

to show what could be done by paying cash instead of buying on the instalment plan. In every room the board of health regulations which could any way apply to that house were posted, and we felt that the neighbors learned a great deal from this, especially since we always had some one at home there in the afternoon to talk over with them the application and relation of our problems and successes with our landlord to their own.

The tenants upstairs became interested, and the people soon asked if they might use the rooms for meetings, which of course gave the tenement a larger influence at once. The whole

house took on a different aspect, and one tenant moved because she said she "could not keep up to the pace." This was considered a real fall on her part by the people in her neighborhood, and she has never really been reinstated socially because of it.

The landlord has acquired two other houses, and remodeled them, and he frankly admits that he has been influenced in his ideas by what he learned from having us as tenants.

The tenement was not "given up" in one sense, but after three years was moved on to another part of the city. We felt that it had taught a real lesson here.



## THE SPECIFICITY OF DISINFECTANTS AND ITS BEARING ON THEIR STANDARDIZATION.

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Read before the Laboratory Section, American Public Health Association, October 20, 1917,  
at Washington, D. C.

THE standardization of disinfectants has been an annual subject of discussion before health congresses and societies since the advocacy of the "drop method" proposed in 1903 by Rideal and Walker. During these years many refinements, changes and additions have been made to this method, the latest being the control of the acidity of the media by determining the H-ion concentration. There is no doubt that the method has been improved but the question arises has not the broad purpose of the test been overlooked in searching out the details.

There was a time when with limited bacteriological knowledge, it was reasonable to suppose that all disinfectants would bear the same germicidal relation to phenol, no matter on

what organism tested. This time has now passed and when a label bears the statement that a certain preparation has a phenol coefficient of 2, it cannot be taken literally, but must be interpreted in the light of the method used in determining this coefficient. If this method is that of the hygienic laboratory, it means that this preparation under the conditions of that test is twice as germicidal as phenol *on the bacillus typhosus, or a particular strain thereof*. No conclusions should be drawn as to its germicidal action on the staphylococcus, streptococcus or any other organism, not excepting the bacillus coli, or paratyphosus. Naturally the closer the chemical relation between the disinfectant and phenol, and the closer the bacteriological relation of the organisms concerned in

the comparison, the more nearly correct will be any conclusion drawn from such a test. On the other hand, it is only rational to suppose that disinfecting agents of widely different character will vary greatly in their germicidal relation to phenol when tested on organisms of different genera or species. Since the advent of synthetic dyes in preparing differential culture media and later the production of diarseno-benzol, the specificity of drugs for certain microorganisms should not be lost sight of. Chick and Martin (1) recognize this specificity of disinfectants when they say, "Some disinfectants are more efficient against a particular organism than against any other." Churchman's (2) work was quite definite in showing the selective affinity of gentian violet for certain organisms. In the main he could divide bacteria into two classes, those which were inhibited in their growth by very weak dilutions of this dye and those which were unaffected by even much stronger solutions. As a rule closely related bacteria reacted similarly to gentian violet but he found a marked exception to this in one strain of *B. enteritidis*. This strain was indistinguishable by ordinary cultural and staining methods from four other strains of *B. enteritidis*, yet it was completely inhibited by weak dilutions (1:80,000) of gentian violet, whereas the other four were not. Churchman states that this instance is significant because it establishes the fact that chemical substances may be so specific in their selective affinity for microorganisms as to distinguish among strains of bacteria otherwise

indistinguishable; and further that it indicates to what extent our ideas of bactericides must be modified by the conception of chemical affinity, and that the fact that a given bactericide kills a given organism does not justify the conclusion that it will kill all closely related organisms, or even all strains of the same organism.

Browning and Gilmour (3) examined a number of organic and inorganic compounds for their bactericidal action toward different species of bacteria, with a view to discovering—(a) substances possessing specific bactericidal properties for particular organisms and—(b) relationships between chemical constitution and bactericidal action. They found *staphylococcus aureus* and *B. anthracis*, organisms more resistant to phenol than is *B. typhosus*, less resistant than the colityphoid group of organisms to the action of certain basic benzol derivatives among which may be mentioned fuchsin, hexamethyl violet, methylgreen, malachite-green and brilliant green. The amount of hexamethyl violet required to inhibit *B. typhosus* was 150 times that required for *staphylococcus aureus*. Furthermore these authors state that whereas the action of malachite green shows no marked difference between its effect on *B. typhosus* and *B. coli*, brilliantgreen exerts a more marked bactericidal action on *B. coli* than on *B. typhosus*. In other words, the bactericidal action of a disinfectant on one organism is not necessarily a measure of its action on another, even when these organisms belong to the same genus or closely related group.

Another very interesting feature of Browning and Gilmour's work is that they found the presence of serum to increase the antiseptic action of flavine for both staphylococcus and *B. typhosus*. This is certainly contrary to the action of serum on the effect of any other class of disinfectants and should be taken into consideration when giving them a valuation or phenol coefficient.

Flavine is an example of a valuable disinfectant which if gauged by its phenol-coefficient would be practically worthless. Pine oil, on the contrary, is an example of one which, based on its phenol coefficient, should be of considerable importance, but which in practice is deficient in some respects as a general disinfectant. This deficiency was brought to my attention after the issuance of the hygienic laboratory's report (4) on pine oil as

an efficient liquid disinfectant when I was asked by a dentist how long a time would be required to sterilize instruments in this Pine Oil Disinfectant. The Pine Oil Disinfectant was made by incorporating 62.5 per cent. of pine oil in soap according to the directions for making the "Hygienic Laboratory Pine Oil Disinfectant." The phenol coefficient of this solution was determined and found to be 3.8. Several different samples of pine oil purchased on the open market varied in their phenol coefficient. This variability of pine oil was mentioned by Hamilton (5) before this association last year.

In testing the ability of this Pine Oil Disinfectant to sterilize dental or other instruments, small steel blades (scarifiers),  $\frac{1}{2}$  x  $3\frac{1}{2}$  centimeters were contaminated with various organisms and subjected to the action of the dis-

TABLE I.  
CULTURES OF STAPHYLOCOCCUS AUREUS—DRIED.  
RECENTLY ISOLATED CULTURES.

Time	2% pine oil disinfectant	4% pine oil disinfectant	5% phenol	Water control
5 minutes	+ + +	+ + -	+ - -	+ + +
10 minutes	+ + +	+ + +	- - -	+ + +
15 minutes	+ + +	+ + +	- - -	+ + +
30 minutes	+ + -	+ + +	- - -	+ + -
1 hour	+ + -	+ + -	- - -	+ + +
3 hours	- - -	- - -	- - -	+ + +
20 hours	- - -	- - -	- - -	+ + +
OLD STOCK CULTURES.				
5 minutes	+ + +	+ + +	- - +	+ + +
10 minutes	+ + +	+ + +	+ + -	+ + +
15 minutes	+ + +	+ + +	- - +	+ + +
30 minutes	+ + +	+ + +	- - +	+ + +
1 hour	- - +	+ + +	- - -	+ + +
3 hours	- - -	+ + -	- - -	+ + +
20 hours	- - -	- - -	- - -	+ + +

infectant, using phenol as a control, in the following manner:

EXPERIMENT I.

Twenty-four hour broth cultures of recently isolated and old stock cultures of staphylococcus aureus were each used to inoculate 84 metal scarifiers. Both lots were placed in sterile petri-dishes to dry over night.

With sterile distilled water, 2 per cent. and 4 per cent. emulsions of Pine Oil Disinfectant and 5 per cent. solution of phenol were made. Sterile distilled water was used as control. Five per cent. phenol was taken because it is the strength commonly used in sterilizing instruments.

The scarifiers were placed in the above solutions and also in sterile water as a control, 21 in each, and allowed to remain for periods of 5-10-15-30 minutes, 1 hour, 3 hours, and

over night. Those placed in the Pine Oil Disinfectant were removed, 3 at the end of each period of time, washed in sterile water and placed in fermentation tubes of broth.

Those in 5 per cent. phenol were removed and washed in 95 per cent. alcohol before placing in fermentation tubes. Those in sterile water were placed directly in fermentation tubes without washing.

After 24 hours' incubation, transplants were made from the fermentation tubes to agar slants. The results are given in Table I in which the plus sign indicates growth of the organisms. The three columns following each interval of time represent the three scarifiers used in each solution.

By the hygienic laboratory method of testing disinfectants, Pine Oil Disinfectant would be classed as having 4 times the germicidal power of phenol.

TABLE II.  
CULTURES OF STAPHYLOCOCCUS AUREUS—NOT DRIED.  
RECENTLY ISOLATED CULTURES.

Time	2% pine oil disinfectant	4% pine oil disinfectant	5% phenol	Water control
5 minutes	+ + +	+ + +	- - -	+ + +
10 minutes	+ + +	+ + +	- - -	+ + +
15 minutes	+ + +	+ + +	- - -	+ + +
30 minutes	+ + +	+ - -	- - -	+ + +
1 hour	+ + -	+ + -	- - -	+ + -
3 hours	+ - -	- - -	- - -	+ + +
18 hours	- - -	- - -	- - -	+ + +
OLD STOCK CULTURES.				
5 minutes	+ + +	+ + +	+ - -	+ + +
10 minutes	+ + +	+ + +	- - -	+ + +
15 minutes	+ + +	+ + -	+ - -	+ + +
30 minutes	+ + +	+ + +	- - -	+ + +
1 hour	+ - -	- - -	- - -	+ + +
3 hours	- - -	- - -	- - -	+ + +
18 hours	- - -	- - -	- - -	+ + +

From the above experiment in which staphylococci were used as test organisms, Pine Oil Disinfectant, 2 per cent. or even 4 per cent. does not destroy the organisms in one hour; whereas they are usually killed by 5 per cent. phenol within 15 minutes. Another striking point brought out in Table I is that Pine Oil Disinfectant, 4 per cent., is no more effective than the 2 per cent. dilution.

In order to check these results a second series was carried out differing from the former only in that the inoculated scarifiers were not dried before placing them in the disinfectant solutions and that the fermentation tubes were incubated 48 hours before transplants were made from them.

These results confirm those of Experiment I, and again show that Pine Oil Disinfectant diluted with water to make a 4 per cent. mixture or emulsion is little, if any, more effective than the 2 per cent. emulsion.

Other similar tests were made using *B. typhosus*, *streptococcus pyogenes*, and *B. diphtheria* and showed that in practically all instances the test organisms were killed within 15 minutes by the disinfectants employed; and that of the four bacteria used in the experiments, the staphylococcus alone stood out as being disproportionately resistant to pine oil solutions in soap.

Pine oil cannot, then, be termed a general disinfectant of high germicidal value, for although showing to advantage when tested on the typhoid bacillus, it is much less efficient than phenol or cresol when the infecting organism is the staphylococcus aureus. This is another instance of the fallacy

of the present generally accepted method of determining the value of disinfectants, namely, the so-called hygienic laboratory method. The physician or dentist who does not know the intricacies or inaccuracies of the present methods of testing or standardizing disinfectants is justified in thinking that a phenol coefficient of 4 means that the preparation is four times as active as phenol, and that a 1 or 2 per cent. solution of it will sterilize his instruments within 5 or 10 minutes. He is further justified in reposing confidence in the phenol coefficient of a disinfectant because the method of determining this is sanctioned by the government and by the Council on Pharmacy and Chemistry of the American Medical Association. One state requires that disinfectants must have the phenol coefficient on the label, and before a disinfectant will be admitted to New and Non-Official Remedies, this same condition must be complied with. Defects of this method have been repeatedly pointed out, but have been apparently disregarded by its advocates and entirely overlooked by others interested in having it made a legal method for standardizing disinfectants. Hamilton and Ohno (6) have shown that this method in different hands gives widely varying results when testing the same disinfectant on the same strain of typhoid organism. If comparable results cannot be obtained when the same organism is used, one would certainly expect a disinfectant to vary widely in germicidal power when tested on different organisms. In fact, Anderson and McClintic, the

originators of the Hygienic Laboratory Method, say (7) "Unless different observers use the same species of organism, there can be no possibility of uniformity in results. The coefficient obtained with different species may vary as much as 300 per cent." If this be the case, what is the practical value of the phenol coefficient as determined by the Hygienic Laboratory Method? Certainly only a small proportion of any commercial disinfectant is employed to destroy typhoid bacilli.

In the case of pine oil, we have an agent considerably more germicidal than phenol when acting on the typhoid bacillus, and very much less active than phenol on the staphylococcus. This proves conclusively that the comparative disinfectant value of pine oil and phenol cannot be determined by any one test.

Other defects in the Hygienic Laboratory Method have been pointed out. Duyser and Lewis (8) call attention to three factors which cause unreliable results. As stated they are "(a) The use of an excessive number of bacteria depletes the disinfecting solution before the culture is rendered sterile. (b) An unknown volume is withdrawn for testing, in that the volume of the standard loop is not constant. (c) It is impossible to determine from the broth tube inoculated, how complete the killing was at the time the sample was withdrawn." Duyser and Lewis showed that the so-called standard loop, which is supposed to transfer a constant amount of culture from a tube or flask to the disinfectant, varies under the best

conditions at least 30 per cent. from the average, and when not very carefully used may vary as much as 80 per cent. This inaccuracy of the standard loop was verified by A. D. St. John (9) who was able to obtain with the same loop a variation of 300 per cent. by slowly withdrawing the loop edgewise and quickly withdrawing it parallel with the surface. The lack of uniformity of different loops in the hands of different bacteriologists can readily be appreciated.

So that, as a matter of fact, the stating of the phenol coefficient on a label may be misleading and, instead of informing the public as to the value of any particular agent, may misinform and give a false sense of security. At the same time it would afford ample opportunity for litigation and a wide difference of opinion among experts.

The value of any method of testing disinfectants has been well stated by Phelps (10), "The results of any disinfection experiments are fundamentally influenced by such conditions as temperature, character of the organism employed, number of organisms in unit volume, and character of the medium. In the absence of complete data covering these points results are practically worthless, at least for purposes of comparison. Even with such data given, it is still impossible owing to the variable conditions obtaining in practice, to establish any relationship, or order of excellence, among the various disinfectants. At best we can only hope to establish such relationship under specified experimental conditions." He further says "It seems at present to be quite

necessary to determine the relative germicidal values of two disinfectants upon the actual kind of germ upon which they are to be employed and to qualify the final comparative results accordingly." In no case has the practical value of this dictum been so clearly exemplified as in the present investigation. Based on the report of Stevenson from the Hygienic Laboratory entitled "An Efficient Liquid Disinfectant," one would be led to think that "Pine Oil Disinfectant" is 4 to 6 times as effective a germicide as phenol, without any exceptions. That this is not the case has been clearly shown by the preceding experiments and contrary to the statement in the above report, Pine Oil Disinfectant cannot be satisfactorily used to replace the ordinary coal tar compounds commonly employed as disinfectants.

There is another phase of the question brought up by these discrepancies, namely, legalizing the Hygienic Laboratory Method as a standard method under which prosecutions may take place. That this is not desirable is readily understood when the inaccuracy of the method is considered. Almost every factor concerned is variable and is difficult or impossible to control. The temperature, the media, the number of bacteria used, the change in resistance of the organisms, and the technique are some of these factors already mentioned. These have been well discussed in the paper of J. T. Ainslee Walker (11).

Aside from these considerations, the true value of a disinfectant can only be stated in terms of the organism or organisms on which it is to be used in actual practice. To state that Pine Oil Disinfectant is "an efficient liquid disinfectant" which "may be used wherever the ordinary coal tar compounds are used" is certainly drawing erroneous conclusions from a phenol coefficient determination.

The paradoxical action of pine oil in being markedly more germicidal than phenol on the typhoid bacillus and decidedly less germicidal on the staphylococcus is an interesting phenomenon deserving further study and is apparently illustrative of specificity in disinfectants.

#### REFERENCES.

1. CHICK and MARTIN, Jr. *Hygiene*, 1908, 8, 654.
2. CHURCHMAN, Jr. *Exper. Med.*, 1912, 16, 221 and 822.
3. BROWNING and GILMOUR, Jr. *Path. and Bact.* 1913, 18, 144.
4. STEVENSON, *Public Health Reports*, 1915, 30, 3004.
5. HAMILTON, Am. Jr. *Public Health*, 1917, 7, 282.
6. OHNO and HAMILTON, Am. Jr. *Public Health*, 1914, 4, 486.
7. ANDERSON and McCLINTIC, Jr. *Infectious Diseases*, 1911, 8, 1.
8. DUYSER and LEWIS, Jr. *Industrial and Engineering Chem.*, 1914, 6, 198.
9. ST. JOHN, Jr. *Industrial and Engineering Chem.*, 1914, 6, 940.
10. PHELPS, Jr. *Infectious Diseases*, 1911, 8, 27.
11. WALKER, N. Y. *Medical Jr.*, 1916, 103, 500.