LXVIII. THE INFLUENCE OF ALKALI CATIONS ON THE FERMENTATION CAPACITY OF YEAST.

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(*Received December 30th, 1934.*)

In a recently published communication [Lasnitzki and Szörényi, 1934] experiments on the influence of different cations on the growth of yeast cells were described. They showed that growth, under the experimental conditions used, was greatly increased by potassium and rubidium, and very little, if at all, by lithium, while sodium and caesium had an intermediate action. The influence of these five alkali cations thus agreed with their position in the Hofmeister series. A growth-promoting influence was also found for magnesium, while calcium, under the same conditions, had hardly any action.

We have been occupied with a second parallel series of investigations on the influence of alkali cations on the fermentation capacity of yeast. The object of these researches was to find out whether the energy metabolism of yeast cells, which must be considered as a necessary condition of growth, is increased by alkali cations and whether a similar order of action may be found in this case as in the activation of growth. It is these investigations which form the subject of this paper.

The influence of alkali salts on alcoholic fermentation has, like their influence on growth of yeast, up to the present time not been very systematically investigated. Harden [1917] found when using washed acetone-yeast and dried yeast that the activation of glucose fermentation in a medium containing phosphate by pyruvic acid or acetaldehyde only took place in the presence of potassium (or ammonium) and not at all or at the most to a slight degree in the presence of sodium. On the other hand Harden and Henley [1921], in confirmation of corresponding investigations of Meyerhof [1918], found that a surplus of potassium phosphate in the medium inhibited the fermentation of glucose and fructose by (unwashed) acetone-yeast or yeast-juice, and that other alkali salts (sodium and potassium sulphate, sodium and potassium chloride) in a high concentration acted in a similar manner. There is a doubt, however, whether the effects obtained can be ascribed to the cations. Contrary to the above authors the following workers used living yeast cells. Boas [1921] tested the action of lithium, sodium, potassium and ammonium nitrates (0·1 and 0·2 *M*) on the fermentation of sucrose by a suspension of brewer's yeast in pure water. The addition of the salts, with the exception of lithium nitrate, led to an increase of fermentation intensity, which, however, as a rule was near the limit of experimental error, and in no case was the increase more than 30 %. Also the difference in the action of the individual alkali salts was but little evident. Speakman *et al.* [1928] investigated the influence of sodium chloride on fermentation by brewer's yeast and found that weak concentrations somewhat accelerated whilst stronger concentrations inhibited. Since, however, the yeast cells under the experimental conditions used (wort as medium, and long duration of experiments) grew considerably the results were evidently not very clear. Finally Lindahl [1933; 1934], who worked

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under conditions similar to those of Boas, found that the fermentation of glucose by brewer's yeast was inhibited by lithium chloride, the amount of this inhibition being increased as the concentration of the salt was augmented, while the addition of potassium at the same time to a certain extent modified this inhibition. These results agreed with the findings in corresponding experiments upon the respiration of fertilised eggs of sea-urchins. Lindahl further stated that the oxidation of glucose and the dephosphorylation of hexosediphosphoric acid was inhibited by lithium chloride while the oxidation of glycerophosphoric acid and ethyl alcohol was accelerated.

Methods.

Alcoholic fermentation was measured by estimating the amount of carbon dioxide developed, Warburg's [1926] manometric method being used. The culture medium was similar to that used in our growth experiments, but in order to exclude the effect of respiration, fermentation was carried out under anaerobic conditions.

We proceeded as follows. 10 to 30 mg. of fresh baker's yeast was accurately weighed and shaken for about 10 minutes with 10 to 20 ml. of "suspension fluid". The suspension fluid had a similar composition to that used in the growth experiments. 0·1 g. diammonium hydrogen phosphate, 0·03 g. magnesium sulphate ($MgSO_4$, $7H_2O$), 7·5 g. glucose, 0·75 g. asparagine, 0·15 g. tartaric acid, distilled water to 100 ml. The homogeneous cell suspension was centrifuged at a high speed for 5 minutes, the supernatant fluid poured off and the sediment diluted with suspension fluid so that the quantity of cells per ml. corresponded to 0·5–1·5 (usually 1·0) mg. of fresh yeast. 2 ml. of the suspension (kept constantly shaken) were measured into medium sized metabolism troughs (in one experiment 1 ml. in smaller troughs also). 1 ml. of "addition solution" (or 0·5 ml. respectively) was then added, and as control the same volume of distilled water. The addition solutions were 0·03 *M* solutions of lithium, sodium, potassium, rubidium and caesium chlorides. Having mixed these with the cell suspension in the proportion of 2:1, we had as in the growth experiments an alkali chloride concentration of 0·01 *M*. As regards the hydrogen ion concentration (p_H of the control medium about 3·1), it was probably near the upper limit of the zone which allows an optimum fermentation by living yeast. Before beginning the experiment the medium was saturated with nitrogen. The temperature employed was as a rule 21°, but in some cases a little higher. The readings were mostly made at intervals of 15 minutes. The time between the addition of the alkali chlorides and the first reading was about 1 to 1½ hours.

The experimental conditions in the fermentation experiments differed in three respects from those of the growth experiments: (1) much smaller quantity of cells; (2) shorter duration of experiments; (3) anaerobic conditions instead of the aerobic (or partial anaerobic) conditions in the growth experiments.

Results.

Our results are expressed by the "fermentation quotient" which is defined as

$$Q_{CO_2} = \frac{\text{carbon dioxide developed, in } \mu\text{l (0°, 760 mm. Hg)}}{\text{mg. yeast}^1 \times \text{time (in hours)}}.$$

The quotient is calculated to within 0·5.

The individual experiments which will now be considered are divided into three groups. In Exps. 1 to 3 (Table I) the influence of sodium and potassium

[1] Here as fresh yeast.

Table I.

Exp. no.	Date (1932)	Temperature	Duration (mins.)	Q_{CO_2} Control	Q_{CO_2} Addition of NaCl	Q_{CO_2} Addition of KCl	Time interval (mins.)
1.	9. v	25°	40	32·5*	46·5	84·5*	20–40
2.	29. vi	21°	75	31·0	58·5	75·0	30–75
3.	11. vii	21°	135	23·5	43·5	55·0	75–135

* Mean of two parallel estimations.

chlorides, acting for varying time periods, was examined. The fermentation intensity tended to alter during the observation time. With the exception of one control estimation of Exp. 1, in which there was a small decrease, this change consisted of a more or less distinct increase, apparently up to constant values. For comparison we will therefore consider, where possible, only those time intervals in which the fermentation intensity in all parts of the experiment was practically constant. It is to be noted that, compared with the figures of the corresponding control estimations, the addition of sodium chloride to the medium gives a definite increase of the fermentation intensity, while the addition of potassium chloride gives a still higher increase.

In Exps. 4 to 8 (Table II), again for varying time periods, the actions of sodium, potassium, rubidium and caesium chlorides were compared. Here the fermentation intensity as a rule increased during the observation time, but it appeared that the addition of potassium or rubidium chloride tended to bring it more rapidly nearer to a constant value. In one of the control estimations of Exp. 4 only did the fermentation intensity decrease until it gradually approximated to the value of the other control. For comparison those periods in which the fermentation intensity was not sufficiently constant in all parts of the experiment are again excluded. We see that all four alkali chlorides increase the fermentation intensity. Potassium and rubidium chlorides have always the same effect, and

Table II.

Exp. no.	Date (1932)	Temperature	Duration (mins.)	Q_{CO_2} Control	Q_{CO_2} Addition of NaCl	KCl	RbCl	CsCl	Time interval (mins.)
4.	28. v	25°	90	28·5*	61·0	79·0*	83·5	33·5	60–90
5.	8. vi	21°	120	12·0	28·5	35·0	36·0	36·0	60–120
6.	9. vi	21°	240	28·0	48·0	60·0	62·5	57·5	195–240
7.	20. vi	21°	180	15·0	48·0	79·0	73·5	32·5	135–180
8.	18. vii	24°	120	35·0	56·5	69·5	73·0	66·0	60–120

* Mean of two parallel estimations.

that of sodium chloride is always weaker than either. The position of caesium chloride is, on the other hand, variable. While in three experiments its influence is as strong, or nearly as strong, as that of potassium and rubidium chlorides, in two experiments it is weaker than that of sodium chloride. In one of these the increase obtained is near, if not within, the experimental error.

Lastly in Exps. 9 to 11 (Table III) we investigated the influence of lithium chloride, comparing it with that of potassium chloride. In Exps. 9 and 10 we also again investigated the effect of caesium chloride and in 11 that of sodium chloride. In Exp. 11 we tested in addition the effect of ammonium chloride. It may be noted that there was a striking decrease of the fermentation intensity

in one control estimation of Exp. 9. Gradually the values became comparable with those of the other control in which there was only a slight decrease. Otherwise the fermentation intensity tended to increase or, notably in the tests with potassium chloride, to remain fairly constant. For comparison we have again selected only the time intervals above specified. As we see, the action of lithium chloride is in two experiments within the experimental error. The same can be said as regards ammonium chloride. In the third experiment with lithium chloride the effect is larger, due to the low value of the corresponding control;

Table III.

Exp. no.	Date (1932)	Temperature	Duration (mins.)	Q_{CO_2} Control	Addition of NH_4Cl	LiCl	NaCl	KCl	CsCl	Time interval (mins.)
9.	2. vi	21°	195	13·5*	—	23·5	—	42·0	43·5	135–195
10.	3. vi	21°	165	28·0*	—	29·0	—	47·5	52·5	90–165
11.	13. vi	21°	135	26·0	29·0	28·0	42·5	65·5	—	75–135

* Mean of two parallel estimations.

nevertheless the position of lithium chloride relatively to potassium chloride remains unaltered. Caesium chloride acts similarly to, or a little more strongly than, the latter. Sodium chloride stands in Exp. 11 approximately between lithium and potassium chloride. This is also shown especially clearly in Fig. 1, in which the whole course of fermentation in the individual tests of this experiment (with the exception of that with ammonium chloride) is given.

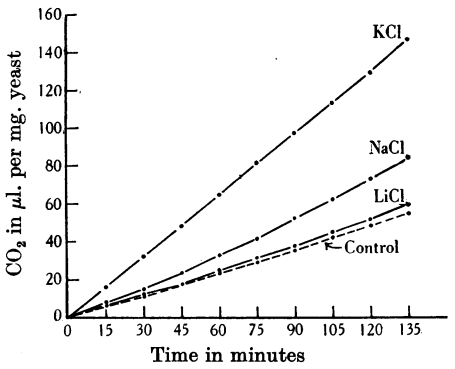

Fig. 1. Influence of 0·01 *M* lithium, sodium and potassium chlorides on fermentation of baker's yeast at 21°.

The observation that the fermentation intensity tended to increase during the course of experiments, together with the fact that in media containing potassium or rubidium chloride it was either fairly constant or showed a comparatively slight rise, indicates that when not completely activated the induction period of the fermentation was delayed, the time utilised in preparing the experiment having been insufficient to enable the fermentation intensity to reach a constant value. On the other hand the fermentation decrease, sometimes observed in the control, requires a different explanation.

The mean increase in fermentation intensity by the addition of potassium chloride, the effect of which was tested in eleven experiments, was about 150 %. In order to find the mean position in which the other alkali chlorides stand in

relation to potassium chloride we cannot compare the mean values one with another, in view of the very unequal number of estimations made. For this purpose we have chosen rather the "relative mean increase" which we may define as

$$\frac{\text{mean increase by alkali chloride}}{\text{mean increase in the corresponding tests with potassium chloride}} \times 150.$$

We, in this way, obtain the following figures:

Relative mean increase in fermentation intensity by

LiCl	NaCl	RbCl	CsCl
about 20 %	about 80 %	about 150 %	about 100 %

While rubidium chloride acts as strongly as potassium chloride the mean action of sodium and caesium chlorides is only about one-half to two-thirds as large. Lithium chloride acts very feebly, if at all. It is evident that these differences must be ascribed to the influence of the cations, the chlorine ion being a common constituent. As regards the individual figures there was considerable variation in the increase of fermentation by the addition of each alkali chloride, but meanwhile we are unable to give a definite explanation for this. However large the variation may be, it will be noted that there is in each individual experiment a similar order of action of the alkali chlorides as shown by the relative mean values. The only exception to this rule is caesium chloride.

Discussion.

The object of our researches was to show whether alkali cations can augment the fermentation capacity of yeast and whether there is a parallelism between the influence on fermentation and that on growth. We have seen that our results confirmed our anticipations. The potassium ion greatly increases fermentation. Rubidium acts similarly, while lithium, on the contrary, either only increases it a little or not at all. Sodium and caesium have intermediate effects. Thus it is found that the order of action is similar both for growth and fermentation, and this supports strongly the theory that increase of growth occurs as a result of an acceleration of energy metabolism. Complementary researches on the influence upon respiration appear therefore to be necessary. One must be guarded, however, about drawing conclusions as regards caesium. Its mean effect on fermentation is slightly stronger than that of sodium while the relation is rather the reverse as regards the amount of growth activation. On the other hand caesium was the only alkali cation of which the relations to potassium were not similar in all fermentation experiments. Further investigations are needed in order to explain the irregular action of caesium.

Among the five alkali cations tested potassium is probably the only one which is of importance for the life of the yeast cell under normal conditions, the sodium content of yeast being apparently very small. Potassium may thus be considered as a natural activator of yeast fermentation (and very likely of the whole energy metabolism). In view of this fact the very important question arises as to whether the observed activation of fermentation is in effect the result of the addition of different alkali cations to the potassium originally present in the yeast cell, or whether the original potassium content was lessened by our preliminary manipulation, and the subsequent addition of alkali cations compensated in a varying degree for this deficit. A satisfactory answer to this question can only be given by making ash analyses. Meanwhile we may be permitted to draw attention to the following point. The ordinary method of preparing fermentation experiments consists in washing the yeast with distilled or tap water in order to free it from

adhering substances. On electrostatic grounds, under this condition a large outward diffusion of potassium is only possible if the surface of the yeast cell is permeable to cations and anions to a similar degree. That this is not so, and that the potassium content of yeast is therefore only slightly or not at all lessened has been shown by Genaud [1929]. He found that the potassium and likewise the chlorine content of wine and baker's yeast was hardly changed by washing with distilled water. If the yeast cells were, however, washed in 0·01 *M* ammonium chloride solution (with a p_H of 7·0) potassium and calcium ions came out of the yeast into the medium from which an approximately equivalent quantity of ammonium ions disappeared. The chlorine content of the yeast here also remained approximately constant.[1] Thus it is probable that the surface of the yeast cell is permeable with ease to cations but with difficulty to anions. The outward diffusion of cations must therefore be more rapid in an electrolytic medium than in the practically electrolyte-free water, for in the former an interchange of the cations takes place. But, in our investigations the yeast was washed with a relatively large amount of suspension fluid, rich in ammonium and hydrogen ions. The condition for a more rapid outward diffusion of potassium was thus present, and it is probably for this reason that we obtained, by the subsequent addition of alkali cations, a significantly greater increase of fermentation intensity than had Boas who, before commencing his experiments, only washed his yeast in distilled water.[2] Meanwhile it is questionable whether, with a single and short washing as carried out by us, there is a very significant diminution in the potassium content of the yeast. It is important that this point should be investigated, and especially also to find out whether any fermentation at all is possible in the complete or almost complete absence of potassium.

When we consider that the potassium content of yeast cells suspended in water is relatively high and that it can only be diminished by the addition of another kind of cation we must assume that the inhibitory effect of lithium, which Lindahl observed in his researches, was indirect. It may be that a certain part of the intracellular potassium is replaced by the almost inert lithium. It remains for further researches to show whether this conception is correct, and whether in addition to the indirect action there may not be a direct inhibiting influence of lithium, at least when in a high concentration.

If, as appears probable, potassium is a natural activator of alcoholic fermentation it will be interesting to try and find out at what stage of the glucose disintegration the potassium works. This can be done by the investigation of partial reactions; lines formerly worked along by Harden, and recently also by Lindahl.

Appendix: The influence of magnesium.

We also investigated the influence of magnesium in three further experiments, using methods similar to those already described. The suspension fluid contained, as in the corresponding growth experiments, 0·015 *M* potassium chloride, and was free from magnesium. Also, as before, magnesium was added as magnesium sulphate and magnesium chloride. Further parallel estimations were made with the addition of sodium sulphate in order to test the influence of the SO_4 ions. The

[1] Similar results were obtained with magnesium by Lohmann [1931] and by Euler and his collaborators [1931] during the course of their experiments on the activation of alcoholic fermentation by magnesium. These authors, however, worked with dried yeast.

[2] However, in comparing our results with those of Boas we must also remember the differences in the conditions of the media during the fermentation experiments themselves, especially the higher concentration of the salts added by him.

concentration of the addition solutions was 0·06 *M*. After mixing with the yeast suspension in the proportion of 2:1 the concentration of the added salts was therefore 0·02 *M*, $2\frac{1}{2}$ times as high as the concentration of magnesium sulphate in the former experiments. The concentration of potassium chloride was as before 0·01 *M*. In two experiments each estimation was duplicated.

Of the three experiments, the duration of which was between 60 and 120 min., two showed no influence of magnesium. In the third there was an increase in fermentation intensity of about 30 % for both magnesium salts, while sodium sulphate was without influence. Thus it appears that in the given conditions magnesium exerts little or no action on fermentation.

According to the researches of Lohmann [1931] and Euler *et al.* [1931] magnesium is an activator of alcoholic fermentation as well as of lactic acid formation in the animal tissue. It represents the inorganic part of the "co-enzyme system". Thus we cannot conclude from our result that the magnesium ion is without essential importance for the fermentation capacity of yeast. The best explanation for the indifference of magnesium in our experiments would appear to be that the intracellular magnesium was not sufficiently washed away by our technique, so that even in the control medium the activating amount of magnesium was at a maximum. The fact that magnesium ions, in consequence of their lower mobility, diffuse more slowly out of the cells than do potassium ions may account for this. On the other hand there is in our investigations the remarkable difference between the lack of dependence of the fermentation on the presence of magnesium and the activating effect of the latter in the growth experiments made under similar conditions as to media. Perhaps this difference can be ascribed chiefly to a subsequent outward diffusion of a large amount of magnesium, due to the longer duration of the growth experiments, in spite of the fact that otherwise the conditions for diffusion were less favourable.

Summary.

In the present investigations we have tested the influence of alkali cations on the alcoholic fermentation of baker's yeast. The fermentation was carried out under anaerobic conditions. Our results show that the alkali cations on the whole facilitate fermentation. Individually, however, their actions are very different, as was the case in the growth experiments previously described. For instance, with potassium chloride there is a mean increase of about 150 %. Rubidium acts as strongly as potassium. On the contrary the effect of lithium is slight or zero. Sodium stands between lithium and potassium while the position of caesium is somewhat doubtful. Although in five experiments its influence was about as strong as that of potassium, in two experiments it was perceptibly weaker than that of sodium. If one leaves caesium out of account for the moment, the results of both series of investigations show a clear parallelism between growth and fermentation activation. This parallelism indicates that the activation of growth is to be attributed to an increase of the fermentation capacity (or very likely of the whole energy metabolism) of the yeast cell. In some experiments (with the addition of potassium to the medium) the influence of magnesium was also tested. Its action proved to be very slight or zero. This is explained on the grounds that the intracellular magnesium was not sufficiently washed away during the preparation of the yeast.

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