

Quarterly Report

- I. Contract Number: N00014-90-C-0042
- II. Project Title: An Endotoxin Binding Protein for Treatment of Septic Shock
- III. Grantee: Norman R. Wainwright, Associates of Cape Cod, Inc. *Wainwright*
- IV. Grant Period: 16 OCT 1989 to 15 OCT 1992
- V. Quarter Covered by this Report: 16 OCT 1989 to 31 DEC 1989
- VI. Summary of Progress to Date:

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In the first quarter of this project, our primary goal was to purchase the equipment and supplies necessary to assemble the scaled-up extraction process for the endotoxin binding protein. Two large, 150 liter, stainless steel tanks were fitted with sterile connections to serve as pyrogen free buffer reservoirs. Now, buffers are prepared in advance to fill the 150 liter polypropylene extraction tank with its motor driven propeller. To date, one half of the frozen cell debris has been extracted in 6 Molar Urea and clarified by diatomaceous earth filtration.

A housing and membrane cassettes for ultrafiltration have also been purchased and used to filter the clarified extract. We have processed all of the extract to date through the 30K dalton filter and concentrated it with a 8K dalton filter. *(See report)*

The first step in chromatography has been scaled-up to a 1500 ml cation exchange bed in a radial flow column. The processed, concentrated extract has been applied and eluted from this column and found to be biologically active. One problem was noticed with a batch of resin which was stored in 0.2 Molar NaOH. The apparent column capacity was diminished 40-50 percent. This result is being confirmed and we may have to decrease the concentration of NaOH for longer term resin storage.

VII. Projections for Next Quarter

We will process the remainder of the cell debris through extraction, clarification and cation exchange. The cation exchange column purified protein will be stored in 3 Molar Urea, 1 M NaCl at 4 degrees Celsius. Next quarter we will begin the second column purification step over the C-4 reversed phase column.

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Norman R. Wainwright
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Scientific Officer. *1/10/90*
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