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Fungal Peritonitis Caused by *Lecythophora mutabilis*

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Fungal peritonitis caused by *Lecythophora mutabilis*, a mold rarely isolated from humans, is described. A patient on continuous peritoneal dialysis developed clinical, microbiological, and serological evidence for peritonitis due to this fungus. In vitro susceptibility testing of the fungus revealed marked differences in the activities of various antifungal agents. Although initially responding to treatment with oral ketoconazole, intraperitoneal miconazole, and catheter replacement, the patient had a documented relapse. The patient was eventually cured following the removal of a second catheter in association with prolonged imidazole treatment.

Peritoneal dialysis patients, and especially those on continuous ambulatory peritoneal dialysis (CAPD), are at risk for fungal peritonitis (R. J. Johnson, P. G. Ramsey, N. Gallagher, and S. Ahmad, Am. J. Nephrol., in press). Most infections are caused by yeast fungi, with species of the genus *Candida* Berkhout being most commonly implicated (1, 8; Johnson et al., in press). However, peritonitis may be caused by saprobic fungi such as *Fusarium* sp. strain Link, *Trichosporon* sp. strain Behrend, *Rhodotorula* sp. strain Harrison, *Acremonium* sp. strain Link, *Penicillium* sp. strain Link, and *Drechslera* sp. strain Ito (3, 7–10, 13; Johnson et al., in press; M. T. Moulsdale, J. M. Harper, and G. N. Thatcher, Letter, Med. J. Aust. 1:88, 1981).

Recently, several taxonomically problematic fungi have been studied, reevaluated, and described, and new combinations have been proposed (4). Among these molds was *Lecythophora mutabilis* (van Beyma) W. Gams et McGinnis, a new combination for the organism known previously as *Phialophora mutabilis* (van Beyma) Schol-Schwarz. *L. mutabilis* has been reported as a rare cause of endocarditis (19; C. A. Pierach; G. Gulmen, G. J. Dhar, and J. C. Kiser, Letter, Ann. Intern. Med. 79:900–901, 1973). This species is morphologically similar to other fungi classified in the genera *Phialophora* sp. strain Medlar and *Phialemonium* sp. strain W. Gams et McGinnis (4, 16). This communication describes a patient on CAPD who had clinical, microbiological, and serological evidence for peritonitis caused by *L. mutabilis*. The clinical course is discussed and mycological investigations, in vitro susceptibility studies, and response to antifungal therapy are emphasized.

CASE REPORT

A 61-year-old woman with polycystic kidney disease was switched from hemodialysis to CAPD in January 1981. Her course on peritoneal dialysis was uneventful except for the development of insulin-dependent diabetes mellitus.

In mid-July 1982, she developed abdominal discomfort, cloudy dialysate, and low grade fever (38°C). A peritoneal fluid sample revealed 333 leukocytes, with 72% neutrophils, 16% monocytes, 11% lymphocytes, and no eosinophils. Intraperitoneal methicillin (200 mg/liter of dialysate) was administered for 5 days. Cultures remained negative, there was no clinical improvement, and tobramycin treatment (8 mg/liter of dialysate) was started. Although peritoneal fluid cultures for bacteria remained negative, a beige-colored mold grew in both the brain heart infusion broth and the blood agar 3 days after the initial culture.

The patient was admitted to the Swedish Hospital Medical Center, Seattle, Wash., on 8 August 1981. She was in moderate distress; her oral temperature was 38°C. There was marked tenderness without rebound over her abdomen which was maximal in the left lower quadrant. The results of the pelvic examination were normal. Hematocrit was 28%; a leukocyte count was 8,000/mm^3, with a differential of 70%, neutrophils, 1% bands, 20% lymphocytes, 6% monocytes, and 3% eosinophils. Blood urea nitrogen was 20 mg/dl, serum creatinine was 4.8 mg/dl, and serum albumin was 1.9 g/dl. Peritoneal cell count showed only 18 leukocytes, with 72% neutrophils and no eosinophils.

The tobramycin was discontinued, and the patient was started on continuous peritoneal lavage with miconazole added to the dialysate at a concentration of 2 μg/ml. Ketoconazole was also given orally in doses of 200 mg twice daily. The patient continued to experience abdominal pain, but two further diagnostic tests, abdominal ultrasound and computerized tomography, were both negative for intra-abdominal pathology.

Of the three peritoneal cultures obtained before the institution of antifungal therapy, only the initial culture was positive for fungal growth. The remaining two cultures were sterile. The mold was initially incorrectly identified as *Aureobasidium pullulans* (de Bary) Arnaud. However, subsequent studies by several mycologists led to agreement that the fungus represented a typical isolate of *L. mutabilis*. In vitro susceptibility studies generated results which reflected a significant discrepancy between institutions as to values obtained for amphotericin B and miconazole (Table 1).

Two weeks after the commencement of therapy with miconazole and ketoconazole, the patient was markedly improved. Because of in vitro susceptibility data, the dosage of miconazole was increased to 6 μg/ml of the dialysate. After the increase in dosage, the patient experienced worsening abdominal discomfort. Peritoneal fluid remained abnormal with a leukocyte count of 940 with 69% neutrophils and 16% eosinophils.

Due to continued abdominal pain and fever, the patient

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TABLE 1. Susceptibility of *L. mutabilis* to antifungal antimicrobial agents in vitro

Laboratory	Inoculum (conidia/ml)	Medium	Type of test	MIC/MLC (μg/ml)[a]			
				AMB	5-FC	MON	KETO
Montana State University, Bozeman, Mont.	10^4	Synthetic amino acid medium-fungal	Macrobroth dilution	1.16/2.31[b]	>322.75/−	>19.16/−	>12.8/−
University of Washington, Seattle, Wash.	—[c]	Yeast nitrogen base agar	Agar dilution	25/ND[d]	100/ND	3.1/ND	ND

[a] Abbreviations: AMB, Amphotericin B; 5-FC, 5-fluorocytosine; MON, miconazole; KETO, ketoconazole.
[b] MICs and MLCs were read at 24 and 72 h, respectively, at Montana State University.
[c] Measured by photometrically comparing inoculum to a laboratory strain of *C. albicans* adjusted to a 0.5 McFarland standard.
[d] MICs were read at 48 h at the University of Washington; MLCs were not done (ND).

underwent a limited laparotomy 17 days after admission. The findings included a small amount of purulent material adherent to the deep cuff of the Tenckhoff catheter, as well as peritoneal adhesions. Cultures grew no microorganisms. The catheter was replaced, and oral ketoconazole (200 mg twice daily) and intraperitoneal miconazole (4 μg/liter of dialysate) were continued. Over the next 2 weeks the patient's symptoms abated, and she was discharged on CAPD on 10 September 1982. Her ketoconazole treatment was discontinued 2 weeks later (total treatment duration, 6 weeks), and the intraperitoneal miconazole was completed in early November 1982 (total treatment duration, 14 weeks). Six weeks after completion of antifungal therapy, she again developed abdominal pain, cloudy dialysate, and a temperature of 38°C. Peritoneal fluid revealed 550 leukocytes, with 20% neutrophils and 70% eosinophils. Although a routine peritoneal culture was negative, culture of a 0.45-μm (pore size) filter (Gelman Sciences, Inc., Ann Arbor, Mich.) through which 100 ml of dialysate had been filtered was positive for *L. mutabilis*. Gram stain was negative. The patient was restarted on ketoconazole orally, 200 mg twice daily, and miconazole intraperitoneally at a dose of 1 μg/ml. Despite some clinical improvement, peritoneal cultures were positive 1 month later. The catheter was removed on 3 February 1983, and the patient was placed on hemodialysis. The ketoconazole treatment was continued for an additional 6 months. The patient has now been on hemodialysis for over 2 years and has had no further evidence of mycotic disease.

MATERIALS AND METHODS

Culture technique. Peritoneal fluid was inoculated, either directly or after concentration, by passing 100 ml of the dialysate through a 0.45-μm (pore size) filter (17) onto the following media: brain heart infusion broth, MacConkey agar, phenylethyl acetate agar with 5% human blood, and Trypticase soy agar (Difco Laboratories, Detroit, Mich.) with 5% sheep blood. After incubation for 48 h at 37°C, the blood agar and brain heart infusion broth were further incubated for 2 weeks at room temperature. After the initial isolation of a mold, the following media were added to all cultures: Sabouraud agar plate, Sabouraud agar plate with chloramphenicol (50 μg/ml, and Mycosel agar plate (BBL Microbiology Systems, Cockeysville, Md.).

Procedures for fungal identification. The organism was plated on 2% malt-extract agar, asparagine-yeast agar, oatmeal agar, or potato flakes agar (15). All cultures were incubated at 30 to 35°C for as long as 6 weeks. Fragments taken directly from mold growth on the various media and Scotch tape preparations were mounted in lactophenol cotton blue and studied by transmitted-light microscopy.

Immunoelectrophoresis. (i) Preparation of crude extracts. The fungal extract of *L. mutabilis* was prepared by grinding, with a mortar and pestle, the growth from a 14-day Sabouraud agar slant in 0.1 M sodium barbital buffer (pH 8.6). The resultant suspension was centrifuged at 3,000 × *g* for 10 min to remove disrupted fungal elements. An extract of *Candida albicans* (Robin) Berkhout was similarly prepared for use as a control.

(ii) Preparation of electrophoresis slides. Glass microscope slides (2.5 by 7.5 cm) were precoated with 1 ml of 0.6% agarose (Bio-Rad Standard; Bio-Rad Laboratories, Richmond, Calif.), dried overnight, and stored at room temperature. The agarose well bed was prepared by mixing 1.5 ml of 1.2% agarose with 1.5 ml of sodium barbital buffer to yield a final agarose concentration of 0.6%. After the agarose solidified, a razor blade was used to remove the agarose from 5.0 cm of the slide, leaving an agarose bed of approximately 2.5 by 2.5 cm at one end of the slide. A sample (0.2 ml) of the patient's acute or convalescent serum was added to 1.8 ml of 0.6% agarose to yield a 10% serum concentration. This suspension was poured onto the shaved section of the precoated slide, forming an agarose bed containing serum of 2.5 by 5.0 cm.

(iii) Rocket electrophoresis. A sample (10 μl) of the *C. albicans* control was also run against the patient's serum. The agar slides were subjected to electrophoresis at 3 V/cm for 12 h at room temperature. The slides were washed overnight in 1× phosphate-buffered saline (pH 7.2), followed by a 4-h rinse in distilled water. After they were stained for 4 min in Coomassie brillant blue (Sigma Chemical Co., St. Louis, Mo.), the slides were destained in ethanol, distilled water, and acetic acid (9:9:2) until precipitin lines were clearly visible.

Antifungal susceptibility testing. Susceptibility studies against antifungal antimicrobial agents were carried out at two institutions. Studies performed at one institution (the University of Washington) utilized inoculum concentrations as determined by diluting a suspension of the fungal isolate to the same optical density as a 0.5 McFarland standard (prepared using an isolate of *C. albicans* maintained in the laboratory as a standard susceptibility testing strain exhibiting in-house reproducibility). An agar dilution methodology was used with yeast nitrogen base (Difco) as the test medium, and MICs were determined at 48 h (18). Tests performed at the other institution (Montana State University) involved the inoculation of 10^4 conidia per ml, as

determined with a hemocytometer and verified by plate counts, into a liquid medium, synthetic amino acid medium-fungal (GIBCO Laboratories, Grand Island, N.Y.), incubation at 35°C, and visible determination of MIC and minimum lethal concentrations (MLCs) (6). The antifungal antimicrobial agents tested at the first institution were amphotericin B, 5-fluorocytosine, and miconazole, while those tested at the second institution were amphotericin B, 5-fluorocytosine, miconazole, and ketoconazole. Antifungal agents were dissolved in the medium used for testing; appropriate control organisms were included with each test run.

RESULTS

Description of the causative fungus. Macroscopic and microscopic descriptions are based on isolates grown on potato flakes agar at 25°C. Colonial growth was relatively slow; e.g., colonies were 3 to 4 cm in diameter after 14 days of incubation. Colonies were initially mucoid and somewhat yeast like, becoming more leathery with age. Young colonies were yellow-beige in color, later becoming centrally brown due to the formation of chlamydoconidia (Fig. 1). Conidiophores were generally simple, smooth, and mainly hyaline in color. Conidiogenous cells were cylindrical adelophialides (reduced phialides that are not delimited from the subtending intercalary hyphal cell by a basal septum) (4). The tips of the phialides were conically tapering and possessed distinct collarettes. Conidia were hyaline, nonseptate, and smooth walled. Conidia tended not to be united as "heads" at the tips of the adelophialides. Germination was frequently observed from mature phialoconidia. In mature cultures, the presence of dematiaceous chlamydoconidia was very common (Fig. 2). Confirmation of species identity was kindly provided by W. Gams (Centraalbureau voor Schimmelcultures, Baarn, The Netherlands) and M. R. McGinnis (North Carolina Memorial Hospital, Chapel Hill, N.C.). The strain is maintained as living cultures 82-72 and 83-3 (Department of Pathology, University of Texas Health Science Center at San Antonio).

Serological studies. Rocket electrophoresis of *L. mutabilis* against the patient's acute and convalescent sera revealed positive precipitin lines. Whereas the acute sera yielded a single tall precipitin "rocket," indicating a relatively low level of specific humoral antibody, the convalescent serum taken 6 weeks later yielded two precipitin rockets. In addition to the single faint rocket observed in the acute serum specimen, a second intense shorter rocket was observed in the convalescent serum sample, indicating an increase in the antibody level in response to the *Lecythophora* sp. Immunoelectrophoresis of the *C. albicans* control against the patient sera revealed no visible precipitin. The peritoneal fluid sample from December 1982 also demonstrated precipitins to *L. mutabilis*. Although no control antiserum for this organism was available as a positive control, these results indicate that there was a dramatic qualitative increase in antibodies against *L. mutabilis* in this patient.

In vitro susceptibility studies. Results of in vitro antifungal susceptibility tests are shown in Table 1. Data obtained at the University of Washington Clinical Microbiology Laboratories suggested resistance of the *L. mutabilis* isolate to 5-fluorocytosine and amphotericin B, whereas the MIC of miconazole (3.1 μg/ml) indicated susceptibility. These results were obtained by an agar dilution technique conducted in yeast nitrogen base medium by using spectrophotometrically prepared inocula. Results generated at Montana State University indicated resistance of the organism to 5-fluorocytosine and miconazole, whereas the MIC of amphotericin B indicated susceptibility. The method of testing at the latter institution was a macrobroth dilution method using a liquid synthetic medium (synthetic amino acid medium-fungal) with an inoculum of 10^4 conidia per ml. The disparity in the results obtained between the two institutions, although a matter of clinical concern, was not surprising or unexpected and is addressed below.

FIG. 1. Maturing *L. mutabilis* colony showing the central brown color due to the formation of chlamydoconidia.

DISCUSSION

The patient described here developed peritonitis caused by a fungus, *L. mutabilis*, which has been very rarely implicated as an agent of human disease. Treatment with oral ketoconazole, intraperitoneal miconazole, and catheter replacement resulted in marked clinical improvement. However, relapse was documented after completion of antifungal therapy, and necessitated the termination of peritoneal dialysis with conversion to hemodialysis.

L. mutabilis is classified in a group of molds which has recently undergone considerable study and taxonomic revision (4). This fungus and related species present difficulties for clinical laboratory personnel, since these organisms are seldom discussed in the arena of classic medical mycology. These organisms exhibit morphologic features and taxonomic nuances presenting difficulties to even those investigators most familiar with this group of molds. Identification of these *Hyphomycetes* may be best accomplished by consultation with mycologists possessing expertise with these fungi.

In this patient, diagnosis of the mycotic nature of the disease was difficult. Only one of three cultures obtained before initial treatment with imidazole antifungal agents was positive. The difficulty in isolating this organism may be similar to that of systemic candidiasis, in which blood cultures are often negative (2, 14). Documentation of infection in this case was verified by a repeat recovery of *L. mutabilis* at the time of relapse and by demonstration of serum precipitins in the presence of negative controls.

The development of peritoneal eosinophilia during treat-

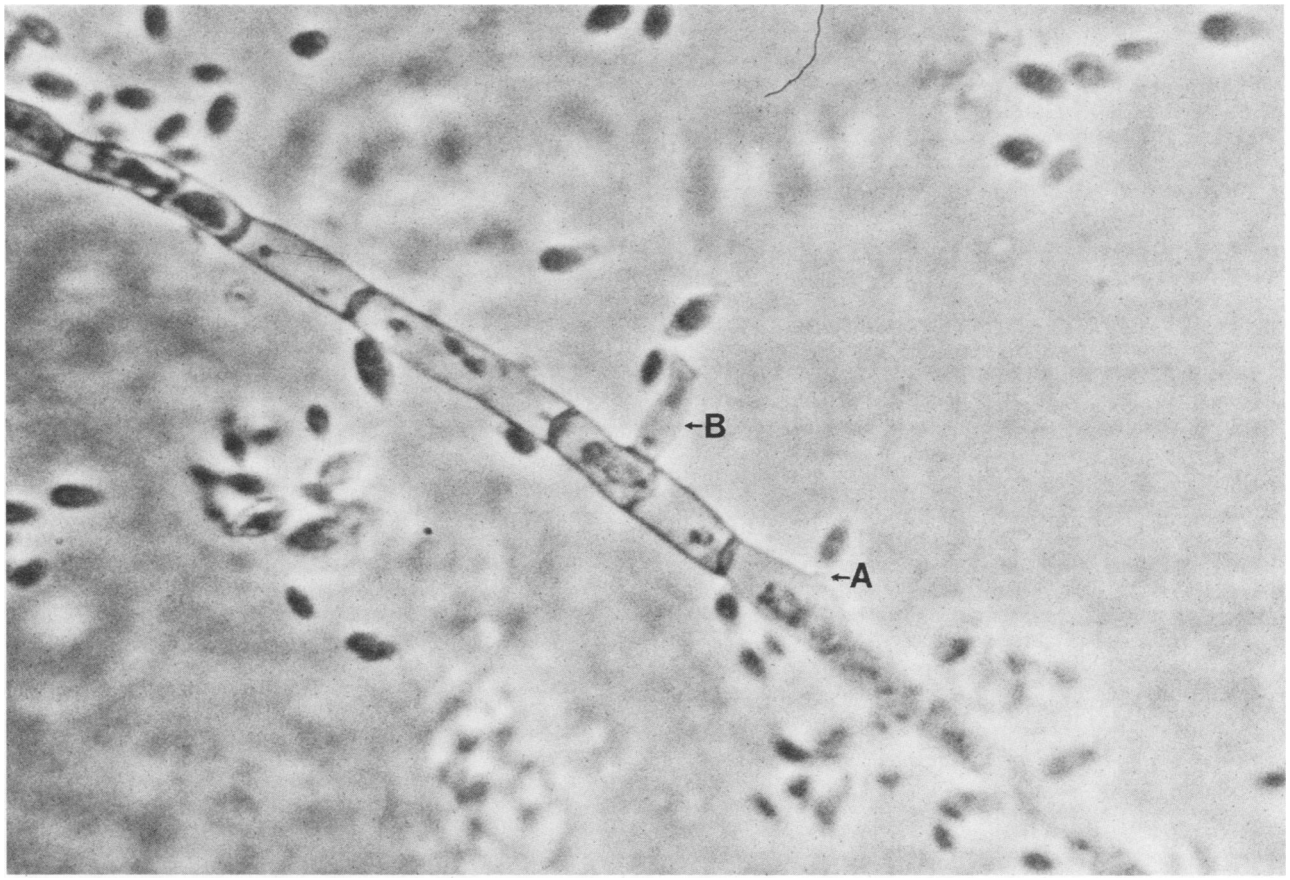

FIG. 2. Micrograph showing (A) dematiaceous chlamydoconidia forming from an immature adelophialide and (B) mature adelophialide with a distinct collarette (magnification, ×250).

ment suggests an allergic hypersensitivity response (5, 20). Peritoneal eosinophilia has been reported in a patient receiving intraperitoneal miconazole (10). Although miconazole could be the sensitizing agent, the maximum eosinophilia in the case reported here was noted 6 weeks after termination of therapy. It is interesting that we have not seen peritoneal eosinophilia in four additional patients we have treated with intraperitoneal miconazole and oral ketoconazole (unpublished observations).

The discrepancy in the results of antifungal susceptibility testing between the two laboratories is not uncommon. In vitro antifungal susceptibility testing is at present unstandardized. Differences in test methodologies, inoculum, concentrations and forms, and media used, as well as a variety of other factors, are known to influence in vitro results (6, 11, 12). The imidazoles are notoriously influenced by test methods (12). Hence, the data presented in Table 1 reflect this variation and serve to underscore the lack of standardization in this area of laboratory medicine. Even more problematic and of greater significance is the lack of correlation of in vitro data with the outcome of therapeutic response. Although laboratory evaluation of antifungal drugs results in the generation of values listing quantitative MICs and MLCs, the numbers may bear little relevance to patient care. We feel that at present there are no strictly right or wrong susceptibility values when contrasting data generated in different laboratories by various methods. Rather, further research is needed to prospectively correlate in vitro values with the outcome of treatment of experimental mycotic infections and, when possible and appropriate, with human mycoses. Research efforts continue in reference laboratories to provide a standardized, reproducible methodology.

Fungal peritonitis is associated with significant morbidity (7). Intraperitoneal amphotericin is poorly tolerated due to infusion-related abdominal pain, and a mycologic cure often requires the discontinuation of peritoneal dialysis (17; Johnson et al., in press). Removal of the infected cathether is critical for therapeutic success, as certain fungi can actually invade the silastic matrix (10). Recently, we managed fungal peritonitis with catheter replacement in conjunction with a course of oral ketoconazole and intraperitoneal miconazole (Johnson et al., in press). Of five patients thus far treated by using this regimen, only the patient reported here has not been able to continue peritoneal dialysis.

The significance of rarely isolated and uncommon fungi, both yeasts and molds, as agents of human disease is genuine. Undoubtedly, clinicians and microbiologists will encounter *L. mutabilis* and related organisms with increasing frequency in contemporary medicine.

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LITERATURE CITED

1. **Bayer, A. S., M. J. Blumenkrantz, J. Z. Montgomerie, J. E. Galpin, J. W. Coburn, and L. B. Guze.** 1976. *Candida* peritonitis: report of 22 cases and review of the English literature. Am. J. Med. **61**:832–840.
2. **Bodey, G. P.** 1966. Fungal infections complicating acute leukemia. J. Chronic Dis. **19**:667–668.
3. **Eisenberg, E. S., B. E. Alpert, R. A. Weiss, N. Mittman, and R. Soeiro.** *Rhodotorula rubra* peritonitis in patients undergoing continuous peritoneal dialysis. Am. J. Med. **75**:349–352.
4. **Gams, W., and M. R. McGinnis.** 1983. *Phialemonium*, a new anamorph genus intermediate between *Phialophora* and *Acremonium*. Mycologia **75**:977–987.
5. **Gokal, R., J. M. Ramos, M. K. Ward, and D. N. S. Kerr.** 1981. "Eosinophilic" peritonitis in continuous ambulatory peritoneal dialysis (CAPD). Clin. Nephrol. **15**:328–330.
6. **Hoeprich, P. D., and P. D. Finn.** 1972. Obfuscation of the activity of antifungal antimicrobics by culture media. J. Infect. Dis. **126**:353–361.
7. **Kerr, C. M., J. R. Perfect, P. C. Craven, J. H. Jorgensen, D. J. Drutz, J. H. Shelburne, H. A. Gallis, and R. A. Gutman.** 1983. Fungal peritonitis in patients on continuous ambulatory peritoneal dialysis. Ann. Intern. Med. **99**:334–337.
8. **Khanna, R., D. G. Oreopoulos, S. Vas, W. McCready, and N. Dombros.** 1980. Fungal peritonitis in patients undergoing chronic intermittent or continuous ambulatory peritoneal dialysis. Proc. Eur. Dial. Transplant Assoc. **17**:291–296.
9. **Landy, M. E., J. H. Greenwald, A. A. Steiner, and D. L. Ashbach.** 1982. *Cephalosporium* (*Acremonium*) in dialysis-connected peritonitis. J. Indiana State Med. Assoc. **75**:391.
10. **McNeely, D. J., S. Vas, N. Dombros, and D. G. Oreopoulos.** 1981. *Fusarium* peritonitis: an uncommon complication of continuous ambulatory peritoneal dialysis. Perit. Dial. Bull. **1**:94–96.
11. **Odds, F. C.** 1979. Problems in the laboratory assessment of antifungal activity. Postgrad. Med. J. **55**:677–680.
12. **Odds, F. C.** 1980. Laboratory evaluation of antifungal agents: a comparative study of five imidazole derivatives of clinical importance. J. Antimicrob. Chemother. **6**:749–760.
13. **Pearson, J. G., T. D. McKinney, and W. J. Stone.** 1983. *Penicillium* peritonitis in a continuous ambulatory peritoneal dialysis patient. Perit. Dial. Bull. **3**:20–21.
14. **Pizzo, P. A., K. J. Robichaud, F. A. Gill, and G. F. Witebsky.** 1982. Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. Am. J. Med. **72**:101–111.
15. **Rinaldi, M. G.** 1982. Use of potato flakes agar in clinical mycology. J. Clin. Microbiol. **15**:1159–1160.
16. **Rinaldi, M. G., E. L. McCoy, and D. F. Winn.** 1982. Gluteal abscess caused by *Phialophora hoffmannii* and review of the role of this organism in human mycoses. J. Clin. Microbiol. **16**:181–185.
17. **Rubin, J., W. A. Rogers, H. M. Taylor, E. D. Everett, B. G. Prowant, L. V. Fruto, and K. D. Nolph.** 1980. Peritonitis during continuous peritoneal dialysis. Ann. Intern. Med. **92**:7–13.
18. **Shadomy, S., and A. Espinel-Ingroff.** 1980. Susceptibility testing with antifungal drugs, p. 647–653. *In* E. H. Lennette, A. Balows, W. H. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
19. **Slifkin, M., and H. M. Bowers.** 1975. *Phialophora mutabilis* endocarditis. Am. J. Clin. Pathol. **63**:120–130.
20. **Steiner, R. W.** 1982. Clinical observations in the pathogenesis of peritoneal dialysate eosinophilia. Perit. Dial. Bull. **2**:118–119.