

THE RHEUMATOID DISEASE FOUNDATION

5140 REVERE ROAD, PARKWOOD, DURHAM, NC 27713 / (919) 544-4040

JOHN R. A. SIMOONS, Ph.D.
President

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Thank you, Dr. Baron for the introduction.

Mr. Chairman, Ladies and Gentlemen:

It is indeed a pleasure and an honor for the invitation to present a paper on the use of Imidazole Compounds for the treatment of Rheumatoid Disease and to give a brief update on the on-going double-blind clinical trial with Clotrimazole at Bowman Gray School of Medicine.

My personal interest in the etiology, pathogenesis and treatment of rheumatoid arthritis started 20 years ago when my daughter, Mavis Vecchio became a victim of this dreadful disease at the age of 24 while living in New Jersey. She had just entered the United States as an immigrant from the Netherlands after spending about one year in Sao Paulo, Brazil and was always in good physical health. From the onset of the disease in 1965 she saw the best rheumatologists in the N.J. and New York area who treated her with the entire range of anti-arthritis drugs known to them. That the disease gradually progressed and destroyed several joints in spite of their treatment could not be blamed on incompetence because they tried every approved medical therapy. She was treated by four rheumatologists in ten years and in 1975 was almost crippled and in agonizing pain, day and night. They finally suggested joint replacements, corrective surgery and the use of analgetics to relieve the pain with which she should learn to live. We refused to surrender and conducted an extensive search in the medical and scientific literature, reviewed new publications and non-conventional treatments, attended seminars on rheumatoid disease here and abroad to save my daughter before it was too late. The Breakthrough came in July 1975 when Dr. Bernard Levine of the New York University Medical Center, informed me that he spoke

THE RHEUMATOID DISEASE FOUNDATION IS A PROJECT OF
THE ROGER WYBURN-MASON & JACK M. BLOUNT FOUNDATION
FOR THE ERADICATION OF RHEUMATOID DISEASE

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with Dr. Wyburn-Mason, a British Scientist, who had just made a startling announcement at the Ninth International Congress of Chemotherapy in London, England. Dr. Wyburn-Mason stated that he believed that rheumatoid arthritis and other rheumatoid diseases are caused by an infection with pathogenic free-living amoebae and that treatment with anti-amoebic compounds will destroy the organisms and cure the disease.

He tested his new concept with the approval from the Committee on the safety of drugs (the equivalent of our FDA) and the support of Bayer UK who supplied the drug in 12 patients with classical active rheumatoid arthritis. These patients had failed to respond to conventional treatment and were now treated with Clotrimazole tablets, a powerful anti-protozoal drug in doses of 25 to 100 mg per Kg bodyweight depending on tolerance. Two patients were withdrawn from the study due to intolerance but the ten patients tolerating treatment showed a rapid improvement after a transient violent increase in joint pain, swelling, pyrexia, gastro-intestinal disturbance and other symptoms of a Herxheimer reaction.

Active disease disappeared within 28 days and after 6 weeks the blood-count and erythrocyte sedimentation rate became normal. Treatment was continued for 6 - 12 weeks and the patients were followed up for 12 - 15 months. In 4 - 6 months the albumin/globulin ratio and the electrophoretic pattern returned to normal and at the end of the follow-up period the rheumatoid factor and autoantibodies had disappeared from the blood. There was no return of disease activity during the period of observing and no other treatment was necessary.

Dr. Levin referred me to Dr. Thomas Kantor, Professor of Medicine and Chief Rheumatology of the New York University Medical Center who expressed much interest in this new concept and promised that he would look into it and do some testing.

In subsequent meetings, telephone calls and correspondence with Dr. Kantor it became obvious that he changed his mind and decided not to pursue Wyburn-Mason's new concept. I then decided to meet with Dr. Wyburn-Mason and together with Dr. Lukas, Medical director of our London office, we had a two hour meeting on October 28, 1975 at his home in Richmond Hill, Richmond. I will never forget my first meeting with this remarkable man who patiently answered all our questions and then lectured us on his fascinating new

medical concept. He described how he succeeded in isolating a small amoeba, named by him as *Amoeba chromatosa*, from the body tissues of man and certain animals by utilizing the property of THERMOTROPISM possessed by many parasites. Thermotropism is the attraction of such organisms to an environment at body temperature to one below this.

SLIDE A

An apparatus was devised in which minced fresh human or animal tissue could be exposed to a temperature gradient between zero and 37degrees C. Across a stainless steel funnel was placed a perforated zinc diaphragm anchored about half way down. To the stem of the funnel was attached a piece of rubber tubing, which was closed by a metal clip. The funnel was filled with Ringer's solution to reach to just above the zinc diaphragm. On the diaphragm was placed a 15cm diameter membrane filter with pores of 0.5 to 1.0 microns in diameter and turned up at the edges. On the membrane was placed a layer of the minced unfixed fresh tissue obtained at autopsy, biopsy or operation. This was spread out to a thickness of about 0.5 cm. On top of this tissue was placed a glass jar or beaker with a diameter almost as large as that of the zinc diaphragm. The beaker was filled with ice, which thus cooled the underlying tissue. The funnel was placed in a water bath maintained at 37degrees C. The level of the water in the bath reaching to the level of the zinc diaphragm. This heated up the Ringer's solution in the funnel in contact with the tissue to the same blood temperature. The whole operation must be carried out in sterile conditions to avoid contamination. The temperature gradient was maintained for 1 - 2 hours, the ice being replaced as it melted and the waterbath temperature maintained with a thermostat. At the end of this period, the Ringer's solution was run off into a centrifuge tube and cautiously centrifuged at 250RPM for 15 minutes. The supernatant solution was decanted and the residue microscopically examined.

SLIDE B

Photograph of a limax amoeba which emigrated from the malignant tissue in a case of carcinoma of the bronchus. X1500. Note single spike-like pseudopodium, typical of many species of *Naegleria*.

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SLIDE C

Here we see a cyst of a limax amoeba besides a trophozoite heavily pigmented as recovered from human malignant tissue. The pigmentation is presumably due to phagocytosis of debris from the minced-up tumor tissue (unstained X2000)

SLIDE D

These are also unstained limax amoebae from malignant tumors. The top picture shows simultaneous cyst formation from a cluster of trophozoites (X950), the other three pictures show the same cyst (unstained) at 1000, 2000 and 4000 magnification. The cysts have pores in their walls giving a reticulated appearance.

After discussing his experiments of the isolation of the limax amoeba from infected tissues he allows us to review several case histories of patients treated with Clotrimazole over the last 24 months. At the Rheumatoid Arthritis Clinic of the Hounslow Hospital he treated the first group of 54 patients using Clotrimazole at various doses to establish the optimal dose which would be effective with the least side-effects to the patient. He arrived at a dose of about 2 gm per day in patients of normal weight which could be increased or decreased to adjust for the weight of the patient. The most common side-effects were nausea, diarrhea, anorexia, lethargy, drowsiness and pain on micturition. Needless to say that my daughter was one of the first patients in this country to be treated with Clotrimazole and the results were dramatic. After the inflammatory process disappeared, swelling, redness and pain dissipated she could gradually reduce the dose of prednisone to re-activate her completely suppressed pituitary-adrenal function which took about one year. She took a total of 200 Clotrimazole tablets of 500 mg over a period of six months and the disease went completely in remission. Her rheumatologist never used the term "cured" but said that it is burned out. Whatever he calls it, we know that she was free of pain for the first time in more than ten years and resumed a normal life raising a family of two healthy boys and attends to all her domestic and social duties. She also underwent several corrective surgeries and Dr. Inglis of the Hospital for Special Surgery in New York confirmed that there are no signs of active disease anymore. We know that we had found the cure we were looking for

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so many years and that the anti-amoebic treatment proposed by Dr. Wyburn-Mason finally did it.

After our first meeting and personal success I became more interested in Roger's hypothesis and offered to assist him with the "in vitro" evaluation of several compounds on their anti-protozoal activity. We screened several compounds for their "in vitro" activity against the *Naegleria Fowleri* as requested by Roger.

Although Roger had used several unrelated compounds such as Copper sulfate, Bile salts, chloroquine, camoquin, suramin and pentamidine he obtained the best results with Clotrimazole and decided that we should evaluate other Imidazole compounds.

A literature search revealed that in 1970, Abdul Rabbo applied a new anti-amoebic Imidazole compound synthesized by MERCK A.G. as BT 985 in a few patients with acute lupus erythematosus and obtained remission which lasted for six months. Encouraged by these results he then tried the compound in 12 patients suffering from active rheumatoid arthritis who had not responded to any treatment and were severely ill. He reported a 90% success rate and described the results as "dramatic" in the American J. of Tropical Med. Hyg. 1972, 75, 64-66. Abdul Rabbo and Merck, however, failed to see the link of an amoebic etiology and the disease.

Since Clotrimazole was not available in this country for oral administration our own Dr. Jack Blount experimented also with another Imidazole compound, Metronidazole (Flagyl) to treat his own rheumatoid arthritis. He applied a much higher dose than recommended by the manufacturer for the treatment of fungal infections and obtained dramatic results in spite of the severe side effects. Dr. Blount is therefore the first physician who used Metronidazole (Flagyl) for the treatment of rheumatoid disease and the first patient was himself.

Research chemists and pharmacologists have long established that there is a certain relationship between the chemical structure and biological activity of a compound and that this can be modified by introducing substitutions at certain positions in the nucleus. Biological half lives, toxicity, therapeutic effect, absorption, excretion and metabolic fate can all be affected by certain chemical manipulations.

As an example we may refer to the Corticosteroids of which the basic structure consists of three 6-carbon rings and a single 5-carbon ring, the Cyclo-pentano-phenantrene nucleus.

Our own system synthesizes corticosteroid hormones from dietary cholesterol which has the same basic structure.

Substitution of a Hydroxyl group at the C-11 position produces Hydrocortisone (Cortisol), the major gluco-corticoid secreted by the adrenals in man. A Ketogroup at the C-3 position is essential for glucocorticoid activity while an Oxy or Hydroxyl group at C-11 is indispensable for anti-inflammatory activity.

We can obtain short acting, intermediate acting and long acting compounds by certain chemical substitutions.

Our research therefore tried to establish which substitution in the Imidazole nucleus would be responsible for the anti-amoebic activity and the toxicity.

IMIDAZOLE compounds remained of great interest to research scientists because of their specific anti-microbial activity against a wide range of micro-organisms unaffected by the more widely used antibiotics. We conducted an extensive literature search of Imidazole compounds to find the relationship between their chemical structure and biological activity and toxicity. Their activity is listed as: anti-fungals; anti-protozoals; anti-amoebics; anti-parasitics etc. By comparing their toxicity expressed as LD₅₀ in animal toxicity studies we could determine which specific substitution would affect an increased toxicity. By also comparing the anti-protozoal activity against *Naegleria Fowleri* in our own experiments and published data we arrived at the following assumptions:

SLIDE 1

METRONIDAZOLE: This slide shows the chemical structure of FLAGYL, Metronidazole, which we will use to explain our findings:

The Imidazole nucleus is a 5-ring with two Nitrogen atoms in the ONE and THREE position. The 3 remaining positions contain Carbon. There are two double-bonds, one between C₂ and N₃ and the second between C₄ and C₅. This is the basic Imidazole Nucleus in which there is a substitution in the ONE position of a 2-hydroxy-ethyl group, which is a short aliphatic chain. We also see a NITRO group at the FIVE position.

Here is what we found:

1) Anti-protozoal and anti-amoebic activity of Imidazole compounds are more pronounced in compounds which have a substitution at the ONE position of chemical moieties and that these substitutions increase the activity if they are higher, which we will see in another slide.

2) The presence of a NITRO group at the 4 or 5 position increases the toxicity expressed as LD₅₀ in rats considerably but does not affect the anti-protozoal activity.

3) Compounds with a substitution at the TWO position have no anti-protozoal activity and are listed as "pesticides".

4) Compounds with a substitution at the THREE position also failed anti-protozoal activity against Naegleria and are listed as active against Candidiasis and Moniliasis infections.

5) The most potent Imidazole compound against Naegleria Fowleri "in vitro" is Clotrimazole, followed by Tinidazole, Nimorazole, Ornidazole and Metronidazole in this order. All these five compounds are substituted at the ONE position and the only one without a NITRO group is Clotrimazole.

I mentioned earlier that Dr. Blount used Metronidazole (Flagyl) because this was the only available Imidazole compound approved by the FDA against Fungal infections for oral administration. I also mentioned that Dr. Blount used a much higher dose to achieve the desired effect. This compound is listed in the Protocol of our Foundation and is now widely used worldwide for the treatment of rheumatoid disease.

SLIDE 2

TINIDAZOLE: Dr. Wyburn-Mason called Tinidazole his most effective compound with the least side-effects. During our review meetings with him in 1978 in which we discussed our findings he selected this compound for the initial treatment of all his patients and would only switch to another compound if the patient failed to respond. Tinidazole or FASIGYN is widely available in Europe for use as an anti-trichomonal drug in strips of eight 500 mg tablets which the patient also takes during two consecutive days.

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SLIDE 3

LEVAMISOLE. Listed as an anthelmintic it has been widely tested since 1975 as a possible anti-arthritic compound. This compound has a beta-thiazole group at the ONE and TWO position and also a substitution at the FOUR position. The compound is extremely toxic and has a narrow therapeutic range just like Penicillamine and Methotrexate with which it is often compared. There are numerous reports of clinical studies in this country and abroad and

SLIDE 4

SIDE EFFECT increases as DOSE increase and Efficacy also increased at a higher dose but patients could not tolerate the therapeutic dose.

SLIDE 5

shows the ratio at two dose levels between number of patients dropped due to ineffectiveness and side-effects.

All clinical studies with this compound were discontinued and the FDA rejected further evaluation in view of the serious side-effects and mortality rate.

SLIDE 6

TIFLAMIZOLE, synthesized by DUPONT Pharmaceuticals has substitutions at the TWO, FOUR and FIVE positions and has NO anti-protozoal activity. In a series of phase one clinical studies this compound was ineffective against rheumatoid arthritis and was also considered very toxic in view of the severe side-effects encountered during these studies. We believe that further evaluation will be halted.

SLIDE 7

CLOTRIMAZOLE. This compound, synthesized by Bayer A.G. is a trityl-imidazole with a bis-phenyl-2-chloro-phenyl substitution at the ONE position. There is no NITRO group in the formula which makes it different from the other Nitro-imidazole compounds and also less toxic. We consider this compound one of the most potent anti-protozoal and anti-amoebic drugs available today.

Clotrimazole is listed as a broad spectrum antimycotic agent, is fungicidal and fungistatic against pathogenic fungi, moulds, protozoan and certain bacteria and therefore not only covers the spectrum of activity of the polyenes (Amphotericin-B, Nystacin and Candicidin) but also that of Griseofulvin.

As reported earlier, Wyburn-Mason was the first to use Clotrimazole for the treatment of rheumatoid arthritis. After the initial group of 12 patients he conducted several open label studies and in the years following his announcement to the ninth International Congress of Chemotherapy he treated hundreds of patients with several other anti-protozoal compounds. Since Clotrimazole was not available and only supplied by Bayer for clinical studies, he used other Imidazole compounds such as Tinidazole, Ornidazole and Nimorazole which are widely used for the treatment of trichomonal, fungal and yeast infections in several European countries. Wyburn-Mason never thought of conducting a double-blind clinical trial because he considered administering a placebo to patients was unethical and refused to withhold treatment for the sake of convincing the medical establishment of the validity of his new treatment. It is, of course, unethical but the FDA and the medical profession do not accept results of open label studies as proof of efficacy and safety of a treatment with a new compound. They insist on controlled studies in which patients and their physicians are blinded in order to obtain objective results which can be statistically evaluated by biostatisticians not connected with the study. The first such controlled study was conducted in England by Dr. Wojtulewski at the St. Mary's hospital in Eastbourne in 1979. A double-blind study in which 24 patients were given Clotrimazole while 23 patients got Ketoprofen a standard non-steroidal anti-inflammatory agent. The study lasted for 8 weeks. Wojtulewski used a relatively high dose of Clotrimazole, i.e. 40mg/kg/day the first week and 80mg/kg/day for 7 weeks. The patients were selected at random and all suffered from active rheumatoid arthritis.

SLIDE 8

This slide shows the incidence of side-effects such as nausea, vomiting, diarrhoea, anorexia besides lethargy, drowsiness and pain on micturition. The Clotrimazole group had considerably more than the Ketoprofen group. Some of these symptoms were definitely caused by the Herxheimer reaction but were listed as side-effects. In spite of these reactions, Wojtulewski retained the majority of the patients in the study because 17 of the original 24 completed the 8 weeks treatment. The 7 withdrawals were mostly by

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gastro-intestinal intolerance. The Clotrimazole group did considerably better than the Ketoprofen group when changes in clinical measurements were compared as shown in the next slide.

SLIDE 9

Please note that significant improvement from pre-entry in the Clotrimazole group was more pronounced than in the Ketoprofen group. Dr. Wyburn-Mason and I, visited with Dr. Wojtulewski in 1980 to review his findings. He informed us that he continued at a lower dose for up to 8 months in an uncontrolled study what we call an open label study (patient and physicians know which drug is taken) and that he had only few side-effects but considerably more improvements than the first 8 week trial. He reported this also in his publication in Annals of the Rheumatic Diseases, 1980, 39, 469-472 and gave us several graphs of which the next

SLIDE 10

shows the results of treatment of up to 36 weeks at a dose of 1 gram Clotrimazole per day.

Wyburn-Mason had also established that the optimal dose for the Imidazole group should be 2 gram per day for 2 consecutive days per week for a period of not less than 6 weeks and up to 12 weeks. At this dose we could expect less side-effects and the patient could cope better with the Herxheimer reaction if taken only 2 days in a row for a week. With this in mind we prepared the protocol for our double-blind study at Bowman Gray School of Medicine by Dr. Robert Turner, Professor of Medicine and Chief Section on Rheumatology. The IND and the Protocol were approved by the FDA and the Supervisory Board of Wake Forest University for Human studies.

SLIDE 11

This slide indicates the basic data of the protocol in which 64 patients with active rheumatoid arthritis are randomly selected into two groups of which one group of 32 patients will receive Clotrimazole tablets at a dose of 20mg/kg for two consecutive days per week for a period of 12 weeks. The other group will receive IDENTICAL Placebo tablets also for two consecutive days per week for 12 weeks. It is a double-blind study and the data are being collected by trained personnel of the Bowman Gray School of Medicine.

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The patients are requested to visit their physicians at the hospital once a week for examination and laboratory tests. They also receive their medication for one week only.

We hope to obtain an objective interpretation of the results of the study when all patients have completed the 12 week treatment. The data will be evaluated statistically by the Biostatistician who is not connected with the investigating team. The first patient was entered into the study on December 19, 1984 and we hope to complete the study in about 18 months when all 64 patients received the treatment. I maintain regular contact with Dr. Robert Turner, the chief investigator and chief of the rheumatology section of the Bowman Gray School of Medicine.

I visited with Dr. Turner on the 16th. of June and we reviewed the data of the patients who have entered the study to date. Please note that it is a double-blind study and that we do not know which patients received Clotrimazole and which the Placebo.

Report on Study

In conclusion, I would like to share with you a letter which I received on June 13, 1985 from a patient who requested information on the anti-amoebic treatment for rheumatoid arthritis after several physicians failed to relieve her from the agonizing pain and suffering in spite of their conventional treatment.

I recommended that she contact Dr. Kirk Morgan of Louisville, Kentucky, one of our cooperating physicians, closest to her home.

Dear Dr. Simoons:

First, let me thank you for all your kindness to both of us. You have been very helpful. Dr. Kirk Morgan has been very kind and helpful. If all of the doctors were as polite and considerate as you and Dr. Morgan it must be a better world. I must write Doctor Morgan too.

Mrs. Ann Land says she is better than she has been in years. She said she would write to you. Mr. Ralph Smith says he would not take anything for his treatment.

I am glad Dr. Burkheart (her family physician) has decided to be interested. I wish I could afford to go to Alabama in July. It would be very helpful and interesting, I am sure.

A report of the treatment:

- Week 1 I took 8 tablets on Tuesday, March 12; Wednesday March 13th., 8 more. At the very beginning I had a heavy coated tongue, a metallic taste. Felt a little sick.
- Week 2 Took 8 tablets on March 19th. That night I got worse, ached all over, was feverish, was restless. Could not go to sleep. On Wed. March 20th., hurt all over. Took one prednisone tablet and the 8 pills that day.
- Week 3 Nothing unusual. March 28 (Wednesday) ached all over. Muscles were almost unbearable. Was not able to work. Thursday got some relief about 7.30, did more housework than usual. Did a lot of sweating especially at night. Nausea all of the time, headache, tightness in the jaws and throat. Pains in around ribs. Upset stomach, vomited on Sunday after lunch. Legs still hurt.
- Week 4 April 2nd. Took the Flagyl and (Zyloprim 3 a day for 7 days) April 3rd., took same. I was too sick to eat. I could not hold my head up. Wanted to vomit. I had a temperature, ached all over and was very weak. I had colitis, cramps in stomach. I felt bad on Thursday. Had runny nose and headache. Felt real good on Saturday. Sunday a little draggy. Thought school on Monday, April 8th. Tuesday, April 9th., I did not feel too well. Friday, April 12, felt better. My joints do not ache any more. The flesh gets sore if I bend or stoop too much. But as far as the rheumatoid disease, I think it is cured. I know I am much better of the aches and pains. If any part of our letters are of any help feel free to use them. If I can answer any questions feel free to ask.

Sincerely,

(Mrs.) Elsie F. Ware
1765 Hoover Pike
Nicholasville, Kentucky, 40356

I believe that this letter typically describes the symptoms of the Herxheimer reaction, which all physicians should closely observe when using the anti-amoebic treatment in rheumatoid disease.

I have a few minutes left and would like to answer any questions which you may have.

