IN THE LABORATORY

False Disinfection Velocity Curves Produced By Quaternary Ammonium Compounds

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Quaternary ammonium compounds are being offered as disinfectants for the medical professions, as bactericides for the sanitization of eating utensils and drinking glasses, and as germicides for general use. The more widely used commercial bactericides of this class are Hyamine 1622 (p-diisobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride monohydrate) and the apparently identical or very similar Phemerol (p-tert-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride monohydrate); Roccal and the identical or closely related Zephiran; Emulsept; Q.A.C.; and Teramine.

Before recommending to the State Department of Health that these compounds be approved as bactericides, additional studies of their efficiencies and limitations were made. When tested by the official Food and Drug Administration method for evaluating antiseptics and disinfectants (*3*), all appear to justify the very high "phenol coefficient" values of 250 to 625 against *Staph. aureus* (*1*) and approximately one-half as great against *E. typhosa* (*2*). The end points obtained when the F.D.A. method was used were very irregular, and plate counts of bacterial populations exposed to quaternary ammonium compounds revealed a very high initial velocity of disinfection:

$$K = \frac{I}{\text{time}} \cdot \log \frac{\text{initial number of organisms exposed}}{\text{survivors}}.$$

This was followed by a rapidly decreasing value of K. When the logarithms of the survivors, as determined by plate counts, were graphed against time, very steep curves were obtained for the first few minutes. The data presented in Fig. 1 are typical. In some instances, particularly when the bactericidal activity was tested in the presence of some additional organic material such as milk, the plate-count numbers again increased after several days and proteolytic decomposition of the milk took place.

Experimentally, it has been found that the presence of sterile evaporated milk allows the growth of organisms exposed to solutions of quaternary ammonium compounds, while parallel inoculations into broth fail to grow.

The shape of the death-rate curve may be somewhat influenced by the rapid removal of the quaternary ammonium compound from the solution. When used in the dilutions their phenol coefficient numbers would indicate them to be efficient bactericides, these compounds are rapidly removed from solution by particulate or organic materials. Also, the hypothesis is advanced that the very high phenol coefficient numbers previously reported for the quaternary ammonium compounds reflect the sum of the agglomeration of the organisms, and their bacteriostatic action in addition to their bactericidal action. These compounds are extremely surface-active cationic detergents, and the clumping of treated organisms, with their adhesion to glass surfaces, has been observed experimentally.

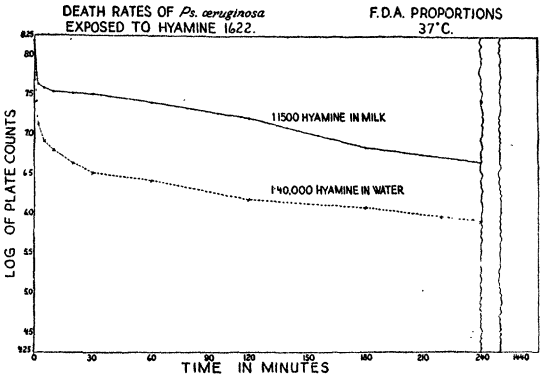

FIG. 1

In tests by the F.D.A. technic, the 1/100-ml. loop might not pick up a clump of agglomerated organisms which tend to adhere to the surface of the tube; if picked up, such a clump might remain adherent to the loop when it was immersed in the broth subculture; and if the coated and agglomerated organisms were placed in broth, in which no additional particulate material was present, they might remain in a state of bacteriostasis and fail to give rise to growth. In the case of plate counts in agar, many viable organisms in one clump would give rise to only one colony and hence would be read as only one viable inoculum.

Since it appears that the tendency of organisms to agglomerate and adhere to surfaces, following exposure to dilute solutions of the quaternary ammonium compounds, has influenced the end points obtained with the F.D.A. method and the apparent velocity of disinfection when plate counts were made, both the bactericidal efficiencies and the very favorable toxicity indices of these compounds must be re-evaluated.

In some clinical applications it is possible that a surface-active bacteriostat, of very low toxicity to tissues, may be more desirable than an active bactericide which decreases tissue vitality. Also, it has yet to be demonstrated whether viable organisms coated with quaternary ammonium compounds are infective or are readily destroyed by the natural defense mechanisms of the body.

The data thus far obtained indicate that, when employed in sufficient concentrations and in the absence of large amounts

of particulate or organic materials, the quaternary ammonium compounds probably do disinfect.

References

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The Maillard Reaction in Microbiological Assay[1]

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In 1912 Maillard (*1*) pointed out that solutions containing amino acids and reducing sugars turn brown when heated. Although this browning reaction has recently been recognized to be of the greatest importance in nearly all industries concerned with materials of biological origin, including foods, it is still little understood because of its complexity. It occurred to the writers that further insight into the Maillard reaction might be gained through use of the microbiological assay technique, since the latter involves the autoclaving of solutions containing amino acids and dextrose (a reducing sugar) under conditions favorable to the Maillard reaction.

Accordingly, the customary casein hydrolysate medium was prepared for tryptophane assay with *Streptococcus faecalis* in such a way that the reducing sugar component of the medium (dextrose) could be autoclaved separately from the rest of the medium for comparison with a "normal" series, using medium containing identical ingredients but autoclaved in the presence of dextrose in the customary manner.

The growth curves obtained are shown in Fig. 1. Good growth was obtained by the standard procedure (Curve A), but still better growth was obtained in the medium prepared by autoclaving the dextrose separately from the rest of the medium and combining aseptically after cooling (Curve B). These results have been repeated and are reproducible.

It thus appears likely that optimal growth may seldom be reached in microbiological assays, due to the effects of the Maillard reaction. This would not be discovered in recovery tests, since both standard and unknown series would be autoclaved alike. The question arises, however, whether this apparent accuracy of the microbiological assay can always be relied upon, in view of the possibility that standard and unknown samples might differ in their ability to alter the influence of the Maillard reaction upon growth.

Since sucrose is not a reducing sugar and does not readily take part in the Maillard reaction, it was thought that sucrose might be preferable to dextrose, if satisfactory growth were obtained in a medium containing sucrose. Accordingly, a third series was prepared and run simultaneously with the assays shown in Fig. 1. In this C. P. sucrose replaced the dextrose ordinarily used but at one-half equimolar concentration (final concentration of sucrose in the medium, 1/12 M). Here again the growth obtained was better than "normal"; in fact, the growth curve obtained with sucrose in the medium was identical with Curve B (Fig. 1).

A certain amount of browning during autoclaving is commonly accepted as being inevitable in microbiological assay; however, our media using either sucrose autoclaved with the medium or dextrose autoclaved separately were *water white*. Compared with these, the normal medium transmitted 84.5 per cent as much light at 610 mμ, the decrease in transmission being due to the browning which occurred during autoclaving.

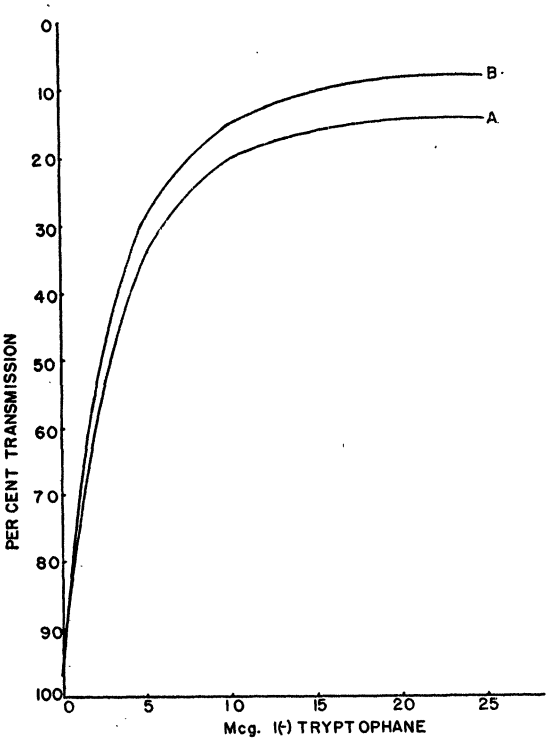

Fig. 1. Growth curves for tryptophane assay using *Str. faecalis*.

Whether the poorer growth obtained by the customary procedure is due to destruction of nutrients or production of growth inhibitors has not yet been determined. The Maillard reaction produces an increase in acidity, and the possibility that this may be responsible for the reduced growth has not been overlooked. The fact that the greatest differential in growth occurs at the higher growth levels lends support to this possibility. The actual difference in acidity due to browning was rather slight, however, amounting to a difference of only 0.96 ml. of 0.1 N acid in the uninoculated blank tubes, which was responsible for a difference in pH of only 0.2 (Curve A medium, pH 6.96; Curve B medium, pH 7.16).

The possibility that these results might be a peculiarity of the tryptophane assay, due to destruction of tryptophane during autoclaving, was ruled out by another series of assays. In these, graded levels of l-tryptophane solution were autoclaved *separately* before adding to the autoclaved media.

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