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Pathogenicity of *Dermatophilus* and *Geodermatophilus*

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Cutaneous infection in laboratory animals could not be induced with any of several strains of *Geodermatophilus*. A model for consistent production of streptotrichosis in rabbits, with cultures of *Dermatophilus congolensis*, is presented.

Luedemann (5) described a group of soil-derived dematiaceous actinomycetes resembling, in their complex life cycle, the species *Dermatophilus congolensis* (3), to which he applied the generic term *Geodermatophilus*. Because of the striking morphological and ontogenic affinities of the two genera and in view of the known pathogenicity of *D. congolensis* (2, 7), which occurs in nature as an obligate parasite or commensal of animals, including man, the question of the possible pathogenicity of *Geodermatophilus* arose. Luedemann provided us with several strains of *Geodermatophilus* for comparison with our own collection of isolates of *D. congolensis*, and the present account describes efforts to induce disease experimentally in laboratory animals.

MATERIALS AND METHODS

The *Geodermatophilus* isolates employed for pathogenicity studies were *G. obscurus*, strains G-7 and G-9; *G. obscurus* subspecies *amargosae*, strain G-12 (ATCC 25081); *G. obscurus* subspecies *utahensis*, strain G-17 (ATCC 25079); and undescribed species G-4 (pink colony) and G-23 (orange colony). These were compared with *D. congolensis* strains Deer (ATCC 14638), A-21, and 461, isolated from skin lesions of deer, sheep, and squirrel, respectively (3, 8).

Cultures of *D. congolensis* were maintained on Difco Brain Heart Infusion (BHI) agar, and inocula for pathogenicity studies were grown either in beef infusion broth (F5A) or on slants of YDC-NZ agar, as described by Luedemann (5), consisting of 0.5% yeast extract (Difco), 0.5% NZ Amine type A (Sheffield Chemical Co., Norwich, N.Y.), 1% dextrose, 1% soluble starch (Difco), 0.1% $CaCO_3$, and 1.5% agar (Difco). Cultures of *Geodermatophilus* were grown and maintained on the YDC-NZ medium. Suspensions of the microorganisms were plated for purity and viability immediately preceding their use for inoculation of animals.

All experimental animals were bred and raised at the Division of Laboratories and Research. Guinea pigs were females of mixed color weighing approximately 300 g; mice were 20-g females of the NYLAR strain; rabbits were of mixed color and of both sexes and weighed approximately 2 kg (1,600 to 2,500 g).

For direct microscopic examination of lesions, the roofs of pustules formed at 48 hr after inoculation were removed with a sharp scalpel and used for the preparation of smears and cultures. Additional tissue was biopsied, fixed in 10% formalin, embedded in paraffin, and sectioned by routine techniques. Stains applied were hematoxylin and eosin, Giemsa, periodic acid-Schiff, and Grocott methenamine-silver (GMS).

RESULTS

Attempts to infect guinea pigs. The Deer strain of *D. congolensis* and strains G-4 (pale orange, turning dark grey), G-12 (black), and G-23 (bright orange) of *Geodermatophilus* were cultured for 5 days at 37 C on slants of YDC-NZ, and homogeneous suspensions prepared in F5A broth were adjusted to an optical density (OD) of 0.12 in a Coleman Jr. spectrophotometer at 650 nm. The animals' hair on both flanks was shorn over an area of about 2 by 3 cm with electric clippers. A pair of animals was used for each microbial suspension. The shorn area on the left side was inoculated intradermally with 0.1 ml of the respective suspensions; that on the right side was scarified with 50 pricks of a 20-gauge needle, after which a gauze pad (4 by 4 inch) was soaked in the suspension of the microorganism and used to rub the material into this scarified area. Aside from a small, transitory, slightly erythematous nodule that appeared at the site of intradermal inoculation on some of the animals, no lesions developed and it was judged that no infection had taken place with the organisms applied.

Attempts to infect mice. *D. congolensis* strains Deer, A-21, and 461 were grown for 3 days at 37 C in F5A broth. For each strain, the entire growth (sediment) from two tubes was distributed among six mice by the following method. The mice were shaved over an area of 1.5 by 2 cm on the right flank, and the skin was scarified by six long scratches with a 20-gauge needle. The culture sediment was dropped onto the site and rubbed in with a gloved finger. A scarified but uninoculated area on the left side served as a control. No

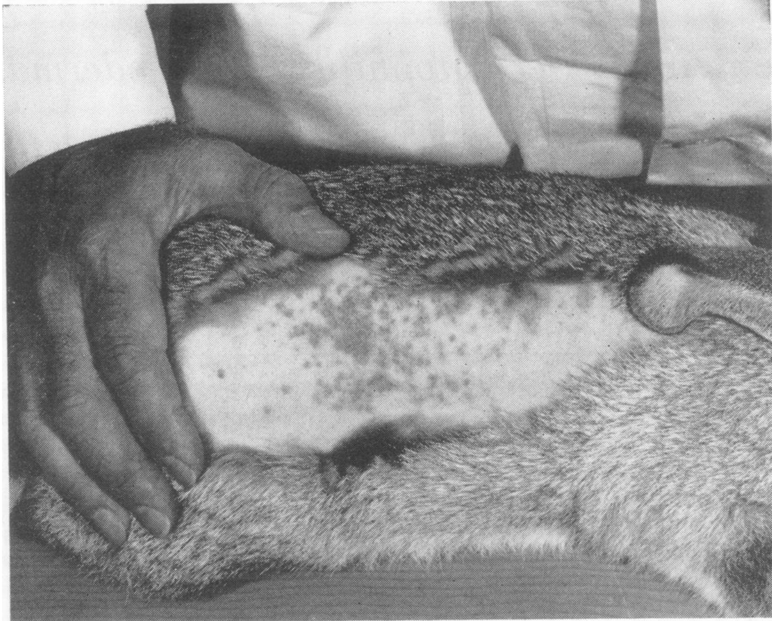

FIG. 1.

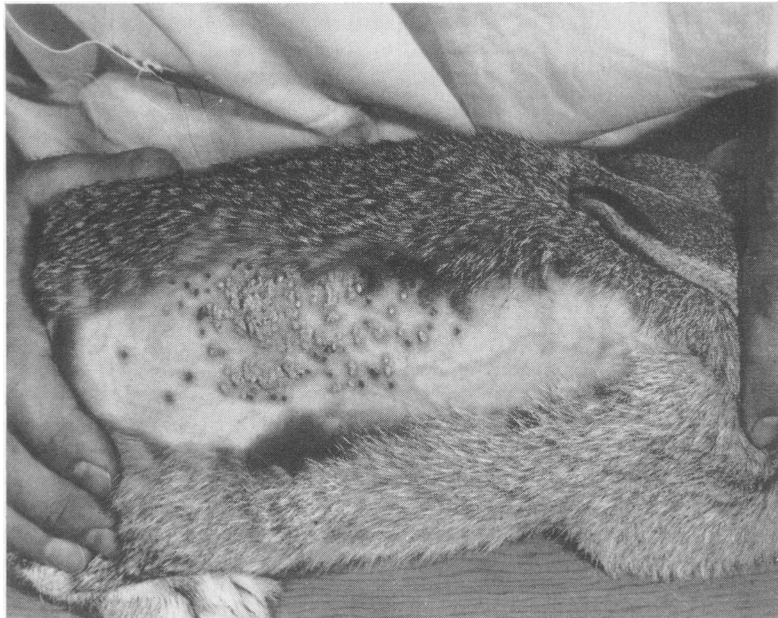

FIG. 2.

FIG. 1 and 2. *Streptotrichosis of rabbit induced with strain A-21 of Dermatophilus congolensis, 2 and 5 days postinoculation, respectively.*

lesions developed during the observation period of 5 weeks.

Infection in rabbits. Streptotrichosis with lesions of characteristic gross appearance and histopathology was consistently induced in rabbits by application of *D. congolensis* strains A-21 (four replications), Deer, and 461. A pair of rabbits was employed in each trial, including each of the four attempts with A-21. The animals' hair was clipped over an area of approximately 8 by 10

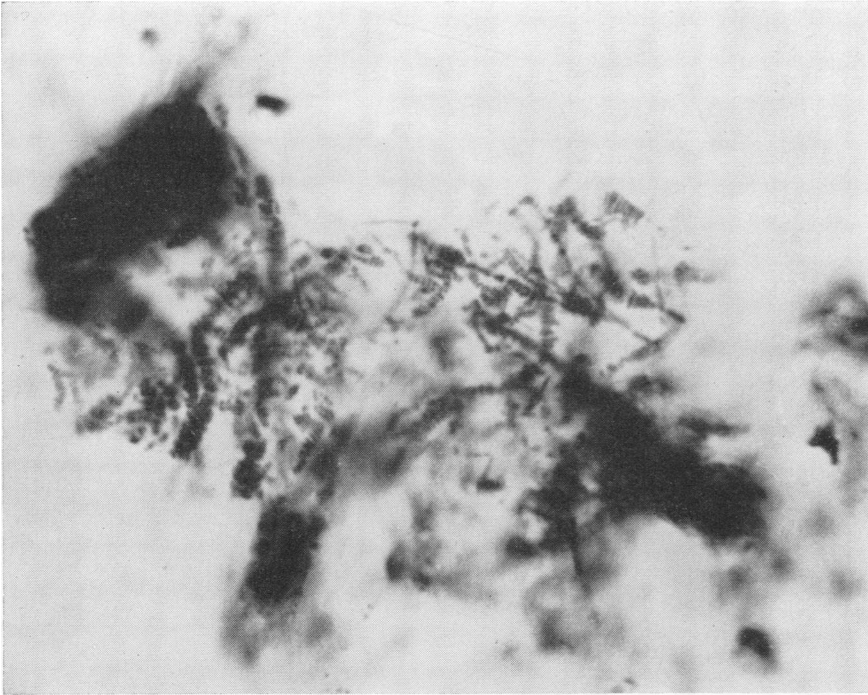

FIG. 3. *Smear prepared from the underside of crust of experimental streptotrichosis of rabbit, 5 days post-inoculation; various stages in life cycle of the microorganism. Giemsa stain, photographed through green filter.* × *908.*

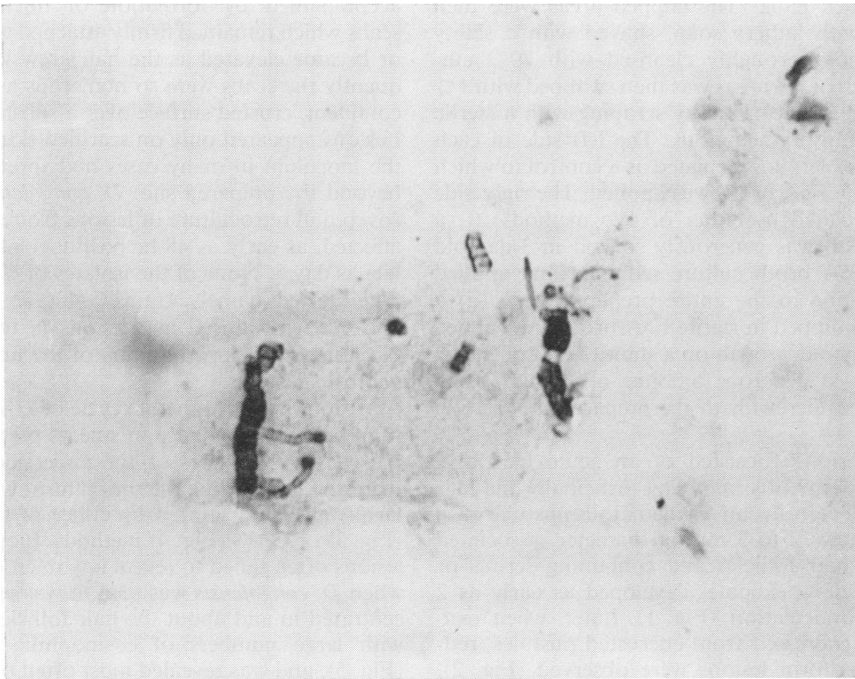

FIG. 4. *Smear from the same source as in Fig. 3 but stained with Grocott methenamine-silver and photographed through red filter.* × *908.*

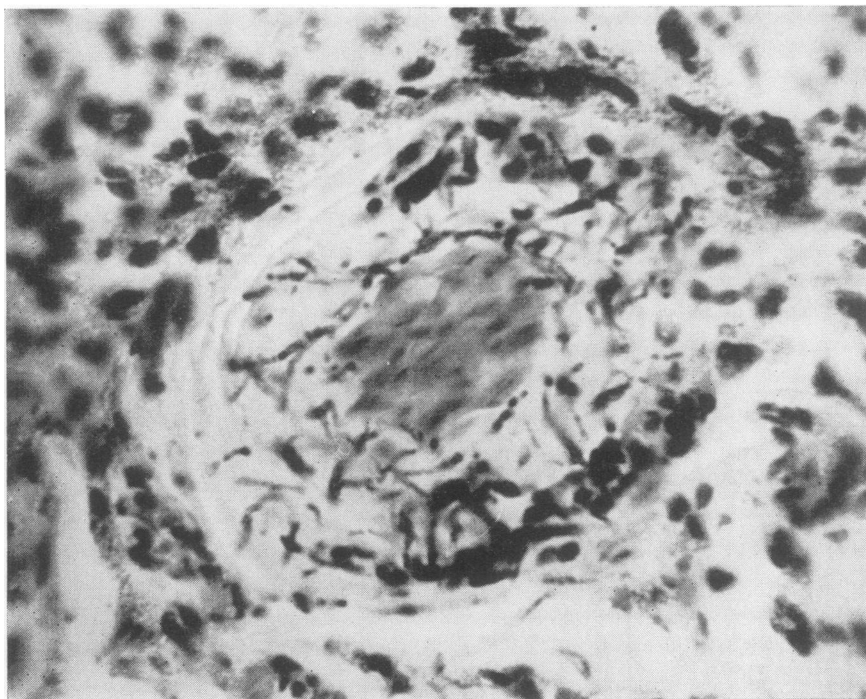

FIG. 5. *Biopsy section of a hair follicle from an experimental rabbit, showing numerous segmenting forms of Dermatophilus congolensis and a heavy concentration of eosinophilic leukocytes. Giemsa stain.* × *908.*

cm on each flank. The clipped areas were then washed with lathery soap, shaved with a safety razor, and thoroughly cleansed with 70% ethanol. Each of the areas was then swabbed with 1% Tween 80 and scarified by scraping with a sterile scalpel until erythematous. The left side of each animal was left uninoculated as a control to which only sterile F5A broth was applied. The right side was inoculated by either of two methods: (i) a sterile swab was generously soaked in 3-day-old (37 C) F5A broth culture sediment and applied with rubbing to the entire prepared area; (ii) a swab was dipped in sterile F5A broth and rubbed over 3-day-old growth on a slant (37 C) of YDC-NZ agar, a generous amount of growth being transferred therewith to the prepared inoculation site.

Skin lesions consisted of an acute ulcerative pustular dermatitis involving principally the hair follicles. Typically, an erythematous pustular rash with pustules 2 to 3 mm in diameter, associated with the hair follicles and containing serous or whitish yellow exudate, developed as early as 2 days postinoculation (Fig. 1). Later, when exudate was expressed from encrusted pustules, reddish crateriform lesions were observed (Fig. 2). In most cases, healing had begun after about 1 week and was complete in about 2 weeks. It was accompanied by formation of thick yellowish scabs which remained firmly attached to the lesion or became elevated as the hair grew longer. Frequently the scabs were so numerous as to form a confluent, crusted surface over a substantial area. Lesions appeared only on scarified skin, although the inoculum in many cases had spread to areas beyond the prepared site. *D. congolensis* was recovered in retroculture of lesions from all animals affected, as early as 48 hr postinoculation and as late as day 8. None of the isolates of *Geodermatophilus* tested, namely strains G-4, G-7, G-9, G-17, and G-23, produced any lesions in the animals. No lesions developed on any of the uninoculated control sites.

Various stages of the life cycle of *D. congolensis* were seen in abundance in smears prepared from the roofs of pustules or the underside of crusts from the lesions; the microorganism was particularly well demonstrated by either of the Giemsa (Fig. 3) or GMS (Fig. 4) methods. Biopsies of the lesions often failed to reveal any microorganisms; when *D. congolensis* was seen, it was usually concentrated in and about the hair follicles, together with large numbers of eosinophilic leukocytes (Fig. 5), and was revealed most often by means of the Giemsa stain. Pier, Neal, and Cysewski (6) found, on the contrary, that "while the organism

was easily located in Giemsa's-stained tissue specimens of the skin biopsy, particularly in the outer regions of the exudate, considerable diligence was necessary in searching out definitive forms in direct smears. Young active lesions proved most rewarding."

DISCUSSION

In contrast to the regularity with which lesions of streptotrichosis developed when properly prepared rabbit skin was exposed to *D. congolensis*, in no case were lesions induced by application of any of various strains of *Geodermatophilus*. The method of inoculation employed in these studies appears to offer a useful model for consistent production of streptotrichosis in experimental animals and gives rise to lesions characteristic of those found in natural infection. Wetting the skin surface by means of Tween 80 appeared to facilitate the infection. According to Roberts (7), *D. congolensis* may be unique as an invader confined to the living epidermis, attacking neither the keratin of the stratum corneum and hair or wool nor the dermis proper. As contrasted with this species, which is either a parasite or commensal of animals, *Geodermatophilus* is apparently a soil inhabitant. Luedemann (5) obtained a number of strains of *Geodermatophilus* from varied soil habitats, including desert areas, and Ishiguro and Wolfe (4) isolated them from high-altitude Mount Everest soil samples. Ahrens and Moll (1) cultured, from salt water of the Baltic Sea, a microorganism apparently identical with *Geodermatophilus* for which they proposed the name *Blastococcus aggregatus*. Relevant to the difference in pathogenicity between *Dermatophilus* and *Geodermatophilus* are the observations that the latter grows at 37 C at a reduced rate from its optimum at 24 to 28 C (5), whereas the former grows better at 37 C than at 27 C (3).

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