

Double-Blind Placebo Controlled Crossover Study of Clotrimazole  
20 mg/kg in Q.I.D. Dosage Two Days Per  
Week for Six Weeks in the Treatment of Rheumatoid Arthritis

Introduction

Clotrimazole has a broad spectrum of anti-mycotic activity and was developed clinically for its control of fungal infections, pathogenic for animals and man. The drug was developed as an agent by Bayer Laboratories from a screening program involving more than 900 substituted imidazoles active against dermatophytes, yeasts, biphasic fungi and molds.

Clotrimazole has also been shown to have anti-protozoal action and it has been suggested that chemical compounds which have anti-protozoal activity are useful when administered internally for treating rheumatoid arthritis (1). It has also been suggested that this may be one of the most effective agents of this class for treating rheumatoid arthritis (1,2).

Background

Clotrimazole is an antimycotic drug, synthesized by the Bayer Laboratories in Elberfeld, Germany, by M. Plempel et al (3), known as Bayb 5097, also known as canesten or lotrimin (Delbay U.S.A.) It is chemically bis-phenyl (2-chlorophenyl)-1-imidazolyl-methane and is approved by the U.S. Food and Drug Administration only for topical treatment of tinea and candida infections of the skin. It is available as a 1% solution or cream for external use. Clotrimazole has an in vitro activity and inhibits growth of most strains of the various dermatophytes that cause tinea pedis, tinea cruris and tinea corporis. It also inhibits the growth of some strains of gram-positive bacteria and has activity against trichomonas species in high concentrations (4). Clotrimazole is also available in 100 mg. vaginal tablets and it is reported to be active against vaginal trichomoniasis (5).

Oral clotrimazole has been used successfully to treat systemic mycotic infections in broad series of clinical trials with divided dosage ranging from 40-100 mg/kg/day (6). Side effects are primarily gastrointestinal with upper GI discomfort, nausea and diarrhea a significant limiting factor in 1/3 of patients. CNS effects are seen in up to 8% of patients consisting of apathy, depression and in rare cases, hallucinations.

Since the anti-helminthic imidazole levamisole has been widely used in the treatment of rheumatoid arthritis, much has been learned which can be applied to studies of the effects of other agents of a similar class in the treatment of patients with this disease (7). Early in the course of trials with this agent, daily dosage was employed with a distressing 10% of patients developing severe neutropenia (8). With the development of regimens utilizing several days per week or one day per week therapy a lower incidence of hematologic problems has been

demonstrated although this drug continues to have too high an incidence of side-effect development to be widely used in the treatment of rheumatoid arthritis at present (9,10). Another imidazole, metronidazole has been tried in rheumatoid arthritis without significant side effects but with doubtful efficacy (11). Clotrimazole in high doses has been shown to be effective in the treatment of rheumatoid arthritis but its use has been associated with an unacceptably high incidence of side effects (2). Since it has been suggested in an uncontrolled study that lower doses may be effective in the treatment of rheumatoid arthritis (1), this trial will be undertaken at lower doses to test the efficacy of this drug in rheumatoid arthritis.

An in depth discussion of the chemistry, toxicology, and pharmacology, of clotrimazole is included in Appendix T. A detailed bibliography for this protocol is included as Appendix J.

#### Objective

To evaluate the efficacy and tolerability of clotrimazole 20mg/kg given in q.i.d. divided dosage for two consecutive days per week for six weeks compared to placebo given in the same schedule in a double-blind study in the treatment of patients with active rheumatoid arthritis.

#### Selection of Subjects

##### A. Inclusion criteria

1. Male and female outpatients
2. Adults (18 to 70 years)
3. Patients with diagnosis of rheumatoid arthritis on stable dosages of aspirin or non-steroidal anti-inflammatory agents for at least one month will be enrolled in this trial. Patients must meet the criteria for definite or classical rheumatoid arthritis as indicated in Appendix A.
4. Patients must have active disease as indicated in Appendix B and must agree to attempt to continue on their nonsteroidal anti-inflammatory drugs at the same dosage during the duration of the study.

##### B. Exclusion Characteristics

1. The presence of arthritis due to the ARA exclusions listed in Appendix A (Section C).
2. Patients who do not show active disease as indicated in Appendix B.
3. Pregnant females or nursing mothers.
4. Patients who have:
  1. Active peptic ulcer, gastritis, or important gastrointestinal diseases.
  - II. Cirrhosis, hepatitis, or liver enzyme abnormalities more than 20% above the upper limits of normal
  - III. Serious cardiovascular disease
  - IV. Serious hematologic disease
5. Patients who are being treated with or who have been treated

in the last three months with immunomodulating agents (gold, penicillamine, antimalarials, corticosteroids, or cytotoxic agents

#### Number of Patients

Forty patients will be entered in this double-blind crossover study.

#### Duration of Study

A. Duration of clinical trial is 1 year.

B. Duration of study for each patient is six months as follows: six weeks on medication or placebo followed by six weeks observation, followed by six weeks on medication or placebo, followed by another six weeks of observation.

#### Procedure

A. History and physical. Complete history and physical examination will be obtained at visits one and nineteen.

B. Patients will be numbered consecutively as they are entered into the study. By random allocation, one-half of the patients will receive clotrimazole in 250 mg. compressed tablets in the dose of 20 mg/kg in four divided doses, taken two consecutive days per week for the period of six weeks. One-half of the patients will receive identical placebo tablets. This will be followed by a six week wash-out/observation period. At the end of 12 weeks, patients will be crossed over and receive the alternative placebo or trial medication. This will also be followed by a six week observation period. The randomization process will be performed at visit one.

At visit one, a bottle containing either active medication or placebo tablets will be dispensed to each patient. Directions on the bottle will read, "Take as directed." The patients will be given written instructions to take six to eight tablets per day, depending on their body weight as equally divided q.i.d. doses. Medications will be dispensed at each visit, and the number of returned tablets will be recorded on the Clinical and Laboratory Assessment of Disease Activity Sheet: (Appendix C) for each visit. No changes in anti-inflammatory agent medication will be allowed during the study. Injectable corticosteroids are not allowed during the study. Pure analgesics such as acetaminophen will be allowed during the study but notation of the number of tablets taken will be noted on the clinical and laboratory assessment sheet.

#### C. Clinical Observations

The same study technician will evaluate a given patient with the help of one of the rheumatology physicians and compile the data in Appendix C at the same time of day at each visit. Demographic data will be entered as indicated giving the patient's name, visit date, unit number, age, sex, and duration of disease. The anti-inflammatory agent medication, analgesic medication, number of study capsules returned and number consumed will be recorded if appropriate, on the Assessment Sheet (Appendix C). The American Rheumatism Association functional class 0 to 4 will be determined as indicated in

Appendix D and recorded on the Assessment Sheet. Morning stiffness will be entered in units of one hour from zero to five. Grip strength readings from each hand will be recorded separately in mm/Hg from 0 to 260. A Total Joint Count Recording Sheet (see Appendix E) will be used to evaluate the total number of joints involved and the value (0 to 60) will be entered on the Assessment Sheet. A joint will be considered affected if objective findings such as tenderness, pain on passive motion, or swelling are noted on examination. Pain intensity, patient assessment, and observer assessment will be quantitated using visual analogue scales. Patients will indicate the severity of their pain at each visit on an 11 point scale (see Appendix F). Patient and observer assessment will be evaluated using similar measurements (see Appendix F). Laboratory data including Westergren ESR, uric acid, alkaline phosphatase, total protein, and rheumatoid factor titer will be recorded on the Assessment Sheet. Peripheral blood WBC/mm<sup>3</sup>, differential leukocyte count, and hematocrit readings will also be entered on the Assessment Sheet. Clinical efficacy and side effect evaluations will be performed at each visit and will be recorded on the Assessment Sheet (Appendix C).

#### D. Laboratory

1. The following laboratory analyses will be performed for safety or efficacy:

a. Blood and urine specimens will be obtained at the initial visits and every two weeks for the following:

CBC

SMAC - See Appendix C for methods and normal ranges Routine urinalysis

2. Hemoccult cards will be given to the patient to test for occult blood, and these cards will be checked at each visit. Results from at least one specimen should be recorded each week. Other results can be recorded if more are obtained.

Westergren sedimentation rates and rheumatoid factors will be done at visit one, seven, ten, sixteen, and E. Adverse Experiences

Adverse experiences are defined as any unwanted signs or symptoms which may in any way be related to pharmacologic action of any of the drugs taken by the patient. All such experiences are to be entered on the Clinical and Laboratory Assessment Sheet (Appendix C) with the following data to be recorded: onset date, duration, frequency, drug relationship, and action taken. Any adverse experiences should be reported immediately to Dr. Robert Turner or the Rheumatology Fellow on call at (919) 748-4209.

#### F. Dropout Patients

An effort will be made to determine the reason for each dropout and complete history and physical examination will be performed at the beginning of the study and at the study termination, listing the

investigators' opinion as to how the patient did on the study, any adverse experiences encountered, and the patient and observer opinion as to the efficacy of the medication utilized at each time period.

#### Statistical Analysis

A. An overall flow chart of the protocol and procedures for this study is depicted in Appendix H.

B. Data analysis will be performed as follows:

Forty subjects ( $S_1 \dots S_{40}$ ) will be randomly divided into 2 groups of 20 (Groups I and II) consisting of subjects  $S^*_1 \dots S^*_{20}$  and  $S^*_{21} \dots S^*_{40}$  respectively (Statistical Tables Appendix K). Group I and Group II). Group I and II subjects will receive drug, placebo or neither according to the scheme outlined in Appendix H. Data collected per Appendix C will be recorded in parallel for each Group at visits 1, 7, 10, 16, and 19. Statistical computations will be performed on an IBM PC-XT computer using LOTUS 1,2,3, electronic spreadsheets for data recording and manipulations. Each measured parameter at visits 7, 10, 16 and 19 will be compared with its value at visit 1 using the paired t-test (19). This test will reveal significant effects of drug or placebo on rheumatoid arthritis activity disease parameters within each group. Each parameter will also be tested for stability by analysis of variance using the treatment-by-subjects design (20) in which the parameter is evaluated over the course of the protocol by computation of an F

statistic. The F value will be computed for data collected on visits 1, 7, 10, 16 and 19. A significant F value indicates that the parameter was not stable during the protocol. These two tests of significance should allow a preliminary evaluation of the effects of drug on the disease activity parameters. Negative results would strongly suggest lack of effect. Positive results in one or more tests would indicate that further data analysis (e.g., intergroup comparisons) is warranted. In this circumstance outside statistical consultation will be sought.

#### Appendix A

A. Criteria for the classical of rheumatoid arthritis

This diagnosis requires 7 of the following criteria. In criteria 1 through 5 the joint signs or symptoms must be continuous for at least 6 weeks. Any one of the features listed under Exclusions (See below) will exclude a patient from this and all other categories.

1. Morning stiffness
2. Pain on motion or tenderness in at least 1 joint (observed by a physician).
3. Swelling (soft tissue thickening or fluid, not bony overgrowth alone) in at least 1 joint (observed by a physician).
4. Swelling (observed by a physician of at least 1 other joint (any interval free of joint symptoms between the 2 joint involvements may not be more than 3 months).

5. Symmetric joint swelling (observed by a physician) with simultaneous involvement of the same joint on both sides of the body (bilateral involvement of proximal interphalangeal, metacarpophalangeal, or metatarsophalangeal joints is acceptable without absolute symmetry). Terminal phalangeal joint involvement will not satisfy this criterion.

6. Subcutaneous nodules (observed by a physician) over bony prominences, on extensor surfaces, or in juxtaarticular regions.

7. Roentgenographic changes typical of rheumatoid arthritis (which must include at least bony decalcification localized to or most marked adjacent to the involved joints and not just degenerative changes). Degenerative changes do not exclude patients from any group classified as having rheumatoid arthritis.

8. Positive agglutination test. Demonstration of "rheumatoid factor" by any method which, in 2 laboratories, has been positive in not over 5% of normal controls, or positive streptococcal agglutination test.

9. Poor mucin precipitate from synovial fluid (with shreds and cloudy

solution). An inflammatory synovial effusion with 2,000 or more white cells/mm<sup>3</sup>, without crystals can be substituted for this criterion.

10. Characteristic histologic changes in synovium with 3 or more of the following: marked villous hypertrophy; proliferation of superficial synovial cells often with palisading; marked infiltration of chronic inflammatory cells (lymphocytes or plasma cells predominating) with tendency to form "lymphoid nodules"; deposition of compact fibrin either on surface or interstitially; foci of necrosis.

11. Characteristic histologic changes in nodules showing granulomatous foci with central zones of cell necrosis, surrounded by a palisade of proliferated mononuclear and peripheral fibrosis and chronic inflammatory cell infiltration.

#### B. Definite rheumatoid arthritis

This diagnosis requires 5 of the above criteria. In criteria 1 through 5 the joint signs or symptoms must be continuous for at least 6 weeks.

#### C. Exclusions

1. The typical rash of systemic lupus erythematosus (with butterfly distribution, follicle plugging, and areas of atrophy).

2. High concentration of lupus erythematosus cells (4 or more in 2 smears prepared from heparinized blood incubated not over 2 hours).

3. Histologic evidence of periarteritis nodosa with segmental necrosis

of arteries associated with nodular leukocytic infiltration

extending perivascularly and tending to include many eosinophils.

4. Weakness of neck, trunk and pharyngeal muscles or persistent muscle swelling or dermatomyositis.

5. Definite scleroderma (not limited to the fingers).

6. A clinical picture characteristic of rheumatic fever with migratory joint involvement and evidence of endocarditis, especially if accompanied by subcutaneous nodules or erythema marginatum or chorea. (An elevated antistreptolysin titer will not rule out the diagnosis of rheumatoid arthritis).

7. A clinical picture characteristic of gouty arthritis with acute attacks of swelling, redness, and pain in 1 or more joints, especially if relieved by colchicine or accompanied by urate crystals.

8. Tophi

9. A clinical picture characteristic of acute infectious arthritis of bacterial or viral origin with: an acute focus of infection or in close association with a disease of known infectious origin, chills, fever, and an acute joint involvement, usually migratory initially (especially if there are organisms in the joint fluid or response to antibiotic therapy).

10. Tubercule bacilli in the joints or histologic evidence of joint tuberculosis.

11. A clinical picture characteristic of Reiter's syndrome with urethritis and conjunctivitis associated with joint involvement, usually migratory initially.

12. A clinical picture characteristic of the shoulder-hand syndrome with unilateral involvement of shoulder and hand, with diffuse swelling of the hand followed by atrophy and contractures.

13. A clinical picture characteristic of hypertrophic osteoarthropathy with clubbing of fingers and/or hypertrophic periostitis along the shafts of the long bones especially if an intrapulmonary lesion (or other appropriate underlying disorder) is present.

14. A clinical picture characteristic of neuroarthropathy with condensation and destruction of bones of involved joints and with associated neurologic findings.

15. Homogentistic acid in the urine, detectable grossly with alkalization.

16. Histologic evidence of sarcoid or positive Kveim test.

17. Multiple myeloma as evidence by marked increase in plasma cells in the bone marrow, or Bence-Jones protein in the urine.

18. Characteristic skin lesions of erythema nodosum.

19. Leukemia or lymphoma with characteristic cells of peripheral blood, bone marrow, or tissues.

20. Agammaglobulinemia.

#### Appendix B

Active RA patients must show at least 3 of the following criteria:

1. Number of tender or painful joints on motion =>6
2. Number of swollen joints =>3
3. Duration of morning stiffness (hrs) >3/4
4. ESR (mm/hr) >28

Appendix C

Clinical and Laboratory Assessment of Disease Activity  
in Rheumatoid Arthritis Patients

Name:  
Date of Visits:  
Unit Number:  
Age:  
Sex:  
Disease duration (months):

	Initial or Subsequent Visit	Subsequent Visit
ARA Functional Capacity (0-4)	[ ]	[ ]
Morning stiffness (hours 0-5)	[ ]	[ ]
Grip strength (mm.Hg) R/L (0-260)	[ ][ ][ ]/[ ][ ][ ]	[ ][ ][ ][ ]/[ ][ ][ ][ ]
Total joint count (0-60)	[ ][ ]	[ ][ ]
Patient assessment of pain (0-10)	[ ][ ]	[ ][ ]
Observer assessment of pain (0-10)	[ ][ ]	[ ][ ]
*ESR (mm/hr)	[ ][ ][ ]	[ ][ ][ ]
Uric Acid (mg/dl)	[ ][ ]-[ ]	[ ][ ]-[ ]
Alkaline phosphatase (U/L)	[ ][ ][ ]	[ ][ ][ ]
Total protein (gm/dl)	[ ]-[ ]	[ ]-[ ]
*Rheumatoid factor (neg. or 1:)	neg/1:[ ][ ][ ]	neg/1:[ ][ ][ ]
Peripheral Blood		
WBC/ $\times 10^6$ /mm <sup>3</sup>	[ ][ ]-[ ][ ]	[ ][ ]-[ ][ ]
RBC/ $\times 10^6$ /mm <sup>3</sup>	[ ][ ]-[ ][ ]	[ ][ ]-[ ][ ]
Differential	NS [ ][ ][ ] L [ ][ ][ ]	NS [ ][ ][ ] L [ ][ ][ ]
	NB [ ][ ][ ] E [ ][ ][ ]	NB [ ][ ][ ] E [ ][ ][ ]
	M [ ][ ][ ] B [ ][ ][ ]	M [ ][ ][ ] B [ ][ ][ ]
Hematocrit	[ ][ ]-[ ]	[ ][ ]-[ ]

Medications

Adverse Experiences

\*To be done only at visits 1,7,10,16,19



## Appendix D

### Classification of Functional Capacity (Capacity in Rheumatoid Arthritis)

Class 1: Complete functional capacity with ability to carry on all usual duties without handicaps

Class II: Functional capacity adequate to conduct normal activities despite handicap or discomfort or limited mobility of 1 or more joints

Class III: Functional capacity adequate, to perform only few or none of the duties of usual occupation or of self care

Class IV: Largely or wholly incapacitated with patient bedridden or confined to wheelchair, permitting little or no self care

## Appendix E

Name:  
Unit Number:  
Date of Visit:

### Total Joint Count

S = Swelling T = Tender or Painful on Passive Motion E = Both Blank = Uninvolved

Upper Extremities		Lower Extremities	
Right	Left	Right	Left
Temporomandibular _____		Hip _____	
Sternoclavicular _____		Knee _____	
Acromioclavicular _____			
Shoulder _____		ANKLE _____	
Elbow _____		HOCKLE _____	
Wrist _____		TARSA _____	
METACARPOPHALANGEAL		METATARSOPHALANGEAL	
First (thumb) _____		First (great) _____	
Second _____		Second _____	
Third _____		Third _____	
Fourth _____		Fourth _____	
Fifth _____		Fifth _____	
PROXIMAL INTERPHALANGEAL		INTERPHALANGEAL (distal and proximal)	
First (thumb) _____		First (great) _____	
Second _____		Second _____	
Third _____		Third _____	
Fourth _____		Fourth _____	
Fifth _____		Fifth _____	

## Appendix F

Name:  
Unit Number:  
Date of Visit:

### Visual Analogue Scales

Indicate the severity of your pain by circling the number on the line with a pencil. (Pain Scale)

No Pain 0 1 2 3 4 5 6 7 8 9 10 Unbearable Pain

Indicate degree of well-being with respect to your rheumatoid arthritis averaged over the past 6 weeks or since the last visit by circling the number on the line with a pencil (Patient Assessment)

Best 0 1 2 3 4 5 6 7 8 9 10 Worst

Indicate degree of rheumatoid arthritis activity averaged over the past 6 weeks or since the last visit by circling the number on the line with a pencil (Observer Assessment)

Best 0 1 2 3 4 5 6 7 8 9 10 Worst

Appendix G  
 SHAC II Methods and Normal Ranges

<u>TEST</u>	<u>METHOD</u>	<u>NORMAL RANGE</u>
Sodium	Ion Selective Electrode	135 - 146 mEq/L
Potassium	Ion Selective Electrode	3.5 - 5.0 mEq/L
Chloride	Mercuric/Thiocyanate	95 - 106 mEq/L
CO <sub>2</sub>	pH/phenolphthalein Indicator	22 - 30 mEq/L
Urea Nitrogen	Diacetyl-oxamine	8 - 24 mg/dl
Glucose	Hexokinase	70 - 110 mg/dl
Calcium	Cresolphthalein complexone	8.5 - 10.5 mg/dl
PO <sub>4</sub>	Reduced phosphomolybdate	2.5 - 4.5 mg/dl
Uric Acids	Uricase	2.5 - 8.0 mg/dl
Cholesterol	Enzymatic	120 - 260 mg/dl
Total Protein	Buret	6.0 - 8.0 g/dl
Albumin	BCG Dye	3.2 - 5.0 g/dl
T. Bilirubin	Jendrasik and Grof with blank	0.1 - 1.2 mg/dl
D. Bilirubin	Jendrasik and Grof with blank	0.0 - 0.4 mg/dl
Alk. Phosphatase	PNPP	30 - 110 U/L
LDH	UV - NAD converted to NADH	90 - 250 U/L
SGOT	UV - Enzyme Conversion	0 - 35 U/L
Creatinine	Jaffe - picric acid	0.5 - 1.5 mg/dl
Iron	FerroZine	40 - 16 mcg/dl

Appendix H  
 Overall Flow Chart of Protocol and Procedures

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
VISITS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19						
Hist. & Phv.	X																								
Wound Factors	X						X					X						X							X
ESR	X						X					X						X							X
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SHAC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
H.A.	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemucult	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluations*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Med-P <sub>1</sub> **		P	P	P	P	P	P						C	C	C	C	C	C							
Med-C <sub>1</sub>		C	C	C	C	C	C						P	P	P	P	P	P							

\*Clinical efficacy and side effect evaluations

\*\*Medications prior to visit: P<sub>1</sub> = placebo first; C<sub>1</sub> = clotrimazole first

## Appendix I

### Detailed Discussion of Chemistry, Toxicology, and Pharmacology of Clotrimazole

#### CHEMISTRY

This drug is a synthetic tritylimidazole.

Chemical: 1-(2-chlorophenylbenzhydryl) imidazole; also bis-phenyl-(2 chlorophenyl-1-imidazolyl methane.

Approved Name: Clotrimazole

Number: FB h 5097

$C_{22}H_{17}ClN_2$  M.P. 143-144 Molecular Weight 344.8

Weakly alkaline, colorless, crystalline substance which forms stable salts with both inorganic and organic acids. It is slightly hygroscopic.

#### TOXICOLOGY

##### Animal Studies

###### Dose

LD 50	700-900 mg/Kg	Mice and Rats
1000-2000 mg/Kg		Rabbits

In mice and rats death is seen in 24 hours. In rats death occurs in 2-6 days of weakness, apathy and muscle spasm.

Dogs and cats vomit the drug and no oral LD50 can be established but it is greater than 2000 mg/Kg.

In rodents 200 mg/Kg/day is tolerated by lavage with minimal weight loss. At autopsy liver and adrenal hypertrophy is seen with associated marked increase in microsomal enzyme induction.

Dogs at 100 or 200 mg/Kg/day show emesis and liver and adrenal enlargement.

SGOT enzyme elevation is seen but no jaundice.

Monkeys receiving 150 mg/Kg/day for 6 weeks develop vomiting, diarrhea and liver enlargement.

Rats on chronic study of 50 and 150 mg/Kg/day show SGPT, Alk Phos elevation, liver enlargement, fatty liver, and adrenal enlargement.

In monkeys and dogs at 12 months post drug liver size returns to normal but the adrenals remain enlarged for a much longer period (12).

##### Human Studies

Thirteen healthy volunteers each received 500 mg. Clotrimazole for 28 days. The preparation was taken orally in capsules soluble in gastric juice. Before the test and on the 3rd, 7th, 14th, 21st and 28th day hepatic function, protein and fat metabolism, renal function and the peripheral blood-picture were examined. At the same time the volunteers were questioned about the subjective tolerance.

Due to gastro-intestinal intolerance, the starting dose of 60 mg/Kg/day could not be maintained. Subjective side effects were less on a lower dose. In one case there was a rise in liver transaminases

and in one there were leukopenia. Both stopped treatment.

There were six males (mean 25 years) and seven females (mean 37 years). The drug was given three times daily after meals. Nine of the volunteers completed the 28 day trial. Of the other four, one stopped on the 3rd day, one on the 4th day and two on the 21st day of the trial. Three groups of side effects were observed.

1) Gastro-intestinal, including nausea, vomiting, anorexia and diarrhea.

2) Central nervous, including lethargy, depression and irritability.

3) Frequency of micturition.

Of the two subjects who discontinued the trial due to changes in biochemical parameters, one showed an increase in SGOT to 36.5 I.U., and in SGPT to 58.2 I.U. on the 21st day of treatment. Seven days later the SGOT had returned to normal and the SGPT was still slightly raised. In the second, the base line leukocyte count was 3,600 which fell to 2,400 on the 21st day. At the same time the alkaline phosphatase fell sharply. Seven days later the blood picture had returned to normal and the alkaline phosphatase was 1 mU/ml.

For those completing the trial there was a change in nearly all parameters, with the exception of  $\alpha_1$ -,  $\alpha_2$ - and  $\gamma$ -globulins, and SGPT and polymorphonuclear leukocytes.

3rd day: Fall in erythrocytes, haemoglobin, leukocytes (with relative lymphocytosis) total cholesterol and total protein; increase in phosphatides, urea, bilirubin, and free glycerine.

7th day: Alterations in the blood picture remained.

Esterified fatty acids, lipoproteins, alkaline phosphatase and SGOT increased; free glycerine, total cholesterol plasma proteins and urea tended to normal.

21st day: Blood picture relapsed, SGOT and bilirubin raised.

Fat metabolism not investigated. Total protein decreased; but relative increase in "pre-albumin."

28th day: Haemoglobin raised, bilirubin normal. Erythrocyte down, white blood count normal. Phosphatides and enzymes normal. Increases in lipid fractions and urea; total protein still low; low "pre-albumin" fraction, raised globulin fraction.

The increase in blood urea is within the norm and is probably without clinical significance, since kidney function remains normal. It is suggested, however, that in patients with impaired renal function, blood urea and creatinine should be checked regularly.

From the measurements of ketosteroid excretion, there is no evidence of interference with adrenal function after administration of clotrimazole

In a second study 7 healthy women received 3.0 G clotrimazole orally/day for 14 days. Two developed anorexia, one with nausea and vomiting. Apart from a rise in the alkaline phosphatase of one

patient to 32.6, monitoring was normal.

#### PHARMACOLOGY

On the isolated guinea pig intestine concentrations above  $3 \times 10^{-7}$  cause inhibition of the contractions evoked by ricotine, acetylcholine, histamine and barium chloride. An intramuscular dose of 10 mg/Kg has no effect on gastric secretion in the rat, nor has an oral dose of 100 mg/Kg any purgative effective. 50 mg/Kg given intraduodenally has no choleric action. An oral dose of 100 mg/Kg has no anti-hypertensive action.

#### Central Nervous System

An oral dose of 1000 mg/Kg in the mouse has no mydriatic action, no anti-convulsive action, and no inhibition of a defense reaction. It has a mild sedative effect. An oral dose of 400 mg/Kg in the rabbit has no hypnotic action as gauged by the EEG response. Clotrimazole given to cats at 100 and 200 mg/Kg produced pronounced central excitation, similar to that of anti-depressants.

#### Carbohydrate Metabolism

Prolonged administration did not alter the blood sugar in rats or dogs. However, in acute experiments high doses lowered the blood sugar. The blood sugar of mice given five doses of 250 mg/Kg falls at 3 hours to 116 mg% compared to a control value of 150 mg%.

In fed but not starved rats there is a significant reduction in blood sugar at 2 and 4 hours after single doses up to 250 mg/Kg, the reduction increasing with increased number of doses and lasting up to 22 hours.

Adrenalectomised rats show a greater fall in blood sugar on corresponding doses. Tolbutamide causes a similar fall (40%) in blood sugar in rats rendered hypoglycemic on clotrimazole to the fall produced in untreated rats. Clotrimazole induced hypoglycemia is not brought about by a release of insulin, as there is no concomitant fall in non-esterified fatty acids.

#### Absorption, Distribution and Excretion in Animals

C-14 labelled clotrimazole was given orally to 46 rats. Absorption of a 30 mg/Kg dose was greater than 90%. One hour after administration radioactivity was concentrated in the skin, liver, adrenal cortex, adipose tissue, gastric mucosa and proximal small intestine. The metabolites are excreted in the bile and 90% is eliminated via the faeces. After a single dose there is only 1% activity in the body after four days. There is no secretion of intact clotrimazole either in the bile or the urine.

The main metabolites are bisphenyl-2-chlorophenylmethane, 2 chlorophenyl-4<sup>1</sup>-hydroxyphenyl-phenyl-methane, bisphenyl-2-chlorophenyl methanol, and 2 chlorobenzophenone.

C-14 labelled clotrimazole given orally to 2 dogs resulted in very low peak concentrations in the blood. The excretion of activ-

ity was predominantly via the faeces.

The excretion of clotrimazole metabolites in mice reached peak values from 10-16 hours after administration. The urinary excretion of metabolites was 5% of the dose administered.

Exception tests were carried out with cats, dwarf hogs and cattle. The results were comparable. None of the animal species tested was found to secrete unaltered clotrimazole in the urine, as shown by microbiological assay. On intra-peritoneal and subcutaneous administration, little of the preparation is absorbed. The resulting urinary excretions are less than 1 µg metabolite/ml urine.

#### Serum Levels in Animals

Serum concentrations were determined after single and repeated administration of clotrimazole in mice, rats, guinea pigs, dwarf hogs, dogs and cattle. Peak serum concentrations obtained in mice and rats after single oral dose of 100 mg/Kg are obtained 3-5 hours after administration.

On the third day of receiving 100 mg/Kg/day, the blood levels of two dwarf hogs were between 6 and 8 µg per ml four hours after the last dose. Serum levels for beagle dogs two hours after the last dose in the 100 mg/Kg group ranged from 3-6 µg/ml, and in the 50 mg/Kg group, from 2-3 µg/ml.

#### Tissue Distribution in Animals

After beagle dogs had received 100 mg/Kg for 13 weeks, clotrimazole (or metabolite) concentrations were determined in tissue extracts by chromatography. The concentration was 2-3 µg/G of fresh tissue for heart, lung and kidney. Liver contained 7 µg/G, approximately two-thirds of which were metabolites. Bile contained 12 µg/ml as metabolites only. Adipose tissue contained 20 µg/G clotrimazole and/or its metabolites.

#### Chronic Toxicity

Five groups of 50 male and 50 female rats were treated with Clotrimazole 0, 10, 25, 60 and 150 mg/Kg/day and examined at 6 months. Up to 6 months no adverse effect was apparent except a slower weight gain in one group.

At 6 months females showed some reduction in haematocrit and haemoglobin and a rise in cholesterol. Males in one group had lowered cholesterol levels. Liver weights were increased in both sexes, the livers being fatty. Microscopy showed hepatocellular swelling, increased granularity of the cytoplasm and hyperchromatic nuclei. There were no mitotic figures nor bile stasis. Other organs were normal.

#### Embryotoxicity and Teratogenicity

None seen at chronically tolerated dosage in mice, rats or rabbits.

#### Fertility

Groups of 10 male and 20 female rats were fed the following doses of clotrimazole: 0, 5, 10, 25, and 50 mg/Kg/day throughout. After

ten weeks two females were put with one male for a week in rotation so that each female was exposed to 3 different males for one oestrus cycle. After successful impregnation or 3 weeks the animals were separated. Some rats were delivered by Caesarean at 13 days, others reared their litter to four weeks. Clotrimazole did not impair the parents' behavior, health or fertility. Pregnancy was unaltered.

#### Oncogenicity

Of 50 rats treated for 348 days (448 life days) with a total dose of 3.45 g/Kg, four died, each with hepatic degeneration. None showed any evidence of tumor formation.

#### Biochemical Data in Dogs

There were no significant differences in blood sugar, urea, BSP retention, SGOT, SGPT, ornithine carbamoyl-transaminase, calcium, total bilirubin, or heparin recalcification time between treated and control dogs, or between treated groups of dogs (4 dogs/group) given 50, 100 or 200 mg/kg for 13 weeks.

The serum alkaline phosphatase rose in a dose dependent manner.

Serum cholesterol was significantly reduced in dogs on 200 mg/Kg and leucine aminopeptidase was significantly elevated.

Alkaline phosphatase values rose during the 13 weeks clotrimazole administration.

Urine analysis was normal except for continuous proteinuria in one female on 200 mg/Kg and transient proteinuria in two males on the same dose and one male each on 100 and 50 mg/Kg.

Final bodyweights and heart sizes were not significantly different at necropsy. There was a dose dependent significant increase in liver and adrenal weights. Kidney weight was only significantly increased in the 200 mg/Kg group.

Histopathology was performed at Huntingdon Research Center with results as follows:

Liver: Hepatocyte enlargement occurred in all animals including controls, being more marked in three of the 200 mg/Kg animals. Degenerative changes seen in rat liver were not seen in the dog liver.

Adrenals: In dogs on 200 and 100 mg/Kg there was cellular enlargement, particularly in the zona fasciculata.

All other tissues were examined and found to be normal.

#### Rat Follow-up Study

A further study with rats was undertaken to assess recovery from clotrimazole therapy.

Three groups of 30 male rats were treated five times per week for six weeks with a single dose of a suspension of 0, 10 and 100 mg/Kg clotrimazole orally. Three rats from each group were killed after 1,2,5,10,20 and 30 daily doses, then 1, 2, 4, and 10 weeks after the period of treatment.

In the 100 mg/Kg group, epithelial hypertrophy of the liver

developed initially, followed by hyperplasia and low-grade parenchymal damage towards the end of treatment. Ten weeks after the termination of treatment these liver changes were not observed. Fatty infiltration of the liver was seen in both test and control groups, being more pronounced in the animals treated with 100 mg/Kg, and increasing throughout the study.

#### Enzyme Induction

Repeated oral doses of Clotrimazole given to mice produce an induction of liver enzymes with a consequent lowering of serum levels of clotrimazole due to a more rapid catabolism.

Direct evidence comes from the sleeping time of mice given hexobarbital. Four groups of 20 mice received i.v. 150 mg/hexobarbital/Kg, and the sleeping time measured. Then each received 100 mg. clotrimazole/Kg orally for five days and on the sixth day a further dose of hexobarbitone, and the sleeping time measured again. Results are shown in Table I.

GROUP	Before Clotrimazole	After Clotrimazole
	1	103
2	98	26
3	119	61
4	112	35

Clotrimazole also induces the parathion-splitting enzyme system, which is blocked by DL-ethionine. This block is not reversed by methionine. Clotrimazole induces enzyme systems in adrenalectomized animals, suggesting that the adrenals are not implicated.

In subacute toxicity tests there was a dose dependent increase in liver weight and urinary excretion of ascorbic acid, suggesting that there was an adaptive rather than toxic mechanism responsible; although the failure of methionine to reverse the effect of DL-ethionine suggests that the ascorbic acid excretion test does not differentiate between hyperfunctional and toxic hepatomegaly.

In man, enzyme induction occurs later, within three weeks of starting therapy.

#### Clinical Pharmacology

C-14 Clotrimazole was given orally in gelatin capsules to hospitalized patients and healthy volunteers. It was well absorbed (90%) from the gastrointestinal tract. The blood levels achieved were dose-dependent, reaching a peak around 3 hours. Serum levels over 1 µg/ml of active substance were reached between the third and sixth hour after dosage. The ratio of active to inactive substance was 1:1.5.

In humans, the active substance was also found in saliva, sputum, sweat, and subcutaneous fat. The active substance has only been



demonstrated in the cerebrospinal fluid in cases of meningitis.

No active substance, and only 10% metabolites are excreted in the urine. In infants active substance can be demonstrated in urine after high doses. Clotrimazole is almost completely metabolized in the human. The metabolites are excreted predominantly in the bile.

Similar metabolic and distribution results have been reported by Duhm et al. (13). In clinical studies these workers have used dosages as high as 3 gms in patients receiving increasing dosages of 25 mg/Kg/day for 12 days with peak serum levels seen in 3 hours. Vaginal application results in serum levels 500x lower.

Leukocyte myeloperoxidase levels rose in 15 of 17 patients in another study (14). Effects were seen on the 3rd day in those receiving clotrimazole. This drug has also been studied in such widely varying settings as a trial of therapy for oral candidiasis in newborn babies (15), treatment of

bronchopulmonary aspergillosis in adults (16), treatment of ocular aspergillosis (17), and treatment of fungal infection in renal transplant patients (18).

#### Appendix J

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Appendix K - Statistical Tables

TABLE - Group I  
Visit

	1	7	10	16	19
**Drug	<u>0</u>	<u>P</u>	<u>0</u>	<u>C</u>	<u>0</u>
Subject					
S* <sub>1</sub>	X <sub>1</sub> (1)	X <sub>1</sub> (7)	X <sub>1</sub> (10)	X <sub>1</sub> (16)	X <sub>1</sub> (19)
.	.	.	.	.	.
.	.	.	.	.	.
S* <sub>20</sub>	X <sub>20</sub> (1)	X <sub>20</sub> (7)	X <sub>20</sub> (10)	X <sub>20</sub> (16)	X <sub>20</sub> (19)

TABLE - Group II  
Visit

	1	7	10	16	19
Drug	<u>0</u>	<u>C</u>	<u>0</u>	<u>P</u>	<u>0</u>
Subject					
S* <sub>21</sub>	X <sub>21</sub> (1)	X <sub>21</sub> (7)	X <sub>21</sub> (10)	X <sub>21</sub> (16)	X <sub>21</sub> (19)
.	.	.	.	.	.
.	.	.	.	.	.
S* <sub>40</sub>	X <sub>40</sub> (1)	X <sub>40</sub> (7)	X <sub>40</sub> (10)	X <sub>40</sub> (16)	X <sub>40</sub> (19)

X = value of test parameter

\*\* 0 = none, P = placebo, C = clotrimazole