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SEP 79 A J SINSKEY, R F GOMEZ

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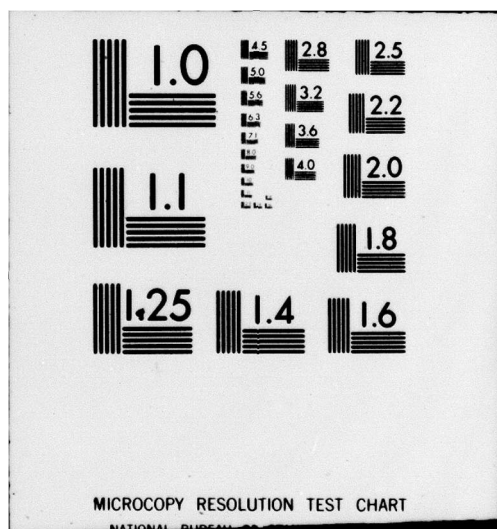
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(C) MECHANISM OF HEAT AND IRRADIATION SYNERGISM.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This research program was undertaken to investigate the mechanism(s) by which bacterial spores can be sensitized to heat by gamma radiation treatments. The study employed spores of the public health significant anaerobic microorganism <u>Clostridium perfringens</u>. Based on biological, chemical and structural evidence, a model was developed to outline the mechanisms of synergism. It is proposed that radiation creates		

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chemical lesions on the spore cortex which result in the loss of ability of this structure to maintain a dehydrated core. Hydration of the core would then result in an increased sensitivity of vital macromolecules found there.

1. Foreword

The following final report is for report work done under contract no. DAAG 29-76-C-0034 and covers all findings made during the contract period, April 15, 1976 to July 14, 1979. All work reported here falls under the general heading of "Mechanism of Heat and Irradiation Synergism".

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3. Body of Report

A. Statement of the Problem Studied

The phenomena of heat resistance had been extensively studied in many bacterial spores, and numerous theories have resulted, including suggestions that heat resistance is due to calcium content, dipicolinic acid, intrinsic heat resistance of spore components, and water exclusion by the spore. Each theory has been tested and found to be less than completely satisfactory.

The phenomena of spore radiation resistance has received less attention, and far fewer theories have been suggested and tested. To date we do not know with certainty which properties of the spore are responsible for this resistance, although it appears that disulfide or cysteine-rich structures in the spore are at least partially responsible. In addition, we do not know which sites, having been affected by irradiation, lead to the spore's inactivation. However, it does appear that there are multiple targets, and that they are not localized in any one area of the spore.

The phenomena of radiation sensitization to heat has been studied least extensively. Consequently, little is known about it as compared to component treatments. Although several researchers have acknowledged the existence of radiation sensitization to heat, few have tried to study or explain the mechanism involved.

Thus our research concentrated on developing basic and model systems for investigating the kinetics and mechanisms of heat and irradiation synergism in spores of Clostridium perfringens. To this end we have established an experimental system which has yielded results consistent with our current theories. In addition, we have developed a unique way of handling DNA from microorganisms, and this

technique has been applied to the study of the effects of heat and ionizing radiation on the DNA of Escherichia coli. The detailed account of our progress is to be found in the following manuscripts and theses based on our results.

B. Summary of Results

Our findings regarding the mechanisms of heat and irradiation synergism indicate:

(1) that C. perfringens spores are sensitized to heat if first treated by gamma radiation

(2) that the sensitization is reversed by high osmotic pressures during the heat treatments

(3) that radiation causes the release of calcium ions from the spores and reduces the retention of both calcium and dipicolinic acid during heat treatments

(4) that spores exposed to gamma radiation suffer structural degradation. These effects include collapsed surfaces as observed by scanning electron microscopy and fragmentation of the core as observed in thin sections by transmission electron microscopy.

Based on these results and current theories on the heat resistance of spores we have constructed the following working hypothesis. We propose that gamma irradiation affects the architecture of the spore so that it loses its ability to maintain a dehydrated state in the spore core. This state could be brought about, for example, by crosslinking of the peptidoglycan components of the cortex. Such chemical change would decrease the expansion properties of the cortex and allow at least partial rehydration of the core, thus increasing the heat sensitivity of vital macromolecules in the core.

C. List of Theses and Publications

Theses

- Gombas, Dave E. 1977. Sensitization of Clostridium perfringens to Heat by Gamma Radiation. Master of Science Dissertation.
- Ulmer, Kevin M. 1978. Rate Zonal Density Gradient Ultracentrifugation Analysis of Repair of Radiation Damage to the Folded Chromosome of Escherichia coli. Doctoral Dissertation.
- Pellon, J.R. 1979. Effect of Heat Treatments on the Folded Chromosome of Escherichia coli. Master of Science Dissertation.

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Gomez, R.F. and D.E. Gombas. 1979. Reversal of Sensitization
of Clostridium perfringens Spores to Heat by Gamma Radiation.
Submitted to Appl. Environ. Microbiol.

D. Participating Scientific Personnel

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