C. FERMENTATION BY DRIED YEAST PREPARATIONS. II.

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It has been shown [Harden, 1925] that the addition of sodium acetate or bicarbonate accelerates the onset of fermentation by zymin in a large volume of sugar solution. Further investigation shows that this acceleration is produced by the presence of other salts, both organic and inorganic, the induction time varying with the concentration and with the specific nature of the salt.

To avoid irregularities previously noticed, the fermentations were always carried out in the following manner: 0.2 g. zymin was wetted for 1 minute with 1 cc. water; to this were added (1) the salt solution, (2) 0.5 cc. 20 % fructose, and (3) water to make a total volume of 2.5 cc. The mixture was saturated with CO_2 and incubated at 29.5° . A period of 5 minutes was allowed for the attainment of an even temperature, after which readings were taken at 5 minute intervals. In each series, a control flask containing zymin, sugar and water was incubated to give the normal induction time for this volume of sugar solution; this varied from 40 to 60 minutes according to the sample of zymin. For the purpose of comparison of the effects of various salts, the induction period has been fixed arbitrarily as the interval of time between the mixing and the attainment of a rate of fermentation of 0.2 cc. in 5 minutes.

The initial p_{H} of the mixtures after saturation with CO_2 was determined by the capillator method in duplicate flasks; the final p_{H} was also determined in the earlier experiments. No difference in the initial p_{H} on the addition of the salts could be detected, while that between the initial and final p_{H} was never more than 0.2, the initial p_{H} being 5.6.

The salts tested and the length of the induction period at the optimum concentrations found are given in Table I. With the exception of sodium arsenate, all the salts tested reduce the induction period materially, the optimum concentration, at least with the inorganic salts, appearing to be dependent on the nature of the anion. Thus the optimum varies from 0.1 M in the case of chlorides to 0.02 M for sulphates and phosphates and 0.002 M for sodium citrate. The last is peculiar in showing a sharply marked optimum concentration, for the other organic salts employed were effective within wide limits of concentration, and with less distinction between uni- and bi-valent anions.

Table I.

Salt				Optimum concentration (molar)	Minimum induction time (min.)
—	...	...	...	—	60
KCl	...	...	...	0.05–0.1	20
NaCl	...	...	...	0.1	25
NH ₄ Cl	...	...	...	0.05–0.1	20
K ₂ SO ₄	...	...	...	0.01–0.05	20
Na ₂ SO ₄	...	...	...	0.01–0.02	20
MgSO ₄	...	...	...	0.01	20
Na ₂ HPO ₄	...	...	...	0.02–0.04	20
K formate	...	...	...	0.2	25
K acetate	...	...	...	0.2	15
K propionate	...	...	...	0.05–0.4	10
K lactate	...	...	...	0.2	15
K succinate	...	...	...	0.05–0.1	20
K tartrate	...	...	...	0.05–0.1	20
Na citrate	...	...	...	0.002	20
Na glycerophosphate	...	...	...	0.04	20
Na hexosediphosphate	...	...	...	0.011 (minimum)	10
Na trehalosemonophosphate	...	...	...	0.016 (minimum)	15

Of the organic salts, potassium formate, tartrate, succinate, lactate and citrate have approximately the same effect as the inorganic salts; that is to say, the induction time is reduced from 60 to 20 minutes in their presence. Potassium propionate and acetate rank with the sodium salts of hexosediphosphate and trehalosemonophosphate in producing an immediate fermentation. The effective concentration of K propionate covers a wide range, 0.05 to 0.4 *M*, but one whose limits have been definitely ascertained. In the case of the hexosephosphoric esters, a minimum concentration of 0.01 *M* is necessary for the immediate onset of fermentation, but it has not yet been possible to determine whether a high concentration will eventually increase the induction time as is the case with other salts.

The position of sodium arsenate (Table II) with respect to this effect of salts is unique, in that it acts in a direction contrary to that of all the other salts tested. In concentrations as low as 0.00005 and 0.0002 *M*, the induction time is normal or slightly increased; as the concentration is raised, the induction time lengthens. This effect is all the more striking as, when fermentation does begin, the rate of fermentation is greatly increased above the normal, as is usual in the presence of arsenate. The length of the induction time has no deleterious effect upon the subsequent fermentation. This is again in contrast with the action of high concentrations of inorganic salts by which the induction time is prolonged and the subsequent rate of fermentation is diminished [Harden and Henley, 1921]. Furthermore, in the presence of arsenate, the beneficial effect of added salts, both organic and inorganic, is partially counteracted. Thus, in the presence of 0.1 *M* sodium chloride and 0.002 *M* arsenate, the induction time is 50 minutes, whereas that with sodium chloride alone is 20 minutes and with arsenate alone, 75 minutes. These results are shown in Table II.

Table II.

Induction time (min.) for 0.2 g. zymin in 2.5 cc. 4 % sugar solution in presence of arsenate ions and added salts.

Concentration of Na_2HAsO_4 (molar)	H_2O	0.1 <i>M</i> KCl	0.2 <i>M</i> Na acetate	0.002 <i>M</i> Na hexose- diphosphate
0	60	10	15	20
0.0002	65	20	—	—
0.002	75	45	45	55
0.01	120	—	—	—

The action of sodium arsenate in prolonging the induction time without any toxic effect on the ensuing fermentation appears to be specific; it is difficult, however, to link this with the only known effect of arsenate ions in the fermentation process, viz. an increase in the rate of decomposition of hexosediphosphate, more especially as the beneficial action of this salt is counteracted equally with that of the inorganic salts by the presence of arsenate in the mixture. It is possible, however, that the action of hexosediphosphate and trehalosemonophosphate is in no way specific but falls in with the general effect of salts.

The phenomenon of induction under discussion presents some analogy to the induction which occurs in maceration extract and which has been studied by Meyerhof [1918]. In the latter case, however, the period of induction is completely abolished by the presence of hexosediphosphate in as small a concentration as 0.0002 *M*. It is moreover greatly diminished by the substitution of sucrose for glucose and fructose, which is not the case with the delayed fermentation of zymin. Meyerhof also found that the induction was removed by grinding the dried yeast with glass powder before preparing the maceration extract; grinding the zymin with glass powder, however, produced no effect on the induction period in the present experiments (see Table III).

Table III.

Induction time (min.) for 0.2 g. zymin in 2.5 cc. 4 % sugar solution.

Variation in conditions	Sugar		
	Fructose	Glucose	Sucrose
0.2 g. zymin unground	60	55	60
0.2 g. zymin + 0.1 g. glass powder	60	—	—
0.2 g. zymin ground with 0.1 g. glass powder	60	—	—
0.2 g. zymin + 0.2 cc. 1 % aldehyde... ..	50	—	—

The "induction" period in the case of zymin placed in a relatively large volume of sugar solution also appears to correspond to some extent with the period which elapses before the attainment of the maximum rate (Meyerhof's "Gäranstieg") in the fermentation of sugars in presence of phosphate by yeast preparations. Unlike this, however, it is very little affected by the addition of aldehyde (see Table III) so that it is probably not due to the same cause.

The results so far obtained render it probable that the induction period under discussion is due to the slow accumulation in the solution of one of the factors necessary for rapid fermentation. Whether this is accomplished by direct extraction from the zymin, a process which is aided by the presence of salts, or whether the process is a more complicated one, involving both the extraction and some subsequent chemical change before fermentation can proceed rapidly, remains to be ascertained.

SUMMARY.

1. It has been found that by adding various inorganic and organic salts to zymin placed in a large volume of sugar solution, the period of induction normally occurring before the onset of rapid fermentation is appreciably reduced.

2. Sodium arsenate is an exception to this general salt effect in that it prolongs the induction period without toxic effect on the fermentation.

REFERENCES.

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