CCXXIII. THE INFLUENCE OF DIFFERENT CATIONS ON THE GROWTH OF YEAST CELLS.

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THE results of previous work, especially that of Loeb and other investigators on marine organisms, have shown the important influence which electrolytes exert on cell growth. Broadly speaking these researches indicate that the influence of the ions in physiological concentration agrees with their position in the Hofmeister series; in other words, it runs parallel with the influence that the ions probably exert on the imbibition of the cell colloids. Ions which favour imbibition, in general, aid growth.

The cations would seem to be of special importance; they apparently play a much more important part in natural cell processes than the anions. It has been frequently demonstrated that as regards potassium there is a direct relation between spontaneous cell growth and potassium content of the tissue. Thus researches on tumours have shown that potassium content and the intensity of cell growth, in general, run parallel. We are thus led to the conclusion that potassium is a natural activator of cell growth. Also it has been shown in recent investigations on animal tissue (chiefly tumours) that potassium increases energy metabolism [see Lasnitzki, 1934]. Energy metabolism (respiration and fermentation) must, however, be considered as the source of that energy which renders possible processes of growth. Thus we are forced to the conclusion that the influence of potassium on growth takes place, wholly or in part, through its influence on the energy metabolism of the growing cell.

While potassium is considered as a natural activator of cell growth its influence, in all its aspects, might be only a special example of a general rule. In this respect it may be asked, therefore, whether not only the growth but also the energy metabolism of the cell may not be subjected to such a general rule. One would expect that the various ions, provided they could enter the cell, would influence cell metabolism and growth in the same direction and to the same degree. We have undertaken comparative researches on this question, especially as regards the group of alkali cations of which the most important representative is potassium.

For investigations of this nature it is necessary to carry out quantitative experiments on growth and energy metabolism on one and the same material and under the best possible parallel conditions. For this purpose we found yeast to be the most suitable material. As regards energy metabolism we limited ourselves to measurements of the alcoholic fermentation. These investigations will, however, be published in a later communication. In the present paper we shall deal only

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with the amount of growth obtained. In the first place we investigated the influence of alkali cations: lithium, sodium, potassium, rubidium and caesium. In addition we also investigated the influence of magnesium and calcium.

Such investigations seemed to us to be necessary in view of the fact that, so far as we are aware, very little systematic research in this direction has been performed. Mitra [1917] investigated the influence of potassium, sodium, calcium and magnesium chloride on the growth of wine yeast. He found that all four salts in a weak concentration accelerated growth, although to a varying degree, while in a stronger concentration they, on the whole, hindered it, again to a varying degree. On mixing two salts there was frequently a definite antagonistic effect. Speakman *et al.* [1928] investigated the influence of sodium chloride on the growth of brewer's yeast. Mitra worked with a synthetic culture medium while these authors grew their yeast on wort. They found that the stronger the concentration of sodium chloride which was added the more was the growth of the yeast hindered.

Technique.

In our researches we used ordinary baker's yeast, and estimated growth volumetrically. We used as culture medium a synthetic medium of which the basis was "Laurent salt solution" [see Koch, 1922]. In view of the absence of specific growth factors vigorous growth could not be expected. The medium, which was fairly acid in reaction to inhibit the growth of bacteria, was prepared by adding together two stock solutions, the standard "suspension fluid" in which the yeast was homogeneously distributed and the variable "addition solution." All stock solutions were conserved sterile, separately on ice. For special details as to concentration *etc.* see later.

We proceeded as follows. A small, accurately weighed amount of fresh yeast was shaken for about 10 minutes with 30–40 ml. of suspension fluid so as to obtain a homogeneous cell suspension. This was centrifuged at a high speed for about five minutes, the supernatant fluid poured off and the solid material diluted with the suspension fluid so as to correspond to 15 mg. of fresh yeast per ml. This cell suspension was distributed, with continuous shaking, in tubes of the form shown in Fig. 1, 2 ml. being placed in each tube. The tube consists of an upper wide, and a lower narrow part, the latter having a capacity of a little over 0.1 ml. and being graduated into 20 parts of 5 μ l. each (not shown in figure). The distance between two graduated marks is great enough easily to allow a reading to 1 μ l. and possibly to 0.5 μ l. The yeast suspension is added in such a way that the under part of the tube is at first empty (Fig. 1, *a*). We then added to each tube 1 ml. of the addition solution¹, and as control 1 ml. of distilled water. Each tube was duplicated. The tubes were then closed with a cellulose plug and left at room temperature for 21 to 24 hours. A longer time than this was unnecessary since growth of the yeast certainly ceases before this. In order to avoid a large drop in temperature during the night the tubes were placed in a wide-necked thermos flask. Our temperature measurements in the whole of the experiments showed maximum oscillations between 20 and 25°.

Fig. 1.

¹ As a result of special tests it appears that there is no difference if the yeast cells are placed at once in contact with the cation to be tested, or (as in the present case) if the latter is only subsequently added to the yeast suspension.

At the end of the experiments a few drops of 40 % formaldehyde solution were added in order to avoid bubbles of gas from fermentation of the yeast, and the narrow under part of the tube was filled with fluid by removal of the air with a fine capillary tube. The tubes were then centrifuged at a high speed for five minutes, whereby the whole of the yeast was concentrated in the narrow part (Fig. 1, *b*). The volume of the yeast column was then immediately estimated, and the mean value of the two estimations taken. The difference between the two parallel estimations was, as a rule, between 0 and 3 μ l. Similarly the volume of yeast at the beginning of the experiment was estimated, with usually two control estimations.

Results.

1. Alkali cations.

The suspension fluid for these investigations had the following composition:

Diammonium hydrogen phosphate, $(\text{NH}_4)_2\text{HPO}_4$	...	0.1 g.
Magnesium sulphate, $\text{MgSO}_4, 7\text{H}_2\text{O}$		0.03 g.
Glucose		7.5 g. ¹
Asparagine		0.75 g. ¹
Tartaric acid		0.15 g.
Dist. water		<i>ad</i> 100 ml.

We utilised as addition solutions 0.03 *M* solutions of lithium, sodium, potassium, rubidium and caesium chlorides. The concentration of alkali chloride after the mixing in the proportion of 2 : 1 was 0.01 *M*, and the concentration of magnesium sulphate (MgSO_4) 0.008 *M*. The p_{H} of the control medium was about 3.1.

The results of all the experiments are given in Fig. 2; the results of individual experiments being also shown in Table I. It is seen that in the

Table I.

Experi- ment no.	Date (1932)	Duration in hours	Original volume of yeast in μ l.	Volume of yeast in μ l. at end of experiments					
				Control	Addition of				
					LiCl	NaCl	KCl	RbCl	CsCl
1	26. v	21	30	36	40	49	69	63	48
1 <i>a</i>	2. vi	21	(37)	(43)	—	—	(69)	—	—
2	9. vi	24	31	39	40	47	70	70	48
3	13. vi	22	29	30	37	54	68	68	48
4	16. vi	22	29	36	37	52	67	69	48
5	20. vi	23	31	39	38	55	69	66	47
6	23. vi	23	28	35	38	48	62	67	46
7	5. ix	22	26	33	31	40	59	53	38
8	7. ix	22	29	31	34	51	67	69	43
9	7. xi	23	26	36	36	53	67	58	43
10	9. xi	23	27	31	39	52	65	70	48
Mean			29	35	37	50	66	65	46
Mean increase				6	8	21	37	36	17

control nutrient solution there is only a slight increase of yeast cells. The increase is about 20 %. On the other hand we see that all five alkali chlorides favour growth. Individually, however, their actions are very different, and we can

¹ As was found in a preliminary experiment with addition of potassium chloride, doubling the concentration of glucose or asparagine had no influence on the result.

separate them into three groups. The first group is represented by lithium chloride, and here the influence, if any, is very slight. Compared with the control solution there is a doubtful increase of only about one-third. The second group, represented by caesium and sodium chlorides, has a medium influence. Caesium chloride gives an increase of about 60 %, and sodium chloride over 70 %. The yeast increases in the caesium solution almost three times and in the sodium solution about three and a half times as much as in the control solution. The third group, represented by rubidium and potassium chlorides, has the greatest influence. The yeast increase amounts in both to about 125 %. The increase is about six times as much as in the control solution.

Fig. 2. Original volume of the yeast: white.
Increase of volume: black.

Fig. 3. Original volume of the yeast: white.
Increase of volume: black.

As the chlorine ion is a common constituent of the addition solutions we must ascribe the difference in action to the influence of the alkali cations. We see that, as a whole, they favour the growth of the yeast, and that their actions correspond with their position in the Hofmeister series of cations. It may be remarked that on microscopical examination the yeast cultivated in the several different media shows no definite morphological differences.

In five experiments (Nos. 3-7) the influence of 0.01 *M* ammonium chloride was also investigated. The average values were:

At the beginning ...	...	...	29
At the end, control ...	...	...	35
At the end with addition of NH ₄ Cl ...	...	...	33

(The values found in order were: 31, 32, 36, 35, 31.)

The addition of ammonium chloride to the nutrient medium, already containing ammonium ions, had thus no growth-promoting influence.

2. Magnesium.

In these experiments the suspension fluid contained 0.015 *M* KCl and was magnesium-free. Otherwise the composition was similar to that of the previous experiments. The addition of magnesium was made as magnesium sulphate and magnesium chloride. Further parallel estimations with the addition of sodium

and ammonium sulphates were made in order to test the influence of the SO_4 ions. The concentration of the addition solutions was 0.024 M in 9 experiments, while in 4 additional experiments performed previously it was $2\frac{1}{2}$ times greater. On mixing in the proportion of 2 : 1 the concentration of the salts added was 0.008 and 0.02 M respectively; and the concentration of potassium chloride, as in the previous experiments, 0.01 M . The p_{H} of the control solution was about 3.4.

Table II.

Experi- ment no.	Date (1932)	Duration in hours	Original volume of yeast in μl .	Volume of yeast in μl . at end of experiments				
				Control	Addition of			
					MgSO_4	MgCl_2	Na_2SO_4 0.008 M	$(\text{NH}_4)_2\text{SO}_4$
1	13. x	22	31	46	53	50	48	45
2	17. x	22	30	43	59	53	44	40
3	24. x	22	31	48	71	58	54	56
4	26. x	23	28	43	71	56	41	41
5	1. xi	23	30	40	72	53	41	37
6	11. xi	23	31	45	70	59	46	47
7	18. xi	23	31	52	66	54	—	52
8	28. xi	21	30	46	58	53	44	—
9	6. xii	21	29	42	73	53	43	—
Mean			30	45	66	54	45	45
Mean increase				15	36	24	15	15
0.02 M								
10	10. v	24	32	47	63	53	49	—
11	20. v	24	31	54	64	58	50	—
12	25. vii	22	32	51	76	58	49	50
13	29. vii	22	33	49	69	54	44	48
Mean			32	50	68	56	48	49
Mean increase				18	36	24	16	17

The total results of the 9 experiments with the weak concentration of added salts are given in Fig. 3, the results of individual experiments being shown in Table II, Nos. 1–9. In the control solution the increase in yeast was 50 %. The addition of MgSO_4 gave an increase of 120 %. This figure corresponds with that of the previous experiments with the same medium (addition of potassium), while the larger figure obtained in the control solution shows that the presence of potassium chloride alone in the medium is more effective than the presence of magnesium sulphate alone. If magnesium sulphate is replaced by magnesium chloride growth-promotion is less. The yeast increase amounts to only 80 %. If magnesium sulphate is replaced by sodium or ammonium sulphate growth is similar to that in the control solution.

In the 4 other experiments with the $2\frac{1}{2}$ times increase in the concentration of the salts we obtained about the same figures (Table II, Nos. 10–13). Therefore it appears that the actions of both magnesium salts are within wide limits independent of their concentrations, and also that sodium and ammonium sulphates remain inactive even when the concentration is augmented.

That magnesium sulphate increases growth more than magnesium chloride cannot be ascribed to a growth-promoting action of the SO_4 ions, in view of the inactivity of sodium and ammonium sulphates. Also a reversal action, which might occur with the increase of Cl ion concentration from 0.01 to 0.026 g.-ion/l., probably does not enter into the question because the further increase to

0.05 g.-ion/l. is without influence¹. Therefore it probably depends upon the SO_4 ions favouring the action of the magnesium without themselves exerting any influence on growth. As a possible explanation of this action, it may be that the ability of the yeast to absorb the magnesium depends upon the nature of the accompanying anions. If this is so SO_4 ions are more favourable to the absorption of magnesium than are Cl ions.

3. Calcium.

In Exps. Nos. 1-7, Table II, we also investigated the influence of 0.008 *M* calcium chloride, in order to test if, and in what degree, magnesium can be replaced in its growth-promoting activity by calcium. The average figures were:

At the beginning ...	...	...	...	30
At the end, control ...	...	...	...	45
At the end with addition of MgCl_2 ...	...	...	...	55
At the end with addition of CaCl_2 ...	...	...	...	47

(The values found in order were: 47, 45, 53, 46, 51, 44, 46.)

From these results we must conclude that magnesium is not, or at the most only to a slight degree, replaceable by calcium. How far the difference in the action of these two bivalent cations is related to their positions in the Hofmeister series must meanwhile remain an open question.

SUMMARY.

The influence of different cations on the growth of baker's yeast has been investigated. The yeast was cultivated in a synthetic medium. For the investigation of the alkali cations it contained magnesium sulphate as a constant constituent. All alkali cations favour growth of the yeast. They differ among themselves, however, their actions agreeing with their positions in the Hofmeister series. Lithium increases growth very slightly, if at all, potassium and rubidium strongly, while sodium and caesium have an intermediate effect. Magnesium also exerts a growth-promoting influence, these investigations being carried out in a medium containing potassium. Magnesium sulphate acts more strongly than magnesium chloride, although this difference in all probability cannot be ascribed to a direct influence of the anions. Magnesium cannot, or at the most can only to a slight degree, be replaced by calcium.

¹ This does not exclude the possibility that addition of Cl ions as such might have a slight growth-hindering influence (see the experiments with ammonium chloride).

REFERENCES.

- Koch (1922). *Mikrobiologisches Praktikum*. (J. Springer, Berlin.)
 Lasnitzki (1934). *Protoplasma*. (In press.)
 Mitra (1917). *Univ. California Pub. Agric. Sci.* **3**, No. 5, 63.
 Speakman, Gee and Luck (1928). *J. Bacteriol.* **15**, 319.