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FINAL REPORT ON CONTRACTS N00014-67-A-0191-0028 AND N00014-75-C--ETC(U)
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BROWN UNIVERSITY *Providence, Rhode Island • 02912*

Division of Biological and Medical Sciences

(11) 9 June 1977

(12) 4 p.

Dr. Robert K. Jennings
Program Director, Biochemistry
Office of Naval Research
Arlington, Virginia 22217

Dear Dr. Jennings: (6)

Enclosed is the Final Report for Contracts N00014-67-A-0191-0028 and N00014-75-C-0471.

In accordance with your request, a copy is being forwarded to the Defense Documentation Center.

Sincerely,

Elizabeth H. Leduc

Elizabeth H. Leduc
Dean of Biological Sciences

EHL:nu

Enclosure

Copy to:
DDC, Alexandria, VA

(15) N00014-75-C-0471,
N00014-67-A-0191-0028

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FINAL REPORT
on
Contracts N00014-67-A-0191-0028 and N00014-75-C-0471

Briefly described, the problem attacked under these contracts can be summarized by the questions, "Are cholesterol oxidation products present in dried foods known to contain cholesterol? Are these oxidation products potentially harmful? Will any of these cholesterol derivatives violate the 'Delaney Amendment' which prohibits the induction within, or addition to, natural foods of carcinogens during processing?"

Since this report is expected to cover findings made in the current contract at the time of writing the report, it can be stated that the answer to all three questions is "Yes." Recent analyses of a commercially available dried egg preparation known as "Instant Custard" repeatedly show the presence of the 5,6 epoxide of cholesterol and 3,5,6 hydroxycholesterol. Both of these substances are listed as carcinogens in classic sterol literature.

Significant Findings

The research was based on a study in which it was shown that a concentrate of the contaminants of USP cholesterol were more potent than highly purified cholesterol in inducing aortal smooth muscle cell death[1]. Pathologists consider smooth muscle cell damage to be the first step in the development of atherosclerosis. Eggs and milk are dried by forcing them in a fine spray into heated air. Evaporation is almost instantaneous. There is a continued exposure to air of the product for a significant period prior to packaging. Cholesterol will spontaneously oxidize when exposed to air unless it is purified to exceptionally high levels. By sterol chemists it is considered to be a very labile substance. Thus it was reasonable to assume that current food processing could produce oxidation products just as occurred upon storage of USP cholesterol.

Thus the first question to be answered was, "What compounds in the concentrate of USP cholesterol contaminants are potent?" Work began on this problem and one group of derivatives--the hydroxy peroxides--was found inactive. As we began our attack on the remaining components, we learned from Dr. John Watson that in his system (cultured hepatoma cells), 25 hydroxycholesterol was the most potent agent among the known spontaneous oxidation products in inducing cell death by stopping cholesterol biosynthesis.

It was tried and found to duplicate in part the pathology induced by the original concentrate. The 3,5,6 trihydroxide was found to be less potent than the 25 OH; the 5,6 epoxide far less, if at all. (These assays were run by our collaborator, Dr. H. Imai.) Meanwhile our extracts of dried foods were quantitatively assayed by Dr. C. Merritt's group and showed the presence of the trihydroxide and the 5,6 epoxide.

Dr. Imai has tested the following mixtures:

(1) A dose of equal parts of 25 hydroxy and 3,5,6 trihydroxy (10 mg/kilo toto).

(2) A dose of equal parts of the preceding two, plus the 5,6 epoxide (10 mg/kilo toto).

The results are (a) that the trihydroxy synergizes the 25 hydroxy and (b) that adding the epoxide increases the response to such an extent that it approximates the potency of the original concentrate.

Purified cholesterol was used as the control substance in all these studies. It had no effect. Final quantitation of these assays is in progress. We expect to have a manuscript completed by August describing the results.

Publications and lectures resulting from this work are as follows:

Lectures:

Dr. N. T. Werthessen delivered a briefing entitled "Potential Atherogenic and Carcinogenic Sterol Derivatives in Desiccated Food Products Derived from Eggs, Milk, and Meat" at the FDA, Bethesda, Maryland, on 24 March 1976.

Publications:

1. Necrogenic Agent Obtained from Cholesterol Used in Dietary Experiments. Third International Symposium on Atherosclerosis, W. Berlin, Germany, 24-28 October 1973. K. T. Lee, H. Imai, N. T. Werthessen, and C. B. Taylor.

2. One-Day Ultrastructural Bioassay of Angiotoxicity: Possible Early Prediction of Atherogenicity. 27th Annual Meeting, Council on Arteriosclerosis, American Heart Association, Atlantic City, New Jersey. N. T. Werthessen, H. Imai, and K. T. Lee. Nov. 1973.

3. Smooth Muscle of the Arterial Wall, Proceedings of Lindau Conference, Heidelberg, Germany, 29-31 October 1973. (Vol. 57 of Advances in Experimental Medicine and Biology.) Edited by S. Wolf and N. T. Werthessen. Plenum Press (1975).

4. Angiotoxicity and Arteriosclerosis Due to Contaminants of USP Grade Cholesterol. N. T. Werthessen, H. Imai, C. B. Taylor, and K. T. Lee. Archives of Pathology and Laboratory Medicine, Vol. 100, November 1976.

5. Arterial Lesions by Oxidation Products of Cholesterol. The IVth International Symposium on Atherosclerosis, Tokyo, Japan, 24-28 August 1976. H. Imai, A. H. Soloway, V. Subramanyam, and N. T. Werthessen.

6. Arterial Wall Injury by Cholesterol Derivatives. H. Imai, M. Kanisawa, K. Kojima, and N. T. Werthessen. Presented at the 61st Annual Meeting of the FASEB in Chicago, Illinois, on 7 April 1977. (Meetings 1-8 April.) Published in Federation Proceedings, Vol. 36, No. 3, 1 March 1977.

7. Dynamics of Arterial Flow, Proceedings of Little Lindau Conference, Delaware Water Gap, Pennsylvania, June 1976. Edited by S. Wolf and N. T. Werthessen. In press.