When the kinetic energy is neglected, the per cent error is clearly too high, and the correction should therefore be applied.

Figure 3 gives the new flow line for this run after the kinetic energy correction has been applied. The points which did not lie on the uncorrected flow line now fall on it or are closer to it. Inasmuch as the kinetic energy correction is appreciable for the first half of the points during a run, the slope of the new flow line is steeper, while the shearing stress intercept is changed only slightly; hence the values for mobility are now higher while the yield values are changed only by a small amount.

If it were necessary to make this kinetic energy correction for each point in each run, the calculations would be too long and the procedure too complicated for control work. Therefore, a capillary is selected which has a diameter small enough to make the rate of flow so low that the kinetic energy correction is negligible. In some of our runs the flow was 1.0 to 0.75 cc. per sec. during the first half of the runs. When the initial rate of flow is not greater than 0.25 cc. per sec. the kinetic energy correction becomes negligible, *i. e.*, about 1.5% at the start and then rapidly decreasing.

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STUDIES ON THE STANDARDIZATION OF GERMICIDES.*[1]

(PRELIMINARY REPORT.)

BY WM. NYIRI, M.D.[2]

Disinfection is one of the main branches of hygiene. To employ chemical disinfection successfully we have to know the properties and, first of all, the efficiency of the different germicidal agents. The standardization of germicides is thus the basis of the subject. This procedure is carried out by exposing bacteria to chemical agents of different strengths and by observing the time necessary to damage or destroy these organisms. The task may appear, at first sight, rather simple as bacteria are organisms representing a low form of life with rather well-known biological characteristics. The experimental conditions would be, therefore, apparently in our hands. Closer familiarity with the subject teaches, however, that there are considerable difficulties to overcome. When we expose bacteria to the action of disinfectants we have to deal in the main with three factors: Germicides, bacteria and culture medium. The strength of the disinfectant we can change readily, but it is not easy to control the experimental conditions presented by the bacteria. We find not only differences in the vitality of the various types of bacteria and great contrasts between spores and vegetative forms, but there are also considerable differences in resistance depending upon the age of the culture. Even within the same culture the vitality of the individual organism may vary

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from day to day. In addition to the above, bacteria become more resistant in adapting themselves to the disinfectant. The temperature of exposure plays, of course, an important rôle, as does the density of the bacterial suspension. The presence of organic matter also greatly influences the efficiency of many disinfectants. The third factor, the culture medium is also likely to cause many variations; thus liquid media differ from solid and present wide variations within the group. The H-ion concentration must also be taken into account. The neutralization of the germicide, whether accomplished or omitted, after finished exposure may have a marked influence on the culture, as even traces of certain disinfectants, if transferred with the bacteria can cause inhibition of growth. Even differences in methods used to overcome the above difficulties introduce elements of variation (garnet-, thread-method, watery suspension, etc.). Additional difficulties are presented by the object aimed at by the investigator, whether inhibition of growth or bacterial death is desired as criterion of the efficiency of the disinfectant, and by the methods used in reading of the results; gross inspection of the culture, counting of surviving bacteria and animal inoculation tests must be rated differently in interpreting results.

The two outstanding methods for the standardization of disinfectants are the *Rideal-Walker Test* and the *Hygienic Laboratory Method.* Both have been in vogue for years. In both of these methods a suspension of *B. typhosus* is exposed to different concentrations of the germicide in question during varying periods of time, and the results of this experiment are compared with those obtained by the action of phenol under the same working conditions. Both tests, especially the more accurate Hygienic Laboratory Method, are of great value and have yielded many important results. A disadvantage of these methods is, that the result (phenol coefficient) expresses a relation between the efficiency of the disinfecting agent in question and phenol, which is used as standard. It is obvious that such data is only of comparative merit as phenol and whatever agent may be selected as standard, are themselves disinfectants and thus open to criticism. The phenol coefficient as a numerical expression shows nevertheless a tendency to accuracy.

Several attempts have been made to express the efficiency of disinfectants by graphic methods, but only Reichel has succeeded in accomplishing a practical result with his "curves and equations of efficiency." From experimental data Reichel was able to establish a certain relationship between Concentration (P) of germicide and Time (T) of exposure necessary to kill a vigorous strain of typhoid bacillus. With these factors he constructed an equation of efficiency: $T.P^n = R$. He calls R the "resistance constant." The graphic expression of this equation is a hyperbolic curve in which R governs the general position of the curve in the coördinate system, while the exponent n is the expression of a more or less marked curvature. The exponent is at the same time responsible for the asymmetric position of the curve in the coördinate system. The advantage of this formula is that it gives us an expression of the efficiency of a germicide agent in absolute terms.

This constitutes an advance over the comparative value of the phenol coefficient. An additional advantage is the following: Given a certain concentration of the germicide we may obtain from this equation the time necessary to kill ty-

phoid bacilli, or, vice versa, we may calculate the concentration of the germicide if the time of action is given. The usefulness of this formula for standardization of disinfectants has appealed to us, and we have made it the point of departure of the following investigations.

Proceeding from the fact that various types of bacteria show different resistance to the action of disinfectants, we exposed several kinds of microörganisms to the germicide, varying both concentration and time. By this means we expected to obtain several curves and equations of efficiency. For every one of the bacterial types we should obtain, of course, two results, *i. e.*, the action of the agent without and in the presence of organic matter. We believe that these several curves and equations all referring to the same disinfectant should characterize this agent rather definitely and independently and should yield more information than does a single equation. These considerations are the basis of our studies.

We have, to date, employed two germicides, phenol and tincture of iodine. We have started with phenol because it has been used in such a large number of previous investigations and is characterized by a rather definite disinfecting action. Our second choice, tincture of iodine, was made on account of the manifold uses of this substance and further on account of its extraordinary potency. The alcohol content of tincture of iodine compels us to take this substance into consideration. Among the microörganisms we selected representatives of different groups; *B. typhosus*, for the coli group; *staphylococci* for the cocci; *B. pyocyaneus* for the dye-producing bacteria and *B. subtilis* for the spore-bearing group, the latter instead of anthrax. *B. diphtheriae*, *streptococci* and *pneumococci* were also studied as test material, but proved too variable in their vitality and too sensitive for the purpose of this investigation. *B. pneumoniae* as an example of the capsule-bearing organisms should properly be included among the types under study, and this will be done in continuation of this work. The above-mentioned five types of bacteria have been selected because they facilitate uniformity of working conditions on account of their luxurious growth on broth.

Each curve and equation expresses the results of several similar experiments. The deviations of individual experiments are insignificant. Up to the present we have obtained the following equations for phenol:

B. typhosus without organic matter ..... $T.P^{5.6} = 3.5$
B. typhosus with organic matter ..... $T.P^{4.8} = 4$
Staphylococcus pyogenes aureus without organic matter ..... $T.P^{4.9} = 12$
Staphylococcus pyogenes aureus with organic matter ..... $T.P^{4.4} = 24$
Pyocyaneus without organic matter ..... $T.P^{5.7} = 3.6$
Subtilis without organic matter ..... $T.P^{5.2} = 0.8$

The corresponding results for tincture of iodine obtained thus far are:

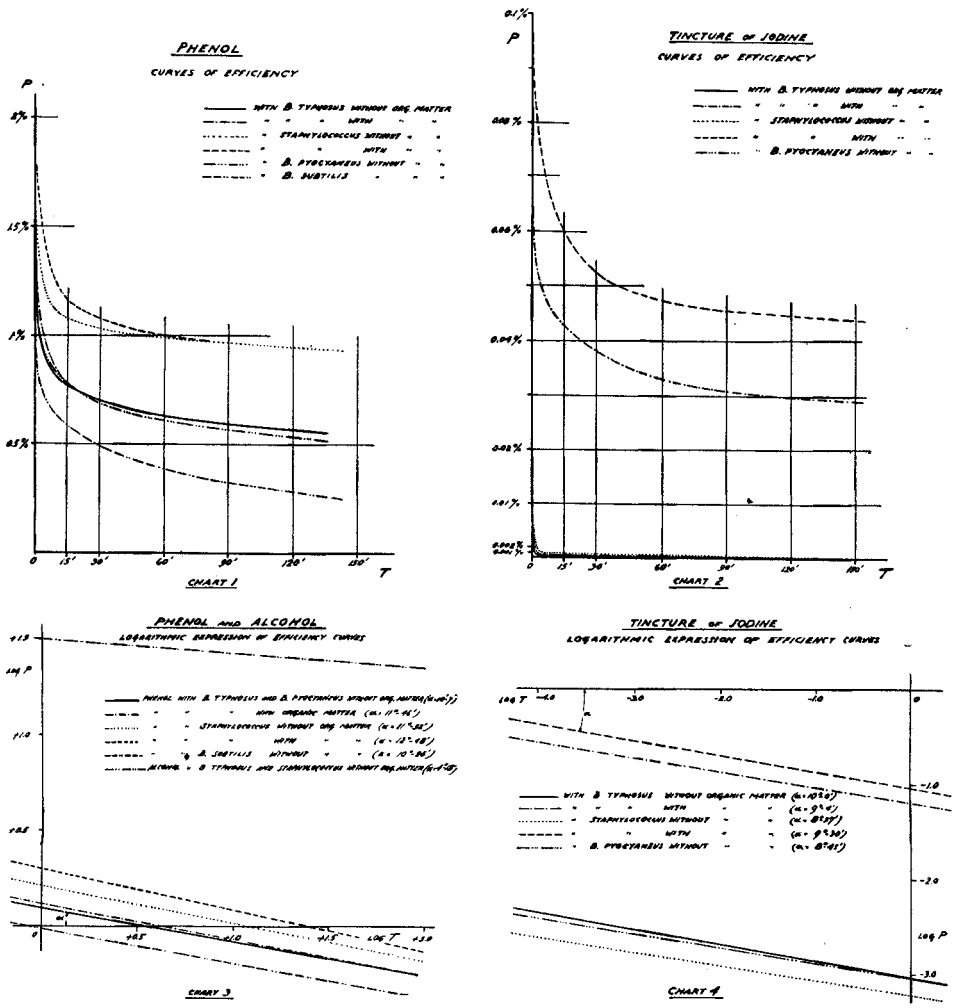
B. typhosus without organic matter ..... $T.P^{6} = 0.82.10^{-18}$
B. typhosus with organic matter ..... $T.P^{6.3} = 0.96.10^{-7.5}$
Staphylococcus pyogenes aureus without organic matter ..... $T.P^{6.6} = 0.74.10^{-21}$
Staphylococcus pyogenes aureus with organic matter ..... $T.P^{6} = 0.61.10^{-6}$
B. pyocyaneus without organic matter ..... $T.P^{5.6} = 8.2.10^{-19.5}$

Alcohol yields with *B. typhosus* and *Staphylococcus* both without organic matter the following equation:

$$T.P^{12.3} = 1.2.10^{+20}$$

The following charts illustrate the curves corresponding to the above equations:

The logarithmic lines to these hyperbolic curves were drawn and seem to yield interesting results as well. They show that all the iodine lines cross the left negative quadrant, while the phenol lines are to be found almost exclusively in the right positive quadrant. Alcohol, which was studied only for its effect on typhoid and staphylococci, results in a log. line far higher on the positive side than the other two agents. These lines illustrate clearly the very high efficiency



of iodine against the four different organisms and the marked loss of its power in the presence of organic matter. They show the different efficiency of iodine, phenol and alcohol. It is noteworthy that the lines of each disinfectant run approximately parallel to each other. It is also remarkable that the logarithmic lines of phenol ($\alpha = 10°$–$12°48'$) almost parallel with those of iodine ($\alpha = 8°\ 37'$ to $10°$), while the slope of the alcoholic line is more gradual ($\alpha = 4°18'$). (The variations of the H-ion concentration were found practically identical whether

the experiment was carried out with phenol, iodine or alcohol.) An explanation of this behavior cannot be given at present. Further work may show its significance.

These investigations are still incomplete and considerable work remains to be done. They promise, however, that the efficiency of germicides may be determined by means of several equations. The equations obtained for each individual disinfectant are expected to show certain similarities which in turn characterize this particular agent independently of standards chosen at random. We have thus far collected data for phenol, tincture of iodine and some for alcohol. Future work should supplement these results and new data on other disinfectants should be collected. We also hope that sufficiently numerous investigations carried out along these lines will give results which may contribute to advance our knowledge of the theory of the action of germicides.

CONTRIBUTION FROM RUTGERS UNIVERSITY,
RESEARCH LABORATORIES OF THE N. J.
COLLEGE OF PHARMACY.

SILVER-ION CONCENTRATION STUDIES OF COLLOIDAL SILVER GERMICIDES. II—CHANGES IN THE SILVER-ION CONCENTRATION OF SOLUTIONS ON STANDING.

BY RALPH B. SMITH.

A method was developed in the first paper of this series[1] for determining the silver-ion concentration of colloidal silver preparations. It seemed desirable to extend the measurements of silver-ion concentrations to solutions of colloidal silver germicides which had stood varying lengths of time; a series was made up and a set of measurements made over a period of 16 months. In order to detect small variations in silver-ion concentration it is necessary to have a method which will give very stable readings. The ordinary methods of plating and short circuiting pure silver wire electrodes, which will give reliable values in concentrations of $N \times 10^{-3}$ or stronger, will not give sufficiently stable readings when the concentration drops to 10^{-6} mols per liter. After much experimentation a method was developed which gives stable and reproducible readings at any silver-ion concentration. The electrode used is an 8-inch piece of No. 14 B & S gage, pure silver wire which is used without cementing into a glass tube, as has been common practice in the past. The electrode is placed in boiling 1% KCN solution for 10–15 minutes and then carefully removed and rinsed without touching any portion of the electrode which will later come in contact with the solution to be tested. The electrode is then stored for at least 15 hours in a solution similar to the one to be tested. It is probable that irregularities on the surface of the electrode are the cause of the unstable readings when the ordinary method of preparation is used but the KCN treatment seems to remove all such difficulties.

The solutions for these tests were made up in 1 per cent concentration for the products of the strong type and in 10% concentration for the other products.

[1] JOUR. A. PH. A., 14 (1925), 10.