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Electroporation of Bacteria and Yeast: An Experimental Study of Molecular Transport

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James Weaver

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Massachussetts Institute of Technology

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The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

12 a. DISTRIBUTION / AVAILABILITY STATEMENT
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13. ABSTRACT (Maximum 200 words)

This research involves a basic study of electroporation with an emphasis on molecular transport across cell membranes. We have explored a new mechanism for drug delivery, the use of electroporation to move molecules into, across and out of tissues. Electroporation of human skin may provide the basis for greatly enhanced transdermal drug delivery, and for non-invasive "sensing" of biochemicals within the body. Another advance is a new general method for rapidly determining the clonal growth of individual cells. For more details, see attached.

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September 20, 2001

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Subject: Grant No. DAAL03-90-G-0218

Dear Ms. Jackson:

On behalf of our Principal Investigator, Dr. James C. Weaver, enclosed please find a copy of the final technical report that was submitted to the subject grant.

Should you have any questions, please let us know.

Sincerely yours,

Mary A. McGonagle

Mary A. McGonagle
Contract Administrator

Enclosure

Text for ARO Final Report

1. (leave blank)

2. 05-17-95

3. 05-17-95 Final report: 1 January 1992 - August 31, 1995

4. TITLE AND SUBTITLE: Electroporation of Bacteria and Yeast: An Experimental Study of Molecular Transport

5. FUNDING NUMBERS: DAAL03-90-G-0218

6. AUTHORS: James C. Weaver

7. NAME OF INSTITUTION: Massachusetts Institute of Technology, Cambridge, MA 02139

8. REPORT NUMBER: none

7.

LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED UNDER ARO SPONSORSHIP DURING THIS REPORTING PERIOD, INCLUDING JOURNAL REFERENCES:

The following papers have been submitted

M. R. Prausnitz, A V. G. Bose, A R. S. Langer and J. C. Weaver "Transtissue Molecular Transport due to Electroporation of Skin" Proceedings, The First World Congress for Electricity and Magnetism in Biology and Medicine, Lake Buene Vista, FL, June 14 - 19, 1992, M. Blank and B. Greenenbaum, Eds. (submitted)

B. S. Lau, C. D. Milano, M. R. Prausnitz, R. S. Langer and J. C. Weaver "Quantitative Determination of Molecular Transport Across Erythrocyte Ghost Membranes by Electroporation" Proceedings, The First World Congress for Electricity and Magnetism in Biology and Medicine, Lake Buene Vista, FL, June 14 - 19, 1992, M. Blank and B. Greenenbaum, Eds. (submitted)

E. A. Gift and J. C. Weaver "Cell Survival Following Electroporation: Quantitative Assessment Using Large Numbers of Microcolonies" Proceedings, The First World Congress for Electricity and Magnetism in Biology and Medicine, Lake Buene Vista, FL, June 14 - 19, 1992, M. Blank and B. Greenenbaum, Eds. (submitted)

8. SCIENTIFIC PERSONNEL SUPPORTED BY THIS PROJECT AND DEGREES AWARDED DURING THIS PERIOD:

Weaver, James C.	Principal Investigator
Vanu G. Bose	Research Assistant

9. REPORT INVENTIONS (BY TITLE ONLY): None this period

BRIEF OUTLINE OF RESEARCH FINDINGS

In the course of continuing our basic study of electroporation of microorganisms, with an emphasis on what is important to molecular transport. **we have also explored what may be a "breakthrough" for drug delivery: the use of electroporation with tissue to move molecules into, across and out of tissues. More specifically, electroporation of human skin may provide the basis for greatly enhanced transdermal drug delivery, and for non-invasive "sensing" of biochemicals within the body.**[00] Prausnitz etal Weaver 1992 transtissue molec trans World Congress trrfxioev[00] This is a partial result of ARO's funding of the basic study, and could have major technological applications.

As noted in a previous progress report, "electroporation involves a rapid membrane rearrangement in response to short (e.g microseconds), strong (e.g. 5,000 volt/cm) electric fields, and the resulting membrane openings ("pores") cause tremendous changes in molecular transport across the cell membranes. If the basic mechanism for molecular transport due to electroporation becomes understood, widespread applications in research, biotechnology and medicine are likely."

In a continuing effort to understand molecular transport across cell membranes due to electroporation, we have recently concluded a study using red blood cell ghosts, in which the number of molecules taken up (or released) by individual ghosts was determined. In addition to determining the magnitude of transport, a significant result is the finding that molecular transport plateaus as a function of electric field strength (for a commonly used exponential pulse shape). A preliminary description of this work has been presented, and will be published in the proceedings of the First World Congress for Electricity and Magnetism in Biology and Medicine, and a complete journal article is being prepared.[0000]

Both the tissue electroporation and red blood cell ghost studies have received critical and timely support from ARO. The results of both, and presently ongoing studies, strongly suggest that electroporation will prove to be a phenomenon of major importance to biology, biotechnology and medicine.

Finally, with previous support from ARO, a new general method for rapidly determinating the clonal growth of individual cells has been developed.[00] Individual microorganisms are incorporated into gel microdrops (GMDs), exposed to conditions which may affect cell growth, and then the degree of microcolony formation is quantitatively determined. For work with microorganisms GMDs about 20 to 40μ in diameter are made from agarose. The GMDs are "diffusionally transparent", capable of gently confining cells upon replication, so that microcolonies are formed. The GMDs are also sufficiently small and robust that they can be handled much like cells, e.g. suspended, pipetted and centrifuged. GMDs also be analyzed by flow cytometry, so that the distribution of microcolony size can be quickly determined, and the distribution of clonal growth within a subpopulation rapidly determined.

Using this "Microdrop Technology", we are continuing to investigate the basic problem of determining the subpopulation of microorganisms which (1) experiences significant electroporative uptake, and (2) survives, as determined by the stringent test of clonal growth. With this in mind, we are using GMDs and fluorescent molecule uptake to see if we can quantitatively determine this critical microbial subpopulation, and the amount of molecular uptake its cells experience. A preliminary report of this effort has been recently submitted.[00]