Histoplasma capsulatum: Occurrence in Soil from the Emilia-Romagna Region of Italy

Abstract. *The existence of an area in Europe in which histoplasmosis is endemic was revealed by the isolation of* Histoplasma capsulatum *from soil. The soil specimen was collected in a chicken yard on a farm near Bologna, Italy. The Emilia-Romagna region had been selected for study because several, apparently autochthonous, human cases of histoplasmosis had originated there.*

Over a period of several years, a number of apparently autochthonous cases of histoplasmosis have been reported from the Emilia-Romagna area of Italy (*1*). The desire to determine whether or not *Histoplasma capsulatum* was endemic in this area prompted a search for that fungus in soil.

Because *H. capsulatum* is known to flourish in avian and chiropteran habitats (*2, 3*), soil samples were collected in sites frequented by bats and by wild and domesticated birds. Ninety-seven such samples were collected from the superficial layers of the ground and placed in plastic bags for transportation to our laboratories in Bologna, Italy, and Atlanta, Georgia, where they were processed for the detection of systemic human pathogenic fungi by two different methods.

At the Communicable Disease Center soil suspensions were prepared by mixing a heaped teaspoonful of soil in 30 ml of physiological saline that contained 1000 units of penicillin and 1 mg of streptomycin per milliliter. The suspension was allowed to settle for 1 hour. A 5-ml sample of the supernatant was then removed with a pipette, and 1 ml of this material was inoculated intraperitoneally into each of four mice. Eight weeks after inoculation the mice were killed and small portions of the livers and spleens were inoculated into tubes of a modified Sabouraud dextrose agar (*4*) containing 0.05 mg of chloramphenicol per milliliter. The culture tubes were incubated at 25°C and examined at intervals over a 4-week period for the development of *H. capsulatum*.

In the Mycology Laboratory of the Medical Clinic of the University of Bologna, soil suspensions were prepared in a different manner in an attempt to isolate *H. capsulatum* directly from soil. With an electric blender, a teaspoonful of soil was thoroughly mixed in 50 ml of sterile physiological saline. The suspension was allowed to settle, and 1-ml portions of the supernatant were used to inoculate several tubes of a liquid medium of the following composition: neopeptone, 10 g; dextrose, 20 g; distilled water, 1000 ml; penicillin G, 2500 units/ml; streptomycin, 250 mg/ml. After incubation for 7 days at 37°C, 1-ml quantities were placed at room temperature or at 37°C on pour plates prepared with neopeptone–antibiotic agar. The plates were examined frequently for the development of colonies of *H. capsulatum*.

On one of the plates incubated at room temperature, colonies of *H. capsulatum* appeared. The soil from which these colonies were derived had been collected in a chicken yard on a farm in the Province of Bologna, 25 km from the city of Bologna between the villages of Mezzolare and San Martino in Argine.

The culture of *H. capsulatum*, as is characteristic of some soil isolates of this fungus, produced macroconidia that were initially smooth. But as the colony aged, the spores became covered with the tubercles characteristic of that species. Only after repeated transfers and incubation at 37°C on blood agar was the mycelial phase converted to its unicellular, budding yeast phase. Tissue-phase cells were also demonstrated in mice injected with a suspension of the mycelial elements. The identity of the fungus was further verified by means of the fluorescent antibody procedure, whereby the yeast cells induced in vitro were brightly stained with a conjugate specific (*5*) for the yeast phase of *H. capsulatum*.

The successful recovery of *H. capsulatum* from soil collected in the Emilia-Romagna area of Italy conclusively demonstrates for the first time the existence of an endemic histoplasmosis area in Europe.

GIULIO SOTGIU
ALDO MAZZONI

Istituto di Clinica Medica, Generale e Terapia Medica, Università di Bologna, Bologna, Italy

ADRIANO MANTOVANI

Istituto di Parassitologia, Università di Roma, Rome, Italy

LIBERO AJELLO
JOSEPH PALMER

U.S. Department of Health, Education, and Welfare, Communicable Disease Center, Atlanta, Georgia 30333

References and Notes

1. G. Sotgiu and G. Corbelli, *Bull. Sci. Med.* **127**, 85 (1955); G. Corbelli, A. Mazzoni, L. Allegri, *Giorn. Malattie Infettive Parasittarie* **9**, 347 (1957); L. Allegri and E. Bottiglioni, *Malattie Infezione* **4**, 135 (1958).
2. L. D. Zeidberg, L. Ajello, A. Dillon, L. C. Runyon, *Am. J. Public Health* **42**, 930 (1952).
3. C. W. Emmons, *Public Health Rept. U.S.* **73**, 590 (1958).
4. L. Ajello *et al.*, *Mycopathol. Mycol. Appl.* **12**, 199 (1960).
5. L. Kaufman and W. Kaplan, *J. Bacteriol.* **82**, 729 (1961).
6. We are grateful to Dr. Leo Kaufman for testing our isolate of *H. capsulatum* with the fluorescent antibody procedure.

1 December 1964

Dimorphic Development of Transplanted Juvenile Gonads of Mosquitoes

Abstract. *Potentially masculine gonads of* Aedes stimulans *(Diptera: Culicidae) can be induced to become ovaries by exposing larvae to elevated temperatures. A juvenile gonad when transplanted into another larva will become a testis or an ovary in response to stimuli which are wholly intrinsic in the implanted gonad. Humoral factors in the hemocoel of the larval host do not influence the direction of morphogenesis of the implanted organ.*

Aedes stimulans which are males according to their genetic constitution develop feminine characteristics when larvae are reared at elevated temperatures (*1, 2*). We have examined the effects of temperature on genetically masculine gonads developing (i) *in situ*, (ii) in other larvae of the same species, and (iii) in larvae of a different species. Our purpose was to determine whether the genic products controlling dimorphism are humoral, or whether they arise and function solely within each imaginal disc.

Sexual dimorphism of most insects is irrevocably determined at fertilization, and morphogenesis of sexual organs is subsequently controlled intrinsically (*3*). However, humoral factors may alter the sexual phenotype of organs of some insects. Two studies have been reported in which plastic or undetermined tissues of potential (according to genotype) females have become masculinized after coming in contact with the blood or gonads of the male insect. Alar discs of female larvae of the lepidopteran, *Orgyia antiqua*, became mas-