A Study of the Stuart Method for the Evaluation of Germicides

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The evaluation of germicides has been the subject of considerable controversy. At the present time, the phenol coefficient method is the official procedure of the Association of Official Agricultural Chemists (1950) for the evaluation of water-soluble germicides. However, Klarmann and Wright (1946), McCulloch (1947), DuBois (1947), McCulloch, Hauge, and Migaki (1948), and Stuart, Boguskey and Friedl (1950) have questioned the accuracy of the results that are obtained when the phenol coefficient method is used for the testing of germicides differing in chemical and physical properties from those of phenolic compounds, such as the quaternary ammonium compounds. Other investigators (Stuart, 1947; Mallman and Leavitt, 1948) have criticized the phenol coefficient method on the basis that the conditions under which this method is carried out do not parallel those of actual use of the germicides, especially where it is desired to disinfect contaminated surfaces. Because of this difficulty with the phenol coefficient method, there has been considerable interest in the so-called "use dilution" methods for the evaluation of germicides.

In the studies described in this paper, the use dilution method of Stuart, Ortenzio, and Friedl (1953), designated as the Stuart Method, was selected as the procedure to be compared with the official phenol coefficient method as it has been accepted as a "first action" method by the Association of Official Agricultural Chemists. In this procedure, the use concentration is determined by that concentration which will disinfect the surfaces of 10 stainless steel penicillin assay cylinders in 10 minutes. The steel cylinders are contaminated by dipping in a broth culture of the test organism and dried before use.

The specific objective of this study was to compare the Stuart method with the official phenol coefficient method by using various types of germicides and test organisms. In addition, studies were also made of factors that might affect the reproducibility of the Stuart method.

MATERIALS AND METHODS

Germicides

Three quaternary ammonium germicides and three germicides representative of phenolic, cresylic, and pine oil types were tested. The quaternaries were Rocal, an alkyl dimethyl benzyl ammonium chloride and two detergent sanitizers, designated "A" and "B". The detergent sanitizers contained 1.5 per cent alkyl dimethyl benzyl ammonium chloride as the active ingredient, a non-ionic detergent, and different proportions of inorganic buffer salts to provide different degrees of alkalinity. A 1.33 per cent solution (200 ppm of the active ingredient) of detergent sanitizers A and B gave pH values of 10.3 and 11.4, respectively. The phenol was reagent grade, mp 39-41 C. The cresylic germicide, Lysol, contained cresylic acid, o-phenylphenol, and soap as active ingredients. The pine oil disinfectant contained pine oil, soap, and isopropanol as active ingredients.

The test concentrations of the quaternaries were selected in the range of those recommended by the manufacturer. The phenol test concentrations corresponded to the phenol resistance values specified for each test organisms prescribed in the official phenol coefficient method of the Association of Official Agricultural Chemists (1950) and in the Stuart method. The test concentrations of the cresylic and pine oil disinfectants were determined by multiplying the Salmonella typhosa phenol coefficient at 20 C claimed on the label by 20. These dilutions were expressed in terms of parts per million.

Test Organisms and Media

The test organisms used were Salmonella choleraesuis ATCC 10708, specified by Stuart et al. (1953); Salmonella typhosa Hopkins 26, specified in the official phenol coefficient method; and Microoccus pyogenes var. aureus FDA 209, specified in both methods. The cultures were maintained as prescribed in the official phenol coefficient method. Fresh broth cultures of S. choleraesuis and S. typhosa were made from the stock cultures after 28 consecutive daily transfers in FDA broth. A fresh broth culture of M. pyogenes var. aureus was made from the stock culture after 21 consecutive daily transfers in FDA broth.

Letheen broth was used for the subculture medium.

1 Sterwin Chemicals, Inc., New York, N. Y.
2 Experimental preparations kindly furnished by Economics Laboratory, Inc., St. Paul, Minnesota.
3 Lehn and Fink, Inc., Bloomfield, N. J.
4 Sergeant Chemical Co., Newark, N. J.
with all of the germicides that were tested. Both lacteal broth and FDA broth were prepared according to the procedure given in the official phenol coefficient method. A single lot of peptone and beef extract was used in the preparation of the media used in these studies.

Methods

The Stuart method was performed according to the procedure of Stuart et al. (1953). The drying time of the inoculated stainless steel cylinder carriers was standardized at 15 minutes at 37°C instead of the 10- to 60-minute period prescribed in the original method with the exception of those studies pertaining to the effect of drying time. The official method of the Association of Official Agricultural Chemists (1950) was used for the determination of phenol coefficient values and use concentrations. All tests were performed at a temperature of 20°C.

In determining the use concentration of a given germicide, the Stuart method and the phenol coefficient method were performed on the same day, with the same culture of the test organism, and the same lot of subculture medium. Replicate determinations were performed on different days. Goetichius and Grinsfelder (1953) found that the day-to-day variations of phenol coefficient test results were no greater than the variations between replicate determinations made on the same day.

Results and Discussion

Comparison of the Use Concentrations Obtained with Each Method

For a number of years it has been generally accepted that the dilution of a germicide equal to 20 times its S. typhosa phenol coefficient at 20°C provided a use concentration with a suitable margin of safety for the disinfection of contaminated surfaces (Varley and Reddish, 1936). However, Stuart et al. (1953) presented results which indicated that such a use dilution might not be a safe guide for use in the disinfection of contaminated surfaces by certain disinfectants.

A comparison of the use concentrations obtained by the Stuart method, using the three test organisms, with those calculated by multiplying the experimentally determined and claimed S. typhosa phenol coefficients at 20°C by 20 is presented in Table 1. These data reveal that the Stuart method use concentrations of the three quaternary ammonium germicides obtained with S. choleraesuis as the test organism were less than the corresponding values calculated from the S. typhosa phenol coefficients. Although S. typhosa was not specified for use as a test organism in the Stuart method, tests were made with this organisms in order to determine if there would be any appreciable differences in the results as compared with those obtained with S. choleraesuis. The results show very little difference between the use concentrations of the quaternaries obtained by the Stuart method with either S. choleraesuis or S. typhosa as the test organism.

None of the use concentrations of quaternaries indicated to be safe by the S. typhosa phenol coefficient could be confirmed by the Stuart method when M. pyogenes var. aureus was used as the test organism. In every case, higher concentrations were required in order to obtain the Stuart method end point. Stuart et al. (1953) stated that none of the quaternary ammonium germicides tested during their studies killed either S. choleraesuis or M. pyogenes var. aureus at the dilutions indicated to be safe by the S. typhosa phenol coefficients claimed or found. This difference in results when S. choleraesuis was used in our studies does not appear to be caused by the detergents in the detergent sanitizers, because similar results were obtained with Rocal, a pure quaternary.

In the case of phenol, the cresylic germicide, and the pine oil disinfectant, the Stuart method use concentrations agreed quite closely with the S. typhosa phenol coefficient use concentrations when S. typhosa was used. However, considerably higher use concentrations of these germicides were obtained with S. choleraesuis as compared with the values calculated from the phenol coefficients.

Stuart et al. (1953) stated that a 48-hour FDA broth culture of S. choleraesuis should have a phenol resistance equivalent to that of a 24-hour FDA broth culture of S. typhosa. However, it was found that S. choleraesuis had a greater phenol resistance than S. typhosa when maintained according to the procedure given in the official method of the AOAC. This increased phenol resistance accounts for the higher use concentrations of phenolic and related germicides that are secured by the Stuart method with S. choleraesuis as the test organism.

With phenol, the cresylic germicide and the pine oil,

<table>
<thead>
<tr>
<th>Germicide</th>
<th>Stuart Method Use Concentration ppm</th>
<th>Phenol Coefficient Use Concentration* ppm</th>
<th>Experimental</th>
<th>Claimed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. typhosa choleraesuis</td>
<td>S. typhosa choleraesuis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detergent sanitizer A</td>
<td>10500</td>
<td>10500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detergent sanitizer B</td>
<td>10250</td>
<td>10250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>10750</td>
<td>10750</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* S. typhosa phenol coefficient × 20 expressed as ppm.
disinfectant, much greater use concentrations were obtained by the Stuart method with \textit{M. pyogenes} var. \textit{aureus} as compared with the other test organism. Here again, the greater phenol resistance of this organism than that of the \textit{Salmonella} may account for this difference.

In this study, reproducible use concentrations were obtained with the Stuart method. The results shown in table 1 represent an average of a minimum of two replications in the case of phenol and a maximum of five replications in the case of Roccal. However, in an independent study carried out at another laboratory (W. G. Mizuno, 1953, private communication) the minimum use concentrations of detergent sanitizers A and B were 15000 ppm when \textit{S. choleraesuis} was used as the test organism. The corresponding average values obtained in this study were 13733 ppm and 11333 ppm, respectively with a range of 1333 ppm between the maximum and minimum values in each case. No comparable tests were made with phenolic, cresylic, or pine oil disinfectants. These results suggest that the Stuart method is not as reproducible as might be desirable for a test to be used for regulatory purposes.

\textbf{Effect of Drying Time of Inoculated Carriers on the Use Concentrations Obtained with the Stuart Method}

Mallman and Leavitt (1948) found that \textit{S. typhosa} was destroyed by drying on glass rods for longer than one hour while little destruction of \textit{M. pyogenes} var. \textit{aureus} took place under the same conditions. In order to determine the effects of drying time of the inoculated carriers on the use concentrations obtained with the Stuart method, the procedure was carried out in the same manner as in the preceding studies except that carriers inoculated with \textit{S. choleraesuis} and \textit{M. pyogenes} var. \textit{aureus} were dried for 10 minutes and 60 minutes in a 37 C forced circulation incubator. The quaternary ammonium germicide Roccal and phenol were used as test germicides. The results are shown in table 2. Two replicate determinations were made, and identical results were obtained in each test.

When \textit{S. choleraesuis} was used as the test organism, there was a decrease in the Stuart method use concentration of both Roccal and phenol when the drying time of the carriers was increased from 10 minutes to 60 minutes. This indicates that some destruction of \textit{S. choleraesuis} may take place when long drying times are used. However, with \textit{M. pyogenes} var. \textit{aureus} as the test organism, there were no differences in the use concentrations of either Roccal or phenol when the carriers were dried for 10 minutes or 60 minutes.

\textbf{Effect of Drying Time on the Numbers of Viable Organisms Recovered from Inoculated Carriers}

Since the drying time of the inoculated carriers used in the Stuart method may affect the use concentration obtained, a study was undertaken to determine the effect of drying time on the number of viable organisms that could be recovered from these inoculated carriers. This information should give an indication of the size and constancy of the inoculum transferred on a single carrier.

The carriers were inoculated with either \textit{S. choleraesuis} or \textit{M. pyogenes} var. \textit{aureus} as prescribed in the Stuart method. Groups of inoculated carriers were dried for 10, 15, and 60 minutes, respectively, in a 37 C forced circulation incubator. At the end of the drying period, each carrier was shaken for 10 minutes in each of four successive sterile tubes containing 10 ml of a 1000 ppm solution of the non-ionic detergent Glim. A reciprocating shaker was used. A 1 ml aliquot was removed from each tube on completion of shaking, diluted in sterile water, and plated with tryptone glucose extract agar (Difco). The plates were incubated for 48 hours at 37 C. Counts were expressed as the number of organisms per tube. A sample of the broth culture of the test organisms was also diluted and plated to determine the number of organisms per ml of broth culture.

The results given in table 3 show that only slight destruction of \textit{S. choleraesuis} took place when the drying time of the inoculated carriers was increased from 10 minutes to 15 minutes. The results obtained by the Stuart method should not be affected to any appreciable extent by this increase in drying time. However, when a 60-minute drying time was used, the total recovery was 19990 organisms as compared with 164500 and 144150 after 10-minute and 15-minute drying times, respectively. Since this organism is destroyed to a considerable extent over a 60-minute drying period at 37 C, the inoculum transferred by the carriers would be greatly decreased in size. These results substantiate those given in table 2. When \textit{S. choleraesuis} was used as the test organism, an increase in drying time from 10 minutes to 60 minutes resulted in a decrease of the Stuart method use concentration.

The results obtained with \textit{M. pyogenes} var. \textit{aureus} show slight increases in the total number of viable organisms recovered after 15-minute and 60-minute

\textbf{TABLE 2. Effect of drying time of inoculated carriers on use concentrations obtained with the Stuart method}

<table>
<thead>
<tr>
<th>Germicide</th>
<th>Concentration in Parts per Million</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{Salmonella choleraesuis}, drying time</td>
</tr>
<tr>
<td></td>
<td>10 min.</td>
</tr>
<tr>
<td>Roccal</td>
<td>220</td>
</tr>
<tr>
<td>Phenol</td>
<td>55555</td>
</tr>
</tbody>
</table>

\footnote{B. T. Babbitt, Inc., New York, N. Y.}
Table 3. Effect of drying time on the number of viable organisms recovered from inoculated carriers

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Number of Organisms per Tube*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella choleraesuis†</td>
<td>Micrococcus pyogenes var. aureus‡</td>
</tr>
<tr>
<td></td>
<td>10 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>1</td>
<td>160000</td>
<td>140000</td>
</tr>
<tr>
<td>2</td>
<td>3800</td>
<td>3200</td>
</tr>
<tr>
<td>3</td>
<td>590</td>
<td>700</td>
</tr>
<tr>
<td>4</td>
<td>210</td>
<td>220</td>
</tr>
<tr>
<td>Total recovery…</td>
<td>164500</td>
<td>144120</td>
</tr>
</tbody>
</table>

* Volume of suspension per tube, 10 ml.
† Initial number of organisms per ml of broth culture: S. choleraesuis, 4.6 × 10⁷; M. pyogenes var. aureus, 6.1 × 10⁶.

Drying times. In this case, this organism was not destroyed to any detectable extent by drying at 37 C for 60 minutes. The size of the inoculum was not significantly different after either 10-minute or 60-minute drying times. Again these results confirm those given in table 2.

General Comments on the Stuart Method

As compared with the official phenol coefficient method, the Stuart method gives quite different results in certain cases. Considerably greater use concentrations of all of the germicides tested than the corresponding values determined from the S. typhosa phenol coefficient were obtained when M. pyogenes var. aureus was used, as compared with the results obtained with the Salmonella species. The Stuart method measures the effectiveness of a germicide in the penetration and killing of organisms in a dried film on a metal surface rather than determining the concentration required to kill organisms in a liquid suspension, as in the phenol coefficient test.

Certain precautions must be taken with the Stuart method that do not enter into the phenol coefficient method. The stainless steel carriers must be carefully maintained in order to prevent damage to the surfaces. When S. choleraesuis is used as the test organism, the inoculated carriers should not be dried any longer than 15 minutes at 37 C. Longer drying times result in considerable destruction of this organism, which will result in lower use concentrations. It is also recommended that one single drying time in the 10-minute to 15-minute range be used for all determinations.

One definite disadvantage of the Stuart method is the increased time and effort required to secure an equivalent amount of information to that furnished by the phenol coefficient method. A minimum of 45 minutes is required to perform the test for one concentration of the germicide. Eight concentrations of the germicide and two phenol control dilutions can be tested by the phenol coefficient method in the same period of time.

The Stuart method would be of value in the confirmation of phenol coefficient values of germicides that are to be used for disinfection where pyogenic organisms such as M. pyogenes var. aureus are of significance. In these applications, an extra margin of safety is desirable. However, for routine disinfection of surfaces where thorough cleaning of the surface to be disinfected is practiced, the values obtained with the official phenol coefficient method should provide a sufficient margin of safety.

Summary

The use dilution method of Stuart, Ortenzo, and Friedl (1953) was compared with the official phenol coefficient method in the evaluation of quaternary ammonium, phenolic, cresylic, and pine oil germicides. The Stuart method use concentrations of the quaternary ammonium germicides were approximately equivalent to those determined by multiplying the Salmonella typhosa phenol coefficient at 20 C by 20, when either Salmonella choleraesuis ATCC 10708 or Salmonella typhosa Hopkins 26 was used as test organism. Greater use concentrations of the phenolic, cresylic, and pine oil germicides were obtained by the Stuart method with S. choleraesuis as the test organism than with S. typhosa. The Stuart method use concentrations of all germicides tested with Micrococcus pyogenes var. aureus FDA 209 were greater than the values calculated from the S. typhosa phenol coefficient.

When S. choleraesuis was used as the test organism with the Stuart method, an increase of the drying time of the inoculated carriers from 10 minutes to 60 minutes resulted in a decrease in the number of viable cells that could be recovered. The use concentrations were correspondingly decreased. A 10-minute to 15-minute drying time is recommended when this test organism is used. Some advantages and limitations of the Stuart method are also discussed.

REFERENCES

Studies on the Efficiencies of Disinfectants for Use on Inanimate Objects

III. Physicochemical Factors Affecting Surface Disinfection

R. L. STEDMAN, E. KRAVITZ AND H. BELL

Industrial Test Laboratory, Philadelphia Naval Shipyard, Naval Base, Philadelphia, Pennsylvania

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In Part II of this series (Stedman, Kravitz and Bell, 1954b), it was shown that the antimicrobial activities of disinfectants vary widely when materials of different porosities are employed as test surfaces. In general, higher concentrations of disinfectants are required to disinfect porous surfaces (battleship linoleum and asphalt tile) than are needed on nonporous (stainless steel). In addition, it was reported that a profound difference occurs with various formulations of phenolics in the degree of retention of antimicrobial activity when changing from a nonporous to a porous test surface. It was suggested that physicochemical factors might play a significant role in the disinfection of porous surfaces.

This report describes a study in which certain physicochemical properties of disinfectants were compared with the degree of retention of bactericidal activity of these agents on changing the porosity of the surface. The relationships of surface tension depression, wetting, and detergency to antibacterial activity have been studied in the past by a number of workers. In general, it is the opinion of many investigators that low surface tension may contribute to bactericidal activity but that it is not solely responsible for effective antimicrobial action (for a summary of pertinent references see Lawrence, 1950). Likewise, a relationship has been shown to exist between bactericidal activity and immersional wetting in a series of homologous alcohols (Cowles, 1940). On the other hand, no correlation was found between detergents activity and bactericidal efficiency in a series of colamino formyl methyl pyridinium chlorides (Epstein, Harris and Katzman, 1943). In these studies, however, conventional survivor curves or bactericidal efficiency tests were employed to obtain antimicrobial data. In the present investigation, it was desired to study the relationship, if any, of these physicochemical factors to differences in disinfectant performance as obtained previously with a simulated use test method.

Experimental Methods and Results

The bactericidal activities of the disinfectants were determined by the performance test method described in Part I of this series (Stedman, Kravitz and Bell, 1954a). In all instances, the dilutions of formulation (in the case of phenolics, cresylic, coal tar, and detergent-sanitizer products) or of active ingredients (quaternary ammonium germicides) required to achieve 99.99 per cent reduction of Micrococcus pyogenes var. aureus in the presence of 10 per cent normal horse serum at 20°C are given as end points, since these were the conditions under which the profound differences in degree of retention of activity had been encountered. All other test conditions were identical with those employed in Parts I and II. In addition to the products designated and described in Parts I and II, four other products were studied in the current phase of the work.

A soluble type phenolic product, containing a mixture