XXI. THE EFFECT OF ACETALDEHYDE AND METHYLENE BLUE ON THE FERMENTATION OF GLUCOSE AND FRUCTOSE BY YEAST-JUICE AND ZYMIN IN PRESENCE OF PHOSPHATE AND ARSENATE.

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It was previously shown by the authors [Harden and Henley, 1920, where the literature is quoted] that, in the fermentation of glucose in presence of phosphate by zymín or yeast-juice, acetaldehyde or methylene blue produced a remarkable acceleration in the early stages of the reaction and it was suggested that the function of these substances was to act as hydrogen acceptors and thus enable the reaction at once to attain a high rate. The experiments have now been extended to fructose, with the objects of ascertaining the maximum rates attainable with or without added acceptors and of comparing these with the corresponding rates yielded by glucose.

I. COMPARISON OF THE MAXIMUM RATES AND THE COURSE OF THE REACTION OBSERVED IN THE PRESENCE OF PHOSPHATE WITH GLUCOSE AND FRUCTOSE RESPECTIVELY IN THE PRESENCE AND ABSENCE OF ACETALDEHYDE.

A. *Zymín*. To 2 g. of zymín were added 10 cc. of a solution containing 1 g. of the sugar and, when acetaldehyde was used, 3 cc. of 1 % acetaldehyde; 0.2 cc. toluene was added in each case. This mixture was incubated at 25° until a constant rate of fermentation had been attained and varying volumes of 0.6 M K_2HPO_4 (previously saturated with CO_2) added. Readings were in many cases made every two minutes but the rates have usually been calculated for five minutes. With the smaller quantities of phosphate repeated additions were made when the rate began to slacken. This procedure was adopted because, especially in the case of glucose, the rate was depressed even by a small excess of phosphate. The final volumes therefore occasionally differ slightly, but this has very little effect on the maximum rate obtainable.

In Table I are given the maximum rates obtained with and without acetaldehyde, and the volumes of phosphate solution added.

Table I.

No.	Sugar	With 3 cc. 1 % acetaldehyde		Without acetaldehyde	
		Max. rate cc. per 5 min.	Additions	Max. rate cc. per 5 min.	Additions
1	Fructose	15	1 of 5 cc.	7.25	1 of 5 cc.
2	"	14	2 of 2 "	9	2 of 2 "
3	"	14	3 of 1 "	9.75	3 of 1 "
4	Glucose	9	2 of 2 "	7	1 of 2 "
5	"	7.5	2 of 1 "	5.5	1 of 1 "
6	"	9	1 of 2 "	—	—
7	"	8.25	1 of 2 " and 1 of 1 "	—	—
8	Cane-sugar	8.75	1 of 2 "	7.5	2 of 2 "

B. *Yeast-juice*. The experiments with yeast-juice were carried out in a similar manner to the above, 20 cc. of yeast-juice and 4 cc. of 1 % acetaldehyde being used, the total volume being 24.6 cc.; 0.2 cc. of toluene was added in each case. The results are embodied in Tables II and III, each of which refers to a different sample of yeast-juice.

Table II.

No.	Sugar	With 4 cc. 1 % acetaldehyde		Without acetaldehyde	
		Max. rate cc. per 5 min.	Additions	Max. rate cc. per 5 min.	Additions
9	Glucose	16	1 of 1 cc.	10.75	3 of 1 cc.
10	"	18.5	1 of 1 " and 1 of 2 "	11.75	2 of 2 "
11	"	21	1 of 5 "	—	—
12	Fructose	29.6	1 of 5 "	24.5	1 of 5 "
13	"	36	1 of 10 "	33	1 of 10 "

Table III.

14	Fructose	17.25	1 of 5 cc.	—	—
15	"	18.25	4 of 2 "	—	—
16	Cane-sugar	8.25	1 of 5 "	5.5	1 of 5 "
17	"	11.25	2 of 2 "	10	1 of 2 "

Owing to the fact that repeated additions of phosphate were made in most of these cases, the course of the reaction was irregular. Fig. 1 however shows the characteristic effect exerted by acetaldehyde on the fermentation of fructose in presence of phosphate (experiment B 13 above). In this case the rates in cc. per 2 min. are plotted against the time, and it is seen that in the presence of aldehyde the rate rises much more rapidly, but only to a slightly higher maximum.

It will be seen from the above that the maximum rate obtainable from glucose under the optimum conditions of phosphate and aldehyde falls far short of that observed with fructose. For the sample of zymine employed for experiments A 1-8 the maximum rates in presence of aldehyde were 15

for fructose and 9 for glucose, a ratio $F/G = 15/9 = 1.67$. In the case of the yeast-juice used in experiments B 9-13 the ratio $F/G = 36/21 = 1.7$, almost identical with that for zymine.

It appears to follow from this, since all these rates were determined in presence of an excess of acceptor, that the difference in the behaviour of fructose and glucose although partly attributable to a difference in the rate of production or of reduction of acceptor, cannot be wholly accounted for in this way, but must extend to some other stage in the reaction.

Fig. 1.

II. RELATIVE EFFICIENCY OF ACETALDEHYDE AND FRUCTOSE AS AGENTS OF "INDUCTION."

It was suggested in the previous paper [Harden and Henley, 1920] that the inductive effect of fructose [Harden and Young, 1909] on a mixture of

glucose and phosphate was due to the more ready production of acceptor from fructose than from glucose. This might occur in either or both of two ways: firstly, by the more rapid production from fructose of a substance capable of acting as an acceptor in the preliminary stage of the reaction (possibly identical with the precursor of glycerol and possibly a different substance from that produced from glucose) or by the more rapid reduction of such a preliminary acceptor under the prevailing conditions; secondly by the more rapid fermentation of the fructose, yielding as intermediate product acetaldehyde (which may be termed the normal acceptor) which would then be available to complete the cycle of fermentation of further molecules either of fructose or of glucose, even in the presence of a considerable excess of phosphate. Under these circumstances it became of interest to ascertain the relative efficiencies of fructose and acetaldehyde in this respect. Experiments were therefore made with yeast-juice and glucose in presence of excess of phosphate by adding varying amounts of acetaldehyde and fructose and comparing their effects, with the result that a concentration of $1/42,200$ of acetaldehyde gave almost exactly the same reaction curve as $1/211$ of fructose (see Fig. 2), this latter being equivalent to 10 % of the glucose present. In other words acetaldehyde is 50 times as effective as fructose, when molecular quantities are compared.

Experiment 18. Six quantities of 25 cc. yeast-juice + 2 g. glucose + 10 cc. $0.6\text{ M K}_2\text{HPO}_4$ + 0.2 cc. toluene were incubated for ten minutes at 25° and then were added:

No.	10 % glucose cc.	10 % fructose cc.	1 % acetaldehyde cc.	Water cc.
1	2	0	0	4
2	2	0	0.1	3.9
3	2	0	0.5	3.5
4	2	0	1.0	3
5	2	0	1.5	2.5
6	0	2	0	4

The course of the fermentations in the various flasks is shown by the curves of Fig. 2, rates in cc. per five minutes being plotted against time. The curve for (4) was almost coincident with that for (5), whilst that for (3) was intermediate between those for (4) and (2). It will be seen that the addition of 0.1 cc. of acetaldehyde solution (2) produced almost exactly the same effect as that of 2 cc. of the fructose solution (6).

III. EFFECT OF ACETALDEHYDE ON THE FERMENTATION OF GLUCOSE AND FRUCTOSE BY YEAST-JUICE AND ZYMIN IN PRESENCE OF ARSENATE AND PHOSPHATE.

It has previously been shown [Harden and Young, 1911] that arsenate greatly accelerates the action of the hexosephosphatase of yeast-juice and zymine so that the fermentation of glucose and fructose proceeds much more rapidly in the presence of a suitable concentration of arsenate than in its

absence. Excess of arsenate, however, produces inhibition. It therefore became of interest to ascertain whether the arsenate, in addition to its effect on the hexosephosphatase, exerted any modifying influence on any other stage of the fermentation and in particular whether it in any way affected the accelerating action of acetaldehyde, a possibility which has not previously been considered.

Fig. 2.

C. Yeast-juice.

Experiment 19. Six flasks were made up as follows:

No.	Yeast-juice cc.	Sugar	Toluene cc.	Water cc.	1 % acetaldehyde cc.	0.3 M Na_2HAsO_4 cc.	0.6 M K_2HPO_4 cc.
1	20	2 g. fructose	0.2	15	0	0	5
2	20	"	0.2	10	5	0	5
3	20	"	0.2	14	0	1	5
4	20	"	0.2	9	5	1	5
5	20	"	0.2	10	0	5	5
6	20	"	0.2	5	5	5	5

The sodium arsenate and potassium phosphate were added to the mixture after it had been incubated for 30 mins. at 25°. The course of the fermentation in the different flasks is shown by the curves of Fig. 3.

A similar experiment was carried out with glucose, a different sample of yeast-juice being employed, which was capable of producing fermentation in presence of an exceptionally high concentration of phosphate.

Fig. 3.

Experiment 20. Six flasks were made up, each containing 20 cc. yeast-juice + 2 g. glucose + 0.2 cc. toluene together with:

No.	Water cc.	1 % acetaldehyde cc.	0.3 M Na_2HAsO_4 cc.	0.6 M K_2HPO_4 cc.
7	12.5	0	0	7.5
8	7.5	5	0	7.5
9	11.5	0	1	7.5
10	6.5	5	1	7.5
11	7.5	0	5	7.5
12	2.5	5	5	7.5

The sodium arsenate and potassium phosphate were added to the mixture after it had been incubated for 30 mins. at 25°.

The course of the fermentation was very similar to that observed in experiment 19 with fructose, as is indicated by the following observations:

No.	Arsenate cc.	Acetaldehyde cc.	Maximum rate cc. per 5 min.	Time (min.) re- quired to attain max.
7	0	0	18	60
8	0	5	33.6	10
9	1	0	16.8	85
10	1	5	35.9	10
11	5	0	(7.1)	90
12	5	5	22.7	10

The small increase of rate in 10 as compared with 8 is probably due to the fact that in 8 the optimum concentration of phosphate was not present, whereas in 10, owing to the action of the arsenate, optimum conditions were maintained.

Two interesting points emerge from these experiments:

1. In presence of excess of phosphate, arsenate does not remove the inhibition, but somewhat increases it (Curves 1, 3, 5, Fig. 3 and experiment 20, Nos. 7, 9, 11).

2. The presence of arsenate does not affect the accelerating action of acetaldehyde in presence of phosphate (Curves 3, 4, 5 and 6, Fig. 3 and experiment 20, Nos. 8, 10).

D. Zymin.

Experiment 21. Twelve flasks were made up each containing 2 g. zymin with the following additions; 0.2 cc. of toluene was added in each case:

No.	Sugar	Water cc.	1 % acetaldehyde cc.	Added subsequently		Water cc.
				0.6 M K_2HPO_4 cc.	0.3 M Na_2HAsO_4 cc.	
1	1 g. glucose	9.4	0	2	0	1.2
2	"	6.4	3	2	0	1.2
3	"	9.4	0	2	0.1	1.1
4	"	6.4	3	2	0.1	1.1
5	"	9.4	0	2	0.2	1
6	"	6.4	3	2	0.2	1
7	1 g. fructose	9.4	0	3	0	0.2
8	"	6.4	3	3	0	0.2
9	"	9.4	0	3	0.1	0.1
10	"	6.4	3	3	0.1	0.1
11	"	9.4	0	3	0.2	0.2
12	"	6.4	3	3	0.2	0.2

The course of the reaction is indicated by the following observations:

No.	Sugar	Arsenate cc.	Acetaldehyde cc.	Maximum rate cc. per 5 min.	Time (min.) re- quired to attain maximum
1	Glucose	0	0	5.9	40
2	"	0	3	6.2	10
3	"	0.1	0	6.8 (average)	45
4	"	0.1	3	9.6 "	10
5	"	0.2	0	6.9 "	45
6	"	0.2	3	9.9 "	10
7	Fructose	0	0	9.8	35
8	"	0	3	12.1	10
9	"	0.1	0	10.1 (average)	35
10	"	0.1	3	14.1 "	10
11	"	0.2	0	9.9 "	40
12	"	0.2	3	14.4 "	10

In its main features the zymin behaves in a similar manner to yeast-juice. Owing to the small concentration of arsenate employed, the inhibiting effect of the arsenate, which was marked in the case of yeast-juice, is here barely perceptible.

Another point of difference is that the maximum rate, especially with glucose, in presence of arsenate is considerably greater than in its absence, the two rates in question being 6.2 and 9.9 or a ratio of 1.6, whereas with yeast-juice and glucose the ratio is $33.6/35.9 = 1.07$. The difference is much less with fructose, the corresponding ratios being 1.2 for zymin and 1.0 for yeast-juice. In order to ascertain whether a real difference of this kind exists between yeast-juice and zymin, experiments were made in which the maximum rate in presence of the optimum concentration of phosphate in presence of acetaldehyde was determined, and arsenate was then added to the same mixture. With glucose this produced, as in the previous experiment, a marked rise of rate, whereas with fructose only a small and insignificant rise was observed. This shows that the arsenate exerts a definite accelerating effect on the fermentation of glucose by zymin apart from its action on the hexose-phosphatase. The exact significance of the phenomenon has not yet been ascertained.

Experiment 22. 2 g. zymin + 1 g. of the sugar (glucose or fructose) + 3 cc. 1 % acetaldehyde + 6.44 water + 0.2 cc. toluene were incubated at 25° until the rate was constant. Successive small additions of phosphate were made, as in experiments 1-8, until the maximum rate obtainable had been observed. 0.2 cc. of 0.3 *M* Na₂HAsO₄ was then added and the average rate attained was noted.

Sugar	Maximum (cc. per 5 min.)	
	In absence of arsenate	In presence of arsenate
Glucose	6.0	8.5
Fructose	13.0	13.5

IV. EFFECT OF METHYLENE BLUE.

It was shown in the previous communication [Harden and Henley, 1920] that methylene blue behaved in a similar manner to acetaldehyde with respect to the fermentation of glucose by zymin in presence of phosphate. These experiments have now been extended to the fermentation of both glucose and fructose in the presence of phosphate, with the result that the methylene blue has been found to behave in a similar manner in all these cases.

The experiments were carried out exactly as in the case of acetaldehyde, but the methylene blue was added in the solid form. No attempt was made to ascertain the optimum conditions, so that the rates observed are not necessarily the maximum obtainable.

Fig. 4.

E. Zymin and glucose and fructose.

Experiment 23. Four flasks were made up containing each 2 g. zymin + 1 g. of sugar + 9.4 cc. water + 0.2 cc. toluene. Nos. 1 and 2 contained glucose, Nos. 3 and 4 fructose. 0.1 g. methylene blue was added to Nos. 2 and 4. These were incubated for 35 mins. at 25° and 2 cc. 0.6 M K_2HPO_4 were then added to each. The course of the reaction is seen from the curves (Fig. 4).

F. Yeast-juice and glucose and fructose.

Experiment 24. Four flasks were made up containing

1.	25 cc. yeast-juice	+ 2 g. glucose	+ 0.2 cc. toluene	
2.	" "	" "	" "	+ 0.2 g. methylene blue
3.	" "	+ 2 g. fructose	" "	
4.	" "	" "	" "	" "

These were incubated for 15 mins. at 25° and 15 cc. 0.6 M K_2HPO_4 were then added. The course of the reaction is shown in the accompanying curves (Fig. 5).

Fig. 5.

In every case the addition of the methylene blue caused a marked acceleration in the rate of attainment of the maximum. The concentration of the phosphate was so high as greatly to inhibit the fermentation of the glucose in absence of methylene blue (curve 1).

SUMMARY.

1. Acetaldehyde diminishes the time required by a mixture of fructose and phosphate to attain its maximum rate of fermentation in presence of yeast-juice or zymin, but does not substantially increase the maximum rate obtainable.

2. In presence of acetaldehyde fructose is more rapidly fermented than glucose in presence of phosphate both by yeast-juice and zymin.

3. Acetaldehyde is about 50 times as effective as fructose (when molecular quantities are compared) in "inducing" fermentation in a mixture of glucose with excess of phosphate.

4. Arsenate does not affect the accelerating action of acetaldehyde in presence of phosphate in the fermentation of glucose and fructose by yeast-juice but considerably increases the rate attained with glucose, and to a less degree that attained with fructose, when zymin is used.

5. Methylene blue produces an effect similar to that of acetaldehyde in the fermentation of both fructose and glucose by zymin and yeast-juice in presence of phosphate.

REFERENCES.

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