FINAL TECHNICAL REPORT

to

The National Science Foundation
Office of Sea Grant Programs

The New England Institute, Ridgefield, Connecticut
Principal Investigator: James H. Green, Ph.D., Sc.D.
Grant No. GH-34
Starting Date: October 1, 1968
Completion Date: September 30, 1970
Grant Title: Useful Biomedical Materials Derived from the Sea: An Inter-Disciplinary Approach
Research and Results:

An informal technical report was sent to NSF in March, 1970. This included a description of the current status of biomedical testing of coenzymes Q, their analogues and biochemical precursors, and a final report on progress in bioluminescence research terminated by the transfer of the investigator (Dr. John Lee) to another institution.

At the time of starting this Sea Grant Program, the coenzymes Q_{10} and Q_9 had been identified in a fraction (separated by solvent partition and thin layer chromatography) from hexane extract of shark livers. A patent application, No. 757,862, had been filed on September 6, 1968, disclosing "a method of controlling the host defense system in animals by introduction into the body of compounds such as certain quinones, carotenoids, tocopherols and structurally related compounds on appropriate dosage schedules." As stated in the proposal: "Biomedical Evaluation: The requested support from the Sea Grant Program is directed toward this aspect of the project..."

Publications related to this research, partially supported by the Sea Grant Office, are listed in the attachment. (This list and copies of the papers have been sent to Leatha Miloy, Office of the Sea Grant Programs, Texas A&M, and to the Pell Marine Science Library, University of Rhode Island.)

Earlier studies had shown that some extracts of nonpolar lipids from lemon shark livers were able to stimulate biological activity in the reticuloendothelial system (RES) of experimental animals (Heller, et al, Proc. IVth Int. Symp. on R.E.S., 375, 1964; Bliznakov, Int. J. Cancer, 3, 336, 1968). Coenzymes Q_{10} and Q_9 were identified as the major constituents of a fraction from lemon shark livers, which showed intense biological activity when tested for RES activity (Casey, Colquhoun and Lee, Lipids, 5, 856, 1970).

Commercial supplies of the pure coenzymes were then obtained and extensively tested to study their effect on various parameters of the RES in animals. It was shown that the administration of coenzymes Q_6 and Q_{10} increases phagocytic activity in rats as measured by the carbon clearance technique. In mice, a two-fold increase in primary hemolytic antibody formation vs. sheep erythrocytes was produced by the administration of coenzyme Q_{10}. This was the first proof of the stimulation of the RES by these coenzymes (Publ. 1 and 9).

Studies were then made of the relationship between the chemical structure of coenzymes Q and biochemically related substances and their effect on phagocytic activity in rats (Publ. 4 and 7). Coenzyme Q_{10} has proved most effective so far, although a eutectic mixture of coenzymes

Q_{10} and Q_9 showed an intense stimulatory effect. A small amount of coenzyme Q_9 (not available commercially) has been isolated form shark liver extract and will be tested further.

The effectiveness of coenzyme Q_{10} in treating disease states was then tested and the results were reported at a recent international meeting (Publ. 8, 5, 6 and 11). Based on the assumption that the therapeutic effect of many drugs, including antimalarial drugs, is not only directed against the infectious agent, but also requires participation of the host, a combination of an antimalarial drug (Aralen - chloroquine hydrochloride) and an RES-stimulating agent (cholesteryl oleate, glucan, or coenzyme Q_{10}) was tried. Mice infected with blood-transferred Plasmodium berghei were given pretreatment with low doses of Aralen combined with the RES-stimulating agents. The results of these combination pretreatments (Publ. 6, 11) were increased survivorship, prolonged survival time and reduced parasitemia compared with the use of Aralen alone. (Stimulation of the RES alone is ineffective against this infection in mice.) Preliminary results of a therapeutic rather than a prophylactic approach are being obtained now.

In extending these studies using emulsions of coenzyme Q_{10}, we were able to demonstrate the following effects on two tumor systems in mice (Publ. 5). Injections of the emulsions in appropriate doses and time schedules substantially decreased splenomegaly and hepatomegaly and increased the survivorship of mice infected with Friend leukemia virus. Similar treatment reduced the percentage of mice developing tumors, increased the survivorship and reduced the tumor size in mice with 3,4,9,10-dibenzpyrene induced tumors. The effect in both tumor systems depended strongly on the

dose of coenzyme Q_{10}. A complete analysis and search for other useful compounds in the above mentioned liver extract is now being completed (Publ. 12, 13).

Scientific Collaborators

John H. Heller, M.D., President
Alan D. Adler, Ph.D., Associate Professor
Emile G. Bliznakov, M.D., Professor
Bruce F. Cameron, M.D., Ph.D., Associate Professor
Adria C. Casey, Ph.D., Associate Professor
John W. Lee, Ph.D., Associate Professor
Eugene Premuzic, Ph.D., D. Phil., Associate Professor
John P. Ransom, Ph.D., Professor
Iain B. Matheson, Ph.D., Post Doctoral Fellow
David J. Wilkins, Ph.D., Associate Professor

Comments

The work on bioluminescence of marine organisms progressed well up to September, 1969, when the scientific collaborator in this program left the Institute. Two years of effort have gone into the product of our interest in elasmobranch research. This led to the identification and biomedical testing of the coenzymes Q in a completely different way from others which were considered (e.g. simply playing a part in electron transport processes in mitochondria). The material and procedures are near to the stage when this "drug from the sea" can be put to use.

Based on this program and the added expertise that we have now developed, a continuation of support from the Sea Grant Office is being asked for research on:

1. "Useful Biomedicals from Marine Animals and Plants" - E. T. Premuzic and J. H. Green.
2. "Metal-Organic Compounds in Marine Flora and Fauna on the Northeast Seaboard" - J. H. Green and E. T. Premuzic.

James H. Green
December 3, 1970

PUBLICATIONS AND CONFERENCE PAPERS
DURING TENURE OF SEA GRANT NO. GH-34

1. Adler, A., E. Bliznakov, E. Premuzic, A. Casey, B. Cameron, J. Green and J. Heller, J. RE. Soc., 5, 592, 1968, Stimulation of Phagocytosis in Rats by Substances with Quinoidal Structures. Abstract. NEINST-R-68-001

2. Premuzic, E., E. Bliznakov, A. Adler, A. Casey, B. Cameron, J. Green and J. Heller, J. RE. Soc., 5, 592, 1968, Factors Influencing the Activity of Shark Liver Extracts in Stimulating Phagocytosis. Abstract. NEINST-R-68-002

3. Premuzic, E., in "Progress in the Chemistry of Organic Natural Products", L. Zechmeister, ed., Springer-Verlag, 1970/71, New York, Chemistry of Natural Products Derived from Marine Sources. In press.

4. Casey, A. C. and E. G. Bliznakov, Presented at December 1969 Meeting of the Reticuloendothelial Society, San Francisco, California, J. RE. Soc., 7, 631, 1970, Effect and Structure to Activity Relationship of the Coenzymes Q in the Phagocytic Activity of Rats and Immune Response of Mice. Abstract. NEINST-R-70-001

5. Bliznakov, E. G., Presented at VI International Meeting of the Reticuloendothelial Society in Freiburg, Germany, July, 1970, Antineoplastic Activity of Coenzyme Q_{10}. Abstract. NEINST-R-70-002

6. Bliznakov, E. G., Presented at VI International Meeting of the Reticuloendothelial Society in Freiburg, Germany, July, 1970, Protective Effect of Reticuloendothelial System Stimulants in Combination with Chloroquine on Plasmodium Berghei Infection in Mice. Abstract. NEINST-R-70-003

7. Casey, A. C. and E. G. Bliznakov, Presented at VI International Meeting of the Reticuloendothelial Society in Freiburg, Germany, July, 1970, Relationship Between Chemical Structure of Coenzymes Q and Related Substances and their Effect on Phagocytic Activity of Rats. Abstract. NEINST-R-70-004

8. Heller, J. H., Presented at VI International Meeting of the Reticuloendothelial Society in Freiburg, Germany, July, 1970, Modification of RES Parameters by Coenzymes Q. Abstract. NEINST-R-70-005

9. Bliznakov, E., A. Casey and E. Premuzic, Coenzymes Q: Stimulants of the Phagocytic Activity in Rats and Immune Response in Mice. Experientia, 26, 953, 1970.

10. Bliznakov, E., To be presented at VII National Meeting of the Reticuloendothelial Society in Augusta, Georgia, December, 1970, Effect of Reticuloendothelial System Stimulants in Combination with Chloroquine on Plasmodium Berghei Infection in Mice. Abstract. NEINST-R-70-006

11. Bliznakov, E., in the series "Advances in Experimental Medicine and Biology", Plenum Press, Protective Effect of Reticuloendothelial System Stimulants in Combination with Chloroquine on Plasmodium berghei Infection in Mice. In press. NEINST-R-70-007

12. Norman, M., E. T. Premuzic, B. B. Premuzic and C. Kelly, Preparative Chromatography of Minor Constituents Associated with Extracts of Lipids. In preparation.

13. Norman, M., E. T. Premuzic and B. B. Premuzic, Constituents of a Polar Fraction Obtained from the Liver of Lemon Shark, Negaprion breviosteris. In preparation.