XVI. ON THE RELATIONS OF THE PHENOLS AND THEIR DERIVATIVES TO PROTEINS. A CONTRIBUTION TO OUR KNOWLEDGE OF THE MECHANISM OF DISINFECTION.

PART II. A COMPARATIVE STUDY OF THE EFFECTS OF VARIOUS FACTORS UPON THE GERMICIDAL AND PROTEIN-PRECIPITATING POWERS OF THE PHENOLS.

By EVELYN ASHLEY COOPER, *Beit Memorial Fellow.*

Lister Institute of Preventive Medicine.

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Evidence set forth in a previous communication on this subject [Cooper, 1912, 2] suggested that the germicidal power of the phenols is due, not to a typical chemical action upon the bacterial proteins, as appears to be the case with formaldehyde, but to a de-emulsifying effect upon their colloidal suspensions as evidenced by the precipitation of proteins when a certain phenol concentration is attained.

Since H. Chick [1910] has shown that disinfection by heat is analogous to the heat-coagulation of proteins, it seems that a similarity in mechanism exists between the germicidal actions of heat and of the phenols.

Hardy [1899] pointed out that the heat-coagulation of proteins consists of two processes, namely, (1) The reaction between the protein and hot water (denaturation). (2) The subsequent separation of the altered protein in a particulate form (agglutination). It is not at present known whether the essential stage in the processes of disinfection by heat and the phenols is a denaturation of the bacterial proteins or a destruction of their colloidal suspension.

The experimental work described in this paper has been undertaken with the object of studying more closely the apparent relationship between the bactericidal action of the phenols and their precipitating effects on proteins.

I. THE INFLUENCE OF CHEMICAL CONSTITUTION UPON THE GERMICIDAL AND PROTEIN-PRECIIPITATING ACTION OF THE PHENOLS.

The experimental method. The minimum concentrations in water of various phenols and certain other substances have been determined at which a visible change in the state of aggregation of the protein occurred. Small alterations in the magnitude of the protein-particles brought about by such concentrations were indicated by a turbidity; larger alterations by a progressively increasing precipitation of the colloid as the concentrations of the phenols rose.

Two proteins were used in the experiments, namely, gelatin and dialysed egg-albumin; the former was employed in the form of small strips and the latter as its colloidal suspension in water. The effect on the gelatin was to produce a turbidity in the strip immersed in the solution under examination. Throughout the experiments the weight of protein, volume of solution, and temperature (20°) were kept constant. Observations of the effects of the various solutions upon the proteins were made immediately after admixture and also after standing one hour. The results tabulated below are based on the observations made after the one hour's standing.

The experimental results. The protein-precipitating powers of the various substances have been compared with their germicidal activities, which are expressed in the table below in terms of the concentrations required to disinfect *B. typhosus* or *Staphylococcus pyogenes aureus* in 15 minutes under the standard conditions employed by Chick and Martin [1908]. The concentrations have been selected from two previous papers on disinfection (1) by Morgan and Cooper [1912] and (2) by the author [1912, 1], to which papers the numbers 1 and 2 in the following table (p. 177) refer.

The following parallelisms can be deduced from these results.

1. The germicidal and protein-precipitating powers of phenol are decreased by the introduction of hydroxyl-groups into its benzene nucleus and increased by the introduction of nitro-groups and also by that of a methyl group.

2. The monohydric phenols are superior to the monohydric alcohols and also to acetone both as germicides and protein-precipitants.

3. The effects of substitution in the molecule of phenol upon its germicidal and protein-precipitating powers are frequently of somewhat similar orders of magnitude. An exact correspondence was not expected, firstly, because it is well known that the carbolic acid coefficient of a

substance varies to some extent with the species of micro-organism employed, and, secondly, because bacterial proteins were not used in the determination of protein-precipitating power.

TABLE I.

Substance	Protein-precipitating power	Germicidal power	Organism
	Minimum concns. at which precipitation was visible. (Percentages in terms of weight per volume)	Concentrations killing in 15 minutes. (Percentages in terms of weight per volume)	
	Gelatin 0.1 g.	Vol. of liquid 10 c.c.	
Phenol ...	2.5	0.8 [1]	<i>B. typhosus.</i>
Trinitrophenol (Picric acid)	0.2	0.1 [1]	"
Resorcinol ...	4.0	2.6 [2]	"
Catechol ...	5.0	1.7 [2]	"
Quinol ...	5.0	0.75 [2]	"
Pyrogallol ...	3.0	1.0 [2]	"
Phloroglucinol	Satd. solns. (5%) had no visible effect	> 2.3 [2]	"
<i>p</i> -Nitrophenol	0.9	0.46 [1]	<i>Staphylococcus py. aur.</i>
<i>m</i> -Nitrophenol	0.6	0.27 [1]	" "
Trinitrophenol (Picric acid)	0.2	0.14 [1]	" "
Ethyl alcohol	50 % and 100 % had no visible effect	35.0 [1]	" "
Acetone ...		> 10.6 [2]	" "
Phenol ...	2.5	1.0 [1]	" "
Egg-albumin $\frac{1}{2}$ c.c. 10 % solution (0.05 g.). Vol. of solution containing precipitant = 4.5 c.c.			
Phenol ...	1.0	{ 0.8 [1] 1.0 [1]	<i>B. typhosus.</i> <i>Staphylococcus py. aur.</i>
<i>m</i> -Cresol ...	0.7	{ 0.5 [2] 0.3 [2]	" " <i>B. typhosus.</i>
Acetone ...	12.0	> 10.6 [2]	<i>Staphylococcus py. aur.</i>
Methyl alcohol	20.0	35.0 [1]	" "
Ethyl alcohol	20.0	{ 35.0 [1] 32.5 [1]	" " <i>B. typhosus.</i>
Propyl alcohol	12.0	14.0 [1]	<i>Staphylococcus py. aur.</i>

II. THE EFFECTS OF VARIOUS SUBSTANCES UPON THE GERMICIDAL AND PROTEIN-PRECIPTATING ACTION OF THE PHENOLS.

The experimental method employed in the investigation of the precipitating action of the phenols was similar to that described in Section I. The various substances were added to the phenol solutions immediately before admixture with the protein. The results recorded in this section are based on observations of the condition of the proteins after one hour's contact with the phenol solutions.

A. SODIUM CHLORIDE.

Reichel [1909] showed that the addition of sodium chloride increased both the bactericidal action of phenol and its solubility in proteins. The germicidal power was about doubled by 10 per cent. salt and quadrupled by 20 per cent. salt, and the effects of salt upon the solubility in proteins were of similar orders of magnitude. Smaller amounts of salt had little influence.

These experiments indicated how in the presence of salt a larger amount of phenol became available for disinfection, but did not throw any light on the mechanism of the germicidal action of this disinfectant. It was therefore of interest to investigate the influence of salt upon the protein-precipitating action of phenol.

Gelatin. The minimum concentrations of phenol, which induced a visible precipitation in the presence of varying amounts of sodium chloride, were determined. The results are tabulated below:

TABLE II.

Percentage of salt present (weight per volume)	Minimum phenol concentrations precipitating the protein
0	3 %
5	2.5
12	1.5
19	0.75

Although saturated aqueous solutions of *m*-cresol (1.25 per cent.) had no visible effects upon gelatin, this protein was precipitated by 0.8 per cent. solutions of *m*-cresol in the presence of 9 per cent. salt and even by 0.4 per cent. solutions in the presence of 20 per cent. of salt.

Dialysed egg-albumin. 3.3 per cent. aqueous solutions of phenol only induced a faint turbidity in a sample of egg-albumin, but in the presence of 6 per cent. salt an extensive precipitation occurred. 1 per cent. solutions of phenol in water and in 6 per cent. salt had no visible effect on the albumin suspension; a turbidity however was induced by the same phenol concentration in 15 per cent. salt, and a marked precipitation in 22 per cent. salt solution.

The precipitating action of phenol upon proteins was thus greatly increased by the presence of salt, but was not appreciably affected until high salt concentrations were attained.

A comparison of the results of experiments with gelatin with those obtained by Reichel [1909] indicates that the germicidal power, the solubility in proteins, and the precipitating action of phenol are increased to very similar extents by the presence of salt.

The increased germicidal power thus appears to be due to an increased de-emulsifying action of the phenol upon the bacterial proteins resulting from the availability within the emulsoid particles of a larger amount of phenol.

B. ALCOHOL.

Kronig and Paul [1897] showed that the germicidal action of phenol upon spores was decreased by the presence of alcohol and that solutions of phenol in 98 per cent. alcohol possessed no bactericidal power.

The author [1912, 2] found that the uptake of phenol by a protein was greatly decreased by the presence of alcohol, the protein absorbing for example eight times as much phenol from aqueous solution as from a solution of phenol in 50 per cent. alcohol. These experiments indicated how in the presence of alcohol less phenol became available for disinfection, but they afforded no explanation of the nature of its germicidal action. It was therefore of some importance to investigate the influence of alcohol upon the protein-precipitating action of phenol.

Gelatin in the form of strips was chiefly employed in the experiments, because it was not precipitated by immersion in alcohol of any strength. Although this protein was precipitated by $2\frac{1}{2}$ per cent. aqueous phenol solutions, there was no indication of precipitation by 5 per cent. phenol solutions in 50 per cent. alcohol, and the extent of the precipitation by 6.25 per cent. phenol solutions in 25 per cent. alcohol was much less than that induced by the above aqueous solutions. Furthermore, 10 per cent. solutions of phenol in absolute alcohol had no visible effect upon gelatin. This is of interest when correlated with Kronig and Paul's observation that solutions of phenol in 98 per cent. alcohol possessed no germicidal power.

A sample of dialysed egg-albumin was immediately precipitated by 1.5 per cent. aqueous phenol solutions, but was not visibly affected by the same concentration in the presence of 10 per cent. alcohol.

Although 0.5 per cent. aqueous solutions of picric acid precipitated gelatin, similar concentrations in 30 per cent. alcohol had no visible effect upon this protein.

The inhibiting effect of alcohol upon the germicidal power of phenol thus appears to be due to a decrease in the de-emulsifying action of the latter upon the bacterial proteins, resulting from a diminution in the amount of phenol available within the emulsoid particles.

C. FAT.

Strips of gelatin were immersed in castor-oil, containing varying amounts of phenol. It was found that solutions of phenol in the fat as strong as 10 per cent. (by weight) had no visible precipitating action upon gelatin, although this protein was precipitated by $2\frac{1}{2}$ per cent. aqueous solutions of phenol.

This can be correlated with the fact discovered by Koch that solutions of carbolic acid in oil possess no germicidal power. The greatly diminished protein-precipitating action of phenol in the presence of fat is probably due, as in the case of alcohol, to a decreased uptake of phenol by the protein, because, while phenol is about three times as soluble in gelatin as in water, the partition-coefficient for phenol between fat and water is six.

D. ALKALI.

Kronig and Paul [1897] showed that, while 4 per cent. solutions of phenol in water exerted a strong germicidal action upon anthrax spores, solutions of sodium phenate equivalent to 4 per cent. phenol possessed no germicidal power.

Morgan and Cooper [1912] showed that *p*-nitrophenol was about five times as powerful a germicide as its potassium salt. The inhibiting action of alkali upon the agglutination of proteins by heat suggested that the decreased germicidal power might be due to the protective effect upon the bacterial protoplasm of the alkali, produced in the hydrolysis of the alkali phenates, against the precipitating action of the phenols simultaneously liberated. Accordingly the effect of alkali on the protein-precipitating action of the phenols has been studied.

Dialysed serum-albumin: Phenol. The albumin was not visibly affected by 0.9 per cent. solutions of phenol, but was precipitated by 1.7 per cent. phenol. The precipitation was not only completely inhibited by N/50 to N/150 concentrations of caustic soda, but even by a concentration as low as N/300.

Gelatin: Phenol. Although gelatin was precipitated by 2·5 per cent. aqueous phenol solutions, this protein was not visibly affected by 4 per cent. phenol in the presence of N/25 caustic soda and was only very slightly precipitated by the same concentration in presence of N/50 caustic soda.

Traces of organic bases, such as ethylamine and ethylenediamine, also inhibited the precipitation of gelatin and egg-albumin by phenol.

Gelatin: p-Nitrophenol. Gelatin was precipitated by 1 per cent. aqueous solutions of *p*-nitrophenol, but was not visibly affected by the same concentration in the presence of N/100 caustic soda, nor even by 1·4 per cent. (N/10) solutions containing N/10 caustic soda.

Not only could alkali inhibit the precipitation of proteins by the phenols, but when present in concentrations varying from N/1 to N/10 it could disperse egg-albumin already precipitated by phenol.

The results indicate that very low concentrations of alkali are sufficient to inhibit the precipitation of proteins by phenol. As alkali combines with the phenols forming salts, which are partially hydrolysed in solution, the inhibiting concentrations must have been even smaller than those stated above.

In view of these results it was of great interest to investigate the influence of alkali upon the germicidal action of phenol in greater detail, and in particular to study the effects of very low alkali concentrations. The experiments consisted in the estimation of the minimum concentrations of phenol required to disinfect in a specified time under standard conditions in the presence of varying amounts of alkali.

The following results were obtained:

TABLE III.

Organism, B. typhosus.

Concentration of caustic soda present	Minimum concentration of phenol required to disinfect in 15 mins. at 20°
0	9·5 in 1000 (N/10)
N/250	" "
N/50	" "
N/25	11 in 1000 (N/8·5)
N/20	" "
N/15	13·5 in 1000 (N/7)

The germicidal powers of the above concentrations of alkali in the absence of phenol were next determined and are compared in the following table with those of solutions containing initially the same alkali concentrations with varying amounts of phenol.

TABLE IV.

Concentration of NaOH	Time required for complete disinfection
N/500 NaOH	> 5½ hours
N/250 „	> 5½ „
N/100 „	45 minutes
N/50 „	20 „
N/25 „	< 3 „
N/15 „	< 3 „
N/10 „	< 3 „
N/25 „ + N/10 phenol	> 15 „
N/25 „ + N/8·5 „	15 „
N/20 „ + N/10 „	> 15 „
N/20 „ + N/8·5 „	15 „
N/15 „ + N/10 „	> 15 „
N/15 „ + N/8·5 „	> 15 „
N/15 „ + N/7 „	15 „
N/10 „ + N/12 „	< 15 „
N/10 „ + N/10 „	15 „
N/10 „ + N/9 „	> 15 „
N/10 „ + N/7 „	> 15 „

The above results indicate that alkali decreased the germicidal power of phenol when present in moderately low concentration. While, however, the precipitation of serum-albumin by phenol was inhibited by an initial concentration of N/300 caustic soda and that of gelatin by N/50 caustic soda, bactericidal power was not measurably affected until the initial alkali concentration was raised to N/25. The precipitating action of phenol was therefore much more sensitive to the inhibiting effect of alkali than its germicidal action. This discrepancy is not sufficiently explained by the slight acidity of the broth culture, nor by the toxic action of the alkali, since an analysis of the results tabulated above indicates that not only did small concentrations of alkali such as N/250 exert a very feeble germicidal action, but furthermore the bactericidal action of the alkali was greatly decreased by the presence of phenol. Owing to this depreciating effect N/10 solutions of alkali, containing initially N/7 or N/9 concentrations of phenol, possessed a smaller germicidal power than those which initially contained lower phenol concentrations such as N/10 and N/12.

It is possible that a larger amount of alkali is required to preserve the colloidal suspension from the action of the phenol in the case of the bacterial proteins than in the cases of gelatin and albumin.

E. ACIDS.

Hailer [1910] showed that acids, arranged according to their germicidal power and also according to their efficiencies in increasing the bactericidal power of phenols, were not in the same order. Thus sulphuric acid exceeded oxalic acid in bactericidal power, but was less effective in increasing the germicidal power of phenol. Some of the experimental results described in the previous pages suggested that the discrepancy might be due to differences in the effects of the acids upon the protein-precipitating action of phenol. This, however, seems not to be the case because it was found that, although acids greatly increased the precipitating action of phenol, sulphuric acid was effective in much lower concentration than oxalic acid.

The effects of alcohol and salt upon the precipitating action were shown to be due to a redistribution of phenol between the water and proteins. The greatly increased precipitating action in the presence of acids however is not sufficiently explained in this way, as it was previously shown [Cooper, 1912, 2] that the addition of acid only very slightly increased the solubility of phenol in protein. It is probably due to the formation of an acid salt of the protein, which is more readily precipitated than the protein itself.

DISCUSSION OF RESULTS.

The parallelisms deduced from the study of the effects of changes in the chemical constitution of phenol and of the presence of certain substances (e.g. salt, alcohol, fat) upon its germicidal and protein-precipitating powers support the conclusion arrived at in the previous communication, namely, that the germicidal action of phenol is associated with an alteration in the condition of the colloidal suspensions of the bacterial proteins as evidenced by the precipitation of such proteins as gelatin and albumin when certain phenol concentrations are attained.

It has already been pointed out that the heat coagulation of proteins consists of two stages.

1. A reaction between the proteins and hot water (denaturation).
2. A separation of the protein in a particulate form (agglutination).

It is therefore of interest to attempt to characterise the essential process in disinfection by the phenols as either a denaturation of the bacterial proteins or a de-emulsification of their colloidal solution. Chick and Martin [1911; 1912] have shown that the process of denaturation by heat

is accelerated by acid and alkali, and retarded by salt, while the subsequent process of agglutination is facilitated by low concentrations of acid and salt, but inhibited by high concentrations and also by alkali.

It was shown in Section II of this paper that the bactericidal power of phenol, its protein-precipitating action and solubility in proteins were increased to equal extents by the addition of salt. The latter thus appears to have no *direct* effect upon the germicidal and precipitating actions of phenol, but to increase them merely by raising the phenol concentration within the emulsoid protein-particles. It is therefore not possible by a study of the effects of salt to correlate these properties of phenol with either the denaturing or agglutinating action of heat upon proteins.

The fact, however, that the germicidal action of phenol is inhibited by low concentrations of alkali, although to a less extent than its precipitating action upon proteins, suggests that the essential process in disinfection by phenol resembles the final phase in the heat-coagulation of proteins and consists in the destruction of the colloidal suspensions of the bacterial proteins.

Since the process of disinfection by hot water was found [Chick, 1910] to be accelerated by alkali as well as by acid, it is possible that the essential stage in this process is the denaturation of the bacterial proteins by the action of the water and not the subsequent precipitation. A study of the effects of salt upon the velocity of disinfection by hot water will throw light on this problem.

SUMMARY.

1. The germicidal and protein-precipitating powers of phenol are similarly affected by the entrance of various chemical groups into its molecule.

(a) The introduction of hydroxyl groups decreases and that of nitro-groups and of a methyl group increases the bactericidal and protein-precipitating powers of phenol.

(b) The monohydric phenols are superior to the alcohols both as germicides and protein-precipitants.

2. Sodium chloride increases the germicidal and protein-precipitating action of phenol through increasing its solubility in proteins.

3. Alcohol, on the other hand, decreases the germicidal and protein-precipitating action of phenol through diminishing its solubility in proteins. Solutions of phenol in absolute alcohol exert no germicidal action upon spores and have no visible precipitating action on gelatin.

4. Solutions of phenol in fat also possess no germicidal power and do not visibly precipitate gelatin.

5. While the presence of very small amounts of alkali is sufficient to inhibit the precipitating action of phenol upon proteins, only moderately low-alkali concentrations can effect a measurable decrease in its germicidal power. The explanation of this discrepancy is not apparent.

6. The precipitating action of phenol is increased by the addition of acids.

MAIN CONCLUSION.

From the evidence set forth in the previous communication and in the present paper it is concluded that the absorption of phenols by bacteria is merely the initial stage in the process of disinfection, and that the germicidal action which follows is due, not to a typical chemical union of the phenols with the bacterial protoplasm, as appears to be the case with formaldehyde, but to a de-emulsifying action upon the colloidal suspension of some constituent protein or proteins essential for the vitality of the organisms.

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