3. Consideration of the metabolism of polyols in different organisms leads to the conclusion that there are at least three types of polyol dehydrogenases (L-iditol, D-mannitol and galactitol dehydrogenases) distinguishable by their stereochemical affinities.

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A Sodium-Yeast and some of its Properties

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An observation made in this laboratory by Winder (1950) showed that if baker's yeast (*Saccharomyces cerevisiae*) be allowed to ferment in the presence of sodium citrate and glucose there is a large entrance of sodium ions into the cell. It appeared, therefore, that it would be possible to prepare a 'sodium-yeast', i.e. a yeast in which all or nearly all the potassium would be replaced by sodium, such a yeast being analogous to the 'ammonium-yeast' described by Conway, O'Brien & Boyle (1941) and Conway & Breen (1945). A comparison of the properties of such a yeast with those of a control 'potassium-yeast' and with those of the ammonium yeast might well throw light on the role of these ions in metabolic processes. It proved possible by the methods here described to prepare, as required, a yeast in which 98% of the cellular potassium was replaced by sodium and to measure its growth, fermentation and oxygen consumption rates.

METHODS

Preparation of a sodium-yeast. A fresh twice-washed sample of baker's yeast (usually 10 g. fresh wt.) was used; 1 vol. of the centrifuged material was suspended in 20 vol. of a medium containing 5% (w/v) glucose and 0.2M sodium citrate and fermentation was allowed to proceed for 2 hr. The yeast was then centrifuged, washed with 20 vol. of distilled water and resuspended in fresh fermentation medium. This procedure was repeated until the K content of the yeast was reduced as far as possible. As a control, a sample of the same fresh yeast was treated similarly, using a medium containing potassium citrate instead of the sodium salt. The yeast which has been treated in this way is referred to as 'K-yeast'. Whenever an analysis was to be carried out the yeast was twice washed with 20 vol. of distilled water to remove Na or K from the interspaces.

The fermentations were carried out under three different sets of conditions: (a) at room temperature with occasional stirring, (b) at 37° with occasional stirring, (c) at room temperature with continuous shaking. In the final stages of

preparation of an Na-yeast K was taken up so readily that the flasks in which the fermentation was carried out and the centrifuge cups used had to be coated on the inside with paraffin wax.

Sodium and potassium analyses. These were carried out with a flame photometer using an internal Li standard. The procedure consisted in taking a sample of twice-washed yeast, boiling in 10–20 vol. of distilled water and diluting as required to obtain a convenient reading on the instrument. Centrifuging the solutions before atomizing in the photometer appeared to make no difference to the results and the practice was not maintained.

Growth experiments. The medium for Na-yeast was 2.6 g. glucose, 0.57 g. NaH_2PO_4 , 2H₂O, 0.4 g. $(\text{NH}_4)_2\text{SO}_4$, 0.1 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg. thiamine, 1 mg. *p*-aminobenzoic acid, 2 mg. riboflavin, 2 mg. nicotinic acid, 2 mg. calcium pantothenate, 4 mg. pyridoxine hydrochloride, 0.05 mg. inositol and 0.004 mg. biotin, made up with water to 100 ml.

For K-yeast the medium was the same as above but an equivalent amount of KH_2PO_4 was substituted for the NaH_2PO_4 . The pH of such media was 3.6. In investigating the effect of added K on the growth rate of Na-yeast varying amounts of KCl were incorporated in the medium.

In each growth experiment 0.1 ml. of a 1:100 suspension of the yeast was added to 100 ml. of sterile growth medium in a Pasteur flask and incubated at 28°. Such an inoculum contained approximately 100–400 cells. Every 12 or 24 hr. two small samples were withdrawn from the medium after shaking and a count made on each with a haemocytometer, the mean being used to plot the result. Sterile procedures were used throughout.

Fermentation experiments. The microdiffusion procedure described by Conway (1950) was followed. 1 ml. of 1:100 yeast suspension + 1 ml. of 5% (w/v) glucose were placed in the outer chamber and the CO_2 evolved was absorbed in 1 ml. of 0.1N-NaOH containing a mixed phenolphthalein and thymolphthalein indicator. After the requisite time, 0.5 ml. of saturated BaCl_2 was added and a back titration was carried out with 0.05N-HCl. Blanks in which the glucose was omitted were run for the same times in each case. All units were set up in duplicate or in triplicate.

In investigating the effect of the B-group of vitamins on the fermentation rate the following substances were incorporated in the 5% glucose solution (amounts in mg./100 ml.): pyridoxine hydrochloride 4, nicotinic acid 2, riboflavin 2, calcium pantothenate 2, thiamine 1, *p*-aminobenzoic acid 1, inositol 0.05 and biotin 0.0001. The procedure with this fermentation medium was the same as above.

Oxygen consumption. Oxygen consumptions were measured by the Warburg manometric method at 30° and 120 strokes/min. 3 ml. of a 1:100 suspension of yeast were used. The suspending medium had the following composition: 0.095% Na_2HPO_4 , 0.080% NaH_2PO_4 , 0.6% NaCl. The pH of the suspension was 6.63. No glucose was added.

RESULTS

The sodium and potassium content of the sodium-yeast preparations

The effects of seven fermentations (2 hr. periods) at room temperature in the sodium citrate medium on the Na and K content of a yeast sample are shown in Fig. 1. After one fermentation of 2 hr. the K

content of the yeast is reduced to about half its normal value and after the first few fermentations the rate of replacement of K by Na becomes greatly reduced. In this sample the sum of the Na and K contents of the original fresh yeast in this example was $5.2 + 133.7 = 138.9$ m-equiv./kg. wet yeast, the mean value for the sum of the two throughout being 140.7 m-equiv./kg., the figures ranging from 133.0 to 146.0, indicating very clearly that under these conditions there was an equivalent replacement of K^+ by Na^+ .

For somewhat different conditions as listed in Table 1 the gain in Na^+ may be appreciably greater than the loss of K^+ .

As has been mentioned under 'Methods', vigorous shaking at room temperature and a temperature of 37° were examined. The results were very similar but appear significant for the last traces of K remaining in the yeast. The raised temperature, or vigorous shaking at room temperature seem to reduce the K content more quickly to somewhat lower levels as shown in Table 1. Thus after thirteen to eighteen fermentations the mean K content under such conditions was 3.6 m-equiv./kg. of centrifuged yeast, whereas at room temperature with occasional stirring the mean value after twenty-one to twenty-two fermentations was 4.4 m-equiv./kg.

The effect of waxing the interior of the vessels. Under 'Methods' it was mentioned that waxing the interior of the vessels gave a greater K depletion of the yeast in the final stages. Table 2 shows the marked differences that result, indicating at the same time the effect of mere traces of K in the external medium.

Fig. 1. Changes in the K (x) and Na (o) contents of a yeast sample fermented for seven successive 2 hr. periods in 5% glucose + 0.2M sodium citrate. The dotted line represents the mean Na + K content throughout (+).

Table 1. *Sodium and potassium content of sodium-yeast preparations*

Preparation	No. of 2 hr. fermentation periods	Na content m-equiv./kg. wet yeast		K content m-equiv./kg. wet yeast	
		Before	After	Before	After
Room temperature, occasional stirring					
I	7	—	—	138.7	9.5
II	7	5.2	133.7	133.3	8.0
III	21	—	160.9	—	3.0
IV	22	—	140.5	—	4.1
V	21	0.6	137.9	143.3	6.0
Room temperature, vigorous shaking					
IX	6	2.4	198.5	144.7	22.5
X	16	2.4	184.9	144.7	4.4
XI	18	2.1	138.6	128.1	3.0
At 37° with occasional stirring					
VI	12	5.0	134.8	148.1	8.3
VII	13	1.3	96.4	168.0	4.0
VIII	15	—	131.9	162.8	3.0

Table 2. *The effect of coating the fermentation flasks and centrifuge cups with paraffin wax on the final potassium concentration in the yeast*

No. of fermentation periods	K content of the yeast in m-equiv./kg. wet yeast			
	Unwaxed	Waxed	Unwaxed	Waxed
0	122.7	—	154.1	—
7	23.5	25.2	22.0	11.4
8	21.1	—	21.4	—
9	19.2	16.0	17.3	—
10	19.0	14.7	17.6	5.8
11	15.1	—	—	—
12	13.9	—	15.2	—
13	12.8	5.7	16.0	—
15	—	—	—	3.6
21	—	—	—	3.0

Table 3. *Replacement of sodium of a sodium-yeast by potassium*

	m-equiv./kg. wet yeast	
	Na	K
I. Fresh yeast	2.1	128.1
II. Sodium-yeast prepared from I	141.6	4.1
III. After 2 hr. fermentation of sample II in potassium citrate solution	48.4	144.3
IV. Sample II after two 2 hr. fermentation periods	5.9	173.1

In the investigation of growth, fermentation and oxygen consumption rates described below, only samples of 'Na-yeast' with the lowest K levels were used.

Reversal of the process. If a 1:20 suspension of Na-yeast be allowed to ferment as described in the

Fig. 2. Growth curves of normal (●), sodium (×) and potassium (○) yeasts at 28° in a low-K medium. C = cells/cu.mm. (The count at zero time is taken as 100 per cu.mm.)

preparative method for 2 hr. in 5% (w/v) glucose + 0.4M potassium citrate, K is found to enter very rapidly, replacing the Na^+ . Two such fermentation periods are sufficient to restore the Na-K balance to normal levels. Results of such an experiment are given in Table 3.

Growth curves for the sodium-yeast

Growth experiments were carried out as described under Methods. Normal, Na- and K-yeasts were grown in the low-potassium medium already described, which had a potassium content of

0.55 mg./100 ml. derived from the added biotin solution which was available only in a phosphate buffer (British Drug Houses' preparation). It will be seen from Fig. 2 that the preparative method has of itself no effect on the growth rate of the yeast as the curves (log. (count) against time) for normal untreated yeast and for the K-yeast are identical. The growth of the Na-yeast is, however, much slower. It may be noted also that the maximum cell population attained is approximately the same in each case, but is approached more slowly in the case of Na-yeast.

In Fig. 3 an interesting observation is illustrated. When repeating such growth experiments with samples from another preparation, it was found that whereas the normal and K-yeast curves practically overlap, as before, the Na-yeast obviously did not begin to grow for more than 24 hr. and the final level was much lower than that of the other curves. Examination of the Na-yeast sample from which the inoculum was made in this case revealed that it was almost entirely in a spore form. No reason for this difference can be suggested as the yeast samples were in all cases prepared and stored under exactly the same conditions. The experiment described below would seem to indicate that during these 24 hr. the yeast was accumulating K^+ from the medium in which it was present in low concentration. Microscopic examination after 60 hr. growth failed to reveal any spore forms.

The effect of added potassium on growth of sodium-yeast. The Na yeast sample used in this experiment was the spore form mentioned above. The growth medium was the same as before, but for samples A and B potassium was added in amounts as indicated on Fig. 4. Curve C has been transferred from Fig. 3 for comparison. It is seen that in the presence of the added K the samples grow from the beginning, and with the higher K content a somewhat higher total cell population is finally reached.

Fermentation studies

A series of experiments on the rate of production of CO_2 by these yeasts is summarized in the graphs of Fig. 5. The Na-yeast and the control K-yeast give practically the same rate which is only about half of that of the normal untreated yeast. The rate for K-yeast is slightly higher than that for Na-yeast but by an amount so small as to be scarcely significant. In order to test the idea that this reduction in the fermentation rate following the preparative method used might be due to loss of coenzyme factors from the yeast, the fermentation studies were repeated incorporating vitamins of the B-complex in the medium as described under 'Methods'. No significant difference from the curves illustrated was observed.

Fig. 3. Growth curves of further samples of normal (●), sodium (×) and potassium (○) yeasts at 28° in a low-K medium from spore inoculum (see text).

Fig. 4. Effect of added K on the growth rates of Na-yeast. Curve A, growth in a medium containing 40.04 mg. K/100 ml.; curve B, 0.94 mg. K/100 ml.; curve C, 0.55 mg. K/100 ml. This last curve has been transferred from Fig. 3 for comparison. Temp. 28°.

Fig. 5. Fermentation rates measured by microdiffusion method for CO_2 evolution the small blank values being subtracted. A (3 expts.), normal yeast; B (4) K-yeast; and C (5) Na-yeast.

Table 4. *Summary of O₂ consumption experiments*

Sample no.	Oxygen uptake (μ l./hr./30 mg. centrifuged yeast)		
	Fresh yeast	Na-yeast	K-yeast
4	46.1	29.5	61.6
5	20.4	17.1	62.3
6	39.3	63.0	—
7	45.3	—	—
8	52.0	—	—
9	43.0	—	—
10	41.5	31.8	122.5
13	—	—	62.8
14a	—	10.6	99.9
14b	—	13.3	108.1
Mean	41.7 (7)	27.6 (6)	86.2 (6)
Mean dry wt. (mg.)	6.32	6.84	6.98
Mean Q _{O₂}	-6.5	-4.0	-12.35

Oxygen consumption rates

The oxygen consumptions of the resting yeast samples showed a linear relation with time and are summarized in Table 4. The rate for Na-yeast is only about two-thirds that of the rate for normal fresh yeast, while that for K-yeast is about twice the latter. The difference between the K- and Na-yeast rates is very striking, the former being more than 3 times the latter.

Physical characteristics

Samples of each yeast were examined microscopically. The K-yeast showed no visible difference from the normal fresh yeast. All the cells were clear, oval and of about the same size in each case, indicating that the preparative method, the repeated fermentations and washings and the effect of the high external concentration of the potassium citrate produced no apparent morphological changes when so examined. The Na-yeast cells, on the other hand, were more opaque to light and contained more clearly distinguishable granular material. They had a smaller average size and a much more irregular, almost angular, outline than in the other two samples.

Also, on centrifuging during the preparative stages the Na-yeast always packed more closely and occupied a smaller volume than the corresponding K-yeast being centrifuged at the same time. (In the various studies here described the amount of yeast refers to the centrifuged material. The average dry weights of 30 mg. of centrifuged Na-yeast and K-yeast were very similar, being 6.84 and 6.90 mg. respectively.)

It was also noticed that if samples of the two yeasts were left exposed to air, the Na-yeast dried much more quickly, becoming rapidly reduced to a tough glue-like consistency.

DISCUSSION

The fact that waxing the interior of the fermentation and centrifuging vessels allows of a very appreciable ultimate reduction of the K content of the Na-yeast (which could be finally reduced to about 3 m-equiv. K/kg. centrifuged yeast 98% of the cellular K⁺ being lost and replaced by Na⁺) suggests that rigorous purification of the sodium citrate from traces of K may give a greater K depletion.

Pending further possible reduction in the K values results of interest appear in the comparison between the Na-yeast and a preparation to which K⁺ is added and one treated in a similar manner but with potassium citrate instead of sodium citrate.

From the results it is evident that there is no appreciable difference in the fermentation rates when K is replaced by Na. There are, however, large differences in oxygen consumption and in growth rates and also in the physical characteristics of the Na-yeast. There are many observations in the literature concerning effects of K⁺, NH₄⁺ and Na⁺ on enzyme actions. Here the subject is confined to the yeast cell. Using quickly dried yeast, Meyerhof & Kaplan (1951) found for instance that Na⁺ alone is unable to allow fermentation but is slightly harmful if enough K⁺ and NH₄⁺ are present—an observation that does not apply to the living yeast cells investigated here.

Similar investigations were previously carried out on an ammonium-yeast in which practically all the K⁺ was replaced by NH₄⁺ (Conway & Breen, 1945). It is of interest to compare the results with the Na-yeast.

The substitution of K by NH₄ caused a marked decrease in growth rate, while fermentation rate was decreased to 40%. Also the substitution of K ions by NH₄⁺ does not decrease the oxygen consumption but markedly increases it.

It is true that in the Na-yeasts examined there is a small concentration of K⁺ present and this small concentration may be significant in action. But where large differences in oxygen consumption and growth rates exist, a further depletion of K ions may be expected only to increase such differences somewhat further. On the other hand, a further K depletion may show effects on fermentation rates which are not now apparent.

SUMMARY

1. A method is described for replacing up to 98% of the cellular potassium of yeast by sodium, thus forming a 'sodium-yeast' analogous to the ammonium-yeast previously described.

2. The process can be easily and rapidly reversed.

3. The 'sodium-yeast' grows in a medium containing as little as 0.55 mg. K/100 ml. but at a much slower rate than a control 'potassium-yeast'.

4. The fermentation rate does not differ significantly from that of a control 'potassium-yeast'.

5. The resting oxygen consumption rate is only 32% of that of the control 'potassium-yeast' and about 62% of that of a fresh yeast sample.

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