The Alcoholic Ferment of Yeast-juice. Part III.—*The Function of Phosphates in the Fermentation of Glucose by Yeast-juice.*

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In a previous communication the authors have shown* that when a soluble phosphate is added to a fermenting mixture of glucose and yeast-juice the following phenomena are to be observed: (1) The rate of fermentation is at once greatly increased. (2) This acceleration lasts for a short time and the rate then falls off, and returns approximately to its original value. (3) During this period the extra amount of carbon dioxide evolved and alcohol produced are equivalent to the phosphate added. (4) The phosphate is converted into a form which is not precipitable by magnesia-mixture, and is then probably present as a salt of a hexosephosphoric acid.

(1) *Effect of the Addition of Phosphate on the Total Fermentation.*

The addition of phosphate, however, does not simply produce this initial decomposition of an equivalent of glucose, but also, as a rule, a greater total fermentation, after allowance has been made for the amount decomposed during the initial period.

This is clearly shown by the results embodied in the following table.

In each experiment two or more portions of 25 c.c. of yeast-juice were taken, a solution of glucose alone, or one of glucose and phosphate, added, and the total volume made to 50 c.c. The solution of phosphate employed had a concentration of about 0·3 molar, and the concentration of glucose was 20 grammes per 100 c.c. in experiment 8, and 10 grammes per 100 c.c. in all the others. The fermentation was carried out at 25° in presence of toluene until the evolution of gas ceased. The numbers in the last column show the increase in the total fermentation produced during the period subsequent to the initial acceleration.

It will be seen that the increase which occurs after the initial period varies from about 10 per cent. of the original fermentation to as much as 150 per cent.

* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 405.

Experiment.	CO_2 evolved without phosphate.	Cubic centimetres of phosphate solution added.	CO_2 evolved in presence of phosphate.	Increase due to phosphate.	CO_2 equivalent to phosphate.	Increase after initial period.
	grammes.	c.c.	grammes.	gramme.	gramme.	gramme.
1	0·484	10	0·717	0·233	0·132	0·101
2	1·280	5	1·584	0·304	0·066	0·238
3*a*	0·422	10	0·634	0·212	0·132	0·080
3*b*	0·422	20	0·748	0·326	0·264	0·062
4	0·440	10	1·258	0·818	0·132	0·686
5	0·405	5	0·515	0·110	0·070	0·040
6	0·603	5	0·735	0·132	0·066	0·066
7	0·438	5	0·593	0·155	0·057	0·098
8	1·016	15	1·632	0·616	0·198	0·418
	c.c.		c.c.	c.c.	c.c.	c.c.
9	369	10	629	260	63	197
10	337	10	569	232	56	176

(2) *Recurrence of Phosphate.*

The reason for this increase in the amount of sugar decomposed in the long period following the short initial period of acceleration appears to be that the phosphorus compound first formed, which is a hexosephosphate of the formula $C_6H_{10}O_4(PO_4R_2)_2$,* is slowly hydrolysed, probably by an enzyme, with the production of a phosphate and a hexose. The phosphate is thus slowly regenerated and then again undergoes the reaction, causing an increased fermentation in the same manner as when it was originally added.

This recurrence of phosphate is clearly shown by the following experiment. A known amount of phosphate was added to yeast-juice containing glucose, and the mixture incubated at 25° with toluene. At the close of the initial period a sample was removed, boiled and filtered, and the free and total phosphate present in it estimated, and this process was repeated at stated times. The results obtained are given below, the amounts of phosphate being expressed in grammes of $Mg_2P_2O_7$ per 10 c.c.

Experiment 11.—215 c.c. of yeast-juice + 20 grammes of glucose were made to 375 c.c. with a solution of potassium phosphate, the amount of the latter being equivalent to 0·133 gramme $Mg_2P_2O_7$ per 10 c.c. of the resulting liquid.

The slight increase in the total phosphate present is due to a corresponding degree of evaporation during the experiment. It will be seen that the free phosphate per 10 c.c. gradually increases from 0·021 to 0·226, so that 0·205 gramme is regenerated. Since the total phosphorus, expressed as phosphate in

* Young, 'Chem. Soc. Proc.,' 1907, vol. 65.

Time in hours.	Free phosphate as $Mg_2P_2O_7$ per 10 c.c.	Total phosphate as $Mg_2P_2O_7$ per 10 c.c.
	gramme.	gramme.
5·5	0·021	0·266
18·0	0·093	0·269
66·0	0. 133	
138·0	0·175	
426·0	0·226	0·273

the original juice, was 0·266−0·133 = 0·133 gramme, it follows that at least 0·205−0·133 = 0·072 gramme of this has been derived from the hexose-phosphate produced during the initial period from the added phosphate.

Even in the absence of added phosphate a gradual production of free phosphate occurs when yeast-juice is incubated at 25°. In the absence of glucose, when the fermentation is very small, the increase in the amount of free phosphate is comparatively rapid, whereas, in the presence of glucose, the fermentation lasts for a considerable period, and the appearance of free phosphate is delayed, since it continually enters into fresh combination as rapidly as it is formed.

Examples of this are the following. The various materials were digested at 25° with toluene for the time shown, and were then boiled and filtered and the free phosphate estimated.

Experiment.	Material digested.	Time of digestion.	Free phosphate as $Mg_2P_2O_7$ per 25 c.c.
		days.	gramme.
12	(*a*) Yeast-juice alone	0	0·128
	(*b*) " "	2	0·284
	(*c*) " "	10	0·283
	(*d*) Yeast-juice + glucose	2	0·123
	(*e*) " "	10	0·255
13	(*a*) Yeast-juice alone	0	0·024
	(*b*) " "	1	0·053
	(*c*) " "	2	0·063
	(*d*) " "	4	0·069
	(*e*) Yeast-juice + glucose	1	0·011
	(*f*) " "	2	0·025
	(*g*) " "	4	0·051
		hours.	
14	(*a*) Yeast-juice + glucose + phosphate (0·276 gramme per 25 c.c.)	1·25	0·063
	(*b*) " " "	46·0	0·138
	(*c*) " " "	96·0	0·387
	(*d*) " " "	168·0	0·453
	(*e*) " " "	240·0	0·489
	(*f*) " " "	336·0	0·501
	(*g*) " " "	456·0	0·523

In this last experiment (No. 14) sample (*a*) was taken at the close of the initial period and sample (*b*) immediately after the cessation of fermentation, these points being determined by observation of another sample of the yeast-juice.

It is to be noted that during the fermentation only a small increase occurs in the amount of free phosphate (0·075 gramme), while after the cessation of fermentation the increase amounts to about three times as much (0·249 gramme) in approximately an equal time. The total phosphate of the original juice was 0·350 gramme per 25 c.c. and the amount added was 0·276 gramme. Since only 0·104 gramme of phosphate remains combined at the close of the experiment, it follows that at least $0·276 - 0·104 = 0·172$ gramme has been regenerated from the hexosephosphate.

The recurrence of phosphate in these cases appears to be due to the action of an enzyme—whether a special enzyme or one of those already known to occur in yeast-juice has not yet been determined.

After yeast-juice containing the sodium salt of hexosephosphoric acid has been boiled the amount of free phosphate remains practically unaltered when the liquid is incubated at 37°, as is shown by the following experiment.

Experiment 15.—To yeast-juice containing glucose and an amount of free phosphate corresponding to 0·184 gramme of $Mg_2P_2O_7$ per 25 c.c. was added sodium phosphate equivalent to 0·285 gramme of $Mg_2P_2O_7$ and the mixture incubated at 37° in presence of toluene. A sample was taken at the close of the initial period, and as soon as fermentation had almost ceased the whole was boiled, the free phosphate estimated and the remainder of the boiled liquid preserved at 37°, samples being taken at intervals.

At the close of the initial period, 25 c.c. yielded only 0·04 gramme of $Mg_2P_2O_7$, so that practically the whole of the added phosphate must have been converted into a salt of hexosephosphoric acid. At the cessation of fermentation the same volume yielded 0·131 gramme of $Mg_2P_2O_7$, so that at least 0·15 gramme was still present as a hexosephosphate.

After incubation of the boiled liquid at 37° for an additional 144 hours, the amount of free phosphate had only increased to 0·138 gramme, so that the action practically ceased when the liquid was boiled.

(3) *Nature of the Chemical Change which occurs in the Fermentation of Glucose by Yeast-juice.*

The cycle of changes which is undergone by a phosphate in the presence of yeast-juice and glucose appears from the foregoing to be as follows :—

(1) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O.$

(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4.$

The first of these equations does not include the fermenting complex, without which, however, the change does not occur, and it is probable that both the glucose and the phosphate form an intermediate association with this complex, which then breaks down, giving rise to the substances on the right-hand side of the equation, and at the same time regenerating the fermenting complex.

Since free phosphate and a hexosephosphate are invariably present in the yeast-juice prepared by grinding yeast, it follows that at all events some portion of the fermentation is always due to the foregoing reactions. During the initial period of rapid fermentation, as long as free phosphate is still present, the greater part of the change is certainly due to this reaction, whilst in the succeeding period of slower fermentation the constant production of free phosphate by the enzymatic hydrolysis of the hexosephosphate already formed, or by the action of proteoclastic enzymes on phosphoproteins, renders it equally certain that some portion of this greatly diminished fermentation must also be ascribed to the same reaction.

The question at once arises whether it is not possible that the whole of the fermentation is due to this reaction. So far, no fact has been encountered which is inconsistent with this view. In previous communications* the authors have shown that at least two substances are concerned in the production of carbon dioxide and alcohol from glucose: the ferment, which is thermolabile, and the coferment, which is thermostable; and, further, that phosphates are incapable of producing any fermentation when they are added to a mixture of the ferment and glucose in the absence of the coferment.

If the theory suggested above be found to be correct, it will be necessary to complete this statement. Two possibilities present themselves. Either a third substance, a soluble phosphate, is also necessary, and in the presence of the fermenting complex, made up of ferment and coferment, undergoes the reaction under discussion; or the coferment may itself be a complex substance containing a group of unknown composition, united with the phosphoric acid group. The latter would then be passed on to the glucose during fermentation, and a new group taken up from the phosphate, a continuous conversion of phosphate into hexosephosphate being thus effected.

If this cycle of changes correctly represents the reaction which occurs, it follows that the rate of fermentation after the initial period of acceleration depends, in the first instance, on the rate at which phosphate is liberated according to equation (2).

The most satisfactory method of testing the accuracy of this view would be to free both ferment and coferment from phosphate and materials

* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 405; B, vol. 78, 1906, p. 369.

capable of yielding phosphates under the influence of the enzymes which are present, and then to ascertain whether the mixture of these purified materials would ferment glucose; and, further, if they did not, whether the addition of a phosphate would bring about fermentation. Many attempts have been made to realise these conditions, but hitherto without success. It is obvious that if the coferment be a hydrolysable derivative of phosphoric acid, as suggested above, success in this direction cannot be anticipated.

Some idea of the extent to which alcoholic fermentation is due to this recurrence of phosphate could also be gained by ascertaining the amount of free phosphate produced from hexosephosphate in yeast-juice in a given time in the absence of glucose, and comparing this with the amount of carbon dioxide evolved in the presence of glucose under otherwise identical conditions.

In practice, however, two difficulties present themselves. In the first place, in the absence of fermentable sugar, or when the concentration of this is very low, free phosphate accumulates, and the rate of hydrolysis of the hexosephosphate is thus diminished; whereas in the presence of glucose the concentration of phosphate remains constant at a low value during fermentation, and the rate of hydrolysis of hexosephosphate accordingly remains at its maximum for a considerable period.

This inhibitory effect of phosphate on the hydrolysis of hexosephosphate in the absence of all fermentation is shown in the following experiment, which also indicates the extent to which this hydrolysis occurs under these conditions.

Experiment 16.—In order to avoid all fermentation, the inactive residue obtained by filtering yeast-juice through a Martin gelatin-filter was employed, and equal weights of this were incubated for 20 hours with: (1) water; (2) a solution of sodium hexosephosphate free from glucose and free phosphate; (3) a solution of sodium hexosephosphate + an equivalent amount of sodium phosphate. A solution of hexosephosphate was also incubated alone, the conditions of concentration and alkalinity being identical in all four solutions. At the expiration of 20 hours, the solutions were all boiled, and the free phosphate in the filtrates estimated. In the following table the numbers represent the weight of $Mg_2P_2O_7$ found.

The numbers in the last column are obtained by adding together the amount of phosphate produced separately by the incubation of the hexosephosphate and the residue, and subtracting their sum from the free phosphate produced in the other two solutions.

It appears from this that in this particular instance the enzymatic

Solution.	Amount of free phosphate present.		Amount of hexosephosphate hydrolysed by enzyme.
	Before incubation.	After incubation.	
1. Hexosephosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·0	0·0089	
2. 0·75 gramme residue + water	0·0	0·0166	
3. 0·75 gramme residue + hexosephosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·0	0·1068	0·0813
4. 0·75 gramme residue + hexosephosphate as above, + sodium phosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·1626	0·1884	0·0003

decomposition of the hexosephosphate is almost completely arrested by the presence of an equivalent of free phosphate.

The amount of phosphate actually produced in solution 3 from all sources is 0·1068 gramme $Mg_2P_2O_7$, which corresponds to an evolution of about 22 c.c. of carbon dioxide, and this in spite of the fact that the phosphate has been allowed to accumulate. The amount which would be evolved in presence of glucose and coferment would naturally exceed this, owing to the continued reconversion of the phosphate into hexosephosphate.

This experiment also shows that the ferment which brings about the hydrolysis of the hexosephosphate is present in the residue obtained by filtering yeast-juice through a Martin-filter, and does not require a dialysable coferment.

The second difficulty arises from the fact that when yeast-juice or a mixture of ferment and coferment is employed, a certain amount of fermentation always occurs, even in the absence of added sugar. This is due to sugar formed in the liquid, in part by the hydrolysis of glycogen and dextrins and in part by the hydrolysis of the hexosephosphate itself, which yields a fermentable sugar as one of its products. The practical result is that if an actual comparison be instituted between the production of phosphate in the absence of added glucose and the evolution of carbon dioxide in the presence of glucose, it will be necessary to take the sum of the carbon dioxide actually evolved and the carbon dioxide equivalent of the phosphate produced in the absence of added glucose, and this will always be found to be less than the volume of carbon dioxide observed in the presence of glucose. The result of such a comparison is shown in the two following experiments.

A mixture was made of yeast-juice with a solution containing a suitable amount of sodium phosphate and just sufficient glucose to bring about the conversion of the greater portion of the phosphate into hexosephosphate, this

being found by experiment to be rather more than an equivalent of glucose. The mixture was incubated until this conversion had been accomplished and the rate of fermentation had become steady. A sample was then taken and boiled and the free phosphate estimated in the filtrate. The remainder was incubated for a further period, the evolution of gas being noted, and a second sample was then taken.

A parallel experiment was carried out with yeast-juice containing the same amount of sodium phosphate in presence of 10 grammes of glucose per 100 c.c., and the evolution of gas during the same period was observed. The solutions used and the results obtained were as follows:—

Experiment 17.—(1) 100 c.c. yeast-juice + 30 c.c. of a solution containing 1·3 grammes sodium phosphate and 2·62 grammes glucose. (2) 100 c.c. yeast-juice + 30 c.c. of a solution containing 1·3 grammes sodium phosphate and 13 grammes glucose.

Experiment 18.—(1) 100 c.c. yeast-juice + 40 c.c. of a solution containing 1·7 grammes sodium phosphate and 3·16 grammes glucose. (2) 100 c.c. yeast-juice + 40 c.c. of a solution containing 1·7 grammes sodium phosphate and 14 grammes glucose.

Experiment.	Solution 1 (low concentration of glucose).						Solution 2 (excess of glucose).
	Original phosphate.	Final phosphate.	Phosphate produced.	CO_2 equivalent.	CO_2 evolved.	Total CO_2 equivalent.	CO_2 evolved.
				c.c.	c.c.		
17	0·0559	0·1052	0·0493	10·7	39·8	50·5	93·9
18	0·1036	0·1701	0·0665	14·6	27·7	42·3	69·2

These experiments show that although the sum of the carbon dioxide evolved and that equivalent to the phosphate produced in absence of glucose is invariably less than the amount of carbon dioxide evolved in the presence of glucose, yet the difference is no greater than might be expected from a knowledge of the prevailing conditions, and is quite consistent with the view that the whole of the fermentation proceeds according to the equation proposed.

(4) *Influence of Concentration of Phosphate on the Course of the Fermentation.*

When a phosphate is added to a fermenting mixture of glucose and yeast-juice, the effect varies both with the concentration of the phosphate and with the particular specimen of yeast-juice employed. With low concentra-

tions of phosphate the acceleration produced is so transient that no accurate measurements of rate can be made. As soon as the amount of phosphate added is sufficiently large, it is found that the rate of evolution of carbon dioxide suddenly increases from 5 to 10 times, and then rapidly falls approximately to its original value.

As the concentration of phosphate is still further increased, it is first observed that the maximum velocity, which is still attained immediately on the addition of the phosphate, is maintained for a certain period before the fall commences, and then, as the increase in concentration of phosphate proceeds, that the maximum is only gradually attained after the addition, the period required for this increasing with the concentration of the phosphate. Moreover, with these higher concentrations the maximum rate attained is less than that reached with lower concentrations, and, further, the rate falls off more slowly. The concentration of phosphate which produces the highest rate, which may be termed the optimum concentration, varies very considerably with different specimens of yeast-juice.

All these points are illustrated by the accompanying tables and curves.

Experiment 19.—The following solutions were employed:—

(1) 25 c.c. yeast-juice + 15 c.c. sodium bicarbonate solution.

(2) 25 c.c. yeast-juice + 10 c.c. of potassium phosphate solution (0·3 molar) + 5 c.c. sodium bicarbonate solution.

(3) 25 c.c. yeast-juice + 15 c.c. potassium phosphate solution. All the solutions contained 4 grammes of glucose, and the experiment was carried out at 25° in the presence of toluene. The solution of bicarbonate added contained an amount of this salt equal to that formed by the action of carbon dioxide on an equal volume of the solution of potassium phosphate employed.

Time after addition in minutes.	Carbon dioxide evolved in preceding 5 minutes with n cubic centimetres of 0·3 molar potassium phosphate added.		
	n = 0 c.c.	n = 10 c.c.	n = 15 c.c.
5	4·0	11·1	7·7
10	3·2	16·0	9·7
15	4·2	20·2	12·1
20	3·6	**22·4**	16·1
25	4·3	17·4	18·4
30	3·6	6·6	19·4
35	4·3	4·6	**20·4**
40	3·2	4·7	16·7
45	—	4·5	12·7
50	—	4·2	6·0
55	—	4·1	4·0

Experiment 20.—

(1) 25 c.c yeast-juice + 5 c.c. phosphate + 15 c.c. bicarbonate.
(2) 25 c.c. " + 10 c.c. " + 10 c.c. "
(3) 25 c.c. " + 15 c.c. " + 5 c.c. "
(4) 25 c.c. " + 20 c.c. " + 0 c.c. "

The concentration of glucose was 4·5 grammes per 45 c.c., and the experiment was carried out at 25° in presence of toluene.

Time after addition in minutes.	Carbon dioxide evolved in preceding 5 minutes with *n* cubic centimetres of 0·3 molar phosphate added.			
	n = 5 c.c.	*n* = 10 c.c.	*n* = 15 c.c.	*n* = 20 c.c.
5	7·1	2·7	1·6	1·6
10	12·7	6·2	2·4	1·7
15	**16·3**	8·1	3·0	2·0
20	7·7	9·6	3·3	2·0
25	1·9	12·4	4·0	2·3
30	1·8	14·5	5·1	2·6
35	—	**16·8**	6·0	2·7
40	—	15·0	6·5	3·1
45	—	2·9	7·3	3·5
50	—	1·7	8·5	3·8
55	—	—	**8·7**	4·4
60	—	—	8·5	4·9
65	—	—	8·6	5·6
70	—	—	8·1	5·7
75	—	—	7·6	5·6
80	—	—	7·0	**6·0**
85	—	—	6·0	6·0
90	—	—	—	6·0
95	—	—	—	5·5
100	—	—	—	5·4
105	—	—	—	5·0

Curves A, B, C, and D (fig. 1) show the rate of evolution per five minutes for the four solutions in experiment 20. The time of addition is taken as zero, the rate before addition being constant, as shown in the curves.

It will be observed that in experiment 19 practically the same maximum is attained with 10 and 15 c.c. of phosphate, whereas, in experiment 20, 5 and 10 c.c. give the same maximum, whilst 15 c.c. produce a much lower maximum, and 20 c.c. a still lower one, the rate at which the velocity diminishes after the attainment of the maximum being correspondingly slow in these last two cases. By calculating the amount of phosphate which has disappeared as such from the amount of carbon dioxide evolved, it is found that the maximum does not occur at the same concentration of free phosphate in each case.

These results suggest that the phosphate is capable of forming two or

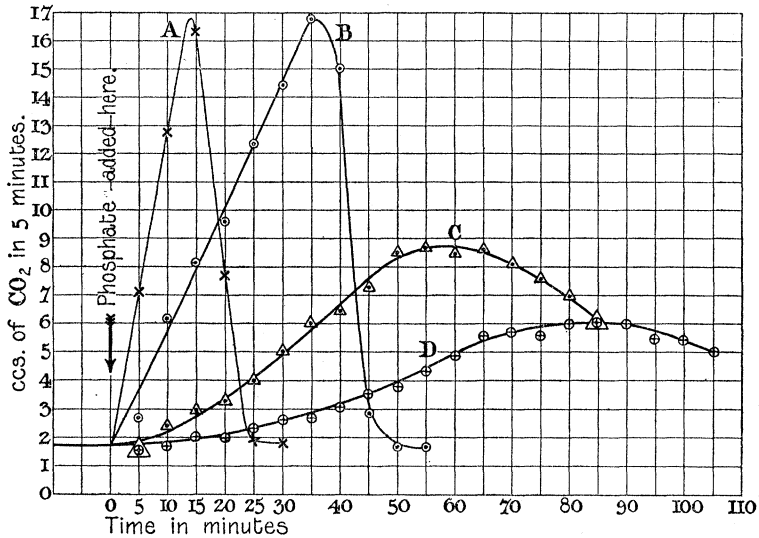

FIG. 1.

more different unstable associations with the fermenting complex. One of these, formed with low concentrations of the phosphate, has the composition most favourable for the decomposition of sugar, whilst the others, formed with high concentrations of phosphate, contain more of the latter, probably associated in such a way with the fermenting complex as to render the latter partially or wholly incapable of effecting the decomposition of the sugar molecule. As the fermentation proceeds slowly in the presence of excess of phosphate, the concentration of the latter is reduced by conversion into hexosephosphate, and a redistribution of phosphate occurs, resulting in the gradual change of the less active into the more active association of phosphate with fermenting complex, and a consequent rise in the rate of fermentation.

In those cases in which the maximum rate corresponding to the optimum concentration of phosphate is never attained, some secondary cause may be supposed to intervene, such as a permanent change in a portion of the fermenting complex, accumulation of the products of the reaction, etc. Experiments on this point are being carried out by varying independently the concentration of the ferment, the coferment, the phosphate, and the hexosephosphate.

In agreement with these conclusions it is found that a high rate of evolution of carbon dioxide can be maintained for a considerable period by the gradual addition of phosphate in such a way that the concentration of free phosphate remains approximately at the optimum value. This may be effected by

adding in every interval of five minutes the amount of phosphate equivalent to the carbon dioxide evolved in excess of the normal rate of fermentation of the yeast-juice during the same interval of time.

Experiment 21.—25 c.c. of yeast-juice + 2·5 grammes glucose were incubated at 25° in the presence of toluene until the rate became constant. A solution of potassium phosphate (2 mol. K_2HPO_4 and 1 mol. of KH_2PO_4) of 0·3 molar concentration was then gradually added from a graduated pipette provided with a tap and passing through the cork of the fermentation flask. Column 2 shows the c.c. of this added in each period of five minutes, and column 3 the evolution of carbon dioxide during each period of five minutes. The normal rate of the yeast-juice, in the absence of added phosphate, was 3 c.c. per five minutes. It will be seen that an average rate of about 15 was maintained for an hour and a quarter, 32 c.c. of the phosphate solution being added in all.

1. Time in minutes.	2. Cubic centimetres of phosphate solution added in each 5 minutes.	3. Evolution of carbon dioxide in each 5 minutes.
	c.c.	
5	5·0	3·1
10	1·5	15·4
15	2·0	16·2
20	1·0	20·2
25	2·5	17·2
30	1·5	12·7
35	2·0	15·7
40	0·0	17·4
45	0·0	9·6
50	3·0	8·4
55	3·0	15·4
60	3·0	20·2
65	3·0	17·2
70	3·0	14·8
75	1·5	14·9

Further experiments on this subject are in progress, particularly with respect to the relations between phosphate and coferment, and the bearing of these phenomena on the fermentation of sugars by living yeast.

Summary.

1. The addition of a phosphate to a fermenting mixture of glucose and yeast-juice not only produces a temporary acceleration in the rate of fermentation, but, in addition to this, an increased total fermentation.

2. This last effect is due to the fact that the hexosephosphate formed during

the period of temporary acceleration is continually hydrolysed by an enzyme, with production of free phosphate, which again enters into reaction and thus brings about an increased fermentation.

3. It appears probable that the presence of phosphate is essential for the alcoholic fermentation of glucose by yeast-juice, the reaction which occurs being the following :—

(1) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O$.

This reaction is only realised in the presence of the ferment and coferment discussed in previous communications, phosphate alone being unable, in the absence of coferment, to bring about fermentation in a mixture of ferment and glucose.

The hexosephosphate thus formed is then hydrolysed :

(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4$.

The rate at which this second reaction occurs determines the rate of fermentation observed when glucose is fermented by yeast-juice.

4. An optimum concentration of phosphate exists which produces a maximum initial rate of fermentation. Increase of concentration beyond this optimum diminishes the rate of fermentation.