263. *The Action of Inhibitors on the Decomposition of Hydrogen Peroxide.*

By Derek Richter.

I. *Photochemical Decomposition.*

Christiansen has suggested that, in reactions which show marked negative catalysis, the high sensitivity to inhibitors may be due to the latter acting by breaking reaction chains (Christiansen and Kramers, *Z. physikal. Chem.*, 1923, **104**, 451; Christiansen, *J. Physical Chem.*, 1924, **28**, 145; *Trans. Faraday Soc.*, 1928, **24**, 596). This suggestion has been used to explain the action of inhibitors in many gas reactions, and also in a number of reactions in the *liquid phase*, such as the autoxidation of aldehydes and of sodium sulphite; but there still remains considerable doubt as to how far this explanation is applicable to reactions in solution (Bäckström, *J. Amer. Chem. Soc.*, 1927, **49**, 1460; Alyea and Bäckström, *ibid.*, 1929, **51**, 90; Richter, *Ber.*, 1931, **64**, 1240).

It is also uncertain whether the inhibitors reduce the average chain length of a chain reaction by breaking the reaction chains when once they are started, or by preventing the initiation of the chains by some type of interaction with the heavy-metal ions or other active centres at which the chains start (Baur, *Z. physikal. Chem.*, 1932, *B*, **16**, 465; *Z. Elektrochem.*, 1928, **34**, 595). Traces of heavy-metal ions have been shown to be an important factor in many autoxidation reactions which are sensitive to the action of inhibitors (Titoff, *Z. physikal. Chem.*, 1903, **45**, 641; Reid, *Ber.*, 1930, **63**, 1920; Harrison,

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Biochem. J., 1924, **18**, 1009; Krebs, *Biochem. Z.*, 1929, **204**, 343; Wieland and Richter, *Annalen*, 1931, **486**, 226).

In the following investigation of the action of inhibitors on the *photochemical* decomposition of hydrogen peroxide, the action of heavy-metal ions can be excluded, since the reaction chains are started by the absorption of light quanta. The activated conditions of molecules produced by the action of light are similar to, if not identical with, those which must be assumed to occur in the dark reactions, so that the behaviour of the photochemical reaction towards inhibitors is also of interest in interpreting the mechanism of inhibition in the thermal reaction.

That the photochemical decomposition of hydrogen peroxide is a chain reaction has been established by a number of measurements of the quantum efficiency of the reaction (see summary by Allmand and Style, J., 1930, 606, 659). Anderson and Taylor (*J. Amer. Chem. Soc.*, 1923, **45**, 650, 1210) found that the photodecomposition was inhibited by a number of organic substances, which in general were effective owing to their acting as internal filters for the ultra-violet light; but they also found that the reaction was strongly inhibited by substances, such as alcohols and amines, which absorbed only slightly in the ultra-violet region used. They concluded that " the inhibitory power of amines is due most probably to the action of the hydroxide-ion concentration produced when an amine is dissolved in water. . . . The behaviour of alcohols is not understood."

In the present investigation it was found that the inhibition by amines was still observed when the p_H was maintained constant by the addition of phosphate buffer (p_H 6·8). The results are consistent with the view that the reaction chains are broken by deactivating collisions between chain members and inhibitor molecules, in agreement with the theory of Christiansen.

Experimental.

The hydrogen peroxide, prepared from sodium peroxide by the method of Kilpatrick, Reiff, and Rice (*J. Amer. Chem. Soc.*, 1926, **48**, 3019), was purified from traces of chloride by distillation from silver sulphate, and twice redistilled in a vacuum. The inhibitors, usually Kahlbaum specimens, were freshly recrystallised or redistilled before use. All the solutions used were made up in twice-distilled water, and special precautions were taken to keep them as far as possible free from traces of dust.

The reaction-velocity measurements were carried out in plane-sided quartz cells, 2 cm. thick, and the reaction was followed by measuring the oxygen evolved in a Haldane-type microvolumeter, to which the cells were connected by ground quartz-glass joints. Instead of the usual chromate mixture, a mixture of hot nitric and sulphuric acids was used for cleaning, since chromium salts are powerful catalysts for the decomposition of hydrogen peroxide.

As a source of ultra-violet light, a K.B.B. atmospheric-type mercury-vapour lamp was used, and by the use of a nickel chloride filter (70 g. $NiCl_2,6H_2O$ and 30 c.c. conc. HCl per litre in a 500 c.c. quartz flask; cf. Bowen, J., 1932, 2236) the light was restricted to the 300 mμ region.

M/10-Hydrogen peroxide solution containing *M*/150 Sörensen phosphate buffer p_H 6·8 was used. Control experiments showed that the addition of this buffer had little effect on the reaction velocity, and had the advantage of making the measurements more easily reproducible. The inhibitor solutions were neutralised with sulphuric acid or sodium hydroxide before addition, so the bases used were present as the sulphates, and acids as their sodium salts. All experiments were carried out at 20°. The duration of the insolation was 10 mins., and as only a small amount of hydrogen peroxide was decomposed in this time, its concentration remained nearly constant during the experiment. Vigorous shaking was required to overcome the supersaturation of the solution with oxygen.

In the experiments of Rice and his collaborators (*J. Physical Chem.*, 1927, **31**, 1352, 1507; *J. Amer. Chem. Soc.*, 1926, **48**, 3019; 1929, **51**, 1376) the reaction was found to be influenced by small traces of dust, and it was concluded that the reaction chains are normally started by the absorption of light at the surface of the particles of dust, which were assumed to be present even in highly purified specimens of hydrogen peroxide. Although their experimental data show that under certain conditions the photosensitising action of dust particles is an important factor, yet it has not been shown that the chains can start only in this way, and until further experimental evidence is available, the alternative view, *i.e.*, that most of the chains start by the direct activation of peroxide molecules through light absorption, is simpler.

The inhibitors used were selected with a view to giving low absorption coefficients in the 300 mμ region, so as to reduce the internal-filtering effect to a minimum. Control experiments in which the inhibitors were used as external filters in a quartz cell of 2 cm. thickness showed that for most of them the internal filtering was small. The figures in Table I give the approximate value of the fraction of the total inhibition which can be ascribed to the internal-filtering effect when the total inhibition is 50%.

Variation of Reaction Velocity with Inhibitor Concentration.—The inhibition did not increase linearly with the inhibitor concentration (Fig. 1), but for most of the inhibitors investigated fell off at higher concentrations according to the relation $V = U/(1 + D\beta)$, where the reaction velocity V is expressed in terms of the rate of the uninhibited reaction U, the inhibitor concentration D, and a constant β.

The curves for pyridine and aniline showed very close agreement with this relation, which was found empirically by Bäckström to hold for a number of other chain reactions, and has been given a theoretical interpretation by Baur (*Z. physikal. Chem.*, **1932**, *B*, **16**, 465). With most of the other inhibitors approximate agreement was obtained. Only the curves for glycine, which has a very low inhibitory power, and for benzyl alcohol and *n*-hexoic and -octoic acids showed marked deviation from it. With the last three compounds the velocity decreased more rapidly at higher inhibitor concentrations than is indicated by this relation.

FIG. 1.

In each experiment: M/10-H_2O_2 and M/150-phosphate buffer p_H 6·8. *Total volume,* 10 *c.c. Temperature,* 20°. *Ordinates give reaction velocity during first* 10 *minutes of reaction.*

(1) *Amyl alcohol.* (4) *Phenol.*
(2) n-*Hexoic acid.* (5) *Aniline.*
(3) ***Benzyl*** *alcohol.*

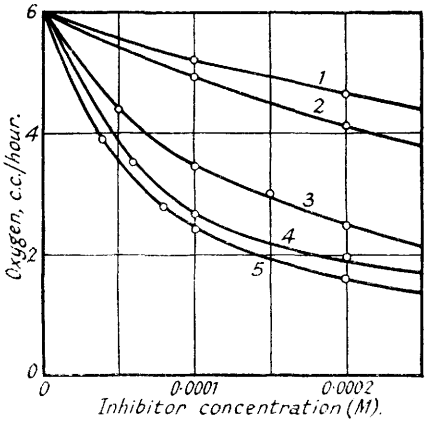


TABLE I.

Inhibitor.	Photochemical. Concn. for 50% inhibition, $M \times 10^{-4}$.	Photochemical. % Internal filtering.	Photochemical. I_{50}.	Catalytic. L. I_{50}.	Catalytic. Y. I_{50}.	Catalytic. H. I_{50}.
Methyl alcohol	22·8	—	219	< 0·1	< 0·1	< 0·1
Ethyl alcohol	16·3	—	306	< 0·1	< 0·1	< 0·1
*iso*Propyl alcohol	14·4	—	347	< 0·1	< 0·1	< 0·1
Amyl alcohol	6·24	—	802	< 3	< 3	< 3
Octyl alcohol	3·32	—	1505	<75	<75	<75
Benzyl alcohol	1·42	0·3	3520	< 2	< 2	< 2
Pyridine	1·61	1·6	3100	< 2	< 2	< 2
Piperidine	8·40	—	595	< 2	< 2	< 2
Aniline	0·72	4·8	6940	< 5	—	—
Glycine	203	—	24	< 2	< 2	< 2
Acetic acid	66·0	—	76	< 5	< 5	< 5
n-Hexoic acid	3·9	—	1280	< 5	< 5	< 5
n-Octoic acid	2·19	—	2280	< 5	< 5	< 5
Acetaldehyde	62·0	2·8	81	5·4	5·6	6·6
Phenol	0·79	0·7	6320	46	5·1	20
Resorcinol	0·70	1·7	7140	294	4·2	625
Pyrocatechol	0·94	1·3	5320	67·7	3·5	244
Salicylic acid	0·62	12·5	8200	< 3	< 3	< 3

I_{50} = Molar inhibitory power at 50% inhibition.
L = Liver catalase. Y = Yeast catalase. H = Hæmatin.

If the reaction chains started at the surface of dust particles, as Rice concluded, it might be expected that the inhibitors would act by blocking the active surface, but the experimental results show that there is no direct connexion between the inhibitory power and the surface activity of the inhibitors.

In order to express the results in a form convenient for comparison, the molar inhibitory powers of the various inhibitors at 50% inhibition (I_{50}) are given in Table I, where

$$I = \frac{\text{Fractional inhibition}}{\text{Molar inhibitor concn.}} = \frac{U - V}{U \times (\text{Molar inhibitor concn.})}$$

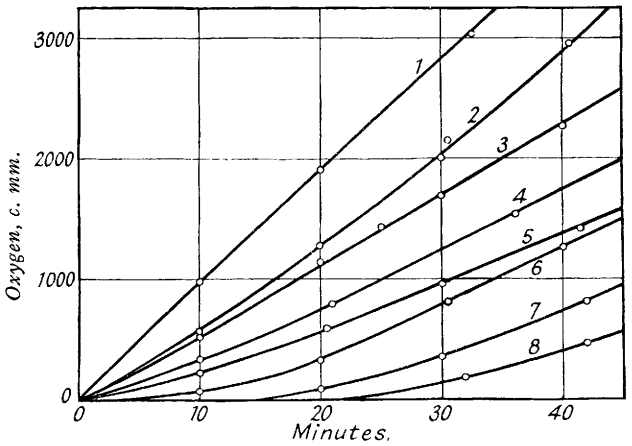
The inhibitors which contain an aromatic nucleus appear to have, in general, higher inhibitory powers than the fully saturated molecules; *e.g.*, pyridine has an inhibitory power of 3100, compared with 595 for piperidine, and benzyl alcohol is more than twice as powerful an inhibitor as octyl alcohol; but the mechanism by which the reaction chains are broken by the inhibitor molecules is as yet obscure.

Chain Length.—Quantum-efficiency measurements do not provide a method of determining the chain length in the photochemical decomposition of hydrogen peroxide, since there is reason to believe that only a small proportion of the quanta absorbed are effective in starting chains (Allmand and Style, *loc. cit.*).

In the presence of an inhibitor, the inhibited reaction velocity was observed to increase as the reaction proceeded, owing to the slow induced oxidation of the inhibitor. This is shown by the curves in Fig. 2. Whatever may be the mechanism by which the inhibitor molecules break the chains, Bäckström's work has shown that there is some justification for the assumption that for each chain broken one inhibitor molecule is destroyed. On this assumption it is possible to estimate the chain length from the inhibition data, since the number of inhibitor molecules destroyed during the reaction can be deduced from the observed increase in the reaction velocity which occurs as the reaction proceeds. The curves already discussed (Fig. 1) give the required relationship between the inhibitor concentration and the reaction velocity. A similar method of calculating the chain length has been used by Jen and Alyea (*J. Amer. Chem. Soc.*, **1933**, **55**, 575).

FIG. 2.

Experimental conditions as in Fig. 1.



(1) *Uninhibited.*
(2) 0·002*M-Methyl alcohol.*
(3) 0·00016*M-Pyridine.*
(4) 0·003*M-Ethyl alcohol.*
(5) 0·006*M*-n-*Octoic acid.*
(6) 0·0005*M-Phenol.*
(7) 0·0008*M-Resorcinol.*
(8) 0·001*M-Benzyl alcohol.*

Phenol, resorcinol, and benzyl alcohol were selected as suitable inhibitors for estimating the chain length in this way, as they have high molar inhibitory powers and a low internal-filtering effect. Further, the kinetic curves for these substances justify the assumption that the inhibitory power of any oxidation product formed in the oxidation of the inhibitor is small in comparison with the inhibitory power of the inhibitor itself.

Starting with a high initial inhibitor concentration, the reaction was allowed to proceed until the velocity had increased to 2000 c. mm. of oxygen/10 c.c./hour. The final inhibitor concentrations corresponding to this reaction velocity were obtained from the curves (Fig. 1), and are given with the calculated values for the chain length of the uninhibited reaction as follows:

Inhibitor.	Initial concn., $M \times 10^{-4}$.	Duration (mins.).	Final concn., $M \times 10^{-4}$.	H_2O_2 decomp., M.	Chain length.
Benzyl alcohol	10·0	41	2·3	0·0341	38
Resorcinol	8·0	29	1·8	0·0241	33
Phenol	5·0	17	1·6	0·0142	35

It is probable that at the commencement of the insolation, when the inhibitor concentration was high, nearly all the chains were broken by collisions with inhibitor molecules, and the number broken in collisions with the walls of the reaction vessel, etc., was negligible; but at the end of the insolation, when the reaction velocity had risen to 2000 c. mm. of oxygen/10 c.c./hour, only two-thirds of the chains were broken by collisions with inhibitor molecules. The proportion of the chains thus broken during the whole period of insolation therefore averaged about 85%, and this figure was taken in calculating the chain length.

The amount of peroxide decomposed was taken from the rate of oxygen evolution, 6000 c. mm. of oxygen/10 c.c./hour. Control experiments to test the extent of any direct oxidation of the inhibitors in the dark by the peroxide showed that this is small in comparison with the

oxidation induced by the photochemical decomposition. Although the values calculated in this way can give only a very rough estimate of the chain length, yet the closeness of the agreement for the three different inhibitors is of interest.

II. *Catalytic Decomposition of Hydrogen Peroxide.*

Haber and Willstätter have suggested that in reactions catalysed by enzymes the active surfaces may function by starting reaction chains (*Ber.*, 1931, **64**, 2844). It appeared to be of special interest to investigate whether reaction chains occur in the decomposition of hydrogen peroxide by the enzyme catalase, since the reaction is known to proceed as a chain reaction when brought about by other means.

If the enzymic reaction has a chain mechanism, it is clear that it must show the high sensitivity to inhibitors which is one of the most characteristic properties of chain reactions (cf. Schwab, Rosenfeld, and Rudolph, *Ber.*, 1933, **66**, 661). The exact chain mechanism for the photochemical decomposition has not been established, but it is probable that the activated chain carriers are the free radicals –OH and –O·OH :

$$H_2O_2^* \longrightarrow -OH + -OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$
$$H_2O_2 + -OH \longrightarrow -O{\cdot}OH + H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$
$$H_2O_2 + -O{\cdot}OH \longrightarrow -OH + H_2O + O_2 \quad . \quad . \quad . \quad . \quad . \quad (3)$$

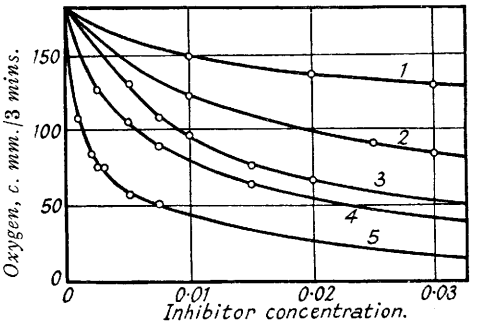
An identical mechanism for the *enzymic* decomposition would imply that the two reactions should be equally sensitive to the action of inhibitors, and should show quantitative agreement in the degrees of inhibition effected by definite inhibitor concentrations, provided that the inhibitors act primarily by breaking the reaction chains.

The decomposition catalysed by liver catalase, yeast catalase, and hæmatin was investigated, the same conditions and inhibitor solutions being used as for the photochemical decomposition. The molar inhibitory powers of the inhibitors, obtained from the inhibition curves (Fig. 3), are given for comparison with the photochemical data in Table I.

The results show that, in agreement with the conclusions of Schwab, Rosenfeld, and Rudolph, the enzymic is much less susceptible than the photochemical reaction to the action of inhibitors, and in this respect resembles more closely the catalysis by hæmatin. It must be concluded that the enzymic decomposition does not involve the formation of reaction chains of the type occurring in the photochemical decomposition.

FIG. 3.

In each experiment : M/10-H_2O_2 and M/150-phosphate buffer p_H 6·8. Total volume, 5 c.c. Temperature, 20°. The reaction velocity (c.mm. oxygen/5 c.c./min.) was measured for the first 3 minutes of the reaction.



(1) *Yeast–phenol.* (4) *Catalase–pyrocatechol.*
(2) *Hæmatin–phenol.* (5) *Catalase–resorcinol.*
(3) *Catalase–phenol.*

EXPERIMENTAL.

The reaction velocity was determined by measuring the oxygen evolved at 20° in a differential-type Barcroft apparatus (Dixon and Elliott, *Biochem. J.*, 1930, **24**, 820). 5 C.c. of the reaction mixture contained, besides the inhibitor, 0·5 c.c. of M/15-phosphate buffer, p_H 6·8, and 2·5 c.c. of 0·2M-hydrogen peroxide. The catalyst was added by means of a Keilin tube after the mixture had been shaken long enough for equilibrium to be attained.

The catalyst concentration was adjusted so that in the absence of an inhibitor the oxygen evolved in 3 mins. was approximately 180 c. mm./5 c.c. The inhibition curves were obtained by plotting the oxygen evolved during the first 3 mins. of the reaction against the inhibitor concentration (Fig. 3). For a number of the inhibitors the values of I_{50} could not be determined accurately owing to the fact that the inhibitor concentration required for 50% inhibition was so high as to exceed the solubility of the inhibitor or interfere with the p_H. For these inhibitors approximate limiting values for I_{50} are given in Table I.

The three catalysts used showed marked specific differences in their sensitivity to inhibitors; *e.g.*, liver catalase was strongly inhibited by resorcinol (I_{50} **294**), whilst yeast catalase was comparatively little affected by it (I_{50} **4·2**). The inhibition curves obtained for acetaldehyde were of a different type from those obtained for the phenols, which indicated that the mechanism of the inhibition was also different. With the hæmatin system some of the inhibitors were partly oxidised during the experiment owing to the catalyst's acting as a peroxidase, but for most of the inhibitors the amount oxidised during the experiment was small.

The liver catalase was prepared from ox liver by the method of Zeile (*Z. physiol. Chem.*, **1931**, **195**, 39), and was purified by precipitation with alcohol and adsorption on calcium phosphate. The yeast suspension was prepared by treating 5 g. of baker's yeast with 97% alcohol for 15 minutes, decanting the clear liquid, and pressing the residue on filter-paper. It was then made up as a 5% suspension. The hæmatin, which was a very pure specimen prepared by the late Mr. N. U. Meldrum, was made up as a 0·1% solution immediately before use.

SUMMARY.

(1) Measurements of the inhibitory power of inhibitors in the photochemical decomposition of hydrogen peroxide have been made.

(2) The chain length of the photochemical reaction has been calculated from the inhibition data.

(3) The inhibitory power of inhibitors in the enzymic decomposition of hydrogen peroxide catalysed by liver catalase, yeast catalase, and hæmatin has been measured.

A comparison of the action of inhibitors in the photochemical and enzymic reactions showed that the enzymic reaction is relatively insensitive to the action of inhibitors, and therefore does not involve the formation of reaction chains of the type that occur in the photochemical decomposition.

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