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Effect of Oxidation-Reduction on Germicidal Efficiency of Some Metallic Salts.

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It is well known that the inorganic ferrous and ferric salts, when tested individually, exhibit very little or no germicidal activity against *Eberthella typhosa* and *Staphylococcus aureus*. However, if an oxidized and a reduced salt, such as ferric and ferrous chlorides, are mixed to form an oxidation-reduction system a pronounced germicidal action occurs.

The salt pairs cannot be combined haphazardly but must be mixed in certain definite proportions for maximum germicidal activity to occur. In the case of the chlorides the most effective germicidal action occurred when the salts were mixed in the proportion of 2 moles of ferric chloride and one mole of ferrous chloride. Using a mixture of the 2 salts in this proportion a dilution of 1:100 was germicidal against *Staphylococcus aureus* in 10 min at 37°C.

The sulfates of the 2 iron salts behaved in a similar manner, indicating that the positive radical is the effective portion of the molecule. In this case the most effective combination was in the proportion of one mole of each salt. The results are given in Table I.

TABLE I.

Effectiveness of Ferrous and Ferric Salts Used Singly and in Combinations Against *Staphylococcus aureus.*

Salt	Killing dilution in 10 min at 37°C*
$FeCl_2$	1:10 failed to kill
$FeCl_3$	1:25
$FeCl_2 + 2FeCl_3$	1:100
$FeSO_4$	1:10 failed to kill
$Fe_2(SO_4)_3$	1:30
$FeSO_4 + Fe_2(SO_4)_3$	1:75

*Dilutions of the combination of chlorides were prepared by mixing 1 g mole of $FeCl_2$ (198.8 g) and 2 g moles of $FeCl_3$ (540.6 g). A 1:100 dilution means 1 g of the mixture diluted to 100 cc with distilled water.

The most effective combination of the sulfates was 1 g mole of each salt.

Continuing with this same line of reasoning it was found that salts of tin and manganese used singly and in combination gave results similar to the iron compounds. The results of tests on the tin salts are given in Table II. It may be seen that stannous and stannic chlorides tested individually exhibited only a mild germicidal action whereas a more pronounced killing effect occurred when the two salts were mixed in proper proportion.

The germicidal effect is not limited to salts having a common metallic ion. Salts of different metallic cations and anions may be crossed with the same general effect. The important point is to have an oxidation-

TABLE II.

Effectiveness of Stannous and Stannic Chlorides Used Singly and in Combination Against *Staphylococcus aureus.*

Salt	Killing dilution in 10 min at 37°C*
$SnCl_2$	1:40
$SnCl_4$	1:50
$SnCl_2 + SnCl_4$	1:140

*The most effective combination of the salts was 1 g mole of each.

TABLE III.

Effectiveness of Various Metal Salts Tested Singly and in Combinations Against *Staphylococcus aureus.*

Salt	Killing dilution in 10 min at 37°C*
$FeCl_2 + SnCl_4$	1:130
$FeCl_3 + SnCl_2$	1:120
$AgNO_3$	1:100
$AgNO_3 + 2Fe(NO_3)_3 + FeSO_4$	1:800 of silver nitrate
$HgCl_2$	1:16,000
$HgCl_2 + FeSO_4$	1:40,000 of mercuric chloride
$MnSO_4$	1:10 failed to kill
$MnSO_4 + SnCl_4$	1:120
$MnSO_4 + Fe_2(SO_4)_3$	1:100

*The salts in the various combinations were mixed in the proportions given in the table. Unless otherwise noted the dilutions refer to the mixtures.

reduction system for increased germicidal efficiency to occur. The results of tests on a number of metallic salts used singly and in combinations against *Staphylococcus aureus* are given in Table III. It may be seen that in every case a mixture of two salts, one in a higher state of oxidation than the other, resulted in an increased germicidal action over that of the constituent salts tested singly.

The increased killing power of the combinations is essentially a function of the positive metallic ions. A mixture of sodium sulfite and sodium sulfate exhibited no increased action over that of the same salts tested singly.

The results indicate that the phenomenon is a general one. There was not a single exception to the rule that 2 salts mixed to produce an oxidation-reduction system increased markedly the germicidal efficiency of the same salts when tested individually.

Summary. Inorganic metallic salts, which are not germicidal or only slightly so, may exhibit a pronounced killing effect when mixed in certain combinations and proportions. In order that increased germicidal action may occur the salts must be mixed to produce an oxidation-reduction system. The phenomenon is a function of the positive metallic ions; the negative ions apparently play no part in the reaction.

In a later paper it will be shown that the phenomenon is not limited to inorganic compounds only but may be applied to mixtures of inorganic salts and organic compounds.

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A Rapid Test for the Activity of Certain Antibiotic Substances.

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The need for a rapid assay of activity of antibiotic substances was emphasized during investigations on such substances. Such an assay of the substrate of mold growth or of chemical fractions would greatly facilitate work.

In working with aspergillic acid, active crystalline material isolated from a strain of *Aspergillus flavus*,[1,2] an antibacterial serial dilution test with *Streptococcus pyogenes* was used. To replace this test, it was thought that aspergillic acid might interfere rapidly with the luminescence produced by luminescent bacteria to a degree which could be directly correlated with the antibacterial activity. Accordingly, a culture of *Photobacterium fischeri* was obtained from the National Type Collection. This culture grew well at room temperature in ocean water with 0.2% peptone or on artificial sea water,* viz.:

NaCl	26.7 g
KCl	0.71 g
$CaCl_2 \cdot 2H_2O$	1.52 g
$MgCl_2 \cdot 6H_2O$	5.11 g
$MgSO_4 \cdot 7H_2O$	6.81 g
Distilled water	1000 ml

again with 0.2% peptone. On such fluid media faint luminescence was visible in 6 hr and maximal luminescence in about 24.

It was soon found that this antiluminescent test was applicable for the assay of aspergillic acid, but not for penicillin. The work here reported deals largely with aspergillic acid.

For the test 0.5 ml of sea water media was placed in a series of 13 x 100 mm tubes. To the first was added 0.5 ml of the solution to be tested. The contents of this tube were mixed and 0.5 ml transferred to the next, the process being continued to give 2-fold dilutions. To each tube was now added 0.5 ml of an undiluted 24-hr culture of

[1] White, E. C., *Science*, 1940, **92**, 127.

[2] White, E. C., and Hill, J. H., *J. Bact.*, in press.

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