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Experimental Dermatophyte Infection. The Extent of the Fungal Invasion

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A spore suspension from a granular strain of *Trichophyton mentagrophytes* under occlusion for 4 days was used to produce two fungal lesions on the upper arm of the Trichophytin-negative author. The material for culture was obtained by stripping across the visible lesions and several centimetres into the surrounding skin. Already when the occlusion was removed, the whole stratum corneum was heavily invaded up to 20 mm into the surrounding, normal-looking skin. When the intensity and the size of the visible lesions topped after about 2 weeks, the culture positivity reached 45 mm into the perilesional skin. One week later the spontaneous involution had eliminated nearly all fungal organisms through the entire horny layer of both the visible lesions and their surroundings. After 42 days the lesions were culture-negative. It was concluded that the growth pattern of an experimental infection could be as observed in natural infections. *Key words: Superficial fungal infections; Delayed hypersensitivity; Experimental dermatology.*

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According to the literature (1–4), artificially produced dermatophytosis is comparable to the natural infection in all essential respects. In a natural dermatophyte infection the mycelia invade the stratum corneum (5, 6) and its horizontal extent reaches several centimetres outside the peripheral demarcation of the visible changes (6).

In the present study, stratum corneum strippings were cultured to investigate whether a similar invasion, including the perilesional extension, could be observed during a primary experimental infection.

MATERIAL AND METHODS

The experimentally infected person (the author) had no previous dermatophytic infection and was Trichophytin-negative.

Preparation of inoculum

1/4 ml of material from a 3-week-old culture of a granular strain of *Trichophyton mentagrophytes* (isolated from lesions in a man infected by a guinea pig) was suspended in 2 ml of sterile 0.9% NaCl, homogenized by shaking with glass beads, filtered through Whatman 43 paper to remove mycelia and resuspended in 0.9% saline with chloramphenicol 100 mg/100 ml, adjusted to contain 600 spores/ml by haemocytometer count.

Inoculation of test sites

Two circular fields, 1 and 2, diameter 30 mm, were delineated on the inside of the right upper arm, 5 cm apart. Without any prior preparation of the skin, two drops (0.07 ml) of the spore suspension, containing about 40 spores, were applied to each field, which then were covered with 30 mm pieces of sterile gauze moistened with three drops of sterile water and occluded with Steridrape and Durapore (from 3M, Minnesota Mining & Manufacturing Co., Los Angeles, Calif., USA) for 4 days.

The size of the visible infection during the period of observation was based on the shortest and longest diameter for each field and determined as the average diameter of the two lesions. The severity of the visible infection was graded at the same time as follows: + (erythema), ++ (erythema and edema), +++ (erythema, edema and vesiculation).

Trichophytin test: 0.1 ml of a commercial product (Dermatophytin 1 : 30, Hollister-Stier, USA) on the upper arm, read after 72 h.

Culture study

The material was obtained by stripping with a 1 cm wide vinyl tape (Scotch tape 681, 3M) perpendicular to the arm across the middle of the visible lesions, outlined with a ballpoint pen, and some centimetres into the surrounding normal-looking skin.

On days 0 and 13 (Fig. 1) the strippings were taken from field 1, on days 7 and 20 from field 2. Stripping was repeated in the same track until the surface appeared glossy after 12–14 strippings. The three most superficial, three from the middle and the last three of the strippings were kept for culturing.

On days 25, 35 and 42, culture material was obtained from both fields. Only the three most superficial strippings were taken and used from each of the fields. The pieces of tape were placed, adhesive side down, on the surface of Sabouraud glucose agar containing chloramphenicol (40 mg/l) and cycloheximide (500 mg/l). The plates were incubated at 26°C and

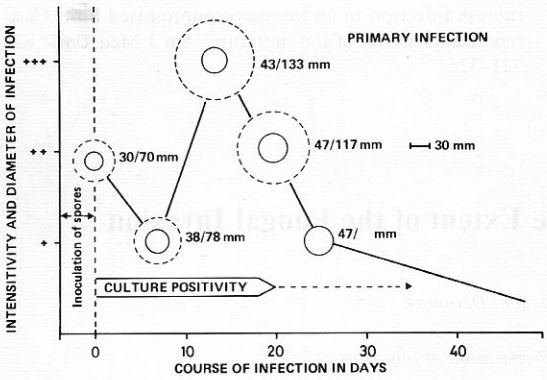

Fig. 1. The unbroken circles illustrate the visible fungal lesion, the broken circles the whole culture-positive area. The dotted horizontal line marks the growth of very few colonies from day 20 until the fields became negative on day 40. The declining line after day 25 indicates that the lesions became increasingly pale and diffuse.

examined for growth for up to 3 weeks. The estimation of the average diameter of the culture positive area was based on measurement of the distance in mm between the edge of the visible lesion and the centre of the most peripheral colony which appeared along the tapes (Fig. 2).

RESULTS

Diary

Day 0: When the occlusions were removed, the fields appeared red, with a pattern very much like a plaster reaction. Culture from field 1 gave growth from the superficial, the middle and the deepest part of the horny layer after 2 days from the visible lesion and up to 20 mm into the surrounding, normal-looking skin (Fig. 1).

Days 2–6: The follicular character of the infection was replaced by a slight but continuous erythema. Periotic itching had started.

Day 7: The lesions had increased a little in size and field 2 was culture-positive at least (the pieces of tape were no longer) 20 mm (78–38/2) peripheral to the new borderline after 3 days. The Trichophytin skin test proved positive: 10 mm.

Day 11: One to two small satellite lesions were observed a few mm from the borderline of fields 1 and 2, which they rapidly merged with.

Days 13–14: The intensity of the infection culminated, a 10 mm satellite lesion appeared in the right fossa cubiti, about 7 cm apart from field 2, and the culture

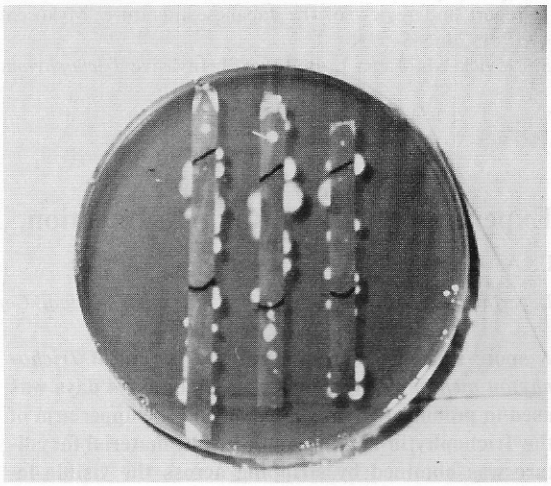

Fig. 2. Experimental infection with *Trichophyton mentagrophytes*. Culture on Sabouraud glucose agar from strips 6, 7 and 8, showing colonies up to 45 mm outside the ballpoint pen markings of the visible infection.

positivity through the entire horny layer reached 45 mm into the normal-looking skin of field 1 (Fig. 2).

Day 16: Slightly decreased inflammation.

Day 20: Central healing has started. Visible lesions had reached their maximum size of 47 mm. The culture positivity still reached 35 mm peripheral to the visible lesion of field 2, but only tapes 3, 9, 11 and 12 were culture-positive, with just one colony from each. Growth appeared after 7–9 days.

Day 25: More pronounced central healing and the fading erythema showed a follicular pattern. Growth of one colony from each field. No growth now from the surrounding, normal-looking skin.

Day 35: Growth of one colony from field 1, none from field 2 and the satellite.

Day 42: The three strips taken were culture-negative, and the skin normalized slowly during the following weeks.

DISCUSSION

The conclusion drawn from the investigation is that the growth pattern of an experimental infection can be the same as in natural infections (6).

The observed fast killing of nearly all fungal organisms at all three levels of stripping and the regression of the mother lesions at the same time as the much

younger satellite lesion on the elbow, make it more likely that the elimination of the infection is due to some direct chemical action rather than an effect of increased turnover rate or destruction of stratum corneum as a substrate, discussed as possible reasons for the spontaneous healing (1, 2, 7).

The investigation concerns only one person. The maximum size of an experimental infection, including the perilesional invasion of the horny layer observed in this study, will certainly differ from person to person, depending on the rapidity with which delayed sensitivity develops.

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Cyclosporin A Does Not Modify Langerhans' Cell Number and Distribution in Normal Human Skin

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We used the model of human skin graft on nude mouse to investigate a possible influence of systemically administered cyclosporin A (CsA) on epidermal Langerhans' cells (LC). This experimental model has the advantage of being independent of the systemic humoral and cellular influences of a human host. No change in the human LC distribution or number could be observed after 3 weeks of CsA therapy as revealed by anti-CD1 and anti-HLA-DR immunohistochemical stainings and the cell counting, despite the evidence of the drug effect on the keratinocyte proliferation. However, our findings do not rule out the possibility that CsA influences the LC functional capacities responsible for the local cell-mediated immunity responses. *Key words: Langerhans' cell counting; 'Nude' mouse; Skin graft.*

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Cyclosporin A (CsA) is an immunosuppressive agent which has been demonstrated to inhibit T-lymphocyte-mediated immune reactions (1). A recent report indicated that topically applied CsA inhibited a local contact hypersensitivity to nickel (2). Since this type of immune response is known to be mediated by Langerhans' cells (LC), the question of CsA influence on the LC population has been raised (2–4). The results of LC quantitation after topical application of CsA were rather contradictory. No influence of the drug on the number of LC could be observed in the human patch-test experiments (2), although a significant reduction of ATPase-positive dendritic cells in CsA-treated mice has been reported (3).

Systemically administered CsA did not modify LC number in mice (3, 4) nor in CsA/prednisone immunosuppressed renal transplant patients (5). The turnover of epidermal LC (and also their number) is maintained in normal conditions by recruitment of circulating LC precursors from the monocyte–macrophage lineage and, to a lesser degree, by an *in situ* slow-rate LC division (6, 7). Since the systemic phenomena may influence the epidermal LC number, we used a model of human skin graft to nude mice, which is free of the human systemic control mechanisms, for studies on the direct effect of CsA on human epidermal LC distribution and density.

MATERIALS AND METHODS

Normal human skin was obtained from mammoplasty and whole thickness grafts (approximately 1 cm^2) were performed