Pharmacological Studies with 5-Fluorocytosine

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A cylinder plate bioassay for 5-fluorocytosine (5-FC) is described which permits determination of 5-FC concentrations in biological fluids in the presence of amphotericin B. Using this assay, we determined serum concentrations in 12 patients after a single dose of drug and in 8 patients receiving daily 5-FC at 6-hr intervals (4 to 120 days). Drug was detectable in serum as early as 0.5 hr and concentrations were measurable as long as 24 hr after a dose, regardless of renal function. Peak concentrations occurred 6 hr after the initial drug dose, but were seen between 1 and 2 hr after a dose in all patients receiving a minimum of 4 days of therapy. Mild to moderate renal impairment produced marked increases in peak 5-FC concentrations in the serum of a group of eight patients on three different dosage schedules. Five patients were studied before and after amphotericin B-induced renal insufficiency. Peak concentrations increased from 14 to 142% concomitant with the change in renal function. Parallel studies in rabbits confirmed the results in our patients. 5-FC half-life in the serum of 10 rabbits increased from 3.35 ± 0.27 to 24.63 ± 0.70 hr after experimentally induced acute renal failure. Concentrations of 5-FC in the cerebrospinal fluid of five patients ranged from 17 to 62 $\mu\text{g/ml}$ and were $74.4 \pm 5.6\%$ of simultaneously determined serum concentrations. The effect of renal function on 5-FC concentrations in cerebrospinal fluid was similar to that seen with serum.

5-Fluorocytosine (5-FC) is a new antifungal agent synthesized in 1957 and shown in 1963 to be effective in the treatment of mice infected experimentally with *Candida albicans* and *Cryptococcus neoformans* (6). Use of 5-FC for the treatment of fungal infections in man first appeared in the literature in 1967 (5), since which time 10 articles in the English literature have reported experience with a total of 22 patients with cryptococcal disease, 19 with *Candida* infection, and 1 with *Torulopsis glabrata* septicemia (3, 4, 8-10, 15-19). Although this agent is undergoing clinical investigation in a number of centers at the present time, relatively little is known about its pharmacological properties. To date, 5-FC concentrations in serum have been reported for 22 patients by six different groups, and concentrations in cerebrospinal fluid (CSF) have been reported for only 13 patients (Table 1). Interpretation of these studies has been handicapped by the failure of many investigators to supply important details. The failure to record recency of amphotericin B therapy in most patients was regrettable from two points of view. Amphotericin B persists in blood up to 6 weeks after therapy (2) and may have interfered with the bioassay for 5-FC. In addition, renal function may have been reduced by amphotericin B therapy.

We developed a cylinder-plate bioassay system for measuring concentrations of 5-FC in sera and CSF in the presence of amphotericin B. To our knowledge, this is the first assay system which permits independent measurement of amphotericin B and 5-FC concentrations in biological fluids. In this paper, we describe in detail our assay and report on 5-FC concentrations in serum and CSF of 12 patients with systemic mycoses. In addition, we have investigated the effect of renal function upon serum and CSF concentrations of 5-FC in both man and an experimental rabbit model.

MATERIALS AND METHODS

Bioassay of 5-FC. Previous studies with 5-FC have demonstrated that maximal in vitro activity can be achieved only in a completely synthetic medium (11, 12). Yeast Nitrogen Base (YNB; Difco) was supplemented with L-asparagine and dextrose as described by Shadomy (11). The YNB concentrate was diluted 1:10 with melted sterile 2% agar immediately prior to use in the bioassay. The medium was buffered at $\text{pH } 7.10 \pm 0.10$ with phosphate (0.01 M).

The indicator organism was *Saccharomyces cerevisiae* ATCC 9763. Suspensions of this organism were prepared by harvesting in Sabouraud broth 48- to 72-hr-old cultures grown on Sabouraud agar slants at 30 C. The *S. cerevisiae* cells were centrifuged ($750 \times g$

TABLE 1. Serum and CSF concentrations of 5-FC reported by previous investigators

Investigator	No. of patients	5-FC concn Serum ($\mu\text{g/ml}$)		Daily dose of 5-FC	Time after 5-FC dose	Renal function	Method of assay	Recency of amphotericin B
		Serum	CSF					
Shadomy (11)	2	11-32	8-26	100 mg/kg	NS ^a	NS	Bioassay	Absent
Shadomy (12)	5	2-33	8-28	100 mg/kg	3-4 hr	NS	Bioassay	Absent
	4	23-86	18-40	150 mg/kg				
Warner and Shadomy (17)	2	6-78	ND ^b	100 mg/kg	NS	NS	Bioassay	Absent
Webb et al. (19)	1	10-20	ND	200 mg/kg	NS	NS	NS	Absent
Record et al. (9)	1	100-180	ND	8.0 g	NS	BUN ^c = 130	NS	Within 2 weeks
		40-45		8.0 g		Improved		NS
	1	110-130	ND	12.0 g	NS	BUN = 87-160	NS	Concomitant
		20-40		8.0 g		BUN = 87		NS
	1	20-100	ND	8.0 g	NS	NS	NS	Within 2 weeks
Davies and Reeves (3)	1	30-150	ND	50 mg/kg	NS	BUN = 39-150	NS	Absent
	1	38-165		55 mg/kg		Renal failure		
Fass and Perkins (4)	1	94	65	4.0 g	NS	BUN = 31	Bioassay	Absent
	1	88	78	4.0 g		BUN = 10		NS
Richards et al. (10)	1	50	ND	200 mg/kg	NS	Mild azotemia	NS	Within 1 week

^a NS, not stated.^b ND, not determined.^c BUN, blood urea nitrogen.

for 20 min), the supernatant fluid was discarded, and the pellet of cells was resuspended in Sabouraud broth. Studies in our laboratory showed that subsequent bioassay results were identical when *S. cerevisiae* was grown on YNB agar and harvested, washed, and resuspended in normal saline. This suggested that there was no significant carry-over of cytosine or other 5-FC antagonists by the test organism from the Sabouraud agar or the broth in which it was harvested.

The washed and resuspended *S. cerevisiae* cells were adjusted to an optical density of 0.890 at 600 nm (5.0×10^7 viable cells/ml) with Sabouraud broth used as a blank. A 1-ml amount of this suspension was added to 99 ml of melted YNB agar (43 to 47 C), and the agar was then dispensed in 20-ml volumes into sterile plastic petri dishes (100 by 15 mm square, Integrid Petri Dish, Falcon Plastics). This seeded agar was allowed to harden, and then steel assay cylinders (internal diameter, 5 mm) were placed on the plates and filled with 100 μl of either the standard solutions or unknowns. Standard 5-FC solutions in serum were prepared in pooled, normal human serum (Microbiological Associates, Inc.) diluted 50% with sodium phosphate-buffered normal saline (0.15 M saline, 0.0008 M phosphate buffer, pH 7.4; PBS). Standard solutions in CSF were prepared by dissolving 5-FC in human CSF. Identical bioassay results were obtained if PBS was substituted for CSF as the solvent used to prepare these standards.

The bioassay plates were allowed to incubate at 37 C for 24 hr. A standard curve was constructed by plotting $\log_{10}$ concentration of the standard solutions as a linear function of the diameter of the zone of inhibition. 5-FC concentrations in patient's sera or CSF were determined by using the corresponding

standard curve. Standard curves were constructed for each individual assay, and all unknown samples were run in duplicate. Pretreatment sera and CSF from all patients were studied and failed to demonstrate any bioactivity in our assay.

Because *S. cerevisiae* is susceptible to amphotericin B in vitro, determinations of 5-FC concentrations in the serum of patients receiving both antifungal agents would be difficult to interpret. To circumvent the problem of amphotericin B interfering with the assay, we undertook to study the ability of both drugs to pass through a porous membrane. Patients' serum containing in vivo amphotericin B concentrations within the reported clinical range (0.1 to 2.5 $\mu\text{g/ml}$) or pooled normal human serum containing 0.1 to 10 μg of amphotericin B per ml, added in vitro, was filtered through a collodion membrane (Collodion Bag Filter; Arthur H. Thomas Co.). This filter will permit passage of molecules with a molecular weight less than or equal to 70,000. Ultrafiltrates of all amphotericin B-containing sera possessed no bioactivity as measured by a previously described amphotericin B assay system with a lower limit of sensitivity of 0.1 $\mu\text{g/ml}$ (2). When serum from patients receiving 5-FC alone or serum to which 5-FC had been added in vitro was ultrafiltered, the bioactivity of the ultrafiltrate was identical to that of the original serum specimen. Moreover, when both 5-FC and amphotericin B were added to serum in vitro such that 5-FC concentrations ranged from 10 to 50 $\mu\text{g/ml}$ with and without 0.5 or 2.5 μg of amphotericin B per ml, the ultrafiltrates of sera containing equal concentrations of 5-FC possessed identical 5-FC bioactivity irrespective of whether amphotericin B was present in the original serum specimen. Thus, by utilizing the differential ultrafiltration char-

acteristics of 5-FC and amphotericin B, we obtained an ultrafiltrate of serum containing all of the bioactive 5-FC in the absence of amphotericin B. Because the 5-FC standard curve over the range of 1 to 50 $\mu\text{g/ml}$ was identical whether the solvent was 50% serum or the ultrafiltrate of 50% serum, all 50% serum and ultrafiltrate unknowns were read from a simultaneous standard curve with the use of 50% serum only. The lower limit of sensitivity of this assay system is 1 $\mu\text{g/ml}$ in 50% serum.

CSF samples from our patients receiving both 5-FC and amphotericin B were similarly filtered through a 70,000 molecular weight collodion membrane. No detectable amphotericin B ($< 0.1 \mu\text{g/ml}$) was found in these ultrafiltrates. Concentrations of amphotericin B $\geq 0.5 \mu\text{g/ml}$ were required to produce any zone of inhibition against *S. cerevisiae* in our bioassay.

To determine serum concentrations of amphotericin B independent of 5-FC, cytosine was used to block the action of 5-FC in vitro. The indicator organism in the amphotericin B bioassay previously alluded to was *Paecilomyces varioti*. This fungus was quite sensitive to 5-FC in vitro. Cytosine added to the media, to bring the final concentration to 50 $\mu\text{g/ml}$, prevented the formation of any zone of inhibition by serum containing 5-FC in concentrations at least as great as 300 $\mu\text{g/ml}$. There was no cytosine effect upon amphotericin B zone sizes in this system.

Patient material. All but 1 of the 12 patients in this study were hospitalized at the National Institutes of Health, Bethesda, Md. There were seven men and five women ranging in age from 23 to 61 years. Six of the eight patients receiving daily 5-FC therapy were being treated for culture-proven *C. neoformans* meningitis, a seventh had *C. neoformans* osteomyelitis, and the eighth patient had disseminated sporotrichosis. The four patients who received only a single dose of 5-FC prior to determinations of 5-FC in serum were follow-up patients, of whom three had previously treated, inactive cryptococcal meningitis and the fourth had chronic mucocutaneous moniliasis. Hepatic function was normal in 11 patients; 1 patient had a chronically active, but stable, cirrhotic process of unknown etiology. Informed consent was obtained from all patients prior to institution of 5-FC therapy.

The drug 5-FC was provided through the courtesy of Edward Miller, Hoffmann-La Roche, Inc., Nutley, N. J. In those patients receiving daily 5-FC therapy, it was administered in the form of 250- or 500-mg capsules, or both, orally at 6-hr intervals. Patients received the dose being studied while in the fasting state; they were then permitted to eat breakfast. In all patients, blood for 5-FC determinations was drawn 1, 2, and 6 hr after the previous 5-FC dosage. In half of the patients, blood was also drawn at 0.5- and 4-hr intervals. CSF samples were obtained via lumbar puncture simultaneously with the 0.5-, 1-, or 2-hr blood samples. All serum samples were diluted 50% with PBS at the time of collection and stored at -70°C .

Experimental rabbit model. Ten New Zealand white male rabbits weighing 2 to 3 kg were given 5-FC (50 mg/kg) intravenously as a single bolus. Blood samples were then drawn every 30 min for 2 hr, hourly for 4 hr, and then at 12 and 24 hr after the 5-FC dose. Five to

10 days later, these same 10 rabbits were given 250 mg of cephaloridine (Loridine; Eli Lilly & Co.) per kg per day intravenously (iv) on 2 consecutive days. This has been reported to cause acute proximal tubular necrosis in the rabbit, evident histologically by 16 hr (1, 14). On the fourth and fifth days after the first dose of cephaloridine, five rabbits each were rechallenged with 50 mg of 5-FC per kg iv. Serial blood samples were collected during the first 24 hr as before and then at 48 and 72 hr. Serum concentrations of drug were measured with the use of pooled, normal rabbit serum for the standard solutions. 5-FC concentrations for each rabbit were done in triplicate with an error in the method of $\pm 20\%$, which is comparable to results with the human serum determinations. Sera from all 10 rabbits drawn immediately prior to each 5-FC dose demonstrated no bioactivity in our assay. Blood urea nitrogen (BUN) in these 10 rabbits was measured prior to the first 5-FC dose, on the day of the second dose, and daily thereafter for as long as the rabbit survived.

RESULTS

Serum concentrations of 5-FC. Figure 1 shows the peak concentrations of 5-FC in the serum of a group of eight patients with normal renal function on three different dosage schedules. All patients were receiving 5-FC for a minimum of 4 days prior to serum determinations, and peak concentrations occurred at 1 or 2 hr after the previous 5-FC dose in all patients. Although there was a progressive increase in the mean peak serum concentrations with increasing 5-FC dosage, one could not reliably predict exactly what the result would be in an individual patient with a change in

FIG. 1. Peak 5-FC serum concentrations on three different dosage schedules. Dashed lines connect determinations done on the same patient on different dosages. One patient in the middle dosage range is represented twice. The number of patients is given in parentheses.

dosage. In two patients on a constant 5-FC dose given for 3 and 6 months, respectively, with stable renal function, there was no statistically significant change in mean or peak serum concentrations of 5-FC compared with initial serum concentrations (measured after 4 days of drug). These results suggest no tendency for drug concentrations in the blood to rise spontaneously after the first 4 days.

The effect of renal function on peak 5-FC concentrations in serum is depicted in Fig. 2. Peak

concentrations were again seen at 1 or 2 hr. One patient with normal renal function and all but two azotemic patients were receiving concomitant amphotericin B at the time of the determinations of 5-FC in serum. The increased 5-FC concentrations were not due to the presence of amphotericin B in the serum, for this agent had been removed by ultrafiltration prior to use in the bioassay (see Materials and Methods). In five of the patients shown in Fig. 2, serum concentrations were determined after 4 to 5 days of 5-FC therapy alone and then after identical 4- to 5-day therapeutic trials in the presence of stable, amphotericin B-induced renal insufficiency (Fig. 2). In 10 instances in these five patients, peak serum concentrations were seen to increase 14 to 142% concomitant with a change in renal function ($P < 0.0005$ by Student's paired sample t test). (Peak concentration with impaired renal function - peak concentration with normal renal function $\times 100 \div$ % peak concentration with normal renal function = % peak concentration with normal renal function.)

5-FC concentrations were studied in 12 patients after they had received a single dose of drug. 5-FC was detectable in the sera as early as 0.5 hr, peaked at 6 hr, gradually declined thereafter, and was measurable as long as 24 hr after the dose, regardless of renal function. Peak serum concentrations after a 1.5-g oral 5-FC dose were $45.8 \pm 7.1 \mu\text{g/ml}$ in four patients with normal renal function and $46.3 \pm 7.2 \mu\text{g/ml}$ in three patients with mild to moderate renal insufficiency. Studies on six patients receiving a single 2.0-g oral dose, two with normal and four with abnormal renal function, revealed peak serum concentrations of

FIG. 2. Effect of renal function on peak serum concentrations of 5-FC on three different dosage schedules. Solid lines connect determinations done on the same patient on a given 5-FC dosage before and after amphotericin B-induced renal insufficiency. The number of patients is given in parentheses.

FIG. 3. Comparison of serum 5-FC concentrations in five patients after the initial dose (solid line) and after a minimum of 4 days of therapy (dashed line). Each specimen was analyzed three times, and the mean and range of values are shown.

48.5 ± 2.5 and 37.8 ± 5.3 µg/ml, respectively, not a statistically significant difference. Five patients had blood studies both after a single dose and after a minimum of 4 days of 5-FC wherein the six hourly dose was identical to the original single dose (Fig. 3). The reason for the delay in appear-

ance of the peak concentration after a single dose is unclear, but does not appear related to the proximity of meals. In three patients with normal renal function, serum concentrations of 5-FC 12 hr after a single dose ranged from 44 to 52% of the peak value and at 24 hr were 8 to 15% of the peak serum concentration.

TABLE 2. Comparison of simultaneous serum and CSF concentrations of 5-FC in five patients with active cryptococcal meningitis

Pa-tient	Mean 5-FC concn (µg/ml)		CSF/serum × 100 ^a (%)	Serum creatinine (mg/100 ml)	Daily dose of 5-FC (mg/kg)	Time of sampling after 5-FC dose (hr)
	Serum	CSF				
2	80	27	33	0.9	100	1
2	101	62	62	2.4	100	1
3	82	40	49	0.9	140	2
3	65	62	95	1.5	140	2
3	54	45	83	1.4	62.5	2
3	44	31	70	1.3	62.5	1
3	93	55	59	2.2	62.5	1
5	60	52	87	0.8	100	2
8	24	18	75	1.1	50	1
8	23	17	72	1.0	50	1
8	34	34	100	1.3	100	1
8	48	48	100	1.1	130	1
9	53	44	83	2.1	80	2

^a Mean ± SE of mean = 74.4 ± 5.6.

CSF results. 5-FC concentrations in 13 CSF samples from five patients with cryptococcal meningitis are depicted in Table 2, along with simultaneously determined serum concentrations. CSF concentrations ranged from 17 to 62 µg/ml and were 74.4 ± 5.6% of simultaneous blood determinations. There appeared to be no correlation in our patients between CSF protein and 5-FC concentrations. In two patients, patients 2 and 3, CSF concentrations while on a constant drug dosage were seen to rise markedly with mild to moderate changes in renal function, although no consistent changes in the CSF-serum ratio of 5-FC concentrations were observed.

Experimental rabbit model. All 10 rabbits in our study had normal renal function with BUN equal to 17.8 ± 1.4 mg/100 ml at the time of the first iv 5-FC challenge. At 4 and 5 days after the initial dose of cephaloridine, BUN levels were 166.0 ± 16.0 and 230.5 ± 10.7 mg/100 ml, respectively. Liver function tests and electrolytes remained normal throughout this test period.

FIG. 4. Serum concentrations in 10 rabbits after a single intravenous dose of 5-FC before (solid line) and after (dashed line) cephaloridine-induced renal insufficiency. The 24-hr serum concentrations in the pre-cephaloridine rabbits were undetectable.

Figure 4 shows the striking differences in serum concentrations between the rabbits before and after renal failure. Between 6 and 72 hr after the 5-FC dose in the azotemic rabbit, serum concentrations seemed to exhibit an exponential decay. The mean half-life in the 10 normal rabbits was 3.35 ± 0.27 hr, whereas the half-life was 24.63 ± 0.70 hr in the postcephaloridine rabbits, a 7.5-fold increase. There was no statistically significant difference in serum half-life between the rabbits rechallenged with 5-FC on the fourth or fifth day after the first cephaloridine dose.

DISCUSSION

Serum and CSF concentrations of 5-FC reported by previous investigators were fairly comparable to our results. Record et al. (9), Davies and Reeves (3), Fass and Perkins (4), and Richards et al. (10) reported serum concentrations in the range of 20 to 180 $\mu\text{g/ml}$ for a total of 8 patients. This is similar to our observations with 12 patients. Shadomy (11, 12) found serum concentrations of 16.78 ± 7.81 and 43.89 ± 18.96 $\mu\text{g/ml}$, respectively, in five patients receiving 100 mg/kg daily and four patients on 150 mg/kg daily. These concentrations are significantly lower than those seen in our patients on comparable 5-FC dosages. However, the discrepancy may be attributable to differences in both the assay employed and the time of sampling, since Shadomy collected his specimens between 3 and 4 hr after the previous 5-FC dose.

CSF concentrations of 5-FC previously reported (4, 12) are similar to ours, and we have confirmed the high CSF-serum ratio of 5-FC concentrations noted by others. These observations suggest good penetration of the blood-brain barrier by 5-FC in the presence of inflamed meninges.

With the limited data currently available, it is not possible to discuss optimal serum or CSF concentrations of 5-FC. Nevertheless, therapeutically effective concentrations of 5-FC should exceed the minimal inhibitory concentrations (MIC) for the pathogen involved. In vitro susceptibility studies have shown that the usual MIC for 5-FC for both *C. neoformans* and *C. albicans* ranges from 0.46 to 3.9 $\mu\text{g/ml}$ (11, 13). Based on the original MIC for the infecting organism, clinically significant concentrations of 5-FC in serum and CSF were achieved in all of our patients on the three different dosage schedules. Yet, three patients in our series treated with 5-FC alone for cryptococcal meningitis developed resistant strains of *C. neoformans* (MIC > 320 $\mu\text{g/ml}$).

Since 5-FC has been and will most probably continue to be used in conjunction with amphotericin B in the treatment of *Candida* and crypto-

coccal infections, because of the well-documented nephrotoxicity from amphotericin B, and because patients with systemic mycoses often have underlying illnesses which may include some renal insufficiency, the relationship between renal function and serum concentrations of 5-FC is of considerable clinical relevance. To our knowledge, previous reports concerning this relationship have not appeared in the literature. Record et al. (9) did note in one of their patients that the serum concentration of 5-FC on a given dosage was 2.5 to 4 times greater in the presence of marked azotemia than when renal function was "improved." The results of our studies confirm this observation and quite convincingly demonstrate the profound effect which renal function has on serum concentrations of 5-FC. These results are in keeping with expectations, since 85 to 95% of an oral dose of 5-FC is excreted unchanged in the urine of humans (7).

Gastrointestinal disturbances, bone marrow suppression, and abnormal liver function tests (primarily elevated serum transaminases and alkaline phosphatase) have been the major toxic effects reported after 5-FC administration in man. Although experience with 5-FC remains limited, it seems that toxicity in man may be dose-related and appears more frequently in the presence of renal insufficiency. Record et al. (9) described three patients with *Candida* endocarditis treated with 5-FC, two of whom developed 5-FC marrow and liver toxicity in the presence of moderate renal insufficiency. Davies and Reeves (3) described an additional patient with urinary candidiasis and azotemia who developed neutropenia on a daily 5-FC dose of 55 mg/kg which disappeared with cessation of the drug. In three of our eight patients, two of whom had mild renal insufficiency, toxicity related to 5-FC developed and was reversed by reduction in drug dosage. The relationship between 5-FC toxicity and serum concentrations remains unclear, since serum levels were unfortunately not obtained at the time of toxicity in these patients.

In view of the evidence presented in this paper documenting the effect of renal function on 5-FC concentrations in serum and the impression from clinical experience that 5-FC toxicity may appear more readily in the presence of renal dysfunction it is clear that one must exercise great care in choosing 5-FC dosage in those patients with impaired function. Patients requiring 5-FC who have renal failure or are receiving a nephrotoxic agent such as amphotericin B should have serum concentrations serially monitored to avoid potentially toxic levels.

From the foregoing, it seems clear that more knowledge of the relationships between 5-FC

dosage, serum concentrations, therapeutic efficacy, and toxicity is necessary. Further experience with this drug will be needed to provide this information.

LITERATURE CITED

1. Atkinson, R. M., J. P. Currie, B. Davis, D. A. H. Pratt, H. M. Sharpe, and E. G. Tomich. 1966. Acute toxicity of cephaloridine, an antibiotic derived from cephalosporin C. *Toxicol. Appl. Pharmacol.* 8:398-406.
2. Bindschadler, D. D., and J. E. Bennett. 1969. A pharmacologic guide to the clinical use of amphotericin B. *J. Infect. Dis.* 120:427-435.
3. Davies, R. R., and D. S. Reeves. 1971. 5-FC and urinary candidiasis. *Brit. Med. J.* 1:577-579.
4. Fass, R. J., and R. L. Perkins. 1971. 5-FC in the treatment of cryptococcal and *Candida* mycosis. *Ann. Intern. Med.* 74:535-539.
5. Grunberg, E., H. N. Prince, and J. P. Utz. 1967. Observations on the activity of two newer antifungal agents saramycetin and 5-fluorocytosine. *Int. Congr. Chemother.*, 5th, Vienna, p. 69-76.
6. Grunberg, E., E. Titsworth, and M. Bennett. 1964. Chemotherapeutic activity of 5-fluorocytosine. *Antimicrob. Ag. Chemother.* 1963, p. 566-568.
7. Koechlin, B. A., F. Rubio, S. Palmer, T. Gabriel, and R. Duchinsky. 1966. The metabolism of 5-fluorocytosine-2-¹⁴C and of cytosine-¹⁴C in the rat and disposition of 5-fluorocytosine-2-¹⁴C in man. *Biochem. Pharmacol.* 15:435-446.
8. Pankey, G. A., W. R. Lockwood, and J. M. Montalvo. 1970. 5-Fluorocytosine: A replacement for amphotericin B in the treatment of *Candida* and *Cryptococcus neoformans* infections. *J. La. State Med. Soc.* 122:365-369.
9. Record, C. O., J. M. Skinner, P. Sleight, and D. C. E. Speller. 1971. *Candida* endocarditis treated with 5-FC. *Brit. Med. J.* 1:262-264.
10. Richards, A. B., B. R. Jones, J. Whitwell, and Y. M. Clayton. 1970. Corneal and intraocular infection by *Candida albicans* treated with 5-FC. *Trans. Ophthalmol. Soc. U.K.* 89:867-885.
11. Shadomy, S. 1969. In vitro studies with 5-fluorocytosine. *Appl. Microbiol.* 17:871-877.
12. Shadomy, S. 1970. Further in vitro studies with 5-fluorocytosine. *Infect. Immunity* 2:484-488.
13. Shadomy, S., H. J. Shadomy, J. A. McCay, and J. P. Utz. 1969. In vitro susceptibility of *Cryptococcus neoformans* to amphotericin B, hamycin, and 5-fluorocytosine. *Antimicrob. Ag. Chemother.* 1968, p. 452-460.
14. Silverblatt, F., M. Turck, and R. Bulger. 1970. Nephrotoxicity due to cephaloridine: a light and electron-microscopic study in rabbits. *J. Infect. Dis.* 122:33-44.
15. Tassel, D., and M. Madoff. 1968. Treatment of *Candida* sepsis and *cryptococcus* meningitis with 5-fluorocytosine. *J. Amer. Med. Ass.* 206:830-832.
16. Utz, J. P., B. S. Tynes, J. Shadomy, R. J. Duma, M. M. Kanan, and K. N. Mason. 1969. 5-Fluorocytosine in human cryptococcosis. *Antimicrob. Ag. Chemother.* 1968, p. 344-346.
17. Warner, J. F., R. F. McGehee, R. J. Duma, S. Shadomy, and J. P. Utz. 1971. 5-Fluorocytosine in human candidiasis. *Antimicrob. Ag. Chemother.* 1970, p. 473-475.
18. Watkins, J. S., M. J. Campbell, D. Gardner-Medwin, H. R. Ingham, and I. G. Murray. 1969. Two cases of cryptococcal meningitis, one treated with 5-FC. *Brit. Med. J.* 3:29-31.
19. Webb, A. J., D. C. E. Speller, and K. G. Buckler. 1970. *Torulopsis glabrata* septicemia treated with 5-FC. *Lancet* 1:839-840.