

the main stem only bear three glands (1-3), appear to be impure. Such plants are produced by crossing the two pure forms (0) and (3-1). Type 2 appears to be an exception, being a pure form with leaf glands (1), while Types 3 and 10 have only been observed in the pure (3-1) condition. Type 1 has not been observed sufficiently to justify a definite statement.

The Alcoholic Ferment of Yeast-juice. Part VI.—The Influence of Arsenates and Arsenites on the Fermentation of the Sugars by Yeast-juice.

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The rate of fermentation of a simple sugar by yeast-juice undergoes a temporary acceleration when a soluble phosphate is added.* The close chemical analogy which exists between phosphates and arsenates suggested the idea that this similarity might extend to the effect of arsenates on fermentation, and accordingly experiments were made to test the point. It was thus found that arsenates have a great effect on fermentation, but subsequent work has shown that it is of a totally different nature from that exerted by phosphates.

I. Influence of Arsenate on the Rate of Fermentation.

When a solution of sodium arsenate is added to a mixture of glucose, mannose or fructose with yeast-juice, which has attained a steady rate of fermentation, an immediate large increase in the rate of evolution of carbon dioxide is produced. The rate, as a rule, rises for a short period, attains a maximum, and then very gradually diminishes. A typical example is the following.

Experiment 1.—The experiments throughout were carried out at 25° in presence of toluene, the solutions being previously saturated with carbon dioxide. The standard solution of sodium arsenate employed was approximately 0.3 molar, and was prepared by dissolving 5.312 grammes of crystallised sodium arsenate in water, and making up to 100 c.c. Two

* Harden and Young, 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 410.

separate mixtures were made, each containing 25 c.c. yeast-juice and 2.5 gm. of glucose, and were incubated until a steady rate of fermentation had been attained. There were then added (*a*) 25 c.c. of water and 2.5 gm. glucose; (*b*) 20 c.c. of water + 5 c.c. standard arsenate solution and 2.5 gm. glucose.

The rates of evolution of carbon dioxide were then observed, the apparatus previously described being employed.

Time.	<i>a.</i>		<i>b.</i>	
	Total gas.	Average rate per 5 minutes during interval.	Total gas.	Average rate per 5 minutes during interval.
5	2.1	2.1	23.3	23.3
10	4.9	2.8	47.8	24.5
15	8	3.1	72.5	24.7
20	10.7	2.7	99.5	27
30	15.9	2.6	154.1	27.3
40	21.4	2.8	208.4	27.15
50	26.5	2.55	259.3	25.45
65	34.5	2.7	332.2	24.3
95	50.6	2.7	459.0	21.1

A striking feature of the effect of the addition of a phosphate to yeast-juice is that the marked acceleration only continues until an amount of carbon dioxide has been evolved which is chemically equivalent to the phosphate added. Moreover, at the close of this period of enhanced fermentation, the added phosphate is no longer present in a form precipitable by magnesium citrate mixture, but has become converted into a hexose-phosphate. Neither of these phenomena occurs when an arsenate is substituted for the phosphate. The enhanced rate of fermentation continues long after an equivalent of carbon dioxide has been evolved, and no organic combination of arsenic is formed.

The first of these statements follows from the results of Experiment 1. Here the arsenate added was equivalent to 36 c.c. of carbon dioxide in the ratio $\text{Na}_2\text{HAsO}_4:\text{CO}_2$, whilst the extra carbon dioxide evolved in the presence of the arsenate amounted to 408.4 c.c. in 95 minutes, and the rate at the end of that time was still more than seven times that of the juice without arsenate.

The sharp contrast between the actions of arsenate and phosphate is clearly shown when the effects of yeast-juice are directly compared, as is done in the following experiment.

Experiment 2.—(1) 20 c.c. yeast-juice + 5 c.c. of 0·3 molar phosphate ; (2) 20 c.c. yeast-juice + 5 c.c. of 0·3 molar arsenate ; (3) 20 c.c. yeast-juice + 0·75 c.c. of 0·3 molar arsenate.

Excess of sugar was present in each case, and the solutions of phosphate and arsenate were each equivalent to 36 c.c. of carbon dioxide for 5 c.c. of the solution. The normal rate of fermentation of the juice was 1·8 c.c. per five minutes.

Time.	1.		2.		3.	
	Total.	Rate per 5 minutes.	Total.	Rate per 5 minutes.	Total.	Rate per 5 minutes.
5	7·1	7·1	11·4	11·4	21·7	21·7
10	19·8	12·7	26·5	15·1	46	24·3
15	36·1	16·3	43	16·5	71	25
20	43·8	7·7	59	16	95·3	24·3
25	45·7	1·9	75	16·3	119·8	24·5
30	47·5	1·8				

Calculating the extra amount of carbon dioxide evolved in each case beyond that due to the normal rate of the juice, it is found that, in presence of phosphate, this amounts to 36·7 c.c., and that the rate has fallen to that of the original juice. In presence of arsenate, although the extra amount of carbon dioxide is 66·3 c.c., the rate has remained unaltered at the high value of 16. The disparity between this extra amount of carbon dioxide and the equivalent of the arsenate added is still more strikingly illustrated when only a small amount of arsenate is added. This is shown in No. (3) above, to which only 0·75 c.c. of arsenate, equivalent to 5·4 c.c. of carbon dioxide, was added. In spite of this the extra amount of carbon dioxide evolved in 25 minutes was 120·8 c.c., and the rate at the end of that time was still 24·5 c.c. per 5 minutes.

In order to show that the whole of the arsenate is still present in its original state after the evolution of an equivalent amount of carbon dioxide, the following experiment was made:—

Experiment 3.—Four quantities of 25 c.c. of yeast-juice + 2·5 gm. of glucose were incubated until a steady rate had been attained.

To these were added:—

(1) 5 c.c. water.

(2) 5 c.c. of sodium arsenate solution, yielding 0·2211 gm. of $\text{Mg}_2\text{As}_2\text{O}_7$, and equivalent to 35 c.c. of carbon dioxide.

(3) 5 c.c. of sodium arsenate, as above, and 2·5 grm. of glucose.

(4) 5 c.c. of sodium phosphate equivalent to the arsenate added to (2), and yielding 0·1553 grm. of $\text{Mg}_2\text{P}_2\text{O}_7$.

The fermentations of 1 and 2 were then observed until the evolution of carbon dioxide from (2) was approximately equal to that from (1) + 35 c.c., and the two liquids were then boiled and filtered and the weight of precipitate yielded by magnesium citrate mixture ascertained.

Liquid (3) was allowed to ferment until a total of 713 c.c. had been evolved, and was then boiled and treated as above.

Finally (4), which was intended to demonstrate the different behaviour of phosphate, was allowed to ferment until the rate again became normal, and was then boiled and precipitated with magnesium citrate mixture.

The following table shows the total weight of precipitate produced in each case by magnesium citrate mixture:—

	Original juice.	Added.	Total before incubation.	Total after incubation.	Difference.
1	0·0678	0	0·0678	0·0678	0
2	0·0678	0·2211	0·2889	0·3007	+0·0118
3	0·0678	0·2211	0·2889	0·3513	+0·0624
4	0·0678	0·1553	0·2231	0·1025	-0·1206

These numbers clearly show that the whole of the arsenate remains precipitable by magnesium citrate mixture, even after prolonged fermentation, whereas the phosphate is converted into a non-precipitable form.

This fact is, however, not sufficient to exclude the possibility of the formation of an arsenic analogue of a hexosephosphate, since this might be precipitable by magnesium citrate mixture.

Experiment 4.—The precipitate obtained from another experiment conducted on similar lines to (2) was therefore carefully washed, dried at 100°, mixed with copper oxide, and submitted to combustion in a current of oxygen; 0·3621 grm. gave 0·0069 grm. of CO_2 , corresponding with 0·052 per cent. of carbon. A magnesium hexose-arsenate would contain 15·3 per cent. of carbon, so that the result of this analysis shows that no appreciable amount of such a compound is present. The trace of carbon actually found was no doubt derived from occluded matter from the boiled yeast-juice in which the precipitate was produced. The arsenate therefore remains free throughout the fermentation.

2. *The Products of Fermentation in Presence of Arsenate.*

In order to ascertain whether the reaction which occurred in the presence of arsenate was the normal decomposition of sugar into alcohol and carbon dioxide, the ratio of these products was determined for an arsenate fermentation.

Experiment 5.—75 c.c. of yeast-juice were incubated with 8 gramm. of glucose and 15 c.c. of 0.1 molar sodium bicarbonate solution in order to remove all free acid and free phosphate. In one portion of this the alcohol was estimated at once by distillation. To each of two other equal portions, 1 c.c. of arsenate solution was added, and both were incubated at 25° for 1.5 hours. During this period 185.3 c.c. of pure carbon dioxide, measured moist at 20.8° and 763.6 mm., were evolved, weighing 0.3303 gramm. At the end of this time the total alcohol was estimated in one of the portions, and the alcohol produced in presence of arsenate thus ascertained by subtracting that originally present. The following were the results:—

	Before incubation.	After incubation.	Difference.	Ratio.
Alcohol	0.4898	0.7924	0.3026	0.92
Carbon dioxide	0	0.3303	0.3303	

The theoretical ratio is 1.04. The value obtained agrees well with the ratio previously found by similar methods for the normal fermentation of glucose by yeast-juice.

3. *Effect of the Concentration of Arsenate on the Maximum Rate of Fermentation Attainable.*

The rate attained increases with the concentration of arsenate until a certain limit is reached. A further increase beyond this point diminishes the rate of fermentation, so that just as in the case of phosphate there is a certain optimum concentration of arsenate, corresponding with which there is a maximum rate of fermentation.

In carrying out these estimations of rate of fermentation, a mixture of glucose with yeast-juice, 10 gramm. to 100 c.c., was incubated until the rate of evolution of carbon dioxide had become constant, and all free phosphate had been converted into hexosephosphate. The standard sodium arsenate solution (0.3 molar), containing 10 gramm. of glucose per 100 c.c. and saturated with carbon dioxide at 25°, was then added in the desired quantity. Since sodium arsenate solution after saturation with carbon dioxide contains an equilibrium mixture of the two arsenates and sodium

bicarbonate, the amount of the latter present in the arsenate solution used was previously estimated by neutralising a known quantity with hydrochloric acid and measuring the carbon dioxide evolved. A solution of sodium bicarbonate, also containing 10 grm. of glucose per 100 c.c., was then made up which yielded the same amount of carbon dioxide when neutralised as the arsenate solution. The liquid in each flask was made up to the same volume with this sodium bicarbonate solution. The fermentation flasks containing the proper quantities of arsenate solution and bicarbonate were then incubated at 25° and the rates of fermentation observed. The effect of bicarbonate is to cause a slight increase in the rate of fermentation, owing to a diminution of the acidity of the juice (Buchner).

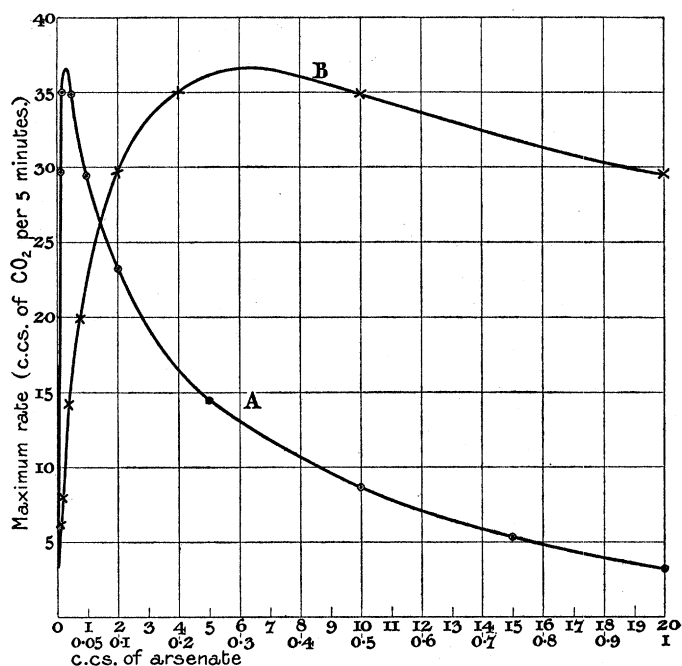
The following results, all obtained from a single sample of yeast-juice, are typical, and show the nature of the relation between concentration of arsenate and rate of fermentation.

When the concentration of arsenate is very low, the rate falls off very rapidly from the maximum, and the numbers obtained in these cases can only be regarded as approximate. This is the case in the present set of observations for amounts of arsenate less than 0.1 c.c.

Experiment 6.—20 c.c. of yeast-juice + x c.c. of 0.3 molar arsenate + $(20-x)$ c.c. sodium bicarbonate solution. Glucose, 4 grm.

Arsenate solution per 40 c.c. mixture.	Molar concentration of arsenate in fermenting mixture.	Maximum rate of fermentation.
c.c.		c.c. per 5 minutes.
0	0	3.5
0.005	0.000037	6.3
0.01	0.000075	8.0
0.02	0.00015	14.2
0.04	0.0003	19.9
0.1	0.00075	29.7
0.2	0.0015	35.0
0.5	0.00375	34.9
1	0.0075	29.5
2	0.015	23.2
5	0.0375	14.5
10	0.075	8.7
15	0.1125	5.3
20	0.15	3.2

The same results are exhibited graphically in the accompanying curves, in which the rate is plotted against the cubic centimetres of arsenate present in the mixture. Curve B represents the earlier portion of A, plotted with a twenty-fold scale of abscissæ.



The striking feature of the curve is the rapid rise of rate with the concentration, followed by a fall, at first rapid and then gradual. The reason for this fall is discussed later on.

The smallest amount of arsenate recognisable by a change of rate of fermentation in this experiment corresponded with the presence of 0.11 mgrm. of arsenic in the fermenting liquid.

Both the optimum amount of arsenate and the maximum rate of fermentation produced by it vary with the sample of yeast-juice employed, and the following numbers illustrate this point:—

Experiment.	Volume.	Juice alone.	Maximum obtained.	Ratio.	Optimum molar concentration of arsenate.
		c.c. per 5 minutes.			
7	20	3.5	36.5	10.4	0.00225
8	20	1.3	14.2	10.9	0.01125
9	20	1.6	25	15.6	0.005625

4. *Effect of the Addition of Arsenate on the Total Fermentation produced by Yeast-juice.*

When arsenate is added to yeast-juice and sugar, the rate of fermentation more or less rapidly reaches the maximum corresponding with the

concentration of arsenate employed. This rate is maintained for a varying time, according to the amount added, and then slowly falls off until ultimately fermentation ceases.

When the concentration of arsenate is comparatively high, fermentation ceases much sooner than in the absence of arsenate, and this appears to be due to some direct action on the fermenting complex, similar to that which was observed by Buchner as resulting from the addition of potassium arsenite, and which is discussed later on. When, on the other hand, a small concentration of arsenate is used a high rate is produced, fermentation continues for a longer period than in the absence of arsenate, and a greatly increased total fermentation results. The total amounts of carbon dioxide and alcohol produced therefore depend on the particular concentration of arsenate employed, and may be either higher or lower than that given by the juice in absence of arsenate.

Experiment 10 is a typical example of the phenomena observed when a moderate quantity of arsenate is used, while Experiment 11 exhibits the effect of variation of concentration of arsenate both on the rate and the total fermentation.

Experiment 10.—Parallel experiments were made with juice alone, and in the presence of arsenate, the solutions having the following compositions:—

1. 20 c.c. yeast-juice + 15 c.c. sodium bicarbonate + 5 c.c. arsenate.
2. 20 c.c. yeast-juice + 20 c.c. sodium bicarbonate.

Each solution contained 4 gramm. of glucose.

Time.		1.		2.	
		Rate per 5 minutes.	Total evolved.	Rate per 5 minutes.	Total evolved.
h.	m.	c.c.		c.c.	
	15	9.5		2.1	
	30	13		2.1	
	45	14.2		1.9	
1		13.9	142.5	1.8	22.2
1	15	12.8		1.6	
1	30	11.9		1.5	
2		9.8	288	1.2	39.5
4		3.4	436	0.9	65.2
6		1.7	498	0.7	85
10		0.2	544	0.41	108
14		0	550	0.33	122
35				0	133

It will be seen that in the presence of arsenate (1) the maximum rate is attained in about 45 minutes, and amounts to 14.2 c.c. per 5 minutes; the total carbon dioxide evolved is 550 c.c., and fermentation ceases in about

12 to 14 hours. In the absence of arsenate (2), the maximum is attained at once and is 2.1, the total evolved is 133 c.c., and the duration of the fermentation is about 24 to 30 hours.

Experiment 11.—The totals evolved by equal volumes of the juice employed in the foregoing experiment were determined with various additions of arsenate, and are quoted along with the maximum rates and the approximate duration of fermentation.

No.	Arsenate added.	Maximum rate.	Total evolved.	Duration of fermentation.
	c.c.			hours.
1	0	2.8	133.3	24—30
2	0.5	25	1024	55—60
3	5	14.2	550	12—14
4	10	5.9	190.6	9—10
5	20	1.5	47.8	7

4 gramm. of glucose were present in each case, and it is probable that fermentation in No. 2 came to a close on account of exhaustion of the sugar.

5. *The Fermentation of Mannose and Fructose by Yeast-juice in the Presence of Arsenate.*

The fermentation of mannose by yeast-juice is also accelerated by the addition of arsenate to about the same extent as that of glucose. In the case of fructose, however, a difference, similar in character to that previously noted in the presence of phosphate,* is also observed with arsenate, viz., the optimum concentration of arsenate is greater and the maximum rate attainable higher than in the case of glucose.

These facts are well brought out by the results of the following two experiments.

Experiment 12.—A series of different volumes of the standard arsenate solution were added to yeast-juice in presence of 1 gramm. of the sugar in question, all the liquids being made up to the same volume, and the maximum rate attained in each case observed.

20 c.c. yeast-juice + x c.c. arsenate + $(26-x)$ c.c. of a solution containing 1 gramm. of sugar.

* Harden and Young, 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 336.

x.	Maximum rate for 5 minutes.		
	Glucose.	Mannose.	Fructose.
0	0·63	0·65	0·65
0·2	7·0		
0·5	9·1		11·7
1	9·8	8	14·4
2	9·7	6·6	16
4	4	4·5	14·8
8			8·4
16			2·4

Experiment 13.—A similar experiment was made with a mixture of 20 c.c. of yeast-juice and 2 gramm. of the sugar, the total volume being 40 c.c.

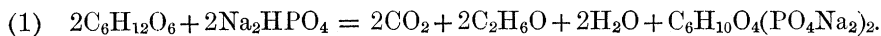
Arsenate.	Maximum rate in 5 minutes.	
	Glucose.	Fructose.
c.c.		
0	1	1·2
0·2	7·2	
1	12·1	26·6
2	12·4	
3	13·4	
5	13·4	43·6
7		45·8
10		42·4
15	5·1	39

In this case the optimum concentration of arsenate in the presence of glucose is between 3 and 5 c.c., and the maximum rate is about 13·4, which is 13·4 times the normal rate. In the presence of fructose, on the other hand, the optimum concentration is about 7 c.c., and the maximum rate 45·8 c.c., which is 38·2 times the normal rate.

In Experiment 12 the juice evidently contains less fermenting complex, and there is not such a great disparity between the optima. The maximum rate for glucose is 9·8 c.c., for mannose, slightly less, 8 c.c., and for fructose, 16 c.c.

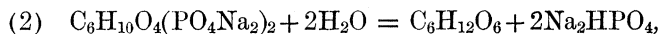
6. *Nature of the Effect of Arsenate on Fermentation by Yeast-juice.*

In considering the nature of the action of arsenate in accelerating the fermentation of sugars by yeast-juice two possibilities present themselves. In the first place the arsenate may replace the phosphate in the main reaction :



The facts that much more than an equivalent of carbon dioxide is evolved at the accelerated rate and that the arsenate is found in the free state at every stage of the fermentation render it necessary to suppose that any arsenic compound which is formed is of a very unstable nature, and only exists at any moment in a very low concentration.

On the other hand, it is also possible that the arsenate accelerates the fermentation by increasing the rate at which free phosphate is produced from the hexosephosphate. This substance is hydrolysed by the enzyme hexosephosphatase according to the equation :



and it is the rate of this reaction which is the controlling factor of the rate of fermentation of sugar by yeast-juice in the absence of added phosphate.*

Acceleration of this change would therefore result in a constant increased supply of phosphate, and would enable reaction (1) to proceed at a correspondingly greater rate. In other words, the effect of the arsenate would be to produce a condition similar to that existing when free phosphate is added gradually to yeast-juice so that its concentration is maintained at the optimum, and the reaction proceeds at its maximum velocity as long as this condition is maintained.†

It also follows from the fact that the rate of fermentation is dependent upon the rate of production of free phosphate that the acceleration produced by the addition of arsenate cannot be due to an increase in the activity of the zymase alone. In a mixture of fresh yeast-juice and sugar this enzyme is not producing its maximum effect, as is shown by the fact that the addition of phosphate causes a large increase in the rate of fermentation. In other words there is an excess of enzyme already present in the mixture. An increase in the activity of the zymase, therefore, without an increase in the supply of phosphate, could not be expected to cause any acceleration in the rate of fermentation. It is possible, however, that the activity both of the hexose-phosphatase and of the zymase might be simultaneously increased by the addition of arsenate. In this case it would be possible to obtain a higher maximum by the addition of arsenate in presence of the right amount of phosphate, than could be obtained by adding phosphate alone.

1. *Proof that Arsenate cannot replace Phosphate in the Fermentation of Sugars by Yeast-juice.*—The first of these alternative explanations, according to which arsenate is supposed to take the place of phosphate and to react with the sugar to form an unstable hexose-arsenate, can be investigated in

* Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

† Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 309.

the following way. A mixture of ferment and co-ferment can be prepared as previously described, which is free from phosphate and hexosephosphate,* and will not ferment sugar without the addition of phosphate. If now arsenate can replace phosphate, such a mixture should be rendered active by the addition of arsenate as well as by that of phosphate.

In order to realise these conditions zymin was freed from phosphates and co-ferment by repeated washing with water, until it was incapable of fermenting sugar. To this was added a solution of co-ferment, free from mineral phosphates and hexosephosphate, as previously described. This mixture was almost incapable of fermenting sugar, whilst the addition of phosphate, either in the form of mineral phosphate or hexosephosphate, readily caused fermentation. When, however, arsenate was substituted for the phosphate no fermentation ensued. This was not due to any inhibiting action of the arsenate, as in the presence of phosphate the same quantity of arsenate did not interfere with the fermentation.

Exp.	Zymin suspension.	Co- ferment solution.	Water.	Fructose.	Phosphate, 0·3 molar.	Hexose- phosphate.	Arsenate, 0·3 molar.	Gas evolved.
	c.c.	c.c.	c.c.	grm.	c.c.	c.c.	c.c.	c.c. hrs. mins.
16. 1	10	20	1	2	0	0	0	7 in 24
2	10	20	0	2	1·0	0	0	67 „ 24
3	10	20	0·75	2	0	0	0·25	0·2 „ 2
4	10	20	0	2	0	0	1	2·7 „ 24
5	10	20	0·25	2	0·5	0	0·25	51·6 „ 24
17. 1	10	20	6·3	0·7	0	0	0	1·5 „ 19
2	10	20	0	0·7	0	6·3	0	132·7 „ 19
3	10	20	6·3	0·7	0	0	0·5	1 „ 19
4	10	20	0	0·7	0	6·3	0·5	133·5 „ 19
18. 1	10	15	0	0·5	0	0	0·2	2·0 „ 20
2	10	15	8·8	0·5	0	8·8	0	25·8 „ 1 50
3	10	15	0	0·5	0	8·8	0·2	47·8 „ 1 50

The hexosephosphate solution used was 0·21 molar for Experiment 17 and 0·14 molar in Experiment 18.

It will be seen that in no case was arsenate able to take the place of phosphate, and it must therefore be concluded that the action of arsenate is fundamentally different from that of phosphate.

Incidentally the foregoing experiments afford further evidence that phosphate is indispensable for the alcoholic fermentation of sugar. Comparing Nos. 1 and 2, Experiment 17, it is seen that in the almost complete absence of available phosphate only 1·5 c.c. of carbon dioxide were

* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 322.

evolved, whereas in the presence of hexosephosphate 132·7 c.c. were obtained. The ratio of the totals produced in presence and in absence of phosphate is thus $132\cdot7/1\cdot5 = 88$, a much greater value than had previously been observed.*

2. *Acceleration of the Action of Hexosephosphatase by Arsenate.*—The direct replacement of phosphate by arsenate being excluded, the alternative explanation, that the arsenate accelerates the action of the hexosephosphatase, remains to be considered. This question can readily be investigated experimentally, and the result of such an examination is to show that the arsenate does in fact produce a very marked effect of this kind, and that it is to this acceleration that the enhanced rate of fermentation in the presence of arsenate is due. This has been proved separately for yeast-juice and zymïn.

In the three experiments carried out with yeast-juice, a comparison was made between the rates of fermentation in mixtures of yeast-juice with (*a*) hexosephosphate and arsenate; (*b*) water and arsenate; (*c*) hexosephosphate alone.

In experiments such as these, done in the absence of added sugar, the rate of fermentation is controlled by the production of sugar (1) from the hexosephosphate; and (2) from the glycogen and dextrins of the juice. Hence, by subtracting the rate of auto-fermentation in presence of arsenate (*b*) from the rate of fermentation of hexosephosphate also in presence of arsenate (*a*) a difference is obtained which represents the share of this enhanced rate which is due to the hydrolysis of the hexosephosphate. This is given by $a-b$, and a comparison of this with the rate of fermentation of hexosephosphate in absence of arsenate shows whether the effect of the arsenate has been to increase the rate of hydrolysis of the hexosephosphate. (In order to obtain the true rate of evolution due solely to the hexosephosphate in absence of arsenate the rate of auto-fermentation of the juice should be subtracted from that observed in presence of hexosephosphate (*c*). As, however, it is not certain what effect the presence of hexosephosphate may have upon the enzymic hydrolysis of glycogen, etc., this has not been done in the present instance. The effect of making this subtraction would be considerably to increase the cogency of the argument.)

In order as far as possible to diminish the auto-fermentation, the yeast-juice employed in Experiments 19 and 20 was incubated for some time before the addition of the hexosephosphate and water, and the yeast employed for the preparation of the juice for Experiment 21 was treated for the removal of glycogen by the method described by Buchner and Mitscherlich, but still yielded a juice which gave a considerable amount of auto-fermentation.

* 'Roy. Soc. Proc.' B, 1908, vol. 80, p. 324.

Experiments 19, 20, 21.—In each case 25 c.c. of yeast-juice + 0.5 c.c. of 0.3 molar arsenate + 20 c.c. of water or a normal solution of sodium hexose-phosphate (containing 8.5 gm. of the acid per 100 c.c.) were employed, the liquids being made up to 45.5 c.c.

No. of experiment.	Time after addition of arsenate, in minutes.	Total c.c. evolved in the time <i>t</i> .			
		<i>a.</i> Arsenate + hexosephos- phate.	<i>b.</i> Arsenate + water.	<i>a</i> - <i>b</i> .	<i>c.</i> Hexosephos- phate + water.
19	15	40.5	26.2	14.3	6.3
	30	77.7	50.5	27.2	12.1
	75	147.9	90.6	57.3	22.3
	205	311.7	165.9	145.8	55.4

No. of experiment.	Time after addition of arsenate.	Rate in c.c. evolved in preceding 5 minutes.			
		<i>a.</i>	<i>b.</i>	<i>a</i> - <i>b</i> .	<i>c.</i>
20	h. m.				
	5	2.9	0.5	2.4	1.4
	10	3.7	0.6	3.1	0.9
	15	3.7	0.4	3.3	0.8
	20	3.7	0.5	3.2	0.9
	25	3.9	0.3	3.6	0.7
	30	3.5	0.4	3.1	0.7
	Total...	21.4	2.7	18.7	5.4
21	5	9.1	3.0	6.1	3.1
	10	8.6	2.3	6.3	2.9
	15	7.3	1.9	5.4	0.9
	15 20	6.8	1.2	5.6	1.2
	35	6.4	1.3	5.1	1.3
	40	6.9	1.7	5.2	1.6
	45	6.1	1.3	4.8	0.9
	50	5.6	1.0	4.6	0.8
	Total...	56.8	13.7	43.1	12.7

It will be seen that in every case the fermentation in presence of hexose-phosphate and arsenate, less the auto-fermentation in presence of arsenate (*a* - *b*) is considerably greater (two to four times) than the rate of fermentation in absence of arsenate (*c*).

As this increased fermentation is carried on at the expense of the hexosephosphate, it follows that the hexosephosphate is being more rapidly

decomposed than in the absence of arsenate, and that therefore free phosphate, which is also a product of the hydrolysis of this compound, must also be liberated in greater quantity. Since, moreover, hexosephosphate yields two equivalents of phosphate for one of sugar the phosphate must accumulate, and accordingly the rate of decomposition of the hexosephosphate can also be gauged by the rate of accumulation of free phosphate.

Experiment 22.—This has been ascertained by estimating the amount of free phosphate in the boiled and filtered solutions from Experiment 19. The following are the results, expressed in grams of $\text{Mg}_2\text{P}_2\text{O}_7$, the equivalent of the arsenate present having been subtracted in each case :—

(a)	Hexosephosphate + arsenate.....	0·6136
(b)	Auto-fermentation + arsenate	0·1144
(c)	Hexosephosphate without arsenate	0·0707

Hence, in presence of arsenate, $0·6136 - 0·0707 = 0·5429$ grm. has been liberated more than in its absence, and of this amount $0·5429 - 0·1144 = 0·4285$ grm. has been formed from the hexosephosphate which was added.

This result is therefore in entire agreement with the observations of the rates of fermentation.

Experiments with zymín confirm the conclusion that the arsenate accelerates the action of the hexosephosphatase in an equally striking manner. Zymín washed free from soluble phosphates and co-ferment was used, and a solution of co-ferment also almost free from available phosphate was added, together with an excess of fructose. This mixture was almost incapable of producing fermentation, owing to lack of phosphate, and as a matter of fact gave a total of only 1·5 c.c. of carbon dioxide, whereas when phosphate was added as much as 132 c.c. were involved. Solutions of hexosephosphate were added to two equal volumes of this mixture, and to one of these arsenate was also added. The sole source of phosphate in these two liquids was the hexosephosphate, and the rate of fermentation would therefore be controlled by the rate of production of phosphate from this. Any increase of fermentation in the presence of arsenate must therefore be due to the accelerating action of this salt on the hexosephosphatase.

Experiment 23.—The zymín and co-ferment solution were prepared in the same manner as those used in Experiment 17.

1. 10 c.c. zymín suspension + 15 c.c. co-ferment solution + 0·5 grm. fructose + 8·8 c.c. of 0·14 molar hexosephosphate + 0·2 c.c. arsenate.

2. The same mixture but containing 0·2 c.c. of water instead of arsenate.

Here the rate of fermentation, and consequently of hydrolysis of hexosephosphate, in presence of arsenate is about double that in its absence.

Time after addition of arsenate, in minutes.	C.c. evolved in preceding 5 minutes.	
	1.	2.
5	2·8	1·4
10	2·7	1·4
15	2·5	1·2
20	2·3	1·0
25	2·3	—
65	2·4	1·3
90	2·2	1·3
115	1·84	1·04
Total in 115.....	47·8	25·8

Whether the arsenate also accelerates the action of the zymase has not been decided with certainty. If this were so, the rate of fermentation attainable by the proper mixture of arsenate and phosphate would be greater than that given by phosphate alone. Since, however, the rate of fermentation in presence of phosphate falls off with diminishing concentration of this salt extremely rapidly and according to a law which has not yet been ascertained, it is impossible at present to institute an accurate comparison between the velocity constants of the two reactions. It may, however, be noted that cases have been observed in which the evolution of gas in five minutes was greater in presence of a certain quantity of arsenate than could be obtained with phosphate alone.

7. *Effect of Arsenate on the Auto-fermentation of Yeast-juice.*

When arsenate is added to yeast-juice which is undergoing auto-fermentation, a very considerable rise is produced in the rate of evolution of carbon dioxide; the rate attained is, however, much lower than that produced in presence of excess of glucose. The extent of this enhanced auto-fermentation is shown by the following (Nos. 1 and 2), whilst the effect of the same quantity of arsenate in presence of excess of glucose is given in columns 3 and 4.

Experiment 14.—Four solutions were incubated, all being made to the same volume of 30·5 c.c.

1. 20 c.c. yeast-juice + 10·5 c.c. water + 0 c.c. arsenate.
2. " " + 10 " + 0·5 "
3. " " + 9·3 " + 0 " + 2 gm. glucose.
4. " " + 8·8 " + 0·5 " + 2 "

The following rates were observed:—

	No. 1.	No. 2.	No. 3.	No. 4.
Initial rate per 5 minutes	1·5	8·5	2·5	36·5
Total in 1 hour 40 minutes	27·2	81·2		

The rate of auto-fermentation of yeast-juice is not, like the rate of fermentation of sugar, controlled by the rate at which phosphate is produced from the hexosephosphate, but by that at which sugar is formed by the hydrolysis of glycogen and dextrins, as is shown by the well known fact, confirmed in the foregoing experiment, that the rate of auto-fermentation is usually considerably less than that of the fermentation of sugar.

Consequently, any increase in the rate of auto-fermentation must be primarily due to an increased supply of sugar. This may be derived from two sources. A portion is supplied, as has been shown (Experiments 19–23), by the increased rate of hydrolysis of the hexosephosphate originally present in the juice. This continues until the whole of the sugar obtainable from this source has been converted into carbon dioxide and alcohol and has the effect of temporarily increasing the rate of fermentation. This acceleration is independent of the presence or absence of glycogen, and hence the effect of arsenate on the juice in the absence of added glycogen gives a superior limit to the extent of its influence.

After this point has been reached, the sugar formed by the hydrolysis of complex carbohydrates becomes the sole source of carbon dioxide. As fermentation then proceeds this supply also gradually becomes exhausted and the rate falls off slowly. In presence of added glycogen, on the other hand, the rate falls off more slowly.

In order, therefore, to ascertain whether the rate of hydrolysis of glycogen is increased by the addition of arsenate, it is necessary to determine whether the effect of adding glycogen and arsenate together to the juice is greater than the sum of the effects of adding glycogen and arsenate to separate portions of the juice.

This is done in the following experiment:—

Experiment 15.—Five quantities of 20 c.c. of yeast-juice were mixed with the following solutions and incubated for 1 hour 45 minutes, the total volume in each case being 30·5 c.c.—

1. 2·5 grm. glucose in 10·5 c.c. solution.
2. 10·5 c.c. water.
3. 10 c.c. water + 0·5 c.c. arsenate.
4. 0·5 grm. yeast-glycogen in 10·5 c.c. solution.
5. 0·5 grm. yeast-glycogen in 10 c.c. solution + 0·5 c.c. arsenate.

	1.	2.	3.	4.	5.
Initial rate (c.c. per 5 minutes)	2.5	1.5	8.5	1.9	10.0
Rate after 1 hour	—	1.4	2.9	1.6	7.2
Rate after 1 hour 40 minutes... ..	—	1.1	1.85	1.35	4.45
Total evolved in 1 hour 45 minutes... ..	—	27.0	81.2	32.4	141.2

It is to be noted, in the first place, that the rate in presence of glycogen (1.9) is less than in presence of sugar (2.5), so that the rate of fermentation in No. 4 is actually controlled by the rate of production of sugar.

The effect of adding glycogen to the juice increases

the total by (4)-(2) $32.4 - 27 = 5.4$ c.c.

The effect of adding the arsenate to the juice alone

increases the total by (3)-(2) $81.2 - 27 = 54.2$ „

Sum of the separate effects of glycogen and

arsenate $54.2 + 5.4 = 59.6$ „

Effect of adding glycogen and arsenate

together (5)-(2)..... $141.2 - 27 = 114.2$ „

Hence at least $114.2 - 59.6 = 54.6$ c.c. more carbon dioxide have been derived from glycogen in the presence of arsenate than in its absence. Comparing the rates per five minutes given in columns 3 and 5, showing the effect of arsenate on the fermentation in absence and in presence of added glycogen, it is to be noticed that the initial rates are not greatly different (8.5 and 10). This is due to the fact that at the commencement of the auto-fermentation there is an ample supply of glycogen. This, however, soon begins to fail, and consequently the rate falls off more rapidly than in the presence of added glycogen, the relative rates after 1 hour being 2.9 and 7.2, and after 1 hour 40 minutes, 1.85 and 4.45. For this reason the comparison is best made between the totals evolved after some time and not between the initial rates.

Further evidence in support of the conclusion that the hydrolysis of glycogen is accelerated by the addition of arsenate is afforded by the effect of the addition of glycogen to Solutions 2 and 3 in the foregoing experiment, which had been allowed to auto-ferment in absence and presence of arsenate respectively.

	No. 2.	No. 3.
5 minutes' rate before addition of glycogen	1.1	1.85
„ „ after	1.4	4.4

Even this enhanced rate of glycogen hydrolysis is not nearly rapid enough to supply sufficient sugar for the fermenting complex in presence of arsenate. The maximum rate obtained with glycogen was 10, whereas the addition of 0.5 c.c. of arsenate to Solution 1 containing excess of glucose produced a rate of 36.5 c.c. in five minutes.

It follows from the foregoing that arsenate has the power of greatly accelerating the action of the diastatic enzyme in yeast-juice.

8. Action of Arsenite on the Fermentation of Sugar by Yeast-Juice.

Buchner* made a number of observations on the effect of arsenite on the fermentation of sugars by yeast-juice. The presence of 2 per cent. of arsenious oxide, in the form of the potassium salt, was found in some cases completely to inhibit the fermentation of glucose, but not of saccharose, its effect on fructose being of an intermediate character, whilst, in other cases, the same concentration caused a marked increase in the total fermentation of all three sugars.

A powerful inhibitory effect was also produced by this concentration of arsenite, even on the fermentation of saccharose by (a) juice which had been prepared from yeast which had been kept at 8° for three days, (b) juice which had been dialysed against water for 16 hours, (c) juice which had been evaporated at 35°, (d) juice diluted with one volume of water.

It was further observed, in many cases, that the presence of arsenite caused a considerable increase in the fermentation during the first 16 hours, even when the total fermentation was less than in the absence of arsenite. It was found that this inhibitory action was not due to the conversion of the arsenite into arsenate; and, further, that it was lessened by the addition of heated yeast-juice or blood serum, either heated or unheated. The inhibitory action, moreover, was greater when the arsenite was incubated with the juice in the absence of added sugar. Buchner advanced no explanation of the preliminary acceleration caused by arsenite, and regarded the inhibitory effect as due to some change in the colloidal state of the enzyme, against which the presence of other colloidal substances partially protected it. It was, according to this, the diminution in the amount of these protecting colloids which made the samples of juice submitted by him to various operations so much more susceptible to the action of arsenite.

A careful experimental comparison of the effects of arsenite and arsenate on fermentation by yeast-juice shows that these two actions are precisely similar in kind, but that the acceleration produced by arsenite is markedly

* 'Die Zymasegärung,' pp. 184—205.

less than that caused by an equivalent concentration of arsenate. This similarity of the action of arsenite to that of arsenate is shown in the experiments summarised below.

(1) Arsenite produces an acceleration which continues long after an extra amount of carbon dioxide equivalent to the arsenite added has been evolved.

Experiment 24.—Yeast-juice containing 10 grm. of glucose per 100 c.c. was incubated until a steady rate of fermentation had been attained. To 20 c.c. of this were added 5 c.c. of a 0.3 molar solution of sodium arsenite, equivalent to 33.6 c.c. of carbon dioxide at N.T.P., and the rate of fermentation observed.

Time after addition, in minutes.	Rate in c.c. of carbon dioxide evolved in preceding 5 minutes.	Total.	Time after addition, in minutes.	Rate in c.c. of carbon dioxide evolved in preceding 5 minutes.	Total.
0	1.7		25	6.2	32.8
5	7.0	7.0	30	5.9	38.7
10	7.1	14.1	45	5.6	56.5
15	6.2	20.3	60	5.6	73.2
20	6.3	26.6	85	3.3	89.7

The total evolved in 85 minutes is thus 89.7 c.c., whereas in absence of arsenite it would have been $17 \times 1.7 = 28.9$, and hence the extra carbon dioxide is $89.7 - 28.9 = 60.8$, which is largely in excess of the equivalent, 33.6.

(2) The acceleration produced by arsenite is much less than that obtained with an equivalent amount of arsenate.

Experiment 25.—20 c.c. of yeast-juice, containing 2 grm. of glucose, gave a rate of 1.7 c.c. in five minutes. The addition of 1 c.c. of a 0.3 molar solution of arsenite raised the rate to 3.1 c.c. in five minutes, whilst the addition of the same volume of 0.3 molar arsenate to the original juice gave a rate of 27.5 c.c.

(3) The rate attained increases with the concentration of the arsenite until a maximum is reached, so that, as in the case of arsenate, there is an optimum concentration of arsenite.

Experiment 26.—Varying quantities of a 0.3 molar solution of sodium arsenite were added to 20 c.c. of yeast-juice, the total volume being maintained at 40 c.c., and containing 10 per cent. glucose.

(4) The maximum rate attainable and the optimum concentration of arsenite are both greater in the case of fructose than in that of glucose.

C.c. arsenite added.	Rate in c.c. of carbon dioxide in 5 minutes.
0	2.1
1	3.4
3	6.4
5	6.9
13	4.5
20	0.8

Experiment 27.—The numbers in the following table refer to 20 c.c. of yeast-juice and 1 gram. of the sugar in 35 c.c. total volume.

C.c. molar arsenite added.	Rate in c.c. per 5 minutes.	
	Glucose.	Fructose.
0	0.34	0.5
2.5	1.17	1.45
5	2.7	3.5
10	2.7	7.96
15	1.48	9.8

It will be noted that the optimum concentration was not attained for fructose, whilst for glucose it was between 5 and 10 c.c.

(5) When a mixture of ferment, co-ferment, and sugar is rendered so free from phosphate that no fermentation will take place without the addition of phosphate, this added phosphate cannot be replaced by arsenite. Arsenite therefore cannot exercise the same function in alcoholic fermentation as phosphate.

Experiment 28.—The following experiment was carried out in a similar manner to Experiment 17 dealing with arsenate.

To equal volumes of a mixture of 10 c.c. of washed zymin suspension + 20 c.c. co-ferment solution free from phosphate + 0.22 gram. of fructose were added the following:—

	C.c. CO ₂ in 18 hours.
1. 6.5 c.c. water	1.5
2. 6 „ + 0.5 c.c. phosphate	38
3. 6 „ + 0.5 „ arsenite	1.4
4. 6 c.c. normal hexosephosphate + 0.5 c.c. arsenite	33

The result of (4) shows that the amount of arsenite present was not sufficient to inhibit the action of the enzyme, whilst (3) shows that arsenite alone produced no increase in fermentation.

(6) Arsenite accelerates the rate of hydrolysis of glycogen, and hence the auto-fermentation of yeast-juice.

Experiment 29.—The following solutions were incubated and the rates of fermentation observed :—

1. 20 c.c. of yeast-juice from well-washed yeast + 14 c.c. water.
2. 20 c.c. of the same juice + 9 c.c. water + 5 c.c. of 0.3 molar arsenite.

Experiment 30.—In this case 25 c.c. of yeast-juice were used along with (1) 15 c.c. of water; (2) 10 c.c. water and 5 c.c. of 0.3 molar arsenite.

Experiment.	Time, in minutes.	Auto. + water.		Auto. + arsenite.	
		Rate in 5 minutes.	Total.	Rate in 5 minutes.	Total.
29	5	0.6	0.6	2.6	2.6
	10	0.5	1.1	2.2	4.8
	15	0.8	1.9	2.2	7.0
	20	0.5	2.4	2.4	9.4
	60	0.55	6.8	2.6	26.7
30	5	0.9	0.9	4.5	4.5
	10	1.0	1.9	4.0	8.5
	15	1.0	2.9	—	—
	20	1.4	4.3	3.8	16.1
	60	1.3	9.8	4.3	33.5

That this increase is, at all events in part, due to an acceleration of the rate of hydrolysis of glycogen is shown in precisely the same manner as for arsenate (Experiment 15). The results obtained are as follows :—

Experiment 31.—Five quantities of 25 c.c. of yeast-juice were incubated with the following solutions and the fermentations observed :—

1. 0.5 gram. glucose in 15 c.c. solution.
2. 15 c.c. water.
3. 10 c.c. water + 5 c.c. of 0.3 molar arsenite.
4. 0.5 gram. glycogen in 15 c.c. solution.
5. 0.5 gram. glycogen in 10 c.c. solution + 5 c.c. arsenite.

	1.	2.	3.	4.	5.
Initial rate (c.c. per 5 minutes) ...	3.0	1.3	4.0	1.6	4.2
Total evolved in 5 hours	—	67.8	118.3	73.7	152.1

Here, as in the experiment with arsenate, the rate in presence of glycogen (1.6) is less than that in presence of glucose (3.0), so that the rate of the fermentation in (4) is actually controlled by the rate of production of sugar.

The effect of adding glycogen to the juice is to

increase the total by $73.7 - 67.8 = 5.9$

The effect of adding the arsenite alone to the juice

is to increase the total by $118.3 - 67.8 = 50.5$

Sum of separate effects of glycogen and arsenite ... $50.5 + 5.9 = 56.4$

Effect of adding glycogen and arsenite together is $152.1 - 67.8 = 84.3$

Hence at least $84.3 - 56.4 = 27.9$ c.c. more carbon dioxide have been derived from the glycogen in the presence of arsenite than in its absence in the same time.

(7) Arsenite produces its effect by accelerating the action of the hexose-phosphatase.

Experiment 32.—The experiments on this point were carried out exactly as described under arsenate (Experiments 19, 20, 21), but, in addition, the auto-fermentation of the juice (*d*) was observed—

<i>t.</i>	Total c.c. evolved in time <i>t.</i>					
	<i>a.</i>	<i>b.</i>	<i>c.</i>	<i>d.</i>	<i>a-b.</i>	<i>c-d.</i>
5	4.1	2.6	1.0	0.6	1.5	0.4
10	7.7	4.8	2.2	1.1	2.9	1.1
15	11.2	7.0	3.4	1.9	4.2	1.5
20	14.8	9.4	4.6	2.4	5.4	2.2
25	17.9	11.7	5.6	2.8	6.2	2.8
55	35.7	24.2	10.7	6.2	11.5	4.5
60	38.7	26.7	12.3	6.8	12.0	5.5
65	41.7	28.8	13.2	7.3	12.9	5.9

As already explained, the original fermentation due to hexosephosphate alone is given by *c-d*, and the enhanced fermentation due to hexose-phosphate by *a-b*. The effect of arsenite on the hexosephosphatase is very much less than that of arsenate, as was to be expected from the relative effects of the two on the fermentation of sugar, and is only clearly visible when the original auto-fermentation is subtracted from the fermentation in presence of hexosephosphate. The result in this case therefore depends on the assumption that the presence of the hexosephosphate and the products of its hydrolysis does not seriously affect the rate of hydrolysis of the glycogen.

Experiment 33.—An experiment was also carried out with washed zymin in precisely the same way as described for arsenate (Experiment 23), hexosephosphate being made the sole source of phosphate.

10 c.c. of washed zymin suspension + 15 c.c. of co-ferment solution free

from phosphate + 0.5 grm. of fructose + 9 c.c. of hexosephosphate solution + 1 c.c. of water, on addition of—

(1) 1 c.c. water gave 9.5 c.c. of carbon dioxide in 25 minutes.

(2) 1 c.c. of 0.3 molar arsenite solution gave 11.1 c.c. in 25 minutes.

In this case the rate was only increased about 20 per cent., whereas the addition of arsenate doubled the rate.

9. *Inhibitory Action of Arsenate and Arsenite.*

Both arsenate and arsenite may exert an inhibitory action on alcoholic fermentation, and this may manifest itself in two ways. If a sufficient excess be added, no fermentation at all occurs; in presence of a smaller amount than is necessary to produce this total inhibition, the effect is manifested by the low rate and shortened duration of fermentation. An experiment showing this behaviour of arsenate in a typical manner has already been quoted (No. 11). Even when the rate of fermentation is considerably increased by the presence of an amount of arsenate in excess of the optimum amount, it frequently happens that this rate falls very rapidly and that all fermentation ceases in an hour or two. The exact conditions under which this occurs have, however, not been ascertained. It is as yet uncertain whether this inhibitory action is due, as suggested by Buchner, to changes in the colloidal state of the fermenting complex or to the formation of inactive combinations of the complex with the arsenate and arsenite, in a similar manner to that suggested by the authors in the case of inhibition produced by excess of phosphate.* In all probability the phenomenon is a complex one in which several factors participate.

The total inhibition produced by arsenite which was observed by Buchner (p. 469) with juice which had been submitted to dialysis, evaporation, etc., may not improbably be ascribed to a loss of fermenting complex during these operations, so that the amount of arsenite added was greatly in excess of the optimum. It is, however, quite possible that the proteins accompanying the ferment present in the juice do exert a protective influence, as suggested by Buchner, and that a diminution in their amount may also play some part in the phenomenon.

10. *Experiments with Various Salts.*

No other salts capable of bringing about results in any way comparable with those produced by arsenates and arsenites have been found. Experiments with the sulphate, orthovanadate, antimonate, tungstate, chromate, stannate, phosphite, and citrate of sodium, and with tartar emetic and zinc sulphate, were all negative.

* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

Summary.

1. When a suitable amount of arsenate is added to a fermenting mixture of yeast-juice and a sugar, it causes a large acceleration in the rate of production of carbon dioxide and alcohol. This enhanced rate differs from that produced by phosphate, inasmuch as it continues long after a chemical equivalent of carbon dioxide has been evolved. The arsenate, moreover, is found in the free state throughout the fermentation.

2. The rate attained increases rapidly with addition of arsenate, until an optimum concentration is reached, after which it decreases, at first rapidly, and then more slowly.

3. The total fermentation produced depends on the particular concentration of arsenate employed, and may be either higher or lower than that given in the absence of arsenate. As the high rate produced by a suitable quantity of arsenate persists for a long time, very considerable increases in the total fermentation may be observed.

4. Glucose and mannose are similarly affected by yeast-juice in presence of arsenate, whereas fructose is much more rapidly fermented than these two sugars, and the optimum concentration of arsenate in its presence is greater.

5. The increased rate of fermentation of sugars in presence of arsenate is due to an acceleration of the rate of action of the hexosephosphatase of the juice, whereby an increased supply of phosphate is afforded. The action is therefore essentially different from that of phosphate, and it has been found that arsenate cannot replace phosphate in the fundamental reaction of alcoholic fermentation.

6. Arsenate also causes a considerable increase in the rate of the auto-fermentation of yeast-juice, and in the rate of the fermentation of glycogen. This is mainly due to an acceleration of the rate of action of the diastatic enzyme of yeast-juice (glycogenase).

7. The action of arsenites is similar to that of arsenates, but is much less marked.

8. Both arsenate and arsenite cause total inhibition of the fermentation when they are present in a high concentration, but the nature of this effect has not been ascertained.
