[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

On the Mechanism of the Catechol–Tyrosinase[1] Reaction

BY CHARLES R. DAWSON AND J. M. NELSON

The enzymatic oxidation of catechol and related substances has attracted the attention of a large number of workers since the pioneer work in this field over two decades ago by Bach and Chodat. Robinson and McCance[2] in 1925, working with an enzyme preparation from the mushroom, *Lactarius vellereus*, showed that in the aerobic oxidation of catechol two atoms of oxygen were consumed per mole of the dihydric phenol. Szent-Györgyi[3] about the same time suggested *o*-quinone to be the oxidation product when catechol is oxidized by this type of enzymatic action. Since the formation of *o*-quinone from catechol obviously would use up only one atom of oxygen, the question naturally presented itself as to what becomes of the second atom of oxygen consumed in the oxidation of the catechol. Onslow and Robinson,[4] accepting the claim made by Szent-Györgyi that *o*-quinone is produced, tried to show that the second atom of oxygen consumed in the oxidation of the catechol resulted in the formation of hydrogen peroxide.

$$C_6H_4(OH)_2 + O_2 \longrightarrow C_6H_4O_2 + H_2O_2$$

Pugh and Raper[5] expressed doubt concerning the formation of hydrogen peroxide in the reaction, pointing out that some of their enzyme preparations used for oxidizing catechol by air, also contained catalase. The presence of the latter obviously would tend to decompose any peroxide formed, thereby returning the second atom of oxygen back to the reaction mixture. In order to reconcile their claim that *o*-quinone is the oxidation product of catechol with the consumption of two atoms of oxygen per mole of catechol, they suggest that the quinone suffers further oxidation to a higher oxidation product, the chemical nature of which is still undetermined.

In the light of what has been stated above, it is quite evident that more information as to the fate of the catechol when oxidized enzymatically in the presence of air is desirable. Since so much of the previous experimental data on this enzymatic oxidation of catechol has been obtained by following the uptake of oxygen as the reaction progressed, it was deemed desirable to follow, if possible, the oxidation products instead. Since it has been demonstrated repeatedly in the previous studies that quinone or quinone-like bodies are formed when the catechol is oxidized under these conditions, and since quinones are known to liberate iodine from acidified potassium iodide solutions, the determination of the liberated iodine by means of standard thiosulfate solution[6] suggested itself as a means of following the enzymatic oxidation of catechol.

Briefly, the method adopted was to add a given amount of enzyme to an aqueous buffered solution of catechol constantly being stirred by a stream of air, and then to remove at definite time intervals 25-cc. portions of the reaction mixture. These samples were added to dilute sulfuric acid to stop the enzymatic action. The last operation was followed immediately by the addition of potassium iodide and the iodine set free by the quinone bodies was then determined by titration with thiosulfate solution.

Oxidation of Catechol with Varying Amounts of Enzyme.—In Fig. 1 is shown graphically the amounts of thiosulfate solution required to take care of the iodine set free by the quinone bodies formed at definite time intervals in the oxidation of catechol by given amounts of enzyme at *p*H 6.6. The amount of standard thiosulfate solution required by the iodine set free from a 25-cc. sample taken from the reaction vessel, when 10 mg. of catechol in 250 cc. of buffered solution is completely oxidized to quinone, is 1.97 cc. It is evident, therefore, that in the case of curve 1, corresponding to 3.5 units[7] of enzyme per mg. of catechol, the catechol has been oxidized completely to a quinone[8] state in less than three

(1) Due to the existent confusion concerning the naming of the phenolase obtained from the common mushroom (*psalliota campestris*), the authors are using, for the time being, the name Tyrosinase, which is consistent with the term employed in previous publications from this Laboratory.

(2) M. E. Robinson and R. A. McCance, *Biochem. J.*, **19**, 251 (1925).

(3) A. von. Szent-Györgyi, *Biochem. Z.*, **162**, 399–412 (1925).

(4) M. W. Onslow and M. E. Robinson, *Biochem. J.*, **20**, 1138 (1926).

(5) C. E. M. Pugh and H. S. Raper, *ibid.*, **21**, 1370–1383 (1927).

(6) A. Valeur, *Compt. rend.*, **129**, 552 (1899).

(7) The units of tyrosinase used are those proposed by Graubard and Nelson.[13] Likewise, the method of preparation of the enzyme used was that described by them.

(8) The authors have used the term quinone to represent a substance which will liberate two atoms of iodine per mole of oxidized catechol.

minutes. The slight drop in titratable iodine beyond the three-minute period undoubtedly is due to the instability of the quinone at this *p*H, 6.6. It might be well to mention at this point that when the complete conversion of the catechol to quinone is brought about at lower *p*H values, 4–5 (see curves 2 and 3, Fig. 3), then the subsequent drop in titratable iodine is slightly less with the expiration of time. This latter information is in agreement with the observation of Szent-Györgyi that the quinone produced in this enzymatic oxidation is more stable at these lower values for *p*H. The observations of Ball and Chen[9] as well as experiences in this Laboratory, however, indicate that the stability of *o*-benzoquinone in dilute aqueous solutions at approximately these values for *p*H is much less than that shown by the data represented by curves 1, 2, 3 and 6 in Fig. 1 and curves 2, 3 and 4 in Fig. 3. This fact therefore suggests that if *o*-benzoquinone is the initial product in enzymatic oxidation of the catechol, it is either being stabilized by the air and enzyme or being changed into, possibly, a hydroxy quinone, as postulated by Wagreich and Nelson.[10] From the reduction potentials of hydroxy quinones reported by Conant and Fieser[11] it is to be expected that such a higher oxidation product of *o*-benzoquinone would be more stable. It should be mentioned, however, that the data represented by these curves do not preclude the possibility that the initial product from the enzymatic oxidation of the catechol may be the hydroxy derivative of the *o*-quinone rather than the *o*-benzoquinone itself.

To make certain that the use of large excess of enzyme could not give rise to any oxidation product from the catechol which could set free more iodine than that corresponding to the conversion of all the catechol to quinone (1.97 cc. of thiosulfate solution), the run corresponding to curve 6 was made in which 10.5 units per mg. instead of 3.5 units per mg. of catechol were used. It can be seen that the only way that curve 6 differs from curve 1 is that it shows that the complete oxidation took place in less time. When, however, the oxidation of the catechol is permitted to take place more slowly by using less enzyme than 3.5 units per mg. of catechol, then it will be observed, as shown in the remaining curves 2, 3, 4 and 5 of Fig. 1, that not only is the oxidation incomplete but there is a greater rate of falling off of the titratable iodine with the expiration of time beyond the maximum shown on the curves. This failure of the enzyme to bring about complete oxidation of the catechol naturally suggests either gradual inactivation of the enzyme, or the occurrence of secondary reactions between the quinone and unchanged catechol.

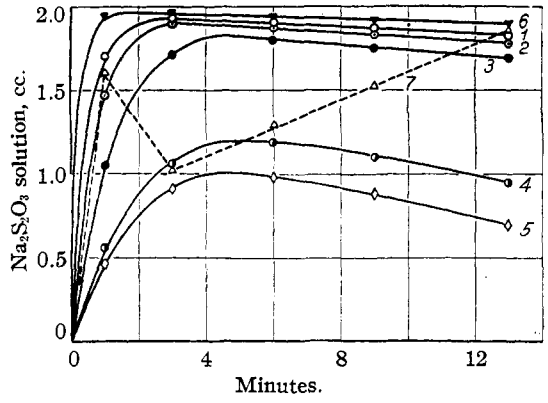

Fig. 1.—Curves showing the effect of varying the amount of enzyme on the production of quinone from the catechol–tyrosinase reaction at *p*H 6.6. Units of enzyme per mg. of catechol: 1, 3.5; 2, 2.5; 3, 1.5; 4, 1.0; 5, 0.5; 6, 10.5; 7, 150 mg. of catechol + 35 units of enzyme. The enzymatic oxidations were run at 25° in a total reaction volume of 250 cc., of which 25 cc. was 0.2 *M* phosphate buffer; 5 cc. was 0.2% freshly prepared catechol solution (10 mg.), and 5 cc. was enzyme solution. The enzyme was added at zero time. With air bubbling through the reaction solution, 25-cc. samples were removed at the time intervals noted and added to 25 cc. of 2 *N* H_2SO_4. After adding 10 cc. of 10% KI they were allowed to remain in the dark for fifteen minutes before titrating the liberated iodine with standard thiosulfate solution. Thus the plotted volumes of thiosulfate solution required to take care of the iodine liberated in each sample are proportional to the amounts of quinone causing this iodine liberation. 1 mg. of catechol per 25 cc. (0.1 reaction volume) when completely oxidized is equivalent to 9.10×10^{-6} moles of quinone which in turn is equivalent to 1.97 cc. of 0.00928 *N* $Na_2S_2O_3$ solution. The curve shown in broken lines is the original data of curve 5, Fig. 2, and is introduced here to show the effect of large excesses of catechol on the production of quinone from the tyrosinase–catechol reaction. To obtain these data the method described directly above was employed on a solution buffered to *p*H 6.6 and containing 150 mg. of catechol to 35 units of enzyme. The sharp break in the curve after the initial production of quinone is attributed to a secondary reaction between quinone and excess catechol. The subsequent rise in titratable iodine beyond the three-minute point can be explained in several ways, but in the absence of more definite information the authors hesitate to make any interpretation at this time.

The Effects of Excessive Amounts of Catechol on the Production of Quinone in the Enzymatic Oxidation of Catechol.—The curves shown in Fig. 2 indicate the effects of increasing amounts of catechol at *p*H 6.6 on the percentage of catechol enzymatically oxidized to the quinone state at various time intervals. When the proportion between enzyme and catechol is 35 units to 9.4 mg. in 250 cc. of reaction mixture, or about 4 units

(9) E. G. Ball and T. T. Chen, *J. Biol. Chem.*, **102**, 691 (1933).
(10) H. Wagreich and J. M. Nelson, *ibid.*, **115**, 459 (1936).
(11) J. B. Conant and L. F. Fieser, THIS JOURNAL, **46**, 1858 (1924).

to 1 mg., the oxidation of the catechol to quinone is about 98% complete in less than three minutes (curve 1, Fig. 2). When more catechol is used, *i. e.*, 18.8 mg. to 35 units of enzyme, and the ratio between enzyme and catechol drops to about 2 units per mg. of the substrate, only about 88% of the catechol is oxidized as indicated by the iodine set free (curve 2, Fig. 2). When the

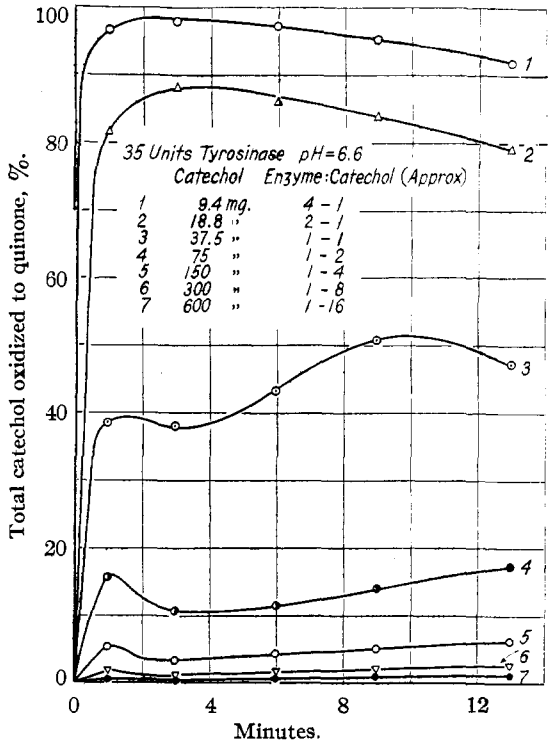

Fig. 2.—Curves showing the effect of large excesses of catechol on the production of quinone from the enzymatic oxidation of catechol. The data shown in these curves were plotted in this manner to indicate more clearly the extent to which excess catechol in the reaction mixture cuts down the percentage oxidation of the catechol to the quinone state. The original data for these curves were obtained in the manner described in the legend of Fig. 1, and curve 5 in this Fig. is plotted as a broken line in Fig. 1 using the original data. It will be observed that in curve 1 where the ratio between enzyme and catechol is about 4 units to 1 mg., the oxidation is practically complete within the first minute. However, it will be noticed that the percentage of total catechol oxidized to quinone decreases as the ratio of catechol to enzyme is increased, thus indicating that secondary reactions become more prominent in the presence of large excesses of catechol.

amount of catechol in the reaction mixture is increased still more, but the amount of enzyme remains constant (35 units), the shapes of curves 3, 4 and 5 in Fig. 2 indicate that not only is the percentage conversion of the catechol to the quinone state increasingly lower but suggest that the large amounts of unoxidized catechol present have so affected the stability of the quinone as to result in too low titratable iodine values. This effect is brought out even more strikingly by replotting the data of curve 5 in Fig. 2 as curve 7 in Fig. 1. Furthermore, it has been shown recently by the present authors[12] that progressively increasing the amount of catechol with respect to a given amount of *o*-benzoquinone results in a progressively increasing rate of the disappearance of the quinone, and that this effect becomes more pronounced in concentrated solutions of quinone and also increases rapidly with increase in *p*H. Curves 6 and 7 in the same Fig. 2 show that when still larger amounts of catechol (300 and 600 mg. per 35 units of enzyme) are used, then the removal of quinone by the large excess of unchanged catechol, *i. e.*, a secondary reaction, appears to be only slightly slower than the enzymatic oxidation reaction.

Variations in the Course of the Reaction with *p*H.—It has been pointed out already (curve 1, Fig. 1 and curve 4, Fig. 3), that at *p*H 6.6 the use of 3.5 to 4 units of enzyme per mg. of catechol in the reaction mixture effected the complete and rapid oxidation of the substrate. Using the same amounts of enzyme and catechol but permitting the reaction to take place at *p*H 5.3 and 4.2, the results (curves 3 and 2, Fig. 3) were much the same as those corresponding to *p*H 6.6 except that the enzyme appeared to be slightly less efficient at *p*H 4.2. The results represented by curve 1 of Fig. 3 were obtained by using again the same concentrations of enzyme and catechol, but the *p*H of the reaction being lower, 3.3. The shape of the last mentioned curve shows that not only was the formation of quinone (titratable iodine) less rapid but the oxidation of the catechol never reached completion. Judging from the fact that in the case of dilute solutions containing less than 10 mg. of quinone per 250 cc. of solution and low *p*H (below 4) the accelerating effect of catechol on the rate of disappearance of *o*-benzoquinone is only slight,[12] the slow and incomplete oxidation of the catechol is probably attributable to a weak catalytic action of the enzyme at this *p*H rather than to any secondary reaction between the unoxidized catechol and quinone. The results as shown by curve 5, Fig. 3, obtained when the same enzymatic oxidation of the catechol was run at *p*H 8.0, are especially interesting. It is known from the work of Ball and Chen[9] that the stability of *o*-benzoquinone diminishes very rapidly as the *p*H of the aqueous solution is increased beyond

(12) C. R. Dawson and J. M. Nelson, THIS JOURNAL, **60**, 245 (1938).

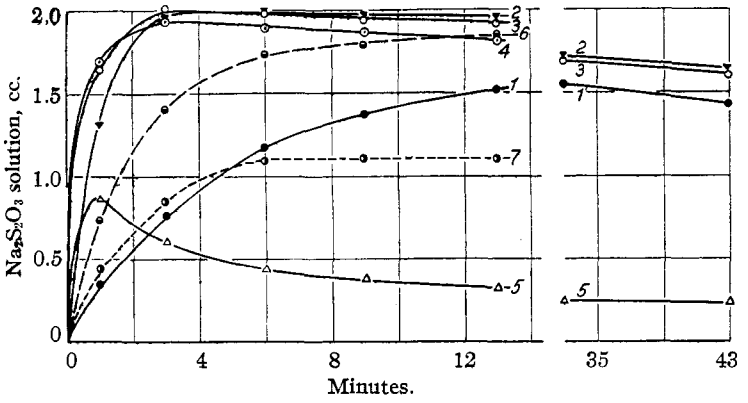

Fig. 3.—Curves showing the effect of *p*H on the production of quinone from the catechol-tyrosinase reaction: enzyme per mg. of catechol 1, *p*H 3.3, 3.5 units; 2, *p*H, 4.2, 3.5 units; 3, *p*H 5.3, 3.5 units; 4, *p*H 6.6, 3.5 units; 5, *p*H 8.0, 3.5 units; 6, *p*H 5.3, 1.0 units; 7, *p*H 5.3, 0.5 units. The data shown in these curves were obtained in the manner described in the legend of Fig. 1. The broken lines, curves 6 and 7 obtained when the amount of enzyme at *p*H 5.3 was decreased, show that no secondary reaction between the quinone and unchanged catechol is apparent as was indicated by a subsequent drop in titratable iodine in the case when analogous runs were made at a higher *p*H, 6.6. Compare with curves 4 and 5 of Fig. 1.

6.8. Likewise from the observations made by the present authors[12] that the effect of catechol on the stability of the quinone also increases rapidly with *p*H, it therefore is not surprising that there should be a sharp drop in the amount of quinone in the solution under these conditions of high *p*H.

Inactivation of the Enzyme.—In the foregoing sections data are presented in the form of curves 1, Figs. 1 and 2, and curves 2, 3, and 4 of Fig. 3, showing that the use of at least 3.5 units of enzyme per mg. of catechol in aerated solutions buffered to *p*H values between 4.2 and 6.6 caused the complete and rapid oxidation of the catechol. In all such cases where enough enzyme was used to cause the complete and very rapid oxidation of the catechol, the enzyme seemed to suffer no appreciable inactivation, as evidenced by the fact that when these experiments were repeated and 10 mg. more catechol was added after the initial 10 mg. had been completely oxidized, another rapid and practically complete oxidation of the added catechol resulted. The data in support of this statement are given in the form of curves 1 and 3 of Fig. 4. The data presented in these two curves up to the seven and one-half-minute period are those obtained from repeating the runs represented in curves 3 and 4 of Fig. 3, and therefore represent the results obtained from the enzymatic oxidation of catechol in solutions buffered to *p*H values of 5.3 and 6.6, respectively. As has been stated already, under these conditions 1.97 cc. of thiosulfate solution corresponds to all of the catechol being oxidized to the quinone state, and it is therefore apparent that the oxidation of the catechol was complete in both cases in about three minutes. On adding 10 mg. more catechol to the remaining 175 cc. of reaction mixture after 3 samples had been withdrawn, the calculated volume of thiosulfate now corresponding to another complete oxidation to quinone is 2.70 cc. It will be noticed in the case of the reaction at *p*H 5.3 (curve 1, Fig. 4) that the second maximum reaches 4.38 on the ordinate axis. This increase of 2.40 cc. of thiosulfate beyond the 1.98 value of the first maximum indicates therefore that the enzyme was still active at the end of seven and one-half minutes or the catechol added could not have been oxidized to this extent. Furthermore, the fact that the rate of oxidation of the second 10 mg. of catechol was practically the same as

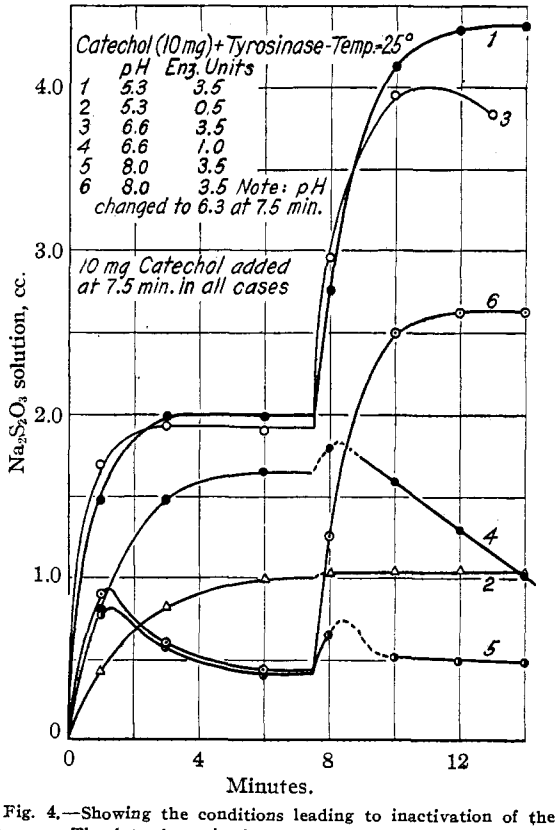

Fig. 4.—Showing the conditions leading to inactivation of the enzyme. The data shown in these curves up to the seven and one-half-minute period were obtained by following the procedure described in the legend of Fig. 1. In order to ascertain whether or not the enzyme had suffered inactivation, 10 additional mg. of catechol was added at this time, and in the case of curve 6 the *p*H was first changed from 8.0 to 6.3.

that of the original 10 mg. is additional evidence that the enzyme must have been practically intact after the complete oxidation of the first 10 mg. of catechol. The fact that the second maximum is not so great in curve 3, Fig. 4, representing the run made under the same conditions except at *p*H 6.6, should probably not be interpreted to mean that there has been more inactivation of the enzyme before the seven and one-half-minute period at *p*H 6.6 than in the case of the enzymatic oxidation at *p*H 5.3, but that the secondary reaction between quinone and unoxidized catechol has been favored at this *p*H by the increase in catechol concentration.

Adopting the same procedure for determining the fate of the enzyme when less enzyme was used than above, *i. e.*, 1.0 unit instead of 3.5 units per mg. of catechol, but otherwise the conditions and *p*H of 6.6 being the same as for curve 3, the results represented by curve 4 were obtained. As shown in this curve, when 10 mg. more catechol was added to the reaction which was still incomplete after the expiration of seven and one-half minutes, the very slight increase followed by the rapid decrease in titratable iodine indicates that not only had the enzyme previously been inactivated nearly completely but the increase in catechol concentration caused the secondary reaction between quinone and catechol to be more pronounced. When this method of testing for the activity of the enzyme during the course of the reaction was repeated at *p*H 5.3 under the same conditions as for curve 1, Fig. 4, but using still less enzyme, *i. e.*, 0.5 unit of enzyme per mg. of catechol, the results shown in curve 2, Fig. 4, were obtained. It will be noted that the addition of 10 mg. more catechol at the seven and one-half-minute period caused no appreciable increase in titratable iodine. These results, in contrast to those shown by curves 1, 3 and 4 of this figure, emphasize that the incomplete oxidation of the original 10 mg. of catechol used for curve 2, Fig. 4, was due to the fact that the enzyme had been inactivated completely under these conditions (very slow rate of oxidation).

In the discussion of the reaction course of the enzymatic oxidation of catechol in alkaline solutions, data were presented in the form of curve 5, Fig. 3, indicating that after the initial production of quinone by the enzyme, the subsequent drop in titratable iodine or quinone is due to the inherent instability of the quinone in more alkaline solution especially in the presence of unoxidized catechol. To determine what influence these secondary actions might have on the inactivation of the enzyme, the experiment represented in curve 5, Fig. 3, *i. e.*, 3.5 units of enzyme per mg. of catechol at *p*H 8.0, was repeated, and 10 mg. more catechol was added after the expiration of seven and one-half minutes. The results are shown in curve 5, Fig. 4. The slight rise and subsequent decrease in titratable iodine observed indicates that these secondary effects continue to obscure the activity of the enzyme to such an extent at this *p*H that no definite conclusions as to the activity of the enzyme can be drawn. Recalling that the quinone is more stable in more acid solutions, the experiment was repeated but at the expiration of seven and one-half minutes the reaction mixture first was made acid (*p*H 6.3) and then 10 mg. more catechol was added. As shown by curve 6, Fig. 4, the amount of titratable iodine or quinone rapidly rose to an equivalence of 2.62 cc. of thiosulfate solution. This increase of 2.25 cc. of thiosulfate solution corresponds to about 84% complete oxidation of the 10 mg. of catechol subsequently added and therefore indicates that the enzyme had not been inactivated appreciably.

It therefore appears that when the enzyme is present in sufficient quantity to bring about a rapid and complete oxidation of the catechol, then, as the results represented by curves 1 and 3, Fig. 4, show, the final completely oxidized catechol seems to exert little inactivating influence on the enzyme. On the other hand, the fact that the enzyme is inactivated when an insufficient amount is used to bring about complete oxidation of the catechol rapidly, indicates strongly that the oxidation process involves more than one step and that some intermediate oxidation product of the catechol, possibly *o*-quinone, is responsible for the inactivation. This interpretation is in agreement with the results discussed in reference to curve 6, Fig. 4, that is, that the enzyme appears to be protected in alkaline solutions where the secondary actions rapidly remove the *o*-quinone. These results suggest the answer to the experimental fact reported by Graubard and Nelson[13] that the enzyme appears to be less rapidly inactivated as the *p*H of the catechol-enzyme system is increased. This pro-

(13) M. Graubard and J. M. Nelson, *J. Biol. Chem.*, **111**, 757 (1935).

tection of the enzyme activity is therefore different from the protection noted in the experiments corresponding to curves 1 and 3, Fig. 4, where the protection seems to be due to the quinone being rapidly oxidized to a higher oxidation product—possibly 4-hydroxy-*o*-quinone as suggested by Wagreich and Nelson.

Since both of these quinones have the same equivalency to iodine and therefore to thiosulfate solution it is obvious that the oxidation of *o*-quinone to 4-hydroxy-*o*-quinone would not be apparent in the complete oxidation curves that were obtained by this method. However, it did seem possible that this two-stage oxidation process would be evidenced in oxygen uptake measurements if they were run under analogous conditions.

Using a reaction mixture identical in composition to that employed for obtaining the results corresponding to curve 1, Fig. 4, but in a Warburg respirometer so that the rate of oxygen uptake could be followed, additional evidence to support our view that the complete enzymatic oxidation of catechol involves more than one step was obtained. This evidence is presented graphically in Fig. 5. It will be noted that the uptake of oxygen quickly reaches 27 mm.[3] which corresponds, within experimental error, to an uptake of 1 atom of oxygen per mole of catechol, and it is only after the expiration of seventy minutes that the second atom of oxygen which is known to be necessary for the complete oxidation of catechol is taken up. The reader may wonder as to the reason for the longer time required for complete oxidation in the case of the Warburg method as compared to that for complete oxidation in the method used for obtaining the results shown by curve 1, Fig. 4. This marked difference in rate of oxidation can be accounted for by recalling that in the Warburg respirometer the air is only in contact with the surface of the reaction mixture, whereas in the other method the reaction mixture is agitated continuously by a stream of air.

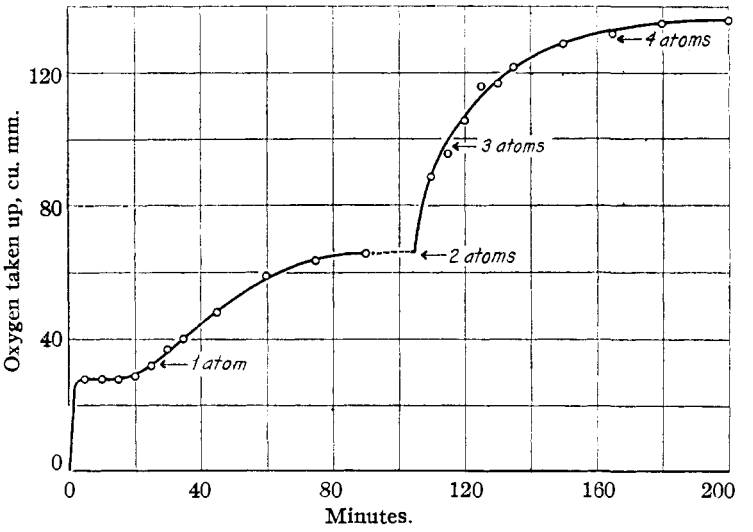

Fig. 5.—Showing that the enzymatic oxidation of catechol takes place in at least two consecutive steps. The data for the above curve showing the rate of oxygen uptake during the enzymatic oxidation of catechol were obtained by using a Barcroft–Warburg respirometer. The total reaction volume was 8.0 cc. and consisted of 2 cc. of 0.2 *M* phosphate buffer (*p*H 5.0) + 1 cc. of freshly prepared aqueous catechol solution (0.32 mg.) + 3.5 cc. of water + 1 cc. of enzyme solution (1.3 units) + 0.5 cc. of concentrated potassium hydroxide solution in the center well of the reaction flask to take care of any carbon dioxide that might be evolved. These conditions are comparable to those used when the complete enzymatic oxidation of catechol was effected as shown by the iodometric method previously described in the case of curve 3, Fig. 3. For this amount of catechol 32.6 cu. mm. oxygen uptake corresponds to 1 atom of oxygen per mole of catechol. At the one hundred and five-minute period, when 2 atoms of oxygen had been consumed, an amount of catechol equivalent to that originally used was again added in order to make sure that the enzyme was still active.

Summary

Some of the information gained concerning the mechanism of the enzymatic oxidation of catechol may be listed as follows:

1. The two atoms of oxygen taken up per mole of catechol in the complete enzymatic oxidation involve at least two consecutive reactions.

2. The concentration of the substrate with respect to that of the enzyme influences the mechanism or course of the reaction. When insufficient enzyme is used to promote rapid and complete oxidation of the catechol, especially in the more concentrated solutions of the latter, the course of the enzymatic oxidation is affected by secondary reactions between the unoxidized catechol and quinone.

3. The most rapid and complete oxidation of the catechol as measured by the extent of quinone formation occurs in slightly acid solutions (*p*H 5–6) when sufficient enzyme is used.

4. In alkaline solutions, however, under similar circumstances secondary reactions effecting the stability of the quinone become prominent.

5. When the amount of enzyme used is insufficient to cause rapid and complete oxidation of the catechol to the quinone state then in acid

solution inactivation of the enzyme becomes more pronounced.

6. The inactivation of the enzyme appears to be brought about by the earlier oxidation product of the catechol (possibly *o*-quinone).

7. The inactivation of the enzyme appears to be offset either by further oxidation of the initial oxidation product of the catechol in acid solutions or by the removal of the early oxidation product of the catechol by secondary reactions in more alkaline solutions.

8. Evidence that no hydrogen peroxide is formed as an initial reaction product of this enzymatic oxidation of catechol was also obtained but is being withheld for a later publication.

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[CONTRIBUTION FROM THE AVERY LABORATORY OF CHEMISTRY OF THE UNIVERSITY OF NEBRASKA]

Brominations with Iodine Monobromide

BY WALTER MILITZER

In the course of some iodine number determinations on certain oils, the effect of the iodine monobromide solution as a substituting reagent was observed. In contrast to iodine monochloride, which is an iodinating reagent,[1] iodine bromide proved to be a brominating agent. Thus, phenol was brominated readily in carbon tetrachloride solution to give good yields of *p*-bromophenol, according to the equation

$$C_6H_5OH + IBr \longrightarrow p\text{-}Br\text{-}C_6H_4OH + HI$$
$$HI + IBr \longrightarrow I_2 + HBr$$

The greater part of the iodine precipitated from solution and could be recovered by filtration. The mechanism of the above reactions is substantiated by the reaction with aniline in which the *p*-bromoaniline hydroiodide precipitates from solution as follows

$$C_6H_5NH_2 + IBr \longrightarrow p\text{-}BrC_6H_4NH_2HI + HBr$$

The theoretical aspect of the reaction is of interest in view of the fact that substitution is quite often considered an addition of halogen and subsequent removal of hydrohalogen. The iodine bromide reaction apparently does not follow this mechanism. The addition of iodine bromide to double bonds is quite slow and selective, whereas its substitution reaction is very rapid. It will not add to cyclic double bonds such as are found in cholesterol and adds only slowly to cyclohexene. Furthermore, the iodine in iodine bromide is positive and the bromine negative, as shown by electrolysis studies.[2] Any subsequent elimination of hydrohalogen would, therefore, tend toward the elimination of hydrogen bromide rather than of hydrogen iodide. These facts seem to contradict the addition–elimination hypothesis.

As a preparative method for *p*-bromophenol and *p*-bromoaniline, iodine bromide cannot be recommended in preference to the existing methods for brominating and, indeed, is inferior; but for certain other compounds it possesses decided advantages over bromine. Thus, in the bromination of α-naphthol iodine bromide brominates quickly and smoothly to give good yields of 4-bromo-1-naphthol, whereas bromine is not applicable to the reaction. Iodine bromide is a milder reagent than bromine and is more easily handled. It can be made quickly in solution just prior to use.

In the preparation of α-bromonaphthalene, iodine bromide presents an advantage over bromine. The usual method of brominating naphthalene[3] with bromine involves a time consuming reaction; the iodine bromide method is rapid. In the action of bromine on naphthalene a by-product is formed which slowly liberates hydrogen bromide and in order to remove this by-product a four-hour heating of the intermediate with sodium hydroxide is necessary. With iodine bromide a similar by-product is formed which slowly liberates iodine, but its removal is accomplished by refluxing the original solution for thirty minutes with a small quantity of zinc dust.

Due to the sluggish addition of iodine bromide to olefinic linkages, it should prove a valuable brominating reagent for aromatic substances containing unsaturated side chains. Its effect in this respect is being investigated.

(1) Hickinbottom, "Reactions of Organic Compounds," Longmans, Green and Co., 1936, p. 293.

(2) Finkelstein, *Z. physik. Chem.*, **124**, 285 (1926).

(3) "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1932, p. 116.