

August 22, 1985

Dr. John Simoons, President
The Rheumatoid Disease Foundation
5140 Revere Road, Parkwood
Durham, NC 27713

Dear Dr. Simoons:

At our recent meeting on August 20, 1985 in Winston-Salem, we discussed in some detail the benefits of expanding the scope of my proposed work to include an evaluation of the levels of PLA₂ activity in resting and stimulated neutrophils from patients in Dr Turner's trial studies with clotrimazole. Since this involves additional expenses at Bowman Gray for the RDF (ie \$ 10,000), to justify these additional costs I detail below what we intend to do and what we hope to accomplish.

Because our previous studies indicate that the appearance of cell-free PLA₂ activity in inflammatory fluid is associated with the coincident accumulation of neutrophils, we suspect that the human synovial fluid enzyme could be derived from circulating neutrophils. In addition, since the neutrophilic PLA₂ is thought to be the major enzyme system involved in the mobilization of arachidonate, we plan collaborative studies with Drs Turner and Smith at Bowman Gray to:

1. compare the resting and stimulated levels of PLA₂ in
 - a. normal humans n = 5
 - b. patients with arthritis n = 5
 - c. 10 patients with arthritis in Dr Turner's protocol + clotrimazole

(these studies will be done in my laboratory and do not involve costs above that already approved by the RDF)

2. at Bowman Gray, Dr. Duane Smith will measure 14-C arachidonate mobilization in prelabelled neutrophils from the same patients. These studies will assess basal and stimuli-induced turnover of arachidonate. The additional \$ 10,000 required for these studies will offset costs at Bowman Gray for the preparation and shipment of neutrophils to Richmond as well as the additional in situ measurement of arachidonate mobilization.

By use of this coordinated approach, at MCV I will measure in vitro levels of PLA₂ and determine whether there are measurable differences in normals vs arthritics + clotrimazole and if there are differences in resting vs stimulated PMNs in each case. These in vitro observations will be greatly strengthened by the in situ mobilization studies done at Bowman Gray by Dr. Smith. In terms of the known literature, studies correlating in vitro and in situ activity in the area of arachidonate

metabolism is seldom if ever done. Collectively this information will most certainly be publishable regardless of the results obtained. Thus, I believe the additional expenses are justified since they insure a solid, comprehensive scientific effort and should result in very useful information.

In addition to the above studies, we plan to examine the effect of clotrimazole and related compounds on the phospholipases derived from the pathogenic amoeba, Naegleria fowleri. As our publications with this pathogen indicate, phospholipases are present in extraordinary quantities in pathogenic amoeba, but are barely if at all measurable in the non-pathogen. Thus, an effect of clotrimazole on these amoebal phospholipases would suggest a potential mode for the anti-inflammatory activity of these compounds. And in doing so would lend credence to the concept of amoebal induction of arthritis and possibly other inflammations.

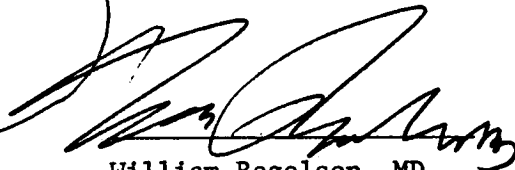
We look forward to initiating these exciting experiments.

Sincerely yours,

Richard C. Franson

8/22/85

Richard C. Franson, PhD



William Regelson, MD

cc: Mr. Perry Chapdelaine