



**Medical College of Virginia
Virginia Commonwealth University**

June 7, 1985

Mr. Perry Chapelaine, Sr.
The Rheumatoid Disease Foundation
Route 4, Box 137
Franklin, TN 37064

Dear Mr. Chapelaine:

Enclosed please find a grant application-request to the Rheumatoid Disease Foundation entitled "The Effect of Clotrimazole and Related Agents on Cell-free, Calcium-dependent Phospholipase A₂ Activity in Human Synovial Fluid". We believe that this work represents an exciting step forward in understanding and perhaps intervening in the inflammation associated with active arthritic disease. We hope that the Foundation feels similarly.

Sincerely yours,

Richard C. Franson

Richard C. Franson, PhD
Associate Professor of Biochemistry



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RHEUMATOID DISEASE FOUNDATION GRANT REQUEST:

Effect of Clotrimazole and Related Agents on Cell-free, Calcium-dependent Phospholipase A₂ Activity in Human Synovial Fluid

Support Requested: from July 1, 1985 through June 30, 1986

Itemized Budget:

technical salary + fringe benefits	\$ 18,200
chemicals, radioisotopes & glassware	\$ 2,500
	<hr/>
	\$ 20,700

Rationale and Objectives

We have found that phospholipase A₂ (PLA₂) activity, an enzyme system involved in inflammation and corticosteroid action, is elevated in the synovial fluid of patients with active arthritic diseases. Based on this observation, we have focused our studies in recent years on the occurrence and regulation of Ca²⁺-dependent and neutral-active PLA₂s in human synovial fluid and human polymorphonuclear leukocytes.

Despite a lack of funding for this project, we have examined the synovial fluid from over 50 patients with a variety of arthritic diseases. As the accompanying reprint describes, we have found a potent Ca²⁺-dependent PLA₂ activity in human synovial fluid. The enzyme is cell-free in this fluid and is directly inhibited by some, but not all, nonsteroidal anti-inflammatory agents.

This PLA₂ enzyme system is also of general interest to the study of inflammation because one of the known mechanisms of action of steroids is the induction (by protein synthesis) of an anti-PLA₂ protein, referred to as lipomodulin or macrocortin. Thus, it is believed that PLA₂s are regulated in situ by endogenous as well as exogenous agents.

Clotrimazole: As an Antagonist of PLA₂s

Recently, at the suggestion of Dr. William Regelson, we tested the anti-fungal drug, clotrimazole for its ability to influence the human synovial fluid PLA₂. This drug, an imidazole derivative, has received recent attention because of its anti-inflammatory activity in patients with arthritis, raising the possibility that either the etiologic agents producing this inflammatory disease could be amebal or fungal, or that a more general mechanism is involved. In this regard, and of importance to our work, immunologic studies now indicate that antigens of the pathogenic ameba, Naegleria fowleri are present in human synovial fluid, and in past work (see enclosed reprints) we have demonstrated that during growth, these ameba secrete large quantities of phospholipases spontaneously, and that these cell-free enzymes degrade the phospholipids of human myelin suggesting a possible relationship to multiple sclerosis.

Our preliminary studies with clotrimazole are shown in figure 1 (page 5): clotrimazole produces dose-dependent inhibition of the synovial fluid PLA₂. It is particularly interesting that the inhibition is inversely related to the concentration of added CaCl₂. Thus, no inhibition is noted with 5mM added CaCl₂, a concentration typically used for the in vitro assay of PLA₂ activity. As the concentration of added CaCl₂ decreases to more physiologic levels (10-100 uM) clotrimazole produces dose-dependent inhibition with an IC₅₀ in the micromolar range.

In summary, these preliminary studies indicate that clotrimazole has direct inhibitory effects on human synovial fluid PLA₂ activity and the data suggests that this inhibition may constitute a direct mechanism for the anti-inflammatory or anti-rheumatoid arthritic activity of this drug.

Discussion

A main feature of inflammation and cell injury in arthritis is thought to involve the generation of leukotrienes and prostaglandins as a result of mobilization of the fatty acid precursor, arachidonate, from membrane phospholipid. Arachidonate does not exist as a free fatty acid to any appreciable extent in biological systems, and it is predominantly esterified in the 2-position of mammalian phospholipids. Therefore, arachidonate must be released from membrane phospholipid in response to various stimuli by the activation of phospholipases. Two pathways are thought to contribute to mobilization of arachidonate in mammalian systems: direct deacylation of the 2-position of phospholipids by a phospholipase A₂ (PLA₂) and the concerted action of a phosphatidylinositol-specific phospholipase C (PI-PLC) and a diglyceride lipase. Because our preliminary studies indicate that large quantities of PLA₂ activity are found in human synovial fluid with little or no PI-PLC activity, the above observations are pertinent to our area of interest and hopefully those of the Rheumatoid Disease Foundation. Thus, we have shown that

- 1) Naegleria fowleri is a potent producer of phospholipase A₂.
- 2) phospholipase A₂ activity is elevated during active joint inflammation in the synovial fluid of rheumatoid arthritic joints.
- 3) clotrimazole, an anti-amebic and anti-fungal agent thought to induce remission in rheumatoid arthritis, is a potent inhibitor of phospholipase A₂.

Request: In view of the above, we request support from the Rheumatoid Disease Foundation to:

- 1) carefully study the mechanism of action of clotrimazole on the inhibition of the human synovial fluid PLA₂
- 2) examine the effects of this drug on other phospholipases associated with human PMNs (polymorphonuclear leukocytes)

- 3) determine the levels of PLA₂ in synovial neutrophils and if possible in synovial fluid from patients treated with clotrimazole (cooperative effort with Dr. Turner at Bowman Gray, Winston-Salem, NC)
- 4) study the effect of other imidazole compounds, such as histamine, on synovial fluid PLA₂
- 5) examine human synovial fluid for the presence of endogenous components that share anti-PLA₂ and anti-inflammatory activities, and
- 6) collaborate with Dr. Susskind (Department of Surgery, MCV) in his Rheumatoid Disease Foundation study attempting to isolate Naegleria or other infectious organisms from rheumatoid synovia.

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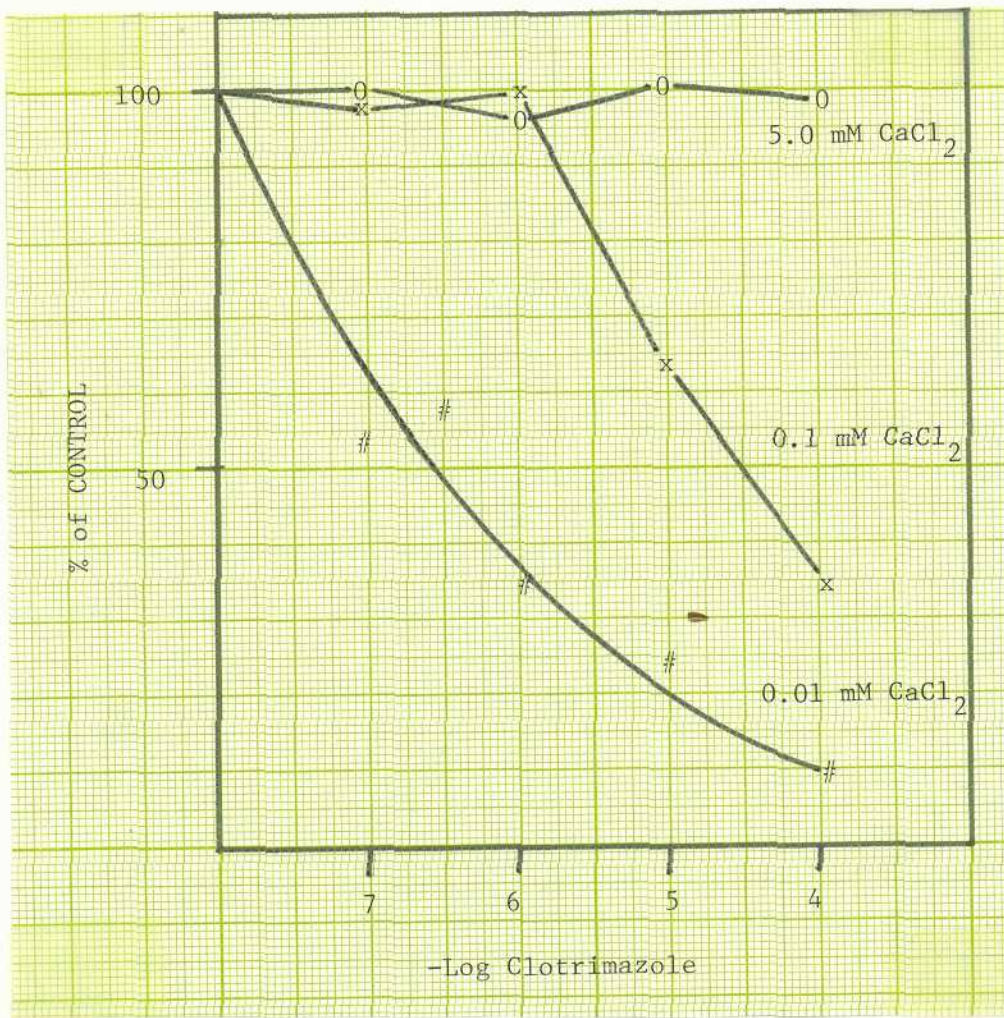


Figure 1

Inhibition of Human Synovial Fluid Phospholipase A₂ Activity
by Clotrimazole as a Function of Added CaCl₂

CURRICULUM VITAE

1. PERSONAL INFORMATION

NAME: Richard C. Franson

DATE OF BIRTH: 12/14/43

PLACE OF BIRTH: Woburn, Massachusetts

CITIZENSHIP: U.S.A.

SOCIAL SECURITY NUMBER: 016-34-3082

MARITAL STATUS: Married, three children

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OFFICE TELEPHONE: (804)786-4117

2. EDUCATION

Bowman Gray School of Medicine, Winston-Salem, NC

1972 Ph.D. Biochemistry under the supervision

1970 M.S. Biochemistry of Dr. Moseley Waite

University of Massachusetts, Amherst, MA

1965 B.S. Botany

3. MILITARY SERVICE RECORD

1966-68 Hospital Corpsman - Clinical Chemist, U. S. Navy

4. POSTDOCTORAL TRAINING or SPECIAL EXPERIENCE

1979-80 Educational leave with Professors H. van den Bosch and
L.L.M. van Deenen, Laboratory of Biochemistry, University
of Utrecht, The Netherlands

1972-74 U.S.P.H.S. Postdoctoral Fellow, New York University Medical
Center under the supervision of Dr. Peter Elsbach

5. ACADEMIC APPOINTMENTS - WORK EXPERIENCE

Associate Professor of Biochemistry, Medical College of Virginia,
4/82 - present

Associate Professor of Biophysics and Biochemistry, Medical College of
Virginia, 7/79 - 4/82

Assistant Professor of Biophysics and Biochemistry, Medical College of Virginia, 9/77 - 7/79

Assistant Professor of Biophysics, Medical College of Virginia, 9/75-9/77

Instructor of Experimental Medicine, N.Y.U. School of Medicine, 9/74-9/75

6. MEMBERSHIPS - SCIENTIFIC, HONORARY & PROFESSIONAL SOCIETIES

Sigma Xi, Harvey Society, American Association for the Advancement of Science, American Heart Association, New York Academy of Sciences, The Biophysical Society, American Society of Biological Chemists

7. HONORS - NIH Research Career Development Award 1980-84

NIH Young Investigator Award 1976-79

8. BIBLIOGRAPHY

A. Papers Published: Richard C. Franson, Ph.D.

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17. Franson, R., Pang, D. and Weglicki, W. Modulation of lipolytic activity in isolated canine cardiac sarcolemma by isoproterenol and propranolol. *Biochem. Biophys. Res. Commun.* 90:956, 1979.
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B. Chapters

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