# A POLAROGRAPHIC SEARCH FOR HYDROGEN PEROXIDE IN THE TISSUES OF THE ALBINO RAT

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Hydrogen peroxide is produced as a by-product of the action of those enzymes which catalyze the direct addition of oxygen to the substrate, such as d-amino acid oxidase, xanthine oxidase, and uricase (1). Pneumococci, which lack catalase, produce hydrogen peroxide in amounts as great as 80% of the amount of oxygen consumed (2). In the conversion of glucose to gluconic acid by the glucose oxidase of molds, if the catalase present is blocked by hydrogen sulfide, the formation of one mole of hydrogen peroxide for each mole of oxygen consumed can be demonstrated (3).

The accumulation of hydrogen peroxide in most living organisms gives rise to harmful effects, so that the widely distributed enzyme, catalase, serves the important physiological function of destroying hydrogen peroxide as rapidly as it is formed. The accumulation of hydrogen peroxide is one of the reasons for the difficulty in growing anaerobic bacteria under aerobic conditions (2). Hydrogen peroxide effects the direct oxidation of the biologically important sulfhydryl compounds such as glutathione (4). In blood, hydrogen peroxide would have the undesirable effect of converting the hemoglobin to methemoglobin (5). The biological advantage of catalase, which is abundant in blood, liver, and kidney, consists of the detoxification of hydrogen peroxide by converting it to oxygen and water.

The authors (6) have recently shown that the use of 0.1 M sodium phenobarbital as the supporting electrolyte converts the usual flat polarographic wave of hydrogen peroxide to a sharply defined wave. Since the procedure can yield quantitative results at concentrations of hydrogen peroxide as low as 0.00003 M and qualitative results as low as 0.000,001 M, it seemed desirable to apply this method to a search for hydrogen peroxide in the tissues of the rat. Lichstein and Soule (7) have recently reported the failure to detect hydrogen peroxide in 11 species of catalase producing

bacteria when the catalase was blocked by 0.003 M sodium azide, a concentration which was shown by Keilin (8) to inhibit completely liver catalase, and to inhibit nearly compeletely yeast catalase (94%), indophenol oxidase (87%) and peroxidase (70%). In the current work, the authors considered it desirable to work with higher concentrations of sodium azide of the order of 0.1 M. Preliminary experiments indicated that 0.1 M sodium azide completely blocked the catalase activity of liver catalase without interfering with the polarographic determination of hydrogen peroxide by the sodium phenobarbital method.

**Experimental**

A 150 gm. rat was killed by subcutaneous injection of 5 ml. of 0.4 M sodium azide, equivalent to a concentration of 0.15 M sodium azide in an estimated 12 ml. of blood. Death occurred by cyanosis in 2 minutes. The neck muscles, including the thyroid gland, were homogenized in 10 ml. of 0.4 M sodium azide, diluted to 40 ml. with sodium phenobarbital solution and centrifuged, making a final 1:20 solution of the tissue that was 0.1 M with respect to both sodium azide and sodium phenobarbital. After 30 minutes passage of nitrogen, the solution was run in the polarograph. The results are indicated in Fig. 1.

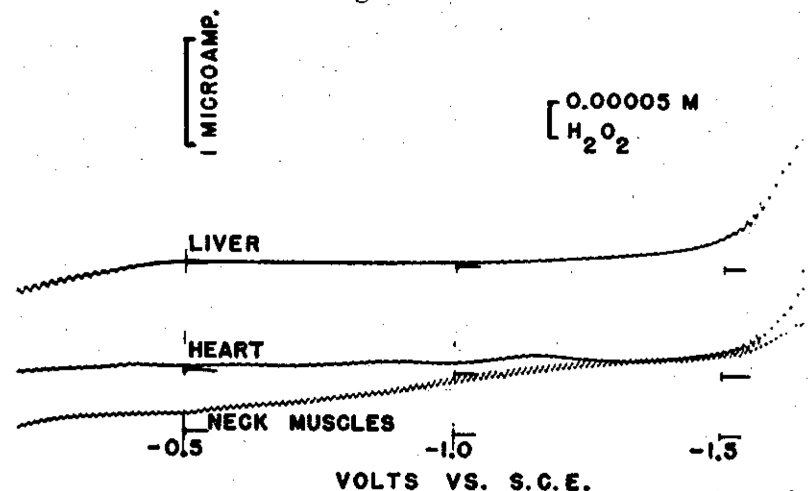


Fig. 1. The absence of hydrogen peroxide in liver, heart and muscle.



A flat polarographic wave occurs between —0.5 and —1.25 v., but there is no definite evidence of hydrogen peroxide, which must therefore have been less than 0.00016 M in the muscle tissue. Heart tissue was prepared similarly. The polarographic wave in Fig. 1 shows a slight tendency toward maximum formation at —0.90 v. and —1.10 v., but a repetition of this curve in Fig. 2 indicates these to be artifacts. Liver was prepared similarly. On the basis of a 1:10 dilution of the liver tissue, it can be seen from the flatness of the polarograph curve in Fig. 1 that the hydrogen peroxide content of the tissue, if any, must have been less than 0.000,08 M.

A 200 gm. rat was killed by subcutaneous injection of 2 ml. of 0.4 M sodium azide, equivalent to a concentration of 0.05 M sodium azide in an estimated 17 ml. of blood. Death occurred by cyanosis in five minutes. The heart, kidney and lung were immediately removed and immersed respectively, in 8 ml. of a solution that was 0.1 M with respect to both sodium azide and sodium phenobarbital. The tissues were homogenized in this same solution, centrifuged, and run in the polarograph after 30 minute passage of oxygen-free nitrogen. Polarographic detection of 0.000,005 M hydrogen peroxide would have been possible, corresponding to 0.000,05 M hydrogen peroxide in the original tissues. In-

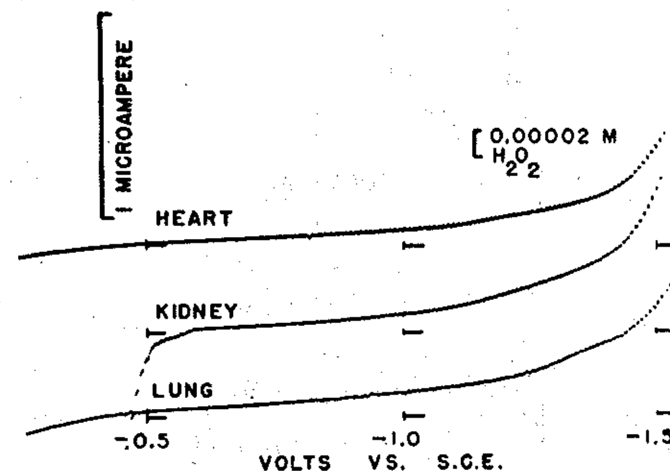


Fig. 2. The absence of hydrogen peroxide in heart, kidney and lung.

spection of Fig. 2 indicates that the concentration of hydrogen peroxide in the heart, kidney and lung must have been lower than 0.000,05 M, corresponding to less than 0.05 vols.% of oxygen available from this source, or approximately 1% of the oxygen available to normal tissues through the hemoglobin and cytochrome mechanisms.

In view of the failure to demonstrate significant amounts of hydrogen peroxide in muscle, heart, liver, lung, or kidney, an experiment was undertaken to determine the possibility of recovery of added hydrogen peroxide from heart tissue homogenate. Results are indicated in Fig. 3 and in Table I.

Inspection of Fig. 3 indicates the complete failure to recover any hydrogen peroxide from the first 0.000,13 M addition after 80 minutes. Table I indicates an overall rate of destruction of hydrogen peroxide equivalent to 0.000,025 M per hour, a rate which is very much slower than the initial rate of destruction which corresponds to at least 0.000,15 M in the second 40 minute period. It is evident that some constituents of the heart homogenate utilize hy-

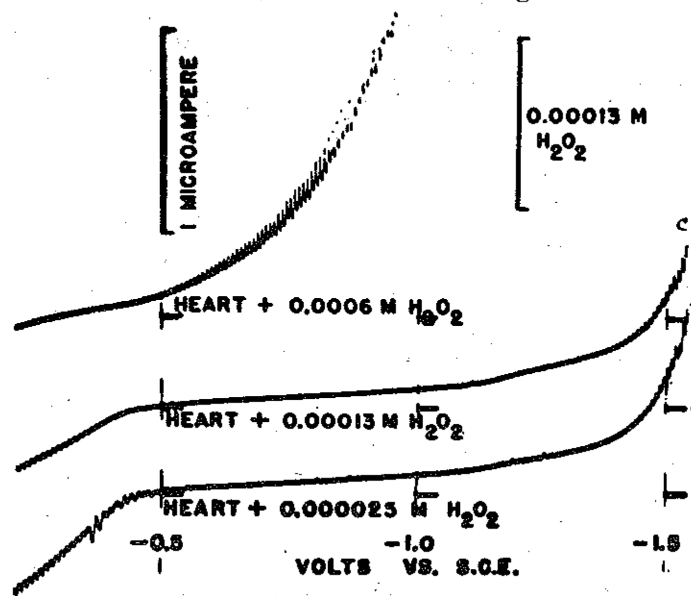


Fig. 3. Failure to recover 0.000,13 M hydrogen peroxide from heart homogenate in 80 minutes.



TABLE I

| Total Added Hydrogen Peroxide moles per l. | Sodium Azide moles per l. | Hydrogen Peroxide Recovered moles per l. | Duration of Experiment minutes | Hydrogen Peroxide Demand by Heart moles per l. |
|---|---|---|---|---|
| 0.000,025 | 1 | below 0.000,01 | 40 | over 0.000,015 |
| 0.000,13 | 2 | below 0.000,01 | 80 | over 0.000,12 |
| 0.000,60 | 3 | 0.000,55 | 120 | 0.000,05* |
| 0.000,60 | 3 | 0.000,09 | 1200 | 0.000,51 |

drogen peroxide rapidly and other constituents utilize it slowly. On the basis of the large amount of sodium azide present and the slower rate of utilization of hydrogen peroxide when the larger amounts are present, it would appear that this is not residual catalase action, but more probably a slow oxidation of the protein materials present.

Experiments on protein-free filtrates from heart and liver, made by precipitation of the proteins from homogenates in a solution containing 5% metaphosphoric acid in 10% acetic acid, indicated the absence of hydrogen peroxide in the protein-free filtrate and nearly quantitative recovery of added peroxide, a loss of only 16% of an original 0.0006 M addition of hydrogen peroxide in 17 hours.

**Summary**

Using a polarographic method for the detection of hydrogen peroxide, it has been shown that the hydrogen peroxide concentration of muscle, liver, heart, kidney and lung in the rat is less than 0.000,05 M. Heart tissue showed an initial demand for hydrogen peroxide as great as 0.000,15 M per hour with a slowing of the demand to 0.000,025 M per hour with larger amounts of hydrogen peroxide over a 20

*The error in measuring large amounts of hydrogen peroxide polarographically accounts for the 0.000,07 difference between this figure and the one above it.

hour period. Protein-free filtrates of heart and liver showed no significant amounts of hydrogen peroxide and a per hour demand of less than 0.000,005 M over a 17 hour period.

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