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.

* We express our gratitude to Dr. W. H. Cole of Rutgers University for furnishing this formula.

Photobacterium fischeri and the mixtures shaken. The tubes were again shaken just before the test was read to oxygenate and produce maximal luminescence. Readings were made in the dark-room with the dark-adapted eye and the degree of luminescence recorded as from 1+ to 4+. A solution of purified aspergillic acid with 256 units per mg was always included and the results of all unknowns were read in comparison with this standard. Tests have proven clear cut, and, except in certain early filtrates, there was at most only one tube between full luminescence and complete blacking out.

TABLE I.

Comparison of Antiluminescent and Antibacterial Activities of Various Antibiotic Substances.

Substance	Smallest amount in γ showing activity	
	Antiluminescent test	Antibacterial test using 1,500,000 *Streptococcus pyogenes*—Strain C203
Aspergillic Acid	15	2
Gliotoxin	18	2
Clavacin*	12	63
Actinomycin A*	47	0.7
Penicillin†	1650	0.06
Gramicidin‡	> 500	0.002
Gramidinic Acid‡	> 500	0.23
AP 21‡	> 500	0.31

*Supplied through the courtesy of Dr. S. A. Waksman of the N.J. Agricultural Experiment Station, Rutgers University.

†This preparation of penicillin was the purest tested and had 350 Florey units per mg.

‡Supplied through the courtesy of Dr. J. C. Hoogerheide of the Biological Laboratories of E. R. Squibb and Sons. AP 21 is the active material obtained from a spore forming soil anærobe.

Readings made after the mixtures had stood for 30 min at 25°C were the most significant. At shorter intervals interference with luminescence was still proceeding.

Comparison of the two tests showed that in 100 consecutive tests on crystalline aspergillic acid or on crude mold substrates, 33 gave identical titers, while 33 were lower with the antiluminescent test and 34 higher. Of the latter 67 tests only 9 showed a variation as great as 2-fold and none showed greater, a degree of variation encountered in a series of antibacterial tests run in duplicate.

Evidence that the antiluminescent test can replace the antibacterial, is shown by the fact that results obtained on the basis of the first test have been in practice just as satisfactory as those based on the second. In some filtrates from old cultures of the mold on brown sugar-tryptone medium the results obtained in the antiluminescent test differ greatly from those obtained in the antibacterial. Such results indicate the presence of unknown active substances other than aspergillic acid.

An attempt has been made to apply this rapid test for activity to the assaying of other antibiotic substances (Table I). Gliotoxin behaves like aspergillic acid. Clavacin from *(Aspergillus clavatus)* and actinomycin A (from *Actinomyces antibioticus*) show somewhat similar correlation. Products from certain soil bacteria show little if any antiluminescent activity. The same is true of penicillin which substance, although derived from a mold, is very different in its properties from aspergillic acid, clavacin, or gliotoxin. Tests have indicated that crude filtrates from *Penicillium notatum* contain some factor other than penicillin which has some power to destroy luminescence.

Summary. A rapid method for the assay of aspergillic acid in crude or pure state has been described. This method, based on antiluminescent activity, may have wider application in the investigation of many antibiotic substances derived from molds and actinomyces.