[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, ST. PETER'S COLLEGE]

The Catalytic Properties of Charcoal. I. "Peroxidase" Activity

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Active charcoal has been found to influence the oxidation of uracil by hydrogen peroxide in a peculiar way. In the absence of charcoal this pyrimidine is completely oxidized at 95°, in its presence the isolation of several intermediate products of biological importance is possible.[1] This action is not strictly analogous to the catalysis of some oxidative reactions by peroxidases, but it suggested an investigation of the behavior of active charcoal in some reactions commonly used to evaluate the activity of peroxidases.

The charcoal used in this investigation was one known to modify the uracil oxidation as above.[2] Although all charcoals tested in this Laboratory gave the usual qualitative tests for peroxidases, this one was chosen because of its neutrality, that is, it did not change the *p*H of buffer solutions in several hours, thus permitting work at constant *p*H.[2] However, there is no reason to suspect that, in other respects, the other charcoals would not behave very much as this one.

The first test used was the indophenol reaction, recently applied to peroxidases.[3] The indophenol produced was found to be strongly adsorbed by the charcoal, so that the latter and the filtrate were analyzed separately. Figure 1 shows the amount of indophenol formed in various times. For the present, we have no explanation to offer for the shape of the curves. The oxygen evolved during the reaction, due to the "catalase" action of the charcoal, was measured and gave a perfectly smooth curve when plotted against time. The behavior of the curve showing the amount of indophenol in the filtrate leads to interesting speculation. One of the hypotheses still under consideration, but still to receive experimental support, is that some of the charcoal had been reduced to colloidal size, and that, under the conditions of the experiments, this colloidal part underwent oxidation by the peroxide after one-half minute. We have observed the destruction of charcoal in colloidal solution at 60°, but not at room temperature with sufficient velocity to account for the rapidity of the disappearance of the indophenol from the filtrate. It may be, however, that the other components of the reaction mixture are a factor affecting this speed. The oxidation by hydrogen peroxide of indophenol adsorbed on charcoal has never been observed by us. It seems possible that the colloidal charcoal is destroyed and that the indophenol thus liberated by loss of its adsorbent is then adsorbed by the "massive" particles of charcoal. This transfer of indophenol would explain the fact that the additive curve shows no discontinuity at this point (curve 3, Fig. 1).

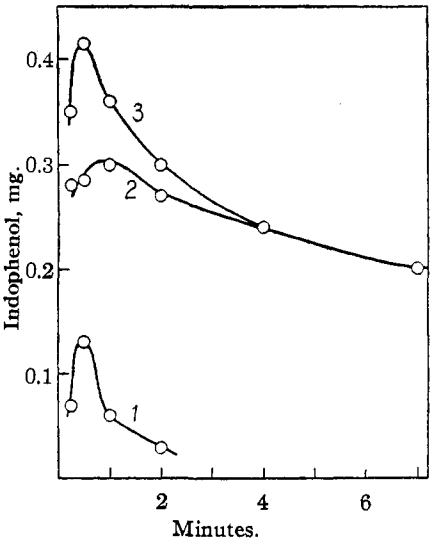

Fig. 1.—Curve 1, indophenol in the filtrate; curve 2, indophenol obtained from the charcoal. The amount remains constant at 0.20 ± 0.02 mg. from seven to at least forty minutes. Curve 3, total amount of indophenol obtained.

Colloidal charcoal was next tested by means of this same reaction. In the literature we were unable to find a method for the preparation of a colloidal solution of active charcoal. Von Weimarn's procedure[4] for obtaining sols of various substances by means of hand grinding with solid diluents was adapted to the problem in hand. We were aided in this preparation by the fact that charcoal sols, like some sulfur sols, seem to belong to the intermediate class of hydrosols, and are somewhat reversible. In fact, although the

(1) Schwob and Cerecedo, THIS JOURNAL, **56**, 2771 (1934).

(2) Schwob, Dissertation, Fordham University, 1933.

(3) Guthrie, THIS JOURNAL, **53**, 242 (1931); Pack, *Ind. Eng. Chem., Anal. Ed.*, **6**, 170 (1934).

(4) Alexander, "Colloid Chemistry," The Chemical Catalog Co., New York City, 1926, Vol. I, p. 660.

colloidal properties of the charcoal sols so obtained have not as yet been fully ascertained, they are apparently similar to those of sulfur sols.

The behavior of this charcoal sol in catalyzing the formation of indophenol is shown in Fig. 2.

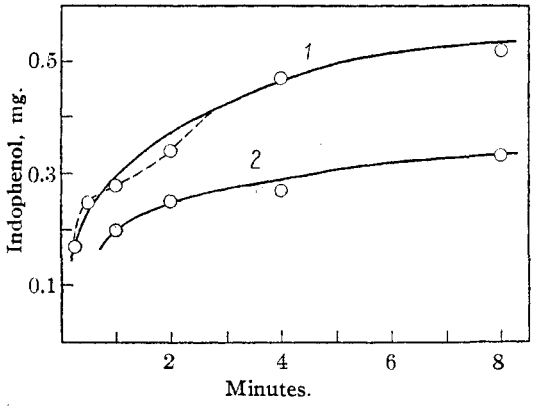

Fig. 2.—Curve 1, amount of indophenol obtained with charcoal hydrosol. For portion in dotted line see text. Curve 2, result obtained when the sol is replaced by distilled water.

No break in the curve, due perhaps to the destruction of the charcoal micelles, is apparent. A slight irregularity did show up in three of the five runs, the results of which have been averaged to give Fig. 2. These irregularities, however, were apt to cancel out in averaging, although if the average curve is redrawn as shown by the dotted line it assumes a form similar to, but less extreme than, each of the three "abnormal" curves. The point is still to be settled, however, since destruction of the charcoal would not necessarily lead to a great diminution in the indophenol yield.

An interesting fact observed was that the charcoal sol liberated no appreciable volume of oxygen from peroxide of the concentration used in these experiments over a period of several hours. Not only is this an added similarity of charcoal to natural purified peroxidase, since, until now, charcoal has been considered as having inherent "catalase" properties, but it tends to show that the suggested "peroxidase" activity is not due to a combined "catalase-oxidase" action. Accordingly, it seems untenable that the "catalase" activity serves to decompose the hydrogen peroxide to give oxygen which then forms indophenol under the influence of the "charcoal-oxidase."[5]

(5) In fact, we were unable to oxidize uracil in the presence of charcoal with either air or oxygen, although this pyrimidine is strongly adsorbed by charcoal.[2]

Hence, it is probable that the charcoal activates the peroxide in some way. Ort and Bollman[6] have shown by electrometric means that some amino acids promote the oxidation of glucose by hydrogen peroxide. The effect of the charcoal sol on the voltage produced by hydrogen peroxide between a bright platinum electrode and an 0.1 *N* calomel electrode was therefore determined. While in the work of Ort and Bollman the activating effect of the amino acids may have been exercised on either the peroxide or the glucose, it seems that, in this case, it is the hydrogen peroxide that is activated. Figure 3 gives the curve obtained when there were added varying amounts of charcoal sol to peroxide solutions of fixed concentration (approximately 0.5%). By means of

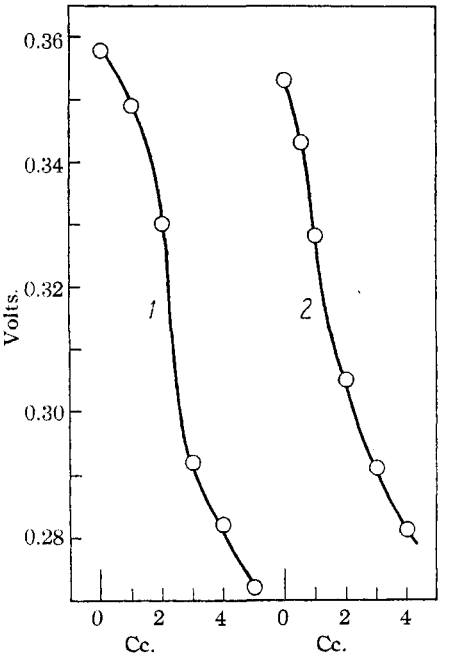

Fig. 3.—Curve 1, voltage obtained with hydrogen peroxide and varying amounts of charcoal hydrosol. Curve 2, result when diluted potato extract replaces the charcoal sol.

a blank run the effect of the small amount of gelatin used to stabilize the sol was found to be negligible for these rather qualitative results. It was thought interesting to find out whether a natural peroxidase gives a similar curve. The result for aqueous filtered potato extract (Fig. 3, curve 2) was found to have much in common with that obtained with charcoal, although the peroxide used in this case was not only from a different

(6) Ort and Bollman, THIS JOURNAL, **49**, 805 (1927).

batch, but was not of the same strength (about 0.6%). No attempt was made to obtain any but relative values, and no corrections have been applied to the voltages as read on the potentiometer. It is projected to elaborate on this method in order to attempt applying it to the determination of peroxidase activity.

Experimental

The charcoal was a commercial animal charcoal purified by refluxing with four times its weight of constant boiling hydrochloric acid for twenty hours. It was then washed with distilled water until free of chlorides. Twice its weight of hot 95% alcohol was then used to extract further impurities (mostly consisting of a greenish gum), followed by treatment with the same amount of ether. This process reduced the ash content from 45 to 1.67%, although the ash still gave a positive test for iron with thioglycolic acid.

Peroxidase Activity of "Massive" Charcoal.—A combination of the methods of Guthrie and of Pack[3] was used. A 0.500-g. sample of the charcoal was suspended in 10 cc. of distilled water, and 5.75 cc. of a solution of *p*-phenylenediamine hydrochloride containing 0.5265 g. per 100 cc. was added. To 8.75 cc. of citrate buffer of *p*H 4.5 in a 10-cc. graduate, 0.6 cc. of 4% alpha-naphthol in 50% alcohol was added carefully so as to float on top of the buffer. The contents of this cylinder were added to the flask containing the charcoal simultaneously with 10 cc. of 1% hydrogen peroxide (commercial, containing acetanilide). All chemicals were at about 25°. The flask was then shaken by hand or machine and, at the end of the required time, 2 cc. of a 2% potassium cyanide solution was added to slow up the reaction. The suspension was then filtered through a fluted filter paper and the charcoal washed with three 10-cc. portions of water, the last portion being used to force the charcoal to the bottom part of the filter. The wash-water was added to the filtrate and it was immediately extracted with 25 cc. of toluene in a separatory funnel for thirty seconds. The toluene layer was then compared with a standard indophenol solution in a colorimeter. The charcoal was dried on the filter paper in the funnel at 100° (higher temperatures tended to destroy part of the indophenol). Four 6-cc. portions of toluene were poured over the charcoal. The last drops filtering through were quite colorless. This solution was also analyzed colorimetrically. A concordance of results of 5 to 8% was usually obtained. Figure 1 represents the average of six such trials.

Preparation of the Charcoal Hydrosol.—One gram of the charcoal was ground by hand in a glass mortar with 9 g. of pure sodium chloride for twenty minutes. Two grams of the resulting mixture was ground with 18 g. of sodium chloride for a further twenty minutes. This was then dissolved in 500 cc. of distilled water. The high salt concentration precipitated the charcoal. This suspension was then filtered through asbestos in a Gooch crucible and washed with one liter of distilled water. The entire contents of the Gooch were then suspended in 500 cc. of water and, for the sol used in this investigation, 4 cc. of a 1% gelatin sol was added. The suspension was then filtered through ordinary filter paper after "aging" six to ten days at room temperature. A fine deposit formed overnight and was filtered off, after which the sol is stable for more than three months. The resulting sol contained approximately 0.003 mg. of charcoal per cc. and was brownish by transmitted light and grayish-black by reflected light. A sol of lesser concentration may be obtained without the use of gelatin.

"Peroxidase" Activity of the Charcoal Hydrosol.—These experiments were performed as with the "massive" charcoal, except that 15 cc. of the sol was used instead of the charcoal suspension. Of course, the resulting solution was extracted with toluene directly. For the blank 15 cc. of distilled water was used instead of the sol.

Electrometric Measurements.—A 15-cc. weighing bottle was used as the cell. The voltage was measured between an uncoiled bright platinum wire immersed 2 cm. and an 0.1 *N* calomel electrode. In order to obtain reproducible results, the wire was heated in a Bunsen flame before each reading. Equilibrium seemed to be reached in five to ten minutes, the calomel electrode being negative. A total volume of 10 cc. was used in each case. Five cc. of 1% hydrogen peroxide was placed in the bottle and, if necessary, water so that when either the charcoal sol or the potato extract was added the resulting solution had a volume of 10 cc. The results were duplicable to 2–3 millivolts.

Summary

1. The "peroxidase" activity of charcoal in the indophenol reaction has been measured at room temperature and *p*H 4.5. The yield of indophenol is at a maximum at one-half minute.

2. A hydrosol of charcoal has been prepared.

3. This charcoal sol has been found to exhibit "peroxidase" activity in the formation of indophenol. No "catalase" activity has been observed.

4. The charcoal sol affects the voltage of the Pt, H_2O_2 || KCl (0.1 *N*), Hg_2Cl_2, Hg cell in a manner similar to that of potato peroxidase.

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