HYDROQUINONE AS AN OXIDATION CATALYST

Thesis for Degree of M. S.

Aubrey May Bacot

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Thesis

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Introduction.

The widespread occurrence of the phenols in plants has led to a number of investigations of their function in metabolism. The commonly observed browning on injury to fruits and the browning of solutions of phenols on oxidation is considered to be the same reaction. Since the phenols are quite easily oxidized substances, their function as reducing agents or oxygen carriers seems to be their most logical one. Phenols occur in animals as well as in plants. Knowledge of the function of the phenols in animals is, as in plants, obscure. Some of the phenols (hydroquinone, catechol, and pyrogallol) have been found by Huston and Lightbody (Unpublished Work) to be related to the prevention of rickets. The question of whether or not this is an oxidation and reduction phenomena arises. The present problem was undertaken with a view of studying the oxidation properties of hydroquinone.

The peroxide theory seems a possible mechanism for the catalytic action of the phenols, especially in the light of recent investigations of Onslow, Biochemical Journal 13; 1-9; (1919), Biochemical Journal 14; 535; (1920), Gallagher, Biochemical Journal 18; 29; (1924).

H. A. Spoehr has recently, Jour. A.C.S. 46; 1494;(1924), 48; 236; (1926), 48; 107; (1926), catalyzed the oxidation of glucose with iron. The iron serves as an activator of oxygen by oscillating between the ferric and ferrous iron. The oxygen of the air oxidizes the iron to the ferric, which the glucose subsequently reduces. His method of carrying out the determinations was adopted with a few modifications. The solution to be oxidized was placed in a kjeldahl in a thermostat at 38°. Air was drawn through the solution after it had been passed through soda lime and a saturated solution of $Ba(OH)_2$ to remove carbon dioxide. The amount of oxidation taking place in the flask was measured by the amount of carbon dioxide given off. This carbon dioxide was determined by bubbling the gas through a standard

solution of $Ba(OH)_2$ and titrating the excess of $Ba(OH)_2$, using Phenolphthalein as indicator. Instead of the rotary pump used by Spoehr, a constant level water pump was used, i.e., an ordinary water suction pump with the water supply to it coming from a constant level reservoir. This plan was adopted because the water pressure in the pipes varied so as to make the air supply and agitation in the flask untrustworthy. The air passed through at the rate of between 28 and 34 liters per 24 hours. Instead of the Meyer 10-bulb absorption tube an absorption tower of glass beads 65 cms. long and 2 cms. in diameter was used.

Experimental Part

In the first solution to be oxidized equimolar concentrations (M/9) of glucose and hydroquinone were used. The reagents were dissolved in 150 c.c. of a buffer solution of sodium hydroxide and mono-potassium phosphate of pH 7.2. Air was drawn through the apparatus and determinations of carbon dioxide given off were made at 24-hour intervals. Figure 1.

Figure 1

M/9 Glucose Curve (1)

24 Hours	.0000 g. CO_2
48	.0038
72	.0015

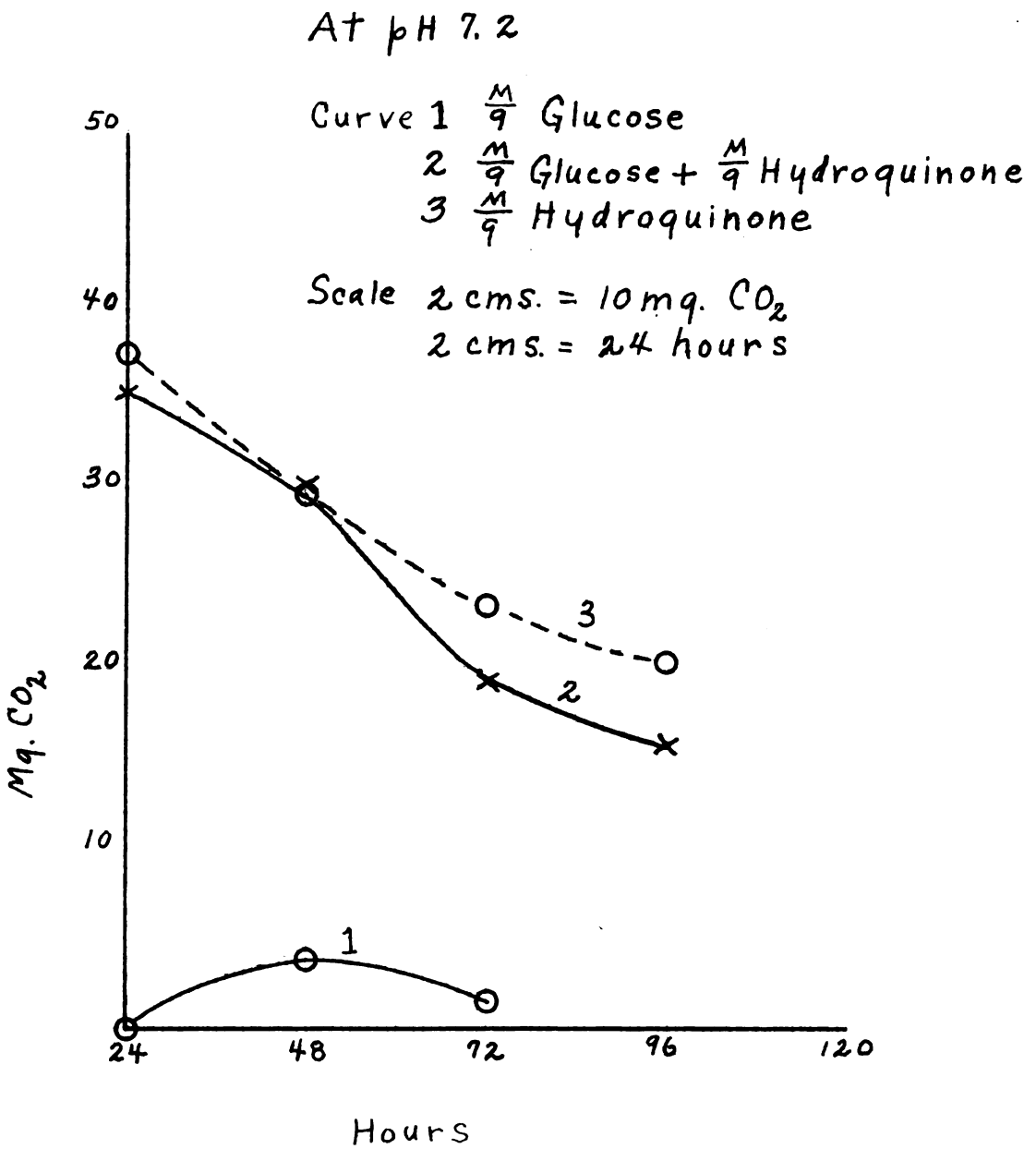
M/9 Glucose plus M/9 Hydroquinone Curve (2)

24 Hours	.0353
48	.0302
72	.0193
96	.0157

M/9 Hydroquinone Curve (3)

24 Hours	.0374
48	.0296
72	.0235
96	.0203

Fig 1


At pH 7.2
Curve 1 M/9 Glucose
2 M/9 Glucose + M/9 Hydroquinone
3 M/9 Hydroquinone
Scale 2 cms. = 10 mg. CO2
2 cms. = 24 hours
50
40
30
20
10
Mg. CO2
1
2
3
24
48
72
96
120
Hours

It will be seen from the graph that the amount of carbon dioxide from the glucose and hydroquinone and from the hydroquinone alone is practically the same; it is even greater in the case of the hydroquinone alone. Moreau and Dufraisse, Jour. Chem. Soc. Vol 127 (1925), have observed that the presence of some gases, that is oxidisable gases, will hinder the catalytic action of oxidation catalysts. They call this action poisoning of the catalyst. The property is not peculiar to the gases. The gases poison the oxidising action of the catalyst by forming oxides that are of approximately the same stability as the oxides of the catalyst so that the oxide of the catalyst and the oxide of the gas react to re-form the gas and catalyst plus oxygen. Where A is gas and B the catalyst:

$$A + O_2 \longrightarrow AO_2$$
$$AO_2 + B \longrightarrow AO + BO$$
$$AO + BO \longrightarrow A + B + O_2$$

One of the conclusions they draw is that every oxidisable substance should show some antioxygenic properties and that a given catalyst should be able to function either as a positive catalyst or as a negative one, the direction of the catalysis being positive if the peroxide of the catalyst attacks the oxidisable substance in preference to the oxide or peroxide of the foreign, or poisoning, substance, and negative if

the converse is true. If A is oxidisable substance or poisoning substance and B the Catalyst,

$$B + O_2 \longrightarrow BO_2$$
$$BO_2 + A^2 \longrightarrow BO^2 + \underset{\text{stable}}{\overline{AO}} \quad \text{positive oxidation}$$
$$BO + \underset{\text{unstable}}{\overline{AO}} \longrightarrow A + B + O_2 \quad \text{negative oxidation}$$

Or, taking the above theory as applied to the oxidation mixture in the flask,

$$\text{Hydroquinone} + O_2 \longrightarrow 2\ \text{Hydroquinone(O)}$$
$$\text{Hydroquinone(O)} \longrightarrow 6O_2 \quad H_2O$$

But when glucose was present,

$$\text{Hydroquinone(O)} + \text{Glucose} \longrightarrow \text{Hydroquinone} + \text{Glucose(O)}$$

The oxygen from the Hydroquinone(O) possibly was used in oxidising the glucose to intermediate oxidation products and thus decreased the amount of carbon dioxide formed in the case where only the hydroquinone was present, or the glucose was serving as a poisoning catalyst to the hydroquinone. Hydroquinone(O) Glucose(O) Hydroquinone Glucose O_2 The blank determination of the glucose gave very little oxidation, (Curve 1).

If hydroquinone can act as a catalyst it should do so in less than equimolar concentrations. Hydroquinone is apparently a more easily oxidized substance than glucose at pH 7.2. Any peroxide

formed through the oxidation of hydroquinone in the above solution would probably attack the excess of hydroquinone before it would the glucose. This would at least be the case until practically all of the hydroquinone had been oxidized. Peroxide formation and consequent oxidation catalysis could better be observed by changing the proportion of glucose and hydroquinone so as to have the glucose considerably in excess.

As a preliminary to this solutions of concentrations of M/200 and M/1000 hydroquinone were tried alone to get some idea as to whether or not a stable peroxide would be formed at these concentrations. The results are plotted on curves on Figure 2. Curve (2) is a check curve to determine the correct position of the second point on (1) and it was stopped at the second point. Curve (4) is M/1000 hydroquinone plus 1 1/2 g. glucose. The amount of carbon dioxide formed is less in (3) and (4) but the general direction of the curves is the same. Hydroquinone was evidently oxidized as before at that pH (pH 7.2). The negative amount of carbon dioxide is due to the subtraction of too great a blank for amount of carbon dioxide from the air that passed through.

Figure 2

M/200 Hydroquinone Curve (1)

24	.0032
48	.0047
72	.0020
96	.0007

M/200 Hydroquinone Curve (2)

24	.0043
48	.0037

M/1000 Hydroquinone Curve (3)

24	.0003
48	-.0008

M/1000 Hydroquinone plus M/18 Glucose Curve (4)

24	.0020
48	-.0008

Fig. 2

At pH 7.2

Curve 1 $\frac{M}{200}$ Hydroquinone

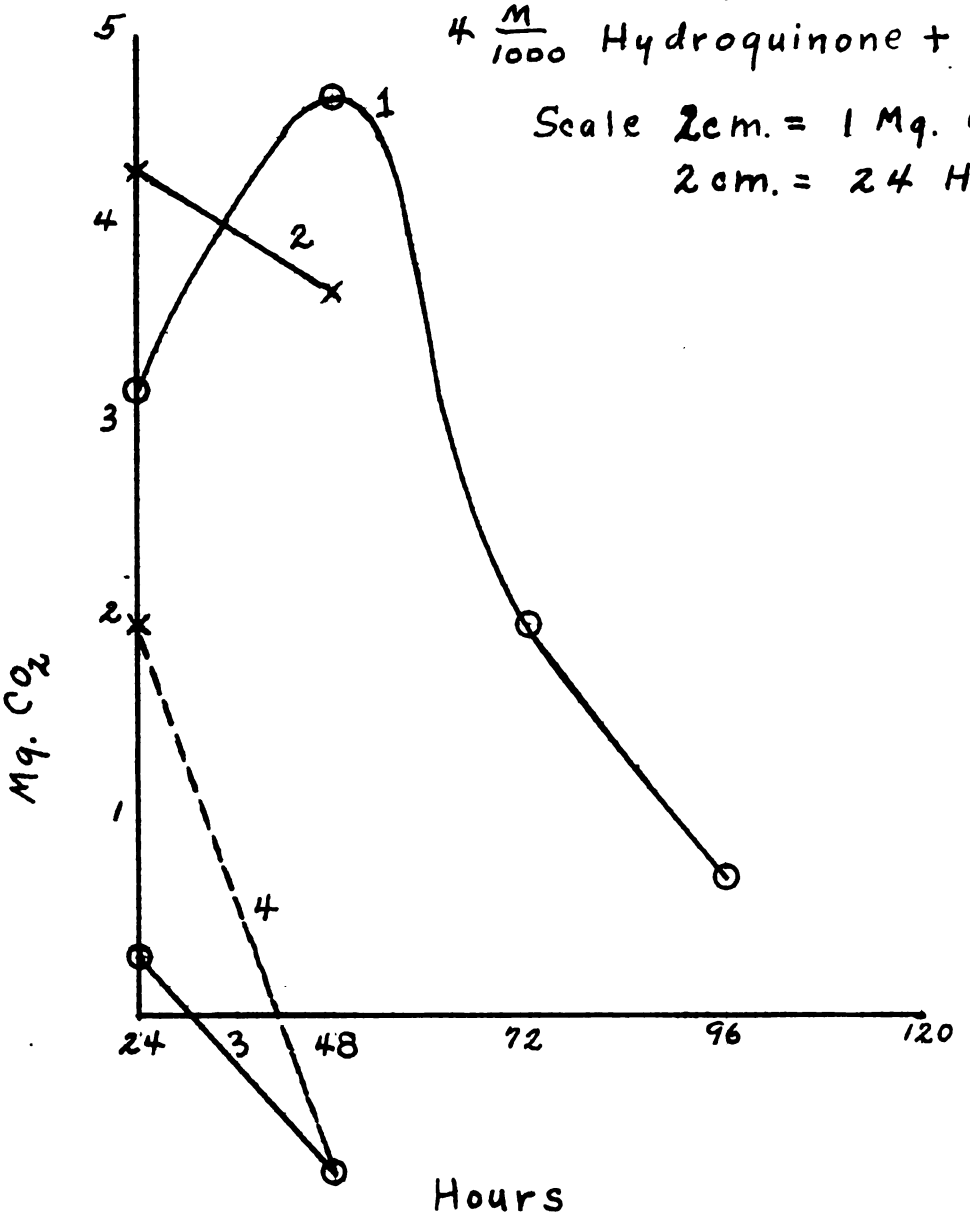
2 $\frac{M}{200}$ Hydroquinone

3 $\frac{M}{1000}$ Hydroquinone

4 $\frac{M}{1000}$ Hydroquinone + 1½ g. Glucose

Scale 2 cm. = 1 Mg. CO_2

2 cm. = 24 Hours



Whenever the phenols are to be oxidized an alkaline pH is generally used. If they form a peroxide during their oxidation as an intermediate it is perhaps possible that the peroxide would be more stable in a more acid pH. Glucose is more difficultly oxidized at low pH than at high pH, but it is not improbable that the more stable peroxide would partially compensate for this increased difficulty of oxidation, i.e., that the more stable peroxide formed, if a more stable peroxide is formed in the more acid pH, would oxidize the glucose even though it is more difficultly oxidized at this pH. An acidity of pH 6.8 was taken as possibly a more favorable one for this stability.

The blank on the hydroquinone gave a gradually rising curve (1), Figure 3, as compared with a falling one in the pH 7.2. The amount of oxidation that took 96-120 hours at pH 6.8 took place in 24 hours at pH 7.2. The blank on the glucose gave a falling curve, (2). In curve (3), the mixture of M/9 glucose and M/200 hydroquinone, there

Figure 3
(pH 6.8)

M/200 Hydroquinone Curve (1)

24	.0004
48	.0004
72	.0008
96	.0011
120	.0021
144	.0011
168	.0027
192	.0015
216	.0015

M/9 Glucose Curve (2)

24	.0038
48	.0025
72	.0015
96	.0013
120	.0015

M/200 Hydroquinone plus M/9 Glucose Curve (3)

24	.0000
48	.0011
72	.0008
96	.0015
120	.0029
144	.0040
168	.0017

Fig. 3

At pH 6.8

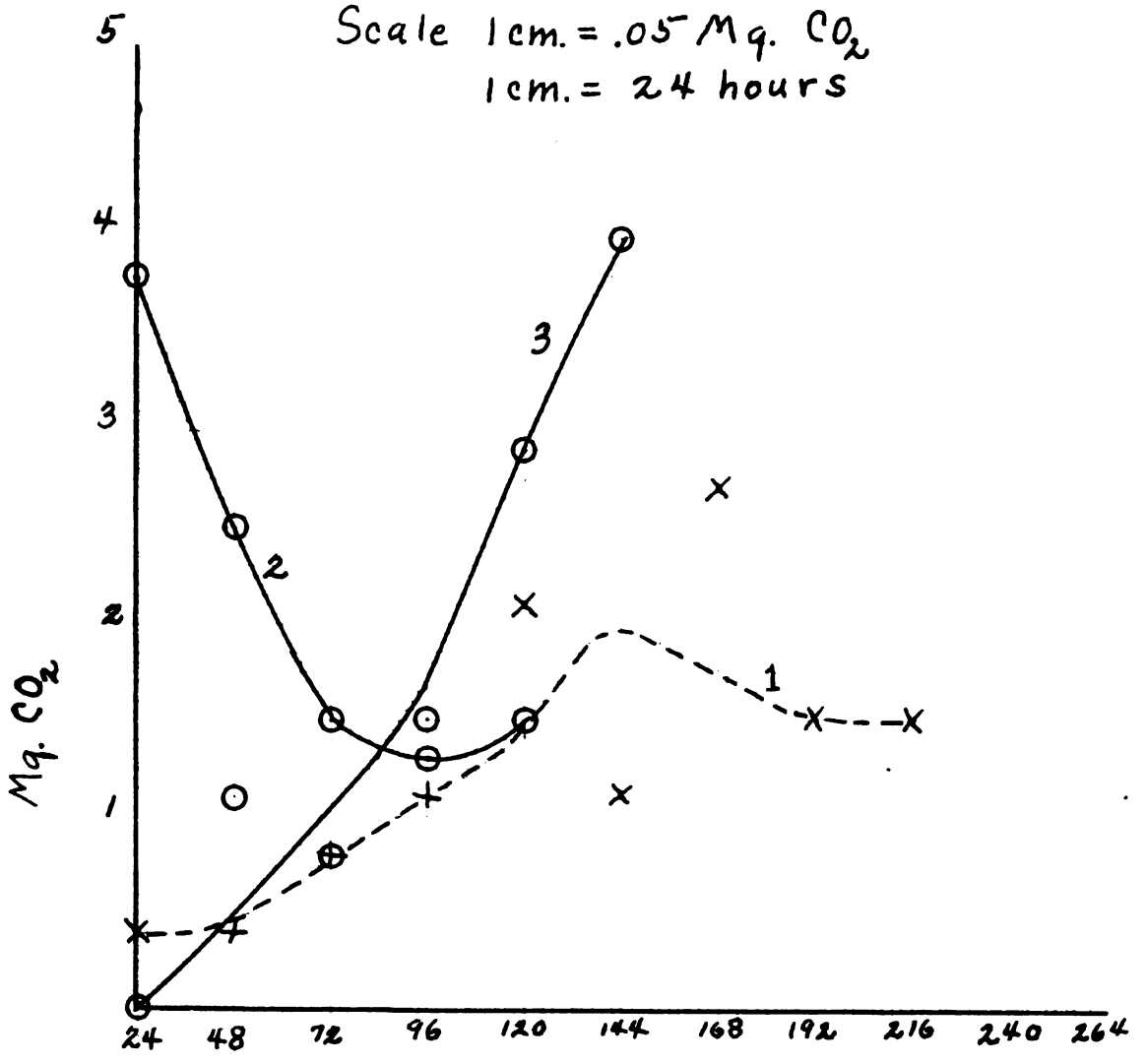
Curve 1 $\frac{M}{200}$ Hydroquinone

2 $\frac{M}{9}$ Glucose

3 $\frac{M}{200}$ Hydroquinone + $\frac{M}{9}$ Glucose

Scale 1 cm. = .05 Mg. CO_2

1 cm. = 24 hours



is increased oxidation, but there was a fungus growth in the flask when it was removed from the bath. The determination was repeated and 1 c.c. of chloroform added to prevent the formation of the fungus growth. The chloroform seemed to delay the rise in the curve somewhat (4) but it began to rise between the 144-168 hour period. There was a fungus growth as befofe. The same determination was run again with 1 c.c. of chloroform added every other day, (5). Curves (4) and (5) on Figure 4. Onslow, Biochem. Jour. 13; pp 1 and 8; (1919), states that chloroform vapor will have the same effect upon the browning of plants and fruits as mechanical injury, i.e., that it initiates or promotes the oxidation of the "catechol-like" compound. The added chloroform probably accounts for the hectic behavior of curve (5).

Qualitative Oxidation Reactions

Some attempts to find the probable reactions taking place in the flask were made with hydroquinone and its oxidation product, quinone. Quinone was found to function as an oxidase in the bluing of guaiacum, that is, it will blue the guauacum in water solution without the addition of a peroxide. The

Figure 4
(pH 6.8)

M/200 Hydroquinone plus M/18 Glucose Curve (4)
1 c.c. $CHCl_3$ introduced

24	.0000
48	.0002
72	.0010
96	.0013
120	.0010
144	.0013
168	.0021
192	.0021

M/200 Hydroquinone plus M/18 Glucose Curve (5)
1 c.c. $CHCl_3$ introduced every other day

24	.0040
48	.0042
72	.0048
96	.0074
120	.0095
144	.0070
168	.0063
192	.0025
216	.0034
240	.0021
264	.0036
288	.0051
312	.0051

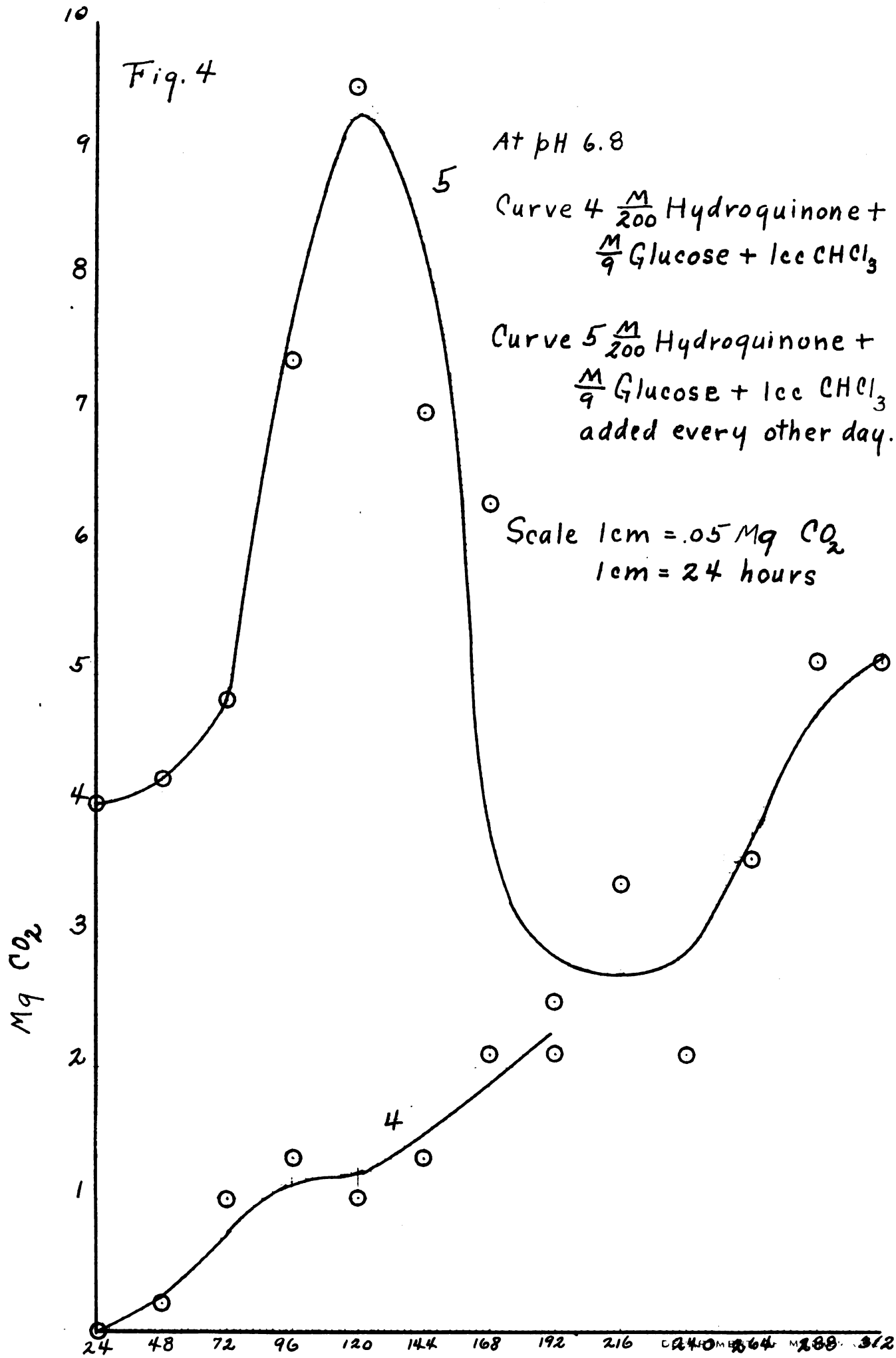


Fig. 4
At pH 6.8
Curve 4 $\frac{M}{200}$ Hydroquinone + $\frac{M}{9}$ Glucose + 1cc $CHCl_3$
Curve 5 $\frac{M}{200}$ Hydroquinone + $\frac{M}{9}$ Glucose + 1cc $CHCl_3$ added every other day.
Scale 1cm = .05 Mg CO_2
1cm = 24 hours
Mg CO_2
10
9
8
7
6
5
4
3
2
1
5
4
24
48
72
96
120
144
168
192
216
240
264
288
312

The oxidase system is composed, according to the Bach-Engler conception, of an oxygenase, a peroxide, and a peroxidase, the oxygenase being an enzyme that forms a peroxide and the peroxidase an enzyme that decomposes the peroxide liberating atomic, or active, oxygen. The quinone, therefore, must form a peroxide that it decomposed, or the peroxide that it forms is easily decomposed in the presence of the oxygen acceptor guaiacum. This seems to be a necessary assumption if all of the substances -- the oxygenase, the peroxide, and the peroxidase -- of the Bach-Engler system are to be included.

There is the possibility that the quinone, being a strong reducing agent, serves as a hydrogen acceptor and liberates the oxygen from the water.

Quinone + water $\longrightarrow$ hydroquinone + oxygen

According to H. Wieland (Chem. Ber. 47; 2085; (1914) the mechanism of many oxidation and reduction reactions can proceed through the intermediate action of the water. The probability of such a reaction here is not without evidence. When a solution of .0044 g. quinone was rocked in a Bunzell apparatus

with 5 c.c. water at 37° it gave an expansion in the manometer of .8 cm. in 8-10 hours (that did not increase in 24 hours) due to liberation of oxygen. If a solution of quinone is allowed to stand for 24 hours at room temperature, or if it is heated to boiling 1-2 minutes, it will give Folin's test for phenols.

It is a question at this point whether the oxygen that is oxidizing the guaiacum is coming from the oxygen absorbed in the water or from the water or from the air at the surface of the water. A small quantity of quinone was placed in a bottle and a stream of nitrogen passed over it for 30-45 minutes to displace the air. The water to be added boiled 30 minutes and a fresh tincture of guaiacum was used. When the water was added to the quinone and then the guaiacum tincture, as soon thereafter as possible, there was an immediate blueing of the guaiacum. This seems to obviate the likelihood that the sole source of oxygen, or even a small part of it, is absorbed oxygen or oxygen at the surface of the water.

It seems doubtful, too, that if it takes 24 hours for the quinone to form sufficient hydroquinone to give a Folin test that it would liberate sufficient oxygen to account for the almost immediate blueing of the guaiacum when the quinone is added to a solution of guaiacum.

The following tests give some idea of the rapidity of this reaction. A solution of quinone made up and tested for hydroquinone in 4,7,10,15, and 30 minutes, and at 4 and 5 hours, gave a greenish coloration but no characteristic phenol test. Solutions of hydroquinone of 1 part in 1000, 1 part in 2000, 4000, 8000, 16000, 32000, 64000, and 128000, gave gradations in color of blue to very slight blue but in no case was a shade of green produced, Still the former test, the test of the hydroquinone produced from the quinone, was made in the presence of the quinone and the green color may be a test for hydroquinone but in a lesser degree than the second phenol test, i.e., with only the hydroquinone. For if a solution of hydroquinone is made up, Folin's reagent added and the blue phenol test obtained, and if, in addition,

small quantities of quinone are added successively with shaking to this solution, the same green color will be formed that is obtained in the solution of quinone "alone" although it is known that there was hydroquinone originally present. If the hydroquinone originally present combined with the added quinone and the resulting quinhydrone is responsible for the green color produced, then in the case where quinone was added to a water solution some phenol must have been produced to combine with the quinone to form the same reaction. If the quinone was reduced to hydroquinone some oxygen must have been liberated that would be effective in oxidizing guaiacum.

The blue color of the Folin test is due to a colloidal oxide of Tungsten reduced by the phenol. The color can be lost by oxidation with bromine-water. A Folin phenol test made with hydroquinone and small portions of bromine-water added with shaking will change the blue color to the green color observed upon the addition of quinone to the test on hydroquinone. Both reactions are therefore oxidation reactions and indicate that hydroquinone is formed

upon the addition of quinone to water, because if no hydroquinone were formed no reduction at all would take place, but as the case is hydroquinone is produced in such small quantities that only a slight reduction occurs -- the extent to which the two phenol tests were oxidized after they had been reduced considerably past this point. Colloidal Molybdenum blue , which is prepared by reducing Molybdic acid with H_2S, gives a colorless solution with quinone as it does with other oxidizing agents.

According to Wieland's theory of water entering into the reaction, the blueing of guaiacum, and possibly the oxidation taking place in the flask, can be accounted for without the necessity of peroxide formation. To see if quinone would form a peroxide and also to see if water is necessary to the blueing of guaiacum, the guaiacum reaction was carried out in anhydrous acetone. First a blank determination was made by bubbling air through the acetone solution of guaiacum, after the air had passed through H_2SO_4, for 30 minutes and no blueing of the guaiacum took place. A small amount of the guaiacum separated on the inside of the tip of the tube where the air was entering. A small quantity of quinone added to the

solution produced practically immediate blueing of the guaiacum that had separated at the tip, and the solution itself blued in about 10-15 minutes. The blueing became more pronounced in 20-25 minutes. The experiment was repeated twice with the same results.

The "anhydrous" acetone used had been standing for 5 months since it was prepared. A test with anhydrous $CuSO_4$ gave no indication of water but the test with $KMnO_4$ did. Ether-over-Sodium was substituted for the acetone and the experiment repeated with the added precaution of two $CaCl_2$ U-tubes in series with the H_2SO_4 bubbling bottle. The guaiacum blued as before, first at the tip of the air inlet tube and then the solution. The solution blued only very slightly but the guaiacum in the bottom of the container blued, even though it was not in direct contact with the incoming air. The only remaining source of water is water in the guaiacum. A small bit of quinone, guaiacum, and 5 c.c. of the dried ether in a stoppered test tube under nitrogen gave the blue guaiacum as before. The guaiacum to be used in the next test dried in an oven at 40° and 200 m.m. pressure for two days. A portion of this with the quinone and ether did not blue, while

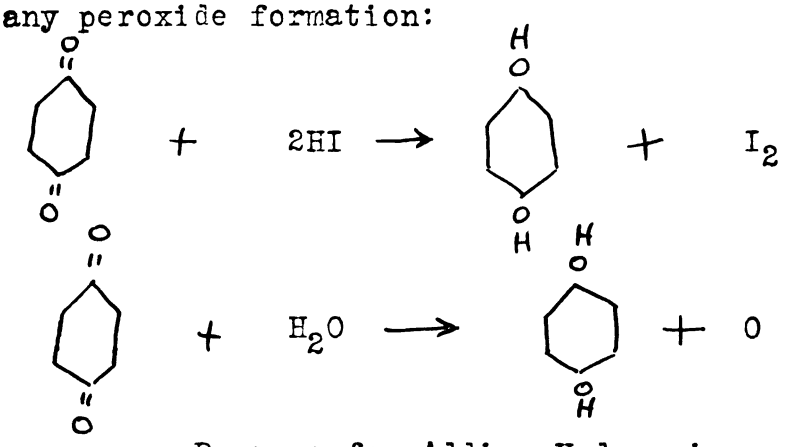
a portion of it moistened with water plus quinone and ether did give immediate blueing.

Catechol and other compounds with two OH groups in the ortho position occurring in plants may give the same reactions as the oxidase system of Chodat. There is present in the juice of some plants, for example the onion and horse radish, an enzyme which will decompose a peroxide to liberate atomic or nascent oxygen. The catechol, or catechol-like compound, is capable of being oxidized to a peroxide upon injury to the plant, and this peroxide in the presence of the peroxidase is decomposed into an oxide and active oxygen. Such a system of peroxidase and peroxidase constitutes an oxidase system according to Chodat's definition of an oxidase. Chodat's oxidase system does not include a catechol or catechol-like compound. (Handbuch der Biochemieschen Methoden Vol 3 pp 42-47 (1910). Because the plant juices containing the catechol or catechol-like compound give an analogous reaction Onslow concludes that the catechol compound is oxidized upon injury to a peroxide which the peroxidase present decomposes. She uses the starch-iodide test for the presence of a peroxide:

$$AO_2 + 2HI \longrightarrow AO + H_2O + I_2$$

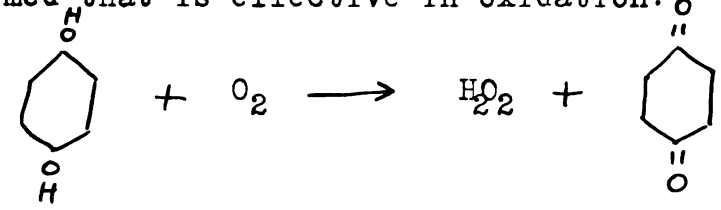
Quinone, however, will give the same reaction independently

of any peroxide formation:

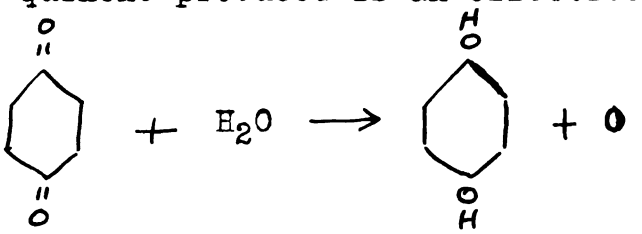


Reasons for Adding Hydroquinone to Spoehr's Sodium-Ferro-Pyrophosphate

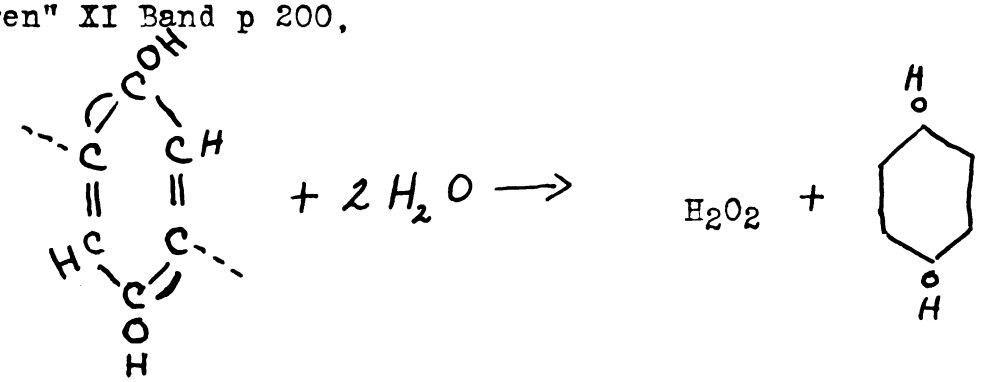
In none of the above oxidation curves has complete oxidation of the hydroquinone occurred. Every oxidation curve of hydroquinone alone in pH 7.2 falls from the initial point and every curve in pH 6.8 rises from the initial point. There is apparently a greater rate of oxidation in the pH 7.2 solution than in the pH 6.8 solution so that the major part of the oxidation occurs in the first 24 hours. There being less hydroquinone present for subsequent oxidation the curve falls. In the pH 6.8 the oxidation is slower and the rate of oxidation increases as the oxidation products of hydroquinone accumulate. For as hydroquinone oxidizes hydrogen peroxide possibly is formed that is effective in oxidation:



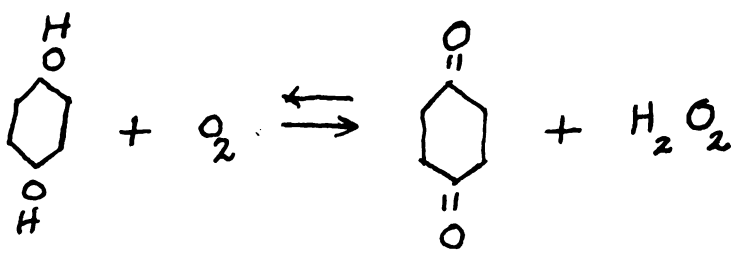
Also as was shown in a former part of the discussion the quinone produced is an effective oxidizer:



Or, according to Pfeiffer in "Chemie in Einzeldarstellungen" XI Band p 200,



As more hydroquinone is oxidized to quinone the rate of oxidation in the flask would be expected to increase. The hydroquinone and quinone are oxidized to carbon dioxide and water at the same time the hydroquinone is being oxidized to quinone. (This is the source of carbon dioxide on the hydroquinone-alone curves).
All of the hydroquinone is not oxidized to quinone before the oxidation to carbon dioxide and water occurs because the reaction is reversible:

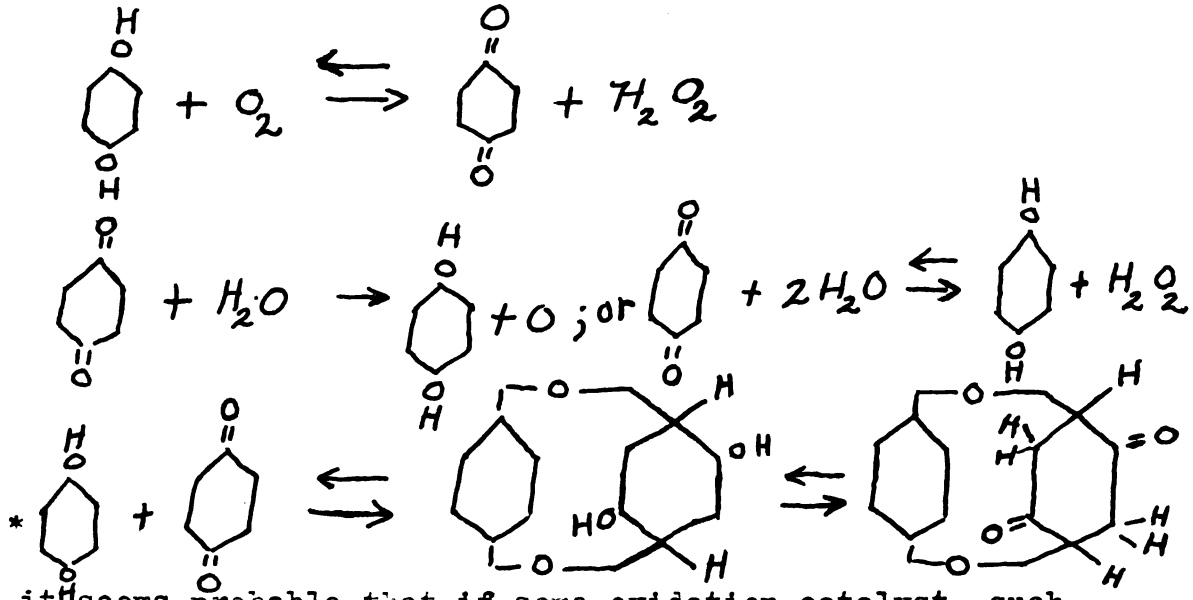


Also because quinone is a strong reducing agent and reacts with the water:

Quinone + water ⟶ hydroquinone + oxygen

Hydroquinone in the presence of quinone forms quinhydrone, a molecule for molecule uniting to form an addition product. The compound is unstable in dilute solutions and dissociates readily into hydroquinone and quinone so that the reactions occurring in the flask are not affected materially by the small concentration of quinhydrone.

Since the hydroquinone has never been oxidized completely, and the system is composed of the equilibrium mixture,



it seems probable that if some oxidation catalyst, such as iron, is introduced to increase the rate of quinone

*Pfeiffer -- same ref. "Chemie in Einzeldarstellungen"p200

formation the oxidation rate of the glucose should increase also, because the rate of oxidation, according to these data, is dependent upon the concentration of quinone or upon the rate of quinone formation. This increase in the rate of oxidation of hydroquinone would also increase the possibility of quinone peroxide formation.

Spoehr's work was repeated, therefore, with the variations as set forth in Figures 5 and 6. His oxidation mixture was 6.7 g. $Na_4P_2O_7.10H_2O$ in 150 c.c. water, 1.0 g. $FeSO_4.7H_2O$, 17.0 g. $Na_2HPO.12H_2O$, and 3.0 g. glucose, added successively. In obtaining the curves on Figure 5 the 150 c.c. of water were added to the mixture of the dry reagents. No mention was made of the order in the original article. The curves on Figure 6 were obtained after the reagents had been added in the order mentioned in the beginning of the paragraph. The difference is due, presumably, to a greater concentration of the catalyst in Figure 6. In Spoehr's second article he gives the precaution to let the Sodium Pyrophosphate dissolve and then the Ferrous Sulphate in that before adding the other reagents because in this order of procedure the maximum amount of the catalyst, Sodium-Ferro-Pyrophosphate, can be formed.

Figure 5

Spoehr's Mixture Curve (1)

24	.0167
48	.0376
72	.0460
96	.0502
120	.0521
144	.0660
168	.0795
192	.0753
216	.0488
240	.0748
264	.0697
288	.0530
312	.0437
336	.0390
360	.0358

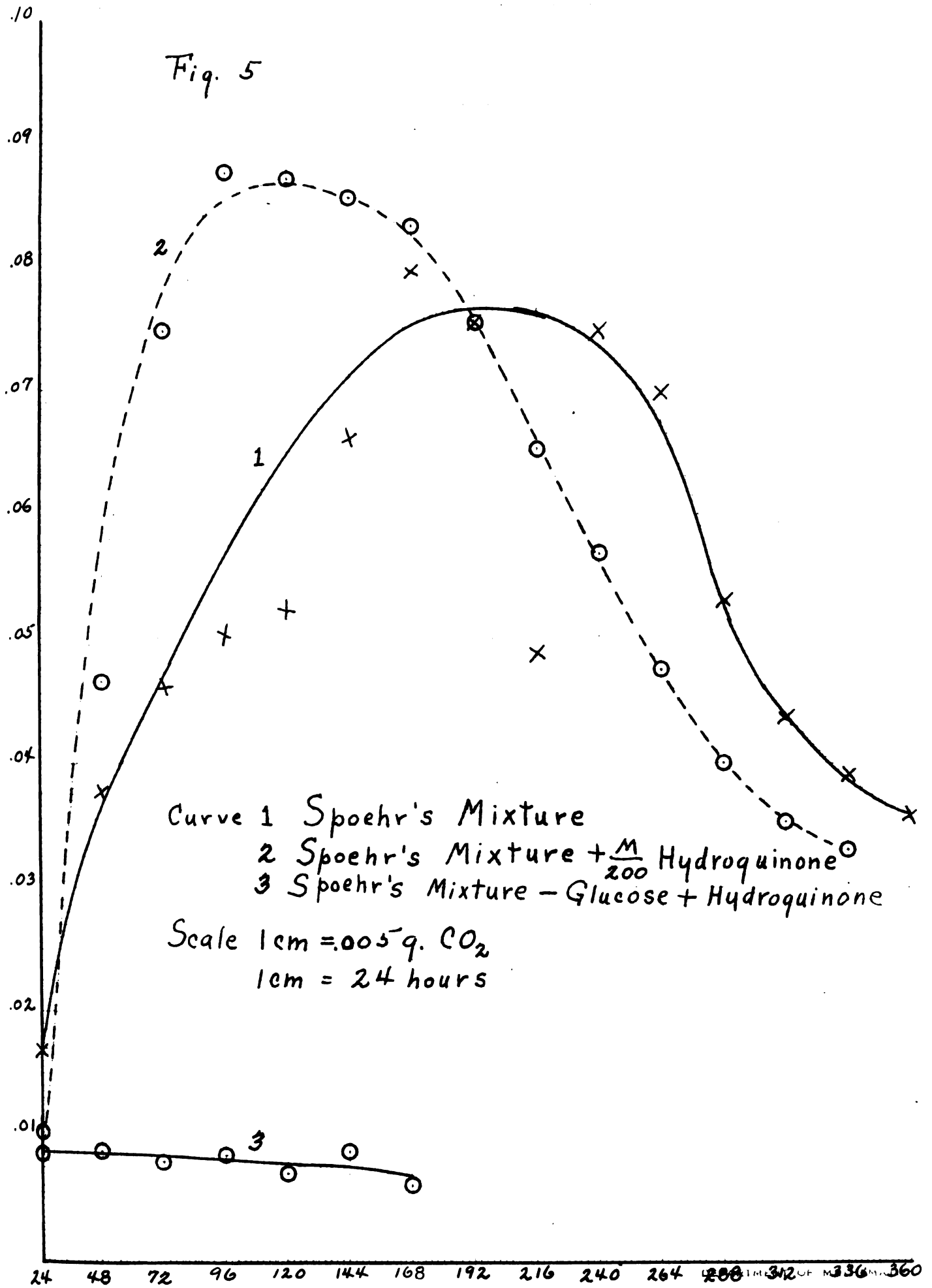
Spoehr's Mixture plus M/200 Hydroquinone Curve (2)

24	.0102
48	.0465
72	.0748
96	.0874
120	.0870
144	.0855
168	.0832
192	.0753
216	.0651
240	.0567
264	.0474
288	.0399
312	.0353
336	.0330
360	

Spoehr's Mixture minus Glucose plus M/200 Hydroquinone Curve (3)

24	.0084
48	.0088
72	.0079
96	.0084
120	.0070
144	.0088
168	.0060

Fig. 5



Curve 1 Spoehr's Mixture
2 Spoehr's Mixture + $\frac{M}{200}$ Hydroquinone
3 Spoehr's Mixture – Glucose + Hydroquinone

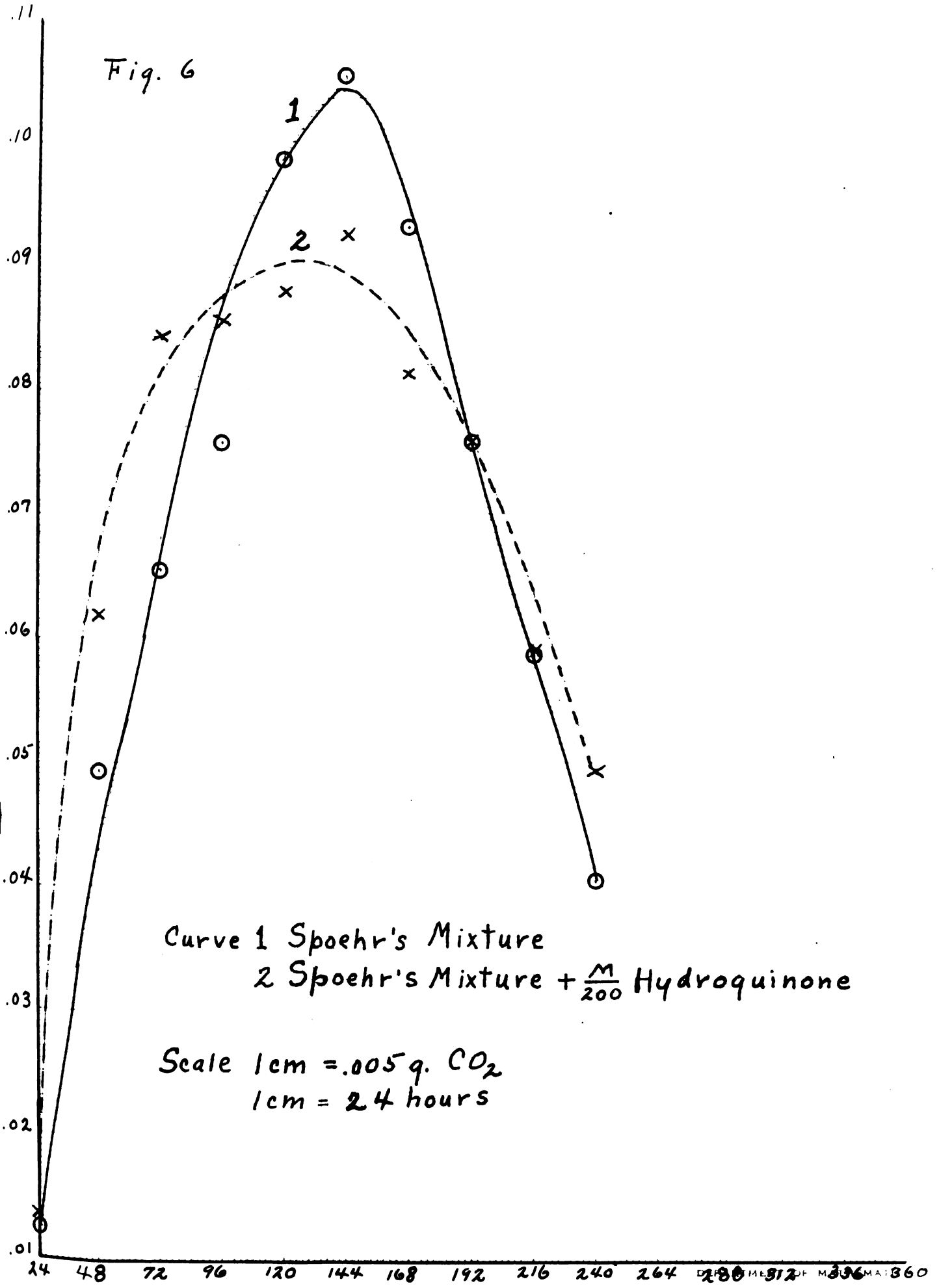
Scale 1cm = .005 g. CO_2
1cm = 24 hours

Figure 6

Spoehr's Mixture	Curve (1)
24	.0125
48	.0493
72	.0655
96	.0758
120	.0986
144	.1051
168	.0930
192	.0758
216	.0586
240	.0404

Spoehr's Mixture plus M/200 Hydroquinone	Curve (2)
24	.0135
48	.0628
72	.0842
96	.0855
120	.0879
144	.0925
168	.0823
192	.0753
216	.0590
240	.0493

Fig. 6



Curve 1 Spoehr's Mixture

2 Spoehr's Mixture + $\frac{M}{200}$ Hydroquinone

Scale 1cm = .005 g. CO_2

1cm = 24 hours

Conclusions

In high concentration of hydroquinone there seems to be a Moreau and Dufraisse effect with glucose in which the glucose acts as the poisoner. In low concentration of hydroquinone there is no such effect noticeable.

The oxidation properties of hydroquinone are due possibly to the reducing action of the quinone formed rather than any peroxide formed.

Hydroquinone does not increase the oxidation rate of glucose by Spoehr's Sodium-Ferro-Pyrophosphate.