THE REACTION OF ASCORBIC ACID WITH FORMALDEHYDE

by

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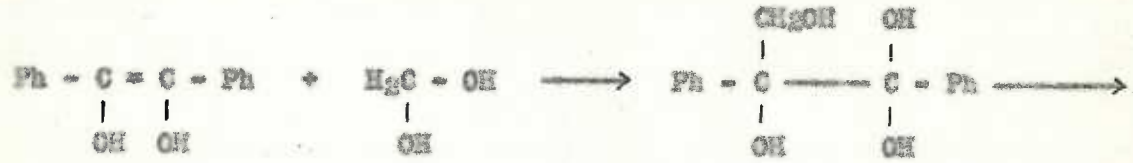
Introduction

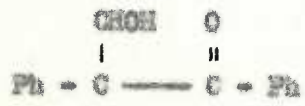
In previous work (1) it was discovered that formaldehyde interfered in the titration of ascorbic acid with 2,6 dichloro phenol indophenol or iodine. This phenomenon appeared to occur even at a low pH. In the dilute solutions used the titratable acidity of the ascorbic acid was found to be unchanged and further, the formaldehyde could be recovered from the solution quantitatively with dimedon.

In more concentrated solutions, especially in the presence of calcium carbonate, ascorbic acid and formaldehyde seemed to form an addition compound which could be obtained as a syrup. No formaldehyde could be recovered from this syrup with dimedon nor did it reduce indophenol. Analysis suggested the addition of two molecules of formaldehyde to one molecule of ascorbic acid. It resembled a sugar in some of its chemical properties and tasted sweet. The specific rotation in water varied from $[\alpha]_D^{21^\circ}$ -10.2° to -12.8°. The acetyl number varied from 761 to 850. The reducing power toward the Shaffer-Somogyi reagent (2) varied from 52-57% of that of glucose. A benzoylated derivative obtained had a molecular weight of about 630.

According to Kusin (3) the condensation of formaldehyde is catalyzed by benzoin. He postulates the mechanism of the reaction thus:

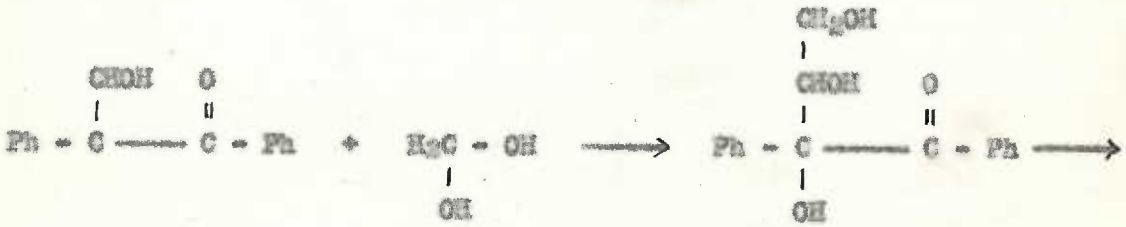


(1) (2)



3

This intermediate product (3) , hydroxy methyl benzoin, he succeeded in isolating. According to his theory, it now adds another molecule of formaldehyde to form another intermediate compound (4) . This splits to yield glyceraldehyde and the original benzoin.

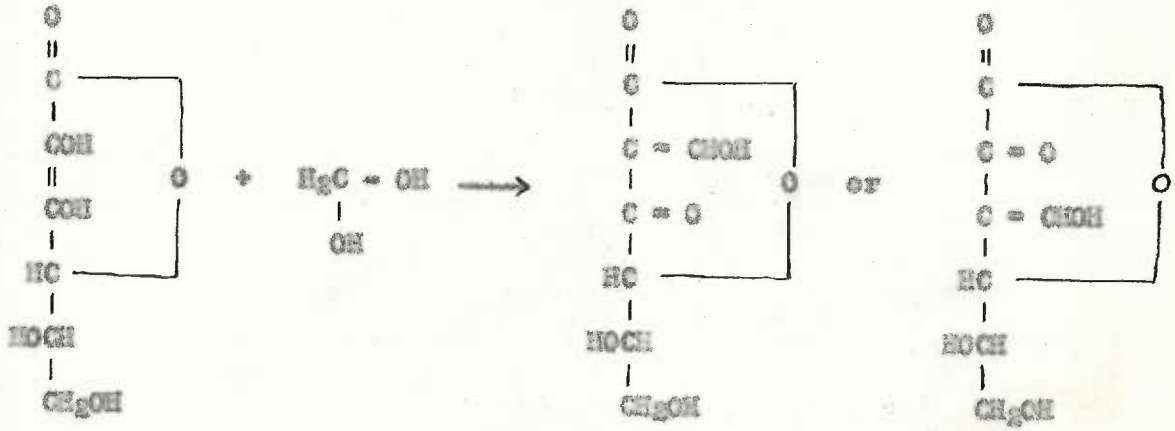


(3) (4)

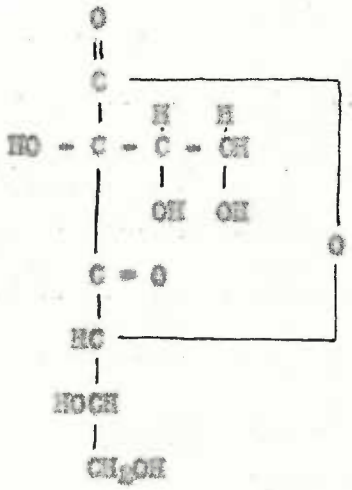
(1)

Since the addition product of ascorbic acid and formaldehyde was found to be a splendid catalyst for the polymerization of formaldehyde

in alkali, it was thought that the formula might resemble, in type, that of Kusin's second intermediate product (4).



This primary addition product might then add another molecule of formaldehyde to form



Such a formula would account for the molecular weight found and the loss of indophenol reducing power.

The work presented in this thesis was an attempt to clarify the mechanism of the reaction between ascorbic acid and formaldehyde, and to isolate a pure addition product whose properties could be investigated.

First, the effect of formaldehyde on the pH of ascorbic acid, reductone, dihydroxy maleic acid and diethyl dihydroxy maleate was determined. Each of these four compounds has an ene-diol grouping which is responsible for the indophenol reducing power and the acidity. In the case of dihydroxy maleic acid, however, the acidity of the ene-diol group is less than that of the two carboxyls.

Second, the time, temperature, concentration and pH relationships of the reaction between ascorbic acid and formaldehyde were studied.

Third, carbon dioxide was found to be produced in this reaction. A study of this was also made.

Fourth, the addition compound was prepared as a syrup by a method different from that used previously.

Experimental

The effect of HCHO on the pH of ascorbic acid solutions.

A 0.1000 N. solution of ascorbic acid* was titrated in 1 ml. steps with 0.1000 N. NaOH. All water used throughout this work was distilled in Pyrex and deaerated with N_2. A Beckmann pH meter (glass electrode) was used for the pH determinations.

ml. NaOH	ml. Ascorbic Acid	pH	23°
0	---	2.65	
1	9	3.32	
2	8	3.71	
3	7	4.08	
4	6	4.46	
5	5	6.30	
6	4	11.17	
7	3	11.68	
2.5	5	4.03	(pK)

Another similar experiment was made using a solution of ascorbic acid (0.1000 N.) in 4% HCHO. The 4% HCHO used had a pH of 3.95 and a negligible buffer capacity.

ml. NaOH	ml. Ascorbic Acid	pH	27°
0	---	2.69	
1	9	3.35	
2	8	3.79	
3	7	4.10	
4	6	4.48	
5	5	7.80	
6	4	10.62	
7	3	11.30	
2.5	5	6.21	(pK)

---

*All ascorbic acid used was Vitamin C "Roche" (synthetic l-ascorbic) made by Hoffman-LaRoche, Inc.

Further data were obtained using ascorbic acid (0.1000 N.) in 38% HCHO. The 38% HCHO used had a pH of 3.69 and negligible buffer capacity.

ml. NaOH	ml. Ascorbic Acid	pH 23°
0	---	3.11
1	9	6.53
2	8	6.92
3	7	7.19
4	6	7.47
5	5	8.40
6	4	10.02
7	3	10.80
8	2	10.89

The effect of HCHO on the pH of reductone solutions.

A 0.1000 M. solution of reductone (CHO - CHOH - CHO) in water was titrated in a similar manner.

ml. NaOH	ml. Reductone	pH 23°
0	---	3.60
1	9	4.70
2	8	7.54
3	7	7.96
4	6	8.32
5	5	9.89
6	4	11.17
7	3	11.51
2.5	5	8.03 (pK)

When 0.1000 M. reductone in 4% HCHO was used the following data were obtained.

ml. NaOH	ml. Reductone	pH	27°
0	---	3.60	
1	9	4.70	
2	8	7.54	
3	7	7.96	
4	6	8.32	
5	5	9.89	
6	4	11.17	
7	3	11.61	
2.5	5	8.10	(pK)

Results obtained using 0.1000 M. reductone in 38% HCHO.

ml. NaOH	ml. Reductone	pH	27°
0	---	5.13	
1	9	6.60	
2	8	7.19	
3	7	7.50	
4	6	7.70	
5	5	9.46	
6	4	10.12	
7	3	10.51	
8	2	10.90	
2.5	5	7.70	(pK)

pK values of ascorbic acid and reductone.

Ascorbic acid in H_2O	4.06
Ascorbic acid in 4% HCHO	6.21
Ascorbic acid in 38% HCHO	7.23
Reductone in H_2O	5.03
Reductone in 4% HCHO	8.10
Reductone in 38% HCHO	7.70

pH values on syrup of addition compound.

The syrup used was prepared by L. F. Hoy.

One and seventeen hundredths grams of syrup in 50 ml. distilled H_2O had a pH of 5.22 at 25°. The addition of 1 ml. 0.100 N. NaOH changed the pH to 10.38.

The effect of HCHO on the pH of dihydroxy maleic acid.

Dihydroxy maleic acid was prepared by Fenton's method (4).

ml. 0.020 N. NaOH	ml. 0.020 N. Acid	pH 25°
0	---	2.20
5	10	3.10 (pK) (5)
5	5	7.6#
5 ml. 0.020 N. acid	+ 5 ml. 4% HCHO	2.29
5 ml. 0.020 N. acid	+ 5 ml. 38% HCHO	3.15

Titration curves were not run in full because the amount of acid available was small and solutions of the acid were so unstable that reliable pH values were difficult to obtain.

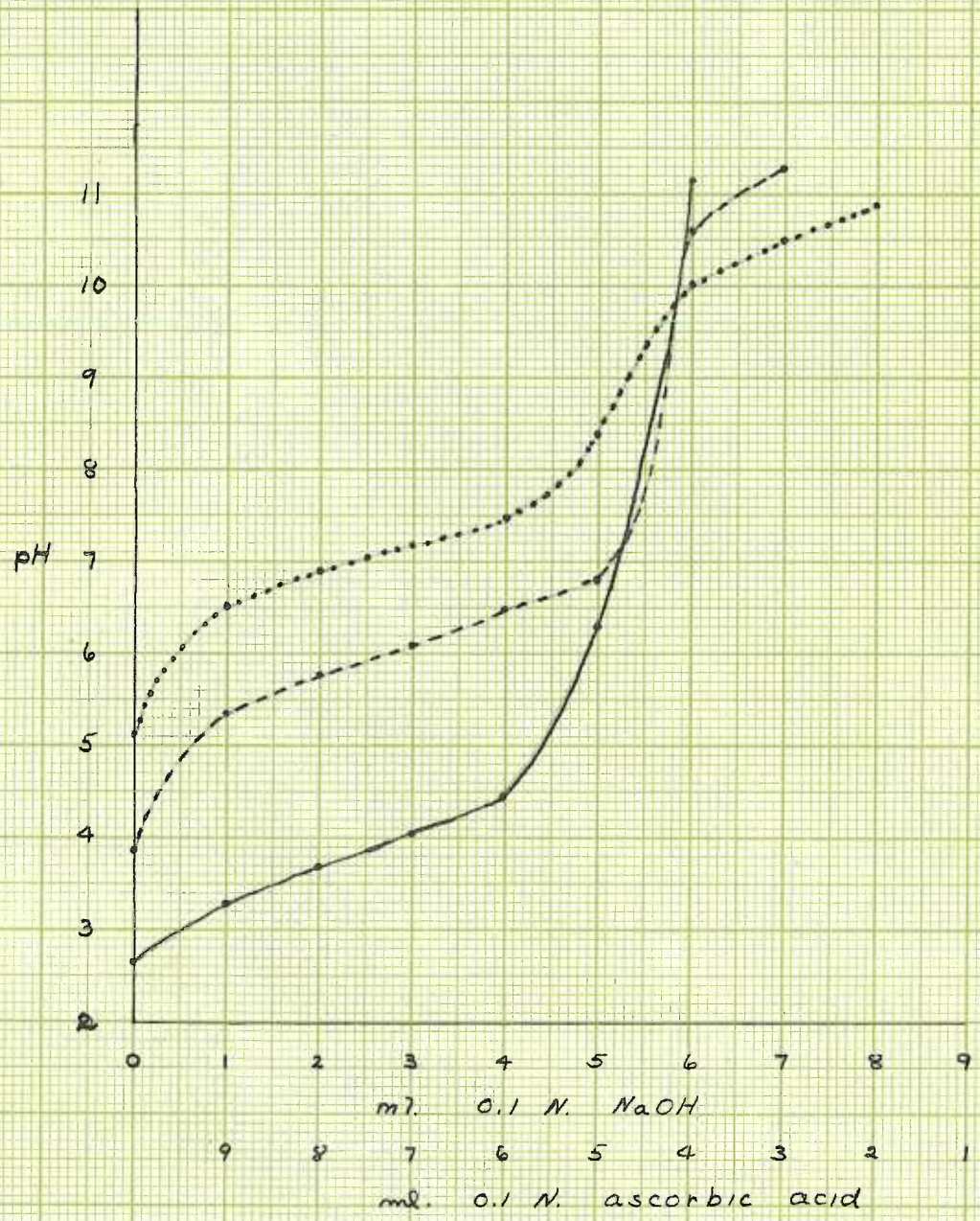
The effect of HCHO on the pH of diethyl dihydroxy malonate.

Diethyl dihydroxy malonate was prepared by Fenton's method (6). Since this acid is rather insoluble in water, a saturated solution, which was used, is only about 0.01 N.

ml. 0.010 N NaOH	ml. saturated solution of acid	pH 25°
0	---	3.08
2	8	3.78
5	5	9.60
5 ml. saturated solution	+ 5 ml. 4% HCHO	3.85
5 ml. saturated solution	+ 5 ml. 38% HCHO	4.95

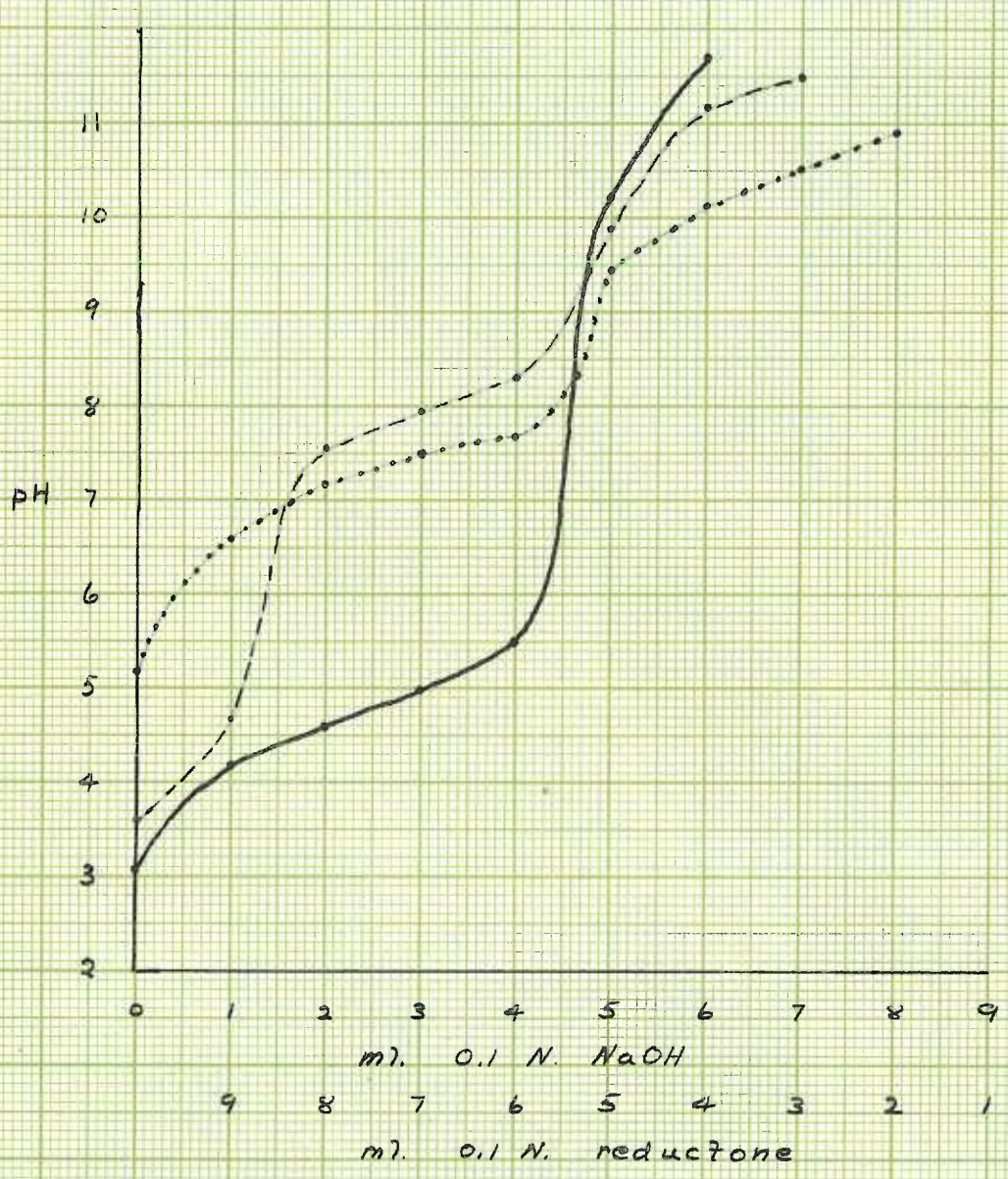
Effect of HCHO on the pH of Ascorbic Acid Solutions

—— ascorbic acid in H_2O 23°
--- ascorbic acid in 4% HCHO 27°
···· ascorbic acid in 40% HCHO 27°



Effect of HCHO on the pH of Reductone Solutions

—— reductone in H_2O 27°
--- reductone in 4% HCHO 27°
···· reductone in 40% HCHO 27°



Rate of reaction between ascorbic acid and HCHO in the presence of $CaCO_3$.

In order to facilitate the preparation of the pure addition product it was thought advisable to study the reaction in detail and to determine, if possible, optimum conditions. The first eight experiments were made as follows: 0.300 g. ascorbic acid, 0.5 g. $CaCO_3$, and 50.0 ml. of 4% HCHO were mixed in a large test tube, placed in a thermostatically controlled water bath, and stirred continuously by an electric stirrer. Analysis was made for HCHO and reducing substances. Temperatures from 31° to 50° were used. In none of these experiments were results obtained which could be interpreted or which could be checked.

Determination of HCHO.

The dimedon (7) method was used. A one ml. sample was taken, acidified to phenol red, and 100 ml. of a saturated solution of dimethyl dihydro resorcinol (dimedon) was added. This was heated on a steam bath for a few minutes, then allowed to stand over night. The fluffy white precipitate was collected by filtration through a weighed Gooch crucible. After drying for at least six hours in an oven at 98° the final weighing was made. Weight of precipitate x 0.1027 equals the weight of HCHO in a one ml. sample.

Determination of reducing substances.

A one ml. sample was diluted to 30 ml. and neutralized to methyl red; 0.5 g. dimedon was added, the mixture heated and allowed to stand

over night. The mixture was shaken at intervals to provide better contact between the solid dimedon and the solution. This completely removed the HCHO. Dimedon and ascorbic acid were removed by the following treatment. A fritted glass filter funnel was employed in removing the precipitate. Four ml. of a precipitating agent (containing 30% $Fe_2(SO_4)_3$ and 9% $MgSO_4$ in 0.25 N. H_2SO_4) were added. The solution was diluted to 50 ml. and allowed to stand ten minutes. After transferring to a 250 ml. flask, $BaCO_3$ was added to the filtrate and H_2S bubbled through. After blowing out excess H_2S with air the mixture was again filtered. The filtrate was neutralized to phenol red. The reducing value was determined by use of the Shaffer-Somogyi reagent #50.

Experiments on the determination of reducing substances.

Since the method just described was so long and laborious another method was sought to replace it.

First, an extraction of excess dimedon with benzene was attempted. The HCHO was removed from a sample of the reaction mixture as before, then extracted with benzene (Figure I).

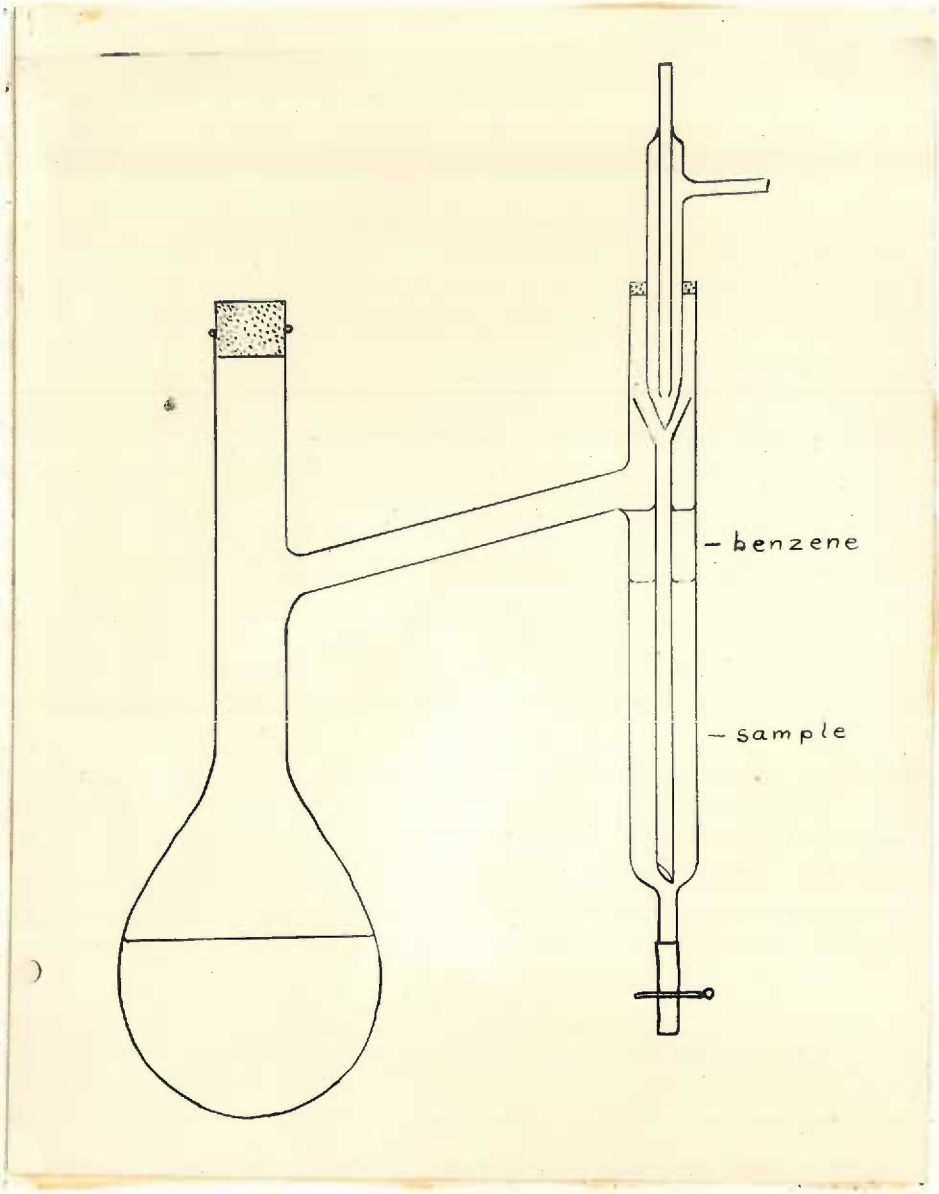
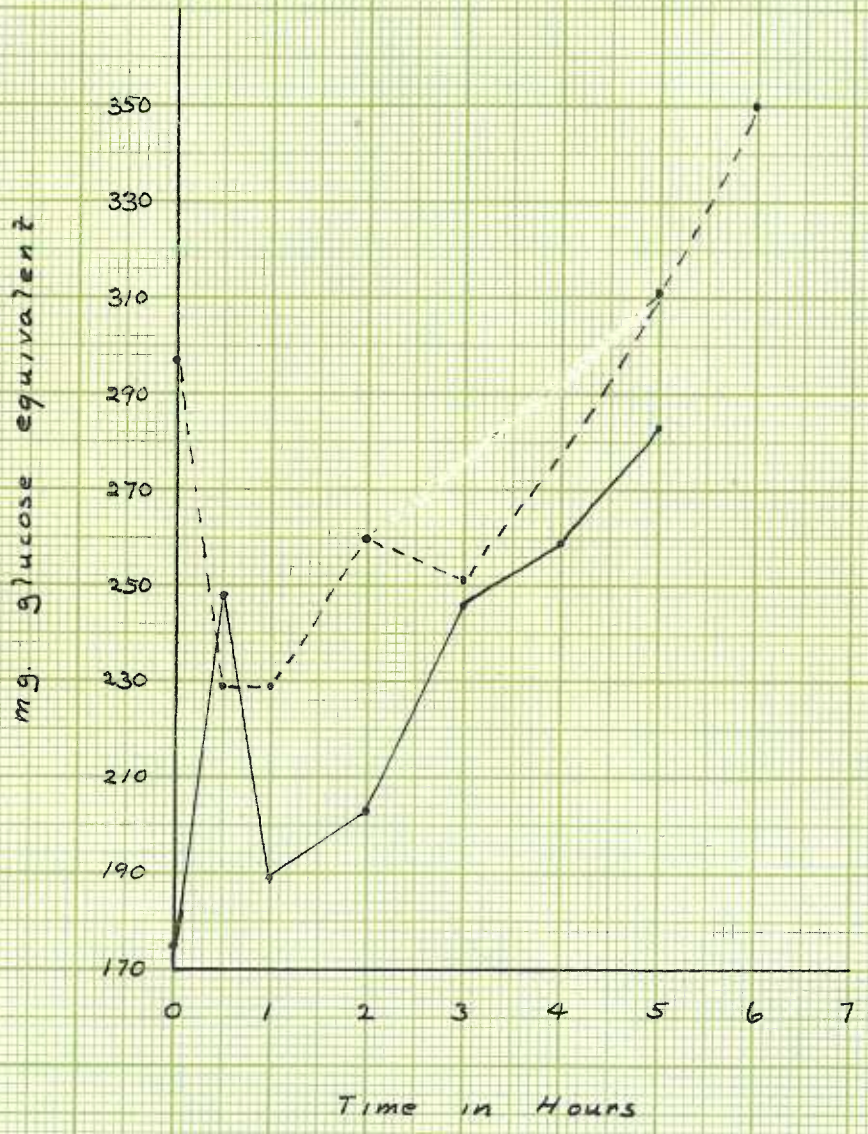


Figure 1

In this apparatus the benzene was vaporized, condensed on the small water condenser, led down through a glass tube, allowed to bubble up through the solution, and returned to the boiling flask by overflow into the side arm. Two trial experiments gave values which failed to check.

Reducing Values obtained after Extraction of Dimedon with Benzene

0.5 g. ascorbic acid 50 ml. 4% HCHO
0.5 g. $CaCO_3$ in air at 35°



Second, reducing values were determined without the removal of HCHO or ascorbic acid. 0.1000 g. ascorbic acid was dissolved in 50 ml. distilled H_2O. A one ml. sample of this mixture was diluted to 5 ml. and its reducing value determined with the Shaffer-Somogyi reagent. The reducing value was equivalent to 1.58 mg. of glucose.

0.1000 g. ascorbic acid was dissolved in 5 ml. 4% HCHO, then diluted to 50 ml. One ml. of this mixture had a reducing value equivalent to 1.71 mg. of glucose.

One ml. 4% HCHO was diluted to 50 ml. A one ml. sample of this solution had a reducing value equivalent to 0.02 mg. glucose.

This showed that variation in reducing power could be followed during the reaction rather easily. Apparently the HCHO undergoes a Cannizzaro reaction and thus has a low reducing power.

The technique used for determining reducing substances throughout the rest of the work was this following. A one ml. sample was taken from the reaction mixture, neutralized to phenol red with 0.5 N. HCl and diluted to 50 ml. Five ml. of this plus five ml. of the Shaffer-Somogyi reagent were heated on a boiling water bath for 15 minutes. After cooling, one ml. of a 4% potassium oxalate - potassium iodide reagent and five ml. of 1 N. H_2SO_4 were added. This was then titrated to the phenol red - starch endpoint with fresh 0.005 N. $Na_2S_2O_3$. Blank titration minus titration of sample equals titration value. One ml. titration is equivalent to 0.113 mg. glucose in the five ml. sample. Data obtained by this method checked better than those of previous methods.

Determination of ascorbic acid.

In order to follow the disappearance of ascorbic acid during the reaction, indophenol and iodine titrations were compared as to applicability. The usual test experiments were made (using 0.500 g. ascorbic, 0.5 g. $CaCO_3$, and 50 ml. 4% HCHO) at 35°. One ml. samples were taken, acidified with 10% acetic acid and titrated.

Titration With 0.009 N. Aqueous I_2
(1 ml. = 0.787 mg. ascorbic acid)

Hours	ml. I_2 Used	Ascorbic Acid Equivalent
0	5.43	4.28 mg.
0.5	5.60	4.40
1	5.45	4.30
2	4.55	3.58
3	3.25	2.56
4	2.15	1.69
5.5	1.15	0.91
6	0.93	0.73
7	0.60	0.47

Titration With Indophenol
(1 ml. = 0.23 mg. ascorbic acid)

Hours	ml. indophenol used	Ascorbic acid equivalent
0	19.55	4.50
0.5	23.25	5.35
1	21.00	4.83
2	17.05	3.92
3	13.20	3.04
5	6.15	1.41
6	3.85	0.89
7	2.30	0.53
12	0.20	0.05
24	0.00	0.00

A control was also run on 0.500 g. ascorbic acid in 50 ml. 4% HCHO (omitting $CaCO_3$) using the same solution of indophenol.

Hours	ml. indophenol used	Ascorbic Acid Equivalent
0	5.50	1.27
0.5	0.95	0.21
1	0.75	
2	0.70	
4	0.65	
5	0.70	
6	0.65	
24	0.90	

In comparing these data it must be noted that the reaction in the presence of $CaCO_3$ went on at a pH of 7.3 while the control without the $CaCO_3$ was at a pH of about 4.2

Iodine titration was preferred because of the greater stability of the solution on standing.

Titration of Ascorbic Acid

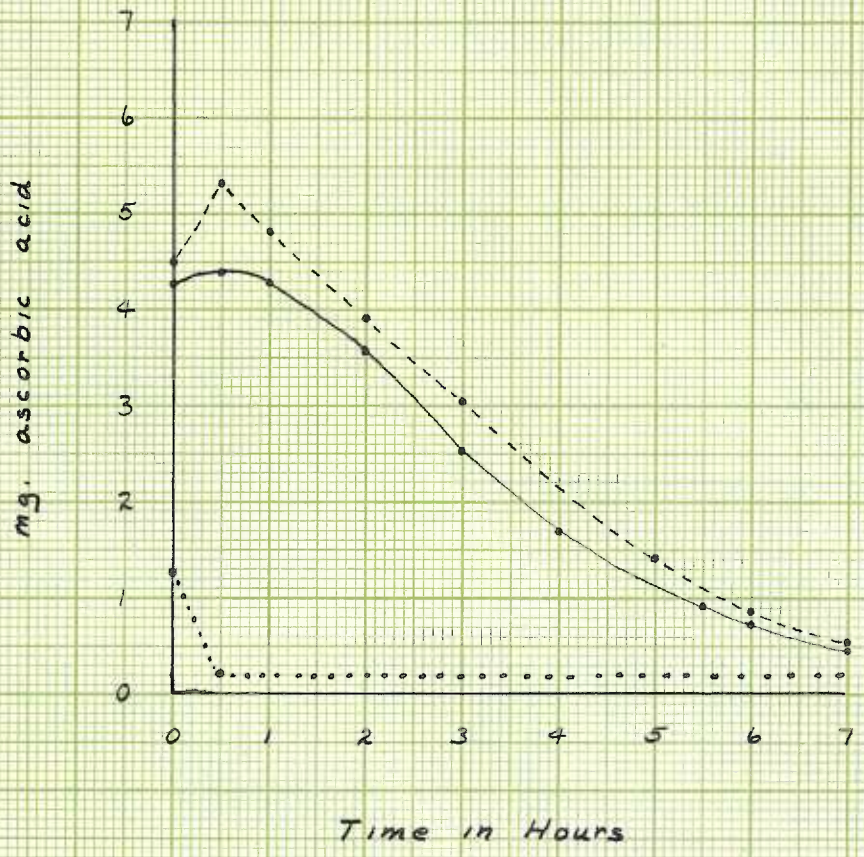
0.5 g. ascorbic acid – 0.5 g. $CaCO_3$ – 50 ml. 4% HCHO allowed to react in air at 35°

0.5 g. ascorbic acid – 50 ml. 4% HCHO used as control under same conditions

— Iodine titration

--- Indophenol titration

···· Indophenol titration of control



Rate of reaction experiments in N_2.

Experiments carried out using the foregoing methods yielded results which could not be interpreted. In order to eliminate oxidation as a factor, further experiments were carried out in N_2. The data obtained were much more satisfactory and could be checked more closely. In the following experiments, HCHO, ascorbic acid and reducing substances were determined by the aforementioned methods.

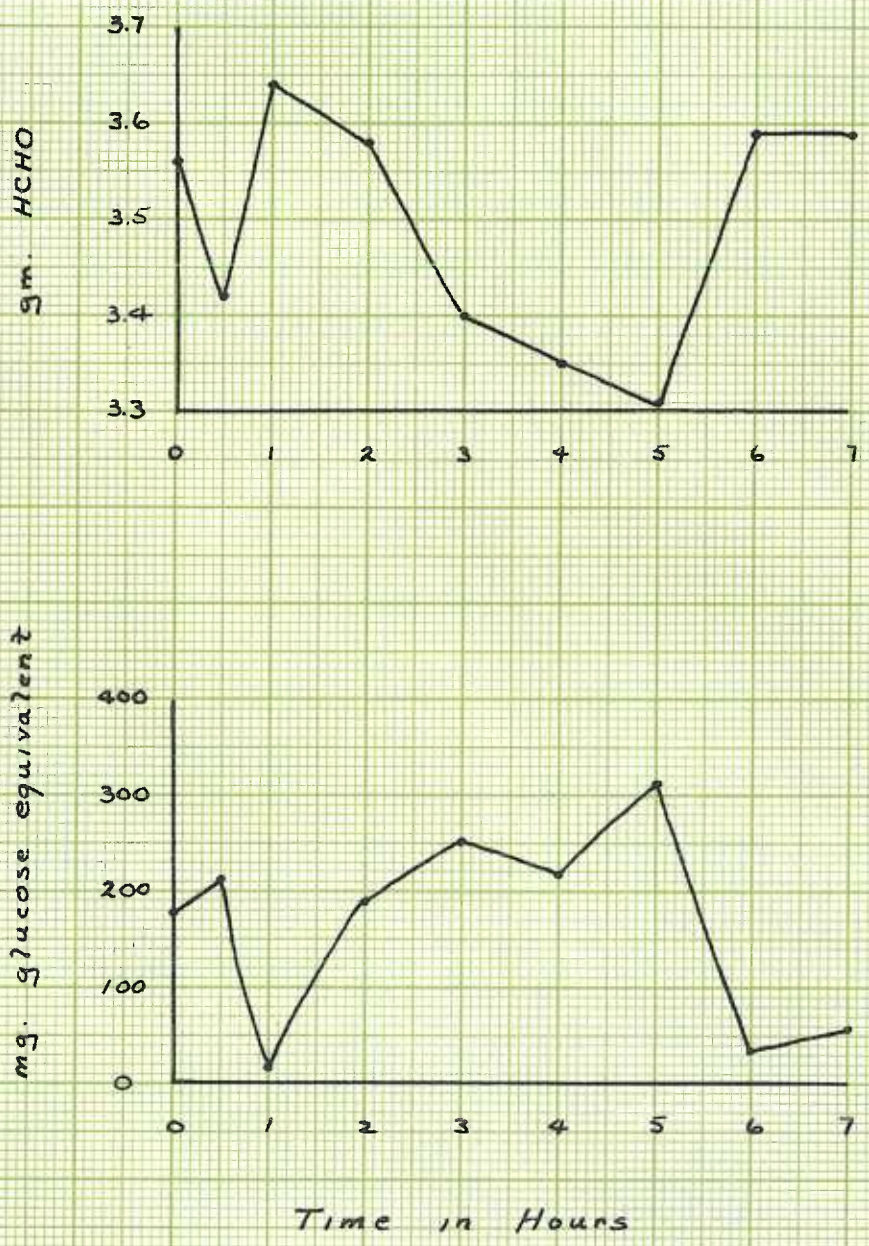
0.500 g. ascorbic acid, 0.5 g. $CaCO_3$, and 50 ml. 4% HCHO were mixed and allowed to react in a 100 ml. Kjeldahl which had a side tube on the bulb for taking samples. A stream of N_2 excluded O_2 and served as an agitator. Runs were made at 35°, 46° and 60°. One experiment was run at 35° using 36% HCHO instead of 4% HCHO.

In order to observe the effects of pH, the following was done. 0.500 g. ascorbic acid, 0.5 g. MgO and 50 ml. 4% HCHO were mixed and run at 35°. MgO maintained a pH of 10.4. An identical experiment was carried out using KOH to obtain a pH of 9.0.

The results of these last two experiments indicated that the function of the $CaCO_3$ was merely that of maintaining a pH rather than that of exerting a catalytic effect. To confirm this, calcium ascorbate was used. 0.500 g. ascorbic acid was dissolved in 25 ml. MgO and excess $CaCO_3$ added. After filtering, 25 ml. 8% HCHO were added and the mixture allowed to react as before. The results were very similar to those obtained when an excess of $CaCO_3$ was present. It was observed that $CaCO_3$ was precipitated during the reaction. This suggested a liberation of CO_2 from the ascorbic acid. Further work showed this to be true.

Sample Curves obtained by allowing the Reaction to proceed in Air

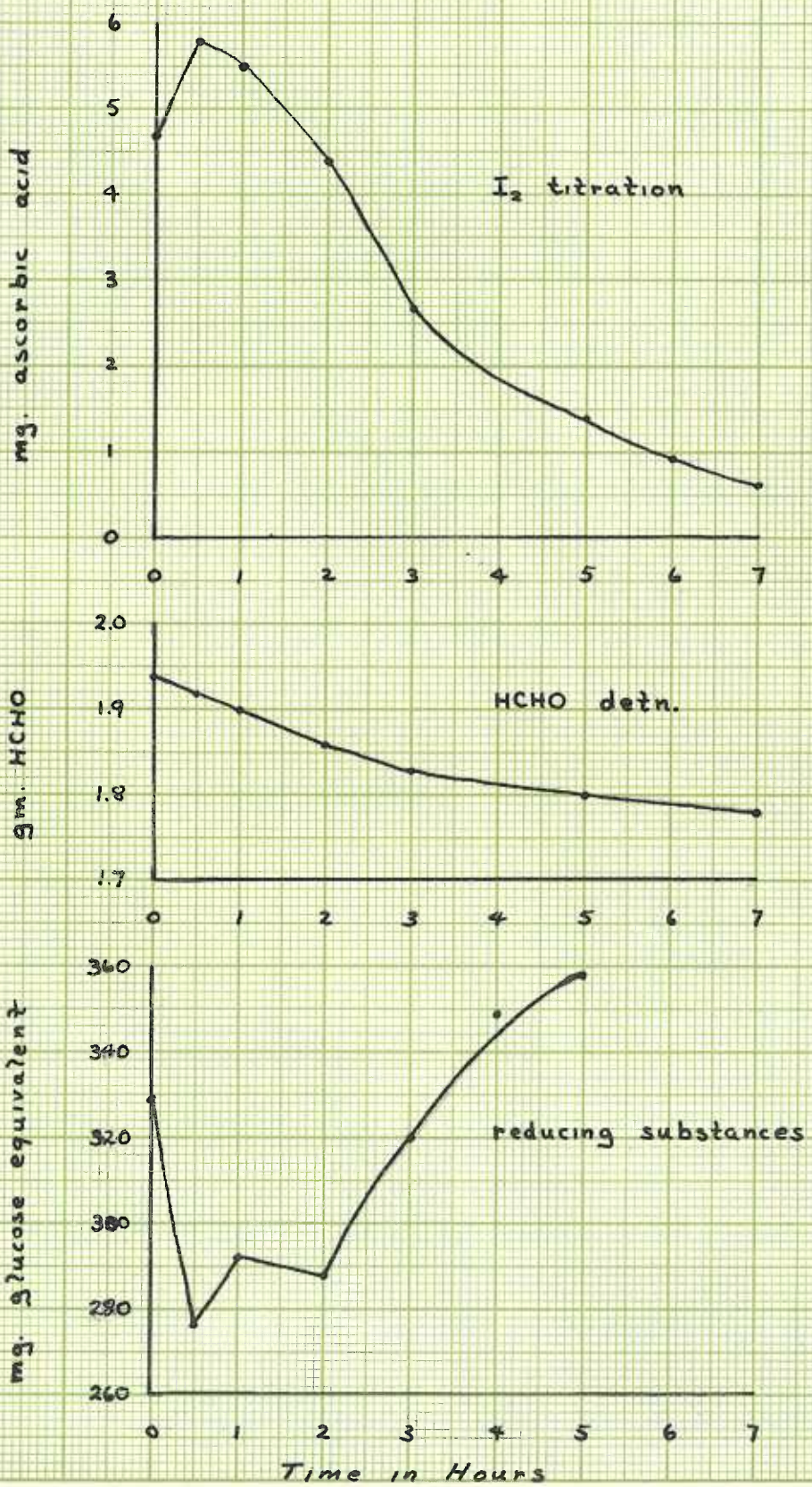
1.0 g. ascorbic acid 1.0 g. $CaCO_3$
100 ml. 4% HCHO in air at 33°



Rate of Reaction in N_2 at 35°

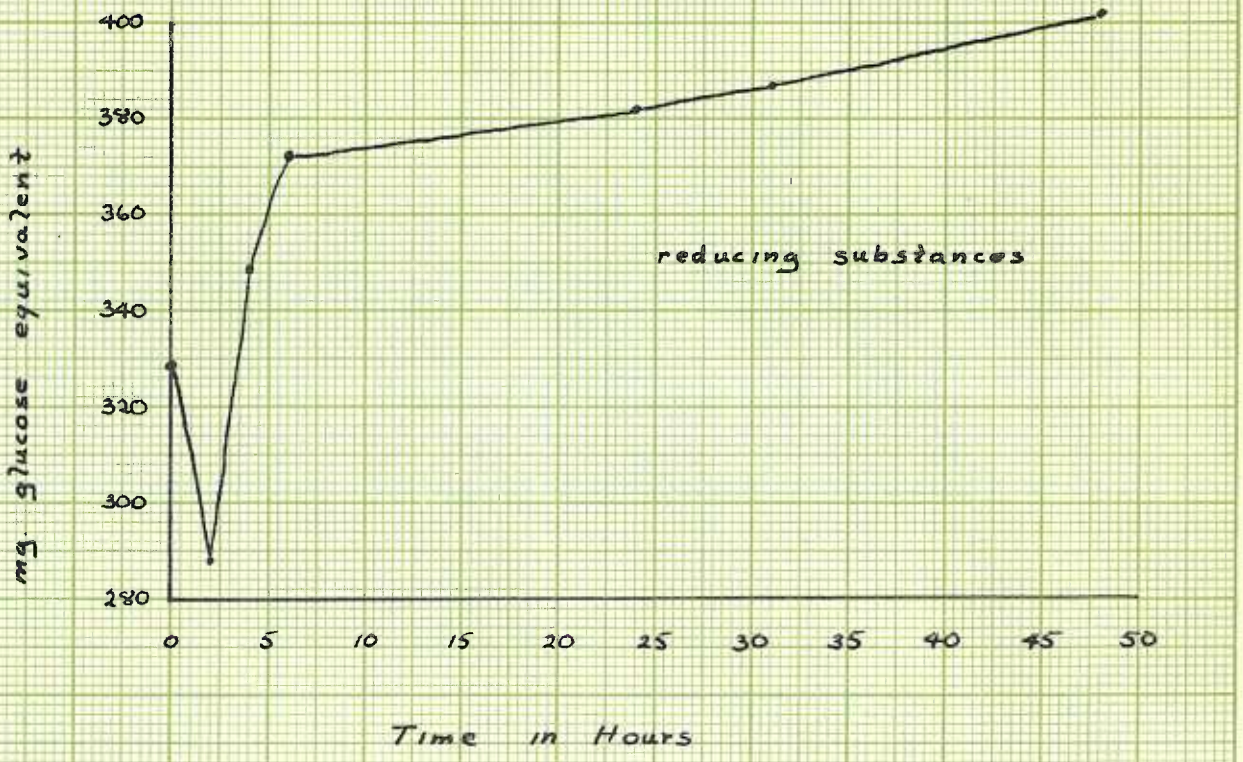
0.500 g. ascorbic acid 0.5 g. $CaCO_3$

50 ml. 4% HCHO pH 7.3



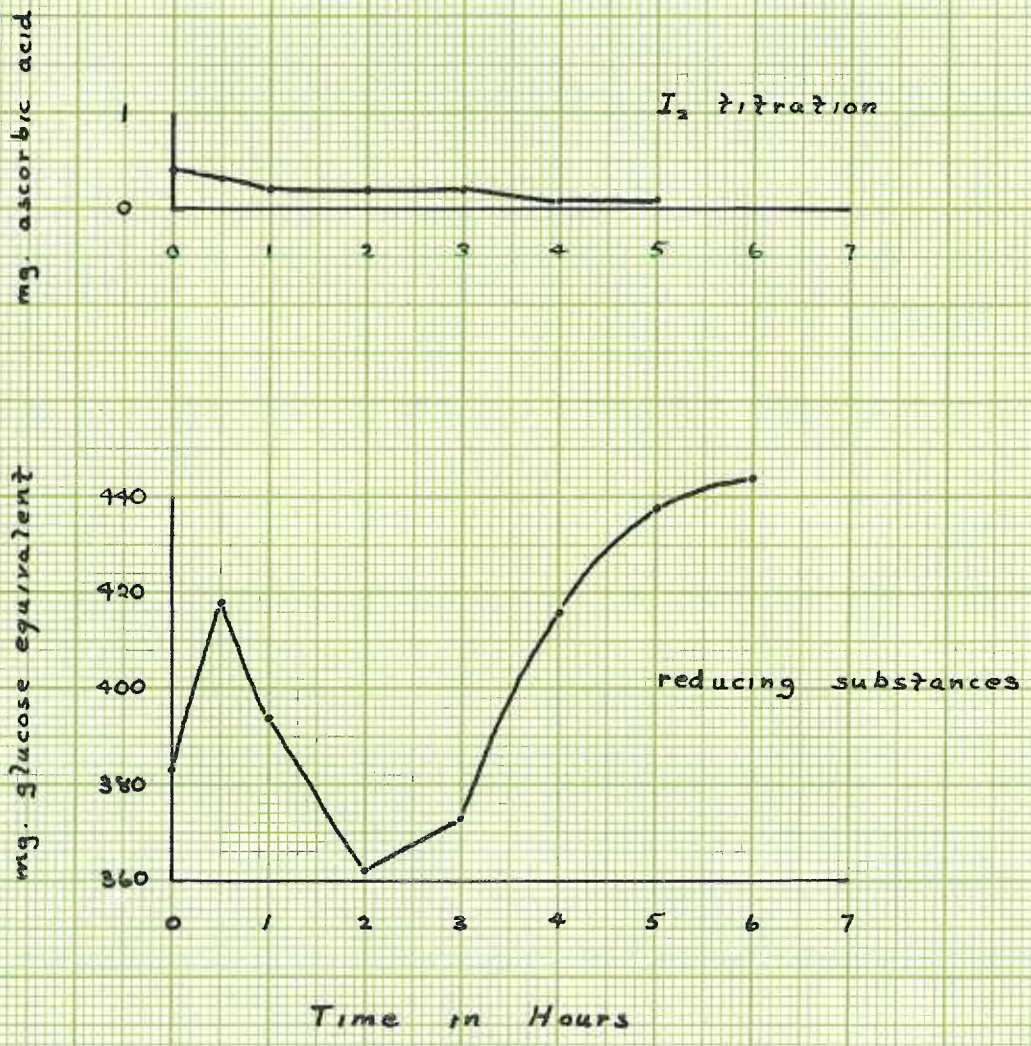
Rate of Reaction in N_2 at 35°

0.500 g. ascorbic acid 0.5 g. $CaCO_3$
50 ml. 4% HCHO



Rate of Reaction in N_2 at 35°

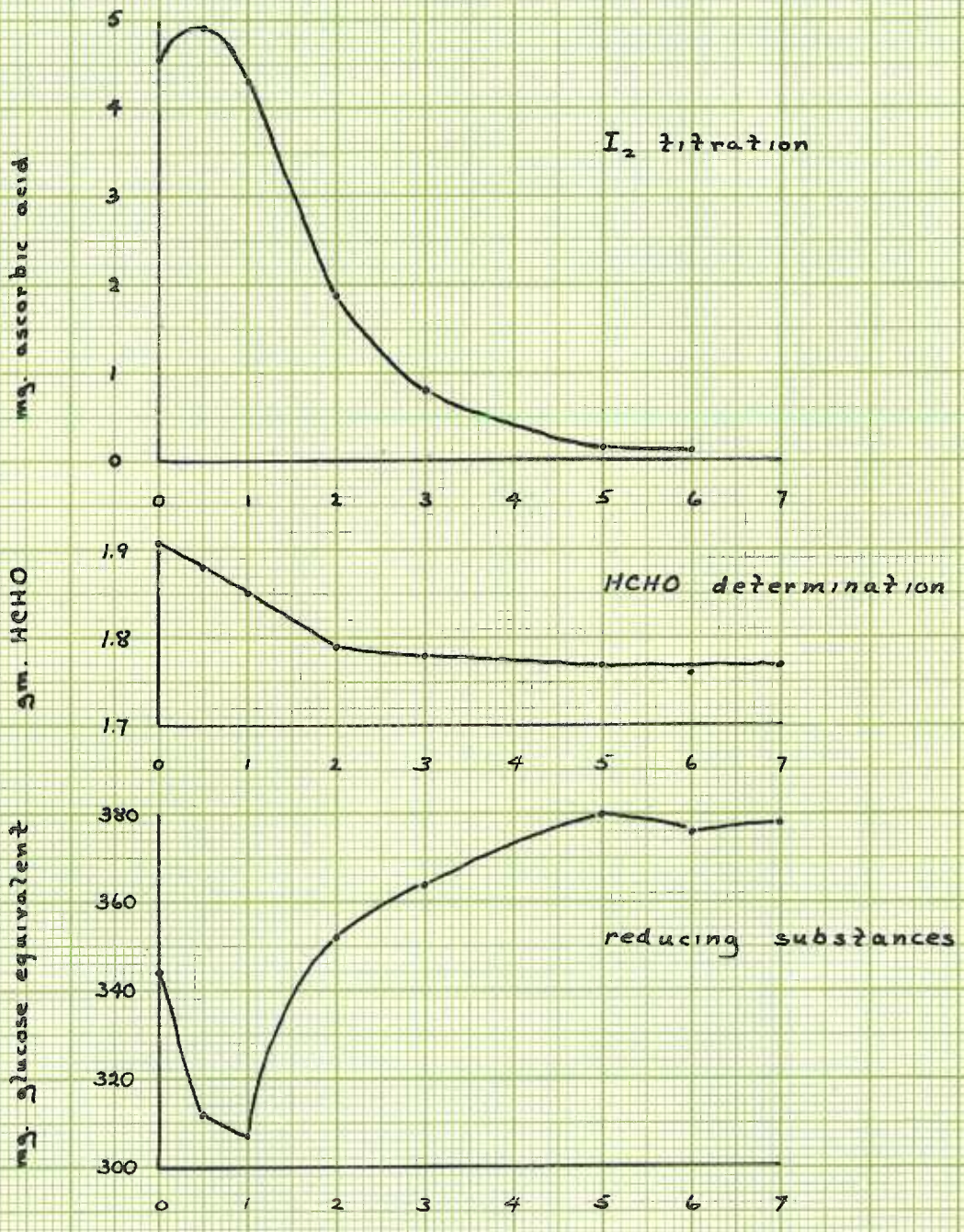
0.500 g. ascorbic acid 0.5 g. $CaCO_3$
50 ml. 38% HCHO



Rate of Reaction in N_2 at 46°

0.500 g. ascorbic acid 0.5 g. $CaCO_3$

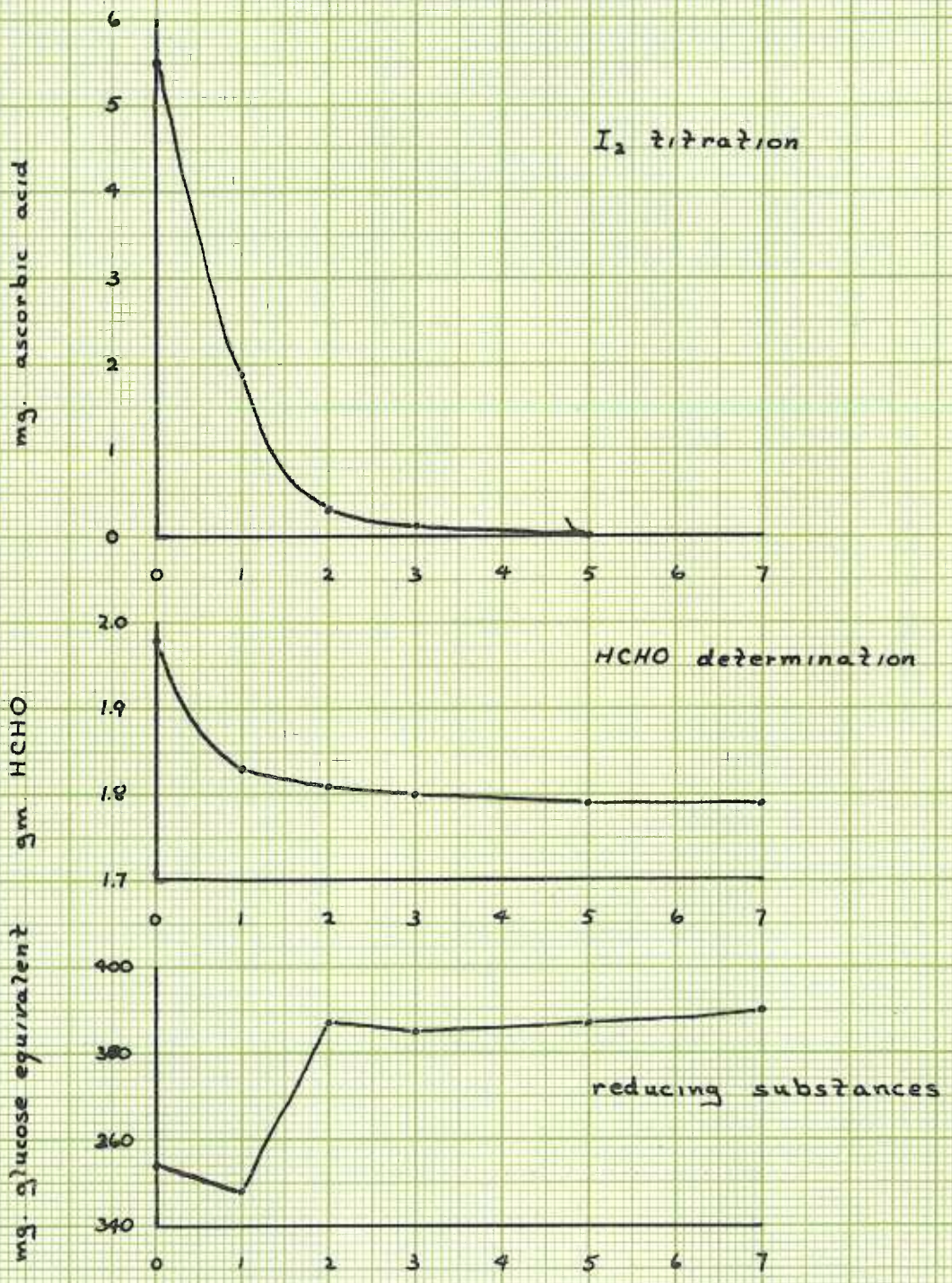
50 ml 4% HCHO



Rate of Reaction in N_2 at 60°

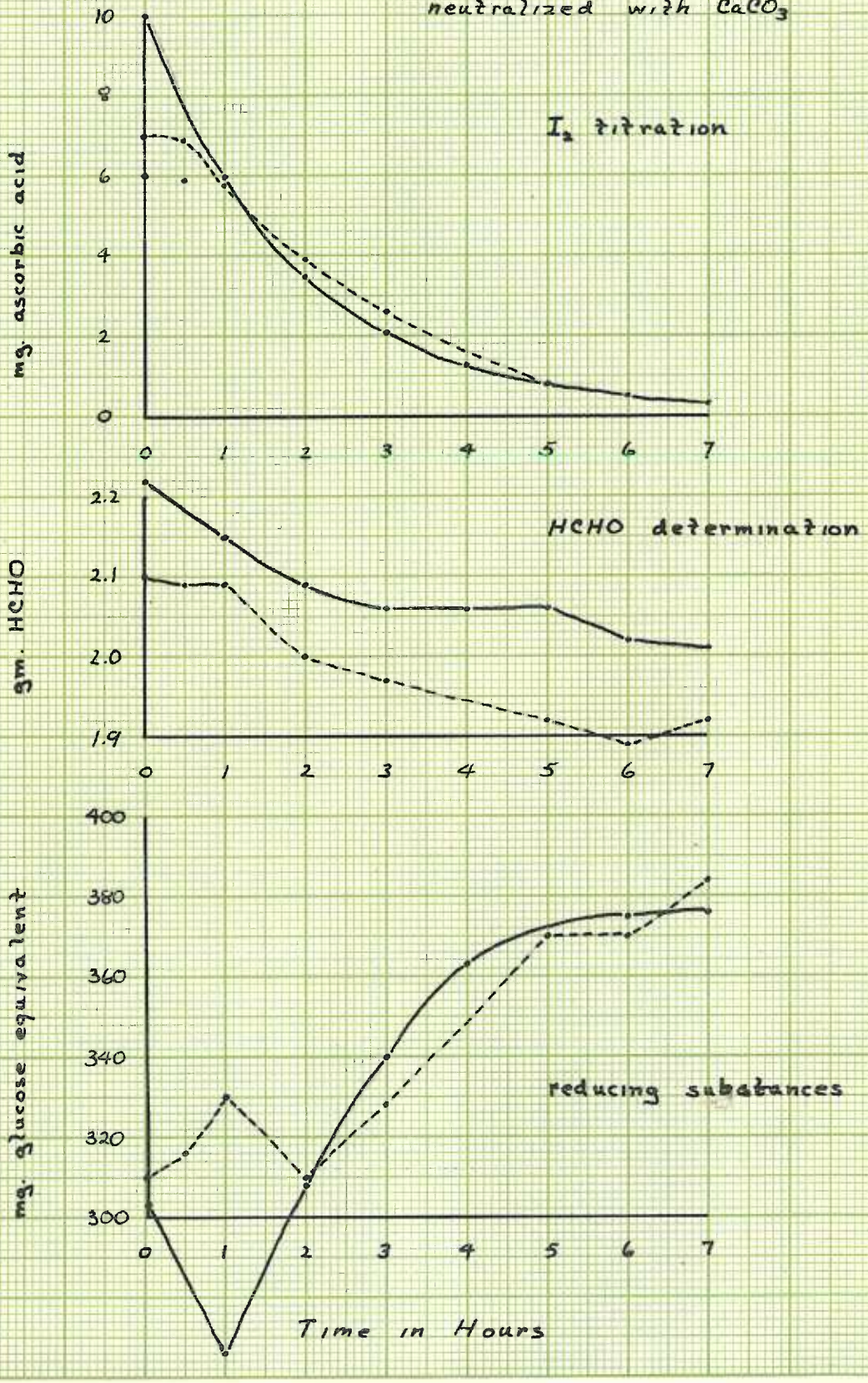
0.500 g. ascorbic acid 0.5 g. $CaCO_3$

50 ml. 4% HCHO



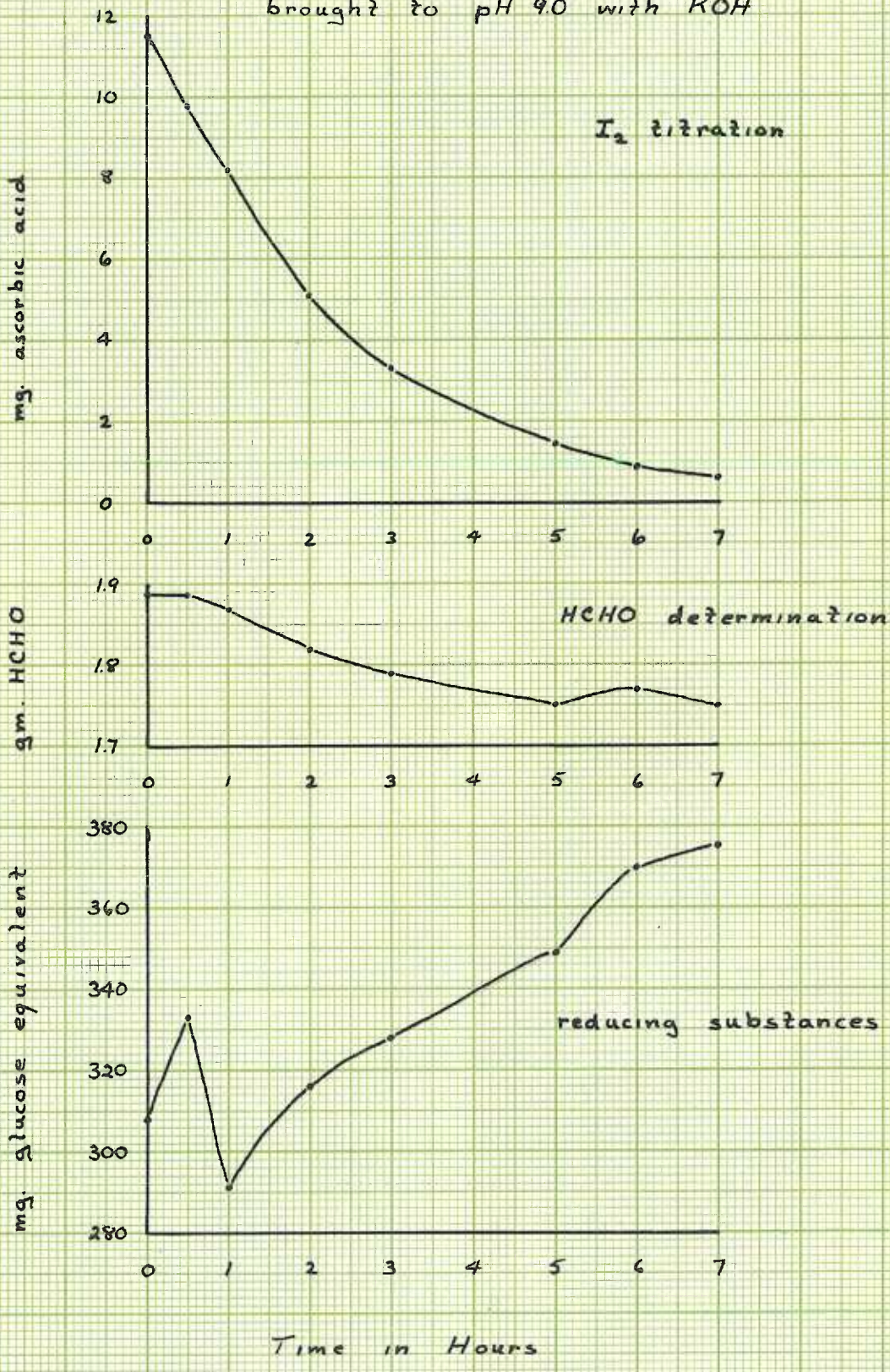
Rate of Reaction in N_2 at 35°

50 ml. 4% HCHO 0.500 g. ascorbic acid neutralized with $CaCO_3$



Rate of Reaction in N_2 at 35°

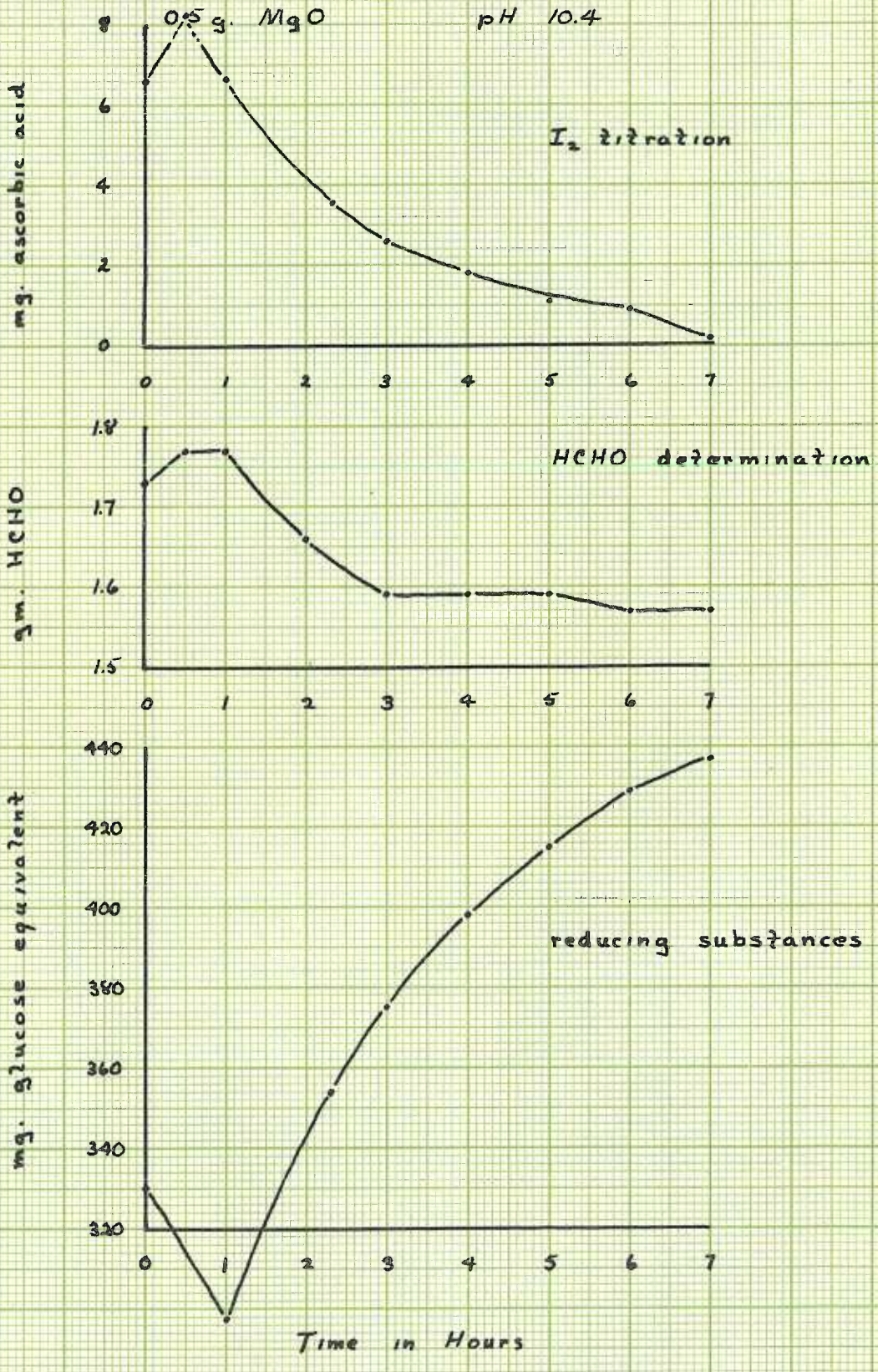
50 ml. 4% HCHO 0.500 g. ascorbic acid brought to pH 9.0 with KOH



Rate of Reaction in N_2 at 35°

0.500 g. ascorbic acid 50 ml. 4% HCHO

0.5 g. MgO pH 10.4



Experiments on the decarboxylation of ascorbic acid.

The apparatus used in this work is shown in Figure II.

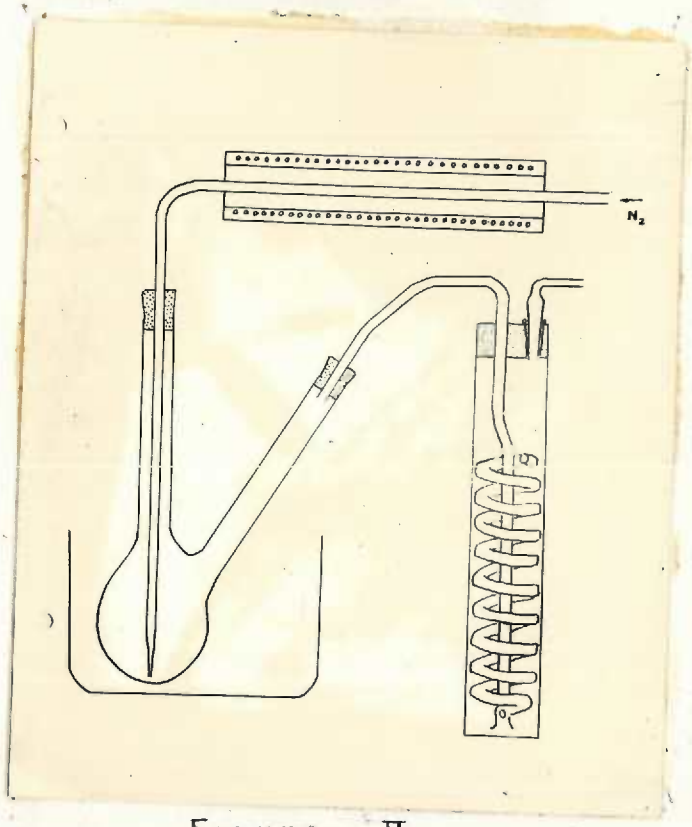


Figure II

The CO_2 produced in the reaction flask was swept into the absorption spiral by a stream of N_2 (purified by passing over hot copper). It was absorbed in the spiral by 0.2 N. $Ba(OH)_2$. At the end of the determination, excess $Ba(OH)_2$ was titrated with 0.1 N. HCl to a phenolphthalein endpoint.

The effect of pH on the reaction was first studied. 0.250 g. ascorbic acid in 25 ml. CO_2-free distilled H_2O was brought to pH 7.2 with NaOH. 2.5 ml. 38% HCHO was added, making the concentration of HCHO about 4%.

The mixture was allowed to react for four hours at 60°, then acidified. Theoretical CO_2 -- 0.0625 g. Found -- 0.0528 g. % theoretical -- 84.

In an exactly similar experiment $Ca(OH)_2$ was used to bring the pH to 7.65. The mixture was allowed to react for four hours at 60°, then acidified. CO_2 -- 0.0577 g. % theoretical -- 91. Next a phosphate buffer was used to maintain a pH of 5.6. The reaction proceeded for six hours at 60°. CO_2 found -- 0.0577 g. Lastly, the pH of an exactly similar mixture was brought to a pH of 10.5 with NaOH and allowed to react for four hours at 60°, then acidified. CO_2 found -- 0.0599 g. % theoretical -- 95.8.

Since CO_2 was produced at a pH of 5.6, it seemed probable that ascorbic acid and HCHO would react without the addition of any base. 0.250 g. ascorbic acid was dissolved in 25 ml. CO_2-free H_2O and 25 ml. 38% HCHO added. This was allowed to react for five hours at 60°. CO_2 found -- 0.0622 % theoretical -- 100. At the end of the reaction the pH was 3.6. Blanks were run under the same conditions. No appreciable CO_2 was evolved. More than theoretical amounts of CO_2 could not be obtained by running the same experiment for 24 hours. Further, the same experiment was carried out at 30° for 23 hours. CO_2 found -- 0.0265 g. % theoretical -- 41. This mixture was run for a further 24 hours. CO_2 found -- 0.0190 g. % theoretical -- 29. For the total 47 hour period CO_2 found -- 0.0455 g. % theoretical -- 70.

The effect of varying the concentration of HCHO was next studied. In each experiment 0.250 g. ascorbic acid was dissolved in 25 ml. H_2O and sufficient 30% HCHO added to obtain the desired concentration.

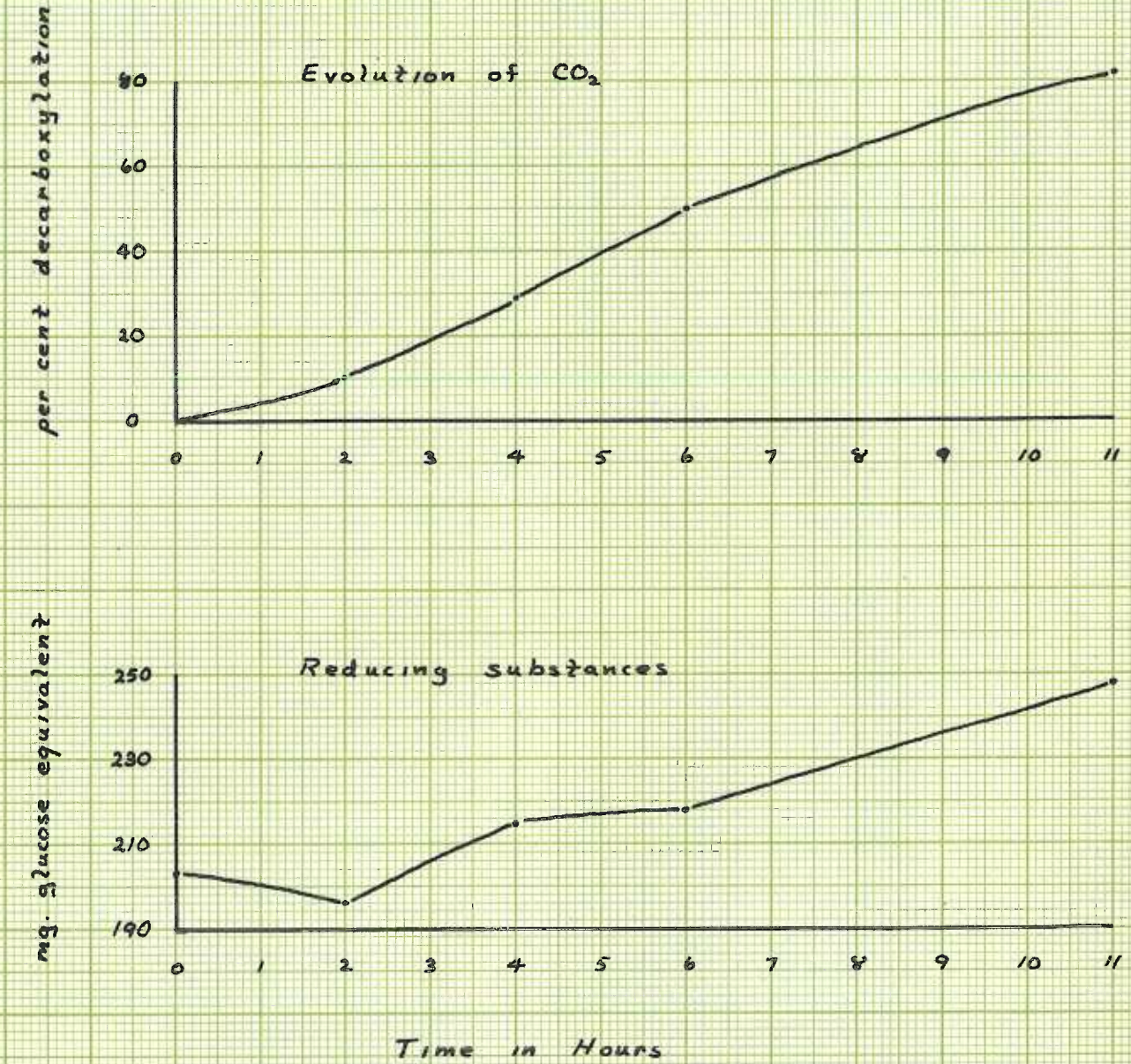
Concentration Of HCHO in %	Time in Hours	C.°	c. CO_2 Produced	% Theoretical
2	24	50	0.0271	40
4	22	60	0.0442	70
6	22	60	0.0493	80
9	6	60	0.0340	55
11	6	60	0.0354	56
14	5	60	0.0433	70
16.5	5	60	0.0574	92
19	5	60	0.0581	93

Further, the speed of the reaction was determined. In this set of experiments 0.250 g. ascorbic acid was dissolved in 25 ml. H_2O and 10 ml. 30% HCHO added to make a concentration of about 11%. The reactions were carried out at 53°.

Time in Hours	c. CO_2 Produced	% of Theoretical	Reducing Substances in mgs. (Glucose Equivalent)	0.01 N. I_2 ml. Titration
2	0.0066	10.5	195	0.25
4	0.0178	28.6	212	0.35
6	0.0279	45.0	218	0.25
11	0.0506	81.0	249	0.15

Rate of Reaction in N_2 at 53°

0.250 g. ascorbic acid 25 ml. H_2O
10 ml. 38% HCHO – total concentration – 11%



Experiments on the addition compound.

Syrup was prepared as follows: 5.00 g. ascorbic acid was dissolved in 100 ml. H_2O and 125 ml. 38% HCHO added. This mixture was allowed to react in N_2 at 60° until no more CO_2 was evolved. Excess HCHO was removed by evaporating in a vacuum at 45°, adding H_2O repeatedly, and evaporating repeatedly until the distillate gave no precipitate with dimedon solution. A golden brown color appeared as soon as the HCHO disappeared. The syrup was taken up in absolute alcohol, decolorized with charcoal, and the alcohol evaporated by a stream of air. Last traces of alcohol were removed by an oil vacuum pump. A thick, sweet, light yellow syrup was obtained. The whole process took about two weeks. This syrup did not reduce indophenol, formed no precipitate with dimedon, and reduced Shaffer-Somogyi sugar reagent very slowly in the cold. A yellow color was obtained in White and Green's aniline test. A color similar but not identical with fructose appeared in the Seliwanoff test. Insoluble white crystals were obtained by oxidation of the syrup with HNO_3 as in the formation of mucic acid. An osazone could not be prepared. Curves obtained (J. Van Bruggen in this laboratory) when the syrup was used to catalyze the condensation of HCHO indicate the presence of a keto group. Boiling with 12% HCl splits out furfural.

Discussion and Conclusion

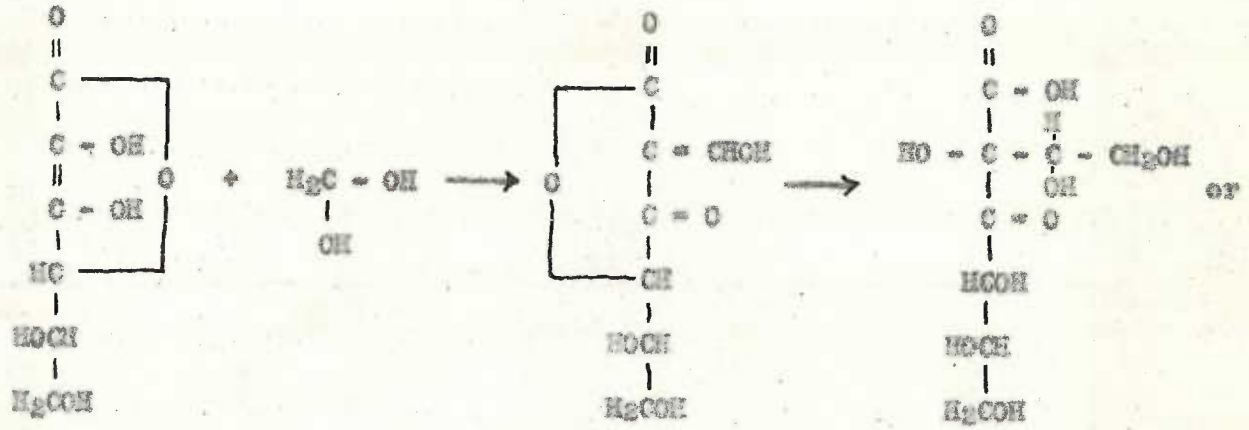
The experimental work presented shows definitely that formaldehyde has the property of blocking the ionization of the ene-diol group. As shown, the pH is raised although there is no effect on the titratable acidity. That the formaldehyde blocks the ene-diol group is also indicated by the fact that the indophenol reducing power disappears. This was determined in the case of ascorbic acid by L. F. Wey and in the case of reductone by R. W. Leong in this laboratory. There is probably a loose addition compound formed.

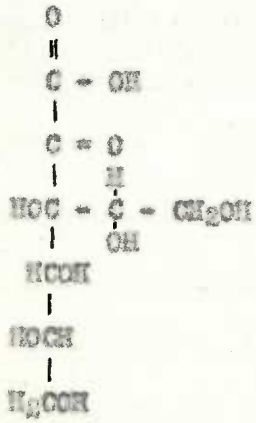
It has been found that calcium carbonate does not have a catalytic effect on the reaction between formaldehyde and ascorbic acid, nor is the presence of the calcium ion necessary. The rate of the reaction is increased by heat, up to 60° at least, and alkali. However, when the alkalinity is raised too greatly, especially at higher temperatures, the formaldehyde undergoes condensation to sugars. The effect of heat and alkali is not simply that of acceleration, however. In the curves of both the iodine titration and reducing substances there appears to be an induction period at the start. The iodine titration first rises, then falls. The reducing substances decrease, then increase. This first portion of both curves tends to be rather unstable due, probably, to the fact

that the ascorbic acid is very loosely combined. The induction period tends to be shortened by a rise in temperature or alkalinity. Formaldehyde curves are not felt to be reliable as a measure of the reaction. It is possible that formaldehyde undergoes side reactions, it is carried over in some part by the stream of N_2, and there is a possibility that dimedon forms a compound with the addition compound which would result in erroneous values.

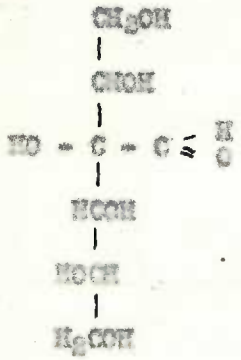
Most important of all is the fact that ascorbic acid appears to be decarboxylated by formaldehyde even in the absence of alkali. As shown previously, theoretical yields of carbon dioxide have been obtained. Further, this reaction occurs even at room temperature and in rather dilute solutions. Haworth (8) mentions that the lactone structure is more easily broken when the ene-diol group is etherized. It is possible that the addition of formaldehyde has a similar effect, causing the lactone ring to break, and followed by decarboxylation.

Following Kusin's ideas, the reaction between formaldehyde and ascorbic acid may be visualized as follows:

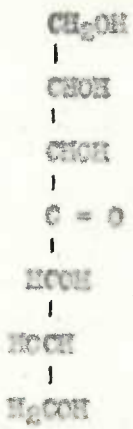




which on decarboxylation would give



or



Isomers of these molecules would undoubtedly be present. No critical experiment has been devised to determine the number of molecules of formaldehyde which reacts with one molecule of ascorbic acid. This is difficult to determine since large amounts of formaldehyde must be used to obtain complete reaction, therefore relative changes in concentration are small. Further, side reactions of formaldehyde can not be ruled out as yet.

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