



**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 A<sub>2</sub> 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 A<sub>2</sub> 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 A<sub>2</sub> (PLA<sub>2</sub>) 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 Ca<sup>2+</sup>-dependent and neutral-active PLA<sub>2</sub>s 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 Ca<sup>2+</sup>-dependent PLA<sub>2</sub> 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 PLA<sub>2</sub> 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-PLA<sub>2</sub> protein, referred to as lipomodulin or macrocortin. Thus, it is believed that PLA<sub>2</sub>s are regulated in situ by endogenous as well as exogenous agents.

## Clotrimazole: As an Antagonist of PLA<sub>2</sub>s

Recently, at the suggestion of Dr. William Regelson, we tested the anti-fungal drug, clotrimazole for its ability to influence the human synovial fluid PLA<sub>2</sub>. 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 PLA<sub>2</sub>. It is particularly interesting that the inhibition is inversely related to the concentration of added CaCl<sub>2</sub>. Thus, no inhibition is noted with 5mM added CaCl<sub>2</sub>, a concentration typically used for the in vitro assay of PLA<sub>2</sub> activity. As the concentration of added CaCl<sub>2</sub> decreases to more physiologic levels (10-100 uM) clotrimazole produces dose-dependent inhibition with an IC<sub>50</sub> in the micromolar range.

In summary, these preliminary studies indicate that clotrimazole has direct inhibitory effects on human synovial fluid PLA<sub>2</sub> 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 A<sub>2</sub> (PLA<sub>2</sub>) 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 PLA<sub>2</sub> 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 A<sub>2</sub>.
- 2) phospholipase A<sub>2</sub> 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 A<sub>2</sub>.

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 PLA<sub>2</sub>
- 2) examine the effects of this drug on other phospholipases associated with human PMNs (polymorphonuclear leukocytes)

- 3) determine the levels of PLA<sub>2</sub> 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 PLA<sub>2</sub>
- 5) examine human synovial fluid for the presence of endogenous components that share anti-PLA<sub>2</sub> 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.

Richard C. Franson, PhD  
Associate Professor of Biochemistry  
Department of Biochemistry  
Box 614  
Medical College of Virginia  
Richmond, VA 23298  
Telephone # 804-786-4117

Richard C. Franson 6/5/85

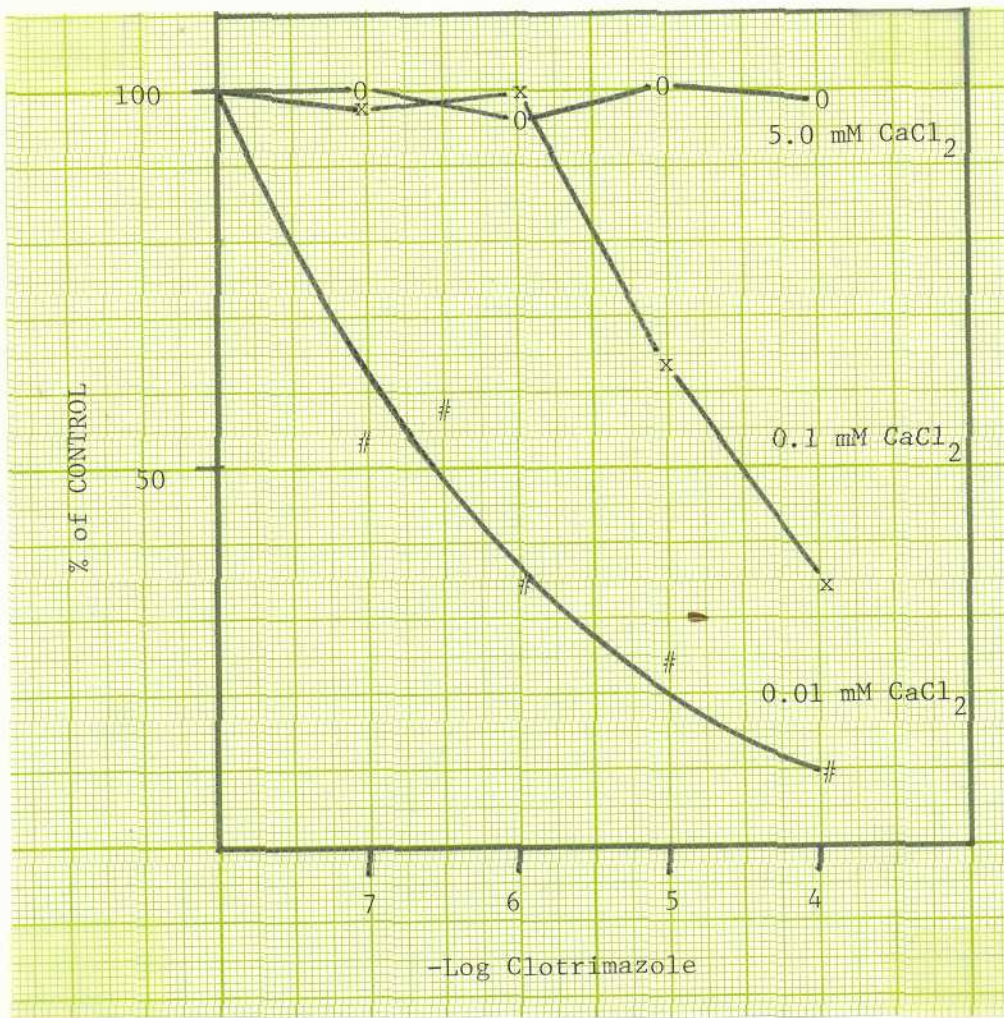


Figure 1

Inhibition of Human Synovial Fluid Phospholipase A<sub>2</sub> Activity by Clotrimazole as a Function of Added CaCl<sub>2</sub>

## 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

HOME ADDRESS: 3010 Comet Road, Richmond, VA 23229

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### 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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3. Wang, P., Franson, R., DeChatelet, L., Waite, M. and McCall, C. Effect of methylprednisolone on some BCG-mediated changes in rabbit alveolar macrophages. *Infect. Immun.* 6:982, 1972.
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11. Weiss, J., Franson, R. and Elsbach, P. Granulocyte phospholipase A-induced permeability alterations in E. coli. Biochim. Biophys. Acta. 436:154, 1976.
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14. Franson, R., Dobrow, R., Weiss, Jr., Elsbach, P. and Weglicki, W. Isolation and characterization of a phospholipase A<sub>2</sub> from an inflammatory exudate. J. Lipid Res. 19:18, 1978.
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## B. Chapters

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