

A COMPARISON OF THE BACTERICIDAL ACTION OF QUINONE WITH THAT OF SOME OF THE COMMONER DISINFECTANTS.*

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This work was undertaken to furnish a basis of comparison of the bactericidal action of quinone and other phenol-oxidation products with some of the commoner disinfectants.

It is only in comparatively recent years, since the Rideal-Walker method for standardizing disinfectants was brought forward in 1903, that there has been in existence a bacteriological method which in the hands of different workers has given consistent results allowing of comparison. The principle of this method has been elaborated and further refined by the Lancet Commission and Anderson and McClintic of the Hygienic Laboratory, Washington, so that the latter method may be considered as being one of scientific exactness.

With these trustworthy means of investigation at our disposal, new and startling facts have been brought forward as regards both the efficacy and the inefficacy of many substances in common use as antiseptics and disinfectants. The Hygienic Laboratory workers from their studies of various proprietary disinfectants on the market have come to the conclusion of the fraudulent nature of many of them, and have undertaken an exhaustive investigation of these materials. The surgical profession has begun to discard some of its old antiseptic stand-bys, because of practical and common-sense reasons, even before modern research has shown the advantage that other substances and a simpler technic have over those formerly used. The surgeons are, of course, far removed from the days of "anti"-sepsis and the carbolic spray. The recognition of the impossibility of sterilizing the deeper layers of the skin has relieved most of them of the necessity of chlorinating the skin, etc. They

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are slow, however, in getting away from potassium permanganate and oxalic acid, and especially from bichloride of mercury.

The previous standardization of the bactericidal action of various substances done by Sternberg, Welch, and others will all have to be revised by our new methods and many steps in this direction have already been taken, to some of which we wish to call attention and compare with our own results. It must be realized that the figures give merely a comparison, under the special conditions of the test, of the bactericidal action of the various substances with phenol, but we are still left in the dark as to the efficiency of these materials under practical conditions. This very seeming defect makes the result more reliable as a bactericidal index, inasmuch as these special conditions admit of being made fixed and the same by different workers in different laboratories, thereby allowing a direct comparison of their results. These figures are, therefore, undoubtedly a rough indication of the practical value of the disinfectants, and as such, an indication for their practical use. Much work still remains to be done in the investigation of the practical side of this problem and many factors will have to be studied, such as the nature of the solvent or emulsion, the effect of the presence of organic matter, the effect of temperature, the relation of disinfection to the time element and to the nature of the bacteria to be destroyed, etc. Some of these points are already on a scientific basis. Thus, Lister pointed out that a solution of phenol in oil has little or no antiseptic action and this observation was confirmed by Koch. Chick and Martin have shown that the minute particles of a satisfactory emulsion exhibit a Brownian motion, are densest in the vicinity of the comparatively large bacteria, actively bombard them and are adsorbed by them, and that when free access to the bacteria is possible, substances show a greater bactericidal action when in the state of an emulsion than in aqueous solution. Other organic materials show similar and equal adsorptive actions, and so reduce by their presence the bactericidal action of this type of preparation, while aqueous solutions that do not coagulate organic matter are better able to penetrate through it to the bacteria and destroy them. Whatever the characteristics of these products, their essential basis must be an efficient bactericide.

These same observers have shown "that the reaction velocity of disinfection increases with the rise of temperature in a manner similar to that of chemical reaction." The importance of the time element in the disinfection process is also recognized and put by some on a similar chemical basis, this factor being taken advantage of in the recent methods of standardization. The difference in resistance of various bacteria, from the standpoint of obtaining results which will admit of comparison, will be an especially difficult problem to investigate.

These problems, however, belong to the future, and, with the general activity everywhere present in public health work, we trust that their investigation will be in the near future. They belong to the large, well equipped, resourceful municipal laboratories of our large cities.

In the review of recent work on standardization of disinfectants the report of the Lancet Commission¹ stands out as a stimulus to other workers and as an exhaustive

¹ See the original articles for description of the Rideal-Walker, Lancet, and Hygienic Laboratory methods of determining the phenol coefficient of a disinfectant.

work on the theory of action of bactericidal substances and the chemical and bacteriological nature of their composition and action. This report is an investigation of nineteen proprietary coal tar disinfectants marketed in England. It divides these disinfectants into two groups, those which form emulsions with water and those forming clear solutions. The first group comprises 14 substances and is subdivided into two classes, those with high and those with low phenol coefficients. There is a sharp difference between these, the high varying between 9.8 and 6.4 and the low between 2.2 and 0.75. The report states that no coefficient was obtained higher than 13. The second group includes five substances and these disinfectants forming clear watery solutions have, under the conditions of the test, relatively low coefficients compared with those of the emulsifying group. Crude carbolic acid (which did not form a clear solution for us) leads with a coefficient of 4.2, trikresol gives 2.5, and lysol 1.7. These results bring out in a striking way the relatively marked bactericidal action of the emulsifying coal tar disinfectants as compared with those forming a clear solution in water. In light of this we wish to note that in some of our tests with similar emulsions we have several times observed more activity in a 1-200 dilution than in a dilution of 1-100, a result we have attributed to increased penetrating power due to the more perfect emulsion at the higher figure. Of course this does not continue farther but shades off when the dilutions become excessive.

Recently some very interesting work has been reported by Post and Nicoll, which, however, greatly loses in its value because they have not used any of the standard methods. Their method, which is a modification of that of Rideal-Walker, is as follows: 0.5 c.c. of disinfectant and one loopful of a broth suspension, made from blood agar tube of the organism to be tested, are brought together; and inoculations of blood agar tubes, from which plates are poured, are made at 1, 10, and 30 minutes and 20 hours. These are incubated and the number of colonies are then counted and compared. They used four organisms: typhoid, pneumococcus, gonococcus, and streptococcus. No phenol coefficient was devised.

Blood agar is certainly an unnecessary refinement when working with the typhoid bacillus, which is the only organism which should be considered, as the other three are too variable in their different strains to be used by different workers. The small amounts of materials used must permit large percentage errors in the results. In spite of these and other defects in the method the uniformity and consistency of the results speak for themselves.

They come to the following conclusions:

1. "The reliability of the prompt action of a few simple germicides such as tincture of green soap, alcohol in dilutions of 50 per cent or over, silver nitrate solution as dilute as 1-1,000, iodine solutions, either as tincture or in aqueous solutions along with potassium iodide, and phenol in 5 per cent solution.
2. "The unreliability of many agents prevalently supposed to be effective germicides.
3. "The slow action of mercuric chloride, although when given hours to act it is effective in great dilutions."

Their tables must be consulted to appreciate the above.

Of the inefficient substances the slow action of bichloride of mercury stands out as the most important practical result. It would seem that this must soon be appreciated by the surgical profession, and that this substance will pass into retirement with

its predecessor, the carbolic spray. Already great advances have been made toward a simplified technic with proved, efficient antiseptics. Formaldehyde or rather formalin was shown not to kill the typhoid bacilli in 30 minutes in 1 per cent solution. This result we have been able to confirm. Argyrol 50 per cent and protargol 10 per cent are much less efficient than silver nitrate, 1-1,000.

Among the substances which stand out as sufficiently efficient to warrant attention and also wider adoption of their use are iodine, 50 per cent alcohol, silver nitrate, and tincture of green soap. They found complete killing of *B. typhosus* in one minute by tincture of iodine, by 1-400 solution of iodine in potassium iodide and water, tincture of green soap, and 50 per cent alcohol and great efficiency of 1-1,000 silver nitrate. The action of the tincture of green soap, as the authors suggest, is undoubtedly due to the alcohol present. We have been able to follow the aqueous solution of iodine in high dilutions with similar positive results. This latter substance is being used more and more in practice, such as application on the skin to prepare fields for operation, in solution for irrigation of infected wounds, the tincture painted on sluggish ulcers, for preparation of sterile iodine catgut, etc. The valuable work of A. V. Moschcowitz on the experimental and practical investigation of iodine catgut has shown this to be a most satisfactory preparation and has resulted in an extremely simple method for its preparation.

Seelig and Gould have recently reported some very interesting results on "Osmosis as an Important Factor in the Action of Antiseptics," and here again show the efficiency of iodine. Working with typhoid cultures contained in celloidin capsules, pouches of living rabbit mesentery, omentum, and skin, and excised rabbit diaphragm, and surrounded by various antiseptics, it was found that alcohol was most efficient as a bactericide, because of its combined bactericidal action and its rapid ability to osmose, which later property varied directly as its strength (up to 95 per cent). Tincture iodine acted even more quickly than plain alcohol. Mercury bichloride acted very poorly, showing a low grade of osmosis. Iodine penetrated the entire thickness of skin in one hour, but did not kill the typhoid bacilli. Rubbing skin with castor oil aided penetration of alcohol, and was used as proof that oil in the skin helps the penetration of alcohol and iodine and for this reason should not be removed by washing before this method of preparing a surgical field. This penetrating power of iodine seems to us most important.

Another ingenious method of testing the bactericidal and penetrating powers of disinfectants is that of Kendall and Edwards, which is briefly as follows: A mixture of definite quantities of a 24 hour broth culture of *B. coli* and sterile 1.5 per cent agar medium is allowed to harden in a sterile tubular mold 1.5 cm. in diameter. Pieces are then cut with a sterile knife at 2 cm. intervals and dropped into a beaker containing the disinfectant solution. These are removed at hour intervals with a special wire gauze forceps, washed with sterile water, and a core 3 mm. in diameter is removed in turn from their center and placed in No. 1 lactose broth fermentation tubes. These are incubated and the results as to growth after several days read and tabulated. A 5 per cent solution of phenol is used for comparison and a phenol coefficient is determined. The following table copied from their article is the report of all the substances they have tested. The relative efficiency of formalin is to be noted but the long time element does not indicate it to be very active. Bichloride acted only in strong solutions.

TABLE SHOWING COMBINED GERMIDICAL AND PENETRATING POWERS OF SOME COMMON DISINFECTANTS (KENDALL AND EDWARDS).

Agar 1.5 per cent. 72 hour incubation at body temperature. Temperature of exposure 20 degrees.

DISINFECTANTS	DILUTION	TIME OF EXPOSURE		
		1 hour	3 hours	5 hours
Carbolic acid.....	5 per cent	+	+	-
Carbolic acid.....	1 " "	+	+	+
Formalin.....	4 " "	+	-	-
Formalin.....	1 " "	+	-	-
Formalin.....	0.25 " "	+	+	-
HgCl.....	0.1 " "	+	+	+
HgCl.....	1 " "	+	-	-
Chloride of lime.....	10 " "	+	+	-
Chloride of lime.....	4 " "	+	+	+
Hyco.....	2 " "	+	+	+
Cresol.....	1 " "	+	+	+
Sulphonaphthol.....	2 " "	+	+	+

+ equals growth.
- equals no growth.

Further evidence of the bactericidal activity of mercuric chloride is given by Reymann and Nyman, who, using the method of Krönig and Paul, found its action less than that of silver nitrate.

H. C. Wood in a recent résumé of the subject of intestinal antiseptics mentions beta-naphthol as the substance best adapted for this use. He states that it is bactericidal at a dilution of 1-10,000, but does not mention the nature of the test for determining this figure, or the time it takes for this action to occur. We refer the reader to our data on this subject.

Our results with a few substances, using the Rideal-Walker method as outlined in a previous article, we submit in the following tabulated form.

TABLE 1. APRIL 23, 1911.

ORGANISM: *B. typhosus*.

Sample	Dilution	Time of Culture Exposed to Action of Disinfectant in Minutes:						Rideal-Walker Phenol Coefficient
		2½	5	7½	10	12½	15	
Hydrocyanic acid.....	1-100	+	+	+	+	-	-	110)100 9
	1-100	+	+	+	+	+	+	
Potassium cyanide.....	1-100	+	+	+	+	+	+	Coefficient not determined but less than 1
Formalin (Sat. sol. of formaldehyde in water....	1-100	+	+	+	+	+	+	(Ditto) 90)200 2.2
Triresol.....	1-200	+	+	+	+	-	-	
	1-200	+	+	+	+	-	-	70)200 2.8
Parakresol.....	1-100	-	-	-	-	-	-	
	1-200	+	-	-	-	-	-	
Phenol.....	1-70	+	-	-	-	-	-	
	1-90	+	+	+	+	-	-	
	1-100	+	+	+	+	+	-	
	1-110	+	+	+	+	+	+	

+ equals growth.
- equals no growth.

TABLE 2. MAY 4, 1911.

ORGANISM: *B. typhosus*.

Sample	Dilution	Time of Culture Exposed to Action of Disinfectant in Minutes:						Rideal-Walker Phenol Coefficient
		2½	5	7½	10	12½	15	
Crude phenol	1-300 1-400	— +	— —	— —	— —	— —	— —	100)400 4
Iodine and potassium iodide.....	1-100 1-8,000 1-10,000	— — +	— — +	— — +	— — +	— — +	— — +	80)8,000 100
Sodium hydroxide.....	$\frac{n}{10}$ = 1-250 $\frac{n}{20}$ = 1-500 $\frac{n}{50}$ = 1-1,250 $\frac{n}{100}$ = 1-2,500	— — + +	— — — +	— — — +	— — — +	— — — —	— — — —	110)2,500 23
Ethyl alcohol.....	95 per cent 25 per cent	— +	— +	— +	— +	— +	— +	
Beta-naphthol in NaOH, $\frac{N}{10}$	1-100	—	—	—	—	—	—	100)200 2
Beta-naphthol in NaOH, $\frac{N}{20}$	1-200	+	—	—	—	—	—	
Beta-naphthol in NaOH, $\frac{N}{50}$	1-500	+	+	+	+	+	+	
Beta-naphthol in 25 per cent alcohol.....	1-1,000 1-2,000	— +	— +	— +	— +	— +	— +	80)1,000 12.5
Phenol.....	1-80 1-90 1-100 1-110 1-120	— + + + +	— + + + +	— + + + +	— + + + +	— + + + +	— + + + +	

TABLE 3.

(Extracted from table in previous article.)

Sample	Dilution	Time of Culture Exposed to Action of Disinfectant in Minutes:						Rideal-Walker Phenol Coefficient
		2½	5	7½	10	12½	15	
Quinone.....	1-16,000	+	+	+	+	+	—	160 coefficient
Hydroquinone.....	1-200	+	+	+	—	—	—	2.5 coefficient
Phenol-quinone.....	1-4,000	+	+	+	+	—	—	44 coefficient

This rather heterogeneous group of substances was studied in relation to the bactericidal action of phenols colored by exposure to sunlight and the oxidation products quinol and phenol-quinol thus formed.

We were interested in the phenol coefficient four (4), for crude carbolic acid, thus confirming that obtained by the Lancet Commis-

sion and therefore admitting our results to comparison with theirs. This activity in excess of that of pure phenol is probably to be attributed, as the report of this commission pointed out, to phenoid substances which occur in crude phenol and many coal tar disinfectants as well. The pungent odor of quinone suggested to us that of formalin which, because of very irritating effects of the latter on mucous membranes and its being a gas in watery solution, we expected to be strongly bactericidal. Our findings of its slight activity here agree with those of others. The cresols were tested because of their resemblance and relation to phenol. The coefficients 2.2 for trikresol and 2.8 for parakresol are interesting, for they disprove the statement that the mixture of the three cresols, para-, meta-, and ortho-, is more efficient than any one of them. Iodine was studied because of our interest in the recent reports about it, and because of its present wide popularity in surgical technic and surgical dressings. Its high phenol coefficient and its efficacy in killing typhoid bacilli in $2\frac{1}{2}$ minutes under the conditions of the test are further proof of its value as a practical antiseptic.

The potency of hydrocyanic acid and potassium cyanide as organic poisons and the fact that they are not oxidizing agents led us to be curious as to their capacity as disinfectants. They are seen to be less active against bacteria than is phenol.

We studied beta-naphthol in order to compare its action with that of quinone and were led to make this investigation because of the high efficiency claimed for this substance by H. C. Wood. We found that this material is not soluble in water, is soluble one part in three hundred parts of 25 per cent alcohol, forming a colorless solution, and is easily soluble in tenth normal sodium hydroxide, one part in one hundred, forming a dark yellow solution on standing. The table shows the efficiency of alcohol in strong solutions but not in weak ones and that of beta-naphthol (in alcoholic solution) in a dilution of 1-1,000, but certainly not as high as 1-2,000. The test with sodium hydroxide shows marked activity even in a dilution of 1-2,500 and also shows the interesting fact that this activity rather than being increased by a solution in it of beta-naphthol is, on the contrary, diminished. This can be attributed

to the neutralization of this substance by the weakly acid beta-naphthol, and this indicates two things: first, that the activity of the former is due to its hydroxyl group, and second, that of the latter to its acid radical. As this process of neutralization would occur in the alkaline juices of the intestine we believe that the action of this substance as an intestinal antiseptic would thereby be seriously if not entirely destroyed. Certainly with its activity cut down to that of a dilution of one to two or three hundred (see table) a sufficiently large dose could not be given to be efficient. From this consideration it would seem that beta-naphthol is certainly not an ideal substance for an intestinal antiseptic, if indeed it is at all potent in this capacity.

In light of these results, the bactericidal potency of quinone in high dilutions has suggested to us the possibility of using it as an intestinal antiseptic, because one could expect a marked action from it on the intestinal bacteria when given in the small doses in which it is necessary to administer a drug for this purpose. Similarly it would seem that it is a good agent for irrigating infected wounds, treatment of urethritis, etc. Some experiments have been begun to investigate these subjects and will be reported later. The effect of quinone on the alimentary canal of dogs will have to be thoroughly studied before one is justified in treating patients with it.

CONCLUSIONS.

1. Under the conditions of the above tests, quinone is the most efficient bactericidal substance yet reported; phenol-quinone is less active than quinone, and hydroquinone still less so.
2. Many of the commonly used disinfectants and antiseptics such as bichloride of mercury, formalin, argyrol, protargol, etc., are relatively inefficient in strengths used.
3. Iodine, alcohol in strengths above 50 per cent, silver nitrate in solution of 1-1,000 are very efficient bactericidal agents.
4. Potassium cyanide and hydrocyanic acid, although powerful organic poisons, have a weak bactericidal action.
5. The phenol coefficient of crude phenol is 4, of trikresol is 2.2, and of parakresol is 2.8, thereby disproving the statement that a

combination of all three cresols is more powerful than any one of them.

6. Beta-naphthol and sodium hydroxide neutralize one another's bactericidal action, and this would also occur in the intestine and so render the former substance useless as an intestinal antiseptic.

7. The work of recent observers and the above results indicate the advantage of getting away from the use of bichloride in surgical technic and the substitution for it of tincture iodine and 50 per cent alcohol.

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