

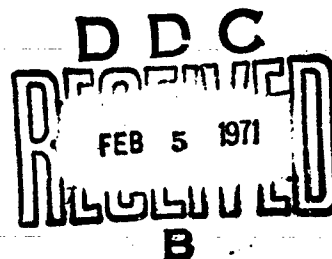
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## FINAL REPORT

### EFFECT OF SALMONELLA ENDOTOXINS ON LYMPHOCYTE CULTURES GROWN IN THE ECOANALYZER

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Work Unit No. NR 136-787

Contract N00014-68-C-0226

## OBJECTIVES

(a) To characterize the endotoxins produced at pH 4.2 and at pH 7.0 biochemically and serologically, and (b) to assay the relative toxicities of these two endotoxin preparations on lymphocytes.

## ABSTRACT

Two distinct endotoxins are produced by Salmonella typhimurium, one at pH 7.0 and the other at pH 4.2, as determined by gel precipitin reactions and by biochemical analyses. pH 4.2 endotoxin lacks heptose that is present in pH 7.0 endotoxin, and the 2-keto-3-deoxyoctonate moiety of endotoxin has been modified in a fashion that causes displacement of the usual absorption peak for this structure.

The pH 4.2 endotoxin is much less toxic for mice than is the pH 7.0 endotoxin. Under tissue cell culture conditions, swine buffy coat cells and human lymphocyte RPMI 1788 cell line cells showed cytopathic effects from 5 µg/ml endotoxin in the culture medium. pH 7.0 endotoxin was clearly more toxic at this dose level than was pH 4.2 endotoxin, as pH 7.0 endotoxin was lethal to exposed lymphocytes, at 48 hours, at which time 4.2 endotoxin showed only slight cytopathic effects on the cells.

## PLANS FOR FUTURE

Research on the effects of environment on the immunochemical properties of S. typhimurium endotoxin has been completed under this contract. A request for research support on our program of growth of normal human lymphocytes in the Ecoanalyzer has been submitted to ONR.

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Summary Report:

A. Effect of pH of culture medium on physiology of Salmonella typhimurium.

1. In nutrient broth at pH 4.2, S. typhimurium is barely able to survive, as indicated by growth curve studies; whereas at pH 7.0 this organism has a generation time of about 20 minutes.
2. Physiological processes of S. typhimurium are markedly impaired at pH 4.2, with consequent loss or reduction of various characters, such as flagella formation, diagnostic antigens, and enzyme systems responsible for H<sub>2</sub>S production, glucose fermentation, and virulence factors.
3. pH 4.2 grown S. typhimurium produced less than 10% dry weight yield of endotoxin as that of pH 7.0 grown cells.

B. Modification of tertiary structure of S. typhimurium endotoxin by pH 4.2 grown cells.

1. Specific antisera to the two endotoxin preparations were prepared in rabbits, and were used to demonstrate that the pH 4.2 endotoxin contained one determinant that was antigenically different from the pH 7.0 endotoxin, by gel precipitin analyses. A common antigenic determinant was shared by the two endotoxins.
2. Biochemical analyses on the two endotoxin preparations gave the following data:
  - a. Both pH 4.2 and pH 7.0 endotoxins have nearly the same nitrogen content (0.2%) and phosphorus content (1.8%), but pH 4.2 endotoxin lacks heptose sugar. Both endotoxins contain galactose, glucose, rhamnose, and mannose, pH 7.0 endotoxin also contains heptose.
  - b. 2-keto-3-deoxyoctonate (KDO) with normal absorption at 548 nm is present in pH 7.0 endotoxin, but with pH 4.2 endotoxin, the KDO absorption peak is displaced to 530 nm. This seems to indicate a modification of KDO structure in pH 4.2 endotoxin.
3. The total carbohydrate in pH 4.2 endotoxin is only 50% of that in a similar weight of pH 7.0 endotoxin.

C. Comparison of toxicities of pH 4.2 and pH 7.0 endotoxins.

1. At a concentration of 5 µg/ml, both pH 4.2 and pH 7.0 endotoxins were lethal to tissue cultured swine lymphocytes, but the pH 7.0 endotoxin produced cytotoxicity within 24 hours, whereas the pH 4.2 endotoxin required 48 hours for production of similar cytotoxicity. At comparable dosage, a similar pattern in lethal effects on mice was produced, with pH 7.0 endotoxin causing rapid death, and pH 4.2 endotoxin producing delayed death.
2. At less than the lethal dosage, both endotoxins acted as partially effective immunogens; subsequent injection with virulent strains of S. typhimurium resulted in survival of test mice for much longer periods than normal mice were able to live after exposure to this pathogen. Apparently, the endotoxins are only part of the pathogenicity pattern of S. typhimurium, as injection of pH 4.2 grown cells confers solid immunity on test mice, rather than the partial immunity produced by the endotoxins.

- D. Ecoanalyzer (computerized continuous cell culture apparatus) research is still in the developmental stage. A description of the current status of this program is presented in the research grant application recently submitted to ONR.

#### PUBLICATIONS

Christopher, John. 1971. Biological and chemical characteristics of endotoxins produced under different cultural conditions. Ph.D. Thesis, Iowa State University, Ames, Iowa.

A manuscript for publication of this thesis research is in preparation.

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