Michael R. McGinnis
Marcel Borgers
Editors

# Current Topics in Medical Mycology

## VOLUME 3

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## VOLUME 3

With 93 Illustrations



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# Current Topics in Medical Mycology 3

## *Editorial Board*

# Series Preface

*Current Topics in Medical Mycology*, is intended to summarize current research areas in medical mycology for medical mycologists and other scientists who are working in microbiology and immunology. Topics to be included in each volume will serve as contemporary reviews, summaries of current advancements and future directions, and mechanisms to enhance the interdisciplinary use of medically important fungi in understanding pathogenesis, epidemiology, mycotoxins, taxonomy, and other areas where basic, applied, and clinical sciences are used.

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# 1—Animal Models for Dermatomycotic Infections

JAN VAN CUTSEM

Under natural conditions the skin of man and animals is exposed to numerous agents and may be subject to infections by various pathogenic organisms, including viruses, bacteria, and fungi. In predisposed individuals or those exposed to large concentrations of pathogens, the infection is more pronounced, more invasive, more extended, and less susceptible to therapeutic agents. There are a number of uncommon organisms that are often considered to be saprophytic yet turn to be infectious agents. In this chapter the most common fungal pathogens used as test species in animal models for dermatomycoses are discussed, and the usefulness of the animal models for studying infection and screening antifungal compounds is evaluated.

Most dermatomycoses are caused by dermatophytes and yeasts, mainly *Candida* spp. and *Malassezia* sp.. The increasing incidence of acquired immunodeficiency syndrome (AIDS) and its secondary infections have led to other skin infections, e.g., cryptococcosis, becoming more common. These fungi are the ones most frequently used in experimental animal models of dermatomycosis. Infection by the dermatophytes *Candida* spp., *Malassezia* sp., *Cryptococcus* sp., and miscellaneous agents are discussed in detail in this chapter.

The objectives when using an animal infection model are to study the pathogenicity of the strain and the immunity problems, to evaluate therapeutic agents by screening methods, and to determine the in vivo potency of a compound under experimental conditions in order to obtain information about its antifungal value. The information obtained is used to direct the synthesis of new antifungal agents via substitution or modification of active structures, enabling new candidates to be selected for clinical trials. These models therefore must ensure that extrapolation of the infection and the results after prophylactic or therapeutic treatment to natural and spontaneous infection in man and animals is possible.

A screening model must be standardized to ensure reproducibility, that all animals used in the same experiment have identical lesions, and that the

infection shows the same evolution. No differences in intensity of the infection may occur over a period of years.

First, the animals must be housed in isolated rooms and in separate cages. These cages have smooth walls with the bottom consisting of wire meshes, and they must be easy to disinfect. The next requirement is small, inexpensive animals that are docile, easy to manipulate, and inexpensive to maintain. They must be highly sensitive to the infection, and their body weight must be low so that only a small amount of test compound is needed. Species that make their toilet by licking or that scratch or bite itching or irritated lesions are not used if possible. This requirement disqualifies the mouse, rat, hamster, and rabbit. Taking all these recommendations into consideration, the most valuable candidate is the guinea pig.

Spontaneous cure with persistence or reappearance of chronic lesions may occur, especially in models with high inflammatory reactions. Yet the need for chronic noninflammatory infection models persists. Unfortunately, most animals develop acute inflammatory reactions.

## Dermatophytes

Dermatophytes are keratinophilic fungi that affect mainly the keratinized layers of the skin, i.e., the hair and nails of man and animals. Other parts of the body may be invaded, however, although mainly in predisposed and immunocompromised patients. The geographical distribution of the dermatophytes largely depends on the species. Some species are ubiquitous, whereas others are present in delimited areas. Yet, owing to migrations and traveling, the geographical delimitation of these dermatophytes is becoming increasingly blurred and cosmopolitan. It is especially true for anthropophilic dermatophytes, which are host-specific; only rarely are animal infections caused by true anthropophilic dermatophytes found. Natural occurrence of infections in animals by *Trichophyton rubrum*, *Epidermophyton floccosum*, and others have been described (7,38,41,74,77), but infection with anthropophilic agents in experimental animals is difficult (63) and almost not reproducible. It has been possible to infect rabbits with *T. rubrum* (61) after exposure to irradiation or after castration, but the infection was not homogeneous in all animals.

Fujita and Matsuyama (19) were able to obtain superficial invasion of the upper two-thirds of the horny layer of the plantar part of the hind foot of guinea pigs after inoculation under occlusion with anthropophilic strains of *Trichophyton mentagrophytes*. No inflammatory response was recorded, and hyperkeratosis and desquamation were absent; the infection remained silent. The same authors also used zoophilic isolates of *T. mentagrophytes* and concluded that these strains were consistently more invasive and

spread more intensively, producing a strong inflammatory response, erythema, and formation of thick scales.

It is generally accepted therefore that zoophilic dermatophytes are more pathogenic to laboratory animals than anthropophilic strains. Some geophilic agents are also pathogenic in experimental infections, especially *Microsporum gypseum* and *M. nanum*. *Microsporum canis* or *T. mentagrophytes* are most often selected for animal models of dermatophytosis, and most studies are performed on the abraded or nonabraded skin of guinea pigs. (28,63,76).

The experimental infection of cattle by *T. verrucosum* resulted in a clearing phase after the inflammatory phase (42,43). The evolution of *M. nanum* infection on the pig skin also led to a spontaneous recovery (22). *Trichophyton mentagrophytes* var. *quinckeanum*, the agent of mouse favus, is highly pathogenic for the mouse; but on the abraded and nonabraded skin of mice, resolution of the disease occurred within 2 to 3 weeks, producing scutulum and inflammation in the stratum corneum (30,65). *Trichophyton mentagrophytes* var. *quinckeanum* was also pathogenic in inbred strains of mice: The most sensitive were BALB/K mice (30).

The infection of *T. verrucosum* on guinea pig skin was successful and produced the typical evolution of inflammation, as observed with other dermatophytes (40,46). A guinea pig skin graft on athymic mice infected with *T. mentagrophytes* underwent the same evolution. Yet an acute and a chronic phase did not spread to the mouse skin (27). In guinea pigs, there was no difference between the occluded and the nonoccluded *T. mentagrophytes* infection skin (39).

If corticoids or methotrexate were used or if germ-free animals were infected, the infected was not prolonged (26,31). Delayed hypersensitivity, a specific type of cell-mediated immunity, correlated well with the onset of intense inflammation, limitation of surface spread at inoculated sites, and ultimate rejection of infection. Acquisition of specific delayed hypersensitivity was seen in conjunction with enhanced resistance to reinfection (30,31,35,36,43,46).

Guinea pigs infected with *M. canis* or *T. mentagrophytes* on scarified skin using homogenized infected hairs and scales taken from infected guinea pigs demonstrate irregular skin infections. Moreover, it is difficult to obtain adequate quantities for a large series of animals. The most regular infections are obtained with cultures growing at 25°C for 12–14 days on Sabouraud glucose agar in tubes with low glucose content (1% or 2%). For some slow-developing dermatophytes, 21 days of incubation are needed (64,80). After incubation, the aerial sporulated mycelium is removed and suspended in saline or in a mixture of bees' honey and saline (50:50). A 1-ml aliquot is used for each tube, a quantity sufficient for inoculation of two guinea pigs. Standardization of the inoculum is obtained by pooling the collected material of at least 20 tubes and homogenizing it in an ultra-

turrax (20,000 rpm) for 15–30 seconds. Diluted bees' honey is preferred over pure saline; the inoculum mats better on the scarified skin (although eventually on nonscarified skin), and germination of the conidia is faster. An inoculum of *T. verrucosum* grown at 37°C is moderately more pathogenic then an inoculum produced at 25°C in the guinea pig. It may be due to the more prolific development of the fungus and to a profuse production of conidia, especially chlamydoconidia and aleurioconidia.

Large series of nonpredisposed Swiss mice, Wistar rats, and Fisher rats were infected on clipped abraded and nonabraded skin. The infection on the abraded skin was more regular, but the course of the infection was not as extensive as in guinea pigs. Moreover, both mouse and rat often licked the inoculum, the infection site, and the topically applied preparations. They also scratched the itching lesions, producing satellite lesions.

The rabbit is more sensitive but presents the same disadvantages as the mouse and rat. The higher cost of the animal, its maintenance, and especially the larger amounts of test compound needed for treatment make its use in great numbers unpractical. It is, however, the most sensitive animal for *T. schoenleinii*.

Infection on the comb of cocks can be reasonably obtained with *M. canis*, *T. mentagrophytes*, and *M. gallinae* after scarification, but topical treatment is difficult. If a fluid excipient is used it does not adhere sufficiently, and if a viscous cream is applied the chicken feed forms crusts on the inoculated part.

Infection on scarified skin of dermatophyte-free dogs is uniform, but the dogs have to be muzzled in order to avoid licking. Overall, mongrels seem to be more sensitive than beagles. We found no difference in pathogenicity among *M. canis*, *M. gypseum*, and *T. mentagrophytes* for dogs. Various sites can be inoculated over a large skin surface area (Fig. 1-1), but dissemination and interference make the use of such a model questionable.

We have also inoculated calves with *T. verrucosum* on skin that was either scarified or unscarified. Natural infections were used as controls and persisted longer than the experimental ones.

The albino guinea pig is sensitive to dermatophyte infections with zoopathogenic strains and theoretically may be infected at various sites (19). In this case the animals rub against the walls of the cage; and therefore when different inocula or different medications are applied, they may become mixed. In addition, if various medications are applied, percutaneous absorption may also occur for some substances, and so interference cannot be excluded.

Scarification or abrasion of the clipped skin provides the most uniform and consistent infection (Figs. 1-2 and 1-3). Depilation with sodium sulfide (36 g/100 ml of water) applied for 30 seconds is also useful. A slight abrasion of the skin is obtained, and this method is interesting if occlusive dressing with polyethylene film and sealing with adhesive tape is used. We have compared occluded with nonoccluded skin of guinea pigs infected with *M.*

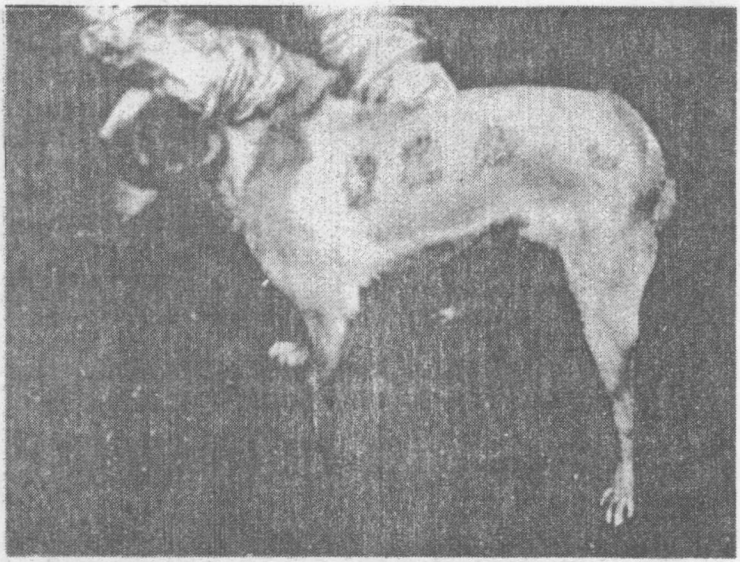

FIG. 1-1. *Microsporum gypseum* infection on the scarified skin of the dog, 14 days after infection.

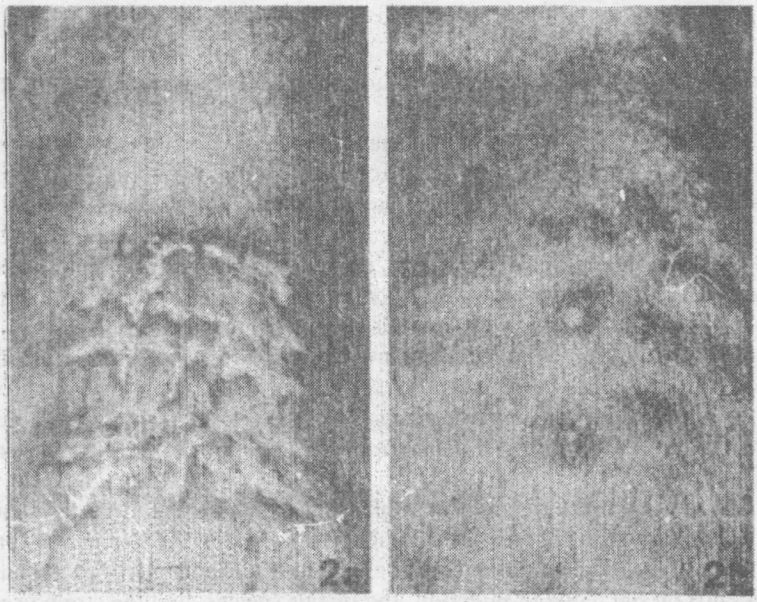

FIG. 1-2. *Trichophyton mentagrophytes* infection on the skin of the guinea pig, 7 days after infection. (a) Scarified skin. (b) Intact skin.

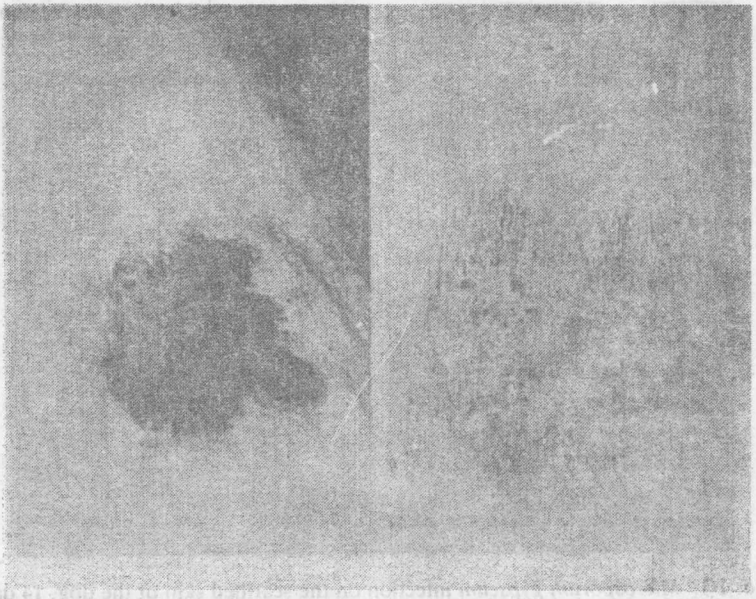


FIG. 1-3. *Microsporum canis* infection on the scarified skin of the guinea pig, 21 days after infection. (a) Inflammatory lesion. (b) Noninflammatory lesion.

*canis* or *T. mentagrophytes*, and major differences in the evolution of the infection were noted.

In other experiments we administered either hydrocortisone acetate or prednisolone acetate daily by the intramuscular route at 10 $mg \cdot kg^{-1}$ from day −7 to day +7; metronidazole daily by the intraperitoneal route at 20 $mg \cdot kg^{-1}$ from day −7 to day +7; alloxan once intramuscularly at 200 $mg \cdot kg^{-1}$ 24 hours before infection; chloramphenicol at 50 $mg \cdot kg^{-1}$ and streptomycin at 40 $mg \cdot kg^{-1}$ orally on alternate days from day −7 to day +14, combined or not with prednisolone acetate intramuscularly at 10 $mg \cdot kg^{-1}$ from day −7; or four subcutaneous injections of estradiol undecylate in female guinea pigs at 2 $mg \cdot kg^{-1}$ weekly, starting 1 week before infection. None of these predisposing factors was able to modify the normal course of *M. canis* or *T. mentagrophytes* infections of the scarified skin of guinea pigs.

The normal evolution of dermatophytosis in the guinea pig may be clearly divided into four phases (35): incubation, spreading, inflammation, and healing or clearing extending from days 25 to 60. The length of each phase depends on the mode of infection, the fungus species, and the strain used. In our experiments, after a second inoculation the onset of the infection was more irregular (occurring 1–2 days earlier), the inflammation was mild-