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The Further Preparation of Inorganic Cationic Yeasts and Some of their Chief Properties

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1. A method is described for replacing the intracellular K^+ of the yeast cell by Rb^+, Cs^+, Li^+ or Ca^{2+} ions. In the formation of a calcium yeast it is necessary to proceed first through a sodium yeast (Conway & Moore, 1954) as in the formation of a magnesium yeast (Conway & Beary, 1962). This concludes the series of such yeasts in which almost all the usual K^+ is replaced by another cation, and for which the effect on the properties of fermentation, oxygen uptake and of growth are described. 2. Previous work has shown that all these inorganic cations that can be accumulated in quantity at pH 7·0 are taken up by the same carrier, that the uptake of Mg^{2+} is almost completely inhibited by anoxia and cyanide (0·2mM) and that in the uptake of Mg^{2+} ions a practically equivalent amount of H^+ ions is excreted. It is suggested that these facts amount to a definitive demonstration that the carrier is a cytochrome.

Work in this Laboratory has shown that it is possible to replace practically all of the intracellular K^+ of the yeast cell, *Saccharomyces cerevisiae*, with various inorganic cations, and metabolic consequences of such K^+ replacement by NH_4^+, Na^+ and Mg^{2+} ions have been investigated. Thus an 'ammonium yeast' (Conway & Breen, 1945) was made by repeated fermentations in ammonium chloride solution, and a 'sodium yeast' (Conway & Moore, 1954) was made by similar fermentation in sodium citrate medium.

For the 'magnesium yeast' (Conway & Beary, 1962) it was found necessary to replace first the K^+ by Na^+ and then on suspending the yeast in 0·2M-magnesium acetate in 5% glucose solution at about pH 7·4 the Na^+ was replaced by Mg^{2+}. In the work reported below the general cation carrier in the yeast cell wall was made to transport large amounts of the univalent cations Rb^+, Cs^+ and Li^+ and the bivalent cation Ca^{2+}. Thus yeasts were produced in which practically all of the K^+ was replaced by Rb^+, Cs^+, Li^+ and Ca^{2+}.

When resting yeast was fermented for about 20hr. in a calcium acetate medium, the K^+ content of the cells did not change appreciably and a maximum of 38·4μmoles/g. was accumulated. When, however, as was done with the magnesium yeast, the K^+ was first replaced by Na^+, it was then found possible to make a calcium-rich yeast by repeated fermentation of the sodium yeast in calcium acetate medium.

Some of the main properties of these four yeasts were then investigated and compared with those of a normal untreated yeast.

With the preparation of the four yeasts described below there is completed the series of cationic yeasts that have had almost all their K^+ replaced by one or other of Rb^+, NH_4^+, Cs^+, Na^+, Li^+, Mg^{2+} and Ca^{2+}. The powers of fermentation, oxygen uptake and growth of these four yeasts are described.

METHODS

Preparation of a potassium yeast, a rubidium yeast, a caesium yeast and a lithium yeast. A twice-washed sample of baker's yeast from the Cork Yeast Co. was allowed to ferment for successive 2hr. periods diluted 1:20 in a 0·2M solution of the particular ion in 5% (w/v) glucose. Lithium citrate was used to make the lithium yeast, and KCl, RbCl and CsCl respectively were used in the making of the potassium, rubidium and caesium yeasts. Where necessary the pH of the fermentation medium was adjusted to 7·4 with 0·2M-tris buffer. The fermenting yeasts were continuously shaken in air at room temperature (18°). All media were inoculated with 10000 units of penicillin and 10000 units of streptomycin/100ml. of medium to prevent bacterial contamination. The yeast was centrifuged, washed and resuspended in fresh medium every 2hr. This prevented a build-up in the suspending fluid of K^+ lost from the cells, which might compete for the carrier. As a control a sample of the same fresh yeast was treated similarly with a medium containing potassium citrate instead of the investigated ion species. Before analysis the yeast was washed twice with 20vol. of distilled water to remove the investigated ion from the intercellular space.

Preparation of calcium yeast. A sodium yeast was made

first according to the method of Conway & Moore (1954). This yeast, in which 98% of the cellular K^+ has been replaced by Na^+, was then suspended in 0·2M-calcium acetate in 5% (w/v) glucose and a series of 2hr. fermentations was carried out as described above. The fermentation medium was continuously bubbled with O_2 at room temperature. The Ca^{2+}, Na^+ and K^+ contents were measured at regular intervals until accumulation ceased.

Preparation of magnesium yeast. This was carried out by the method of Conway & Beary (1962). Again a sodium yeast was made first and this was grown in magnesium acetate medium.

Rb^+, Ca^{2+}, Li^+, Na^+ *and* K^+ *analyses.* These were carried out by flame photometry at wavelengths 795, 852, 670·8, 589 and 769mμ for Rb^+, Ca^{2+}, Li^+, Na^+ and K^+ ions respectively, by using a Beckman model DU spectrophotometer with flame attachment. Samples of twice-washed yeast were boiled in 10–20vol. of distilled water and diluted to give a convenient photometer reading. All samples were centrifuged before reading.

Ca *analysis.* This was carried out by the method of Conway & Armstrong (1961).

Mg *analysis.* This was carried out by using the Titan Yellow method of Young & Gill (1951). All yeast samples were wet-ashed with conc. HNO_3 before determination.

Fermentation experiments. The micro-diffusion procedure of Conway (1962) was used with the standard no. 2 'units'. All 'units' were set up in triplicate.

Growth experiments. The growth medium employed here contained 2·6g. of glucose, 0·48g. of $KH_2PO_4,2H_2O$, 0·4g. of $(NH_4)_2SO_4$, 0·1g. of $MgSO_4,7H_2O$, 1mg. of thiamine, 1mg. of *p*-aminobenzoic acid, 2mg. of riboflavine, 2mg. of nicotinic acid, 2mg. of calcium pantothenate, 4mg. of pyridoxal hydrochloride, 0·05mg. of inositol and 0·004mg. of biotin made up with water to 100ml.

The $KH_2PO_4,2H_2O$ was substituted when growing the lithium yeast by an equivalent amount of $LiOH,2H_2O$, or, for the rubidium yeast, the caesium yeast or the calcium yeast, by equivalent amounts of RbCl, CsCl or $CaCl_2$ respectively.

In all cases the pH was adjusted to 3·6 with tris–phosphate buffer (0·1M-H_3PO_4 and 0·15M-tris). All media and flasks were sterilized. In all growth experiments 0·1ml. of a 1:100 yeast suspension was added to 100ml. of sterile medium in a Pasteur flask and incubated at 28°. Every 12hr. two small samples were withdrawn from the medium after shaking and a count was made on each with a haemocytometer, the mean being used to plot the result. All media were bubbled with O_2 throughout.

Respiration experiments. The endogenous O_2 consumption was measured by the 'direct' Warburg manometric method at 30° with shaking at 120 strokes/min. A 3ml. portion of diluted (1:100, w/v) yeast was suspended in tris–phosphate buffer, pH 5·4 (0·1M-H_3PO_4 and 0·15M-tris). The central well contained 0·2ml. of 20% (w/v) KOH. Oxygen consumption was expressed as μl./hr./30mg. of centrifuged yeast.

Determinations of pH. These were carried out with the Beckman model G meter.

Freezing-point depressions. These were measured by the micro-cryoscopic method of Conway & McCormack (1953). The yeast, having been washed twice with 20vol. of distilled water and packed by centrifugation at 3000 rev./min. for 20min. in an International centrifuge (size L, type SB), was broken up by repeated freezing and thawing in liquid N_2. This procedure gave a fluid mixture very suitable for freezing-point measurements. The mixture was stored frozen in stoppered glass tubes each containing 0·25ml. and these were thawed just before determination. The apparatus had a high sensitivity and successive readings differed very little.

To calculate the intracellular molarity from the readings of freezing-point depression it was necessary to know the total water content of the yeast. This was determined by measurements of the dry weight of the centrifuged yeast. The intracellular water volume was then calculated from this total water content and the extracellular water volume as calculated by Conway & Downey (1950).

RESULTS

Ion content of the different yeasts. Table 1 shows the mean content of ten samples each of a rubidium yeast, a caesium yeast, a lithium yeast and a control potassium yeast after ten 2hr. fermentation periods at room temperature (18°) in the respective fermentation media (see the Methods section). With the rubidium and caesium yeasts practically all of the K^+ has been removed, though with the lithium yeast there always remains, in spite of many repeated fermentations, about 20μmoles of K^+/g.

Fig. 1 shows the time-curves for the replacement of K^+ in the yeast cells by Rb^+, Cs^+ and Li^+. Before the immersion of the washed yeast samples in the solutions of the three salts rubidium chloride,

Table 1. *Mean contents of ten samples each of a rubidium yeast, a caesium yeast, a lithium yeast and their associated* K^+ *contents*

The mean contents were measured after ten 2hr. fermentation periods at room temperature (18°) in the respective fermentation media as described in the Methods section. Also included are the mean contents of ten potassium yeasts, in which K^+ is the only external inorganic cation, in a concentration and media similar to the other cations. The contents of the various cations are expressed as μmoles/g. and are given as means ± S.E.M.

Rubidium yeast		Caesium yeast		Lithium yeast		Potassium yeast
Rb^+	K^+	Cs^+	K^+	Li^+	K^+	K^+
120·1	2·4	100·1	1·7	124	21·0	167·3
±5·3	±0·5	±3·1	±0·3	±2·7	±0·8	±2·4

caesium chloride and lithium chloride the concentrations of K^+ in the three yeast samples were 143·0, 130·0 and 123·8 μmoles/g. respectively. The data for the samples of rubidium yeast and lithium yeast were all multiplied by factors that would have the effect of bringing the washed samples of rubidium and lithium yeast in each case to 130·0 μmoles/g. (which is the median value for the three) for better graphical comparison. For the first 6 hr. of fermentation in producing the rubidium and caesium yeasts the internal K^+ concentration is displaced sharply, there being a quantitative replacement of K^+ by Rb^+ and Cs^+. A number of fermentation periods are required to decrease the internal K^+ concentration further although the cells do not appreciably gain any more Rb^+ and Cs^+. The lithium yeast differs in this respect in that there is a steady loss of K^+ and gain of Li^+ over the 20 hr. period of fermentation; the K^+ concentration, however, rarely fell below 20 μmoles/g. Here the replacement is not quantitative,

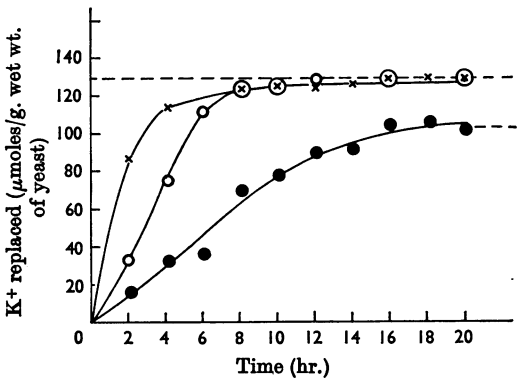

Fig. 1. Time-curves for the replacement of K^+ in the yeast cells by Rb^+ (×), Cs^+ (○) and Li^+ (●). (For better graphical comparison, the Cs^+ and Li^+ data were multiplied throughout by factors bringing their concentrations at zero time to 130 μmoles/g., which is also the value for Rb^+; see the text.)

the final concentration of K^+ plus Li^+ often being as high as 171·0 μmoles/g. wet wt. of yeast. Higher temperatures and vigorous shaking did not significantly alter the rate of K^+ replacement.

Results of suspending the rubidium, caesium and lithium yeasts in 0·1 M-*potassium chloride.* These results are given in Table 2. With the rubidium and caesium yeasts after 6 hr. similar amounts of Rb^+ (48·6 μmoles/g.) and Cs^+ (44·4 μmoles/g.) had come out, the concentrations of Rb^+ and K^+ in the rubidium yeast being 53·2 and 52·6 μmoles/g. respectively, and 42·6 and 42·6 μmoles/g. respectively with the caesium yeast.

The position with lithium yeast is strikingly different, 103·5 μmoles/g. of yeast having come out in 6 hr., with 93·0 μmoles of K^+/g. remaining.

Stages in the production of a calcium yeast. When fresh yeast is fermented in 0·2 M-calcium acetate in 5% glucose and bubbled with oxygen there is a very slow uptake of the Ca^{2+} to a maximum of 38·4 μmoles/g. after 20 hr. There is no K^+ loss from the cells over this period (Table 3). Table 4 summarizes the results obtained in six sets of experiments in which a sodium yeast was prepared first as described by Conway & Moore (1954) and then a calcium yeast prepared from this by repeated fermentation in 0·2 M-calcium acetate in 5% glucose solution. Thus a yeast containing, for example, 94·5 μmoles of Ca^{2+}/g., 10·4 μmoles of K^+/g. and 33·4 μmoles of Na^+/g. could be produced by this method. The progressive displacement of Na^+ by Ca^{2+} is shown in Table 5. Ca^{2+} was not lost from the yeast when the calcium-rich cells were suspended in 0·1 M-potassium chloride and tap water for 90 min. (Table 6).

Fig. 2 shows the entrance rates of Mg^{2+} and Ca^{2+} into yeast. The upper two curves are for entrance into sodium yeasts, formed as described above; the lowest curve is for entrance of Ca^{2+} into yeast without first forming a sodium yeast. All three curves were for the conditions described in the text.

Fermentation studies of rubidium yeast, caesium yeast, lithium yeast and calcium yeast. A series of experiments on the production of carbon dioxide

Table 2. *Effect on the accumulated* Cs^+, Rb^+ *and* Li^+ *of suspending the caesium yeast, rubidium yeast and lithium yeast over 2 hr. fermentation periods in* 0·1 M-*potassium chloride*

The 1:20 suspensions were shaken in air and samples taken every 2 hr. and analysed. Results are expressed as μmoles/g. wet wt. of yeast.

	Rubidium yeast		Caesium yeast		Lithium yeast	
Time (hr.)	Rb^+	K^+	Cs^+	K^+	Li^+	K^+
0	101·5	2·38	87·0	2·6	156·0	16·7
2	66·0	—	86·0	—	104·0	—
4	47·5	28·4	52·1	32·2	75·0	82·0
6	53·2	52·6	42·6	42·6	52·5	93·0

Table 3. *Time-course of* Ca^{2+} *entrance into fresh yeast*

The yeast was fermented in calcium acetate–glucose medium. All fermentations were bubbled throughout with O_2. Results are expressed as μmoles/g. wet wt. of yeast.

No. of 2hr. fermentation periods	K^+	Ca^{2+}
2	135·0	6·4
4	138·0	12·3
6	137·0	21·3
8	147·1	25·4
10	147·1	38·4

Table 4. Ca^{2+}, K^+ *and* Na^+ *contents of calcium yeast prepared from sodium yeast*

Sodium yeast was prepared as described in the Methods section and then suspended in 0·2M-calcium acetate in 5% glucose. Ten 2hr. fermentations were carried out at room temperature and bubbled with O_2. Results, which refer to the last 2hr. fermentations, are expressed as μmoles/g. wet wt. of yeast.

Yeast sample	Na^+	K^+	Ca^{2+}
1	8·8	29·4	110·8
2	16·8	5·01	75·6
3	14·3	23·4	94·14
4	36·2	13·1	93·6
5	21·9	14·2	84·51
6	33·4	10·4	94·5
Mean	21·9	15·92	92·19

Table 5. *Time-course of* Ca^{2+} *entrance into sodium yeast over six 2hr. fermentation periods*

Sodium yeast was prepared as described in the Methods section and then suspended in 0·2M-calcium acetate in 5% glucose. Ten fermentations were carried out over six 2hr. fermentation periods at room temperature, and all were bubbled throughout with O_2. Results are expressed as μmoles/g. wet wt. of yeast.

No. of 2hr. fermentation periods	Na^+	K^+	Ca^{2+}
0	148·2	10·8	4·07
1	65·4	10·0	8·13
2	57·1	11·4	22·83
3	40·0	10·5	40·93
4	27·8	10·1	52·93
5	24·6	11·7	75·93
6	21·9	14·2	84·51

by the different yeasts as determined by the Conway micro-diffusion method is summarized graphically in Fig. 3. The rates of fermentation are

Table 6. *Effect on the accumulated* Ca^{2+} *of suspending the calcium-rich yeast in* 0·1M-*potassium chloride and in tap water*

Samples of twice-washed yeast were diluted 1:20 with 0·1M-KCl and tap water and shaken at room temperature for 90min. Results are expressed as μmoles/g. wet wt. of yeast.

Time (min.)	Ca^{2+}	K^+	Na^+
0	93·1	12·6	8·4
90 (in 0·1M-KCl)	93·5	50·0	13·8
90 (in tap water)	88·5	34·7	10·5

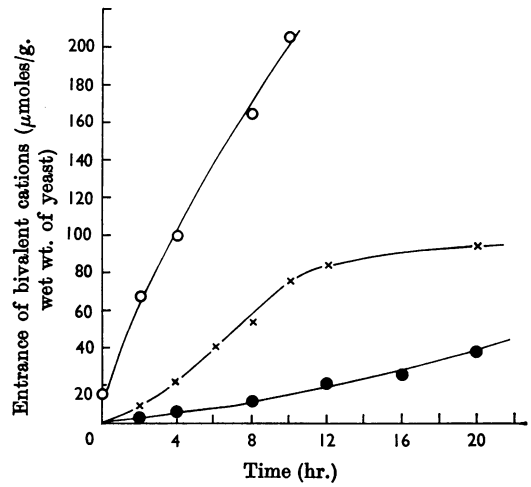

Fig. 2. Entrance of Mg^{2+} and Ca^{2+} into yeast. The upper two curves are for entrance of Mg^{2+} (○) and Ca^{2+} (×) into sodium yeast, and the lowest curve shows the entrance of Ca^{2+} (●) into yeast that has not been first converted into a sodium yeast.

compared with those of a normal resting yeast and a control potassium yeast. The rubidium yeast ferments at practically the same rate as the control potassium yeast, which is 71·4% of the rate of the fresh untreated yeast. The caesium yeast ferments at 17·1% of the rate of the normal, and the lithium yeast and the calcium yeast respectively ferment at 25·0% and 9·29% of the rate of the normal resting yeast.

The addition of 10m-moles of potassium chloride/100ml. to the suspension medium had the effect of increasing the overall fermentation rates of the different yeasts by a slight amount.

Oxygen consumption of rubidium yeast, caesium yeast, lithium yeast and calcium yeast. Table 7 summarizes a number of experiments on the uptake of oxygen by the yeasts. The rubidium

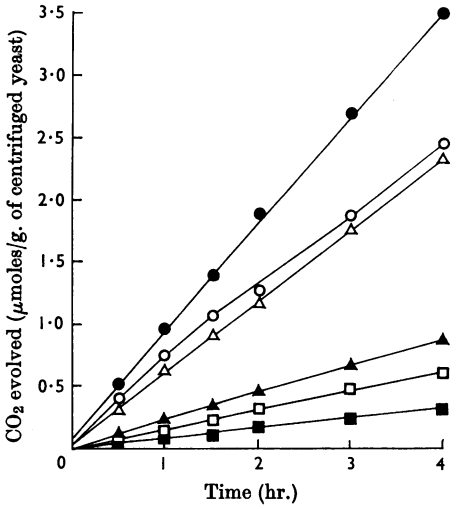

Fig. 3. Fermentation rates of normal, potassium, rubidium, caesium, lithium and calcium yeasts, measured as described by Conway (1962). Evolution of CO_2 is expressed as μmoles/g. of centrifuged yeast. ●, Normal yeast; ○, potassium yeast; △, rubidium yeast; ▲, lithium yeast; □, caesium yeast; ■, calcium yeast.

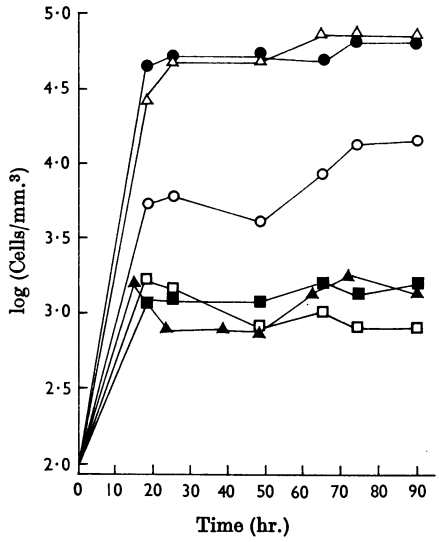

Fig. 4. Growth curves for normal (△), potassium (●), rubidium (○), caesium (□), calcium (■) and lithium yeasts (▲). Growth was at 28° in a low-K^+ medium. The count at zero time is taken as 100 cells/mm.³ for each type of yeast.

Table 7. *Summary of oxygen-consumption experiments*

Endogenous uptakes are expressed as μl./hr./30mg. of centrifuged yeast, and are given as means of the numbers of experiments in parentheses.

Type of yeast	Uptake of O_2
Fresh yeast	44·01 (9)
Potassium yeast	125·95 (9)
Rubidium yeast	121·67 (5)
Caesium yeast	96·34 (5)
Lithium yeast	71·91 (5)
Calcium yeast	18·9 (4)

yeast has the fastest uptake rate, being 96·5% of the rate of the control potassium yeast, then in decreasing order the caesium yeast, lithium yeast and calcium yeast at rates of 76·5%, 67·1% and 15·0% the control potassium yeast. The addition of 1m-mole of potassium chloride/100ml. to the different yeast suspensions increased their oxygen consumption rates by a slight amount.

Growth of rubidium yeast, caesium yeast, lithium yeast and calcium yeast. When the growth of these yeasts was studied in the synthetic media as described in the Methods section the rubidium yeast grew at the fastest rate; the final cell population attained, however, after 4 days' growth was never as high as that of either an untreated yeast or a control potassium yeast (Fig. 4). The caesium, lithium and calcium yeasts grew at approximately the same rate, which was very much slower than the other yeasts. In fact, the cells appeared to grow for the first 15hr. only, after which time there was no further increase in population. These results are represented graphically in Fig. 3. When 10m-moles of K^+/100ml. were incorporated into the growth medium of the rubidium yeast a marked stimulation of growth was apparent; the same effect was observed in the caesium yeast. Only a very slight improvement was found in the growth rate of the calcium and lithium yeasts on the addition of K^+.

Freezing-point depression of frozen-and-thawed yeast and total intracellular concentration. By using the micro-cryoscopic method of Conway & McCormack (1953) and treating the yeast as described in the Methods section the freezing-point depression of frozen-and-thawed resting yeast was examined. From seven experiments an average depression of $0·709 \pm 0·023°$ was obtained. If one assumes a depression of 1·86°/mole for the frozen-and-thawed mixture and an extracellular water volume of 0·301./kg. (Conway & Downey, 1950) from the data of freezing-point depression and total water volume, which was found to be 0·78ml./g., the intracellular molarity of the yeast may be calculated. For normal resting yeast this was found to be $0·616 \pm 0·019$M. From nine experiments an average freezing-point depression of 0·638

Table 8. *Calculated total intracellular molarity of frozen-and-thawed resting baker's yeast, calcium yeast and magnesium yeast*

Details are given in the text. All concentrations are expressed as M.

Yeast sample	Resting yeast	Calcium yeast	Magnesium yeast
1	0·633	0·521	1·010
2	0·624	0·526	1·015
3	0·614	0·531	1·049
4	0·633	0·555	0·947
5	0·624	0·560	0·947
6	0·571	0·560	0·986
7	0·614	0·585	1·015
8	—	0·585	0·996
9	—	0·585	0·947
10	—	—	0·952
Mean	0·616±0·019	0·556±0·024	0·986±0·034

±0·027° was obtained for frozen-and-thawed calcium yeast. This gave a calculated intracellular molarity of 0·556±0·024M. Table 8 compares these experiments with the results from a similarly treated magnesium yeast. This magnesium yeast was made by the method of Conway & Beary (1962) and contained 5·85 μmoles of Na^+/g., 13·3 μmoles of K^+/g. and 274·1 μmoles of Mg^{2+}/g. A freezing-point depression of 1·129±0·039° was obtained with this yeast and an intracellular molarity calculated at 0·986M.

DISCUSSION

At the outset it may be noted that there exists in the yeast cell a general carrier of inorganic cations, which is here termed the 'physiological K^+ carrier' because of all the inorganic cations it has the greatest affinity for K^+.

The affinities of the various cations for the carrier have been given by Conway, Duggan & Kernan (1963) and taking the affinity for K^+ as 100 range from 1350 for H^+ to 0·5 for Mg^{2+}. From the work on Ca^{2+} in the present paper the affinity of this cation can be given as 0·2 by comparing the slopes of entrance of Mg^{2+} and Ca^{2+} into a sodium yeast as in Fig. 2.

When the external pH is 7 or close thereto, the H^+ concentration being therefore comparatively low, and when at the same time one or other of the inorganic cations is present in relatively high concentration (0·2M suffices in all cases) it will be freely accumulated when glucose (5%, w/v) is present.

The fact that these various cations when present together compete for the same active group on the carrier is shown by the facts of mutual competition and the application of Michaelis–Menten kinetics. Data for the competition of K^+ and Na^+ are given by Conway & Duggan (1958*a*), of the competition of Mg^{2+} and Rb^+ by Conway & Beary (1958) and of the competition of K^+ and H^+ by Conway *et al.* (1963), applying the Lineweaver & Burk (1934) equation. [The 'physiological K^+ carrier' is not the only cation carrier in the yeast cell wall. Rothstein (1954) showed the presence of a special carrier of bivalent cations, particularly Mg^{2+} and Mn^{2+}, operative at pH 5·0, which showed no inhibition by K^+.]

Mechanism whereby the various cationic yeasts are formed through the medium of the 'physiological K^+ carrier'. The question arises as to what is happening in these exchanges. First, although the physiological K^+ carrier in the cell membrane can take up large amounts of cations, its active group is relatively a very small quantity, being about 0·12 μmole/g. (Conway & Duggan, 1958*b*). It must therefore act cyclically, the carrier taking up the cation and releasing some inside the cell, being changed thereby into a somewhat different substance, the restoration to the original carrier occurring through metabolic activity so that the cycle is completed. The uptake of the cations may be assumed to occur on anionic groups. These are provided by a cytochrome or cytochrome-like carrier that on receiving metabolic hydrogens, during metabolism, converts these into H^+ ions, which are set free into the external medium, and electrons, which are retained, forming the anionic groups that attach the cations. On transferring its electrons to a system of higher redox potential the attached cations are released into the cell. The cycle is restored by the acceptance of further metabolic hydrogen atoms. In this way the carrier receives cations and provides at the same time the necessary energy to transport them.

In a second type of concept the anionic charges are provided by a complex with adenosine tri-

phosphatase, as in Skou's (1960) enzyme preparation. In this, with the breakdown of the enzyme complex, ATP provides the energy while at the same time anionic charges disappear, releasing the absorbed cations.

The anionic charges disappear in such breakdown by the combination of electrons with protons. From the following argument, there can be little doubt but that the first theory, that of the 'redox pump', is here the correct one and the stages of the argument are as follows.

Taking the whole range of inorganic cations, with regard to active transport by the 'physiological K^+ carrier' it will be sufficient to demonstrate with one of these the operation of the 'redox pump'. Mg^{2+}, the second lowest of the series in terms of affinities, may be selected. At pH 7·0 it is taken up in large amounts from 0·2 M-magnesium acetate. The uptake is almost completely inhibited by anoxia and cyanide (0·2 M). Also, in the uptake of the Mg^{2+} a practically equivalent amount of H^+ is excreted (Conway & Beary, 1958). This is a picture of events that is to be expected when metabolic hydrogen atoms are absorbed by a cytochrome carrier at the edge of the cell, the metabolic hydrogen atoms being split into H^+ ions, released externally, and electrons, which are carried forward to oxygen, forming the requisite transport for cations (in the present case Mg^{2+} ions).

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