40° was observed. The decreased activity above 40° is believed to result from two factors, a slowing of the reaction independent of enzyme destruction, and destruction itself, first found to occur at 50°. The course of the reaction was not appreciably affected by a change in temperature from 25 to 40°. Temperature coefficients and constants for the action of this enzyme were calculated.

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## Germicidal Action of Bromine.

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The halogens, fluorine, chlorine, bromine, and iodine are strong oxidizing agents which combine readily with protoplasm. Chlorine is widely used as a disinfectant in water-treatment while iodine is a much used skin-disinfectant. Fluorine is probably too active to use in disinfection. Less work has been done with bromine, which strongly resembles chlorine in its general properties. Review of the literature gives little information on relative resistance of pure cultures of microörganisms to bromine under known conditions. Beckwith and Moser[1] reported that chlorine, bromine, and iodine were about equally effective toward *Escherichia coli,* that is, 0.2 parts per million of each halogen showed 95 to 97% efficiency in 60 minutes. Wood and Illing[2] found that after 5 minutes, water containing *Escherichia coli* was sterile in presence of 0.15, 0.35, and 0.45 ppm. Henderson[3] patented a device for "antisepticizing" water with bromine. Hildesheim[4] used bromine for treatment of swimming-pool water. Bromine has also been used as a seed-disinfectant by DeZeeuw,[5] Wilson,[6] Matskov,[7] and LaRue.[8] Other work has been

1 Beckwith, T. D., and Moser, F. R., *J. Am. Water Works Assn.,* 1933, **25**, 267.

2 Wood, R. D., and Illing, E. T., *Analyst,* 1930, **55**, 126.

3 Henderson, C. T., U. S. Patent, 1935, 1,995,639.

4 Hildesheim, H., *Tech. Gemeindeblatt,* 1936, **39**, 56.

5 DeZeeuw, R., *Exp. Sta. Rec.,* 1912, **26**, 819.

6 Wilson, J. K., *Am. J. Bot.,* 1915, **2**, 420.

7 Matskov, F., Physiol. Untersuch. Zuckerrübe Erste Artikelserie, Ukrainisches Inst. Angew. Bot. Sect. Pflanzenphysiol. Charkiv 156-190, 1930.

8 La Rue, C. D., *Science,* 1937, **85**.

carried out by Regel,[9] Shuhmacher,[10] Friedenthal,[11] Rupp, Wegner, and Maiss.[12]

The organisms used in this investigation are indicated in the tables. The germicidal value of bromine is expressed in ppm required to kill a suspension of the organisms under controlled experimental conditions. The smallest amount of bromine required to kill each organism within a certain time is the minimal bromine death-point. A stock saturated solution of bromine was prepared

TABLE I.
Amount of Free Bromine Required to Kill Spores of Aërobic Spore-forming Bacteria in 30 Seconds.

| Organism | Parts per million of Bromine | |
|---|---|---|
| | pH 3.5-4.0 | pH 6.8-7.2 |
| *Proteus vulgaris* | 40-60 | 170 |
| *Bacillus megatherium* | 28-35 | 110 |
| *" mesentericus* | 180-300 | over 450 |
| *" subtilis* (565) | 160-200 | over 450 |
| " " (566) | 170-220 | over 450 |

TABLE II.
Amount of Free Bromine Required to Kill Mold-spores, Yeasts, Non-spore-forming Bacteria in 15 to 30 Seconds, at Room Temperature.

| Organism | Bromine ppm |
|---|---|
| Kill mold-spores: | |
| *Aspergillus niger* | 28 |
| " *sp.* | 25 |
| *Oöspora lactis* | 8 |
| *Mucor sp.* | 30 |
| *Penicillium sp.* | 1-5 |
| Yeasts: | |
| Cryptococcus | .3 |
| Mycoderma | .5 |
| *Endomyces albicans* | * |
| *Monilia albicans* | .25 |
| *Saccharomyces sp.* | 3.0 |
| Non-spore-forming bacteria: | |
| *Staphylococcus aureus* (92) | .15 |
| " *albus* (76) | .25 |
| " *sp.* (80) | .20 |
| " *aureus* (77) | .15 |
| " " (79) | .10 |
| " " (89) | .15 |
| *Escherichia coli* (252) | .15 |
| " " (251) | .15 |
| *Eberthella typhosa* (377) | >.03 |
| " " (378) | .06 |

*Results inconsistent.

9 Regel, *Deut. Militärarztl. Z.*, 1914, **43**, 55; *Chem. Zentr.*, 1914, **1**, 581.

10 Shuhmacher, *Schweig. Apoth. Ztg.*, 1915, **53**, 281.

11 Friedenthal, H., Brit. Patent 215,016, 1923.

12 Rupp, E., Wegner, W., and Maiss, P., *Arch. Pharm.*, 1924, **262**, 2.

before each experiment. A suspension of the microörganism under study was treated with the bromine solutions in 50 cc quantities and counts of viable organisms made at regular intervals. A suspension in water was counted as a control at the same time. One cc of the inoculated bromine water was plated at intervals of 30 seconds, 1, 2, 3, and 5 minutes. A small amount of sodium-thiosulfate solution was previously added to the dish to stop the action of the bromine.

Acidity was found to be important. Bromine was more active at a pH 3.5-4.0. When the reaction was changed to neutrality much more bromine was required to kill the organisms. The results with aërobic spore-bearing bacteria are shown in Table I.

Aërobic spore-forming bacteria are almost as resistant to bromine as they are to chlorine. They are also much more resistant to bromine than are non-spore-forming bacteria, mold-spores and yeasts.

Mold-spores are quite resistant to bromine. They survived exposure to concentrations that readily killed yeasts and non-spore-forming bacteria.

*Conclusions.* Bromine is shown to be an effective germicidal agent against non-spore-forming microörganisms. Spore-forming bacteria are much more resistant. The element has marked specificity for *Escherichia coli* and *Eberthella typhosa*. Mold-spores were more resistant than yeasts or non-spore-forming bacteria.