# THE INFLUENCE OF CERTAIN INORGANIC SALTS ON THE GERMICIDAL ACTIVITY OF HYDROGEN PEROXIDE

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Received for publication August 12, 1929

Several investigators have noted during recent years that the decomposition of hydrogen peroxide is accelerated by the presence of certain inorganic salts. The results of earlier investigations have been summarized by Bohnson (1921) who studied the mechanism of the decomposition of hydrogen peroxide by ferric salts, and noted that ethyl alcohol was oxidized to acetic acid by hydrogen peroxide in the presence of a ferric salt, a reaction which was quantitatively studied by Walton and Christensen (1926). Walton and Graham (1928) in a study of the oxidation of certain dicarboxylic acids showed that ferric or cupric salts increased the oxidizing power of hydrogen peroxide. Promoter action[1] in the catalytic decomposition of hydrogen peroxide has been extensively studied by Professor J. H. Walton and his students. Bohnson and Robertson (1923) and Robertson (1925) studied the promotor effect of cupric salts on the decomposition of hydrogen peroxide catalyzed by ferric salts. Robertson (1926, 1927) showed that the decomposition of hydrogen peroxide catalyzed by potassium dichromate was promoted by cobaltous or manganous salts. Walton and Graham (unpublished data) found that the oxidation of hydrazine by hydrogen peroxide was accelerated by both cupric and ferric ions, and that the cupric ions promoted the oxidation catalyzed by the ferric ions.

The germicidal activity of hydrogen peroxide is apparently

[1] A promoter in the broadest sense is a substance whose presence in relatively small amount increases the activity of a catalyst.

due to its oxidizing action, and, if this is true, its effectiveness should be materially increased by any substance which increases its oxidizing power. The present investigation was undertaken to test the accuracy of this hypothesis.

## TECHNIQUE

A modification of the Hygienic Laboratory method (1912) of determining the phenol coefficient was used in measuring the germicidal effect of the hydrogen peroxide with different concentrations of the inorganic salts. Twenty-four-hour cultures of *Es. coli* or *Staph. aureus* were used as test organisms. One-half cubic centimeter of the twenty-four-hour culture was introduced into 9.5 cc. of the disinfectant which was adjusted to a pH of 6.4. Transfers were made with a 4 mm. loop from the disinfectant to the liquid media at intervals of two and one-half minutes during the period of the tests, which were conducted at room temperature.

The medium used for this study had the following composition:

| | | |
|---|---|---|
| Liebig meat extract | 5 grams | + 1.5 per cent agar for slants |
| Pepton (Armour) | 10 grams | |
| NaCl | 5 grams | |
| $H_2O$ | 1000 grams | |

The pH of the medium was adjusted to 6.8.

In order to observe the effect of organic materials, such as carbohydrates and proteins, upon the disinfectant, the following additional experiments were made.

Sterile pieces of number two Whatman's filter paper were immersed in a twenty-four-hour broth culture of the organism and then transferred into tubes of the disinfectant. From this tube they were transferred at intervals of five minutes to a wash tube of sterile broth, and from this to another tube of sterile broth in which they were incubated for forty-eight hours.

A two per cent solution of gelatin was prepared with which the hydrogen peroxide was diluted for the experiments. The organisms were tested in the gelatin dilutions in the same manner as outlined before.

The inorganic salts used were all of C.P. quality which were

recrystallized at least once, while the hydrogen peroxide was of commercial quality. Its concentration was checked by titration with a standard solution of potassium permanganate and it was found to contain 2.5 per cent by weight of $H_2O_2$. All dilutions were made with distilled water.

## EXPERIMENTAL

### *Hydrogen peroxide alone*

The germicidal activity of hydrogen peroxide on *Es. coli* and *Staph. aureus* was determined in accordance with the method described. The results are summarized in table 1. The data

TABLE 1

*Germicidal activity of hydrogen peroxide on Es. coli or Staph. aureus*

| DILUTION OF 2.5 PER CENT $H_2O_2$ WITH WATER | TIME IN MINUTES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 | 30 | 45 |
| 1/1 | + | − | − | − | − | − | − | − | − | − |
| 1/2.5 | + | + | + | + | − | − | − | − | − | − |
| 1/5 | + | + | + | + | + | − | − | − | − | − |
| 1/7.5 | + | + | + | + | + | ± | − | − | − | − |
| 1/10 | + | + | + | + | + | + | − | − | − | − |
| 1/20 | + | + | + | + | + | + | + | − | − | − |
| 1/30 | + | + | + | + | + | + | + | + | + | + |

+ indicates growth, − indicates absence of growth.

given in this and all subsequent tables represent the average of several checks.

When either *Es. coli* or *Staph. aureus* was treated with a one to one dilution of the stock solution of hydrogen peroxide, the organisms were killed within a period of five minutes. Higher dilutions of the hydrogen peroxide decreased the toxicity very markedly until one part of hydrogen peroxide to thirty parts of water exhibited no toxicity during a period of forty-five minutes.

### *Hydrogen peroxide with cupric and ferric sulphates*

The combined effect of ferric and cupric sulphates on the germicidal activity of hydrogen peroxide was determined when the

concentration of each salt was equivalent to 0.1 of a millimol of the metallic ion per 120 cc. of solution. The results are given in table 2 and figure 1. The effect of the ferric and cupric ions is pronounced. In their presence, a dilution of the hydrogen peroxide of 1–50 is equivalent in toxicity to a dilution of 1–1 of hydrogen peroxide alone.

The concentrations of both the ferric and cupric ions were varied and their effect determined. The optimum concentrations of ferric and cupric ions were found to be 0.1 of a millimol per

TABLE 2

*The effect of cupric and ferric sulphates on the germicidal activity of hydrogen peroxide on Es. coli or Staph. aureus*

Concentration of salts—$Fe^{+++}$ 0.1 millimol per 120 cc.; $Cu^{++}$ 0.1 millimol per 120 cc.

| DILUTION OF 2.5 PER CENT $H_2O_2$ WITH WATER | TIME IN MINUTES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 | 30 | 45 |
| 1/50 | ± | − | − | − | − | − | − | − | − | − |
| 1/100 | + | + | ± | − | − | − | − | − | − | − |
| 1/200 | + | + | + | + | − | − | − | − | − | − |
| 1/300 | + | + | + | + | + | − | − | − | − | − |
| 1/325 | + | + | + | + | + | + | − | − | − | − |
| 1/350 | + | + | + | + | + | + | + | + | − | − |
| 1/375 | + | + | + | + | + | + | + | + | + | + |
| $Fe_2(SO_4)_3$ alone | + | + | + | + | + | + | + | + | + | + |
| $CuSO_4$ alone | + | + | + | + | + | + | + | + | + | + |
| $Fe_2(SO_4)_3 + CuSO_4$ | + | + | + | + | + | + | + | + | + | + |

120 cc. of solution. When present in concentrations of 0.01 of a millimol per 120 cc. of solution, their influence had entirely disappeared. Concentrations of 0.25 of a millimol per 120 cc. of solution gave a slight increase in toxicity over hydrogen peroxide alone. When the concentrations of both ions were increased, the toxicity of the peroxide gradually diminished. At concentrations of 0.1 molar (100 millimolar), both the ferric sulphate and cupric sulfate alone were toxic to *Es. coli* and *Staph aureus.*

In a concentration of 0.1 millimol per 120 cc. of solution, neither ferric sulfate nor copper sulfate, used singly with hydrogen peroxide, was effective in increasing its toxicity.

In order to test the hydrogen peroxide under conditions which would more nearly approximate those which it would meet in actual use, the filter paper test and the gelatin dilution tests

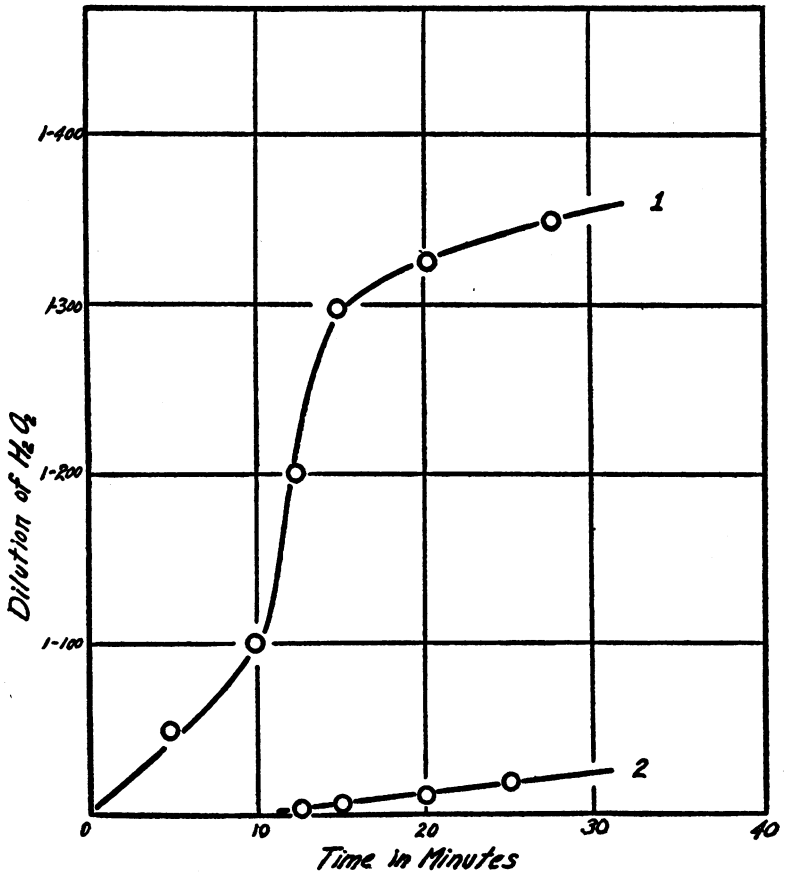


FIG. 1. GERMICIDAL ACTIVITY ON ESCHERICHIA COLI
*1*, $H_2O_2$ plus $Fe^{+++}$ and $Cu^{++}$; *2*, $H_2O_2$ alone

were made. The results are summarized in tables 3 and 4. In the filter paper tests it was observed that the carbohydrate material had little effect upon the toxicity of the hydrogen peroxide for either organisms. The gelatin dilution tests showed

that the germicidal activity of the hydrogen peroxide was destroyed in part by the protein material.

*Hydrogen peroxide with potassium dichromate and manganous or cobaltous sulphates*

The influence of potassium dichromate promoted by manganous sulphate or cobaltous sulphate on the germicidal activity

TABLE 3

*Filter paper tests on Escherichia coli and Staphylococcus aureus*

Concentration of salts—$Fe^{+++}$ 0.1 millimol per 120 cc.; $Cu^{++}$ 0.1 millimol per 120 cc.

| DILUTION OF 2.5 PER CENT $H_2O_2$ WITH WATER | ESCHERICHIA COLI | | | STAPHYLOCOCCUS AUREUS | | |
|---|---|---|---|---|---|---|
| | 5 minutes | 10 minutes | 15 minutes | 5 minutes | 10 minutes | 15 minutes |
| 1/50 | + | − | − | + | − | − |
| 1/100 | + | − | − | + | ± | − |
| 1/200 | + | + | + | + | + | − |
| 1/300 | + | + | + | + | + | + |

TABLE 4

*Gelatin dilution tests on Es. coli or Staph. aureus*

Concentration of salts—$Fe^{+++}$ 0.1 millimol per 120 cc.; $Cu^{++}$ 0.1 millimol per 120 cc.

| DILUTION WITH GELATIN SOLUTION | TIME IN MINUTES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 | 30 | 40 |
| 1/50 | + | + | + | + | − | − | − | − | − | − |
| 1/100 | + | + | + | + | + | + | − | − | − | − |
| 1/200 | + | + | + | + | + | + | + | − | − | − |
| 1/300 | + | + | + | + | + | + | + | + | + | + |

of hydrogen peroxide was also studied and found to be comparable to the combined effect of cupric and ferric sulphates. The increase of toxicity which resulted was practically the same in the higher concentrations of the hydrogen peroxide as in the case of the iron and copper catalysis, but was considerably lower as the concentration of the hydrogen peroxide was decreased. The results are tabulated in tables 5 and 6.

TABLE 5

*The effect of cobaltous sulphate and potassium dichromate on the germicidal activity of hydrogen peroxide on Es. coli*

Concentration of salts—$(Cr_2O_7)^{--}$ 0.1 millimol per 120 cc.; $Co^{++}$ 0.1 millimol per 120 cc.

| DILUTION OF 2.5 PER CENT $H_2O_2$ WITH WATER | TIME IN MINUTES | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 | 30 | 45 | 60 |
| 1/50 | ± | − | − | − | − | − | − | − | − | − | − |
| 1/100 | + | + | − | − | − | − | − | − | − | − | − |
| 1/200 | + | + | + | + | + | + | − | − | − | − | − |
| 1/300 | + | + | + | + | + | + | + | + | + | + | + |
| $CoSO_4$ alone | + | + | + | + | + | + | + | + | + | + | + |
| $K_2Cr_2O_7$ alone | + | + | + | + | + | + | + | + | + | + | + |

TABLE 6

*The effect of manganous sulfate and potassium dichromate on the germicidal activity on hydrogen peroxide on Es. coli*

Concentration of salts—$(Cr_2O_7)^{--}$ 0.1 millimol per 120 cc.; $Mn^{++}$ 0.1 millimol per 120 cc.

| DILUTION OF 2.5 PER CENT $H_2O_2$ WITH WATER | TIME IN MINUTES | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 | 30 | 45 | 60 |
| 1/50 | + | + | − | − | − | − | − | − | − | − | − |
| 1/100 | + | + | + | − | − | − | − | − | − | − | − |
| 1/200 | + | + | + | + | + | + | − | − | − | − | − |
| 1/300 | + | + | + | + | + | + | + | + | + | + | + |
| $MnSO_4$ alone | + | + | + | + | + | + | + | + | + | + | + |
| $K_2Cr_2O_7$ alone | + | + | + | + | + | + | + | + | + | + | + |

TABLE 7

*The effect of phenol on Es. coli and Staph. aureus*

| DILUTION OF PHENOL | TIME IN MINUTES | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Escherichia coli | | | | | | Staphylococcus aureus | | | | | |
| | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 |
| 1/60 | + | − | − | − | − | − | − | − | − | − | − | − |
| 1/70 | + | + | − | − | − | − | + | − | − | − | − | − |
| 1/80 | + | + | + | − | − | − | + | + | − | − | − | − |
| 1/90 | + | + | + | + | − | − | + | + | + | − | − | − |
| 1/100 | + | + | + | + | + | − | + | + | + | + | − | − |
| 1/110 | + | + | + | + | + | + | + | + | + | + | + | − |

### *Phenol Coefficients*

After the germicidal activity of phenol was determined (table 7) the phenol coefficients were calculated in accordance with the method formulated by Reddish (1927)

$$\text{Phenol Coefficient} = \frac{\text{Dilution of } X \text{ which will kill in 10 minutes but not in 5 minutes}}{\text{Dilution of phenol which will kill in 10 minutes but not in 5 minutes}}$$

Using this formula the following phenol coefficients were secured with *Es. coli:*

| | |
|---|---|
| Hydrogen peroxide alone | 0.014 |
| Hydrogen peroxide plus ferric and cupric sulphates | 1.4 |
| Hydrogen peroxide plus potassium dichromate and cobaltous sulphate or manganous sulphate | 1.4 |

With *Staph. aureus:*

| | |
|---|---|
| Hydrogen peroxide alone | 0.012 |
| Hydrogen peroxide plus ferric and cupric sulphate | 1.2 |

SUMMARY

1. The germicidal activity of hydrogen peroxide on *Es. coli* and *Staph. aureus* is greatly increased by the combined presence of ferric and cupric sulfates.

2. The optimum concentrations of ferric and cupric sulfates were found to be approximately one tenth of a millimol of each of the metallic ions per one hundred and twenty cubic centimeters of solution.

3. The influence of these salts on the toxicity of hydrogen peroxide is not altered by the presence of cellulose, but is slightly decreased in the presence of protein matter.

4. Potassium dichromate when promoted with manganous sulfate or cobaltous sulfate has been found to increase the toxicity of hydrogen peroxide for *Es. coli* approximately as much as the ferric and cupric sulfates. The effects of potassium dichromate with manganous sulfate and potassium dichromate with cobaltous sulfate have been measured at only one concentration, namely one-tenth of a millimol of the dichromate and metallic ion per one hundred and twenty cubic centimeters of solution.

5. The phenol coefficients of hydrogen peroxide when measured in relation to *Es. coli* and *Staph. aureus* were 0.014 and 0.012 respectively, but in the presence of the optimum concentrations of cupric and ferric sulphates they were increased to 1.4 and 1.2; while 0.1 of a millimol of dichromate ions in 120 cc. of solution in the presence of the same concentration of cobaltous or manganous ions increased the phenol coefficient of hydrogen peroxide measured on *Es. coli* to 1.4.

6. Since the salts which catalyze and promote the decomposition and increase the oxidizing property of hydrogen peroxide all increase the germicidal activity of hydrogen peroxide, it seems logical to conclude that the toxicity of hydrogen peroxide is dependent upon its ability as an oxidizing agent.

The writers desire to express their appreciation to Professor J. H. Walton for suggesting the problem involved in this investigation.

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