

THE EFFECT OF BORATE ON THE OXIDATION OF GLUCOSE AND OTHER SUGARS.

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INTRODUCTION.

The experiments recorded in this paper were suggested by a consideration of the details of some experiments on carbohydrate metabolism. In order to minimize the liability of the bladder to infection following catheterization, it is common practice to finish the catheterization by irrigation with boric acid solution. The known behavior of boric acid in the presence of polyhydroxy compounds as well as the possibility of pH changes in the reagent used for sugar analysis led us to test the effect of boric acid on the determination of urinary sugar. We have found that the presence of boric acid causes errors of a magnitude which cannot be ignored.

A dog was catheterized and then injected subcutaneously with 1 gm. of phlorhizin dissolved in Na_2CO_3 . The catheterization was repeated at intervals of 2 hours, the bladder being emptied each time as completely as possible but without washing. Sugar was determined in these urines by the Folin and Peck (1919) titration before and after the addition of the amount of saturated boric acid solution which would have been used in washing (25 cc.). The results are shown in Table I.

We have found no mention of the interference of borates with sugar determinations in the handbooks or in the literature of phlorhizin diabetes. While the majority of investigators apparently have not used boric acid irrigation, it has been used by some. It is desirable therefore to call attention to the possibility of errors

in the determination of urinary sugar (see Table I) where proper precautions are not taken to prevent any but a small amount of the boric acid from entering the sample.

These observations on the interference of boric acid with the oxidation of sugar excited our interest to such an extent that we have studied the problem from several angles, but this paper reports in detail only the effects of boric acid on copper and iodometric methods of determining glucose and the common mono- and disaccharides.

Theoretical.

Since the addition of certain organic hydroxy compounds causes an apparent increase in the degree of dissociation of boric acid in

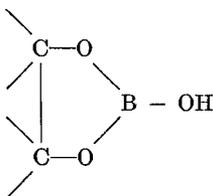
TABLE I.
Urinary Sugar. Error Caused by Saturated Boric Acid.

Urine No.	Volume.	Glucose found.		Error.
		As taken.	With 25 cc. saturated HBO ₂ .	
	cc.	gm.	gm.	per cent
1	50	2.155	1.855	13.9
2	30	1.460	1.350	7.5
3	30	1.235	1.105	10.5

aqueous solution, it is possible after adding glycerol, mannite, invert sugar, or glucose in proper quantity to a boric acid solution, to titrate 1 hydrogen atom of the boric acid quantitatively. The apparently increased dissociation of the acid in the presence of polyhydroxy compounds is indicated by the position of the titration curves (Mellon and Morris, 1924), by the change in conductivity (Magnanini, 1890), and by the change in the reaction to phenolphthalein on adding these compounds to borax solutions (Biot, 1842). In some cases, *e.g.* mannite and sorbite, a change in the optical activity of the polyhydroxy compound may be observed (Fischer and Stahel, 1891).

The investigations of Magnanini have led to the belief that two hydroxyl groups on adjacent carbon atoms are necessary for the increase in conductivity of boric acid, and the work of Boeseken

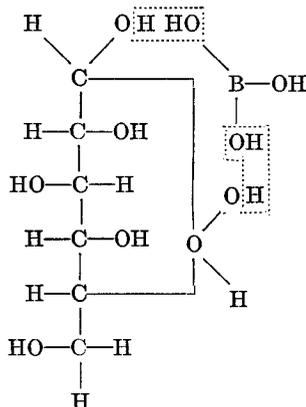
(1913) has further indicated that these hydroxyl groups must be on the same side and in the same plane with respect to the carbon atoms for the most favorable effect. It was assumed that easily saponifiable esters were formed between hydroxy compounds and boric acid (Klein, 1878) and Van't Hoff (1908) gave the following ring structure to such esters:



Interaction of Glucose and Borates.

Boeseken (1913) believed that he had determined which of the two possible isomeric lactonic formulæ should be assigned to α -glucose and which to β -glucose on the following basis. α -Methylglucoside has only a slight negative effect on the conductivity of boric acid, which shows that the aldehydic hydroxyl group is necessary for the reaction with boric acid. While both α - and β -glucose increase the conductivity of boric acid, the α form causes the greater increase and this increase falls off at a rate equal to the rate of mutarotation. On the other hand the conductivity of the solution containing β -glucose increases with time at a rate equal to the rate of mutarotation. The structure with the hydroxyl group of the aldehydic carbon atom on the same side as that of the second carbon atom was therefore assigned to α -glucose. Irvine and Steele (1915) found however that 2, 3, 5, 6-tetramethylglucose (now regarded as 2, 3, 4, 6-tetramethylglucose (Charlton, Haworth, and Peat, 1926)) has practically the same effect on the conductivity of boric acid as glucose itself. The hydroxyl group on the second carbon atom therefore probably has no rôle in the formation of the borate ester, but according to these authors the second hydroxyl group necessary for the increase in conductivity is one formed on the addition of water to the lactonic oxygen atom.

Thus:



This is in conformity with Armstrong's (1903) theory of the transformation of $\alpha \rightleftharpoons \beta$ -glucose through the oxonium derivative and Boeseken's opinion that the transformation occurs without rupture of the lactone ring. In any case, the formation of a borate ester of glucose involves the aldehydic hydroxyl group which is certainly of great importance in the oxidation of glucose.

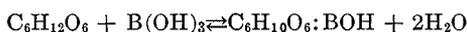
The decrease in the optical activity of glucose on the addition of borax (Rimbach and Weber, 1905; Murchhauser, 1923) may be taken as additional evidence of ester formation, although the former authors ascribe part of the decrease to the effect of the alkaline reaction on the sugar. Boric acid itself has no apparent effect on the optical activity of glucose. If the alkali had an effect on the glucose in the sense of the transformation investigated by Lobry de Bruyn and Van Ekenstein, its neutralization should leave the optical activity unchanged, but if the low optical activity is a specific effect of borax, neutralization might restore the original optical activity of glucose. The data of Table II show the latter to be true, thus proving that under our experimental conditions none of the glucose was converted to mannose or fructose or suffered other irreversible changes in the presence of the alkaline borate.

The change of rotation with time in Solution 5 (Table II) is

very interesting in that it indicates the liberation of an excess of α -glucose. This phenomenon will be the subject of further work.

Conditions Affecting Formation of Glucose-Borate Compounds.

The equation representing the formation of an ester between glucose and boric acid may be written as follows:



That the amount of ester formed will depend on the concentrations of glucose and of boric acid is indicated by measurements of the conductivity at various concentrations of boric acid and of polyhydroxy compounds (Boeseken, 1913; Rimbach and Ley, 1922). The ester which is formed dissociates as a stronger acid than boric acid.



It may be stated as a general principle that reactions favored by the presence of alkali tend to produce acids which neutralize the alkali. Esters of alcohols and acids are hydrolyzed in the presence of alkali to give the free acid which neutralizes the alkali; lactones and acid anhydrides are converted by alkali to the acid forms and alkali brings about the formation of saccharic acids from the sugars. Applying this principle to the above reaction, the addition of alkali to a solution of boric acid and glucose should favor the formation of the ester of these two substances which is a stronger acid than boric acid. It was not surprising therefore to find that borax decreases the specific rotation of glucose but that boric acid has no measurable effect and therefore probably does not combine to an appreciable extent. (Rough calculations from the conductivity data of Boeseken show that probably not more than $\frac{1}{2000}$ of the glucose combines with boric acid, an amount certainly not detectable by the polariscope.)

Oxidation of Glucose.

In the oxidation of glucose by alkaline copper reagents, 5.5 to 6 equivalents of oxygen are utilized per mol of glucose. Numer-

ous products are formed by complex changes of the molecule; but one, gluconic acid, arises by direct oxidation of the aldehyde group of glucose. This acid may be oxidized further or the glucose may be split directly into fragments which are then oxidized. Blocking of the aldehydic hydroxyl group as in methylglucosides has a profound effect on the oxidation. Since boric acid or borates form esters through the aldehydic hydroxyl group, the prediction may be made that boric acid will interfere with the oxidation of glucose and this will be especially evident when the oxidation of glucose gives gluconic acid as the main end-product.

Since the oxidation of glucose by alkaline copper solutions is affected by the reaction of the solution (Visscher, 1926; Somogyi, 1926) and since boric acid as well as the ester of glucose and boric acid will alter the reaction of solutions to which they are added, the pH factor must be controlled before any specific effect may be ascribed to borates.

EXPERIMENTAL.

Oxidation of Glucose by Alkaline Copper Solutions.

Fehling's Solution.—In a series of experiments performed to determine the effect of the addition of boric acid on the oxidation of glucose by Fehling's solution, the Fehling-Soxhlet solutions were used in the manner described by Shaffer and Hartmann (1920-21) for the determination of glucose by the "cuprous" titration. The glucose used was the Difco, Anhydrous, c.p., or Pfanstiehl, c.p., Special Anhydrous. Boric acid was recrystallized three times from water and dried over CaCl_2 . Merck's Blue Label boric acid as well as borax in equivalent amounts produced substantially the same effects. All determinations and blanks were run in duplicate. The results are shown in Table III.

In order to control the effect of neutralization of the alkali in the copper solution, we have determined glucose by Fehling's method after adding sulfuric acid. These results are shown in the last column of Table III. Since boric acid acts as if it were monovalent even in very alkaline solutions (Schmidt and Finger, 1908), we have assumed that the molecular and equivalent weights are the same and on this basis compared the effect of molar quantities of boric acid with 0.5 molar quantities of sulfuric acid. Since the

sulfuric acid is without effect under our conditions, it seems reasonable to believe that the interference of boric acid is not due to a mere change of reaction but is a specific effect of borate ions.

The presence of borate may interfere with the complete oxidation of glucose in the time of heating used, or the compound which

TABLE II.
Influence of Borate on Optical Activity of Glucose.

Solution No.		α	$[\alpha]$
		degrees	degrees
1	0.5 M glucose.....	9.59	53.3
2	0.5 " " + 0.5 M HBO ₂	9.59	53.3
3	0.5 " " + 0.2 " " + 0.1 M NaOH.....	8.66	48.1
4	25 cc. Solution 3 + 25 cc. H ₂ O.....	4.24	47.1
5	25 " " 3 + 25 " 0.1 M HCl.....	4.80	53.3

α was determined at room temperature (25°), a 2 dm. tube and sodium lamp being used. The values given are the final values after mutarotation had ceased.

TABLE III.
Fehling's Solution. Influence of Boric Acid and Sulfuric Acid on Oxidation of Glucose.

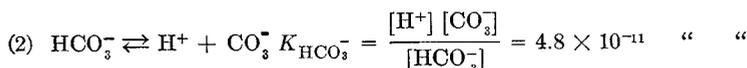
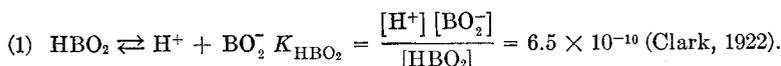
Acid present.	HBO ₂			H ₂ SO ₄
	Glucose used.			
	0.5 mm	0.75 mm	1.0 mm	1.0 mm
<i>m.-eq.</i>	<i>mg. Cu</i>	<i>mg. Cu</i>	<i>mg. Cu</i>	<i>mg. Cu</i>
0.0	181	267.5	344	339
0.5	178		344	
1.0	176		342	
2.5	175	262.0	338	
5.0	173	257.0	332	
10.0	164	247.5	321	339
15.0	151	237.0	309	
20.0	149	225.7	295	338

it forms with glucose may be oxidized to give products which are different from those ordinarily formed. If the first postulate is true, increasing the time of heating should bring the amount of copper reduced closer to the amount reduced in the absence of borate. Table IV shows that increasing the time of boiling does not bring the copper reduced in the presence of borate closer to

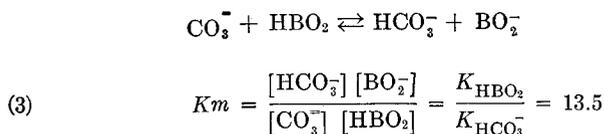
that reduced when borate is not present and it appears, therefore, that the products of oxidation of the glucose in the presence of borate are different and less completely oxidized than in its absence.

The solutions (Table IV) were brought to boiling in 4 minutes and boiled for the lengths of time shown. Blanks and all determinations were run in duplicate under reflux condensers to prevent concentration of the solutions.

Micro Reagent of Shaffer and Hartmann.—Somogyi (1926) has shown that the optimal initial pH of the carbonate-bicarbonate buffer present in the micro reagent of Shaffer and Hartmann (1920–21) is 9.8 to 9.9. In the present work the original Shaffer-Hartmann reagent has been used because on the addition of boric acid the carbonate-bicarbonate buffer will approach the optimal condition rather than recede from it. The change in reaction of the buffer due to the addition of boric acid may be calculated from the dissociation constants of boric acid and the bicarbonate ion.



For the reaction:



In the original reagent (5 cc.) there are 1.5 mm of Na_2CO_3 and 0.5 mm of NaHCO_3 . Assuming complete dissociation for all salts, the concentrations (in mols per liter) will be, after the addition of 1 mm of HBO_2 :

$$\begin{aligned} [\text{HCO}_3^-] &= 0.1 + [\text{BO}_2^-] \\ [\text{CO}_3^{2-}] &= 0.3 - [\text{BO}_2^-] \\ [\text{HBO}_2] &= 0.2 - [\text{BO}_2^-] \end{aligned}$$

Substituting in equation (3) and solving:

$$\begin{aligned} [\text{BO}_2^-] &= 0.19 \\ [\text{CO}_3^{2-}] &= 0.11 \\ [\text{HCO}_3^-] &= 0.29 \end{aligned}$$

$$\text{pH} = 10.3 + \log \frac{0.11}{0.29} = 9.9$$

These calculations take no account of possible loss of CO_2 which, as Somogyi has shown, is a factor affecting the reaction of the mixture; nor do they allow for the dissociation as an acid of the small amount of glucose borate ester which might be present. This latter factor is negligible because if all the glucose (0.005 mM) combined with boric acid, the concentration of this stronger acid would be only 0.001 M in the undiluted reagent. The value of the pH given above is to be regarded only as an approximation which nevertheless is sufficiently exact for our purpose.

Judging by the pH of the buffer alone, the amount of copper reduced when 1.0 mM of boric acid is added should be greater than

TABLE IV.
Fehling's Solution. Influence of Time of Boiling.

Time of boiling.	1 mM glucose.	1 mM glucose + 10 mM HBO_2 .	Difference.
<i>min.</i>	<i>mg. Cu</i>	<i>mg. Cu</i>	<i>mg. Cu</i>
2	334	316	18
5	335	317	18
10	340	322	18

TABLE V.
Shaffer-Hartmann Micro Method. Copper Reduced by 0.005 mM Glucose in Presence of Borate.

HBO_2 present, mM	0.00	0.025	0.050	0.250	0.500	1.000
Cu^{++} reduced, mg	1.99	1.94	1.86	1.81	1.35	0.63

when no boric acid is present. Instead a marked decrease is found which we ascribe to the effect of the borate ion. Table V shows the number of mg . of copper reduced by 0.005 mM of glucose in 15 minutes in this solution when borate is present in varying amounts.

Experiments on the effect of time of heating on the amount of copper reduced showed that the reduction was not complete in 15 minutes when borate was present. Fig. 1 shows that the rate of oxidation as well as the amount of copper reduced are both decreased when borate is present.

The borate has two possible effects: (1) It reduces the concentration of free glucose which may be oxidized at a given time; or

(2) it forms a compound with glucose which may be oxidized directly to give end-products differing from those given by glucose alone and which therefore may reduce a different quantity of copper. The curves of Fig. 1 which show that borate decreases the amount of oxygen utilized by the glucose even though the

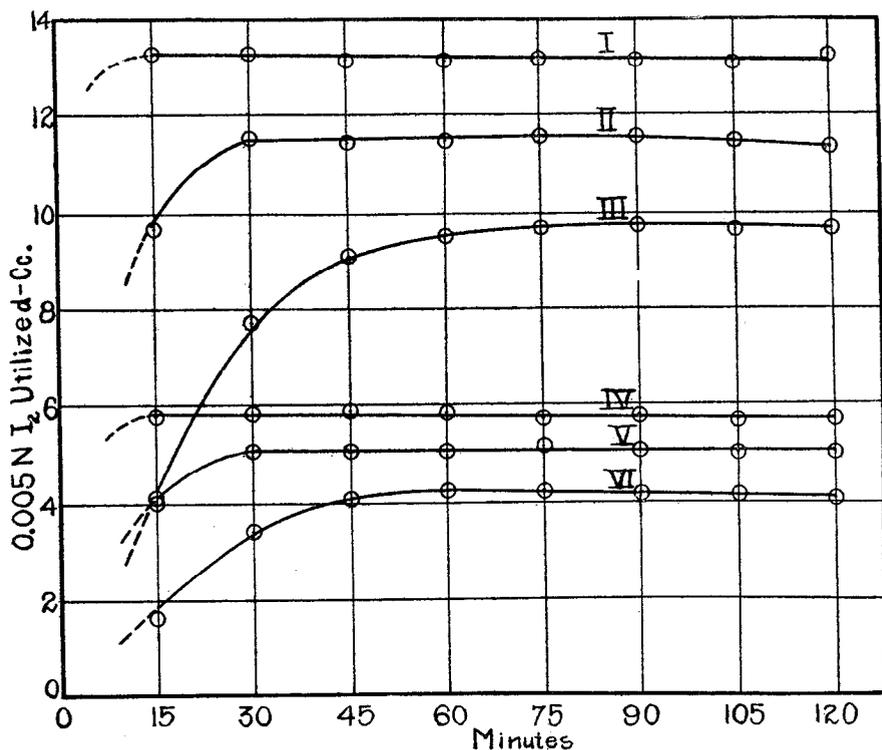


FIG. 1. The effect of boric acid on the reduction of copper in the Shaffer-Hartmann micro reagent. Curves I, II, and III were obtained with 0.01 mm of glucose alone, and in the presence of 0.5 mm HBO₂ and 1.0 mm HBO₂ respectively. Curves IV, V, and VI were obtained with 0.005 mm glucose, without addition, with 0.5 mm HBO₂, and with 1.0 mm HBO₂ respectively.

heating be continued for 2 hours seem to indicate that both effects are present, but that the second postulate is probably more important quantitatively.

Benedict's Qualitative Reagent.—If Benedict's qualitative reagent is used in the manner directed by Shaffer and Hartmann for

quantitative work, an interference with complete oxidation of glucose in the presence of boric acid is encountered.

0.25 mM of glucose and varying amounts of boric acid dissolved in 25 cc. of water were added to 25 cc. of the reagent. The solutions were brought to a boil in a covered flask in 4 minutes and boiling continued for 5 minutes. The results are shown in Table

TABLE VI.

Benedict's Qualitative Reagent. Influence of Boric Acid on Copper Reduced by 0.25 mM Glucose.

HBO ₃ , mM	0.0	0.5	2.5	5.0	10.0	25.0
Copper reduced, mg.....	80.3	78.5	73.2	63.4	40.2	25.0

TABLE VII.

Benedict's Qualitative Reagent. Influence of Borate on Rate of Reduction of Copper.

Time of boiling.	0.25 mM glucose.	0.25 mM glucose + 5.0 mM HBO ₃ .	0.25 mM glucose + 10.0 mM HBO ₃ .
<i>min.</i>	<i>mg. Cu</i>	<i>mg. Cu</i>	<i>mg. Cu</i>
5	80.3	63.4	40.2
10	79.6	66.3	51.5
15	80.3	67.2	56.7
20	80.1	67.5	58.0

TABLE VIII.

Folin Titration. Error Caused by Boric Acid Added to Glucose Solutions.

H ₃ BO ₃	Titration.	Error.
<i>per cent</i>	<i>cc.</i>	<i>per cent</i>
0.0	2.53	
0.5	2.60	2.8
1.0	2.65	4.7
2.0	2.90	14.4

VI. By lengthening the time of boiling, more copper is reduced, but, as in the previous reagents, it does not reach the values found in the absence of borate. The solutions were brought to a boil in 4 minutes and boiling continued the length of time shown. Appropriate blanks were run. The results are given in Table VII.

Folin-Peck-McEllroy Method.—Since the Folin titration has been extensively used in this laboratory for the determination of sugar

in diabetic urines, we have determined the errors produced by the presence of boric acid in glucose solutions. Duplicate titrations usually checked within 0.02 cc. All solutions contained the same amount of glucose but variable quantities of boric acid. The results are given in Table VIII.

Oxidation of Glucose by an Acid Oxidizing Reagent.

Barfoed's Reagent.—Since it was shown in Table II that borax has a marked effect upon the optical activity of glucose but that boric acid produces no change in the specific rotation, it is interesting to compare the oxidation of glucose by an acid copper reagent in the presence and absence of boric acid. As shown in Table IX,

TABLE IX.

Barfoed's Reagent. Influence of Boric Acid on the Rate of Oxidation of Glucose.

The results are expressed in cc. of 0.02 N I₂.

Glucose, mM.	0.025	0.025	0.05	0.05
HBO ₃ , mM.	0	2.5	0	2.5
<i>min.</i>				
15	1.02	1.07	2.10	2.16
30	2.34	2.43	4.31	4.39
45	3.17	3.28	5.53	5.53
60	3.88	3.93	6.32	6.34
75	4.33	4.51	6.88	7.13
90	4.69	4.77	7.34	7.47

the presence of boric acid does not alter the rate of oxidation of glucose by Barfoed's reagent.

In passing, a few remarks on the technique of these determinations are inserted. Since some difficulties were encountered, the conditions under which satisfactory duplicates could be obtained with pure glucose solutions were worked out. The reagent used contained 0.16 mol of copper acetate and 0.16 mol of acetic acid per liter. 5 cc. of this reagent were heated with 5 cc. of the sugar solution and the copper reduced was determined by the "cuprous" titration method of Shaffer and Hartmann. Exactly 5 cc. of a solution containing 30 gm. of KI and 2.7 gm. of KIO₃ per liter (= 0.071 N I₂) were added to the copper solution containing the

Cu_2O followed by 2 cc. of saturated potassium oxalate solution and 1 cc. of 2.5 N H_2SO_4 . After gently stirring the cuprous oxide precipitate with a glass rod to insure complete solution, the excess iodine was titrated with 0.02 N thiosulfate. The blank titration remained constant during the longest heating period but the amount of copper reduced appears to be altered by slight changes of the temperature of the water bath. The solutions containing the boric acid were heated at the same time as the controls containing no boric acid.

These data do not mean that combination between boric acid and glucose does not occur, but that due to rapid hydrolysis the small amount present does not affect the rate or extent of oxidation.

Oxidation of Glucose by Alkaline Iodine Solutions.

The oxidation of glucose to gluconic acid involves only the aldehyde group. When the aldehydic hydroxyl group of the lactone is blocked, as in glucosides, no oxidation occurs. If it is assumed that boric acid or borates react with glucose to form easily saponifiable esters involving the aldehydic hydroxyl group, the prediction may be made that boric acid will decrease the rate of oxidation of glucose to gluconic acid but will not interfere with the final completion of the reaction.

Romijn (1897) who first used alkaline iodine solutions to determine glucose advocated the use of a weak alkali to prevent the formation of iodate from the hypoiodite. Strangely enough he used borax for alkalinity and allowed 24 hours for complete oxidation. However, Willstätter and Schudel (1918) found that quantitative results were obtainable within 15 minutes if the borax was replaced by the gradual addition of an excess of 0.1 N NaOH. Cajori (1922) used sodium carbonate as the alkali and obtained quantitative results in 25 minutes.

Since the addition of boric acid to the carbonate solution will change the reaction of the solution, preliminary experiments were performed to ascertain the effect of the pH of the carbonate buffer on the rate of oxidation. Molar solutions of sodium carbonate and bicarbonate were prepared from the best grades of the salts available in the laboratory and the quantities of each measured and mixed in proper proportion to give several solutions contain-

ing 0.03 N Na⁺ but of different pH. 125 cc. of exactly 0.1 N I₂ solution were measured into a 250 cc. volumetric flask and the glucose (450 mg. = 2.5 mM), which had been weighed and dissolved in a little water, was washed into the flask and the buffer solution added. This was taken as zero time. The flask was

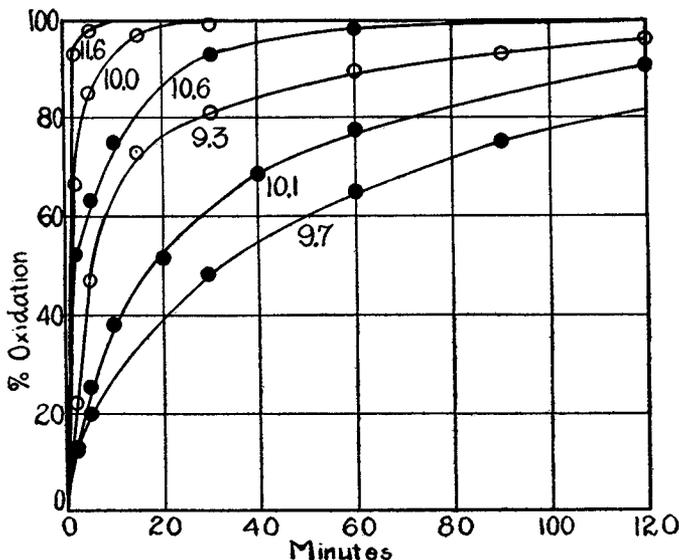


FIG. 2. The effect of boric acid on the rate of oxidation of glucose by alkaline iodine solutions. The figure below each curve represents the pH of the buffer present. The solid circles indicate the presence of borate. The exact compositions of the solutions are as follows:

Calculated pH.....	9.3	9.7	10.0	10.1	10.6	11.6
Na ₂ CO ₃ , M.....	0.025	0.15	0.075	0.15	0.15	0.15
NaHCO ₃ , M.....	0.250		0.150			
HBO ₂ , M.....		0.15		0.10	0.05	

rapidly filled to the mark, stoppered, and the contents mixed by inverting. Beginning at 2 minutes, 25 cc. samples of the solution were removed and immediately run into an excess of H₂SO₄; the iodine remaining in the sample was titrated with thiosulfate solution and the results expressed in per cent of glucose oxidized. Experiments were carried out at room temperature (22–25°).

The pH of the carbonate-bicarbonate buffer was estimated from the data of Auerbach and Pick cited in Clark (1922), but the actual pH of the solution during the oxidation differs from this because of the formation of anions from iodine. The results of this control series are shown with the results with carbonate-boric acid mixtures in Fig. 2. The experiments were performed in the manner described above and the pH of the buffer was estimated as previously described in the section on the micro reagent of Shaffer and Hartmann. Colorimetric tests showed that the pH values were in the calculated order and that the addition of glucose in the concentrations used had no appreciable effect on the pH.

The curves of Fig. 2 show the course of the reactions in carbonate-boric acid mixtures and carbonate-bicarbonate mixtures. Comparison shows that the rate is markedly decreased by the presence of borate. The curve for the carbonate-boric acid mixture of pH 10.1 shows a slower oxidation than the carbonate-bicarbonate of pH 9.3 in spite of the fact that increasing alkalinity favors the oxidation. Borate does not interfere with the completion of the oxidation but does interfere with its rate. The influence of temperature on the rate of oxidation of glucose by iodine has been tested by comparing the rates in carbonate-bicarbonate mixture of pH 10 and in carbonate-boric acid mixture of pH 10.1 in an ice bath, and in the incubator at 36°. The solutions were brought to the temperature of the bath before mixing. A rise in temperature increased the rate in both mixtures but the rate in the carbonate-boric acid mixture was always much slower than in the other buffer.

Influence of Borate on the Oxidation of Other Sugars.

The experimental data cited above would lead one to suspect that most other sugars would respond to the addition of boric acid by a decrease in reducing power toward Fehling's solution, and if they are aldo sugars by a decrease in the rate of oxidation by alkaline iodine solution. Table X shows that the oxidation of fructose, galactose, maltose, and lactose by Fehling's solution is less complete in the presence of borate than in its absence but the oxidation of arabinose seems to be unaltered.

The lactose, maltose, and galactose were the Pfanstiehl, C.P., Special brand, and the arabinose a C.P. Will Corporation sample

which gave the calculated values by Cajori iodine method. 0.5 mm of each of these sugars was used for the determinations given in Table X. Since the fructose was not c.p., the results with this sugar may not be entirely satisfactory.

The effect of borate on the rate of oxidation of various sugars by alkaline iodine solutions is shown in Table XI. The experiments were performed in the manner described previously with

TABLE X.
Influence of Borate on Copper Reduced in Fehling's Solution by Various Sugars.

Sugar, 0.5 mm.	No addition.	With 10 mm HBO ₂ .	Difference.
	mg. Cu	mg. Cu	mg. Cu
Glucose.....	177	167	-10
Fructose.....	314	305	- 9
Galactose.....	162	155	- 7
Arabinose.....	150	151	+ 1
Maltose.....	188	171	-17
Lactose.....	235	224	-11

TABLE XI.
Influence of Borate on Oxidation of Sugars by Alkaline Iodine Solutions.
Results are expressed in per cent of complete oxidation.

Sugar.	Glucose.		Fructose.		Galactose.		Arabinose.		Maltose.		Lactose.	
	CO ₃ ⁻	BO ₂ ⁻										
<i>min.</i>												
2	66.1	22.0	0.0	1.0	88.0	32.7	93.8	38.3	79.5	62.5	77.7	74.7
5	85.0	37.3	0.5	1.4	93.0	56.8	98.5	57.5	89.0	81.0	92.9	91.7
10	97.2	50.8			98.0	73.5	98.6	74.6	97.5	93.8	100.0	98.4
20	99.0	74.0	3.0	2.5	98.2	86.5	100.3	88.0	97.0	97.8	101.0	100.1
60	99.4	86.0	3.5	2.3	98.2	95.2	99.3	98.0	99.3	98.0		
120	99.9	94.5	6.3	2.3	99.0	98.2	101.5	99.3	99.3	100.0		

the carbonate-boric acid mixture (column headed BO₂⁻) of pH 10.1 and the carbonate-bicarbonate mixture (column headed CO₃⁻) of pH 10.0.

The rate of oxidation with hypoiodite of all sugars studied except lactose is definitely decreased; corresponding to this behavior of lactose it was noted by Klein (1878) that lactose does not change the reaction of borax solution to phenolphthalein.

The effect of borate on the oxidation of lactose by Fehling's solution may be accounted for by the effect of borate on the decomposition and partial oxidation products of lactose.

It has been claimed (Cajori, 1922) that pure fructose is not oxidized by alkaline iodine solution but our sample did utilize iodine. This may have been due to impurities or the slow oxidation of fructose itself or to the formation of aldo sugars through the effect of alkali. The oxidation of the latter has been shown to be depressed by borate.

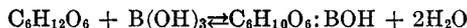
The data of Table XI show that borate interferes to a greater extent with the oxidation of glucose than with the oxidation of galactose or arabinose in alkaline iodine solutions. The interferences in the latter sugars are practically equal and it is significant that each contains a favorably oriented pair of hydroxyl groups in the non-reducing part of the chain on the basis of the amylenic oxide structure, whereas glucose does not contain similarly situated hydroxyl groups. Fischer (1895) found that α -methylgalactoside becomes optically active in borax solution whereas optical activity is not otherwise present (because of internal compensation (Hudson, 1909)). It is conceivable that the concentration of borate ion is reduced by ester formation involving the non-reducing groups of galactose and arabinose and as a consequence less of the ester involving the reducing group is present to interfere with its oxidation. We are aware that this argument requires substantiation at many points and that the extent of ester formation is not necessarily the same wherever the same group is involved but may depend also on the particular configuration of the remainder of the molecule. Thus borate has less effect on the oxidation of maltose which is 4- or 5-glucose- α -glucoside (Irvine and Black, 1926) than on glucose, although the group available for ester formation is the same in both cases. Borate has practically no effect on the oxidation of lactose and since lactose is 4- or 5-glucose- β -galactoside (Haworth and Leitch, 1918), two of the non-reducing hydroxyl groups of the galactose residue may have an effect analogous to the same groups in galactose itself and an interference similar in amount to that found with galactose might be expected. But, as in the case of maltose, disaccharide formation has a further unfavorable effect on the formation of an ester through the reducing hydroxyl group of the

glucose residue, and perhaps by virtue of these two unfavorable circumstances borate forms but a small amount of ester with the reducing hydroxyl group of lactose and consequently does not interfere with its oxidation. We hope that more extended study of the influence of borate on optical activity of the sugars may be correlated with the oxidation data and may help in its elucidation.

DISCUSSION.

Alkaline Copper Methods.—Borate, by forming a compound with the sugar being oxidized, hinders the oxidation of the aldehyde group by cupric ion. This probably changes the relative amounts of the various oxidation products which are formed and accounts for the decreased amount of copper reduced when borate is present. Some of the decomposition products which normally are oxidized further may form compounds with borate and be protected to some degree against this further oxidation. These decomposition products contain groups favorable to reaction with borate such as adjoining hydroxyl groups and α -hydroxy acids (Rimbach and Ley, 1922).

Alkaline Iodine Solutions.—The formation of borate esters interferes with the oxidation of the aldehyde group by decreasing the concentration of sugar available for oxidation by hypiodite and therefore decreasing the rate of oxidation. As glucose is removed by oxidation, the ester is saponified by reversal of the reaction:



making more glucose available for oxidation and allowing the oxidation to proceed to completion.

CONCLUSIONS.

1. The presence of borate decreases the amount of cuprous oxide formed by the oxidation of glucose, galactose, fructose, maltose, and lactose in Fehling's solution and decreases the rate and extent of oxidation in copper reagents which owe their alkalinity to sodium carbonate.

2. In an acid oxidizing reagent (Barfoed's) the presence of boric acid has no effect on the oxidation of glucose.

3. In the oxidation of glucose, galactose, arabinose, and maltose at the aldehyde group by alkaline iodine solutions, boric acid measurably decreases the rate of reaction. This does not appear to be the case with lactose.

4. The improper use of boric acid solution in collecting urines by catheter in the study of carbohydrate metabolism may give rise to serious errors.

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