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TECHNICAL MANUSCRIPT 554

EFFECT OF SMALL AMOUNTS OF SODIUM HYPOCHLORITE ON GROWTH OF EARLE'S L CELLS IN SUSPENSION CULTURES

William C. Jordan
William F. Daniels
Luis H. Garcia
John F. Rosensteel

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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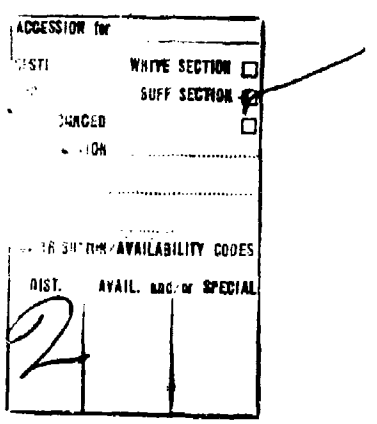

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William C. Jordan

William F. Daniels

Luis H. García

John F. Rosensteel

Process Development Division
AGENT DEVELOPMENT AND ENGINEERING LABORATORIES

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ABSTRACT

Of several candidate disinfectants for use in tissue culture work, especially suspension cultures, sodium hypochlorite (NaOCl) solution was selected to test its effect on growing cells. Metabolizing cells reduce, NaOCl oxidizes; therefore NaOCl leakage into such systems must be neutralized with no untoward effects on the cells.

Dilutions of routine disinfectant-grade NaOCl were tested against cell cultures. Those exposed to 16 to 31 ppm of NaOCl grew with no apparent cell damage.

I. INTRODUCTION*

In past years, several germicides and disinfectants have been employed in our laboratory for sterilizing surfaces of glassware and growth vessels for tissue culture. Most of these have been objectionable or faulty for one reason or another.

As one example, 70% ethyl alcohol solution was used for sterilizing inoculating ports on New Brunswick fermentors, centrifuge spinner bottles, T-60 flasks, and rubber stoppers. Its use posed a fire hazard in enclosed hoods and confined areas.

Formaldehyde was also objectionable; the residual vapors and deposits were so toxic that even a slight amount contacting the cells destroyed them. Thus, this material could be used only on large-scale equipment where the effect of possible leakage into the cell-fermentor system would be minimized by dilution.

Such experiences led us to test a dilute sodium hypochlorite (NaOCl) solution as a germicide for tissue culture work. NaOCl is an oxidizing agent, and cells are reducing in nature when actively growing. The reducing ability of the cells should counteract any of the oxidizing agent leaking into the system, with minimal effect on the propagation of the cells. To test this thesis, we carried out the simple experiments reported here.

II. MATERIALS AND METHODS

The fermentor glassware and all other materials were prepared in the usual manner for tissue culture glassware, assembled, and steam-sterilized for 45 minutes at 250 F.

Two liters of our modification** of Eagle's minimal essential medium for spinners were prepared and fortified with 10% bovine serum. The oxidation-reduction potential of the medium was then stabilized at +75 mv in the manner presented elsewhere;*** the pH ranged from 6.9 to 7.0.

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** Rosensteel, J.F.; Jordan, W.C.; Daniels, W.F. 1969. Growth potential of a variation of Eagle's minimum essential medium for spinner cultures. Biotechnol. Bioengr. 11:263-266.

*** Daniels, W.F.; Fish, D.C.; Garcia, L.H.; Rosensteel, J.F.; Dobbs, J.P. A comparison between tissue cells grown in culture media poised and non-poised by oxidation-reduction potential manipulation. In preparation.

Into each of four spinner bottles, made up from 250-ml centrifuge bottles, enough 5% NaOCl was added to give final concentrations of 16, 31, 63, or 125 ppm when 100 ml of culture were added. Four bottles were used per dilution. A vent with spinner bar was added as described previously.* One hundred milliliters of culture seeded at a count of 1×10^5 cells/ml were then added, and the flasks were placed into the incubator at 37 C. Samples of 2 ml were removed daily and the cells were counted. Four spinner bottles without NaOCl were controls.

III. RESULTS

Initial tests with concentrations up to 2,000 ppm revealed that the cells were not permanently injured and could recover if the NaOCl were removed in time. From these initial concentrations, 16, 31, 63, and 125 ppm were selected to study the effects when the NaOCl was not removed, and the cells were permitted to remain until they had run their normal course of growth compared with controls over a like period.

Figure 1 compares the treatments and controls for pH, per cent viability, and growth curves of the averages for a period of 10 days. From the curves one can deduce that the cells may tolerate between 31 and 63 ppm of NaOCl in the medium with no untoward effects other than a suppressed growth curve compared with the control.

The higher count and faster growth rate of the control could be accounted for by any of several reasons. Therefore, the experiments were carried out further by comparing again the 16 and 31 ppm with controls. The results are shown in Figure 2. Here the control and 16 ppm curves are essentially the same. The 31 ppm treatment, although suppressed at first, ended with a higher count.

No morphological differences were noted in comparing the cells from the controls with those from the treated cell cultures.

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* Daniels, W.F.; Garcia, L.H.; Rosensteel, J.F. September 1969. Oxidation-reduction potential and concomitant growth patterns of cultures of Earle's L cells in centrifuge spinner bottles, (Technical Manuscript 550). Process Development Division, Fort Detrick, Frederick, Maryland.

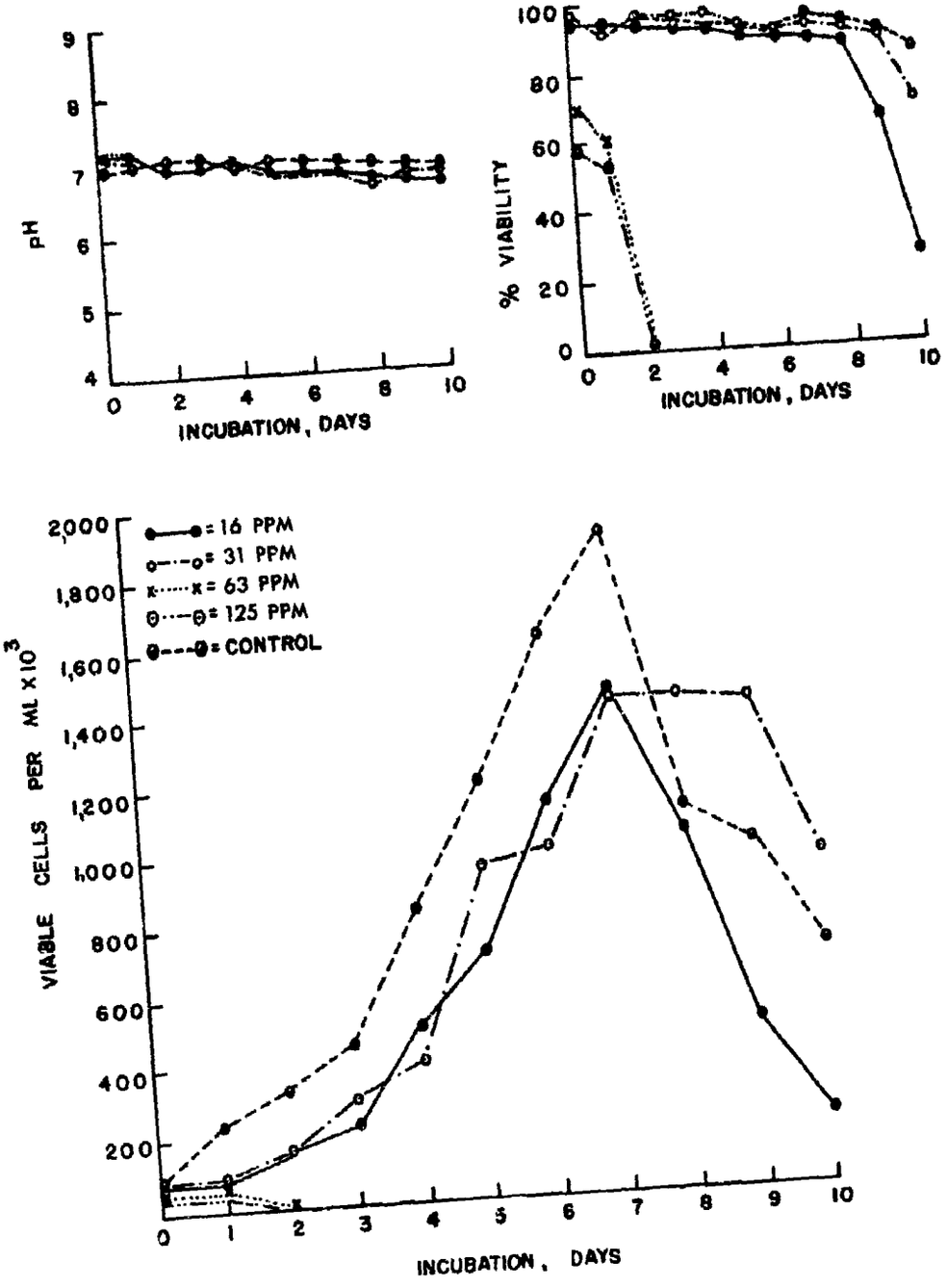

FIGURE 1. Growth Characteristics of Cells in 16 to 125 ppm Sodium Hypochlorite.

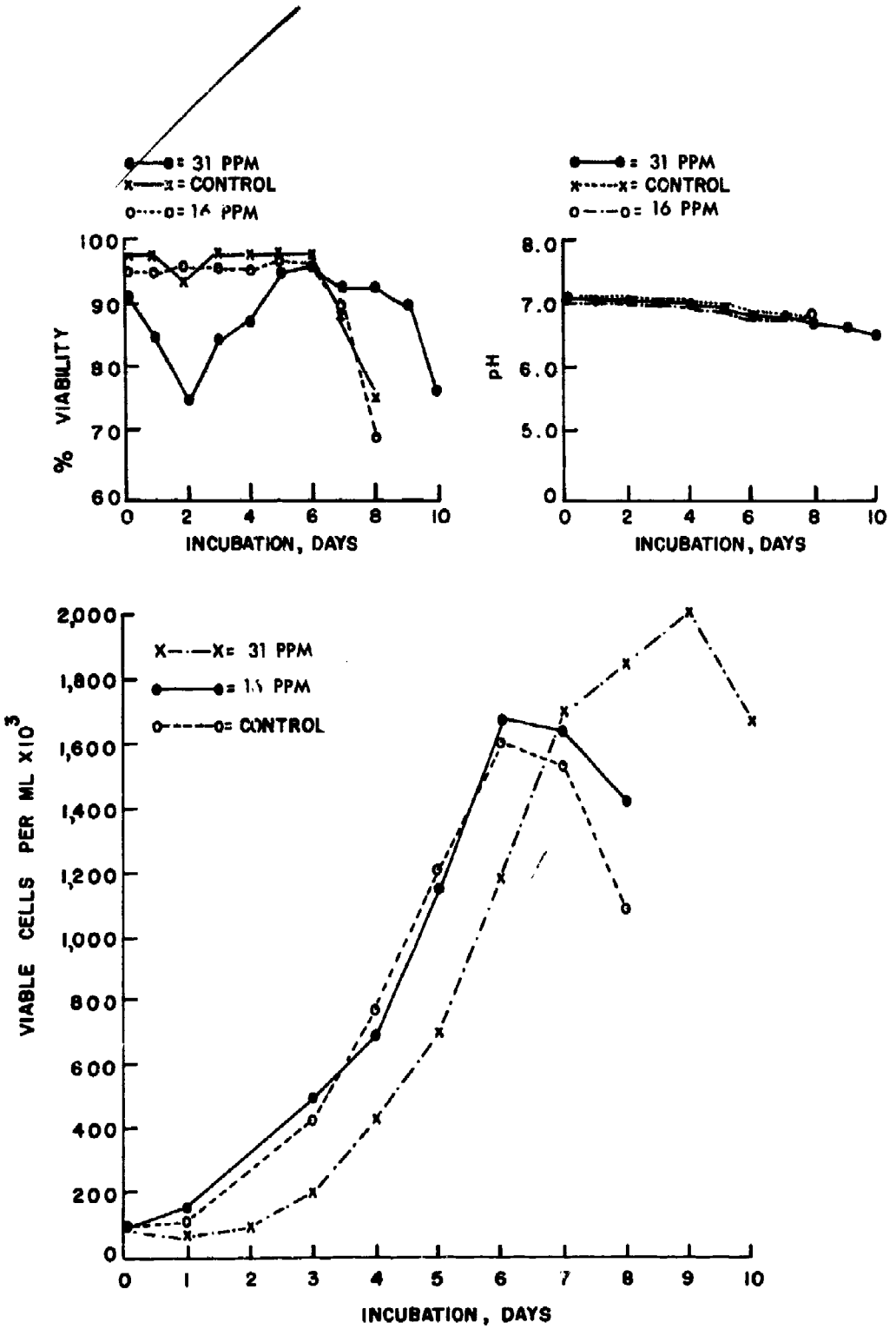

FIGURE 2. Growth Characteristics of Cells Grown in 16 to 31 ppm of Sodium Hypochlorite.

IV. DISCUSSION

From its experience with NaOCl, the Industrial Health & Safety Directorate of Fort Detrick found the following data:* 500 to 5,000 ppm** of NaOCl solution for 5 minutes is enough to kill spores. Vegetative forms of bacteria such as Salmonella, Brucella, and Pasteurella are killed by a concentration of 200 to 1,000 ppm for 1 minute. By these standards, 1,000 ppm of NaOCl can easily be employed to disinfect equipment or materials used in tissue culture work.

An extension of the above application could be the sterilization of such materials as freshly excised tissue membranes. It seems feasible to wash such tissue in a concentration of 2,000 ppm of freshly prepared NaOCl solution for 2 to 5 minutes to kill all forms of bacteria associated with the tissue in its normal habitat. To free the tissue of all the NaOCl, it could be washed with sterile saline solution. Germ-free cultures could probably be obtained by achieving asepsis with NaOCl solutions. An example would be the isolation of cells from human amnion tissue in a sterile state. At 2,000 ppm, 2 minutes of exposure time seem practical for general decontamination. This technique, however, would not free cells from intracellular contamination.

Any of the concentrations that have been mentioned can be utilized to decontaminate external surfaces; the amount of leakage into a vessel would not likely achieve toxic concentrations.

V. CONCLUSIONS

Sodium hypochlorite shows promise as a disinfectant for use in tissue culture systems.

In the event of inadvertent small leakage into a fermentor system, one should not fear toxic or harmful effects to cell growth.

We found no noticeable differences in morphology or cell growth patterns between cells exposed to NaOCl and unexposed controls.

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* Phillips, G.B.; Hanel, E., Jr.; Gremillion, G.G. March 1962. Practical procedures for microbial decontamination, (Technical Manuscript 2). Safety Division, Fort Detrick, Frederick, Maryland.

** Active chlorine.

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William C. Jordan John F. Rosensteel
William F. Daniels
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14. Key Words

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