XX. THE FUNCTION OF PHOSPHATES IN THE OXIDATION OF GLUCOSE BY HYDROGEN PEROXIDE.

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THE conditions under which the oxidation of glucose by H_2O_2 can take place in aqueous solution have been studied by W. Löb and his co-workers [1910, 1911, 1912, 1915]. He believes that the oxidation depends on the hydroxyl ion concentration of the solution, and further that phosphate ions exercise a specific accelerating effect on the reaction. He was unable to produce any oxidation of glucose by H_2O_2 in presence of water alone or when other substances were substituted for phosphates.

For instance neither Sørensen's mixtures of glycol and NaOH nor of borate and HCl could be used to replace the phosphate mixture. Witzemann [1920] confirmed Löb's observations and agreed that phosphate mixtures exercise a specific effect. He was unable to produce the same effect with mixtures of NaHCO_3 and Na_2CO_3 as with phosphate mixtures.

Witzeman [1920] suggested that the phosphates might produce an intermediate compound with glucose of the same nature as the hexosephosphate which has been shown by Harden and Young [1910] to be produced during alcoholic fermentation, and which appears from the work of Embden and others to be connected with the production of lactic acid in muscle.

Witzemann was unable to detect the formation of any compound of glucose and phosphate in absence of H_2O_2 .

As more exact knowledge of the mechanism of this reaction is desirable in view of the part played by phosphate in alcoholic fermentation, further experiments were made to try and ascertain whether an intermediate compound of the nature of a phosphoric ester of glucose was formed during this oxidation. For this purpose free phosphate was estimated at intervals in a mixture similar to one of those used by Witzemann for oxidation of glucose by H_2O_2 at 37° , both by the method of Schmitz [1906] and by the ordinary magnesium citrate method. No change in the amount of free phosphate was observed.

Besides the possibility of the formation of an intermediate compound between phosphate and glucose there are at least two other possible explanations of the effect produced by phosphate in this reaction:

(1) Phosphate may act as a peroxidase, thus rendering possible the oxidation of glucose by H_2O_2 . If this were so phosphate might act in the same way towards mixtures of H_2O_2 and benzidine or guaiacum. No evidence could be obtained of such an action, but it does not follow from this negative result that no such action occurs with H_2O_2 and glucose.

(2) The phosphate mixtures may simply act as buffer substances, the maintenance of the P_H within certain points being essential. If this hypothesis is correct it should be possible to replace the phosphate mixtures by other substances, provided the P_H be maintained within the same limits.

We have carried out experiments on the oxidation of glucose by H_2O_2 with solutions the P_H of which was never over 7.3 and find that glucose is oxidised by H_2O_2 in presence of 0.125 *M* NaHCO_3 saturated with CO_2 ; or 0.25 *M* sodium arsenate saturated with CO_2 ; or 0.25 *M* sodium acetate.

The phosphate ion cannot therefore be regarded as playing a specific part in the reaction. It appears more likely that the buffer action of the salts employed is the important factor, whether as providing and maintaining the most suitable conditions for oxidation or as protecting the H_2O_2 from too rapid decomposition. In this connection the experiment of Witzemann [1920, p. 12, sec. 7] with a mixture of sodium carbonate and bicarbonate should be referred to. This shows that in presence of these salts, at a P_H slightly over 9.3, H_2O_2 is rapidly decomposed and little oxidation takes place, whereas in our experiment in presence of 0.125 *M* NaHCO_3 saturated with CO_2 (P_H 6.8) a considerable amount of oxidation is produced.

Experiments were accordingly made roughly to test the stability of H_2O_2 in solutions the P_H of which was maintained at various levels by the use of buffer solutions. The rate of decomposition was found to increase with rise of alkalinity. In those cases in which the buffer solutions consisted of phosphate mixtures the rate of decomposition of the H_2O_2 was not so rapid as in the corresponding experiments with other buffer solutions; so that in this sense the phosphate ion may be said to have a specific action.

In the experiments of both Löb and Witzemann the P_H of the solutions was only carefully measured in those experiments in which Sørensen mixtures were used. In most if not all of the experiments in which the effects of other substances were compared to that of phosphate mixtures the reaction of the solution was tested with litmus paper only. The glycolol + NaOH and borate + HCl mixtures used are only efficient buffers between P_H 8.0 and 10.2, and at these higher degrees of alkalinity the H_2O_2 is rapidly decomposed. The same applies to the NaHCO_3 + Na_2CO_3 mixture used by Witzemann which must have been much more alkaline than the phosphate mixture.

The experiments in which he compared the effect of Na_2CO_3 and NaOH were not carried out at the same P_H . The reaction of the solution in the latter case was suitable for the oxidation, whereas the alkalinity of the solution used in the former case was too high.

EXPERIMENTAL.

I. *Experiments to determine if mixtures of Na_2HPO_4 and NaH_2PO_4 will act as a peroxidase towards H_2O_2 and benzidine or guaiacum.*

Phosphate mixtures of P_H 9.0, 7.4, 6.9, 6.6, 6.2, 4.8 were tested with H_2O_2 and benzidine or guaiacum. No action was observed in any of the experiments.

To test whether the optimum P_H for the action of a peroxidase lies within the above range, similar experiments were made with the same phosphate mixtures, H_2O_2 and guaiacum with the addition of potato juice. The maximum effect was produced at room temperature from P_H 7.4 to 6.9. A similar result was obtained when benzidine was substituted for guaiacum.

II. *Oxidation of glucose by H_2O_2 in presence of various buffer mixtures.*

Seven flasks were made up as follows:

	Glucose	NaHCO_3	Na_2HASO_4 12 H_2O	$\text{NaC}_2\text{H}_3\text{O}_2$ 3 H_2O	K_2HPO_4	KH_2PO_4	5.7 % sol. H_2O_2	
	g.	g.	g.	g.	g.	g.	cc.	
1	8.75	2.6	—	—	—	—	62	} to 250 cc. with distilled water
2	8.75	—	25.1	—	—	—	62	
3	8.75	—	—	8.5	—	—	62	
4	8.75	—	—	—	8.8	2.2	62	
5	11.25	2.6	—	—	—	—	—	
6	11.25	—	25.1	—	—	—	—	
7	11.25	—	—	8.5	—	—	—	

In Nos. 1, 2, 5, 6 the solution was saturated with CO_2 .

The P_H of each solution was estimated colorimetrically, and the glucose estimated by Bertrand's method and by the polarimeter.

All the flasks were then kept in the incubator at 37° for 24 hours and the P_H and glucose again estimated in each. The results are shown below:

	At start of experiment glucose g. per 100 cc.			After 24 hours			Apparent loss of glucose in g. per 100 cc.	
	P_H	Bertrand	Polarimeter	P	A	B	by A	by B
		A	B					
1	6.8	4.16	3.50	6.7-6.8	3.39	2.99	0.77	0.51
2	6.8	3.96	3.55	6.7-6.8	2.96	2.38	1.00	1.17
3	6.8-7.0	3.64	3.54	5.2	3.01	2.50	0.63	0.53
4	7.0	3.65	3.20	5.6-5.8	2.24	1.85	1.41	1.35
5	6.8	—	4.55	7.6	—	4.55	—	0
6	6.8	—	4.50	7.0	—	4.50	—	0
7	7.3	—	4.25	7.3	—	4.25	—	0

Note. The sample of glucose employed for these experiments showed a 5 % higher content of glucose as estimated by Bertrand's method than when the polarimeter and $[\alpha]_\text{D}$ 52.5° were used. This, and the fact that the Bertrand estimations of glucose at the start of the experiment were carried out before the polarimeter readings were made, may account for the discrepancies between the two sets of figures shown above.

Only traces of H_2O_2 remained in 1, 2 and 4 after 24 hours in the incubator.

No. 3 still contained a considerable amount of H_2O_2 and the action was allowed to proceed for a second period of 24 hours. After this the P_H was

found to be 4.6–4.8 and glucose estimated polarimetrically amounted to 1.76 g. per 100 cc.: a total apparent loss of glucose of 1.78 g. per 100 cc.

These experiments show that in absence of H_2O_2 no loss of glucose has apparently taken place (Nos. 5, 6, 7). Whereas in presence of H_2O_2 (Nos. 1, 2, 3, 4) an apparent loss of glucose has occurred in every case, greatest in the two solutions containing phosphates and arsenates, but considerable in the other two solutions.

III. *Effect of change of P_H on the stability of H_2O_2 .*

All solutions contained 5.0 cc. of 5.7 % H_2O_2 per 100 cc. solution. The H_2O_2 was estimated at the start and again after 24 hours and 48 hours at 37° by titration with standard permanganate.

No.	Mixture	P_H	% H_2O_2 lost	
			after 24 hours	after 48 hours
1	HCl	1.0	—	1
2	Sørensen's glyocol + HCl ...	1.9	1	2.1
3	Na acetate + acetic acid ...	4.6	2	2.1
4	Phosphates	6.8	2	4.2
5	"	7.1*	4.9	7.5 (43 hours)
6	"	7.8*	6.1	7.4 (43 hours)
7	"	8.0*	7.5	13.1
8	Palitzsch's borax + boric acid	6.9	20.2	36.1
9	" " "	7.1	20.2	37.2
10	" " "	7.7	22.3	39.3
11	" " "	7.9	37.2	59.5
12	" " "	8.2	55.3	74.4
13	" " "	8.4	65.9	84.0
14	" " "	8.6	65.9	84.0
15	" " "	8.9	67.0	85.1
16	Sørensen's borate + HCl ...	8.8	—	78.5
17	" + NaOH ...	9.2	—	99.0
18	" " "	12.8	100	—

* In these cases the concentration of the hydrogen peroxide used was 5 % instead of 5.7 %.

IV. *The P_H of some of the solutions tested by Witzemann.*

A solution was made up as described by Witzemann [1920, p. 12, sec. 7]: 2.43 g. $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$; 0.72 g. NaHCO_3 ; 35 cc. H_2O ; 20 cc. (0.2 g.) glucose solution and 20 cc. of 3 % H_2O_2 .

The P_H , estimated colorimetrically, was over 9.3. At this degree of alkalinity H_2O_2 is rapidly decomposed and it is therefore not surprising that little oxidation took place.

To compare the effect of NaOH and Na_2CO_3 Witzemann [1920, p. 14, sec. 9 (2) and sec. 10 (2)] prepared solutions as follows:

P. 14, sec. 9: 32.0 cc. 0.33 M Na_2HPO_4 ; 3.0 cc. H_2O ; 20.0 cc. 1 % glucose; 20.0 cc. 3 % H_2O_2 ; 0.61 g. $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$;
and sec. 10 (3) 25.6 cc. 0.33 M Na_2HPO_4 ; 6.4 cc. 0.33 M NaH_2PO_4 ; 20.0 cc. 1 % glucose; 20.0 cc. 3 % H_2O_2 ; 3 cc. H_2O ; 0.1714 g. NaOH.

The P_H of the former was 9.3–9.6 and of the latter 7.1. The amount of oxidation was greater in the second than in the first case and Witzemann argues that NaOH exercises a less harmful effect on the reaction than does Na_2CO_3 .

It does not seem that this inference can fairly be drawn unless the experiments are carried out under the same conditions of P_H . The acidity of the H_2O_2 solution used by Witzemann is not stated in his paper. It is therefore not possible to say what was the exact P_H in his experiments. But in any case the alkalinity of the solutions used in experiments 7 and 9 (2) must have been much higher than in experiment 10 (3).

SUMMARY.

1. The oxidation of glucose by H_2O_2 takes place in presence of the following buffer substances: $\text{NaHCO}_3 + \text{CO}_2$; $\text{Na}_2\text{HAsO}_4 + \text{NaH}_2\text{AsO}_4$; $\text{NaC}_2\text{H}_3\text{O}_2$; $\text{K}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$, provided the P_H does not rise much above 7.3.
2. The stability of H_2O_2 in aqueous solution is increased by the presence of phosphates which do not however show a specific action in other respects.

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