LXXV. THE SALT EFFECT ON THE INDUCTION PERIOD IN THE FERMENTATION BY DRIED YEAST.

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THE effect of a number of substances on the period of induction during which no fermentation is observed with the mixture of sugar solution and zymase preparations has been observed by many authors. Meyerhof [1918] pointed out that with maceration extract this period was completely abolished in the presence of even a small amount of hexosediphosphate. This was tested first by Harden and Henley [1920] with zymin and later by Mayer [1927] with maceration extract. Raymond and Levene [1928] found that not only natural hexosephosphates (hexosediphosphate, Robison's and Neuberg's monophosphates) but also synthetic phosphates (3-glucose-, 1-fructose-, 3-fructose- and dihydroxyacetone-phosphates) had a marked effect in reducing the period of induction with zymin in large volumes of sugar solution, although hexosediphosphate greatly exceeded all the others.

Neuberg [1918] pointed out a remarkable effect of a number of aldehydes and other hydrogen acceptors in accelerating fermentation with maceration extract. These observations were confirmed by Harden and Henley [1920 and 1921, 1] with zymin.

Recently Harden [1925], and subsequently Harden and Macfarlane [1928], made the interesting observation that the period of induction in fermentation by zymin in large volumes of sugar solution was appreciably reduced by the addition of various inorganic and organic salts.

In the present paper the effect of inorganic and organic salts on the period of induction in the fermentation by dried yeast (prepared from the bottom type beer yeast in Japan) in the presence of phosphate has been studied in order to ascertain which salt would be more potent in reducing the period of induction, and what influence would be exerted on the period of induction when a mixture of salts is employed.

METHODS AND RESULTS.

The apparatus described by Harden, Thompson and Young [1910] was used. The fermentations were carried out at 25° by a suitable amount of dried yeast (about 0.8 g.) with 20 cc. of the various salt solutions containing

2 g. glucose, Na_2HPO_4 (0.04 *M*) and 0.2 cc. toluene, after saturation with CO_2 . The amount of CO_2 evolved was observed at intervals of 10 minutes. The p_{H} values of the mixtures after saturation with CO_2 were determined colorimetrically in the same manner as was described by Billmann and Katagiri [1927]. The p_{H} values were found to be 6.3, and no difference in p_{H} on the addition of the salts could be detected.

For the period of induction, the intersecting point of the two tangents to the curves of normal fermentation and of the initial slow fermentation was chosen as shown in Fig. 1.

Fig. 1.

In order to allow for variation in the period of induction according to the sample of dried yeast, a fermentation was always carried out in the presence of 0.04 *M* phosphate without any other salt. The standard induction period under these circumstances was taken as 100 minutes. When the observed period had not greatly differed from this, the ratio of the two numbers was found and used as a factor to correct all the observations of the series to the standard.

When the sample of dried yeast had a considerably different fermentative power, the amount of dried yeast taken in each series was determined by a preliminary test so as to obtain nearly the same standard induction period (100 minutes). This amount of dried yeast varied from 0.75 to 1.0 g.

In order to test the validity of this method a series of experiments was made with 0.75, 0.8 and 1.0 g. of very different samples of dried yeast (Table I, Exps. 1, 2 and 3).

Table I.

Concentration of Na acetate (<i>M</i>)	Induction period (minutes)			Relative induction period		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
0	73	90	108	100	100	100
0.025	81	100	123	111	111	114
0.05	93	120	128	128	133	119
0.1	95	127	139	130	141	129

It will be seen that the relative values agree well, although the actual values were, in each case, considerably different.

The results of the experiments which are given in Tables I–VI show the average values of more than two duplicated observations. No remarkable difference in the rate of evolution of CO_2 during the rapid fermentation, in each series of experiments, could be detected. However, when still higher concentrations of the salts than the maximum concentration shown in the tables were employed, a reduced rate of fermentation was always observed, as well as a longer period of induction, as was expected from the observations of Harden and Henley [1921, 2].

EFFECT OF SODIUM SALTS.

(a) *Organic salts.* Table II (A) shows the effect of the salts of fatty monobasic and hydroxy-acids.

Table II (A). *Relative induction period with monobasic acids.*

Concentration of Na salt (<i>M</i>) ...	0.025	0.0375	0.05	0.075	0.1	0.2
Formate	98	—	96	—	105	147
Acetate	114	—	119	—	129	178
Propionate	112	123	138	207	>250	—
Butyrate	104	—	121	—	>180	—
<i>iso</i> Butyrate	94	—	115	—	136	—
Glycollate	181	—	196	—	>260	—
Lactate	116	—	150	—	215	—
β -Hydroxybutyrate	102	—	114	—	186	—

It will be seen that the period of induction is prolonged by the addition of these salts and the effect is more potent as the concentration of the salts increases, except formate and *isobutyrate*; with these a slightly reduced period is observed at lower concentrations. However, the observed effects are not the same: among the simple fatty acids formate has the least, while propionate has the greatest effect on the period of induction.

The effect of the hydroxy-acids is in each case greater than that of the corresponding fatty acid. The order of the potency of the effect of hydroxy-acids is inversely proportional to their molecular weights, while in the case of fatty acids no such simple relation could be observed.

Table II (B). *Relative induction period with polybasic acids.*

Concentration of Na salt (<i>M</i>) ...	0.00625	0.0125	0.025	0.05
Succinate	118	166	198	>290
Fumarate	119	182	>280	—
Malate	128	153	>230	—
Tartrate	155	200	>280	—
Citrate	270	>300	—	—

It will be seen in Table II (B) that the effect of succinate, fumarate, malate, tartrate and citrate is in each case greater as the concentration increases. The shortest induction is observed with succinate and the longest with citrate. Fumarate, malate and tartrate produce nearly equal effects.

The hydroxy-acids are more potent than the corresponding fatty acids, and the di- and tri-basic acids have a greater effect than the monobasic acids

in prolonging the induction with dried yeast in presence of 0.04 *M* sodium phosphate and sugar. This variation in the magnitude of the effect on the induction period is probably due to the specific nature of the salts, that is, to the nature of their ions.

(b) *Inorganic salts.* In order to ascertain the effect of inorganic anions, experiments were carried out with NaCl, NaI, NaHCO₃, Na₂SO₄. The results are given in Table III.

Table III. *Relative induction period.*

Concentration of salt (<i>M</i>) ...	0.00625	0.0125	0.025	0.05	0.1	0.2
NaCl	97	100	109	140	272	>300
NaI	136	>215	—	—	—	—
NaHCO ₃	123	134	138	213	>310	—
Na ₂ SO ₄	114	124	131	190	>250	—

It will be seen that these salts produce an effect similar to that of the Na salts of organic acids. The period of induction is again prolonged by the addition of these salts, and the effect is greater as the concentration of these salts increases. The only exception is NaCl, with which a slightly reduced induction period is observed at the lowest concentration.

When the relative induction periods are plotted against the concentration of Na⁺ of the salts added, the curves in Fig. 2 show the relative potency of the effect of their anions on the period of induction.

Fig. 2.

It will be seen that the iodide ion produces the greatest, and the formate ion the least prolongation of the period of induction.

EFFECT OF K, MG, NH₄ AND CA SALTS.

In order to investigate the effect of cations and to obtain further evidence of the specific effect of anions on the period of induction, experiments were made with various salts (Table IV).

Table IV. *Relative induction period.*

Concentration of salt (<i>M</i>) ...	0-00625	0-0125	0-025	0-05	0-1	0-2
KCl	88	90	82	89	110	177*
KI	109	104	177	>220	—	—
KBr	64	38	34	30	26	48
KNO ₃	110	109	133	150	207*	>250
K ₂ SO ₄	67	47	41	35	48*	84*
MgCl ₂	—	85	81	73	91	213*
MgSO ₄	—	67	47	29	25	37*
CaCl ₂	—	102	110	>210	—	—
NH ₄ Cl	89	66	58	60	61	106
(NH ₄) ₂ SO ₄	15	19	19	23	32*	59*
NH ₄ acetate	22	17	13	14	14	13
NH ₄ lactate	32	22	15	15	14	22
NH ₄ succinate	22	20	19	27	32	44*

* Represents the point at which a noticeably diminished fermentation rate is observed.

It will be seen that KI and KNO₃ produce an effect very similar to that of NaI; no reduction in the period of induction is observed at any concentration of these salts and the period is prolonged as their concentration increases. However, with KCl a reduced period is observed at the concentrations of 0-05–0-00625 *M*; the period of induction is gradually reduced until the concentration reaches 0-025 *M* and is then prolonged as the concentration increases. K₂SO₄ is more effective and KBr still more effective than KCl in reducing the period of induction. With these two salts the period is at first very rapidly, then very slowly reduced until the concentration reaches the optimum, after which the period is gradually prolonged as the concentration of these salts increases.

With both magnesium and ammonium salts (chloride and sulphate) a shorter period of induction is observed when these are compared with the corresponding potassium salts, and ammonium salts are more effective than magnesium salts in reducing the period. Among all the salts, CaCl₂ produces the greatest effect in prolonging the period of induction.

These observations show on one hand the specific effect of cations, and on the other that of anions. It is clearly seen, for example, in Fig. 3 in which the relative induction periods are plotted against the concentration of chloride ion, that the calcium ion produces the longest and the ammonium ion the shortest induction period. $\frac{1}{2}$ Ca⁺⁺ > Na⁺ > K⁺ > $\frac{1}{2}$ Mg⁺⁺ > NH₄⁺.

Tables II, III and IV show that this order does not differ greatly when the sulphates are compared, and organic NH₄ salts (acetate, lactate and succinate) are much more effective than Na salts in reducing the period of induction.

The interpretation of this remarkable effect of ammonium salts is not very clear, but it is interesting to note that no specific effect of ammonium ion, as compared with sodium ion, is observed on the rate of normal fermentation of English living yeast [Katagiri, 1926].

As regards the specific effect of anions, it follows from the results in

Tables III and IV that the iodide ion produces the longest and the bromide ion the shortest induction period, the order of the anions being as shown below:

No direct comparison of these results with those of Harden and Macfarlane [1928] is possible, since the conditions of the experiment were different from theirs, dried yeast being used instead of zymin and an addition of 0.04 *M* phosphate being made. However, the results obtained with KCl, NH_4Cl , K_2SO_4 and MgSO_4 coincide with their observations that the induction period is reduced from 60 to 20 minutes in the presence of these salts, although the optimum concentrations of these salts are not quite identical.

Fig. 3.

Fig. 4.

Thus it is concluded that among all the salts employed, NH_4 acetate produces the greatest effect in reducing the period of induction. When its effect is compared with those of Na hexosediphosphate and acetaldehyde, it will be seen (Fig. 4) that NH_4 acetate is a little more potent than acetaldehyde in reducing the period of induction, although Na hexosediphosphate is most effective.

EFFECT OF SALT MIXTURES.

In order to ascertain the combined effect of anions or cations it is necessary to keep the concentration of the accompanying ionic components constant.

Table V.

(A) Mixture of Na_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$ in the presence of $0.04\text{ }M\text{ Na}_2\text{HPO}_4$.

Na_2SO_4 (M)	$(\text{NH}_4)_2\text{SO}_4$ (M)	Relative induction period	$\text{NH}_4^+/\text{Na}^+$
0	0.1	31	5
0.025	0.075	36	1.15
0.05	0.05	43	0.56
0.075	0.025	45	0.22
0.09	0.01	57	0.077
0.095	0.005	88	0.037
0.1	0	>250	0

(B) Mixture of K_2SO_4 and KNO_3 in the presence of $0.04\text{ }M\text{ Na}_2\text{HPO}_4$.

K_2SO_4 ($0.5\text{ }M$)	KNO_3 (M)	Relative induction period	$\frac{1}{2}\text{ SO}_4^{2-}/\text{NO}_3^-$
0.1	0	37	∞
0.075	0.025	51	3
0.05	0.05	66	1
0.025	0.075	128	$\frac{1}{3}$
0	0.1	219	0

Fig. 5.

It will be seen from Table V (A), in which mixtures of Na_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$ in various proportions are taken and the total concentration of sulphate is kept constant, that the period of induction is reduced as the concentration of NH_4^+ increases. When these values are compared with those of the individual effect of these salts, it will be seen (Fig. 5) that NH_4^+ is more potent than Na^+ in controlling the period of induction. In the presence of a small amount of NH_4^+ ($0.01\text{ }M$) the period of induction is remarkably reduced from the expected period (from about 200 to 57). The interpretation of these results is not very clear, but it is probably due to an antagonistic effect of NH_4^+ to Na^+ .

When mixtures of K_2SO_4 and KNO_3 , in which the total concentration of K^+ is kept constant, are employed, the sulphate ion exerts an antagonistic effect on the nitrate ion, although the effect is not so great as was found with NH_4^+ and Na^+ (Table V (B)).

In discussing the combined effect of Na^+ and NH_4^+ , care should be taken to eliminate the effect of the sodium phosphate previously added. With this object, experiments were carried out with a mixture of NH_4 and Na phosphates in which the total concentration of phosphates was kept constant.

Table VI.

Mixture of Na_2HPO_4 and $(NH_4)_2HPO_4$.(A) 0.04 *M* phosphate:

Na_2HPO_4 (<i>M</i>)	$(NH_4)_2HPO_4$ (<i>M</i>)	Relative induction period	NH_4^+/Na^+
0.04	0	100	0
0.038	0.002	33	0.05
0.036	0.004	21	0.11
0.032	0.008	18	0.25
0.02	0.02	11	1
0.008	0.032	9	4
0	0.04	4	∞

(B) 0.08 *M* phosphate:

Na_2HPO_4 (<i>M</i>)	$(NH_4)_2HPO_4$ (<i>M</i>)	Relative induction period	NH_4^+/Na^+
0.08	0	>300	0
0.06	0.02	33	$\frac{1}{2}$
0.04	0.04	28	1
0.02	0.06	21	3
0	0.08	18	∞

It will be seen in Table VI (A) and (B) that the period of induction is reduced in each case (0.04 *M* and 0.08 *M* phosphate) as the concentration of NH_4^+ increases.

When the relative induction periods are plotted against the ratio of NH_4^+ and Na^+ (Fig. 6), the curves are of much the same character. A hyperbolic relation can be supposed to exist between the relative induction period ($= T$) and the ratio of the two ions ($= R$); thus

$$T \times R^{\frac{1}{n}} = \text{constant},$$

where n is a constant according to the nature of the salt; with 0.04 *M* phosphate $n = 3$ and with 0.08 *M* phosphate $n = 4.86$.

If the ratio of NH_4^+ and Na^+ is based on the total concentration of Na ion (added Na_2SO_4 plus sodium phosphate previously added), it will be seen (Fig. 6) that the curve of 0.1 *M* sulphate + 0.04 *M* phosphate (which is incorporated in Table V (A)) is again of much the same character, and the above equation is also applicable when $n = 5$ is chosen as the constant for this mixture. It will be considered from these results that a simple antagonistic relation exists between the two cations NH_4^+ and Na^+ ; NH_4^+ acts in opposition to Na^+ in reducing the period of induction.

Fig. 6 shows that this kind of antagonism is also found between the two anions of sulphate and nitrate.

Fig. 6.

SUMMARY.

1. Fermentation by dried yeast in the presence of 0.04 M phosphate was observed with various concentrations of inorganic and organic salts.
2. The period of induction was found to vary with the concentration and with the specific nature of the salt; both the cation and the anion.
3. The order of the potency of the salts in prolonging the period of induction was found to be $\frac{1}{2} Ca^{++} > Na^+ > K^+ > \frac{1}{2} Mg^{++} > NH_4^+$ with chlorides and sulphates, and $I^- > Cl^- > \frac{1}{2} SO_4^{--}$ with Na and K salts.
4. It was found that NH_4 salts (acetate, lactate, succinate and sulphate) were nearly as potent as acetaldehyde in reducing the period of induction, although they were less potent than hexosediphosphate.
5. The combined effect of the two ions was examined. NH_4^+ produced a remarkable antagonistic action to that of Na^+ , and sulphate ion a slight antagonistic action to that of nitrate ion.
6. A hyperbolic relation may be supposed to exist between the induction period and the ratio of the concentrations of the two ions.

REFERENCES.

- Billmann and Katagiri (1927). *Biochem. J.* **21**, 441.
 Harden (1925). *Biochem. J.* **19**, 477.
 — and Henley (1920). *Biochem. J.* **14**, 642.
 — (1921, 1). *Biochem. J.* **15**, 175.
 — (1921, 2). *Biochem. J.* **15**, 312.
 — and Macfarlane (1928). *Biochem. J.* **22**, 786.
 — Thompson and Young (1910). *Biochem. J.* **5**, 230.
 Katagiri (1926). *Biochem. J.* **20**, 427.
 Mayer (1927). *Biochem. Z.* **186**, 313.
 Meyerhof (1918). *Z. physiol. Chem.* **102**, 185.
 Neuberg (1918). *Biochem. Z.* **88**, 145.
 Raymond and Levene (1928). *J. Biol. Chem.* **79**, 621.