THE DECOMPOSITION OF HYDROGEN PEROXIDE BY BLOOD. GEORGE SENTER'S DISCOVERY OF THE ENZYME INVOLVED

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Discovery of Hydrogen Peroxide and its Catalytic Decomposition

Figure 1. *Jacques Thénard (1777-1857)*

Hydrogen peroxide was discovered in 1818 by Louis Jacques Thénard (Fig. 1), professor at the Collège de France (1). He was examining the action of various acids on barium peroxide and at first thought that he had made modified forms of the acids. Eventually he concluded that the "eau oxygènée" was a new compound that contained more oxygen than water. The addition of various substances, including blood, caused the new compound to decompose, with evolution of oxygen.

The term *catalysis* was coined in 1835 by Jöns Jakob Berzelius to characterize the promotion of chemical reactions by a substance that appears to remain unchanged (2). As an example, he mentioned the decomposition of hydrogen peroxide. Since then, catalysis has been studied by many others, especially Wilhelm Ostwald, whose nomination for the 1909 Nobel Prize for Chemistry was largely based on his work in this field.

In 1863 Christian Friedrich Schönbein (Fig. 2), professor at Basel, found that various other animal and plant extracts, as well as blood, also cause the decomposition of hydrogen peroxide (3). He concluded that *enzymes*, the natural catalysts contained in the additives, were responsible for this effect. Unlike metallic catalysts, enzymes could be deactivated by heating. Certain substances, now commonly termed "catalyst poisons," were found to interfere with enzyme activity.

George Senter

While Ostwald was Professor of Physical Chemistry at the University of Leipzig, he assigned studies that involved catalysis to a number of his English-speaking students. One such recipient was the Scotsman George Senter, who was born in Kildrummy on January 25, 1874. Following primary education, he studied pharmacy in London and graduated from the University of London in 1900. He then entered Ostwald's laboratory, aiming to bring order to a topic with a long history: the

decomposition of hydrogen peroxide by blood. Obviously, Senter had to review the numerous studies that had been carried out during the long time interval since the discovery of the decomposition. He devoted more than one quarter of his very long paper to a survey of catalysis, especially of the catalytic decomposition of hydrogen peroxide by blood and by other substance (4).

Enzyme Action

Other workers had noted that the action of an enzyme is often limited to one or one type of reaction and that the temperature of deactivation varies with the particular enzyme (5, 6). The demonstration that an extract from a living cell can possess enzyme activity dismissed the earlier view that enzyme action required living cells themselves. An example is the extraction of the sugar-fermenting enzyme, zymase, from yeast cells (7). Nevertheless, Senter commented that, owing to the chemical nature of enzymes and the difficulties of their extraction, not one enzyme had been obtained in approximately pure state.

Figure 2. *Christian Friedrich Schönbein (1799-1868)*

Senter viewed the enzymes as belonging to two main classes, the first being hydrolyzing enzymes such as emulsin which, for example, breaks down the glucoside amygdalin. He pointed out that, although these enzymes had been extensively studied, enzymes of the second class, the *oxidases*, had not received much attention. Enzymes in substances that caused the decomposition of hydrogen peroxide were provisionally termed *superoxidases.*

As Berzelius had noted, inorganic substances such as metals can cause the decomposition of hydrogen peroxide. Senter termed such substances *general catalysts*. The utility of platinum as a catalyst was greatly enhanced by the introduction of colloidal platinum by Georg Bredig in 1898 (8). Bredig was a member of Ostwald's staff, so Senter naturally studied the catalysis of hydrogen peroxide both by enzyme preparations and by platinum sol.

Preliminary Experiments

Preliminary experiments on the decomposition of hydrogen peroxide were made, with either defibrinated ox blood or commercial hemoglobin as the catalyst. The results confirmed many of the observations of earlier workers, who had followed the progress of the reactions by measurement of the evolution of oxygen. Because of sources of error such as possible supersaturation, Senter decided to measure the amount of hydrogen peroxide present at any time by titrating it at ice temperature with potassium permanganate solution. Dilute solutions and a low temperature minimized the destruction of hemoglobin by the peroxide.

In a typical experiment, 100 mL each of 0.1 percent hemoglobin solution and 0.01 M hydrogen peroxide were mixed. Samples for titration were withdrawn at timed intervals. Even after 12 hours, only about 3% of the peroxide had been decomposed. When blood solution, corresponding to approximately 1 part in 7,000 with respect to hemoglobin, replaced the hemoglobin solution, all of the hydrogen peroxide had decomposed in about 5 minutes. These results, which showed that hemoglobin itself possesses less than one ten-thousandth of the activity of blood, led Senter to seek the active enzyme in blood.

Isolation of the Enzyme

Various attempts at isolation, including fractional precipitation by sodium chloride or other salts, were unsuccessful. Senter then tried precipitation by ethanol. He had found that the precipitation of hemoglobin from 50% ethanol was negligible. Therefore hemoglobin should stay in solution when equal volumes of blood solution and of 99% ethanol were mixed. The red-brown precipitate that formed was washed with dilute ethanol to remove traces of hemoglobin, then the solid was dried in vacuum. The powdered solid was mixed with water

and left in an ice chest for 1-2 days to extract the enzyme. Filtration gave a slightly yellowish solution that had strong catalytic properties and was stable for weeks at 0° C. At Ostwald's suggestion, the enzyme in the solution was named "Hämase" (I believe that the modern name is *catalase*). Because the ash obtained from a few cc of solution did not give a red color with acidified thiocyanate solution, Senter concluded that the enzyme did not contain iron. This metal was certainly present, but at a concentration too low for detection by his method.

Dynamics of the Hämase-catalyzed Decomposition of Hydrogen Peroxide

Preliminary experiments on the rate of decomposition, carried out with diluted blood, implied that the rate of reaction was of first order with respect to the concentration of hydrogen peroxide. Senter made some measurements at temperatures up to 30° C but, having found that catalysis proceeded satisfactorily at 0° C, he carried out almost all of his subsequent experiments at this temperature.

Having demonstrated that no oxidation of Hämase occurred when the initial concentration of hydrogen peroxide was 1/80 M, he made extensive measurements with fixed amounts of Hämase and various lower concentrations of hydrogen peroxide. Consistent values for the velocity constant, K, were obtained for each of the concentration levels. However, these values depended upon the level chosen. This is indicated in Table 1, which gives the average value of K for each hydrogen peroxide (H_2O_2) concentration.

Table 1. Effect of H_2O_2 concentration on the velocity constant with fixed enzyme concentration

H_2O_2 (M)	1/106	1/126	1/290	1/440	1/460	1/1100
Constant, K	0.0192	0.0175	0.0120	0.0225	0.0188	0.0122

Senter then conducted experiments with a constant initial concentration of peroxide but with variable amounts of Hämase. For purposes of comparison, Senter used velocity constant values that were calculated for an interval in which the H_2O_2 titer fell to approximately one half. Table 2 summarizes the results applicable to the series in which the H_2O_2 concentration was approximately 1/480 M.

Table 2. Effect of relative Hämase concentration with fixed H_2O_2 concentration

Relative Hämase conc.	3:	6:	8:	24
Constant, K	0.0028	0.0058	0.0072	0.0230
Velocity/Hämase conc.	9.33	9.66	9.00	9.6

Obviously, the velocity constant increases with increasing Hämase concentration. However, within experimental error, the velocity of reaction is proportional to the concentration of enzyme. Senter concluded that, in dilute solutions, the rate of decomposition of hydrogen peroxide was proportional to the product of the respective concentrations of peroxide and of Hämase. However, this relationship did not hold for more concentrated solutions. Senter assumed that hydrogen peroxide itself exerted a retarding effect on the reaction. He introduced a correction term to allow for the retardation but could not check this quantitatively. Quoting available results that had been obtained with colloidal platinum as a catalyst (9), Senter noted that, in some respects, the phenomena observed were paralleled by those found with Hämase. In the end Robert Luther, who almost certainly supervised Senter's work, developed a more complicated relationship that satisfactorily accounted for the experimental data.

Effect of Heat and of Various Additives on the Catalytic Decomposition of Hydrogen Peroxide

Parallel experiments with Hämase, carried out at 0° C and 10° C, indicated that the temperature coefficient of the rate of decomposition was +1.50 for this 10-degree interval. Although this coefficient is smaller than in many other reactions, it is in line with +1.7, quoted for the platinum-catalyzed decomposition of hydrogen peroxide.

Senter found that, in decomposition experiments made at 65° C, the activity of blood or Hämase was completely lost in 15 minutes. In experiments at 55° C, only 5% of the original activity remained after 2 hours, while about 60% remained after a heating period of 3 hours at 45° C.

Jacobson had found that small amounts of acids hindered the catalytic decomposition of H_2O_2 (5). Senter extensively examined the very marked hindering effect of submillimolar concentrations of hydrochloric and nitric acids on the Hämase-catalyzed decomposition of

hydrogen peroxide. Results with acetic acid were similar when allowance was made for the incomplete ionization of this weak acid. Senter concluded that the hydrogen ion caused the hindrance, even though hydrogen-ion concentration had no effect in the platinum-catalyzed decomposition of peroxide (9). Following an experiment involving a slightly acidified solution of hydrogen peroxide and Hämase, Senter added sodium hydroxide to neutralize the acid and then added more hydrogen peroxide. A second decomposition could then be performed, thus demonstrating that acidification did not permanently decrease the activity of the enzyme.

Unlike the rapid response to acidification, Hämase deactivation by sodium hydroxide took several hours. However, as with acids, the activity was restored on neutralization. Compound formation between hydrogen peroxide and sodium hydroxide had been reported in 1901 (10), so that hindrance by the alkali might be modified by such formation.

Having shown that salts such as sodium chloride or potassium nitrate also caused hindrance, Senter turned to the well-known general "catalyst poisons," cyanide and aniline. Even at micromolar levels, hydrocyanic acid exerted a hindering effect on the catalysis by Hämase. Senter attributed the smaller effect, when blood was the catalyst, to the binding of the cyanide by hemoglobin. He pointed out that the effect of HCN in catalysis by platinum was about 20 times greater than in catalysis by Hämase. Senter found that aniline has a much weaker poisoning effect than hydrocyanic acid. In experiments with blood solutions that were 2.5 millimolar with respect to aniline, half of the original peroxide remained after 16 minutes.

Senter's paper included a survey of the then prevailing views of enzyme action (4). Concerning living organisms, his provisional theory was that oxygen from the air was carried over to oxidizable substances, whereby oxides and hydrogen peroxide were formed. A reaction scheme for this had been proposed by Fritz Haber in 1900 (11). Senter concluded by summarizing the analogies that exist between catalysis by colloidal platinum and by Hämase. Both substances are colloidal in solution and catalyze the decomposition of hydrogen peroxide at comparable rates. The catalyses have small temperature coefficients and are both poisoned by HCN and aniline. A major difference is sensitivity to temperature. The activity of the enzyme is destroyed by moderate heating.

Senter's Career

Senter received his D. Phil. at Leipzig in 1903 and was appointed lecturer in chemistry at St. Mary's Hospital Medical School in London. Here he continued the work begun in Leipzig, including the study of the hindering effects on catalysis of other compounds (12). He showed

Figure 3. *George Senter (1874-1942) photo provided by B. Hudson of the Royal Pharmaceutical Society of Great Britain.*

that these effects agreed with those expected from the ionic theory and reiterated his belief that enzyme action was most simply explained by the theory proposed by Nernst (11). However, he gave reasons for supposing that this theory was not of general application in heterogeneous systems (13, 14).

Since Senter's pioneering studies, interest in enzymes and enzyme action has grown enormously. By 1992, when the 12th Collective Index of *Chemical Abstracts* appeared, about one hundred pages were devoted to these topics.

Senter's interests eventually turned to areas such as the reactivity of halogens in organic compounds and the Walden inversion. His texts on inorganic and physical chemistry ran through numerous editions. In 1913 Senter became head of the chemistry department of Birkbeck College, which later became part of the University of London. He was appointed Principal of the College in 1918 and became an active member of the University Senate (15). A tribute to his distinguished

service both to the College and to the University marked his retirement in 1939 (16). He died in Pinner, Middlesex, England, on March 14, 1942.

REFERENCES AND NOTES

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John T. Stock, born in Margate, England in 1911, died on February 6, 2005, in Storrs, CT. Professor Emeritus of Chemistry at the University of Connecticut, Dr. Stock held baccalaureate, M.Sc., Ph.D., and D.Sc. degrees from University of London. After an active career in analytical chemistry, Professor Stock turned his efforts in retirement toward the history of chemistry, with particular attention to instrumentation and the legacy of students of Wilhelm Ostwald. He made oral presentations at virtually every national American Chemical Society meeting over a 20-year period and published numerous articles in this field, many in the *Bulletin*. His last paper appears in this issue. The recipient of the Dexter Award of the History of Chemistry Division in 1992, Dr. Stock was a life member of the Royal Society of Chemistry, the Society of Chemical Industry, the Royal Institution, and a 50-year member of the American Chemical Society.