Effects of Extracellular Potassium and Sodium on Intracellular Potassium and Sodium of Baker's Yeast.* (25568)

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In studies of electrolyte exchanges in yeast, it was of interest to learn how the electrolyte content of yeast cells is affected by conditions of growth. Potassium is a major constituent of yeast ash, amounting to 23 to 40% (1). It is known that ash composition is affected by conditions of growth, but few data are available on effects of potassium and/or sodium content of growth medium. These experiments were undertaken to determine how the potassium content of yeast is affected by varying amounts of potassium chloride or sodium chloride in the growth medium.

Materials and methods. The yeast was a pure culture strain of *Saccharomyces cerevisiae* isolated from a cake of Fleischman's yeast. The basal growth medium contained 10 g/l Difco yeast extract and 50 g/l dextrose. This control medium contained approximately 8 meq/l each of potassium and sodium. Sterile salt solutions were added after autoclaving to produce final concentrations of approximately 30, 100 and 300 meq/l potassium or sodium. Each set of cultures consisted of one or, more frequently, 2 flasks of each cation concentration, so that comparisons could be made between cultures grown under same conditions. A uniform inoculum was used for any one set of flasks, and the cultures allowed to grow where temperature usually remained between 21 and 23°C, but might range from 20 to 25°C. Cells were harvested by centrifuging cultures 3 to 6 days after inoculation. It was assumed, from preliminary studies, that cultures had reached the stationary phase of growth at this time. The packed weight of centrifuged cells was used to estimate wet weight, assumed to be 78% of packed weight. This figure of Conway and Downey(2) was corroborated by us by estimating intercellular space (i) from packed weights of known amounts of cake yeast, and (ii) by measuring gelatin space of yeast harvested from laboratory cultures. Yeast was resuspended and an aliquot washed 3 or 4 times with about 10 ml water/g yeast for dry weight and electrolyte determinations. Because preliminary experiments indicated that some intracellular potassium was removed by washing procedure, electrolyte analyses were also made on unwashed aliquots. Corrections for extracellular potassium and sodium were made assuming extracellular solution accounted for the other 22% of packed weight of yeast (see above). Cations were extracted from yeast using Conway's procedure(3). Suspensions of both washed and unwashed cells (about 1 g/10 ml) were boiled 5 to 10 minutes. Supernatant cell extracts were appropriately diluted, as were samples of media, and potassium and sodium determined with a Perkin-Elmer Model 52C internal standard flame photometer. When compositions of cells grown on different media were compared, the data were paired with those of cultures from the same set; *i.e.*, grown at same time. The "t" test was used for calculation of P values.

Results are presented in Table I and summarized in Fig. 1.

Effects of varying potassium. When cultures from the same sets were compared, statistically significant differences could not be observed between Groups I and II. In Groups III and IV, however, there was significant reduction in water content of yeast with each increase in potassium concentration. Potassium content of unwashed cells increased with that of medium, especially at higher potassium concentrations. When yeast was washed, a considerable amount of potassium was removed from high-potassium cells so that Groups III and IV did not differ appreciably from one another, but still contained more potassium than Groups I and II. In all instances in which sodium content of cells was

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TABLE I. Water, Potassium and Sodium Content of Yeast Grown in Various Concentrations of Potassium or Sodium Chloride.*

	K or Na content of medium, meq/l	H_2O content of cells, g H_2O/kg wet cells	K content of cells		Na content of cells	
			Unwashed	Washed†	Unwashed	Washed
			meq/kg wet cells			
	A. Effects of varying potassium in medium; Na = 8 meq/l throughout.					
I.	8 ± .2‡	763 ± 7	101 ± 2	98 ± 3		
II.	29 ± .2	769 ± 6	109 ± 4	100 ± 4		
III.	107 ± 5	747 ± 9 (<.005)§	148 ± 8 (<.025)	119 ± 3 (<.05)		
IV.	303 ± 9	725 ±10 (<.001)	257 ± 9 (<.001)	120 ± 6		
	B. Effects of varying sodium in medium; K = 8 meq/l throughout.					
V.	8 ± .4	767 ±10	112 ± 6	87 ± 4	8.4 ± .6	2.4 ± .6
VI.	34 ± 2	762 ± 8	106 ± 6	81 ± 3 (<.05)	19.9 ± .7	10.9 ± .5 (<.005)
VII.	139 ± 1	743 ± 9 (<.001)	97 ± 5	65 ± 1 (<.001)	78 ± 1	26.4 ± 1.9 (<.005)
VIII.	331 ±15	741 ± 7	104 ± 3	52 ± 5 (<.025)	194 ±17	37.4 ± 1.2 (<.05)

* Data derived from 4 to 6 sets of cultures except for those on sodium content of unwashed cells which were obtained from 2 sets.

† Washed cells were rinsed 3 or 4 times with approximately 10 ml water/g cells.

‡ Mean ± stand. error.

§ Figures in parentheses are P values for differences of results from those of preceding group.

determined, it was very low, usually less than 8 meq/kg wet cells, regardless of potassium content of medium.

Effects of varying sodium. Water content was reduced when sodium chloride concentration was increased above 100 meq/l. With 300 mM NaCl, however, there appeared to be no further decrease in water content. There was relatively little change in potassium content of cells grown in high sodium chloride, except for a slight decrease in Group VII. This difference was significant only in comparison with control Group V; P<.005. However, after washing, there was a marked decrease in potassium content with increasing sodium chloride concentration. With each increment in sodium chloride concentration in the medium, there was a rise in sodium content of both washed and unwashed cells.

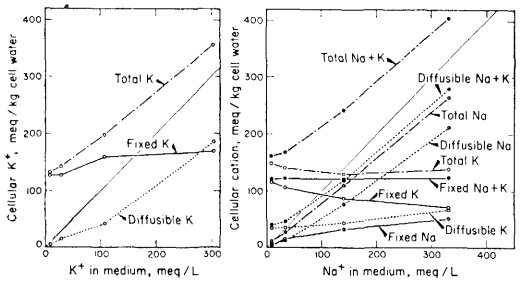

FIG. 1A (left). Effect of K content of growth medium on intracellular K of yeast cells.

FIG. 1B (right). Effect of Na content of growth medium on intracellular Na and K of yeast cells.

Fixed Na or K refers to that remaining after washing. Diffusible Na or K is calculated from difference between total ion and fixed ion concentrations. Straight line drawn at a 45° angle from the origin represents the curve which would be obtained if intra- and extracellular concentrations were equal.

Discussion. Total intracellular K concentration (meq/kg cell water) always increased with and exceeded extracellular K (Fig. 1A). The 2- to 3-fold rise in intracellular K suggests that yeast is poikilosmotic; *i.e.*, its internal osmotic pressure may vary over a wide range, depending on osmotic pressure of the external medium. This poikilosmotic character has been described in various lower organisms, including the bacterium, *Salmonella oranienburg*(4), and the marine alga, *Ulva lactuca*(5), both of which accumulate potassium in response to increased external osmotic pressure. Yeast differed from these organisms in that there was no increase in intracellular K when NaCl was substituted for the KCl supplements to the growth medium (Fig. 1B). However, the total intracellular cation con-

centration, as estimated from the sum of Na and K concentrations, gave a curve remarkably similar to that for K in Fig. 1A. In all cases intracellular Na concentration was lower than that of the medium, while intracellular K concentration exceeded it. The increase in intracellular cation concentration results not only from increase in total cation content, but also from decreased water content of yeast. Nonetheless, the water content was in no case as low as that of commercial yeast.

Yeast grown in high concentrations of either KCl or NaCl lost a considerable amount of potassium when washed with water. Christian(6) found a similar loss of K in washing experiments using *Salmonella oranienburg*. The loss from yeast was too large to be accounted for by possible inaccuracy in estimation of extracellular space, especially in the case of high Na where correction for extracellular K introduced almost no error. Similarly it cannot be accounted for by Conway and Downey's(2) "outer metabolic region" of the yeast cell. The efflux of K from baker's yeast described by Rothstein and Bruce(7) is probably not important in this case for several reasons: efflux rates are too small to account for losses in our experiments, particularly since each washing took 5 minutes or less; only negligible amounts of substrate were present; the efflux described by Rothstein and Bruce would be obliterated by the amounts of potassium in the wash water.

The present data suggest that yeast K exists in 2 fractions. One fraction (diffusible) is easily removed by washing and may account for almost half the intracellular K in cells grown in 300 mM KCl or NaCl. Since this fraction is relatively small (10 meq/kg cell water) in control cells, the increased diffusible K may be a consequence of increased osmotic pressure. The other fraction (fixed) is difficult to remove with washing. Intracellular Na may also be distributed into 2 compartments, although a smaller proportion appears to be in the fixed fraction (Fig. 1B). Fixed K does not change appreciably with increasing extracellular K, but decreases with higher extracellular Na concentrations. However, the sum of the fixed fractions of Na and K is constant over the range of Na concentrations studied, suggesting that Na competes with K for the fixed fraction.

Isotope studies have demonstrated that potassium is distributed in 2 or more fractions in several types of cells(8,9,10). The nature of the 2 fractions in yeast has not been ascertained. Possible factors include intracellular compartments of differing permeability or differential binding phenomena. Conway and Duggan(3) have shown a competitive effect of Na on K uptake by commercial yeast and have prepared Na-rich yeast in which Na has replaced intracellular K.

Summary. Experiments were carried out to elucidate the effects of varying concentrations of potassium and sodium in growth medium on water and respective ion content of yeast. The resulting changes in intracellular potassium and sodium indicate that yeast is a poikilosmotic organism; internal osmotic pressure as indicated by total intracellular sodium and potassium may vary 2- or 3-fold. Sodium and potassium appear to be distributed into at least 2 intracellular fractions; a diffusible fraction which is readily removed by washing, and a fixed fraction relatively constant in amount which is difficult to remove by washing. Sodium can replace over 40% of the fixed potassium at high external sodium concentrations.

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