SUSCEPTIBILITY OF HAMSTERS AND MICE TO *PARACOCCIDIOIDES BRASILIENSIS* USING DIFFERENT ROUTES OF INOCULATION

by

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Abstract

Experiments were made to investigate the efficacy of inoculation of hamsters and mice by different routes, for the recovery of the mycelial phase of *Paracoccidioides brasiliensis*. A clear superiority was found for the intratesticular route in hamsters over the intraperitoneal route in the same animal and also over the intraperitoneal and intravenous routes in mice. The intratesticular route in hamsters is thought to be the most appropriate to be used in future attempts to isolate *P. brasiliensis* from nature.

The natural habitat of *P. brasiliensis* is unknown at present. Publications such as those of Grose & Tamsitt (1965) referring to the isolation of the fungus from bats and of Negroni (1966) from the soil in Argentina, have not been substantiated. Origin of the infection from foci in the soil is thought to be the most probable. An important tool for searching for the natural habitat(s) of pathogenic fungi has been the injection of laboratory animals. As discussed in previous papers on *Blastomyces dermatitidis* (Conti-Díaz, Smith & Furcolow, 1970a; Conti-Díaz, Smith & Furcolow, 1970b), the success of such method rests on the susceptibility of the laboratory animal used for the recovery of the fungus and on the route of inoculation employed. To our knowledge, with only one exception (Salfelder, Schwarz & Johnson, 1968), papers on susceptibility of laboratory animals to *P. brasiliensis* have usually employed massive doses of the pathogen. These methods are obviously inadequate for elucidating the problem of comparative animal susceptibility. Quantitative studies are obligatory.

In the present experiments, hamsters and mice were inoculated through different routes with known relatively small doses of the mycelial phase of *P. brasiliensis*. Mice were chosen as the laboratory

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animals commonly used for the isolation of pathogenic fungi from nature and hamsters because they have been considered the most susceptible animals to this organism.

**MATERIALS AND METHODS**

**First experiment**

**Inocula**

A strain of *P. brasiliensis* (1438, I.H.M.*) isolated in 1957 was grown on sterilized loam soil in flasks placed in humidity chambers. The technique was a modification of that described by HINTON et al. (1957). Macroscopic fungal growth was observed on the surface of the soils since the cultures were 1 month old. Microscopic examination of portions of mycelia showed many aleuriospores similar to those produced by this and other strains of the parasite on some laboratory media. Fifty days after inoculation, 15 ml of soil was added to 100 ml sterile physiologic saline. Processing of this suspension and determination of the number of viable particles (v.p.) were done as in previous experiments (CONTI-Díaz et al. 1970a). In the present experiment, modified SABOURAUD's dextrose agar plates were used instead of yeast extract agar medium. The stock suspension was shown by dilution cultures to contain 4,500 v.p. per ml. It was kept at 4°C until the day of the animal inoculation (40 days later) when similar dilutions were performed to investigate changes in the number of v.p.

**Animal inoculation**

One in 2 and 1 in 10 dilutions of the stock suspension were used for inoculation (suspensions A and B respectively).

Sixty male white Swiss mice weighing 19—23g and 60 male golden Syrian hamsters (*Mesocricetus auratus*) weighing 120—130 g were used. Two groups of mice of 15 animals each were injected intravenously (i.v.) by means of a lateral tail vein with suspensions A and B respectively; two more groups received the same suspensions intra-peritoneally (i.p.). Two similar groups of 15 hamsters each, were injected with the same two suspensions, into the left testicle (i.t.). Finally, two additional groups of 15 animals each, were injected i.p. with the two suspensions. Each animal received 0.1 ml of inoculum in every case. According with the results of the second plate counts, the stock suspension was shown to contain 19,000 v.p. per ml at the time of animal inoculation. There was thus an active multiplication of the fungus in the stock suspension during its preservation at 4°C. So, it was calculated the animals inoculated with suspension A received each 950 v.p. and those with suspension B, 190

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