Laboratory assessment of the antimycotic drug clotrimazole

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SYNOPSIS Laboratory studies on clotrimazole showed that it had marked activity *in vitro* against all the *Candida* spp. and *Cryptococcus* spp. tested, against almost all strains of *dermatophytes*, and against *Aspergillus* spp and other fungal genera responsible for systemic mycoses; it had limited activity towards Gram-positive bacteria. The majority of *Candida* strains required MICs below 1 µg/ml and MCCs below 2 µg/ml.

Serum, urine, and faecal assays of clotrimazole were made by microbiological methods on five children who received 100 mg/kg/day clotrimazole for several weeks. *In-vitro* sensitivity tests and biological fluid drug assays are also reported on specimens from 18 patients in other hospitals receiving clotrimazole for severe candidosis; several were renal transplant cases. Similar investigations are reported on specimens from 18 patients with pulmonary aspergilloses.

The significance of low levels of the drug in body fluids, even after prolonged therapy, is discussed, and it is suggested that clotrimazole may be the first of a long series of imidazole derivatives with varying pharmacological and therapeutic properties.

Until recently the only available therapy for most systemic fungal infections has been with the polyene antibiotics—nystatin, pimarin, candicidin, and amphotericin B. The first three cannot be administered parenterally, and they are not absorbed from the bowel; occasional success when given orally for urinary, pulmonary, and systemic candidoses has probably been due to the eventual elimination of the faecal reservoir. The problem in treating systemic fungus infections arises from the difficulty in achieving an effective serum drug level; amphotericin B can be administered systemically to give good levels but may have severe toxic effects (Andriole and Kravetz, 1962).

Laboratory and clinical reports by Plempel, Bartmann, Büchel, and Regel (1969); Oberste-Lehn, Baggesen, and Plempel (1969) on the antifungal activity of a new synthetic drug, Bay b, 5097, Clotrimazole, and the good results reported by Marget and Adam (1971) in childhood candidosis have prompted further laboratory and clinical studies, some of which are reported in this paper.

Methods and Materials

Clotrimazole is a trityl imidazole derivative—bis-phenyl (2-chlorophenyl)-1-imidazolyl-methane (Fig. 1). A fluffy white powder, insoluble in water, it dissolved freely in organic solvents; a 1% solution in chloroform, stored in the dark at 4°C, was used as stock. Assays against freshly prepared standards, diluted in 0.01M phosphate buffer at pH 7.2, showed

BAY b 5097 'CLOTRIMAZOLE'

bis-phenyl (2-chlorophenyl -1-imidazolyl)-methane

Fig. 1 Structural formula for clotrimazole.

¹Since this paper was written R. L. Newman has died.

no loss of potency over a period of several months. Alternatively, a 1% solution in polyethylene glycol is available (Bayer AG) and dilutions from this gave consistent results on repeated testing. Dilutions from both stock solutions into aqueous vehicles become opalescent immediately on addition, as do the water-insoluble polyene antibiotics, but appear to retain full activity.

Candida and fungi were isolated from clinical specimens by the methods reported earlier (Holt and Newman, 1967); Czapek Dox agar or Sabouraud glucose peptone agar to which was added 100 µg/ml of chloramphenicol or, more recently, gentamicin, proved satisfactory for the recovery of these organisms from specimens initially contaminated with large numbers of bacteria.

Candida isolates were routinely differentiated into *C. albicans* and *non-albicans* by the serum germ tube and chlamydospore production tests; species identification of *Candida* strains and of systemic fungi were kindly carried out by Dr I. G. Murray and Miss C. Philpott (Mycology Reference Laboratory, London) who also supplied many other fungi for sensitivity testing. Sensitivity testing was performed as soon as possible after primary isolation. The same workers kindly tested sera from the cases reported here for immune antibodies.

Administration of the Drug

Adults and babies received 100/kg/day in four six-hourly oral doses; capsules each containing 0.5 g clotrimazole were easily swallowed by adults. These capsules were too large for small babies, and an aqueous suspension of the compound was therefore prepared to contain the calculated six-hourly dose in 1 or 2 ml, measured by the nursing staff from 2 ml syringes. The suspension settled rapidly after mixing, although separation was retarded by the addition of 0.2% gum tragacanth; it was stored at 4° and assays showed no loss of potency after 14 days, the maximum time for which the preparation was stored on the ward.

Sensitivity Tests

BY DISC

Filter paper discs, 9 mm, were impregnated with clotrimazole suspension and rapidly vacuum dried. A 300 µg/ml suspension of clotrimazole in 0.01M phosphate buffer at pH 7.2 was diluted from the 1% stock solution in polyethylene glycol; the discs were sterilized in dry air at 160° for one hr, and 1 drop from a 30 drop/ml pipette was placed onto each. The batch of impregnated discs was immediately vacuum-dried at room temperature over silica gel

and stored in tightly capped containers at 4°. Assays showed that no deterioration occurred under these conditions after three months' storage.

A suspension of *Candida* or *Cryptococcus* sp to be tested was made in infusion broth to an approximate density of 1×10^6 cells per ml. An even lawn of this suspension was sown over an infusion broth agar plate at pH 7.2, and allowed to dry for one hour; clotrimazole discs were then planted onto the agar surface and the plate was incubated for 24 to 48 hours. The diameter of the zone of inhibition was measured; organisms giving zones greater than 20 mm diameter were deemed to be fully sensitive; zones between 12 and 20 mm to indicate relative sensitivity; and less than 12 mm as relatively to fully resistant to clotrimazole. Counted suspensions of mycelating fungi were not feasible, so a very light, well mixed inoculum was prepared; the zones of inhibition were less well defined with these fungi, but still gave reasonable indications of sensitivity to the drug.

Correlation between agar diffusion sensitivity tests by disc and liquid minimal inhibitory concentration (MIC) tests was attempted for suspensions of *C. albicans* at the stated density, and was as follows:

Disc Zone of Inhibition (mm)	MIC (µg/ml)
> 20	< 0.2
20-12	0.2-2.0
< 12	> 2

ESTIMATION OF MINIMAL INHIBITORY AND MINIMAL CICAL CONCENTRATION (MIC AND MCC)

Preliminary studies showed that there appeared to be no differences between the MIC/MCC of clotrimazole for fungi when tested at pH 5.0, 6.0, 7.0, and 8.0; all estimations, and all assays, were therefore performed at pH 7.2 as a biological reality. Similarly a few MIC/MCC estimations for fungi of clinical interest showed no significant differences when performed at 22°, 26°, and 37°; 37° was selected as standard, since this was the temperature at which the drug would be likely to encounter systemic fungi clinically.

Infusion broth or peptone water (Cruickshank, 1965) was used in all experiments; the use of a medium reinforced with carbohydrate was avoided because fermentation with acid products might lower the pH well below 5.0 and tend to reduce the drug's activity.

Drug concentrations in the range 0.05-20 µg/ml were made in 2 ml lots of medium, and 1 drop of standard inoculum was added to each. In the case of

Candida, *Cryptococcus*, *Torulopsis*, and *Geotrichum* strains this inoculum was established by direct counts on 48-hr broth cultures in a haemocytometer chamber, and was calculated to give an initial inoculum of 1×10^5 cells/ml. A non-ionic surfactant, Triton X100, at 0.1% was used to disperse cell aggregates in both counting and seeding (Holt, 1971). Such accurate inocula were not possible with actively mycelating fungi, particularly those forming tough pellicles on the broth surface; attempts were therefore made to prepare a suspension of even turbidity by shaking with sterile glass beads or fragmented glass and a light inoculum was added to each tube, consisting usually of one drop of a 1 in 100 dilution of a well dispersed 48-hr broth culture. The MIC was noted after two and four days at 37°, and the MCC estimated by subculturing into infusion broth from all tubes showing no growth and by planting standard drops onto infusion or Sabouraud agar. In most cases the residuum of the initial inoculum could be seen; this was washed three times with sterile water or infusion broth before subculturing to remove any drug still adherent to the fundal structure. Unpublished observations during work with the halogenated oxyquinolines had suggested that antifungal drugs might otherwise be carried over into subcultures and thus give a fallaciously low MCC.

Sensitivity to clotrimazole was also estimated by the agar dilution technique; light inocula of the fungus were spotted onto a series of infusion agar plates containing different drug concentrations. The end point after two to three days at 37° indicated the MIC; the MCC of the drug was estimated by pad replicating the inoculum site after incubation onto drug-free nutrient agar. The results obtained by this method compared well with those from the liquid methods detailed above.

DETECTION OF EMERGENT RESISTANT MUTANTS
Light inocula of representative strains of each fungal genus were successively passaged across increasing drug gradients on agar layer plates (Szybalski, 1952). In most cases it was necessary to start on plates with a gradient 0.0-5 µg/ml; two to three days were allowed for growth on each plate, and subcultures were taken from the growth edge farthest across the drug gradient. This was continued for 10 to 15 passages, and liquid MICs on the most resistant growth were compared with the values obtained with the original strains before the sequence was started.

EFFECT OF CLOTRIMAZOLE ON MORPHOLOGY OF FUNGI

Candida, *Aspergillus*, and *Trichophyton* species were

grown in broth containing levels of drug previously established as subinhibitory for the individual strain. After three to five days, very thin wet preparations were examined by interference microscopy at $\times 150$ and $\times 600$ magnification.

ASSAY OF CLOTRIMAZOLE IN BIOLOGICAL FLUIDS

Infusion agar or any of the proprietary penicillin assay agars proved satisfactory at pH 7.2. More than 100 strains of *Saccharomyces* and *Candida* were tested as possible indicator organisms, and a strain of *Candida pseudotropicalis* (Carshalton) was selected; it required an MIC of below 0.01 µg/ml clotrimazole, and appeared to be stable after some 100 weekly subcultures. An overnight culture in infusion broth, checked for purity by plate-out when initially inoculated, was used as inoculum. Polystyrene plates, 14 cm diameter, were placed on level surfaces and 50 ml agar base was poured. When set, the base was overlaid with 20 ml agar seeded with 0.1 ml of the overnight broth culture, and the plates were thoroughly dried for two hr at 37°. For larger batches of assays 25 $\times$ 25 cm assay plates were used (Mast); they required 200 ml base and 80 ml seeded overlay.

The range of standards was 0.02, 0.1, 0.5, 2.0, and 10.0 µg/ml clotrimazole, diluted in 90% serum or in 0.01M phosphate buffer at pH 7.2, according to which biological fluids were being assayed. Preliminary studies showed no difference between 90% serum, 50% serum, and aqueous buffer standards; this appears to indicate that no *in vitro* binding occurred in serum, and during the past year aqueous buffer standards at pH 7.2 were therefore used for all assays.

Fish spine beads (no. 3 size) were used as assay reservoirs in the way detailed by Lightbown and Sulitzeanu (1957). After two hr prediffusion at 20°, plates were incubated on a level surface for 24 hr at 37°; the zones of inhibition were of good size, about 30 mm diameter round the 2 µg/ml standard, showing a well defined outer margin of inhibition, with hazy growth perhaps 2 mm wide inside this margin. The outer margin was measured in all cases, the mean of two diameters of each zone being used for subsequent plots on half-log graph paper.

Results

SENSITIVITY OF CLINICAL ISOLATES OF FUNGI TO CLOTRIMAZOLE

These results are shown in Tables I, II, and III.

SENSITIVITY OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA TO CLOTRIMAZOLE

These changes are shown in Table IV.

	Total Tested		Concentration of Clotrimazole (µg/ml)				
			<0.1	0.1-0.5	0.5-2	2-10	>10
<i>C. albicans</i>	108	MIC	48	54	4	2	0
		MCC	0	32	70	3	3
<i>C. non-albicans</i>	116	MIC	68	36	12	0	0
		MCC	19	20	74	3	0
<i>Cryptococcus neoformans</i>	5	MIC		2	3		
		MCC			3	2	
<i>Torulopsis glabrata</i>	3	MIC		1	1	1	
		MCC			2		1
<i>Geotrichum candidum</i>	3	MIC	1	2			
		MCC		3			

Table I Sensitivity of clinical isolates of yeast-like fungi to clotrimazole

	Total Tested		Concentration of Clotrimazole (µg/ml)				
			< 0.1	0.1-0.5	0.5-2	2-10	> 10
<i>Epidermophyton floccosum</i>	5	MIC		4	1		
		MCC			5		
<i>Microsporium audouinii</i>	4	MIC		4			
		MCC			4		
<i>Microsporium canis</i>	4	MIC		2	2		
		MCC		1	3		
<i>Microsporium gypseum</i>	2	MIC			2		
		MCC				2	
<i>Trichophyton mentagrophytes</i> (including integumentum)	5	MIC		2	3		
		MCC		1	4		
<i>Trichophyton rubrum</i>	3	MIC		1	2		
		MCC			3		
<i>Trichophyton sulphureum</i>	4	MIC		2	2		
		MCC			2	2	
<i>Trichophyton verrucosum</i>	2	MIC		2			
		MCC		2			
<i>Trichophyton violaceum</i>	3	MIC		2	1		
		MCC			3		

Table II Sensitivity of dermatophytes to clotrimazole

	Total Tested		Concentration of Clotrimazole (µg/ml)				
			< 0.1	0.1-0.5	0.5-2	2-10	> 10
<i>Aspergillus flavus</i>	3	MIC			3		
		MCC				3	
<i>Aspergillus fumigatus</i>	18	MIC		8	9	1	
		MCC			9	8	1
<i>Aspergillus niger</i>	3	MIC			2	1	
		MCC				3	
<i>Allescheria boydii</i>	2	MIC			2		
		MCC				2	
<i>Madurella mycetomi</i>	2	MIC			2		
		MCC				2	
<i>Mucor mucedo</i>	2	MIC		1	1		
		MCC			2		
<i>Sporotrichum schenkii</i>	3	MIC			1	2	
		MCC				2	1

Table III Sensitivity to clotrimazole of other fungi of medical interest

Organisms (all current clinical isolates)	No. Tested	Disc Sensitivity ¹ of Strains Sensitive to (μ g/disc)			No. Tested	Liquid Sensitivity (μ g/ml)	
		10	50	200		MIC Concentration	MCC Concentration
Gram-positive							
<i>Staphylococcus aureus</i>	38	2	38	38	2	10 50	10 > 50
<i>Staphylococcus albus</i>	40	17	40	40	2	< 5 > 50	10-20 > 50
Haem. strep. group A	17	0	0	0	2	> 500 > 500	> 500 > 500
Enterococci	13	0	0	0	2	200-500 200-500	> 500 > 500
Gram-negative							
<i>Proteus</i> sp.	15	0	0	0	2	> 500 > 500	> 500 > 500
<i>Escherichia</i>	20	0	0	0	2	> 500 200-500	> 500 200-500
<i>Klebsiella</i>	12	0	0	0	2	> 500 > 500	> 500 > 500
<i>Pseudomonas pyocyanea</i>	15	0	0	0	2	> 500 > 500	> 500 > 500

Table IV. Sensitivity of Gram-positive and -negative bacteria to clotrimazole

¹ Disc sensitivity: zone of inhibition diameter over 15 mm round 9 mm disc.

CHANGES IN SENSITIVITY TO CLOTRIMAZOLE AFTER PASSAGE ON GRADIENT PLATES

These changes are set out in Table V.

Fungus	Number of Passages	MIC (μ g/ml) in Liquid Medium	
		Before Passage	After Passage
<i>Candida albicans</i>	15	0.5	1-2
<i>Candida albicans</i>	15	0.2	1-2
<i>Candida albicans</i>	15	0.2-0.5	1-2
<i>Candida albicans</i>	15	0.2-0.5	0.5-1
<i>Candida pseudo tropicalis</i>	15	0.1-0.2	0.5
<i>Candida non-albicans</i>	15	0.5-1	1-2
<i>Candida non-albicans</i>	15	0.5	1-2
<i>Candida non-albicans</i>	15	0.5-1	1-2
<i>Cryptococcus neoformans</i>	15	0.2-0.5	2-5
<i>Cryptococcus neoformans</i>	15	0.5-1	2-5
<i>Aspergillus fumigatus</i>	10	0.2-0.5	1-2
<i>Aspergillus fumigatus</i>	10	1-2	2-5
<i>Aspergillus fumigatus</i>	10	0.5-1	1-2
<i>Madurella mycetomi</i>	10	0.5-1	1-2
<i>Madurella mycetomi</i>	10	1-2	1-2
<i>Microsporon audouinii</i>	10	0.2-0.5	0.5-1
<i>Trichophyton menta-</i> <i>grophytes</i>	10	0.2-0.5	1-2
<i>Trichophyton menta-</i> <i>grophytes</i>	10	1-2	2-5

Table V Changes in sensitivity to clotrimazole after passage

EFFECTS OF SUBINHIBITORY AND INHIBITORY CONCENTRATIONS OF CLOTRIMAZOLE ON FUNGI GROWING IN INFUSION BROTH

Candida spp

Little physical change was apparent in *Candida* spores even at cidal drug levels, but they appeared

not to produce hyphae; control cultures in drug-free broth grew luxuriant hyphae.

Aspergillus spp

The conidiophores when fully mature were very small and showed defective sporulation (Figs. 2, 3, and 4). The hyphae became thin and rarefied, and were frequently fragmented. Interference microscopy revealed small segments of living mycelium within the fragmented hyphae; when the fungus was transferred from broth with subinhibitory concentrations of clotrimazole to drug-free broth, fresh outgrowth grew from these live segments (Figs. 5, 6, and 7).

Similar changes were noted in *Trichophyton* spp.

ASSAYS ON SERUM AND URINE FROM PATIENTS RECEIVING CLOTRIMAZOLE

Five infants received 100 mg/kg/day in six hourly doses of an aqueous suspension of the drug; three of them also had 10 ml of 0.1 % aqueous clotrimazole suspension instilled into a ureteric catheter at eight hourly intervals during the first six days of treatment. A brief summary of the cases is included in Table VI; full clinical details will be reported elsewhere. No clinical, haematological or biochemical side effects were observed.

Clotrimazole was also estimated on five faecal samples from cases 1 and 2; levels of 12, 90, 90, 95, and 110 μ g clotrimazole per g wet faeces were recorded.

Clotrimazole sensitivity tests and assays on serum, urine, and occasionally faeces were made on

Fig. 2.

Fig. 3.

Figs. 2 and 3 Mature conidiophores of *Aspergillus flavus* in drug-free broth

Fig. 4 Conidiophore of *Aspergillus flavus* in broth containing 1 µg/ml clotrimazole

specimens from 18 patients in other hospitals with severe candidosis, frequently with accompanying candidaemia; most cases had received renal transplants and some had received cardiac valve replacements. All the strains of *Candida* required MICs below 1 µg/ml, mostly 0.1 µg/ml, and MCCs below 10 µg/ml, all but three below 2 µg/ml. Twenty-two serum assays ranged between 0.35 and 0.01 µg/ml, and 17 urine levels were estimated between 0.3 and 0.01 µg/ml. Two specimens of faeces contained 15 and 100 µg/g wet faeces. The duration of treatment varied between three and 21 days.

Similar studies were made on specimens from 18 cases of lung aspergillosis, including several with aspergilloma, receiving clotrimazole; three patients also had sarcoidosis. Seventeen strains of *A. fumigatus* were examined; 16 had MICs below 1 µg/ml, the other below 2 µg/ml; 14 strains required MCCs below 2 µg/ml, one strain 2.5 µg, one strain 5-10 µg, and one 10-20 µg/ml. Fifty-four sera were assayed, with levels between 0.4 and 0.01 µg/ml and 38 urine samples had levels between 0.3 and 0.01 µg/ml. Fourteen sputa had clotrimazole concentrations between 0.05 µg/ml and just detectable levels. Several of these patients received treatment for eight to 12 weeks, although where candidosis was associated with terminal renal failure only a short course of therapy was possible.

Fig. 5.

Fig. 6.

Figs. 5 and 6 *Fragmented hyphae of Aspergillus flavus* grown in broth containing 1 µg/ml clotrimazole

Fig. 7 *Fresh outgrowth from segment of living mycelium after transfer to drug-free broth*

Clinical and radiological details of these cases may be presented elsewhere; very frequently they were not available to us.

Discussion

There can be little doubt that clotrimazole has considerable activity *in vitro* towards the *Candida* genus, *Cryptococcus* sp., *Aspergillus fumigatus*, and the dermatophytes, almost all of which require minimal inhibitory concentrations well below 2 µg/ml. Individual strains of the same species, however, require widely differing inhibitory and cidal levels; the MCC/MIC ratio for single strains varied between 1 and 100. For this reason it is strongly recommended that the MIC and MCC should be estimated on all initial isolates and on re-isolates where clotrimazole therapy is contemplated, particularly since microbiological assay of drug concentration in biological fluids has revealed such low levels. Rarely does the serum level estimated in terms of antimycotic activity exceed 0.4 µg/ml.

Marget and Adam (1971) reported serum levels between 5 and 25 µg/ml in their paediatric cases, values several hundred times those obtained in the present study on similar dosage. The discrepancy is explained by the method of estimation; the procedure

Case No.	Sex	Age	Wt (kg)	Clinical Details	Sensitivity to Clotrimazole		Duration of Therapy (days)	Range of Assay Results ($\mu\text{g/ml}$)		Outcome
					MIC	MCC		Serum	Urine	
1	M	5/12	6	Bilateral hydro-nephrosis, due to posterior urethral valves <i>Candida</i> in urine and blood	0.01	1.0	28 plus instillation	0.02-0.15 (9 assays)	0.05-4 (45 assays)	Urine and blood negative after nine days No recurrence
2	M	8/12	8	'Prune belly' syndrome Rt kidney absent, urethral obstruction due to valves, <i>C. albicans</i> in urine and blood	0.11	1.0	14	0.01-0.09 (10 assays)	0.01-0.03 (18 assays)	Urine and blood negative after six days No recurrence
3	F	6/12	5	Lumbar meningomyelocele, dilated ureters, bladder diverticula, <i>C. albicans</i> in both kidneys, and in bladder	0.5	2.5	14 (100 mkd) then 14 (150 mkd) plus instillation	0.01-0.08 (6 assays)	0.0-0.05 (7 assays)	Urine cultures positive till increased dose plus instillation, then negative 16th day onwards Infection recurred three months later
4	M	2/12	3	Left nephroureterectomy transurethral resection of urethral valves, <i>C. albicans</i> in ureteric urine	0.1	1.2	21	0.01-0.08 (10 assays)	0.01-0.04 (11 assays) but no drug in urine during last nine days	Urine cultures remained positive throughout; no change in drug sensitivity Changed to 5 FC—successful
5	M	5/12	5	Nephrectomy, ureteric implants; unhealed, untreated suprapubic catheter track, <i>C. albicans</i> in urine. Failed 5-fluorocytosine therapy	1.2	2.5	63 plus 4 days instillation	0.04 (2 assays)	0.0-0.07 (12 assays)	Urine cultures persistently negative after 45 days' treatment. No recurrence

Table VI Patients treated with clotrimazole

used by Plempel *et al* (1969) to assay sera from the German cases was photocolorimetric, and did not distinguish between active drug and metabolites. The latter were mainly derivatives belonging to the 2-chlorotritylcarbenol series which appeared in urine and serum during continuous medication with the drug, and it would seem that these metabolites were regarded as microbiologically inactive. It is important to establish the relation between the low active levels and the much higher levels of combined active and inactive imidazole derivatives in body fluids. We are certain that the former is the realistic value in the control of therapy.

Because the levels of active drug in body fluids are insufficient to give fungicidal or fungistatic activity, but are enough to discourage fungal growth and further tissue invasion, the success of clotrimazole as a systemic antimycotic agent probably depends on the maintenance of drug levels in the body for several weeks, although assistance from somatic immune response and surgery where indicated is probably an essential factor. The results of the tests for emergence of strains with increased resistance to clotrimazole after multiple subcultures therefore assume importance; the experiments indicate that no great changes in MIC occur, and suggest that prolonged treatment with the drug will not provoke the emergence of resistant strains *in vivo*. At no time during therapy have strains of fungi with greatly enhanced resistance been isolated.

Little has been reported on the fate of clotrimazole after absorption from the intestine, but it seems certain that it is metabolized in the liver (Plempel *et al*, 1969), and unpublished reports on cases treated in Germany suggest that disruption of hepatic enzyme activity may occur; the drug probably acts with non-specific induction of liver enzymes, and for this reason frequent biochemical monitoring of liver function, especially transaminase activity, is essential. In our series, three observations may reflect the heavy involvement of the liver in clotrimazole metabolism. We found repeatedly that serum levels taken at a constant interval after the previous dose fell as treatment continued beyond two weeks. Secondly, during the later weeks of treatment the six-hour curve of drug concentration in serum became increasingly flat, with a vestigial peak in the third hour, whereas estimations during the first few days showed a definite peak at this time. Thirdly, in two children the serum and urine drug levels rose for a few days after treatment was stopped, and only then subsided rapidly. A further unusual finding is that there was no concentration of the drug in urine, in which the clotrimazole content was frequently no higher than the serum level taken at approximately the same time.

Many thousands of imidazole derivatives are theoretically possible, and certainly several hundred have been synthesized and subjected to *in-vitro* tests. Three quite different imidazole derivatives have

undergone *in-vitro* evaluation in these laboratories. Miconazole has about half the activity of clotrimazole over a similar range of fungi but, although clinical trials have been conducted in Belgium with some success on pulmonary candidoses and topical conditions (Brugmans, and van Cutsem, and Thienpont 1970), little is known of its pharmacological fate. Its antibacterial activity was limited to Gram-positive bacteria, and opinions will differ on the value of this particular attribute. Myco-Polycid shows antifungal activity over a wide range, with perhaps one-tenth the activity of clotrimazole, but this is probably sufficient to account for the success of this compound in topical antimycotic therapy; we know of no reports on its systemic use.

Certainly clotrimazole is far from an ideal systemic antimycotic agent; it cannot be given parenterally, it sometimes proves intolerable when given by mouth, and it sometimes disturbs normal liver enzyme activity, which may account in part for the low antimycotic levels detected by us in serum and other body fluids. It is, however, a valuable topical drug, active across a wide fungal spectrum, and it is the only relatively safe drug for the treatment of lung and other deep-seated mycoses caused by *Aspergillus fumigatus*.

Professor Walter Marget of Munich first introduced this drug to us and we acknowledge most sincerely his kind and valuable help then and at subsequent discussions.

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References

- Andriole, V. T., and Kravetz, H. M. (1962). The use of amphotericin B in man. *J. Amer. med. Ass.*, **180**, 269-272.
- Brugmans, J. P., van Cutsem, J. M., and Thienpont, D. (1970). Treatment of long-term *tinea pedis* with miconazole. *Arch. Derm.*, **102**, 428-432.
- Cruickshank, R. (1965). *Medical Microbiology*, 11th ed. Livingstone, Edinburgh.
- Holt, R. J., and Newman, R. L. (1967). Candida in the faeces of children receiving oral tetracycline and phenoxymethyl penicillin. *J. clin. Path.*, **20**, 80-83.
- Holt, R. J. (1970). Studies on the broad spectrum antimycotic agent Bay b5097 (Clotrimazole). In *Proceedings of the 10th International Congress of Microbiology, Mexico City*, p. 149.
- Holt, R. J. (1971). Laboratory and clinical studies on antifungal drugs of the imidazole series. (Abstr.). In *Proceedings of the VIIIth International Congress of Chemotherapy, Prague*, Vol. 1, A.4/7.
- Lightbown, J. W., and Sulitzeanu, D. (1957). The assay of penicillin in blood-serum using *Sarcina lutea*. *Bull. Wld Hlth Org.*, **17**, 553-567.
- Marget, W., and Adam, D. (1971). Bay B5097, a new orally applicable antifungal substance with broad spectrum activity. *Acta paediat. scand.*, **60**, 341-345.
- Oberste-Lehn, H., Baggesen, I., and Plempel, M. (1969). Erste klinische Erfahrungen bei Systemmykosen mit einem neuen oralen Antimykotikum. *Dtsch. med. Wschr.*, **94**, 1365-1367.
- Plempel, M., Bartmann, K., Büchel, K. H., and Regel, E. (1969). Experimentelle Befunde über ein neues oral wirksames Antimykotikum mit breiten Wirkungsspektrum. *Dtsch. med. Wschr.*, **94**, 1356-1364.
- Szybalski, W. (1952). Gradient plate technique for study of bacterial resistance. *Science*, **116**, 46.