189. *The Specificity of Iron as a Catalyst for the Reaction between Hydrogen Peroxide and Pyrogallol.*

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IN the experiments now reported, the reaction between hydrogen peroxide and pyrogallol as catalysed by ferric chloride is studied under various conditions. [Other iron salts have been used as catalyst by Wolf (*Compt. rend.*, 1908, **146**, 784, 1217), Willstätter and Stoll (*Annalen*, 1921, **416**, 64), and Wieland and Franke (*ibid.*, 1927, **457**, 56).]

The main product of the reaction is a brownish dye (or mixture of dyes) soluble in water but only slightly so in ether. Purpurogallin is, at the most, produced in small proportion. If the molar extinction coefficient of the water-soluble dye is taken as of the same order of magnitude as that of purpurogallin, one can calculate (see p. 828) that about 2—5 atoms of oxygen would be required for the formation of 1 mol. of dye. According to Willstätter and Heiss (*Annalen*, 1923, **433**, 27), 3 atoms of oxygen are needed to form 1 mol. of purpurogallin.

During the first stage of the reaction (about 10 mins.) there is an approximate proportionality between the extinction coefficients observed and the iron concentration (see Table II A). It is therefore justifiable to take the extinction coefficients as a measure of the hydrogen peroxide used (cf. papers quoted by Oppenheimer, " Die Fermente und ihre Wirkungen," 5 Aufl., Bd. II, p. 1753). After longer intervals, there is no proportionality; this is connected with the fact that for a given concentration ratio pyrogallol : iron ($9 \times 10^{-2} : 9 \times 10^{-5}$ in Table II A) the colour reaches its maximum after 8—10 mins., and then remains constant for at least 2 hours, even although the greater part of the pyrogallol and the hydrogen peroxide is still unchanged; there is therefore an inhibition of the catalytic activity by the reaction product. The important point is that under the conditions specified no further oxidation of the dye to a colourless substance occurs. But this holds only if the concentration ratio pyrogallol : iron is large, otherwise a fading of the colour is observed. It is probable that the iron is converted by excess of pyrogallol into a relatively inactive complex, incapable of oxidising the dye. This assumption is also in agreement with the fact that the rate of dye formation decreases as the pyrogallol concentration is increased.

It has been observed by Bach (*Ber.*, 1905, **38**, 1878) that the enzyme catalase is inhibited in its activity by pyrogallol. As catalase is an iron compound (Zeile and Hellström, *Z. physiol. Chem.*, 1930, **192**, 171), the inhibition might here too be connected with the formation of an iron–pyrogallol complex.

The dependence of the dye formation on the concentrations of hydrogen-ion and of hydrogen peroxide and on the temperature was also investigated. It is not possible to compare the amount of hydrogen peroxide used under the different conditions, but Tables III—V indicate the minimum hydrogen peroxide consumption.

From the colorimetric measurements, it follows that an extinction coefficient of 5 cm.$^{-1}$ is produced after 10 mins. at 20° in a solution which is 9×10^{-3} and $9 \times 10^{-5}M$ with regard to pyrogallol and ferric chloride, respectively, and $0{\cdot}3N$ in hydrogen peroxide

(p_H 4·1; acetate buffer). For these conditions a hydrogen peroxide consumption of 0·015 ± 0·005 mols. per litre of solution can be calculated by estimating the hydrogen peroxide remaining after completion of the reaction (*Annalen*, 1933, **503**, 265, Table 16). One mol. of iron, therefore, causes about 0·3 mol. of hydrogen peroxide to react with pyrogallol per second, but under the same conditions it decomposes only 10^{-4} mol. of peroxide per second (*ibid.*, p. 261).

The enzyme peroxydase, which, according to Haldane (*Nature*, 1931, **128**, 175) and Kuhn, Hand, and Florkin (*Z. physiol. Chem.*, 1931, **201**, 255), contains iron in the form of a complex, has no measurable effect on the decomposition of hydrogen peroxide, although it is a very efficient catalyst of the pyrogallol–hydrogen peroxide reaction. Haber and Willstätter's theory of enzyme catalysis (*Ber.*, 1931, **64**, 2854) suggests that other iron compounds as well might be specific. The present experiments show that this is the case, a simple iron salt being about 3000 times more effective a catalyst for the hydrogen peroxide–pyrogallol reaction than for the decomposition of hydrogen peroxide.

EXPERIMENTAL.

The water used as solvent and for rinsing the vessels was twice distilled (quartz condenser). Pyrogallol was purified by sublimation, and hydrochloric and acetic acids by two distillations. The other reagents were the purest supplied by Messrs. B.D.H. The measurements of the extinction coefficients, α, were carried out in the Pulfrich Stuphenphotometer (Zeiss), filter LIII being used. The all-glass uncemented cells (Schott and Gen.) provided with ground stoppers were placed in the air-bath described elsewhere (*Nature*, 1934, **134**, 101). The temperature was constant within ±0·1°.

Relative solubility of the dye formed. 35 Cm. of a 0·09M-pyrogallol, 9 × 10^{-5} M-ferric chloride, and 0·3N-hydrogen peroxide solution ($p_H = 4·1$; acetate buffer) were kept at 23° for 2 hours: $\alpha = 0·90$ cm.$^{-1}$. The solution was then extracted three times with 50 c.c. of ether: α of aqueous solution = 0·7 cm.$^{-1}$. The ethereal extracts were concentrated until the volume was 20 c.c.: $\alpha = 0·1$ cm.$^{-1}$.

For the following experiments, solid pyrogallol, the buffer, or the hydrochloric acid and hydrogen peroxide were mixed and brought to the required temperature. At zero time, the ferric chloride solution was added, and the extinction coefficients continuously observed. Addition of ferric chloride before that of hydrogen peroxide does not affect the results.

The following tables show the effect of varying conditions on the extinction coefficients, which are given in cm.$^{-1}$ at the times stated.

TABLE I.

Purity of Water and Previous Treatment of Ferric Chloride.

$H_2O_2 = 0·26N$; $C_6H_3(OH)_3 = 0·090M$; $p_H = 4·1$ (NaOAc = 0·10M; HOAc = 0·30M); $FeCl_3 = 9·0 \times 10^{-6}M$. Temp. = 11·0°. Water: (*a*) ordinary distilled, (*b*) once, (*c*) twice, (*d*) thrice redistilled. Ferric chloride: stock solution 0·01M, prepared from anhydrous salt and stored at 20° in the dark, (*a*) for 1 hr., (*b*) for 8 hrs., (*c*) for 24 hrs., (*d*) last solution heated for 2 hrs. at 55°; after this treatment the catalytic efficiency remains constant and this solution was used in all subsequent experiments.

	Time (mins.).	(*a*).	(*b*).	(*c*).	(*d*).
Water	10	0·120	0·100	0·086	0·092
Ferric chloride	10	0·233	0·218	0·170	0·072

TABLE II.

Variations in Concentration (A) of Ferric Chloride, (B) of Pyrogallol.

(A) Conditions as in Table I. (B) $FeCl_3 = 9·0 \times 10^{-5}M$; other conditions as in Table I.

Time	(A) $FeCl_3$, mols./l.			(B) $C_6H_3(OH)_3$, mols./l.		
(mins.).	9·0 × 10^{-4}.	9·0 × 10^{-5}.	9·0 × 10^{-6}.	0·90.	0·090.	0·0090.
2	3·0	0·38	0·030	0·19	0·38	0·62
4	5·5	0·62	0·042	0·21	0·62	0·79
6	8·2	0·74	0·052	0·23	0·74	0·88
8	10·2	0·81	0·064	0·27	0·81	0·94
10	12·3	0·84	0·072	0·30	0·84	1·00
15	17·0	0·86	0·096	0·37	0·86	1·09
20	—	0·88	0·120	0·42	0·88	1·12
30	—	0·90	0·166	0·58	0·90	1·18

In a 0·0087M-pyrogallol and $9 \times 10^{-4}M$-ferric solution (other conditions as in Table I) after some time a fading of the colour first formed is observed. If, however, the pyrogallol concentration is increased to 0·09M, and the iron concentration decreased to $9 \times 10^{-5}M$, then the colour intensity observed after about 10 mins. remains constant. Under the conditions specified, α (10 mins.) = 0·84 cm.$^{-1}$ and α (240 mins.) = 0·90 cm.$^{-1}$. If, after 240 mins., the solution is made $2 \times 10^{-4}M$ with respect to ferric chloride by further addition, then a fresh increase of the extinction coefficient is observed.

TABLE III.

Effect of Hydrogen-ion Concentration.

p_H 4·6—p_H 3·6, acetate buffer (0·1M in reaction mixture); p_H 2—p_H 0, hydrogen chloride; other conditions as for Table I.

Time (mins.).			p_H.		
	4·6.	4·1.	3·6.	2·0.	0·0.
4	0·021	0·042	0·060	0·42	0·050
10	0·033	0·072	0·100	0·93	0·16
15	0·040	0·096	0·130	1·30	0·26
20	0·045	0·120	0·154	1·60	0·38
30	0·050	0·166	0·198	1·91	0·60

TABLE IV.

Hydrogen Peroxide Concentration.

$FeCl_3 = 9·0 \times 10^{-5}M$; other conditions as for Table I.

Time (mins.).		H_2O_2, N.	
	2·6.	0·26.	0·026.
10	1·33	0·81	0·77

TABLE V.

Temperature.

Conditions as for Table I.

Time (mins.).	11·0°.	25·0°.
10	0·072	0·190

The value of 5 cm.$^{-1}$ for the extinction coefficient of the solution specified on p. 826 was estimated by using the results of Tables I, II, and V. It has been found (*Annalen*, 1933, **303**, 265) that for 17·5 c.c. of 0·26N-hydrogen peroxide, 0·0087M-pyrogallol, and $9 \times 10^{-5}M$-ferric chloride solution (p_H 4·1; 20°) an extinction coefficient of 1·12 cm.$^{-1}$ corresponds to a consumption of 6×10^{-5} mols. of hydrogen peroxide. From this result, the hydrogen peroxide consumption given on p. 827 has been calculated.

It has been found that the molar extinction coefficient of purpurogallin in ether is 800 l./g.-mol./cm. for 470 mμ. This value and the hydrogen peroxide consumption as calculated above were used for the estimation of the number of oxygen atoms which are required for the formation of 1 mol. of the unknown dye (see p. 826).

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