in the Theoretical section).

Table 2. *Effect of structural variation on relative affinity*

Two experiments were carried out with each ion species.

Cation series	Relative transport affinities
Potassium	100
Ammonium	21
Ethylamine	9·2
Ethanolamine	1·4
Taurine	0·2
Acetamide	0·4
Glycine	0·0
Alanine	0·0

Relative transport affinities of 24 amino acids, as measured by the excretion of sodium from sodium-rich yeast

The procedure has been described in Methods, and the results are shown in Table 3. The amino acids examined can be divided roughly into three groups. In the first group, the increased excretion of Na over that into water owing to the presence of the amino acids ranges from 94 to 55 % (average of 77 %) and this group includes only the basic amino acids. Four of the five have isoelectric points ranging from 7·59 for histidine to 10·76 for arginine. Concerning citrulline we have been unable to trace any data for the pK values or the isoelectric point; but from some preliminary titrations the isoelectric point can differ little from 6·0, and so the reason for the position of citrulline among the other basic acids in Table 3 is not at present clear.

Since the increase in excretion of Na with K^+ ions (5 mM) amounts to 160 %, the average relative transport efficiency of this group is as high as 59 (taking the K value as 100).

The third group contains those amino acids causing a percentage increase of excretion of Na over that into water of +28 % to −10 % (average of +11 %). This group includes the dicarboxylic acids aspartic and glutamic, as well as glycine, serine, alanine, threonine, valine and others. Such acids cause a relatively very small or negligible increase in excretion of Na.

The second group, causing an increased excretion of Na ranging from 49 to 29 % (average of 41 %) includes the aromatic amino acids as well as asparagine and glutamine, cysteine and leucine. While such groups tend to merge into each other, one outstanding fact is that the basic amino acids cause very appreciable increase of Na excretion, and the dicarboxylic acids either little or no increase. The exact relative positions of particular amino acids as indicated in Table 3 can be considered as only approximate owing to the inherent variability of the determination and the slight differences in the effects produced by many individual amino acids.

Concentration of the cation carrier

The principle used was to displace K from the carrier by excess of Rb and to measure the displaced K by the increased concentration of K in the suspending fluid. In applying this principle special attention was paid to the following points. (i) It was found essential to use small concentrations of external K, of the order of mM, and a relatively small volume of suspending fluid so that

Table 3. *Amino acids grouped in order of their efficiency in exchanging for sodium ions*

Three experiments were carried out with each amino acid, the average chosen being the median value.

Amino acid	Average Na^+ ion excreted in 2 hr. (m-equiv./kg.)	Increased Na secretion over that into water (m-equiv./kg.)	Na excretion as % of that into water	Isoelectric point
		Group I		
Ornithine	17·5	8·5	194	9·7
Arginine	17·4	8·4	193	10·76
Lysine	15·9	6·9	177	9·74
Citrulline	15·0	6·0	167	—
Histidine	14·0	5·0	155	7·59
		Group II		
Norvaline	13·4	4·4	149	6·08
Glutamine	13·2	4·2	147	5·64
Phenylalanine	13·1	4·1	145	5·48
Cysteine	13·1	4·1	145	5·02
Tryptophan	13·1	4·1	145	5·89
Tyrosine	12·5	3·5	139	5·66
Leucine	12·0	5·0	133	5·98
Asparagine	11·6	2·6	129	5·41
		Group III		
Proline	11·3	2·3	128	6·30
Valine	11·0	2·0	122	5·96
Glycine	10·9	1·9	121	5·97
Threonine	10·4	1·4	116	—
Hydroxyproline	10·3	1·3	114	5·83
Aspartic acid	10·1	1·1	112	2·77
Methionine	10·0	1·0	111	5·74
Isoleucine	10·0	1·0	111	6·02
Serine	9·1	0·1	101	5·68
Alanine	9·0	0·0	100	6·00
Glutamic acid	8·1	−0·9	90	3·22

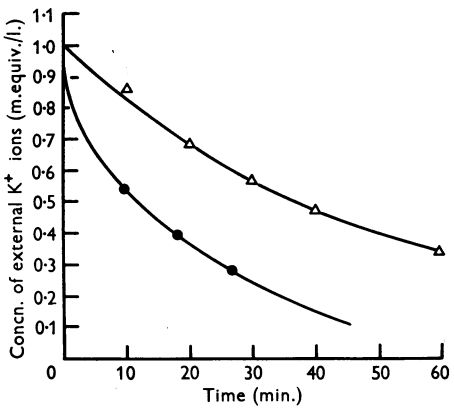

Fig. 7. Rate of uptake of potassium by yeast fermenting 5% glucose at 4–5°, as shown by the decline in the external concentration. △, Values with a 1:2 (w/v) suspension; ●, values for a 1:0·6 (w/v) suspension.

the displaced K would produce an appreciable relative increase in the external concentration. (ii) It was also advantageous to slow down considerably the rate of uptake of K into the yeast cell by reducing the temperature to about 4–5° (Fig. 7). (iii) As excess of Rb would not only displace K from the carrier but also from surface proteins or surface fixed charges, 20 mM-magnesium acetate was incorporated into the suspending fluid to fill these sites with Mg^{2+} ions. This concentration of Mg^{2+} ions in the presence of mM-K would displace K^+ ions from the active carrier site to the extent of only about 5–10%.

The efficiency of Mg^{2+} ions in occupying the fixed surface-anion sites (as distinct from the carrier) was shown as follows. The centrifuged and pressed yeast cells were disrupted by freezing in liquid oxygen and then thawing. There was then free communication between the external and internal fluids, as shown by the fact that inulin diffused very rapidly into all the water of the cells. The disrupted cells were washed four times with water. On suspending the centrifuged residues (corresponding to unit weight of the original centrifuged yeast in 20 vol.) K^+ ions were adsorbed, depending on the pH of the external fluid. At pH 6, 4 m-moles of K^+ ions/kg. of original yeast were so adsorbed; but if 20 mM-magnesium acetate were also incorporated in the suspending fluid only about 0·1 m-mole of K^+ ions was adsorbed. Similar results were obtained with NaCl instead of

KCl. It was considered therefore that, in the experiments for the determination of the carrier, the displacement of K^+ ions by Rb^+ ions (but not by Na^+ ions) could be attributed to the carrier.

The results from seventeen experiments are summarized in Table 4. The mean values of displaced K as m-equiv./kg. of centrifuged yeast was $0{\cdot}104 \pm 0{\cdot}007$ (S.D.M.). In calculating this value, account was taken of the fact that for centrifuged yeast approx. 0·33 l./kg. represents free space for diffusion, made up of 0·23 l. of intercellular fluid and 0·1 l. of outer region of the yeast cell (Conway & Downey, 1950).

Since the affinity of the Rb^+ ion for the carrier is about 0·4 times that of the K^+ ion, under the given conditions about 80% of the active groups of the carrier should be occupied by Rb^+ ions and 20% by K^+ ions. Throughout the 30 sec. interval the transport of K^+ ions may be neglected, but to arrive at a more exact figure for the operative carrier the mean value given above should be multiplied by 1·0/0·8, giving $0{\cdot}13 \pm 0{\cdot}009$ m-equiv./kg. of centrifuged yeast.

Effect of hydrogen on the displacement of potassium by rubidium

The above calculation refers to the carrier as it operates in the yeast suspension shaken in air. Under these conditions it may be expected, assuming the carrier to be a metal–redox system, that the whole of it will not be present in the reduced state which operates as the effective carrier. Similar experiments were therefore carried out in which pure hydrogen gas was bubbled through the suspensions, to produce both anoxia and a reducing atmosphere. The results were compared with suspensions of the same yeast through which air was bubbled. The results are summarized in Table 4, the amount of K displaced from the carrier being increased in the presence of hydrogen. From these results, the total available carrier present (expressed by m-equiv. of operative group) is approx. $0{\cdot}194 \pm 0{\cdot}022$ multiplied by 1·25 ($= 0{\cdot}24 \pm 0{\cdot}03$).

Table 4. *Mean displacement of* K^+ *ions from carrier by excess of* Rb^+ *ions, during aerobic and anaerobic fermentation at 4–5°; also by* Na^+ *ions added similarly to* Rb^+ *ions*

Yeast (5 g.) was suspended in 2·5 ml. of 5% glucose containing 20 mM-magnesium acetate at 4–5°; 0·5 ml. of labelled KCl solution was added to make approx. mM-K^+ ion externally. After 30 sec. 0·1 ml. of water, or 0·1 ml. of RbCl or of NaCl solution, was added to bring the external solution to 10 mM. Samples were then centrifuged after a further 30 sec. and supernatant fluid was removed. In (1) and (3) the conditions were aerobic; in (2) hydrogen was bubbled through the mixture throughout the experiment.

Ion used to displace K^+ ions	Number of experiments	Mean values of displaced K^+ ions (m-equiv./kg. of centrifuged yeast)
(1) Rb^+	17	$+0{\cdot}104 \pm 0{\cdot}007$ (S.D.M.)
(2) Rb^+	11	$+0{\cdot}194 \pm 0{\cdot}022$ (S.D.M.)
(3) Na^+	5	−0·054*

* Each experiment gave a negative value.

DISCUSSION

The carrier considered above, since its affinity for K^+ ions is high compared with that for Na^+ ions and very high compared with that for Mg^{2+} ions, may be regarded as the physiological carrier or mechanism for transport of potassium into the yeast cell, there being a comparatively very slight free diffusion of K^+ ions. Yet it is of interest, in view of the wide range of relative affinities, that the other inorganic cations when present alone (apart from H^+ ions and the balancing anions) can be transported at maximum rates which do not differ much from that of K^+ ions. Such maximum rates, however, require external concentrations which vary inversely as the affinities. At about pH 6 K^+ ions are taken up at a maximum rate when potassium is present at or near to an external concentration of 2 mM. Also, this maximum rate of uptake is maintained only for about 60 min. or so and then comes rather abruptly to an end. The yeast cells from such time onwards may even lose some potassium.

Application of Michaelis–Menten enzyme kinetics to the rates of uptake

It is assumed that the carrier-ion complex here designated as $M_1^- \cdot B^+$ breaks up into $M_1 + B^+ + e$ in proportion to the concentration of $M_1^- \cdot B^+$, just as an enzyme–substrate complex breaks up into free enzyme and the changed molecules of the substrate.

The fate of the carrier-ion complex in this respect depends, however, on its passage through the membrane and the transference of the electrons to an acceptor group, and these two processes are assumed to be proportional to the concentration of the carrier-ion complex. Now, whereas for a single cation species and in a steady state this may be taken as approximately true, it is not true in comparing the rate of uptake of one cation with another. In short, the rate of breakdown of $M_1^- \cdot B^+$ to $M_1 + B^+ + e$ involves movement in the membrane and electron transference, the total rate of which is not likely to be the same from one carrier-ion complex to another.

With regard to the competition of a second cation with K^+ ions for the carrier, it would appear legitimate to hold that if the maximum rate of

uptake of K^+ ions is halved by a certain concentration of a second cation, the K^+ ions are then occupying one-half of the active groups of the carrier. Yet this conclusion is subject to the following qualifications.

(i) If the competing ion results in a more sluggish turnover of the carrier-transporting system, it may lead to a total increase of the reduced form of the redox system, or of the actual carrier molecules, owing to a damming back of the electrons along the line of passage. Thus it would take more of the second ion species to bring about a 50 % reduction of transport than would be expected from a study of the uninhibited transport of the first cation species. Such may cause a shifting upwards of line in the double reciprocal graph as it approaches the ordinate.

(ii) A similar effect may be caused by insufficient oxygen tension where the ion complex needs oxygen for electron acceptance.

(iii) A third point that may lead also to incorrect conclusions is the assumption that the concentrations of external cations remains constant when relatively large uptakes occur.

The conclusions of Epstein and co-workers that there are separate carriers for the potassium group (including rubidium and caesium), and for sodium in barley roots, based on the double reciprocal graphs, may be subject to some such errors as (i). It has been shown here that active transport of Na^+ ions into yeast can be brought about by the physiological carrier of potassium, depending on the conditions, and no special carrier of sodium, bringing the Na^+ cations from without inwards, need be assumed. While this is so for the uptake of Na^+ ions a special carrier must be assumed for their excretion, as shown in a previous paper (Conway *et al.* 1954), and this special sodium-excretion mechanism would appear to be a general endowment of cells.

Concentration of the carrier

The increase in the potassium-combining power of the carrier from $0{\cdot}13 \pm 0{\cdot}009$ (fermenting aerobically) to $0{\cdot}24 \pm 0{\cdot}03$ (fermenting anaerobically) in a current of hydrogen gas accords with the view that the carrier is a metal–redox system.

The yeast cell as a whole contains about 0·60 m-equiv. of iron/kg. and 0·06 m-equiv. of copper (Conway & Ryan, 1954). Most of the iron appears to be bound to or in the membrane or cell wall, as is suggested by the following evidence: when pressed yeast is frozen in liquid air and thawed, this being repeated a few times, the thawed cells are found to have all their cell walls disrupted, as judged by the free diffusion of inulin through all the water present. When the thawed cells are washed repeatedly with water and the iron content of the residue is determined most of this remains.

Foulkes (1956) has recently essayed a determination of the potassium carrier by way of the combination with azide, in accordance with the relation

$$C + n\mathrm{HN_3} \rightleftarrows C(\mathrm{HN_3})_n,$$

where C represents the carrier. By determining the hydrazoic acid concentration necessary to inhibit the transport of potassium by 50 % Foulkes determined the 'K' of this equation at 31° and at 21°, making then a calculation of its value at 0°. At the last temperature the amount of bound azide was determined at pH 5·8. From these data he calculated the total concentration of the carrier to be approximately 0·1 m-equiv./kg. of yeast, but interpreted it as a maximum value 'as other cell constituents may significantly contribute to the binding of the azide'.

The order of the figure obtained by Foulkes is thus the same as that obtained above, but the following points require comment. First, the inhibiting action of the azide may be exerted more on an intermediate electron carrier between the potassium-transporting compound and oxygen, but present in the cell wall or membrane. Secondly, he considers the existence of two potassium carriers, one affected by azide and operative in the non-fermenting sodium-rich yeast, the other being operative during fermentation and azide-insensitive. This statement appeared surprising to us, since we have regularly observed considerable inhibition of transport of potassium by 2 mM-azide in fermenting yeast. As evidence, Foulkes notes very briefly in his paper, after commenting on the absence of inhibition of transport of potassium by cyanide during fermentation with sodium-rich yeast, that 'similar results were produced with 0·0005 M-azide' without giving any details. It may be true that without glucose the effect of azide is greater (a fact probably connected with the necessary passage then of the electrons to oxygen), yet to describe the carrier of potassium during fermentation as being azide-insensitive is quite incorrect.

It will be seen from the results given above that, whether during fermentation with fresh yeast or without fermentation and sodium-rich yeast, the cation carriage shows the same order of affinities, and from the nature of the evidence presented the assumption of two physiological carriers of potassium is unnecessary.

Flux values of the cation transport

The cation net transport may be expressed in terms of maximum flux values obtained with baker's yeast at 18°, such influxes being described as moles/cm.^{2}/sec. By regarding the yeast cell as a

sphere of radius 2·5 μ and with a maximum rate of uptake of 22 m-equiv./kg. during the first 10 min. (Fig. 2) we obtain 4 $\mu\mu$moles cm.$^{-2}$ sec.$^{-1}$ maximum flux of the K^+ ion.

Values for the other cations range from this to about 1 $\mu\mu$mole cm.$^{-2}$ sec.$^{-1}$ for magnesium, though expressed as equivalents for magnesium the range is from about 4 to 2 $\mu\mu$moles cm.$^{-2}$ sec.$^{-1}$. These maximum fluxes for single cation species are reached at very widely varying concentrations, the concentration required being several hundred times greater for magnesium than for potassium.

SUMMARY

1. At about pH 6–7 the physiological carrier of potassium can transport large amounts of other inorganic cations when these are present as the only cation species (apart from the minute H^+ ion concentration) and in sufficient concentration.

2. The relative affinities for the carrier of potassium, rubidium, caesium, sodium, lithium and magnesium, under the conditions investigated (determined by competition with the K^+ ion), were approximately 100:42:7:3·8:0·5:0·5, yet the maximum rates of uptake differed relatively little. Such maximum rates were reached at very different external concentrations; that for potassium was at about 1·6 mM whereas that for lithium was about 300 mM.

3. The double reciprocal graphs of uptake against external concentration ($1/v$ against $1/S$) show that the ions compete for the same carrier, this being illustrated by the competition data for sodium and potassium.

4. The relative affinities of various inorganic cations for the carrier were determined with fermenting yeast and non-fermenting sodium-rich yeast. The same order of affinities was found under both sets of conditions, indicating that the same carrier was operative.

5. Measurement of the competitive effects of some organic cations on uptake of potassium showed that ethylamine had about 9% and ethanolamine 1·4% affinity of potassium for the carrier. Alanine showed no appreciable affinity.

6. Twenty-four amino acids in 5 mM-concentration were investigated for their effect on sodium excretion from sodium-rich yeast. The most effective were ornithine, arginine, lysine, citrulline and histidine, causing increases ranging from 94 to 55%. Alanine and glutamic acid caused no increase.

7. The binding power of the carrier expressed as m-equiv./kg. of yeast was determined by a special displacement of K^+ ions from it with rubidium, and it was found to be 0·13 ± 0·009 m-equiv./kg. in air, increased by anoxia and hydrogenation to 0·24 ± 0·03 m-equiv./kg. of centrifuged yeast.

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