

The antimycotic activity of 5-fluorocytosine

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SYNOPSIS 5-Fluorocytosine (5 FC) showed marked *in vitro* activity against nearly all the clinical isolates of *Candida* and *Cryptococcus* examined; the required minimal inhibitory concentration was usually below 2 µg/ml and the minimal cidal concentration below 10 µg/ml. The drug had limited activity towards *Aspergillus* species and dermatophytes. Strains of *Candida* and other fungi with greatly enhanced resistance were fairly readily obtained by successive cultures on increasingly high concentrations of 5-fluorocytosine.

Serum, urine, and faecal assays of 5 FC were made by a microbiological method on specimens from eight children who received between 100 and 25 mg/kg/day of the drug over several weeks for candidosis of the urinary tract usually associated with congenital anatomical abnormalities. Seven of these cases were successfully treated but in the other the *Candida* became highly resistant and treatment was discontinued. Serum drug levels ranged up to 30 µg/ml; urine levels were frequently between 200 and 500 µg/ml. No 5 FC was detected in faecal samples.

Sensitivity tests and assays were also made on samples from subjects receiving 5 FC in other hospitals; five had systemic *C. albicans* infection, the sixth had cryptococcal meningitis. In the latter case levels up to 34 µg/ml were recorded in the cerebrospinal fluid after oral administration, and an investigation was made into the possibility of competitive antagonism by intrathecal cytosine arabinoside given for Hodgkin's disease. The emergence in two other cases of strains of *Candida* highly resistant to 5 FC is discussed, and it is suggested that careful laboratory monitoring of cases receiving 5 FC must continue throughout therapy and afterwards.

A serious gap has recently become increasingly apparent in our antimicrobial armoury; a wide range of drugs is now used for the safe and effective treatment of almost all bacterial disease, but only one agent, amphotericin B, has hitherto been available for systemic antimycotic therapy. The toxic hazards of this polyene are considerable and it must be used with great care. The lack of safe systemic antimycotic drugs has been emphasized after therapy with potent antibacterial and immunosuppressive agents after organ transplants, and after the use of cytotoxic drugs in leukaemia. Many of these cases ultimately suffer an overwhelming generalized mycotic infection, frequently by fungi normally regarded as feebly pathogenic or not-pathogenic. Extensive reconstructive surgery, particularly when artificial internal prostheses are implanted (Holt, 1969), carries similar risks of complication.

The emergence of new systemic antimycotic agents has therefore aroused great interest; 5-fluorocytosine (5 FC) became available in the early

1960s when it was synthesized in the laboratories of Roche at Basel as an antimetabolite for cytosine during a search for antileukaemic drugs. In this it proved ineffective, but Grunberg, Titsworth, and Bennett (1964) showed that it was active in the control of mouse candidaemia; by the end of the decade it had been used to treat many cases of severe candidosis and cryptococcosis (Utz, Tynes, Shadomy, Duma, Kannan, and Mason, 1969; Record, Skinner, Sleight, and Speller, 1971; Davies and Reeves, 1971; and many others). The mode of action of 5-fluorocytosine has been studied extensively; at first it was suggested that the compound competed with its analogue cytosine, or with related pyrimidines, which were regarded as essential metabolites for sensitive fungal cells, but not for bacteria or mammalian cells (fig 1). Scholer (1968) suggested that sensitive fungi metabolize cytosine to uridine, and that 5 FC interferes with this process; mammalian and bacterial cells on the other hand do not metabolize cytosine in this way and 5 FC is therefore inactive towards them. In 1970 Giege and Weil showed that 5 FC may be deaminated by sensitive fungi, possibly to

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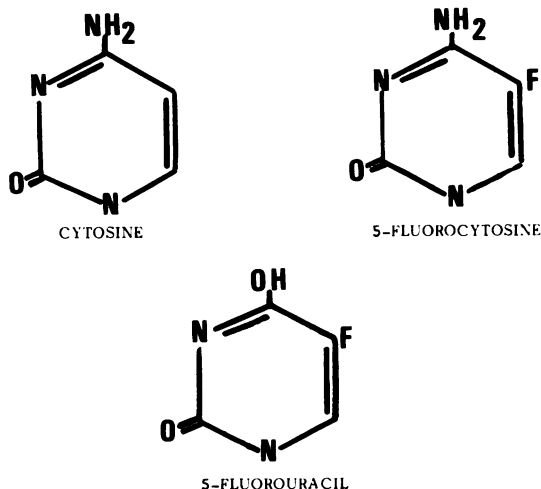


Fig. *Cytosine and analogues*

5-fluorouracil, which is then incorporated into their t-RNA. Up to 50% of t-RNA uracil may be replaced by 5-fluorouracil, with inhibitory effect. Whichever hypothesis is accurate, it seems certain that all experiments *in vitro* with 5-fluorocytosine must be performed with cytosine-free media (Shadomy, 1969); the use of standard bacteriological or mycological media containing the free amino acid invariably gives highly fallacious results in which the drug appears to be relatively inactive against fungi. Furthermore, fungi previously grown in standard media should first be subcultured in cytosine-free medium before subjecting them to sensitivity tests, so that the organisms become depleted of cytosine; washing by suspension and centrifugation in sterile buffer solution may not be sufficient. Shadomy explained the activity of 5 FC in serum and other biological fluids by assuming that these complex mixtures of natural undegraded proteins contain no free cytosine, a suggestion that we have confirmed by chromatographic analysis.

Methods

SOLUTIONS OF 5 FC

A white, finely crystalline powder, 5 FC was dissolved at 1% (10 000 $\mu\text{g}/\text{ml}$) in 0.9% sterile aqueous saline at pH 6.5; this followed the procedure of Shadomy (1969). One ml aliquots of this stock solution were stored at -20° , where tests showed no loss of activity after six months. Appropriate dilutions were made in 0.01M phosphate buffer at pH 7.0.

CLINICAL ISOLATES

Candida and fungi were isolated from clinical specimens by the methods reported elsewhere (Holt and Newman, 1967). Czapek Dox agar or Sabouraud glucose peptone agar, incorporating 100 $\mu\text{g}/\text{ml}$ gentamicin, were used for the recovery of fungi from specimens heavily contaminated with bacteria. Every effort was made to perform sensitivity tests as soon as possible after primary isolation.

Candida isolates were routinely differentiated into *Candida albicans* and *non-albicans* by the serum germ tube and chlamydo-spore formation tests. Speciation of *Candida* strains and of systemic fungi was carried out at the Mycology Reference Laboratory, London, by the kindness of the late Dr I. G. Murray and more recently Miss C. Philpott; this laboratory and that of Dr Y. Clayton also kindly supplied many fungi of medical interest for sensitivity tests.

MEDIA

Shadomy (1969) recommended the use of yeast nitrogen base supplemented with L-asparagine and dextrose, according to the formulae of Wickerham; concentrated media for all tests described in the present study were therefore prepared by dissolving 6.7 g Bacto-yeast nitrogen base (code 0392), 1.5 g L-asparagine, and 10.0 g dextrose in 100 ml glass-distilled water. The solution was sterilized by membrane filtration, and was a 10 \times concentrate, 1 volume of which was diluted with 9 volumes of sterile fluid for use. For liquid tests 0.01 M phosphate buffer at pH 7.0, incorporating phenol red, was used; sterile 1% ion agar (Oxoid L 12) in distilled water, molten, and cooled to 45° , was used as diluent for all plate tests. Chromatography showed no free cytosine present in either yeast nitrogen base or ion agar.

When tests were made with clinical specimens which were possibly contaminated with bacteria, 100 $\mu\text{g}/\text{ml}$ of gentamicin base was included. A series of experiments demonstrated that this antibiotic concentration in no way affected the activity of 5 FC in yeast nitrogen base broth or agar.

SENSITIVITY TESTS

By discs containing 1, 10, and 100 μg 5 FC

Filter paper discs, 9 mm, were impregnated with 5 FC solution and rapidly vacuum dried. The discs were presterilized in dry air at 160° for one hr; dilutions of 5 FC were made in 0.01 M phosphate buffer at pH 7.2 to contain 30, 300, and 3000 $\mu\text{g}/\text{ml}$. Discs of the required strength were prepared by placing one drop from a 30 drop/ml pipette onto the appropriate paper, which was immediately vacuum dried at room temperature over silica gel. The dry discs were

stored in tightly capped containers at 4°, under which condition no deterioration was revealed by assays three months later.

The fungus to be tested was grown overnight in yeast nitrogen base broth; in this and all other subculture procedures used in these studies every care was taken when removing fungi from normal culture media presumed to contain free cytosine to ensure that a minimum of the medium was carried over into cytosine-free cultures. The overnight yeast nitrogen base broth culture was diluted in yeast nitrogen base broth containing 0.1% of a non-ionic surfactant (Triton X100) to a density of approximately 1×10^5 cells per ml for candida or cryptococci; this was impossible with mycelating fungi, so 1/100 dilutions of very well mixed overnight cultures were made. An even lawn of dilute suspension was sown over yeast nitrogen base agar or Czapek Dox plates and allowed to dry for one hour. Discs containing the three concentrations of 5 FC were placed on the agar surface and the plates incubated for 48 hours. Organisms giving zones of inhibition greater than 20 mm diameter round the 1 µg discs were recorded as fully sensitive; zones between 14 and 18 mm diameter round the 10 µg disc were regarded as resistant to the drug.

Because of our interest in the emergence of strains relatively resistant to 5 FC, all sensitivity plates were carefully scrutinized for a minority of colonies growing in the zone of inhibition near the disc; most plates were therefore kept for four to five days before being discarded.

By liquid tests: the estimation of minimal inhibitory and cidal concentrations (MIC and MCC)

All estimations and assays were carried out at pH 7.0 and at 37° to simulate biological conditions. Concentrations of 5 FC in the range 0.05-50 µg/ml were made in 2 ml tubes of yeast nitrogen base broth together with a broth tube without drug. Inocula of Candida and other yeast-like organisms were added to give a final initial concentration of about 1×10^6 cells/ml; mycelating fungi were added as a very light inoculum. All cultures were thoroughly mixed and incubated for 48 hr, after which the MIC was noted by visible turbidity. Standard loop subcultures from each tube were made onto standard infusion broth agar plates, which were incubated for 24 to 48 hr and the MCC recorded by visible growth. This medium contained cytosine, which effectively antagonizes the antimycotic action of 5 FC, thus fulfilling one of the desirable conditions for the test because it is possible to neutralize the inhibitory action of the drug carried over from the first stage. With many antimicrobial agents it is difficult to meet this requirement.

The agar dilution technique was also used to estimate sensitivity to 5 FC; light inocula of fungi were spotted onto yeast nitrogen base agar plates containing a range of drug concentrations; the MIC was read after 48 hours' incubation and the MCC by subsequent velvet pad replication onto drug-free infusion agar. Results from liquid tests compared well with the agar dilution readings.

Induction of Resistant Mutants

Very light inocula of fungi were successively passed across increasing drug gradients on yeast nitrogen base agar plates (Szybalski, 1952), usually starting with plates having a gradient 0.02 µg/ml 5 FC. Each series of plates was given 48-72 hours' incubation for a sequence of 20 passages, and the initial liquid MIC of each strain was compared with the final liquid MIC.

Assay of 5 FC in Biological Fluids

Shadomy (1969) used a *Saccharomyces* sp. (*S. cerevisiae* ATCC 9763) as indicator organism in plate bioassays, and found the lower limit of sensitivity of his method to be about 0.4 µg/ml. We tested this strain and many other strains of *Saccharomyces* and *Candida*, and eventually selected a strain of *C. albicans* (Carshalton 2606) for routine assays; this readily detected 0.1 µg/ml 5 FC. The indicator organism used by us for clotrimazole assays, *C. pseudotropicalis* Carshalton, had a similar level of sensitivity to 5 FC.

The assay strain was maintained in yeast nitrogen base broth by daily subcultures and on each occasion a plate-out was included to check purity. Base layers of 50 ml of 1% ion agar were poured into 14 cm polystyrene plates and after setting they were overlaid with 20 ml of yeast-nitrogen base agar seeded with 0.1 ml of overnight indicator-organism culture; the plates were thoroughly dried at 37°. Larger square assay plates, 25 × 25 cm, were also used, with 200 ml base and 80 ml overlay.

The range of standards was 0.1, 0.5, 2.0, and 10 µg/ml 5 FC prepared in 0.01 M buffer at pH 7.0. Earlier studies had confirmed the observation of Davies and Reeves (1971) that zone diameters for 5 FC in serum were the same as those in aqueous solution, and the latter were therefore used for bioassays of all biological fluids. These were diluted in buffer to final levels of 5 FC within the standard range; sera were diluted 1/5 or 1/10, and urines usually 1/100.

Diluted fluids and standards were applied to the agar surface in fish-spine bead reservoirs (Lightbown and Sulitzeau, 1957), and plates incubated for 24 hr

at 37° on a level surface after prediffusion for two hr at room temperature. Standard curves were constructed on half-log paper and the concentration of 5 FC in each fluid was estimated.

Clinical Experience

CRITERIA FOR TREATMENT OF CHILDREN IN THIS HOSPITAL

Therapy with 5 FC was started only after several urine specimens from each patient were found to contain numerous pus cells and high candida counts; every effort was made to obtain very fresh specimens before regarding the diagnosis as established. Suprapubic aspiration of urine was not attempted because, as will be seen in table IV, many of the children in this series had gross anatomical abnormalities of the urinary tract.

CRITERIA OF SUCCESSFUL THERAPY

Fresh specimens of urine were examined at least once a day throughout treatment and for several weeks after the drug was stopped. General clinical improvement, complete absence of viable *Candida*, and marked diminution of the pus cell count in several successive specimens were required before therapy was stopped. Assessment of complicated cases such as these is notoriously difficult, and bacterial infection sometimes obscured the picture.

ADMINISTRATION OF DRUG

On most occasions adults received tablets containing 0.5 g 5 FC at six or eight hourly intervals to a total of 100 mg/kg/day. The tablets were unsuitable for infants, who were given an aqueous suspension of crushed tablets containing the calculated six hourly dose in 1 or 2 ml. Gum tragacanth (0.2%) was added to the suspension, which was stored at 4°C; assays revealed that it remained stable for three months under these conditions, although no more than 14 days' supply was issued at a time to the wards.

DOSAGE

Our first cases were given 100 mg/kg/day 5 FC as an oral suspension in four six-hourly doses per day. It was soon found that serum levels over 20 µg/ml and urine levels well above 200 µg/ml resulted from this dose; in later cases, therefore, the dose was successively reduced after about a week to 50 mg/kg/day and then to 25 mg/kg/day for the remainder of the course, providing that assays revealed satisfactory levels. None of our patients showed any side effects attributable to the drug.

FAECAL ASSAYS

Aqueous extracts from 10 samples of faeces from

four of the infants receiving 5 FC were assayed. No FC was detected in any. Chromatographic analysis of two of these extracts revealed so confused a picture that it was impossible to say whether free cytosine was present.

PATIENTS IN OTHER HOSPITALS

In addition to the eight children reported above, sensitivity tests and some assays were performed on specimens from subjects receiving 5 FC in other hospitals. In most cases the dose was close to 100 mg/kg/day, although for a few days two patients received approximately 200 mg/kg/day, later reduced to 100 mg/kg/day or less.

Results

EFFECT OF CYTOSINE ARABINOSIDE ON THE ACTIVITY OF 5 FC TOWARDS *CRYPTOCOCCUS NEOFORMANS*

The patient with cryptococcal meningitis was also receiving intrathecal cytosine arabinoside for Hodgkin's disease with meningeal involvement. It seemed important to establish whether this cytosine derivative antagonized the action of 5 FC to the same degree as cytosine. Titrations of both were set up in yeast nitrogen base broth, with a light inoculum of the patient's own strain of *Cr. neoformans*, to give an initial concentration of 1×10^5 cells per ml. Results were read after 48 hr at 37°. Concentrations of cytosine arabinoside between 1 µg/ml and 10 µg/ml were used because it was estimated that the body fluid concentrations of this compound would probably be in that range.

Cytosine arabinoside alone up to 100 µg/ml was found to have no inhibitory action on the *Cr. neoformans*. At a concentration of 1 µg/ml both cytosine and cytosine arabinoside reduced the activity of 5 FC at least one hundredfold. There appeared to be no such interference by procarbazine (Natulan, Roche) at concentrations up to 20 µg/ml in a similar experiment, and the patient later received this drug.

Discussion

The activity *in vitro* of 5-fluorocytosine is confined largely to yeast-like genera, principally *Candida* and *Cryptococcus*, and we confirm the observations of Shadomy (1969) that the drug has only limited activity against *Aspergillus* spp (table I). In the case of *Candida*, fungistatic and fungicidal concentrations were almost always in the range of 0.2 to 2 µg/ml and 2 to 50 µg/ml respectively, and corresponding values of 0.2 to 2 µg/ml and 0.2 to 10 µg/ml were found for a few fresh clinical isolates of *Cr. neoformans* (table II). A small proportion of *Candida* species

Species	Number Tested		Concentration of 5 FC ($\mu\text{g/ml}$)			
			10	10-50	50-200	200
<i>Aspergillus fumigatus</i>	10	MIC	—	1	4	5
		MCC	—	—	—	10
<i>Aspergillus flavus</i>	3	MIC	—	1	2	—
		MCC	—	—	1	2
<i>Aspergillus niger</i>	3	MIC	—	—	3	—
		MCC	—	—	—	3

Table I Sensitivity of *Aspergillus* spp to 5 FC

Species	No. Tested		Concentration of 5 FC ($\mu\text{g/ml}$)				
			<0.2	0.2-2	2-10	10-50	>50
<i>Candida albicans</i>	64	MIC	22	39	3	—	—
		MCC	2	4	28	26	4
<i>Candida non-albicans</i>	19	MIC	12	6	1	—	—
		MCC	—	4	12	3	—
<i>Cryptococcus neoformans</i>	4	MIC	4	2	—	—	—
		MCC	1	2	3	—	—
<i>Torulopsis glabrata</i>	3	MIC	—	3	—	—	—
		MCC	—	—	3	—	—
<i>Geotrichum candidum</i>	2	MIC	1	1	—	—	—
		MCC	—	1	1	—	—

Table II Sensitivity of *Candida*, *Cryptococcus neoformans*, *Torulopsis*, and *Geotrichum* to 5 FC

recovered from clinical sources where the drug had never been used required fungicidal concentrations well above 50 $\mu\text{g/ml}$, probably up to 500-1000 $\mu\text{g/ml}$, and we agree with Shadomy that these must be regarded as natural occurrences. The important practical consequence is that all strains recovered from patients should be tested for sensitivity to the drug, both before and during treatment. Furthermore, we believe that sensitivity tests should be made in liquid medium to reveal the presence of a minority population of fungal cells with greater resistance to 5 FC than the remainder (Holt and Newman, 1972a), and that it may often be a mistake to regard a culture as homogenous in its drug sensitivity.

The serum and urine 5 FC levels in all the patients reported here exceeded the fungal MIC/MCC values at the beginning of treatment (table III). The first cerebrospinal fluid level (34 $\mu\text{g/ml}$) in the patient with cryptococcal meningitis was very much the same as his serum level (26 $\mu\text{g/ml}$), both taken some five hours after an oral dose of 1.5 g (table IV). This suggests that the small molecule diffuses easily through body barriers and that therapeutic levels would be found in sputa of patients receiving the drug for pulmonary mycoses; no such specimens have yet passed through our hands. The second cerebrospinal fluid level was much lower and coincided with the intrathecal use of cytosine arabinoside; tests *in vitro* clearly showed that this agent antagonized the inhibitory action of 5 FC against the patient's strain of *Cr. neoformans*, probably acting in the same way as cytosine.

We were unable to record a coherent series of serum assays to demonstrate peak levels after an oral dose; the adult patients in other hospitals were not accessible to us and the small infants in this hospital were too sick for frequent blood sampling. Our impression is that the peak serum level occurred between one and two hours after a dose, and we tried wherever possible to sample blood at two and six hours. The faeces assayed were all from infants; failure to demonstrate active antimycotic activity in any may have been due to one or more of several causes: technical inadequacy of the extraction process, total absorption of the drug, destruction or conjugation to inactive derivatives are all possible reasons.

A clear warning was sounded by Shadomy (1969) when he noted that resistant strains of *Candida* and *Cryptococci* occasionally emerged during therapy, an observation reinforced in our minds when we found that members of these genera could readily be trained *in vitro* to very high levels of 5 FC resistance (table V). From the start of our therapeutic studies with the drug a careful watch was therefore kept both for infecting fungi with a minority population of greater resistance than the majority and for emergent resistant strains. For a long time none was seen, but during the past few months we have investigated three instances. In the first, reported by Cartwright, Shaldon, and Hall (1972), the sensitivity of *C. albicans* in the urine of a man receiving 5 FC for urinary candidosis after renal transplantation escalated from 0.25 $\mu\text{g/ml}$ to more than 1000 $\mu\text{g/ml}$

No.	Sex	Age	Weight (kg)	Clinical Details	Sensitivity to 5 FC ($\mu\text{g/ml}$)		Duration of Therapy (days)	Range of assay Results ($\mu\text{g/ml}$)		Outcome
					MIC	MCC		Serum	Urine	
1	M	(1/12)	3	Left nephro-ureterectomy, transurethral resection of valves; <i>C. albicans</i> in urine	0.1	2	21 then 16	14-25 (12 tested)	80-200 (23 tested)	Clotrimazole had previously failed. Urine cultures negative after 5th day but <i>Candida</i> reappeared after end of first course. <i>No recurrence.</i>
2	F	1(4/12)	8	Meningomyelocele and hydrocephalus, hydro-nephrosis, ileal loop constructed; <i>C. pseudotropicalis</i> in urine	0.2	1	31	0.9-13 (19)	130-500 (21)	Cultures negative after first 24 hr. <i>No recurrence.</i>
3	F	2(8/12)	16	Meningomyelocele and hydrocephalus; dilatation, upper urinary tract; ileal loop; <i>C. albicans</i> in urine	0.1	0.5	16	7 (1)	80 & 600 (2)	Cultures negative after 5 days. <i>No recurrence.</i>
4	M	(2/12)	4	Nephrostomies, ureteric implants, unhealed suprapubic catheter track; <i>C. albicans</i> in urine	0.1	0.5	77	7-22 (8)	16-500 (48)	Cultures negative after 7 days. Negative till 77 days, then scanty <i>Candida</i> , with greatly increased MCC; <i>persisted.</i>
5	M	(3/12)	3	Urethral valves, trans-urethral resection; <i>C. albicans</i> in urine	0.2	1-2	48	8-21 (3)	30-500 (7)	Cultures negative after 7 days. Remained so. <i>No recurrence.</i>
6	M	(5/12)	7	Meningomyelocele and hydrocephalus, left ureteric reflux; systemic gentamicin for Gram-positive septicaemia; <i>C. albicans</i> repeatedly in urine	0.5	2-5	42	14-24 (4)	150-280 (6)	Cultures negative after 4 days; remained so. <i>No recurrence</i>
7	M	(2/12)	4	Urethral valves; bilateral nephrostomy; dilatation of valves; <i>C. albicans</i> repeatedly in urine	0.1	0.5	40	8-30 (6)	120-600 (12)	Cultures negative after 8 days; remained so. <i>No recurrence</i>
8	M	(6/12)	7	Bilateral nephrostomies, ileal loop; <i>C. albicans</i> from loop urines and catheter urines	0.1-0.2	0.5	23	14-20 (2)	280-800 (5)	Cultures negative after 10 days; remained negative for 30 days.

Table III Children treated with 5-fluorocytosine

No.	Case Description	Age	Sensitivity to 5 FC ($\mu\text{g/ml}$)		5 FC Dose (mg/kg/day)	5 FC Assays			Comment
			MIC	MCC		CSF	Serum	Urine	
1	Cryptococcal meningitis Hodgkin's disease	32	0.01	0.01-0.02	200 then 100 after 3 days	34 3.5	66, 26 3.5	2000 1000 2000	High CSF level before cytosine arabinoside given
2	<i>C. albicans</i> endocarditis	44	0.2-0.5	2	200, later 70		26, 28, 12 22, 9.5, 7	55, 50, 18 300, 300 300	
3	<i>C. albicans</i> endocarditis	67	0.8	5-10	100		14, 27, 22 30	50, 120 70, 75	
4	Renal transplant, <i>C. albicans</i> urinary tract	42	0.05-0.1	1-2					
5	Urogenital surgery <i>C. albicans</i> urinary tract infection	5	0.1-0.2	5-10					

Table IV Details of patients who received 5-fluorocytosine in other hospitals

during six days' treatment. The cultures were kindly referred to us at an early stage, and our impression was that the pretreatment culture contained a minority population of 5 FC-resistant cells, which rapidly emerged despite adequate urine and serum drug levels; in our hands both sensitive and resistant isolates were sensitive to clotrimazole, with which

this patient was successfully treated. The second case was no. 4 in our series, where the strain of *C. albicans* was initially homogenous and fully sensitive to 5 FC (MIC 0.05 $\mu\text{g/ml}$, MCC 1 $\mu\text{g/ml}$). After 20 weeks' treatment, during which high serum and urine drug levels were maintained, *C. albicans* reappeared in the urine, apparently identical to the pretreatment

Strain No.	Species	Number of Passages	MIC/MCC in Liquid Medium ($\mu\text{g/ml}$)	
			Before Passage	After Passage
1	<i>C. albicans</i>	15	0.1/2.0	2.0/50
2	<i>C. albicans</i>	10	1/10	100/> 500
3	<i>C. albicans</i>	10	2/10	100/> 500
4	<i>C. albicans</i>	12	1/20	> 500/> 500
5	<i>C. pseudotropicalis</i>	15	0.1/1	2/10
6	<i>C. non-albicans</i>	15	0.2/2.0	10/50
7	<i>C. non-albicans</i>	10	5/20	> 500/> 500
8	<i>Cryptococcus neoformans</i>	15	1/2	10/100
		15	0.5/5	20/100

Table V Changes in sensitivity to 5 FC after passage on gradient plates¹

¹Strains nos. 2, 5, and 8 were stored in YNB agar stab after the series of passages; after 12 weeks no changes in the enhanced MIC/MCC were noted.

isolates, save that the MIC and MCC were now over 500 $\mu\text{g/ml}$. Both isolates were moderately sensitive to clotrimazole, which successfully eliminated the infection. The third case, referred to us by Dr Logan, was a man with *C. albicans* endocarditis, for which amphotericin B was first given for one month but was discontinued because of thrombocytopenia and anaemia. Blood cultures still grew *C. albicans*, apparently homogenous and fully sensitive to 5 FC, which was therefore given. Within a few weeks no *Candida* was isolated from the blood, which contained high levels of the drug, and the *Candida* serum antibody level was markedly reduced. The drug was continued for six months at 200 mg/kg/day with a view to eventual aortic valve replacement, but the man then relapsed; *C. albicans* was isolated from his blood and throat. We found the isolate to be totally resistant to 5 FC (MIC and MCC above 1000 $\mu\text{g/ml}$) but sensitive to clotrimazole; he was given this for 10 days but then died suddenly after rupture of an aortic valve cusp (Logan and Goldberg, 1972). *C. albicans* was isolated from vegetations on the aortic valve; it is impossible now to ascertain whether the final infection was caused by an entirely different strain of *C. albicans*.

5-Fluorocytosine must be viewed against the limited range of systemic antimycotic agents now available; amphotericin B is frequently toxic and many clinicians, particularly paediatricians, are reluctant to use it. Imidazole derivatives, which share with 5 FC the valuable property of establishing active body fluid levels from oral absorption, have not been administered systemically or intrathecally until very recently and little is known of their efficacy or hazards by these routes. They have, however, a much wider antifungal range than 5 FC (Holt, 1970; Holt and Newman, 1972b) and an almost infinite range of derivatives is possible, with widely differing pharmacological properties. Although recent experiences with 5 FC have naturally led us

to temper our earlier optimism, its success in seven of our eight cases leaves us with little doubt of its potential. Providing searching studies *in vitro* are continued for emergent resistance in each subject treated, the drug will take a valuable share in meeting the ever-increasing demand for systemic antimycotic agents.

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