JOURNAL OF CLINICAL MICROBIOLOGY, July 1975, p. 62-66

Vol. 2, No. 1
Printed in U.S.A.

Wound Infection by *Prototheca wickerhamii*, a Saprophytic Alga Pathogenic for Man

WIE-SHING LEE,* MICHAEL D. LAGIOS, AND RICHARD LEONARDS

Department of Pathology, Children's Hospital, San Francisco, California 94119

Received for publication 3 January 1975

Biopsy of a wound infection of the palmar fascia in a young diabetic woman revealed characteristic periodic acid-Schiff-positive *Prototheca* species cells with a rosette configuration and internal septation. *Prototheca wickerhamii* was cultured repeatedly from the wound drainage and the biopsy tissue. Several diagnostic features distinguishing *Prototheca* species, saprophytic algae, from yeasts are: the formation of endospores by mitosis; greater variation in cell size (2 to 15 μm); the presence of cytoplasmic granules, particularly in old cultures; and the absence of budding forms and pseudomycelia. The organism was resistant to 5-fluorocytosine and the minimal inhibitory concentration of amphotericin B was 12.5 μg/ml. With the exception of the tetracycline group, all other 16 antibacterial agents tested appeared completely ineffective in vitro. A synergism between amphotericin B and tetracycline was clearly demonstrated by the use of the checkerboard method. Infection by *Prototheca* species may be more common than presently realized due to the common expedient of identifying yeast-like isolates as "yeast—not *Candida albicans*."

Protothecosis is an unusual infection caused by achloric algae. The first proven case of algal infection in man was in 1964 (3), and subsequently six more cases of protothecosis have been reported (2, 6, 8, 10, 13, 14; A. G. Smith, Abstr. Annu. Meet. Am. Soc. Microbiol., 1974, Mm22, p. 139). With increasing recent recognition of this infection, four of the cases have been diagnosed during the last two years (2, 10; Smith, Abstr. Annu. Meet. Am. Soc. Microbiol., 1974, Mm22, p. 139). A more thorough understanding of this infection may be expected in the near future with additional clinical studies.

Certain distinct morphologic characteristics of protothecae permit easy identification and distinguish it from yeast, with which it can be confused. These features are discussed in this report.

Previous clinical reports have indicated that many drugs were empirically tried to combat protothecosis, but present knowledge about chemotherapy for the disease is extremely poor. An antibotic sensitivity spectrum for the organism recovered in the present case is presented.

This represents a documented case of protothecosis in man, in which the organism is characterized both histologically and by culture isolation.

MATERIALS AND METHODS

Case Report. The patient is a 30-year-old, single Caucasian woman who had had brittle insulin-dependent diabetes mellitus since age 12. Past complications of her disease included evidence of rare retinal microaneurysms, a peripheral neuropathy, and multiple chronic infections by fungi (vaginal candidiasis and chronic cuticular infections).

In December 1973, the patient noticed a nodular swelling in the right palm, and a diagnosis of early nodular Dupuytren's contracture was made. The patient elected to have excision and release of the contracture, which was performed in late December 1973 under regional anesthesia. Postoperatively, the hand was splinted and initially appeared to be healing well, but in late January a serous, intermittent discharge associated with swelling and tenderness of the palmar fascia developed. Repeated efforts at culture of the discharge failed to reveal a pathogen. The incision was reopened twice in March 1974. At the second procedure tissue was obtained for culture and histology, which revealed the infectious agent.

Methods. All specimens were cultured onto 10% sheep blood plates with Trypticase soy agar base (BBL), Mycosel agar (BBL) slants, and Sabouraud dextrose agar (BBL) slants. The plates and tubes were then incubated at room temperature and 37 C. Biochemical tests for the identification of this organism were done according to the description of Arnold and Ahearn (1).

Disk diffusion antibiotic sensitivity was performed as described in the Kirby-Bauer method (4) except that the plate was incubated for 4 days at room temperature before the zone size was measured.

Tube dilution method was also employed both in Mueller-Hinton broth and in yeast nitrogen broth (YNB). Dextrose (1%) and 0.15% L-asparagine were also added in the YNB as described by Shadomy (12). The inoculum was adjusted according to the

Wickerham card technique (15) and the number of viable cells was further determined by plate count. A 0.5-ml inoulum of 10^4 to 10^5 organisms/ml of broth culture was added to 0.5 ml of broth in tubes containing a serial twofold dilution of the antibiotic. The minimal inhibitory concentration (MIC) of antibiotic agents for this organism was read on day 3. The MIC was defined as the lowest concentration that prevented turbidity upon visual inspection after incubation at room temperature for 3 days. From the clear tubes 0.01 ml of broth was removed via a calibrated loop and streaked on a Sabouraud dextrose agar plate, which was read after 4 days, and the minimal algacidal concentration (MAC) was designated as the lowest concentration in which no viable organisms remained on the plate.

The MICs and MACs of combined amphotericin B and tetracycline for synergism were determined by the standard two-dimensional broth (YNB/dilution) checkerboard method (7). Final concentrations of 0.39 to 50 μg of amphotericin B per ml and 0.20 to 25 μg of tetracycline per ml, alone and in all possible combinations, were obtained by adding 0.5 ml of 10^4 to 10^5 organisms/ml of broth cultures to 0.5 ml of broth in tubes, containing serial twofold dilutions of amphotericin B in rows of nine on one axis and of tetracycline on the other (81 tubes in all).

Starch granules were revealed by the addition of one drop of Lugol iodine to a drop of emulsified organism on the slide and examined under high dry power (40×).

The electron micrograph was prepared according to the method described by Nadakavukaren and McCracken (9).

RESULTS

Isolation and identification. The organism was cultured from the serous wound discharge in five of six specimens and from the biopsy material. Initially, the organism was thought to be a *Saccharomyces*-like fungus since (i) a smooth, moist, cream-colored colony was formed similar to that of yeast; (ii) no hyphae were formed in cornmeal agar; (iii) the organism was urease negative; and (iv) wet mount preparations showed endospores similar to ascospores within the ascus. However, no budding spores developed and the diagnosis of a yeast was subsequently withdrawn. Asexual reproduction in this organism occurs by formation of intracellular endospores (aplanospores within a sporangium). It has been described that the number of endospores vary according to different media used (11). On Sabouraud dextrose agar, the predominant number of endospores ranged from two, three, four, eight, and twelve. The organism varies markedly in size ranging from 2 to 15 μm in diameter as shown in Fig. 1. Intracellular granules are often observed and they seem to be characteristic; these increase in number and size in older cultures. Rather dark brownish cytoplasmic components in most of the cells stain as starch in nature with Lugol iodine solution. The organism grew on most routine media at room temperature and at 37 C. It was susceptible to cycloheximide and failed to grow on Mycosel agar.

The isolate assimilated glucose, galactose, trehalose, and levulose but did not assimilate maltose, sucrose, lactose, xylose, dulcitol, raffinose, inositol, melibiose, adonitol, erythritol, propanol, salisin, and starch. Potassium nitrate was not assimilated. The organism was identified as *Prototheca wickerhamii* based upon mophologic and biochemical studies and was confirmed with further immunofluorescent staining and biochemical tests (done by William Kaplan, Mycology Division, Center for Disease Control, Atlanta, Ga.)

Pathology and ultrastructure. Sections of the tissue excised from the palmar fascia demonstrated granulation tissue with focal necrosis and a fibrinopurulent exudative reaction. Within the exudative portion of the specimen there were numerous periodic acid-Schiff-positive, nonbudding organisms which frequently showed cysts up to 15 μm in diameter. These contained a variable number of endospores ranging from two in some of the smaller sporangia to eight in some of the larger ones (Fig. 2). No mycelia were demonstrated.

Electron microscopy revealed many sporangia containing multiple discrete endospores. The spores contained numerous dense bodies larger than those recorded for *P. zopfii* (9). Characteristic ellipsoidal negative-staining starch deposits were seen with plastids (Fig. 3) and as separate membrane-bound structures. Recently, Nadakavukaren and McCracken (9) stressed that a *Prototheca* species can be identified as an alga because of the distinct membrane-bound starch deposits and the presence of plastids.

Antibiotic sensitivity tests. No inhibitory zone could be demonstrated with the following 16 antibiotic agents: chloramphenicol, streptomycin, kanamycin, gentamicin, penicillin, cephalothin, colistin, erythromycin, furadantin, gantrisin, lincomycin, methicillin, nalidixic acid, ampicillin, carbenicillin, and clindamycin. Moderate resistance was demonstrated to tetracycline and minocycline which were slightly effective with approximate zone sizes of 25 and 15 mm, respectively. The organism would gradually grow back into the tetracycline and minocycline inhibition zones. The precise zone was difficult to measure.

Tetracycline sensitivity was also checked with the tube dilution method in Mueller-Hinton broth. The MIC of tetracycline for this organism was 200 μg/ml and MAC was greater than

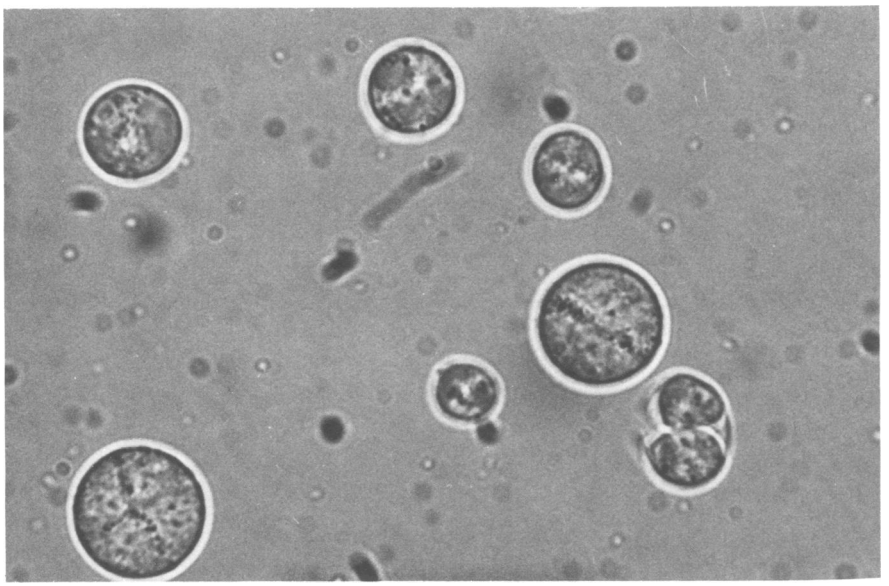

FIG. 1. *Septation of P. wickerhamii demonstrating two and three stages of cell development; also a well-formed sporganium containing two endospores. Wet mount.* ×1,000.

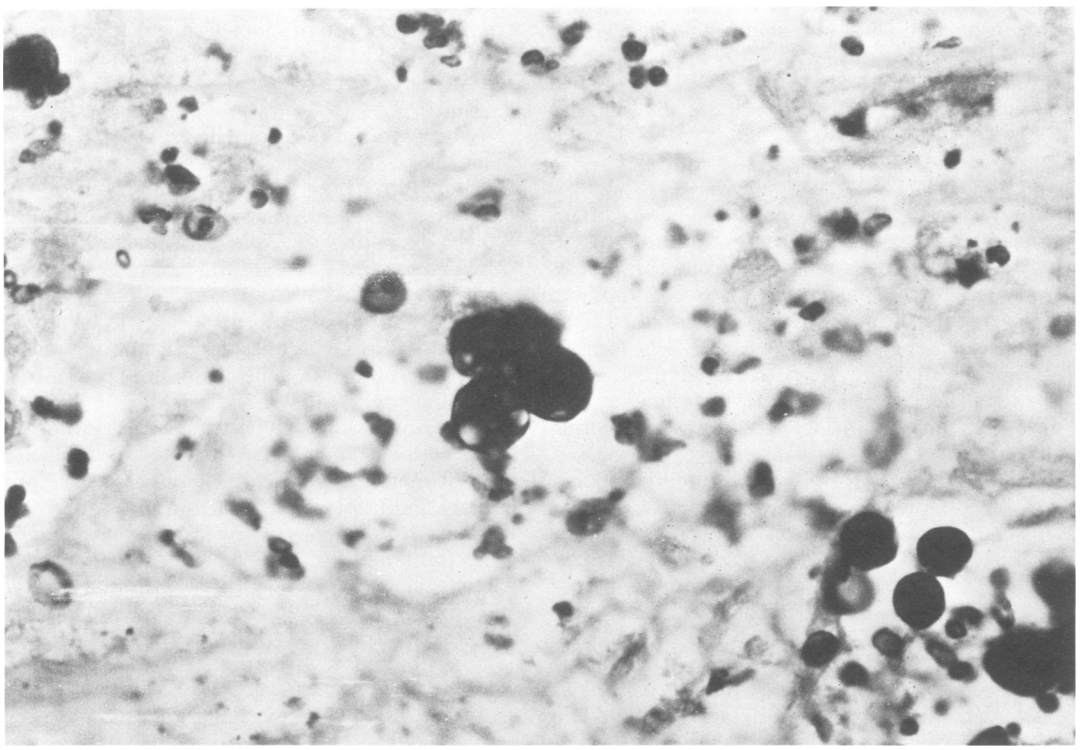

FIG. 2. *P. wickerhamii cells in formalin-fixed tissue stained by periodic acid-Schiff. Note three mature sporangia in the center showing a characteristic rosette configuration with the internally septated endospores.* ×1,000.

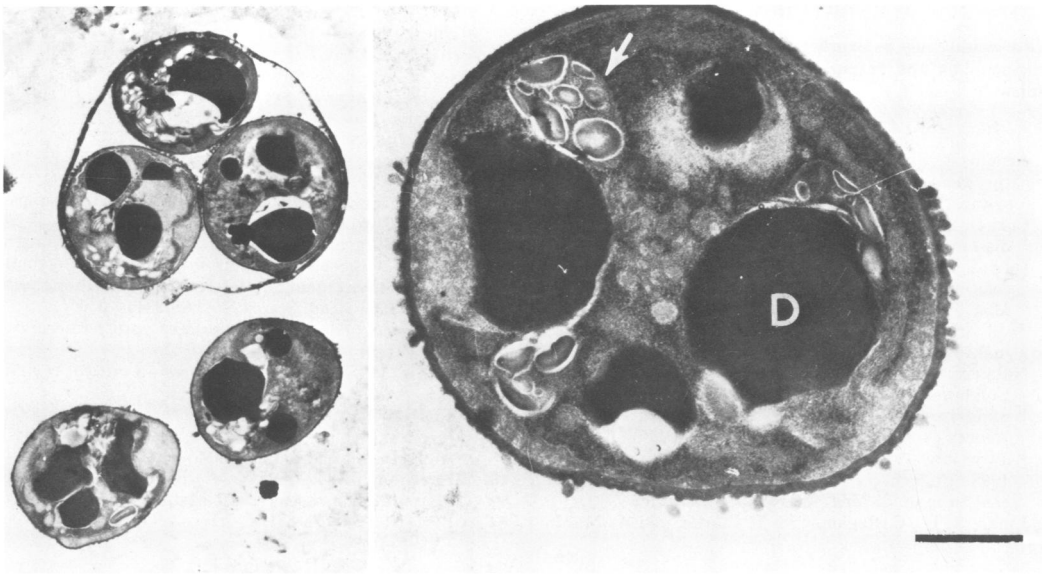

FIG. 3. *Electron micrograph of a 3-day-old culture. Clear ellipsoidal structures within cell membrane (arrow) represent a plastid (amyloplast), a feature unknown in fungi. D, Dense body. Inset demonstrates appearance of sporangium and endospores. Scale = 1 μm.*

200 μg/ml. A similar result was obtained with YNB as the test medium. The reading in YNB was much easier to determine due to relatively good growth. 5-Fluorocytosine and amphotericin B were also tested against this organism in YNB, and no inhibition was demonstrated with 1 mg of 5-fluorocytosine per ml. The MIC and MAC of amphotericin B were 12.5 μg/ml and 25 μg/ml, respectively.

With the checkerboard method (7), the synergism of amphotericin B and tetracycline could be demonstrated clearly. The MIC of amphotericin B and tetracycline was reduced to 0.78 and 1.56 μg/ml; MAC was reduced to 1.56 and 3.12 μg/ml, respectively. This finding demonstrated that the combined antibiotics were effective within acceptable chemotherapeutic levels.

DISCUSSION

Human algal infection is extremely rare. Important aspects of the pathogenicity and epidemiology of protothecosis have been suggested in the literature, but nonetheless, due to the scant number of cases studied, few conclusions can be drawn at the present time. Presumably the potential number of cases that could be reported would increase substantially if isolates ordinarily reported as "yeast—not *Candida* species" were to be re-examined according to the characteristics described in this report. Though colony appearance of this organism was indistinguishable from yeast, morphologically and taxonomically the *Prototheca* species should probably belong to the algae (9). In addition to the usual morphologic characterization, we recommend the employment of Lugol iodine to screen the intracellular components as starch for questionable yeast-like organisms.

The literature on chemotherapy for protothecosis is both scanty and in many instances drugs were employed on an empirical basis (13, 14). Previous reports of partial failure of treatment may represent lack of understanding of the antibiotic spectrum of the organism. In vitro this organism resisted almost all antibacterial agents except the tetracycline group which was also questionably effective. 5-Fluorocytosine, a common anti-fungal agent, was completely without effect and amphotericin B was only minimally effective. Although an opportunity to test the potential synergism demonstrated in vitro between amphotericin B and tetracycline was not provided in this particular case, based on our in vitro sensitivity tests it appears that this combination therapy would be a logical choice. Similarly, a synergistic effect of amphotericin B and tetracycline has been demonstrated to be effective in the therapy of candidiasis and experimental coccidioidomycosis (5). It would be worthwhile to test this potential synergism in the future treatment of protothecosis.

ACKNOWLEDGMENT

We would like to express our appreciation to Sheila Concannon for the preparation of these excellent photographs.

LITERATURE CITED

1. Arnold, P., and D. G. Ahearn. 1972. The systematics of the genus *Prototheca* with a description of a new species, *P. filamenta*. Mycologia 64:265-275.
2. Cox, G. E., J. D. Wilson, and P. Brown. 1974. A case of disseminated algae infection. Lancet ii:379-382.
3. Davies, R. R., H. Spencer, and P. O. Wakelin. 1964. A case of human protothecosis. Trans. R. Soc. Trop. Med. Hyg. 58:448-451.
4. Food and Drug Administration. 1970. Proposed rule making. Fed. Regist. 26:6899-6902.
5. Huppert, M., S. H. Sun, and K. R. Vulovich. 1974. Combined amphotericin B-tetracycline therapy for experimental coccidioidomycosis. Antimicrob. Agents Chemother. 5:473-478.
6. Klintworth, G. K., B. F. Fetter, and H. S. Nielsen, Jr. 1968. Protothecosis, an algal infection: report of a case in man. J. Med. Microbiol. 1:211-216.
7. Libke, R. D., C. Regamey, J. T. Clarke, and W. M. M. Kirby. 1973. Synergism of carbenicillin and gentamicin against enterococci. Antimicrob. Agents Chemother. 4:564-568.
8. Mars, P. W., A. R. Rabson, J. J. Rippey, and L. Ajello. 1971. Cutaneous protothecosis. Br. J. Dermatol. 85(Suppl.):76-84.
9. Nadakavukaren, M. J., and D. A. McCracken. 1973. Prototheca: an alga or a fungus? J. Phycol. 9:113-116.
10. Nosanchuk, J. S., and R. D. Greenberg. 1973. Protothecosis of the olecranon bursa caused by achloric algae. Am J. Clin. Pathol. 59:567-573.
11. Poyton, R. O., and D. Branton. 1972. Control of daughter-cell number variation in multiple fission: genetic versus environmental determinants in Prototheca. Proc. Natl. Acad. Sci. U.S.A. 69:2346-2350.
12. Shadomy, S. 1969. In vitro studies with 5-fluorocytosine. Appl. Microbiol. 17:871-877.
13. Sudman, M. S. 1974. Protothecosis—a critical review. Am. J. Clin. Pathol. 61:10-19.
14. Tindall, J. P., B. F. Fetter, and N. C. Durham. 1971. Infections caused by achloric algae (protothecosis). Arch. Dermatol. 104:490-500.
15. Wickerham, L. J., and K. A. Burton. 1948. Carbon assimilation tests for the classification of yeasts. J. Bacteriol. 56:363-371.