CLXXII. THE EFFECTS OF CYANIDE AND SOME OTHER SALTS ON FERMENTATION BY YEAST PREPARATIONS.

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PARTICULAR interest is attached to the effect of HCN and cyanides on alcoholic fermentation because it is possible that one or other of the component reactions bringing about fermentation might be catalysed by metals and hence be specifically inhibited by cyanide.

Complete inhibition of fermentation with yeast juice by 1.2 % HCN (approx. $M/2$) was observed by Buchner, Buchner and Hahn [1903] but the minimum effective concentration had not been determined.

The effect of cyanides on the rate of hexosediphosphate formation by maceration extract and dried yeast at p_H 8.4 was examined by Neuberg and Kobel [1925]. They found that the reaction was delayed but finally went to completion, the p_H changing to about 6.8. Provided the p_H was not allowed to fall during the reaction, however, there was no observed effect with dried yeast. They were unable to give a definite explanation of the phenomenon.

Further, Neuberg and Perlman [1925] showed that 0.01–0.001 M cyanide slowed down the production of alcohol from sugar by maceration extract and dried yeast and in freshly fermenting yeast preparations completely inhibited it.

Warburg [1925] also investigated the effect of cyanide on alcoholic fermentation to decide whether it acted as a narcotic or by virtue of a chemical reaction. He found with maceration extract and living yeast that 0.01 N HCN diminished the rate of fermentation by 90–50 %, whereas with acetonitrile, a typical weak narcotic, a concentration of 2 M was necessary to bring about a similar effect. He concluded that HCN was not acting as a narcotic.

All these authors found that the concentrations required to bring about these effects were greater than those required to bring about partial or complete inhibition of reactions catalysed by metals, which are of the order of 0.001–0.0001 M .

The yeast preparations, zymen and dried yeast, when mixed with a solution of either glucose or fructose do not begin to ferment immediately. The length of this delay during which there is no production of carbon dioxide varies with the volume of the mixture [Harden and Young, 1911] and is termed

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the induction period. It has now been found that the addition of KCN to the mixture lengthens this period; increasing concentrations of KCN producing a progressively longer induction period until, with sufficiently great concentrations, no fermentation occurs.

EXPERIMENTAL.

Effect of cyanide.

In determining the effect of KCN on the fermentation of glucose by yeast preparations, it was found advisable, because of the gradual removal of HCN gas from the system with the evolution of carbon dioxide, to use the apparatus of Slator [1906], in which fermentation is carried out at almost constant volume and at a reduced pressure.

Estimation of the cyanide content of three similar fermentation mixtures, (a) immediately after mixing, (b) and (c) after fermentation, yielded respectively 96, 89 and 86 % of the KCN added, so that the KCN does not suffer any serious loss.

A uniform method of mixing was adopted. The required volume of water was added to the glucose and yeast preparation and the whole saturated with CO_2 . To this KCN was added, and the bottles were connected to the manometer with the taps shut off and then shaken; this ensured saturation with CO_2 without loss of HCN. The bottles were then placed in a thermostat at 30° and allowed to remain for 5 minutes until temperature equilibrium was attained. The pressure was then reduced and after shaking two or three times during a period of 5 minutes the initial reading was taken; the bottles were then shaken and read every 5 minutes. The period elapsing between the time of placing the bottles in the bath and the time when the rate of production of carbon dioxide became equal to the final steady rate was taken as the induction period.

The p_{H} of the mixture was not seriously affected by the addition of the concentrations of KCN used in these experiments and varied between 5.6 and 6.0, determined by the capillator method.

Fermentation. In fermentation mixtures with zymin, dried yeast and maceration extract, it was found that the presence of cyanide after lengthening the induction period

(a) slowed down the rate of attainment of the maximum rate in the presence of phosphates;

(b) did not affect the maximum rate attained;

(c) lowered the basal rate of fermentation (*e.g.* by 18 % with 0.006 *M* KCN; by 36 % with 0.01 *M* KCN) see Fig. 1.

In fermentations carried out with yeast juice, cyanide produced an induction period which was otherwise absent. This delay was followed by an enhanced rate falling to a basal rate, which was lower than in similar mixtures without cyanide.

These results show that cyanide chiefly affects the mechanism for the liberation of phosphate from hexosephosphate on which the basal rate depends, whilst the processes which occur in presence of free phosphate are unaffected.

Fig. 1. *A* = Fermentation of control mixture after 65 mins. induction period.
B = Fermentation of mixture containing 0.008 *M* KCN after 225 mins. induction period.

Induction period. Zymin (yeast treated with acetone). Fermentation with 2 g. zymin and 2 g. glucose in 15 cc. volume showed a well-marked induction period. The addition of 0.0007 *M* KCN was ineffective, but from concentration 0.006 *M* KCN upwards the induction period was prolonged to an extent varying with the concentration (Table I, Exp. I, 1, 2, 5; 8, 9). As described by Harden and Macfarlane [1928] the addition of specific concentrations of

Table I.

No. of exp.	Yeast preparation	KCN <i>M</i>	K ₂ HPO ₄ <i>M</i>	Potassium acetate	Potassium hexose-diphosphate	Induction period, minutes
11	Zymin (Z 11)	—	—	—	—	35
2	"	0.008	—	—	—	55
3	"	—	0.02	—	—	15
4	"	0.008	0.02	—	—	35
5	"	0.012	—	—	—	60
6	"	—	—	0.2	—	10
7	"	0.012	—	0.2	—	25
8	(Z 12)	—	—	—	—	25
9	"	0.033	—	—	—	120
10	"	—	—	—	0.005	10
11	"	0.033	—	—	0.005	40

either K₂HPO₄, potassium acetate or potassium hexosediphosphate to a mixture of zymin and glucose solution shortened the induction period. In the presence of KCN and one of these salts the induction period was intermediate between that produced by KCN alone and the shortened one due to the salt action (Table I, Exp. I, 2, 3, 4; 5, 6, 7; 9, 10, 11).

Autofermentation. The autofermentation of zymin was also delayed in the presence of cyanide. 2 g. zymin (Z 14) in 30 cc. water commenced to ferment in 60 minutes but was delayed 25 minutes by 0.01 *M* KCN. With 2 g. zymin

(Z 18) in 20 cc. water the delay was increased from 40 to 55 minutes by the addition of 0.013 *M* KCN.

Dried yeast. This preparation behaved in a similar manner to zymin. 2 g. dried yeast and 2 g. glucose in 15 cc. volume showed an induction period of 45 minutes and this was progressively increased by the addition of KCN, being *e.g.* 75 minutes with 0.017 *M* and 100 minutes with 0.033 *M* KCN. Salts had the same effect on the induction period as with zymin.

Maceration extract (Table II). This shows an induction period [Lebedev, 1912] which to some extent resembles that shown by zymin, in as much as it is abolished by hexosediphosphate [Meyerhof, 1918]. The effect of KCN on this was investigated in the same manner as with zymin. Two methods of preparation of maceration extract were employed and the activities of the two solutions were found to differ. Using a dried yeast prepared from a Dutch baker's yeast Lebedev's [1911] method gave a less active solution than the more recent method of Hägglund and Rosenqvist [1927], according to which maceration was carried out in three parts of 0.15 *M* K_2HPO_4 or Na_2HPO_4 solution for 4 hours. This latter preparation had a shorter induction period than the former. The experimental mixtures consisted of 15 cc. maceration

Table II.

Maceration extract	Hexose- diphos- phate <i>M</i>	KCN <i>M</i>	K ace- tate <i>M</i>	Na_2HAsO_4 <i>M</i>	K_2SO_4 <i>M</i>	KCl <i>M</i>	Induction period minutes
Dy. 7 in water	—	—	—	—	—	—	No fermentation
"	0.001	0.005	—	—	—	—	20
"	0.011	0.005	—	—	—	—	145
"	0.011	0.009	—	—	—	—	260
Dy. 9 in water	—	—	—	—	—	—	70
"	0.005	—	—	—	—	—	15
"	—	—	0.1	—	—	—	148
"	0.005	—	0.1	—	—	—	30
"	—	—	—	0.0025	—	—	100
"	0.005	—	—	0.0025	—	—	30
"	—	—	—	—	0.033	—	110
"	—	—	—	—	—	0.075	No fermentation
"	0.005	—	—	—	—	0.075	26
Dy. 11 in phos- phate solution	—	—	—	—	—	—	45
"	0.0033	0.015	—	—	—	—	No fermentation
"	0.0033	0.015	—	—	—	—	15
"	0.0033	0.015	—	—	—	—	100

extract and 2 g. glucose. The addition of KCN (0.005–0.015 *M*) to the mixture inhibited fermentation, which was not observed during 20 hours. When, however, hexosediphosphate was added at the same time as the cyanide fermentation took place, the induction period being always greater than in the presence of hexosediphosphate alone. The presence of Na_2HAsO_4 which prolongs the induction period with zymin [Harden and Macfarlane, 1928] also prolongs it with maceration extract and to some extent counteracts the shortening effect of hexosediphosphate. Such salts as KCl, K_2SO_4 and potassium acetate, which accelerate the onset of fermentation with zymin, delay

the onset with maceration extract both alone and in presence of hexosediphosphate.

By substituting sucrose for glucose Meyerhof [1918] found that the induction period of maceration extract was somewhat reduced. With the preparations used in the present experiments this only happened in the more active extracts macerated in phosphate solution, the induction period being shortened by 20 minutes.

It appears that the salts which diminish the induction period of zymin and dried yeast prolong it with maceration extract. Hexosediphosphate is the one exception to this, being the only substance tested which accelerated the onset of fermentation with maceration extract.

Yeast juice (Table III). Yeast juice together with glucose shows no induction period, but the addition of KCN of sufficient concentration produces

Table III.

Yeast juice	KCN	Induction period,
In all cases	<i>M</i>	minutes
Preparation No. 3	—	0
" " 11	0.075	50
" " 5	0.075	20
" " 5	0.075	35
" " 6	0.10	No fermentation
" " 6	0.075	10
" " 6	0.10	25
" " 14	0.13	55

one. The length of the induction period varies with the concentration of KCN and with each sample of juice.

Changes in the form of combination of phosphorus during the induction period of yeast juice and maceration extract.

Yeast juice. To investigate any change in the form of combination of phosphorus that might be taking place during the induction period produced by the presence of KCN, flasks containing similar mixtures were placed in the thermostat and removed one at a time at intervals during the induction period. The action was stopped immediately by the addition of trichloroacetic acid up to 3 % and the phosphate estimations were carried out in the filtrate by the modified Briggs method (Table IV). The mixture consisted of 15 cc. yeast juice and 2 g. glucose (Exp. IV, 1-6).

During a short induction period produced by the presence of cyanide the acid-soluble organic phosphorus increased in amount at the expense of the inorganic phosphorus as esterification took place.

Exp. IV, 7-13. On the other hand, during the early part of a long induction period produced by KCN, hydrolytic action was observed, the acid-soluble inorganic phosphorus increasing in amount at the expense of the organic phosphorus. Esterification, indicated by increase in acid-soluble organic phosphorus, took place towards the end of the induction period.

During the induction period the total acid-soluble phosphorus remained unaltered.

Exp. IV, 2, 6, 8, 13. After fermentation had proceeded for some time and a steady rate had been established, the acid-soluble inorganic phosphorus was lower in the presence of cyanide than in its absence. At the same time a depressed basal rate was observed.

Maceration extract. In maceration extract the acid-soluble total phosphorus is practically all present as inorganic phosphorus.

Table IV, Exp. IV, 14, 15, 16. A mixture of 10 cc. of maceration extract made from Dy. 11, with 2 g. glucose in 15 cc. volume fermented after 45 minutes. During this time the total acid-soluble phosphorus (60.0 mg.) remained unchanged and the inorganic phosphorus (58.95 mg.) slowly decreased.

Table IV.

No. of exp.	Yeast preparation	Time after mixing (mins.)	KCN <i>M</i>	Hexose-diphosphate <i>M</i>	Acid-soluble phosphorus			Fermentation commenced
					Total mg.	Inorganic mg.	Organic mg.	
IV 1	Yeast juice 13	0	—	—	76.91	23.51	53.40	Immediately
2	"	70	—	—	"	1.20	75.71	—
3	"	0	0.05	—	"	23.51	53.40	—
4	"	10	"	—	"	19.52	57.39	—
5	"	15	"	—	"	17.92	58.99	After 15 mins.
6	"	85	"	—	"	1.11	75.80	—
7	Yeast juice 14	0	—	—	75.63	9.21	66.42	Immediately
8	"	60	—	—	"	1.92	73.71	—
9	"	0	0.13	—	"	9.21	66.42	—
10	"	25	"	—	"	13.22	52.41	—
11	"	35	"	—	"	15.43	60.20	—
12	"	50	"	—	"	9.46	66.17	After 50 mins.
13	"	115	"	—	"	1.52	74.11	—
14	Maceration extract	0	—	—	60.00	58.95	1.05	—
15	(Dy. 11)	20	—	—	"	56.40	3.60	—
16	"	40	—	—	"	53.10	6.90	After 45 mins.
17	"	0	0.015	—	58.80	58.80	—	—
18	"	20	"	—	"	57.75	1.05	—
19	"	40	"	—	"	57.30	1.50	—
20	"	70	"	—	"	57.15	1.65	—
21	"	130	"	—	"	55.95	2.85	No fermentation
22	"	0	—	0.0033	64.65	59.10	5.55	—
23	"	7	—	"	"	59.55	5.10	—
24	"	12	—	"	"	55.95	8.70	After 15 mins.
25	"	0	0.015	0.0033	63.75	58.80	4.95	—
26	"	20	"	"	"	59.10	4.65	—
27	"	40	"	"	"	58.80	4.95	—
28	"	70	"	"	"	55.95	7.80	—
29	"	90	"	"	"	55.65	8.10	—
30	"	100	"	"	"	55.50	8.25	After 100 mins.

Exp. IV, 17-30. Fermentation was completely inhibited in such a mixture by 0.015 *M* KCN. In 130 minutes the acid-soluble inorganic phosphorus decreased by 2.85 mg. The total phosphorus remained unaltered. The addition of 0.0033 *M* potassium hexosediphosphate shortened the induction period considerably from 45 to 15 minutes in the control without cyanide, during which time there was a small amount of esterification shown by a decrease of 3.15 mg. in the acid-soluble inorganic phosphorus. A similar decrease

(3.3 mg.) took place during the 100 minutes' induction period of a mixture containing 0.015 *M* KCN as well as 0.0033 *M* potassium hexosediphosphate. In all these cases the total acid-soluble phosphorus remained constant and no change occurred except a slight esterification towards the end of the induction period.

The induction period of zymin.

In an attempt to arrive at an explanation of the cause of the induction period before fermentation with zymin, the required amount of zymin was ground up in a 12-hour old glucose solution and the desired quantities of the mixture were pipetted into test-tubes which were placed in a thermostat. Determinations of the optical rotation, reducing power and phosphates were made in the trichloroacetic acid filtrates at zero time and subsequently at intervals during the induction period. The reducing power as glucose of the mixture was determined by the Hagedorn-Jensen method (Table V). During the early part of the induction period of 100 minutes of a mixture of 3 g. zymin (Z 18) + 48 cc. glucose solution in a total volume of 60 cc. there were only

Table V.

Mixture	Time after mixing	Optical rotation α_{5461}	Reducing power as glucose mg./1 cc.	Fermentation commenced
3 g. Z 18 + 48 cc. glucose solution in total volume 60 cc.	0	+2.90	44.4	—
	70	+2.81	45.0	—
	80	+2.81	44.6	—
	90	+2.75	42.6	After
	100	+2.68	42.4	100 mins.

slight changes observed in either optical rotation or reducing power. As fermentation commenced these values slowly decreased, as might be expected. In mixtures with 0.13 *M* and 0.2 *M* potassium acetate, which shorten the induction period, and with 0.015 *M* KCN or 0.0067 *M* Na₂HAsO₄ which lengthen the induction period, and with fructose substituted for glucose the same order of changes occurred.

Phosphorus estimations. As before, these estimations were carried out according to the modified Briggs method. On investigating the changes occurring during the induction period when no carbon dioxide was given off, it became evident that the form of combination of the phosphorus altered in an arresting manner [see also Macfarlane, 1931].

Table VI, Exp. VI, 1-7. In the control mixture of 3 g. zymin (Z 18) + 48 cc. glucose solution in a total volume of 60 cc. there was an increase in the total acid-soluble phosphorus from 33.00 mg. to 53.66 mg. during the 100 minutes' induction period. During the early part of the induction period there was an almost corresponding increase in the acid-soluble inorganic phosphorus, followed by a decrease towards the time of commencement of fermentation. The acid-soluble organic phosphorus remained almost constant

Table VI.

No. of exp.	Mixture	Time after mixing minutes	Acid-soluble phosphorus			Fermentation commenced
			Total P mg.	Inorganic P mg.	Organic P mg.	
VI 1	3 g. Z 18 + 48 cc. glucose	0	33.00	10.63	22.37	
2	solution in total volume	20	39.60	18.74	20.86	
3	60 cc.	40	44.63	23.70	20.98	
4		70	47.85	19.47	28.38	
5		80	50.95	13.73	37.22	
6		90	52.80	5.87	46.93	After
7		100	53.66	4.69	48.97	100 mins.
8	3 g. Z 18 + 48 cc. glucose	0	38.35	10.97	27.38	
9	solution + 0.13 <i>M</i> potas-	25	46.79	16.90	29.89	
10	sium acetate in total	35	49.24	5.21	44.03	After
11	volume 60 cc.	40	49.96	5.08	44.88	45 mins.
12	3 g. Z 18 + 48 cc. glucose	0	46.79	11.15	35.64	
13	solution + 0.2 <i>M</i> potas-	7	49.43	13.00	36.43	
14	sium acetate in total	14	50.36	6.14	44.22	
15	volume 60 cc.	21	50.56	3.76	46.80	After
16		28	50.82	3.78	47.04	25 mins.
17	3 g. Z 18 + 48 cc. glucose	0	34.91	10.63	24.28	
18	solution + 0.01 <i>M</i> KCN	20	38.74	14.32	24.42	
19	in total volume 60 cc.	40	41.38	21.12	20.26	
20		70	46.46	26.93	19.53	
21		100	50.86	32.57	18.29	
22		130	52.07	38.48	13.59	
23		150	52.07	38.15	13.82	
24		170	52.20	35.64	16.56	
25		190	52.58	28.18	24.40	
26		210	52.67	26.80	25.87	After
27		230	52.80	13.46	39.34	230 mins.
28	3 g. Z 18 + 24 cc. glucose	0	40.25	11.02	29.23	
29	solution in total volume	25	45.46	11.88	33.58	
30	30 cc.	35	49.39	7.70	41.69	After
31		40	50.29	7.09	43.20	40 mins.
32	3 g. Z 18 + 24 cc. glucose	0	44.03	11.48	32.55	
33	solution + 0.015 <i>M</i> KCN	25	44.28	21.17	23.11	
34	in total volume 30 cc.	35	46.55	23.87	22.68	
35		55	48.78	28.55	20.23	
36		70	52.20	28.12	24.08	
37		80	52.20	15.52	36.68	After
38		100	52.34	7.20	45.14	100 mins.

Z 18 = Zymin No. 18

for the first part of the induction period and then rapidly increased as esterification took place.

Exp. VI, 8-11. In the 45 minutes' induction period in the presence of 0.13 *M* potassium acetate in a similar mixture the total acid-soluble phosphorus increased to almost the same value as in 100 minutes in the control. The absolute increase was, however, less because the total acid-soluble phosphorus at mixing was higher in the presence of acetate than in its absence. As in the control, the acid-soluble inorganic phosphorus at first increased and then later decreased. The organic phosphorus remained almost constant for the first 25 minutes and then increased.

Exp. VI, 12-16. In the presence of 0.2 *M* potassium acetate the induction period of this mixture was further shortened to 25 minutes and when fermentation began the total acid-soluble phosphorus was again approximately the

same; the absolute increase being again less owing to the higher original total acid-soluble phosphorus. Both the inorganic and organic phosphorus behaved as in the former case with acetate present.

If the changes observed in the concentration and rate of extraction of the total acid-soluble phosphorus in zymin bear any relation to the length of the induction period it might be expected that the increase in the rate of extraction of phosphorus coincident with a shorter induction period, which is shown to take place in presence of acetate, would be reversed with cyanide, and this was found to be the case.

Exp. VI, 17-27. The presence of 0.01 *M* KCN in a mixture of 3 g. zymin (Z 18) + 48 cc. glucose solution in 60 cc. volume prolonged the induction period by 130 minutes. The total acid-soluble phosphorus values at mixing and at the onset of fermentation were almost the same as in the control. The absolute increase was therefore identical, but the period required for its production was almost $2\frac{1}{2}$ times as long (see Fig. 2).

Fig. 2. *A* = acid-soluble total phosphorus. *B* = acid-soluble inorganic phosphorus. *C* = acid-soluble organic phosphorus.

Exp. VI, 28-38. This relation was again shown in two mixtures of half the total volume. 3 g. zymin (Z 18) + 24 cc. glucose solution in 30 cc. volume, one with 0.015 *M* KCN and one without. The absolute increase of total acid-soluble phosphorus in the two mixtures was about the same, but its production required 60 minutes longer in the mixture containing cyanide.

As shown in the experiments with and without potassium acetate present, the amount of increase of acid-soluble inorganic phosphorus during the first part of the induction period was approximately the same as that of the total phosphorus. The extra phosphorus derived from the zymin, which was estimated as acid-soluble inorganic phosphorus may have been present in the zymin in that form or as an ester which was hydrolysed; there was, however, no direct evidence of this hydrolysis. On the other hand, in the presence of cyanide the increase in inorganic phosphate was greater than the increase in total phosphorus, which indicates that hydrolysis at the expense of the organic phosphorus, which gradually decreased, was occurring. Esterification occurred towards the end of the induction period as in other cases (see Fig. 2).

In this present set of experiments the only evidence of hydrolysis of organic phosphorus throughout the early part of the induction period of zymin fermentation was obtained in the experiments with cyanide.

This observation that fermentation takes place when an approximately constant concentration of total acid-soluble phosphorus derived from the zymin is reached, throws some light on the nature of the effect of salts. In all the experiments with 3 g. zymin (Z 18) in 60 cc. volume both in the presence and absence of added salts, fermentation began when the concentration of total acid-soluble phosphorus was between 49.0 mg. and 54.0 mg. At mixing the acid-soluble total phosphate was 33.00 mg. in the control, 38.35 mg. in the presence of 0.13 *M* potassium acetate and 46.79 mg. with 0.2 *M* potassium acetate. It was thus seen that of the mixtures in which the same concentration of total phosphorus was reached before fermentation commenced, those in which the total phosphorus at mixing was higher had the shorter induction periods.

In another mixture of 3 g. zymin (Z 18) in 30 cc. volume, *i.e.* half the volume in the former experiments, the total at mixing was 40.25 mg. increasing to 50.29 mg. in 40 minutes when fermentation began. The length of this induction period and the total acid-soluble phosphorus both at mixing and when fermentation began are comparable with those of the mixture in double the volume containing 0.13 *M* potassium acetate. The presence of this salt to some extent counteracts the effect of dilution.

A comparison of the two control mixtures, one in 60 cc. volume and the other in 30 cc., further illustrates this point. At mixing, the total acid-soluble phosphorus of the more dilute mixture was 33.00 mg., this was lower than that of the mixture in 30 cc., which was 40.25 mg. They both began to ferment when the total phosphorus was approximately the same (50.29 mg. in 30 cc. and 53.66 mg. in 60 cc.), but the time taken to arrive at this concentration was 60 minutes longer in the more dilute mixture. Hence dilution retarded the accumulation of phosphate from zymin and increased the induction period.

There still remained a certain amount of phosphorus in the zymin, even after 3 hours' incubation, for the highest value of total acid-soluble phosphorus obtained was never as high as the absolute total phosphorus found. Thus the total phosphorus of 1 g. zymin (Z 18) was 21.0 mg. and the highest total acid-soluble found was 17.5 mg.

Inorganic phosphorus during the basal rate.

It has already been shown that the basal rate of fermentation of sugar solution with zymin was reduced in the presence of cyanide. The acid-soluble inorganic phosphorus of a mixture of 2 g. zymin (Z 14) + 2 g. glucose in 30 cc. volume fermenting at a steady rate was 1.70 mg. By the presence of 0.007 *M* KCN this was reduced to 1.40 mg. and to 1.26 mg. by the presence of 0.01 *M* KCN. These results again show that the presence of cyanide has

interfered with the mechanism by which phosphate is obtained from hexose-diphosphate.

DISCUSSION.

It is inferred, partly from the negative results obtained with regard to the optical rotation and reducing powers of the mixtures and partly from the positive evidence of changes in the form of combination of the phosphorus, that the duration of the induction period of fermentation mixtures with zymin is dependent on changes taking place in the zymin. It is uncertain whether the actual release of phosphate or whether its condition of availability is the necessary factor to bring about the commencement of fermentation. It is also conceivable that the liberation of phosphate may be accompanied by other secondary changes in the zymin by which another substance or group of substances directly influential in bringing about fermentation is made available. At present it is obscure how the liberation of phosphate is hastened or retarded, but the question of an alteration of the permeability of the cell wall of the acetone yeast preparation arises. Potassium acetate which shortens the induction period increases the rate at which the available phosphate becomes acid-soluble. In the presence of cyanide, however, which increases the induction period, and decreases the rate of increase of acid-soluble phosphorus, fermentation does not begin when the concentration of total phosphorus is reached which is effective in the control or in the presence of potassium acetate. The cyanide evidently also exerts some other effect. The essential point does not seem to be the accumulation of organic phosphorus, as this is decreasing throughout nearly the whole of the induction period in the presence of KCN and its increase towards the end is probably the result of the fermentation and not the cause of it, there being always a lag between esterification and evolution of CO_2 [Boyland, 1930].

Cyanide and arsenate.

It having being definitely established that both cyanide and arsenate prolong the induction period of zymin and glucose mixtures, it seemed probable that by introducing these salts independently and together into a mixture some light might be thrown on the nature of their separate actions (see Fig. 3). A mixture of 2 g. zymin (Z 18) with 2 g. glucose in 15 cc. had an induction period of 30 minutes. This was prolonged to about 70 minutes in presence of either 0.013 *M* KCN or of 0.0067 *M* Na_2HAsO_4 (called concentration 1). On doubling and trebling these concentrations (concentrations 2 and 3) almost proportional increases in the induction period were observed with KCN, whereas with arsenate the increases were considerably less (Fig. 3, Curves A and B). It seems, therefore, that the mode of action of the two substances is quite different.

In attempting to investigate this point further the two salts were added together to the mixture and the time effects observed. By adding at the same time the original concentrations (concentration 1) of each salt, the

increase in the induction period (curve *C*) was intermediate between those obtained with concentration 2 of the salts separately, and nearer to the time

Fig. 3. *A* = Induction period due to Na_2HAsO_4 . *B* = Induction period due to KCN. *C* = Induction period due to Na_2HAsO_4 and KCN.

due to the cyanide effect. The addition of 0.026 *M* KCN and 0.0134 *M* Na_2HAsO_4 (concentration 2) together to a mixture produced an induction period, which lies between those which would be expected from the curves of concentration 4 of KCN and Na_2HAsO_4 added separately. The effect was thus found to be more than that of either salt alone but less than the sum of those produced by the two salts alone. At the same time it was more than that produced by the double concentration of arsenate, but less than that due to the double concentration of cyanide. This suggests that interference takes place between the actions of the two substances possibly because they may affect reactions dependent to a certain extent on one another.

Methylglyoxal.

It is supposed that methylglyoxal occurs as an intermediate product of alcoholic fermentation. If this substance is formed towards the end of the induction period as suggested by Boyland [1930], by including it in the original fermentation mixture a shortening of the induction period might be brought about. This has been found to occur in the present experiments with zymine. As the sample of methylglyoxal hydrate¹ used was acid in reaction (p_{H} 1.6) before each experiment the p_{H} was adjusted to 6.0–6.2 with Na_2CO_3 .

¹ I am indebted to Prof. R. Robinson for this specimen of methylglyoxal.

Table VII.

No. of exp.	Mixture	Methylglyoxal <i>M</i>	Induction period, minutes	Reduction of	
				Phosphate rate %	Basal rate %
VII 1	2 g. Z 18 + 2 g. glucose in 20 cc. volume	—	60	—	—
2		0.01	60	—	—
3		0.01*	—	—	—
4		0.02	10	45	40
5		0.02*	—	25	25
6		0.03	15	60	60
7		0.03*	—	35	38
8		0.05	20	Complete	52
9		0.05*	—	52	52
10		0.1	30	Complete	60
11		0.1*	—	60	60
12	2 g. Z 18 + 16 cc. water	—	25	—	—
13		0.05	15	55	60
14	2 g. Z 18 + 26 cc. water	—	70	—	—
15		0.02*	—	30	33
16		0.03*	—	54	55

* Denotes addition at the end of the normal induction period.

Table VII, Exp. VII, 1, 2, 3, 4. The mixture used was 2 g. zymin (Z 18) and 2 g. glucose in 20 cc., which had an induction period of 60 minutes. The concentration of methylglyoxal which had the maximum time effect and shortened the induction period to 10 minutes was 0.02 *M*. After 65 minutes of fermentation at a reduced rate a maximum rate was reached, which was less than in the control without methylglyoxal. The basal rate was reduced by 40 % in the presence of methylglyoxal.

Exp. VII, 6, 8, 10. Concentrations of 0.03 *M*, 0.05 *M* and 0.1 *M* shortened the induction period and increasingly reduced the basal rate up to 60 % in the case of 0.1 *M*. No rise to a maximum rate occurred in the presence of either 0.05 *M* or 0.1 *M* methylglyoxal.

Exp. VII, 5, 7, 9, 11. To determine the effect on fermentation, similar concentrations to the above were added at the onset of fermentation (*i.e.* after the normal induction period of 60 minutes of the mixture). The evolution of CO₂ decreased with increasing concentration of methylglyoxal, the basal rate being reduced 40 % with 0.02 *M* and 60 % with 0.1 *M* methylglyoxal.

Table VIII: On addition of K₂HPO₄ in optimum concentration (0.09 *M*),

Table VIII.

Mixture	K ₂ HPO ₄ <i>M</i>	Methylglyoxal <i>M</i>	Rate of CO ₂ production			Percentage reduction	
			Highest - Basal = Phosphate rate			Phosphate rate	Basal rate
			cc. per 5 minutes				
2 g. Z 18 + 2 g. glucose in 15 cc. volume	0.06	—	12.8	3.2	9.6	—	—
	0.06	0.035	10.9	2.3	8.6	10.4	28.1
	0.09	—	13.6	3.1	10.5	—	—
	0.09	0.02	12.5	2.9	9.6	8.5	6.4
	0.09	0.035	11.5	2.7	8.8	16.1	12.6
	0.09	0.05	9.4	2.1	7.3	30.4	32.2
	0.15	—	13.1	3.4	9.7	—	—
	0.15	0.05	9.0	2.1	7.9	18.5	38.2

together with methylglyoxal in various concentrations at the onset of fermentation, a lower maximum phosphate rate and basal rate were observed than in the absence of methylglyoxal. With 0.02 *M* methylglyoxal the maximum rate was 8.5 % and with 0.05 *M* 43.2 % less than the control, the basal rates of the same mixtures being 6.1 % and 32.2 % lower. In the presence of methylglyoxal the maximum rate was attained about 10 minutes sooner than in its absence.

Exp. VII, 12–16. The autofermentation of zymin (Z 18) in the presence of methylglyoxal showed the same inhibition. With 0.05 *M* methylglyoxal the induction period was shortened from 25 minutes to 15 minutes and the basal rate reduced by 60 %. At the onset of autofermentation the addition of 0.02 *M* and 0.03 *M* methylglyoxal reduced the maximum rates 30 % and 54 % respectively and the basal 33 % and 55 %. There was no indication of fermentation of methylglyoxal as there was a smaller evolution of carbon dioxide in its presence than in its absence.

It appears from these experiments that methylglyoxal exerts an inhibiting influence, either directly or indirectly, both on the process of esterification and on the mechanism which controls the basal rate. At the same time it shortens the induction period.

SUMMARY.

1. In alcoholic fermentation by yeast preparations KCN (minimum concentration 0.006 *M*) slows down the attainment of a maximum rate in the presence of phosphate, without affecting the maximum rate attained. The basal rate of fermentation is lowered.

2. The induction period before the onset of fermentation by yeast preparations (zymin, dried yeast and maceration extract) is increased by cyanide (minimum concentration 0.005 *M*); with yeast juice, cyanide (minimum concentration 0.075 *M*) produces an induction period which is otherwise absent.

3. In the presence of substances such as potassium acetate, potassium hexosediphosphate and dipotassium hydrogen phosphate, which shorten the induction period of zymin and dried yeast, cyanide causes a prolongation of it to a smaller degree than when acting alone.

4. With yeast juice in the presence of cyanide, the total acid-soluble phosphorus remained constant, the organic phosphorus diminished and the inorganic phosphorus increased till near the end of the induction period.

5. With maceration extract the total phosphorus remained constant and there was no change except slight esterification towards the end of the induction period.

6. During the induction period of zymin under various conditions the total and inorganic acid-soluble phosphorus increased by the same amount while the organic phosphorus remained approximately constant till near the end of that period, when it increased as the inorganic phosphorus became esterified.

7. In the presence of cyanide during the first part of the induction period there was a greater increase of acid-soluble inorganic phosphorus than of total phosphorus. This was due to hydrolysis of the organic phosphorus.

8. The optical rotation and reducing power of the trichloroacetic acid filtrates from the fermentation mixtures only showed slight changes until the end of the induction period was approached.

9. In the presence of arsenate, which prolongs the induction period of zymin, cyanide further prolongs it, but the joint effect is less than the sum of the separate effects. The curve connecting increase of induction period with increase of concentration is different for each salt, indicating that their actions are of a different kind.

10. Methylglyoxal shortened the induction period of fermentation with zymin and reduced both the basal rate of fermentation and the rate of esterification.

I desire in conclusion to express my thanks to Prof. A. Harden for his constant advice and help in this work.

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