(Dr. Paul K. Pybus Report at our July 1, 1986 National Rheumatoid Disease Foundation Seminar)

Research Projects in South Africa

We have been studying the configuration of moving objects in sealed coverslip preparations.

We've found that there are definite moving objects in synovial fluid and they are most numerous in RA knees. There are very few in OA effusions. These objects were at first thought to be amoebae due to their appearance of pseudopodic protrusions, size being in the region of 15 microns and characteristics of a single nucleus, but they had a clear cytoplasm and were not of the color described by Wyburn-Mason.

We started to try to culture these in Pennassay amoebic fluid as provided by the Tropical Disease Institute in Durban.

We failed to culture these objects but in our experience we discovered that if left to themselves at room temperature in Pennassay broth the fluid formed a coagulum at the bottom of the test tube and from this coagulum these objects could be recovered for as long as 28 days. During this time they became less numerous and less mobile until after 28 days they hardly moved at all. They did not reproduce.

One year later we tried to see the effect of Flagyl on these objects. This was done on a case not previously treated with Flagyl. Some fluid was first aspirated and examined to show the objects described above.

Flagyl was then inserted into the knees and five minutes later a further specimen was taken. This showed the same cells had become more numerous and had a writhing appearance and moved faster.

After 15 minutes a further specimen was taken to show these objects now less numerous and showing only limited movement of the cell membrane with the occasional pseudopodium.

After 30 minutes a further specimen was again examined and this showed no movement and these cells were now shrunken and crenated.

Most recently we have examined fluids stained with Giemsa stain both before and after Flagyl treatment. This showed these cells to be probably macrophages as they at first stain with a single dark nucleus with bright blue cytoplasm (slide). Examination after Flagyl showed these cells to be more numerous and to have a "traumatized" appearance, namely the nuclei had vacuoles in them and the cytoplasm was usually less and had in places become granular.

We would assume that this phenomenon is due to the Metronidazole which has a lethal effect against the macrophages. We have tried to get the macrophages to go through a filter of 0.5 microns under thermotropism and this they do.

2. Cape Town, Groote Schuur, Prof. O.A. Meyers.

A double blind trial is under way and has been in progress for the past 6 months. So far there are 8 cases in the trial. Two developed a "bad" reaction. This is presumably a good reaction to the Flagyl but we will await further reports. I am informed that they experience difficulty in getting patients to take the drug. This is not, however, our experience when treating patients who are so desperate they will take anything! The Groote Schuur test hopes to be completed at the end of this year.

3.Dr. Jim Grace, Blood Transfusion Center, Dept. Genetics, Pinetown, Natal.

So far Dr. Grace has grown synovial cells (macrophages) in joint fluid (approximately 16 cases). All cases of fluid from patients not previously treated showed an extensive growth of macrophages. The treated ones, however, showed little or no growth. They have been subcultured with success until about 18 days later and then they grew nothing.

He is now proposing to titrate the concentration of Flagyl required to inhibit growth of these cells and other investigations along these same lines; eg: use of other anti-amoebics and drugs that work.

4.Dr. Paul Pybus and Andrew Swan, Edendale Hospital, Pietermaritzburg.

Doubleblind trial in use of intraneural injections for treatment of OA of the knee.

Patients with OA knee only have been selected. They are divided into 4 groups, namely: (1) Intraneural injections with solution of 19.5 ml half-percent Carbocaine and 0.5 ml Aristospan; (2) Intraneural injections using half-percent Carbocaine only; (3) Patients receiving Indocid 50 mg. three times a day; (4) Intra-articular injections of 18 cc half-percent Carbocaine and 2 cc of Lederspan (or Aristospan).

The only double blind part of the test is on grades 1 and 2. Grades 3 and 4 are run as comparisons for the method as such. Fluids made in grades 1 and 2 are all made up in the dispensary. Neither the patient nor the doctor knows which is which. The syringes in which the fluids have been made up are glazed with the use of spray paint prior to leaving the dispensary. Eventually we hope to get an independent assessor.

So far 42 patients have been admitted to the trial but the difficulty is to get a good followup and I should think only about 20 are applicable for analysis.

This test is sponsored by the Association of Orthopoedic Surgeons of South Africa and by Lederle Laboratories. Each contributing R 1000 ($500). This is being used mainly to pay for bus fares for patients, but we must remember that many patients come from very far away and it is difficult to get full cooperation. For this reason the trial may have to go on longer than first envisioned.

Research in the United States

I. Dr. Brian Susskind, Dept. of Immunology, Univ. of Virginia, Richmond, Virginia.

This worker has, using the thermotrophic method of Roger Wyburn-Mason, so far failed to isolate any amoebic organisms in specimens of synovial fluid and in one case of synovial membrane taken from RA joints.

However the search is continuing using certain cancer tissues as advocated by Roger Wyburn-Mason and especially in lymphomatous tissue such as myelomata and lymphomata. He also intends to see the effect on certain cells of various anti-amoebics. The cells that interest him are macrophages, T-lymphocytes, B-lymphocytes, Polymorphs and Eosinophils. He also wishes to see the histologic effects of anti-amoebic treatment on the peripheral nervous system with reference to macrophage invasion.

In addition, an old letter written by Wyburn-Mason to Ray Cursons in New Zealand has recently been unearthed in England and shows that the filter we are using is of too small a diameter and should be the size of 5 microns, and this should be used in the future.

2. Richard Franson, Dept. of Biochemistry, Univ. of Virginia, Richmond, Virginia.

Prof. Franson has investigated the production of Phospholipase A2 (the killer enzyme) that dissolves whole cell membranes and so the destruction of cartilage.

He has discovered that:

1) Naegleria produce a lot of PLA2 by their action and in this way would cause cartilage destruction resulting in deformity.
2) Clotrimazole inhibits the enzyme. This inhibition is proportional to the Calcium ion concentration.
3) Copper ions also have an inhibitory effect on the PLA2.
4) Iron (2 plus) has a marked inhibitory effect on the PLA2 activity.

It would appear therefore that copper in swimming pools is beneficial in the disease as reported by Wyburn-Mason and this should also now be encouraged as it has some scientific background.

5) PLA2 is also produced by macrophages and polymorphonuclear leucocytes and here again copper and iron are excellent in treatment and prevention.

Professor Franson, Dr. Susskind and myself have considered what the implications of this could be and we are presently working on a theory which we think could be very significant. Members are asked if they could possibly provide both synovial fluid and blood from any patient who is on treatment with Flagyl at the time of having a Herxheimer reaction and if these could be left in the original syringe in which they were taken and sent to Prof. Richard C. Franson, Associate Professor of Biochemistry, Medical College of Virginia, P.O. Box 814, Richmond, Virginia 23298 to go with a note as to the time of Herxheimer reaction and the time of taking the specimens. In this way you can assist us very much in helping to determine the action of Flagyl on Rheumatoid Arthritis.

3. Prof. Kwang W. Jeon, Dept. of Zoology, University of Tennessee, Knoxville, Tennessee

You may or may not be aware that research by this very eminent Professor of Amebology discovered two years ago that tissue culture of various synovial fluids showed a very definite difference between those treated with Flagyl and those not. The untreated cases grew many macrophages, and these continued to grow for as long as twenty-eight days; furthermore they could be subcultured. The treated cases, however, grew nothing at all. Professor Jeon is now traveling to the National Institute of Arthritis in Bethesda, Maryland to review the current trends and methodologies in Rheumatoid Arthritis research. I regret to say that he is unable to be with us today.

It would appear from our many research projects that macrophages are definitely destroyed by Flagyl; furthermore when they are destroyed they release PLA2 which as far as we know is neutralized by Clotrimazole.

4. Dr. Robert Turner, Head of Dept. of Research to Rheumatology, Bowman Gray School of Medicine, Winston-Salem, North Carolina.

This project of his is going well if rather slowly. Dr. Turner sees all new Rheumatoid Arthritis cases. They are double-blind, but people are aware of the Herxheimer but still “remain blind.” Most important, Dr. Turner in his final assessment will still remain blind as to which cases have any Herxheimer reaction.

My investigation shows the extreme difficulty of carrying out this trial as forecast by Prof. Wyburn-Mason.

Bowman Gray like other Rheumatological colleges is beset by a large number of patients who have become desperate either by continued failure of their treatments in the past or because of lack of funds and still requiring treatment. Dr. Turner is doing a large number of trials, not only for the RDF, but also for numerous drug firms who are always requiring new trials.

The patients are all volunteers and are able to change from one trial to another and although many can do so, in point of fact, refuse to take advantage of this. Only 13 have dropped out so far from our trial, i.e. about 1 /3, and this is similar to what occurs in several other trials I have looked at. However, it is unfortunate perhaps that we dropped the cross-over part of the trial; this could have been essential as many cases do not show improvement until the treatment has ceased, and this is the price we must pay.

The trial therefore is going slowly and will probably take at least another 1-1/2 years before completion. 23 have finished the study, 4 are still being studied and 27 are still required.

It will probably be at the time this trial is finished that we shall also have a very good, scientifically approved, explanation of the cause of Rheumatoid Arthritis.

It has been strange to think that we have known the treatment for so long and yet because we cannot scientifically prove the ideas it should have taken nearly fourteen years to get it approved by the whole profession. However I am still full of hope that this will occur.