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.

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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.

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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-

culinized when they were implanted into male larvae (*4*). More recently, Naisse (*5*) reported that the placement of a juvenile testis of the firefly, *Lampyris noctiluca,* into a young female larva resulted in an imaginal phenotype which was wholly masculinized. Likewise, the implantation of a juvenile ovary into a male larva resulted in a masculinization of the transplant. Apparently, a hormonal system controls sexual dimorphism in these instances. A seemingly different means controls divergent morphogenesis of genetically masculine gonads of *Aedes stimulans.*

At the beginning of postembryonic development of *Aedes stimulans,* gonadal discs of genotypic males have the capability of developing either into ovaries or into testes. The plasticity of the discs is retained during instars 1 and 2 when the rearing temperature is 26°C or higher (*1, 2*). If larvae are subsequently reared at 18°C for instars 3 and 4, the gonads of genotypic males are testes, whereas ovaries are formed if exposure to the high temperature is continued (*2*). In the following experiments, immature gonads were removed from genotypically male larvae which had been reared at 27°C for instars 1 and 2, and implanted into larvae in the third instar which had also been reared at 27°C during the first two instars.

The first experiments were designed to demonstrate the plasticity of the gonadal discs and to determine the means by which a juvenile gonad is masculinized. Each of the two gonadal discs of a genotypically male donor of *Aedes stimulans* was transplanted into a separate larva of the same species; either both of the host larvae were reared at 18°C, or one of the hosts was held at 27°C, and the other was kept at 18°C throughout development of the last two instars. A gonadal disc transplanted into a larva which was reared at low temperature always developed into a testis without regard to the sex of the host. Gonads belonging to the mosquito host developed normally. A gonadal disc from a genotypic male, when placed into a host larva reared at 27°C, always developed into an ovary irrespective of the genotype of its host. These experiments demonstrate (i) the plasticity of the gonadal discs, (ii) the effect of temperature on morphogenesis of imaginal organs, and (iii) that physiological factors originating from within the transplant are responsible for causing masculinity to be determined at 18°C. If maleness had been under humoral influence, then the gonadal disc when placed into a female larva reared at 18°C should not have developed into a testis, but invariably it did so develop.

Table 1. Differentiation of the two gonadal discs from each genotypically male donor (*Aedes stimulans*) transplanted into separate host larvae of *Aedes vexans*, one of which was subsequently reared at 27.0° ± 0.1°C and the other at 18.0°C ± 0.1°C for instars 3 and 4.

No. of individual gonads transplanted*	Genotype of host	Rearing temperature for instars 3 and 4 (°C)	Phenotype of imaginal gonads	
			Host	Donor
6	♂	27	Testes	Ovary
6	♂	18	Testes	Testis
4	♀	27	Ovaries	Ovary
4	♀	18	Ovaries	Testis
4	♀	27	Ovaries	Ovary
4	♂	18	Testes	Testis
6	♂	27	Testes	Ovary
6	♀	18	Ovaries	Testis

* Each of the two gonadal discs of a donor was placed into a separate host.

The first experiments do not demonstrate the process by which the genetic male transplant becomes feminized at high temperatures. The gonads of both genotypic males and genotypic females of *Aedes stimulans* are wholly feminized at 27°C. Since the host's gonads are always ovaries at 27°C, it is not possible to ascertain whether the implanted disc became feminized independently of factors in the hemocoel. To determine whether femininity is under the influence of physiological factors extrinsic to or intrinsic in each imaginal disc, an exotic species, *Aedes vexans,* was used as a host, because normal males of this species are obtained even at 35°C. A plastic gonadal disc of *A. stimulans* implanted into a male or female larva of *A. vexans* which was reared at 27°C for instars 3 and 4 developed into an ovary (Table 1). The second disc from the same donor implanted into a female or male larva of *Aedes vexans* which was reared at 18°C for the last two instars developed into a testis (Table 1). Feminization is evidently due to physiological factors wholly intrinsic within each imaginal disc. If femininity was under humoral influence, then the disc placed into a male host at 27°C should not have developed into an ovary.

These experiments do not wholly exclude the possibility of a feminizing hormone. It is possible that at 27°C, but not at 18°C, a feminine hormone is secreted in males of *Aedes vexans,* but because of a difference of sensitivity of tissues between the two species, only the implant responds. This alternative interpretation, however, seems highly unlikely. Gonadal discs from genotypically female larvae of *Aedes stimulans* were transplanted into larvae treated in the manner described in the preceding paragraphs. These organs, as expected, developed into ovaries irrespective of the sex of the host or the temperature of the rearing medium.

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References and Notes

1. J. F. Anderson and W. R. Horsfall, *J. Exptl. Zool.* **154**, 67 (1963).
2. W. R. Horsfall and J. F. Anderson, *ibid.* **156**, 61 (1964).
3. A. G. Richards, Jr., *J. N.Y. Entomol. Soc.* **45**, 149 (1937).
4. H. Paul, *Arch. Entwicklungsmech. Organ.* **136**, 64 (1937).
5. J. Naisse, *Gen. Comp. Endocrinol.* **3**, 721 (abstract) (1963).
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Tumor and Virus Antigens of Simian Virus 40: Differential Inhibition of Synthesis by Cytosine Arabinoside

Abstract. *Cells infected with the papovavirus SV40 not only synthesize viral antigen but also synthesize the specific nonviral antigen found in SV40-induced tumors. In the presence of the DNA antagonist cytosine arabinoside, infected cells fail to make viral antigen but still synthesize the tumor antigen. Iododeoxyuridine does not inhibit the synthesis either of tumor or of virus antigen but does prevent the development of infectious virus.*

The development of methods for detecting the tumor antigen induced by simian papovavirus 40 (SV40) both in cells transformed by the virus (*1, 2*) and during the early stages of the replication of the virus in cytolytic systems (*3, 4*) has resulted in an accumulation of data on the tumor and virus antigens in differing experimental systems. During studies of the effects of DNA in-