

From the study of cultures recovered from synthetic media it could be ascertained that variation from "smooth" to "rough" and the appearance of dwarf forms is not markedly influenced as long as a reasonably abundant growth is maintained. In some cases when growth was scant—as was occasionally observed with variants growing without supplementary nicotinic acid—an increased tendency to develop rough and dwarf forms was noticeable. In this case also, however, individual differences are so great that no fixed rule can be given.

Discussion. The distinction between "not exacting" and "exacting" strains has been originally established on the criterion of the ability or inability of a microorganism to satisfy all nitrogen requirements with ammonia nitrogen. In the case of the Flexner bacillus we can, on the basis of our present knowledge, circumscribe the meaning of the term "exacting" somewhat more closely. We know now that there exist at least 3 grades of minimal growth requirements, namely:

1. growth is possible with ammonium salt alone;
2. nicotinic acid has to be provided;
- and 3. nicotinic acid and pantothenic acid have to be available in the medium.

The term "exacting" can, therefore, be more exactly defined as inability to synthesize nicotinic acid and—in case 3—pantothenic acid. Grade (2) will possibly have to be subdivided according to whether or not an intermediary substance (amino acid) will enable the microorganisms to synthesize nicotinic acid.³

Exceptional deficiencies in synthesizing ability do occur.

Summary. 1. Former observations concerning the role of nicotinic acid as an essential growth factor for the Flexner bacillus have been confirmed. Individual strains vary greatly in their requirements and often are able to manufacture this substance themselves or produce variants that have this ability. 2. For certain strains of the Flexner bacillus, pantothenic acid is a second essential growth factor.

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Increased Efficiency of Phenolic Germicides by Addition of Inorganic Salts to Produce Oxidation-Reduction Systems.

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In a previous communication¹ it was shown that inorganic salts of iron, tin, and manganese, when tested individually, exhibited very little or no germicidal activity against *Staphylococcus aureus*. However, when the salts were mixed in certain combinations and proportions to produce oxidation-reduction systems a pronounced germicidal action occurred. The phenomenon was shown to be a function of the positive metallic ions; the negative ions apparently played no part in the reaction.

The addition of an appropriate metallic salt to an inorganic germicide, *e.g.*, ferrous sulfate to mercuric chloride, to produce an oxidation-reduction system, resulted in a greater effectiveness of the latter. The addition of another oxidation-reduction system, such as a mixture of ferrous and ferric sulfates, to the ferrous sulfate-mercuric chloride combination increased still further the efficiency of the mercuric chloride.

The present communication is concerned with the effect of the addition of inorganic metallic salts to the organic phenolic compounds, phenol, cresol, and Hexylresorcinol.

Results from Phenol. An aqueous solu-

¹ Guest, Howard L., and Salle, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 272.

TABLE I.
Effect of Oxidation-Reduction Systems on Germicidal Efficiency of Phenol.

Germicidal sol.		Killing dil. for <i>Staphylococcus aureus</i> in 10 min. at 37°C
(A)	Phenol	1:100
(B)	Phenol	1.25 g
	$\text{Fe}_2(\text{SO}_4)_3 \cdot 5\text{H}_2\text{O}$	3.25 "
	Glycerin	50 cc
	Distilled water to make Phenol concentration—1:400	500 "
(C)	Phenol	1.25 g
	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.60 "
	Glycerin	50 cc
	Distilled water to make Phenol concentration—1:400	500 "
(D)	Phenol	1.25 g
	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.60 "
	FeCl_2	1.70 ""*
	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	7.20 "
	Glycerin	50 cc
	Distilled water to make Phenol concentration—1:400	500 "

*The mixture of ferrous and ferric chlorides represents another oxidation-reduction system.

TABLE II.
Effect of Oxidation-Reduction Systems on Germicidal Efficiency of Cresol.

Germicidal sol.		Killing dil. for <i>Staphylococcus aureus</i> in 10 min. at 37°C
(A)	Cresol	1:300
(B)	Cresol	1.45 g
	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.60 "
	Distilled water to make Cresol concentration—1:345	500 cc
(C)	Cresol	1.45 g
	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	3.60 "
	FeCl_2	1.70 ""*
	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	7.20 "
	Distilled water to make Cresol concentration—1:345	500 cc

*The mixture of ferrous and ferric chlorides represents another oxidation-reduction system.

tion of phenol was found to kill *Staphylococcus aureus* in a dilution of 1:100 in 10 minutes at 37°C.* (Table I-A).

The addition of ferric sulfate to the phenol

* The Food and Drug Administration method for the evaluation of germicides was followed throughout.

in the proportion of one gram mole of phenol to one-half gram mole (one equivalent) of ferric sulfate, and some glycerin to stabilize the preparation, increased the efficiency of the phenol 18 fold (Table I-B). Maximum germicidal activity was obtained by mixing the phenol and iron salt in the above proportion.

TABLE III.
Effect of Addition of Ferric Chloride on Germicidal Efficiency of Hexylresorcinol.

Germicidal sol.			Killing dil. for <i>Staphylococcus aureus</i> in 10 min. at 37°C
(A)	Hexylresorcinol		1:6000
(B)	Hexylresorcinol	1.0 g	
	FeCl ₃ · 6H ₂ O	1.3 "	
	Distilled water to make	1500 cc	1:24,000

In a similar experiment ferric chloride was substituted for the ferric sulfate in the proportion of one gram mole each of phenol and iron salt. In this instance the efficiency of the phenol was increased 15 fold (Table I-C). Maximum germicidal activity was obtained by mixing the phenol and iron salt in the above proportion.

The addition to the solution of phenol and ferric chloride of another oxidation-reduction system, composed of a mixture of ferrous and ferric chlorides in the proportion of one gram mole of ferrous chloride to two gram moles of ferric chloride, increased the efficiency of the phenol from 15 to 45 fold (Table I-D).

Results from Cresol. An aqueous solution of cresol killed *Staphylococcus aureus* in a dilution of 1:300 in 10 minutes at 37°C (Table II-A).

The addition of ferric chloride to the cresol in the proportion of one gram mole of each increased the killing dilution of cresol from 1:300 to 1:4000 or a 13 fold increase in its efficiency (Table II-B). Maximum germicidal activity was obtained by mixing the two compounds in the above proportion.

The addition to the solution of cresol and ferric chloride of another oxidation-reduction system, composed of a mixture of ferrous and ferric chlorides in the proportion of one mole

of ferrous chloride to two moles of ferric chloride, increased the killing dilution of cresol from 1:4000 to 1:12,000 or a 40 fold increase in its efficiency (Table II-C).

Results from Hexylresorcinol. An aqueous solution of Hexylresorcinol killed *Staphylococcus aureus* in a dilution of 1:6000 in 10 minutes at 37°C (Table III-A).

The addition of ferric chloride to Hexylresorcinol in the proportion of one gram mole of each increased the killing dilution from 1:6000 to 1:24000 (Table III-B).

Summary. The addition of an appropriate metallic salt (ferric chloride, ferric sulfate) to the organic compounds phenol, cresol, and Hexylresorcinol to produce oxidation-reduction systems resulted in a great increase in the efficiency of the germicides for *Staphylococcus aureus*. The addition of another oxidation-reduction system to phenol and cresol, composed of a mixture of ferrous and ferric chlorides, increased still further the efficiency of the germicides. It was found that maximum efficiency occurred only if the salts and germicides were mixed in certain definite proportions. The phenomenon applies to oxidation-reduction systems composed of organic germicides with inorganic salts as well as to combinations of inorganic metallic salts and should have far reaching application.