As regards those Flagyl trials I do not think we should be too worried about the fact that either naegleria or acantamoeba are sensitive to Flagyl. I was reading Roger's work again last night and in his original work he never suggests that either of these organisms are responsible for the diseases which he described rather does he only concentrate on this amoeba Chromotosa which he calls a new amoeba. I have also read the piece on culturing this amoeba and therefore the first time it has come to me how simple this really must be. On page 13 on the isolation of the new organism one third of the way down it will be seen that the organism could be preserved on liver infusion agar inn'oculating the centrifuge deposit on a plate of medium and leaving it for 3 to 4 days at room temperature. The organism then forms brownish white shiny cellular dome-shaped separate colonies about 2 -4 mm. Surely somebody can do this without having to pay an awful lot of money for it. I think I must have another go myself.

It is the Sensitivity of the Amoeba chromatosa not the sensitivity of the acantamoeba or naegleria which is so important, I feel sure. Surely this can be done.

As a result of all of our investigations we now either know or can reasonably surmise that:

1. Macrophages and other white blood cells are in large number in Rheumatoid Arthritis synovial fluid.
2. That these macrophages are destroyed by Metronidazole.
3. That a Jarisch Herxheimer reaction occurs as the macrophages are being destroyed by Metronidazole.
4. That [PLA_2] is high in Rheumatoid Arthritis joint fluid.
5. That PLA_2 activity is reduced in a [Ca^{++}] dependent manner by Clotrimazole. It is NOT affected by Metronidazole which has destroyed the macrophages and released PLA_2 from the macrophages and other white blood inflammatory cells by T and B lymphocytes and polymorphs.
6. That Cu^{++}, Fe^{++} and EDTA also inhibit PLA_2 activity in a [Ca^{++}) dependent manner.
7. [PLA_2] is increased in Rheumatoid Arthritis blood and is reduced by Clotrimazole and probably by [Cu^{++}] and [Fe^{++}] as well.
8. That arachnoidoic acid is released from white blood cells by the action of PLA_2.
9. As a final rider I would suggest that the joint symptoms are due to nerve impulses coming from a nerve damaged initially by minimal trauma and then kept going by PLA2. Thus during the Herxheimer the joints (nerves) already affected would be made worse by the increased PLA_2 as a result of the Metronidazole destroying the macrophages. In addition there are joints (nerves) apparently not affected but possibly subclinically affected that will again get worse when the PLA_2 is increased to give pain in previously "unaffected" joints.

This theory has evolved to explain the symptoms: namely that the Jarisch Herxheimer is caused by the increased [PLA_2] **produced by Metronidazole and suppressed by Clotrimazole.**

To put the cap on it, it is only necessary to:

1. Obtain samples of blood from a patient having an Herxheimer caused by Metronidazole.
   a. Before the reaction,
   b. During the reaction,
   c. After the reaction.
   d. Possibly take joint fluids as well, but not essential to this investigation proof.
2. To try Clotrimazole in knee fluids.
3. To possibly try Clotrimazole as a treatment for suppressing the Herxheimer symptoms or alternatively Cu^{++} or Fe

I should now ask delegates if they will:

1. Attend a patient having an Herxheimer reaction to Metronidazole and take blood,
   a. Before the Herxheimer,
   b. During the Herxheimer,
   c. After the Herxheimer.

These specimens should be kept in the original disposable syringe and stored in the deep freeze and then shipped in dry ice. The enzyme PLA_2 is very stable and can be easily kept in the refrigerator, deep freeze, or on dry ice.

   d. Write a good history as to previous treatment and degree of Herxheimer experienced by the patient.

Dr. Paul K. Pybus

All specimens may be mailed directly to:

Richard C. Franson, Ph.D.
Medical College of Virginia
Sanger Hail, Room B1-009
Dept. of Biochemistry
PO Box 614, MCV Station
Richmond, VA 23298
(804) 786-4117

One of us (A.D.H.) having had an opportunity to see some of the clinical results obtained by the other (P.K.P.) with anti-protozoal drugs in Rheumatoid arthritis, undertook to examine synovial fluid from cases of Rheumatoid arthritis. Wyburn-Mason, 1980, and previously Kofoid and Swezy in 1922 had reported the presence of free living amoebae with only 6 chromosomes in body tissues of patients with Rheumatoid arthritis.

Over a hundred specimens of synovial fluid from patients with Rheumatoid arthritis have been examined in sealed coverslip preparations*. Each preparation was examined for objects that moved midst leucocytes and red cells. These objects were scanty and difficult to find. Three grades of activity were observed: changes in surface configuration, the development of a varying outline and finally distinct amoeboidal migratory movement from one part of the microscope field to another. When we compared these stages of activity it was found the changes in surface configuration and outline were features of synovial fluid taken from patients before treatment and that the migratory movement was an added feature of these objects when patients were receiving metronidazole. The time of sampling when metronidazole was being administered seems important because not all specimens reveal these activities.

When water was added to synovial fluids in vitro, movement was induced. Also heat to the stage stimulated further movement if objects were already motile in undiluted synovial fluid.

Efforts were made to cultivate these objects under the assumption they could be a form of amoeba. Synovial fluid was added to Penassey broth freshly innoculated with E. coli. With frequent transfers to freshly innoculated broth, objects that moved could be recorded up to twenty days but were no longer obtainable after ten days in the same media. They did not appear to multiply. We have not been able to carry out chromosome studies.

Concurrently prepared smears of synovial fluid stained with Geimsa's stain failed to highlight any special features amongst the cells that could be linked with the 'mobile' cells, apart from macrophages. No objects similar to stained free living amoebae and Entamoeba histolytica were seen.

After injecting metronidazole into Rheumatoid arthritic effusions, mobile forms were recovered, but caution is required in interpreting this observation because the drug is presumably in an aqueous solution.

It is concluded the objects that move are macrophages and that metronidazole disrupts the activities of these cells as illustrated by their mobility when patients are receiving this drug. It is postulated that the macrophages migration inhibition factor essential for perpetuating the aberrant immune response in Rheumatoid arthritis is blocked at some vital stage and clinical improvement ensures.

* Coverslip preparations should be lightly compressed to ensure cells are in contact with glass surfaces before sealing with heated paraffin wax. Time should be allowed for the heat generated by the wax to dissipate before examination.