GASOMETRIC STUDIES OF CARBOHYDRATE OXIDATION BY HYRDOGEN PEROXIDE[1]

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Published determinations of oxygen consumption and carbon dioxide evolution in the oxidation of carbohydrates by hydrogen peroxide have been unsatisfactory. They involve such procedures as prolonged aeration, which may cause secondary decomposition, or permanganate titration, which is not specific and does not differentiate between conversion of hydrogen peroxide to oxygen and its participation in the oxidation process. The authors have devised a satisfactory gasometric method; it requires no extraneous treatment of the oxidized solutions, other than partial evacuation and the addition of dilute acid to liberate the carbon dioxide. The total available oxygen (including peroxide, and dissolved and atmospheric oxygen) and the carbon dioxide are determined before and after addition of the critical reagent (either the catalyst, the oxidizing agent or the carbohydrate). The difference values represent the oxygen consumption and the carbon dioxide formation attributable to the reaction in question.

The gasometric measurements were conducted in the Van Slyke manometric apparatus, with an accessory calibrated flask of 60 ml capacity. The latter was fitted with a glass stopcock, a measuring cup, and a side

[1] Assisted by a grant from the Research Appropriation of the University of Oklahoma School of Medicine.

tube attached by pressure tubing to the side arm of the manometric apparatus. The typical procedure was as follows: 3 ml of the carbohydrate solution and 1 ml of a solution of the catalytic salt were placed in the flask. The system was closed and evacuated approximately to the 130 mm graduation of the Van Slyke scale, and the pressures were read with the meniscus at the 2 ml level, and again at the 50 ml level. Subsequently, 2 ml of hydrogen peroxide solution were introduced into the flask through a water seal, and the solution was allowed to react during the desired interval. When necessary, excess peroxide was converted to oxygen by introducing 2 ml of a dialyzed manganese dioxide sol, and agitating for several minutes. One ml of N sulfuric acid solution was added through a water seal to release the carbon dioxide, and pressure readings were made as before. Then, 2 ml of N potassium hydroxide solution were introduced into the Van Slyke chamber, the carbon dioxide was absorbed, and a third set of pressure readings was made. In the range used (175 to 400 mm), a correction of +23 mm was applied to the third set of readings, in order to compensate for the effect of the aqueous column above the mercury. Corrections were also applied for the oxygen content of the air above the solution in the flask, and for the gases in the original solutions. All solutions were carefully prepared from boiled distilled water. The calculations were based upon the assumption that, at the pressures used, the gases followed the ideal equation of state.

In applying the method to the iron-catalyzed oxidation of carbohydrates by hydrogen peroxide, it was convenient to employ ferric chloride as the catalyst. The oxygen consumption involved in the oxidation of ferrous iron was thus avoided, and the hydrogen peroxide was completely decomposed within 30 minutes, so that the use of manganese dioxide could be omitted. A series of determinations on mixtures of 0.5615 mM *d*-gluconolactone, 0.05615 mM ferric chloride and 1.123 mM hydrogen peroxide, showed that 0.141 mM O_2 was liberated, 0.42 mM O_2 was used, and 0.034 mM carbon dioxide was formed in 30 minutes at 25° C. The formation of the determined 34 per cent of reducing material (22 per cent *d*-arabinose and 12 per cent keturonic acid and other keto derivatives) should entail an oxygen consumption of 0.0955 mM and a carbon dioxide formation of 0.1235 mM. The extra 0.3245 mM oxygen consumption found, plus 0.0447 mM O_2 corresponding to the carbon dioxide deficit, or a total of 0.3692 mM, could oxidize that fraction of the gluconolactone which was not converted to reducing substances, to dicarboxylic acids. Either the 66 per cent could be oxidized entirely to saccharic acid, or more probably, 22 per cent would remain unoxidized, and the remaining 44 per cent of the gluconolactone could be fragmented to dicarboxylic and monocarboxylic acids. According to the classical idea of decarboxylation, the formation of 22 per cent arabinose should yield 0.1235 mM carbon dioxide. Since only 0.034 mM actually appeared, reduction of carbonic to formic acid, or a similar dismutation between other carbohydrate fragments, is indicated. The presence of formic and other short-chain acids, which could be reduced to aldehydes by metallic magnesium in cold acid solutions, was detected in copper sulfate-calcium hydroxide filtrates. Determinations of these aldehydes by Schiff's reagent revealed the approximate quantities estimated from gasometric analyses. When the concentration of hydrogen peroxide in the oxidation mixture was doubled, carbon dioxide formation increased to 0.21 mM, and oxygen consumption to 0.73 mM. With four times the original quantity of hydrogen peroxide, only traces of carbon dioxide appeared. Similar studies have been made of the iron-catalyzed oxidation of *d*-glucose and of *d*-mannitol. Considerable oxygen was used in both instances; *d*-glucose yielded less carbon dioxide than *d*-gluconolactone, while *d*-mannitol gave only traces.