Infection Caused by *Penicillium marneffei*:
Description of First Natural Infection in Man

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**ABSTRACT**

DiSalvo, Arthur F., Fickling, Agnes M., and Ajello, Libero: Infection caused by *Penicillium marneffei*: Description of first natural infection in man. Am. J. Clin. Pathol. 60: 259-263, 1973. The first isolation of *Penicillium marneffei*, obtained from the liver of a rat captured in Viet Nam, was described in 1956. There have been no additional reports of the isolation of this fungus. Recently our laboratory identified this organism in an isolate from the spleen of a patient with Hodgkin's disease. The distinctive features of *P. marneffei* include the production of the dark red pigment which diffuses into the agar and the ability of this pathogen to exist in dimorphic forms. At 25 C. it develops the structures typical of the genus Penicillium, while at 37 C. the tan, rough colony has a yeast-like consistency, with small, oval or round yeast cells averaging 3 μ in diameter mixed with short, hyphal elements. Histologically, the organism may resemble *Histoplasma capsulatum*.

*Penicillium marneffei* was first reported as an animal pathogen in 1956 by Capponi and associates.1 The initial isolate was obtained from the liver of a bamboo rat (*Rhizomys sinensis*) in Viet Nam. Pathogenicity for the mouse, hamster, and guinea pig was established. A formal description of this new species was published by Segretain* in 1959.

In the course of intensive study of the animal pathogenicity of *P. marneffei*, the investigator acquired a laboratory infection.2 There have been no additional reports of the isolation of this fungus in the literature.

Our case represents the first known natural infection by this fungus in man and the second report of its isolation.

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**Report of a Case**

The patient, a 61-year-old, Caucasian man, a minister, was in apparent good health until April 1967, when a mass appeared on the left side of his neck. Excisional biopsy and lymphangiograms at that time confirmed a diagnosis of Hodgkin's disease with localization in the left supraclavicular area and left axilla. In May 1967, he received cobalt radiation therapy above and below the diaphragm.

The patient did well on this therapy and submitted to biopsy of a right cervical lymph node and repeat lymphangiogram in October 1969. Both examinations were again compatible with a diagnosis of Hodgkin's disease. Consistent with the management of this disease process, the patient was hospitalized for laparotomy, splenectomy, and biopsy of the liver, bone, and lymph...
nodes in May 1971. At operation, the spleen was found to be enlarged and infarcted. The omentum was adherent to the anterior surface of the spleen.

Gross examination of the excised spleen revealed a mass of tan, nodular tissue, 9 cm. in diameter, with a suppurated center. The necrotic material was cultured for bacteria and fungi. A yeast-like growth was isolated on Sabouraud’s dextrose agar (SDA) incubated at 25 C. and on blood agar and thioglycollate broth incubated at 37 C.

Histologic examination of the spleen tissue revealed mononuclear elements, eosinophils, and fairly numerous dysplastic reticulum and giant cells. The excised spleen and lymph nodes had features histologically compatible with Hodgkin’s disease.

Mycology

The hospital laboratory was unable to identify the isolate from the spleen and forwarded the culture to the Bureau of Laboratory Services and Research, South Carolina State Board of Health, for further study. The original isolate on SDA was 13 days old when received, and appeared to be a membranous, confluent, yeast-like growth which adhered to the surface of the medium. Microscopic examination of a lactophenol cotton blue wet mount was suggestive of Geotrichum candidum because of the elongated, rectangular yeast cells and a few septate hyphae. Subcultures were made on various media and incubated at 25 C. and 37 C.

The following description refers to the growth on SDA incubated at 25 C. In 2 days, there was a waxy, membranous, tan colony, 5 mm. in diameter. In 5 days, the colony was 9 mm. in diameter with white, wooly aerial mycelia with a waxy periphery. A faint-rose-colored pigment that diffused into the agar was present on the reverse side. In 10 days, the 25 mm. colony was light brown, and the aerial mycelia were rose-pigmented. The reverse had a rose-red pigment in the center with a light brown periphery. By 15 days, the 30 mm. colony had a heaped, rose-pigmented center, shading from light brown to light green, with a membranous 2 mm. periphery. The aerial hyphae were short, and the colony had striations radiating from its center.

On Wort agar incubated at 25 C. for 15 days, the colony was 22 mm. in diameter, its center was heaped with yellow-green and pink mycelia, the periphery was light green, and the reverse was similar to that on SDA but without the red pigment. Growth was inhibited on SDA with cycloheximide and chloramphenicol.

Microscopic examination of the mycelial form showed metulae, sterigmata, and conidia typical of the genus Penicillium. The chains of conidia were curved, and the individual conidia were spherical, with smooth walls. The initial isolate had coarse septate mycelium and branched dichotomously. There were hyphae with elongated rectangular forms which resembled arthrospores. Occasional spirals were present. As the culture aged, mycelial growth became compatible with typical Penicillium species.

At 37 C., the growth was yeast-like with a cerebriform surface. The friable growth was light tan in color and grossly resembled the yeast form of Blastomyces dermatitidis.

Microscopically, the yeast cells were small, oval to round cells that averaged 3 μ
in diameter, mixed with short, hyphal-like elements. Both the round and hyphal forms divided through formation of a cross-wall.

**Animal Studies**

For the completion of the study, animal inoculations were performed using adult white Swiss mice. Three mice were inoculated intravenously (I.V.) and three intraperitoneally (I.P.) with a 0.5 ml. saline suspension of mycelial fragments of the isolate from the patient. A group of control mice was similarly inoculated with a stock culture of the original isolate of *P. marneffei*. Twenty days after inoculation, one mouse inoculated I.V. and one mouse inoculated I.P. with each strain were autopsied. Lung, liver, and spleen were cultured on SDA, SDA with cycloheximide and chloramphenicol, and Wort agar, and were incubated at 25 C. Wort agar, SDA and brain heart infusion agar were also inoculated and incubated at 37 C. The remaining animals were sacrificed 35 days after inoculation and cultures made in a similar manner.

*P. marneffei* was isolated from the lungs, liver, and spleen of all animals inoculated with the isolate from the patient and with the control strain. Growth was obtained on all media except SDA with cycloheximide and chloramphenicol. At autopsy, tissue impressions were made from the lungs, liver, and spleen and stained by the periodic acid-Schiff (PAS) technic. Round to oval yeast-like cells with occasional cross-walls were present. Elongated, rectangular cells were also seen (Fig. 1).

On the basis of the growth and microscopic features of the isolate at 25 C. and 37 C., it was identified as *P. marneffei*. Cultures and human and animal tissues were sent to the Mycology Section of the Center for Disease Control, Atlanta, Georgia. Studies performed there confirmed our findings and conclusions.

**Histopathology**

Sections of the patient's splenic tissue stained with Gomori methenamine-silver revealed small oval to round yeast cells 2.5 to 4.5 \( \mu \) in diameter (average 3.0 \( \mu \)) (Fig. 2). A distinctive feature of the yeast form of this organism was the dividing septum frequently seen in some cells, indicating that multiplication was by fission rather than a budding process (Fig. 3). With intensive study, long tubular forms 3 to 6 \( \mu \) long by 1 to 2 \( \mu \) wide were also seen (Fig. 1). These elongated forms, with rounded ends frequently had a partition in the middle and resembled arthrospores.

The superficial histologic similarity between this organism and *Histoplasma capsulatum* was striking (Fig. 4). Therefore, selected sections of the spleen, in which yeast cells were demonstrated, were tested with fluorescein labeled *Histoplasma capsulatum* antitoxin. The conjugate did not stain the fungi.

**Discussion**

*Penicillium marneffei* was named for Dr. Marneffe, Director, Institut Pasteur (Indochina), later Director, Institut Pasteur (Paris), according to G. Segretain (personal communication, Dec. 22, 1971). A striking feature of this organism is the soluble red pigment that it produces and its morphologic dimorphism. The yeast form, exhibited during tissue invasion and when grown at 37 C. on various media, has a superficial resemblance to *Histoplasma capsulatum*. A cursory examination will reveal a yeast form, approximately 3.0 \( \mu \) in diameter, that appears to be budding. Closer observation demonstrates that cross-walls are present and that reproduction is by fission and not by a budding process. A diligent search will reveal the presence of long, tubular forms which are not formed by *H. capsulatum*. 
Extensive animal experimentation using mice, guinea pigs, hamsters, and rats demonstrated that the pathologic process is a reticulosis with hepatosplenomegaly, lymphadenopathy, and giant cell formation.\(^2\)\(^3\) Antifungal sensitivity studies by Drouhet demonstrated that \textit{P. marneffei} was susceptible \textit{in vitro} to nystatin and cycloheximide.\(^4\) \textit{In vivo} sensitivity to these agents in hamsters and mice was also studied. Cycloheximide did not alter the course of disease in hamsters or mice. Nystatin modified the mortality rate in mice, while the hamsters survived the 20-day observation period.

Amphotericin B sensitivity studies were performed on our isolate. The minimal inhibitory concentration at 37 C. and 25 C. was 0.78 \(\mu\)g per ml. and the minimal fungicidal concentration was 1.56 \(\mu\)g per ml. at both temperatures.

The only previously recorded case of human infection was the result of a laboratory accident.\(^5\) Nine days after inoculation into the finger, a small nodule appeared at the portal of entry, and axillary lymphadenopathy was present. \textit{P. marneffei} was isolated from the wound. The patient responded to treatment with 20 million units of nystatin per day, administered for 1 month.

The subject of this report was a patient with Hodgkin’s disease; a disease of unknown etiology. Patients with this disease are susceptible to secondary opportunistic infections. Pathogenic organisms that have been isolated from patients with Hodgkin’s disease include \textit{Mycobacterium tuberculosis}, \textit{M. avium}, \textit{Brucella melitensis}, \textit{Cryptococcus neoformans}, various diphtheroids, and viruses.\(^3\) These patients are susceptible to secondary infections because of leukopenia and impaired antibody production. The patient in this report received radiation therapy, which may also have rendered him more susceptible to secondary infection. This patient also had toured Southeast Asia, during the summer of 1970, thus placing him in proximity to the only known area with a recorded infection, but the place and time of his infection with \textit{P. marneffei} remain unknown.

\textit{P. marneffei} has been shown to be a pathogen of man as well as bamboo rats. Clinicians, pathologists, and medical mycologists should be aware of its existence and be alert to its occurrence in patients with impaired resistance.

This culture has been deposited in the American Type Culture Collection and assigned accession number 24100.

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\textbf{References}