PATHOPHYSIOLOGY OF RINGWORM INFECTIONS IN ANIMALS WITH SKIN CYCLES*

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The mouse has the reputation of being resistant to experimental ringworm infection. This is a paradox for in times past spontaneous infections have often occurred in this animal. The purpose of this paper is to demonstrate that ringworm infections can be consistently produced in mice and rats by taking into account the cyclical peculiarities of the skin of these animals.

As a background to this research, I have principally drawn on Buchanan's (1) paper for the references concerned with spontaneous ringworm infection, whereas I shall explicitly cite those having to do with experimental inoculation.

The modern student tends to disregard the mouse as an object of experimental ringworm research for this animal is scarcely mentioned in the recent literature, and with the passage of time, awareness of the commoness of natural infection years ago has dimmed to the vanishing point. Mouse ringworm was first recorded in England in 1850 (1). It was immediately dubbed "mouse favus", appropriately so for there was a striking resemblance to human favus, a disease of antiquity. Typical favus crusts or scutula, composed of masses of hyphae, formed mainly on the head and sometimes on other relatively non-hairy sites such as the extremities and tail, and occasionally on the body. These excrescences sometimes developed hugely, more or less enveloping the head, creating the grotesque appearance likewise seen in humans when favus scutula luxuriate on the scalp. Subsequently, the disease was found to be rampant among mice in dwellings and workhouses in many metropolitan areas of Europe. Humans and domestic animals such as cats and dogs sometimes contracted the disease from infected mice. Transmission to humans was proved experimentally. The disease gained the reputation of being lethal for afflicted animals often died. Doubtlessly the virulence of the fungus was exaggerated. There were even claims that the parasite caused death by penetrating through the skull into the brain and infections could even result in amputation of the extremities. The modern view is that the dermatophytes are limited to the stratum corneum and hair which are dead structures; hence, death must have been an indirect result, perhaps from the interference with vision, nutrition and mobility consequent upon the fungus accretions on the head. Mice, of course, are subject to intercurrent diseases which might have ravished them as well as increasing their susceptibility to the fungus infection. Also it is well known that sick mice may be cannibalized by their not so fastidious brothers: captured mice were observed to nibble on each other's crusts.

Logically enough, some thought that human and mouse favus were caused by the same parasite, namely *Achorion schoenleini*. Indeed, humans who contracted the disease from mice often developed, on the glabrous skin at least, typical favus scutulae. Though he did not achieve definitive classification of the organisms, Quincke (2) was the first to recognize differences, touching off a strenuous dispute which flourished for decades owing to the lack of proper taxonomic standards. But when Bodin (3), an eminent student, endorsed Quincke's view, the argument was weighted in favor of a distinctive organism for mouse favus, which in honor of Quincke's contributions was called *Achorion quinckeanum*. It was

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with this particular parasite that Bloch performed his beautiful guinea pig experiments from which so much of our modern knowledge of the pathology and immunology of ringworm infections has been derived. It was not obvious at first that different organisms could incite the same clinical picture. Sabouraud (4) never isolated any organism other than *A. schoenleini* from hundreds of cases of human favus; this was in conflict with the findings of Bloch (1) whose experience showed *A. quinckeanum* to be the predominant organism. Both were actually right. Sabouraud was dealing with scalp favus which is practically always due to *A. schoenleini* and Bloch's contention was based on glabrous skin favus which can indeed by caused by *A. quinckeanum* contracted directly from mice. This latter parasite could produce two types of lesions: (*1*) typical favus with the pathognomonic scutula, *but only on the glabrous skin* (this response is, incidentally, rare nowadays, but Van Breuseghem (5) has induced it in humans using scales from a mouse naturally infected with *A. quinckeanum*), and (*2*) ordinary eczematous patches of circinate ringworm. *A. schoenleini* infections in mice have been observed only under the most exceptional circumstances. Sabouraud (6) reported the case of a colleague who had had a hedgehog put into a country house to hunt down favic mice but had unwittingly caught the disease from the intended victims. In this case, the organsim proved to be *A. schoenleini* on culture. (A splendid photograph of this hedgehog, and one of the favic mice, appears in Sabouraud's classic, Les Teignes, on page 547.) No less curious is Sabraze's (7) extraordinary tale of the successful inoculation of a mouse with *A. schoenleini*; this particular mouse escaped and transmitted the infection to other mice of the household from whom the organism was duly recovered. Despite these exotic experiences, the usual organism of mouse favus is quite clearly *A. quinckeanum*. At this juncture it is necessary to remark that the species of the genus Achorion have been redistributed to the genus Trichophyton (8) and that *T.* (Achorion) *quinckeanum* has been reduced to synonomy with *T. mentagrophytes* (9), the dermatophyte so often the cause of athlete's foot. The morphologic plasticity of this latter species is sufficiently great that many of its variants were formerly regarded as species, enormously overcomplicating the taxonomy of the dermatophytes.

A few decades ago the most intense efforts were devoted to producing ringworm infection in laboratory animals. In Les Teignes, Sabouraud (6), describing experimental infections with the various dermatophytes in his usual thorough manner, scarcely ever mentions mice, so fruitless were most studies with this particular animal. Similarly, Lucille Georg (10), the most eminent taxonomist of the dermatophytes, in her recent review on the diagnosis of ringworm infections in animals, practically ignores the mouse and its deserved fame as the bearer of "favus". This is all the more noteworthy, since a veritable legion of animals, including cows, dogs, opposums, foxes, rabbits, chinchillas, etc., have acquired *T. mentagrophytes* infections spontaneously. Similarly, Gordon's (11) review of veterinary mycology is disrespectful of the mouse's past glory. Tomasczewski (12) gives a thorough account of the efforts to infect mice with *T. mentagrophytes* and *T. schoenleini*, recording a few erratic successes by previous investigators. He concluded that while *T. mentagrophytes* is clearly the organism of mouse favus, inoculation of that animal usually fails. But the matter cannot be dismissed so easily for we are obliged to explain why Bodin readily infected each of six inoculated mice and Sabouraud (4), using the same culture, failed altogether.

Mouse favus is almost unknown at present, the most recent report being that of Van Breuseghem (5); but while he could transmit the disease to a human, he was unsuccessful in inoculating the organism (*T. mentagrophytes*) back into mice. In Australia, there have been epidemics of favus-type ringworm among wild mice which was said to have reduced their numbers considerably (13, 14, 15). Workers who handled stored wheat contaminated by the mice contracted the disease, usually showing circinate eczematous patches of ringworm, not favus.

Spontaneous outbreaks have been observed among laboratory mice. Parish and Craddock (16) in 1931 observed an astounding incidence of 50% in 2500 breeding stock mice, but again we are confronted with the paradox of only 2 of 30 animals becoming infected

when inoculated; moreover, the experimental disease lacked the classical characteristics of favus. Reports subsequent to this time make it clear that mouse ringworm has tended to become a more benign disease, without the distinctive crusts and with sparing of life. Dubois (17) and also Catanei (18) encountered the disease in white mice; the former observed transmission to man. Booth (19) traced two laboratory epizootics to a commercial breeding house. In this most recent report of 1942 the disease appears to have reached the end of the road in its steady decline in severity, the clinical expression being reduced to some patchy alopecia and scaling, somewhat simulating non-inflammatory tinea capitis in man. I recently had the good fortune to observe several mice which arrived from the breeder with some localized patches of inflammatory dermatitis from which *T. mentagrophytes* was isolated. Healing occurred within 10 days. The rarity of true favus with its imposing crusts in "well bred" mice, especially the laboratory variety, probably reflects superior nutrition and better health, not a change in virulence of the parasite.

The course of the infection and the histopathologic changes are unknown. The most intriguing problem, however, is why the mouse is experimentally resistant and is susceptible in the wild. This question has now been answered, at least in part.

THE PHYSIOLOGY OF MOUSE AND RAT SKIN

An understanding of the cyclical characteristics of mouse and rat skin is the key to producing ringworm infections consistently. In these animals, physiologic and anatomic changes occur in a rhythmic and periodic manner. The most obvious evidence of this is the recurrent waves of hair growth which originate around the throat, sweeping posteriorly and dorsally from this region. The length of the cycle of any individual follicle of a young mouse or rat is about 30–35 days; for the first 17–20 days (anagen), the hair matrix is intensely active and the hair "grows". At the end of this time the follicle, in the remarkably short period of 2–3 days (catagen), transforms itself into a resting structure during which time the club or resting hair is produced. Thereafter for about the next 12–15 days (telogen), the follicle is inactive and there is no growth of hair. The dynamics of hair growth have been reviewed excellently by Chase (20); in his paper can be found a complete description of these events. An important distinguishing feature of mouse and rat skin is that the follicles in any given region are all in the same stage of the cycle.

Not only are the follicles synchronized with each other, but also with regular wave like changes in all the other component structures of the skin. A most remarkable waxing and waning of changes takes place in the thickness of the various layers of the skin, the functioning of the sebaceous glands, vascular supply, water content, oxygen consumption, etc., these coordinated transformations are better signified by the term "skin cycle", which Parnell (21) and ourselves (22) have used, rather than "hair cycles". Because hair growth is the most obvious manifestation of the skin cycle, it serves admirably as a marker of the stages. Representative animals that have skin cycles of this sort are mice, rats, hamsters and rabbits, while humans and guinea pigs do not. This difference perhaps provides a clue to the great susceptibility of the guinea pig to *T. mentagrophytes* infections.

It should come as no surprise, as we learn mostly through the illuminating

studies of Chase, Montagna and their associates (23, 24, 25) that in "skin cycle" animals the responses to various types of stimuli should be strongly influenced by the stage of the skin cycle. It will make a great difference whether the stresses or operations are performed during anagen or telogen. It is not too much to say that the result of any experimentation with mouse and rat skin which does not take into account the stage of the skin cycle must remain in question and, indeed, this has been the source of important errors in the past (26).

EXPERIMENTAL METHODS

New hair cycles may be induced at will by the plucking of telogen hairs (20); in this way, it is possible to prepare the skin so that one knows precisely the day of the cycle and the state of the skin at the time some procedure is performed. Two strains of mice were used: Bar Harbor C-57 black mice and Carworth Farms white mice (other strains of white mice were found equally suitable). In the C-57 strain, telogen can be recognized by inspection for the skin is white then and black at other times. With white mice telogen cannot be determined in this way; it was necessary to shave a small area on the back every day or two; when hair growth ceased, the proximate skin was obviously in telogen and then the hairs were plucked. The skin of the posterior back was used exclusively, an area about one inch square being plucked. Unless otherwise indicated, young mice 12–16 weeks of age were used. Age is a consequential factor for as the animals get older the cycles become longer, somewhat less regular, and the smooth, wavelike progression of the cycles may become so disrupted that irregular islands develop. The same figures as those given for the mouse hold for the first few cycles of young rats (27). Usually in both mice and rats hairs begin to emerge from the follicles on about the 12th day after plucking. To make the inoculations, a small piece of fungus-bearing agar was cut out of a fresh tube culture and smeared gently, *without excoriation*, over the prepared area. Excessive scraping and traumatization of the skin diminishes the opportunity for infection.

Experimental inoculation of mice with T. mentagrophytes

Over 200 young mice of the Bar Harbor C-57 variety were employed in these studies which took place over a period of two years at different seasons. The strain of T. mentagrophytes for the preliminary studies was one which was highly infective for guinea pigs. At least 3 animals were assigned to a group and inoculations were made on each day of the skin cycle beginning with the first day after plucking and extending through the 25th day. Subsequently, about 100 Carworth Farms white mice were inoculated at different stages of the cycle. At various subsequent times the results were checked by "spot" tests in both black and white mice.

Infection took place, with rare exceptions, only when the skin was inoculated between the 12th and 16th day of anagen. The white and black strains behaved similarly in every significant respect; in short, there were only 5 days out of a cycle of 30–35 days that the mice were susceptible. This was confirmed repeatedly with a high degree of reproducibility but it should be noted that an occasional

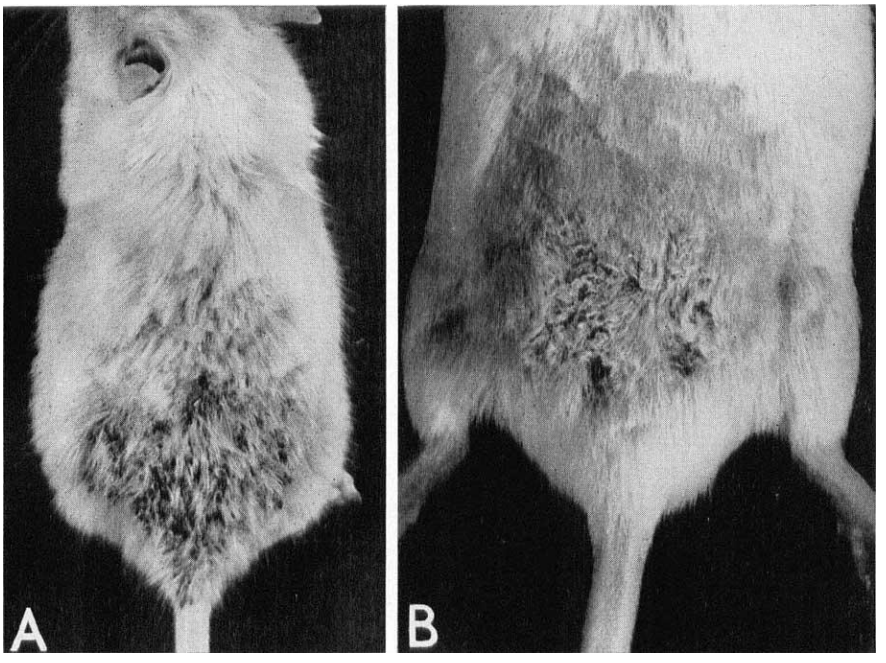

FIG. 1. Gross manifestations of mouse ringworm infection. A. Inflammatory crusted lesion 7 days after inoculation on the 12th day of anagen with Microsporum canis. The hair is matted together but there is no real alopecia. B. T. mentagrophytes infection 5 days after inoculation on 14th day of anagen. There is marked scaling and crusting but no significant hair loss.

animal failed to become infected; unaccountably, possibly because of vagaries in the skin cycle, infection failed in an occasional group which was thought to be in a susceptible period. The course of events following inoculation on the 12th day of anagen was as follows: within 2 to 3 days, a fine, diffuse scaling appeared followed by the rapid development of an inflammatory crust which matted the hairs together (Figure 1). The skin was distinctly infiltrated. When the crust, often quite massive but variable in degree, was forceably pulled off, the hairs came along with it en masse leaving the surface denuded. The peak inflammation was reached on about the 17th or 18th day. After a short stationary phase of 2–3 days, healing was definitely under way, generally being complete in about 5–9 days after climax, in proportion to the intensity of the original dermatitis. Occasionally, the mice exhibited only some moderate scaling without much crusting, but on the whole the infections were of a decidedly inflammatory nature.

It was obvious that the onset of telogen abruptly terminated the infection. No lesion ever advanced or continued to remain inflammatory after the beginning of telogen. Because the hairs were glued into the crust, there was generally no epilation until the crust disintegrated during healing and even then hair loss was usually not striking. With less inflammatory lesions hair loss was not even apparent. Breaking off of the hairs, a cardinal feature of ringworm in humans,

was absent or insignificant for the infection was too short lived for this, and, as we shall see, too few hairs were involved. In general then, alopecia was not a typical trait of these infections except as a result of marked inflammation, corresponding to a kerion in humans. The typical scutula of mouse favus, so characteristic of spontaneous infections among the mice of Europe, were never observed. Permanent hair loss was never observed in our mice, even when the inflammation was extreme. As a rule, the next cycle in a healed area began on time, synchronously with the surrounding area; although sometimes following intense inflammatory changes, the stimulus was sufficient to start a new cycle prematurely. No recurrence of an infection was ever seen. Reinoculation of either healed or previously uninvolved sites produced an infection indistinguishable from the primary one. From this it was concluded that no immunity was induced by the primary infection as in the guinea pig.

The onset of telogen obviously sets an upper limit to the duration of the active disease, which, of course, is longer when the inoculation is made on the 12th day of anagen, the earliest time, at which the animals become susceptible. Quite inflammatory lesions can, however, develop even when the inoculation is at the end of the susceptible period, the 16th day of anagen, leaving only a day or so until catagen (18–19th day) and about 2 more days until telogen. I gained the distinct impression that 16th day anagen infections developed more swiftly and reached the same degree of inflammation.

Mice were inoculated on the 12th day of anagen with four other strains of *T. mentagrophytes* of different degrees of virulence for guinea pigs. Infections resulted with all and more or less to the anticipated degree, the least virulent strain producing the weakest mouse infection and the others behaving proportionately. I had the opportunity to inoculate mice with a strain that had originally been isolated by Sabouraud many years ago, having been maintained on culture under the designation *Achorion quinckeanum*. The infection it produced in mice resembled in every detail, grossly and microscopically, that of one of the less virulent strains of *T. mentagrophytes*. Its identity with that species can hardly be doubted.

Histopathologic findings in T. mentagrophytes mouse infections

Serially sectioned biopsies were examined throughout all stages of the infection and in addition direct mounts were made in 10% KOH solution. The inflammatory reaction was far out of proportion to the number of fungus elements. This was true even in the early stages before the inflammatory upheaval could have sloughed off the parasite. An occasional KOH smear showed an abundance of hyphae in the stratum corneum but more often there were only a few segments to be found in the initial scaling stage and none later: mouse epidermis is thin and probably as soon as inflammation was well under way the parasite was desquamated. Similarly, the great majority of the follicles showed no sign of the fungus at any stage of the infection; the fungus simply failed to enter most follicles, an absolute prerequisite for hair invasion (28).

Morphologically, there are 4 different types of hairs in the furry coat or pelage

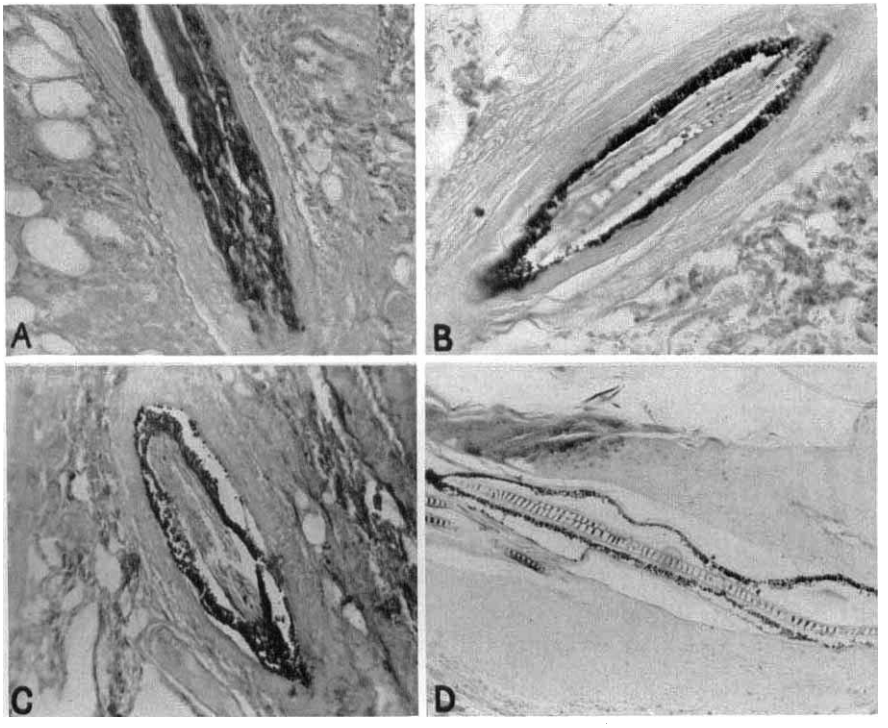

FIG. 2. Appearance of fungi in infected follicles. A. Seven day old T. tonsurans infection in mouse. This segment of hair happened to be non-medullated and a dense bundle of intrapilary hyphae, segmenting into chains of cells, has formed in the *endothrix* pattern characteristic of this organism. (Hotchkiss-McManus stain. Orig. mag. ×300). B. Six day old T. mentagrophytes infection in mouse. A few filaments are present in the cortex of the hair, none in the medulla. The *ectothrix* sheath of spores is highly developed. (Hotchkiss-McManus stain. orig. mag. ×340). C. Ectothrix pattern in 7 day old T. mentagrophytes infection of rat. The intrapilary hyphae are abundant because the hair is non-medullated. The hair has fractured owing to its dissolution by the parasite as in human infections. (Hotchkiss-McManus stain. orig. mag. ×225). D. T. mentagrophytes infection of rabbit. Note almost complete absence of intrapilary hyphae in contrast to highly developed ectothrix sheath of spores (Hotchkiss-McManus stain. orig. mag. ×160).

of the mouse (zigzags, auchenes, monotrichs and awls) (29). The fungus was never found in the follicles bearing zigzag hairs which make up the entire undercoat of hair. The largest follicles containing the longer and thicker overcoat hairs (monotrichs and awls) were clearly preferred although no exact counts were made. Intra-pilary hyphae were scanty (Figure 2B), not forming the dense fascicles characteristic of human hair infections; the parasite, for the most part, avoided the medulla which makes up the greatest bulk of mouse hair, confining itself to the thin outer cortex. Human scalp hair by contrast is mostly non-medullated and, therefore, can support a denser growth of the parasite. In some mouse hairs, however, the medulla is discontinuous and an abundance of hyphae sometimes developed in the non-medullated segments (Figure 2C). In the early

follicular stage of invasion, long filaments grew perpendicularly downwards on the hair surface within the follicular canal, often septating into chains of arthrospores. Sometimes there were rather voluminous chains of fungus cells in this extra-pilary location, crowding the follicular canal with spores; even in these follicles, however, the parasite grew scantily within the hairs, except in the non-medullated portions (Figure 2B). Not infrequently, the infection did not progress beyond the follicular stage of invasion so that filaments were present in the follicles but not in the hair shafts. In classical terms the morphology of the hair infection was of the *ectothrix type.* The parasite was able to penetrate the hair shaft right up to the time of catagen, when involution of the follicle begins. A few days after the onset of telogen, the fungus had all but disappeared, probably for the most part being expelled by the inflammatory reaction.

The trivial invasion of the hair shafts and the sparseness of the infected hairs accounted for the clinical absence of alopecia. In no case was the viable portion of th hair matrix involved nor did the internal root sheath, the cells of which never completely lose their nuclei in mice and rats, become invaded.

The microscopic signs of inflammation were striking (Figures 3A, B). Lymphocytes gathered in large numbers throughout the corium in the first 48 hours, to be joined by progressively increasing numbers of polymorphonuclear leukocytes as the peak of infection was reached. Perifollicular and intrafollicular infiltration by leukocytes was one of the surest signs of the parasites presence within the follicle, though often there was great difficulty in actually visualizing the fungus elements. The ultimate expression of inflammatory violence was collapse of the follicle with the formation of abscesses imperfectly surrounded by irregular sheets of epithelial cells derived from the external root sheath. Such abscesses began intrafollicularly with the congregation of polymorphonuclear leukocytes within the lumen, followed by rupture of the wall, and the pouring out of the follicular contents, including fragments of disorganizing infected hair shafts into the tissue. In this way segments of disorganized hair along with fungus filaments and spores were sequestered into the corium. Unless one searched carefully for hair remnants, it sometimes looked as if there were little colonies of fungus growing in the central portion of these abscesses (Figure 3C). It would be possible to conclude from this evidence, if nothing were known of the evolution of the abscess, that the fungus had violated the law of strict confinement to dead keratinized substrates and had actually invaded the corium by a direct growth into the tissue. In reality, it was passively brought to such an "unnatural" site via its natural vehicle, the hair shaft, which was extruded into the tissue when the follicle collapsed in the violent inflammatory reaction. The hair shaft itself, through digestion and keratinolysis by the fungus, was broken down into bits in which occasionally very little of the original hair substance remained, having all but been lysed, thereby loosing the fungus into the necrotic area. In fact, since the fungus now found itself in the center of a dead, necrotic zone, it is possible that some small active proliferation did occur. I have observed the same phenomenon of sequestration of infected hair fragments in human scalp tissue which is the site of a kerion.

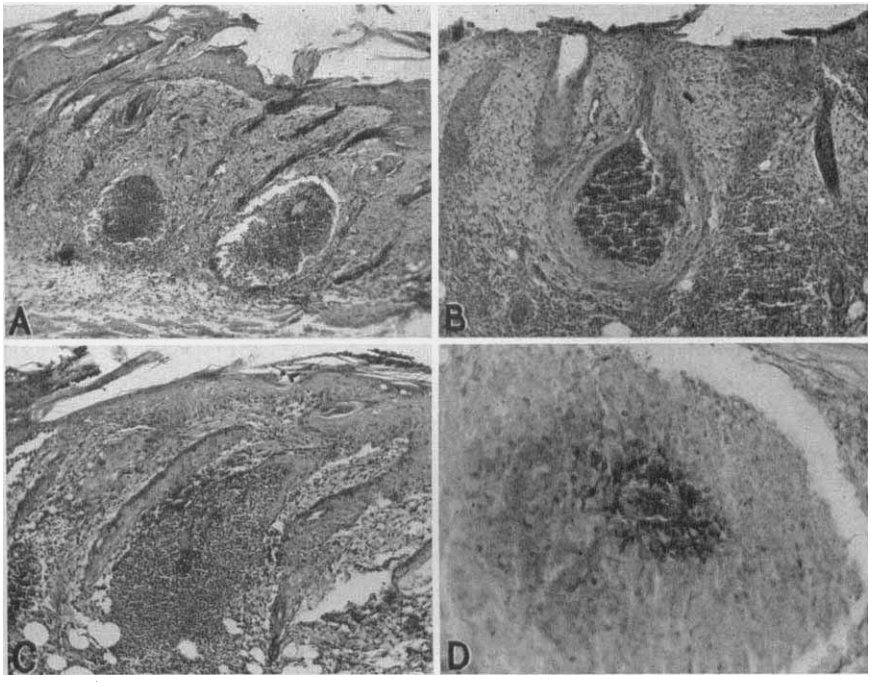

FIG. 3. Histologic changes in mouse skin. A. Six days after infection with T. mentagrophytes. The epidermis is thickened and two abscesses have formed destroying the follicles in which they arose. The one on the right contains in the upper portion a fragment of infected hair. (H & E. orig. mag. ×80). B. Intrafollicular abscess in 5 day old infection with M. canis. Note proliferation of external root sheath cells around abscess as if attempting to contain it and the intense infiltrate in the corium. Despite these major inflammatory changes, no fungi could be found in this section. (H & E. orig. mag. ×130). C. Collapse of infected follicle with outpouring of leukocytes into tissue in 6 day old infection with M. gypseum. Remnants of infected hair fragments remain in abscess. (H. & E. orig. mag. ×130). D. Abscess developed in 6 day old T. tonsurans infection. The appearance is that of a fungus colony growing freely within the abscess but study of serial sections revealed traces of the hair which passively carried the fungus into the tissue when the follicle collapsed. There has possibly been some further proliferation of the hyphae within this necrotic zone. (Hotchkiss-McManus stain. orig. mag. ×130).

Mouse infections with other dermatophytes

The results of inoculating C-57 mice on the 12th day of anagen with other species of dermatophytes is shown in Table 1. Limitations which attach to these purely provisional studies, are: the small number of animals, extremely hot weather at the time, and uncertainty over whether some of the animals classified as having mild infections were actually infected, since the judgment was based principally on clinical grounds.

At the outset it may be pointed out that all these infections whether mild or severe followed the pattern and course of the corresponding *T. mentagrophytes* infections and need no further comment.

It can be seen that the majority of dermatophytes produced infections. It was

TABLE I

Inoculation of mice with dermatophytes on the 12th day of anagen

Species	Strain	No. Animals	Infection Ratio	Extent of Infection*
M. audouini	1A2	3	3/3	Mild to moderate
M. audouini	1A3	4	2/4	Mild to moderate
M. canis	1B1	3	3/3	Severe
M. canis	1B6	3	2/3	Severe
M. gypseum	1C1	3	3/3	Moderate to severe
M. gypseum	1C4	3	3/3	Moderate to severe
T. rubrum (granular strain)	2B7	4	3/4	Severe
T. rubrum	2B2	3	1/3	Moderate
T. tonsurans	2C4	3	2/3	Mild to moderate
T. tonsurans	2C7	3	3/3	Mild to moderate
T. violaceum	2F2	3	0/3	—
T. violaceum	2F4	3	2/3	Mild
T. gallinae	2K1	3	3/3	Moderate to severe
T. gallinae	2K2	3	2/3	Moderate to severe
T. megnini	215	3	1/3	Mild
T. verrucosum (ochraceum)	2H15	3	1/3	Mild
T. verrucosum (album)	2H11	3	0/3	—
T. verrucosum (discoides)	2H12	3	1/3	Mild
T. concentricum	2E1	3	1/3	Mild
T. schoenleini	2D3	3	1/3	Mild
T. schoenleini	2D4	3	1/3	Mild
T. ferrugineum	2G1	3	0/3	—
E. floccosum	3A6	3	0/3	—

* Mild indicates only scaling; moderate indicates slight inflammatory crust; severe indicates marked inflammatory crust.

the zoophilic species which produced the most inflammatory reactions but *T. verrucosum*, which ordinarily elicits the most violent inflammatory reactions in humans, was a notable exception, generally being non-infective. A point to be remembered is that some species do not sporulate in culture (*T. verrucosum*, *T. concentricum*, *T. violaceum*, *T. schoenleini*, etc.), and so the inoculum consisted of hyphal fragments. Spores are hardier and a much greater number of "infectious units" would be available in spore bearing cultures. The distinction between anthropophilic and zoophilic species is a useful clinical concept but does not, as it is becoming increasingly evident, imply an absolute difference in host selectivity. For instance, spontaneous infections with *M. audouini*, an anthropophilic species have occurred in dogs and monkeys (11). That the mouse can be infected with anthropophilic species is again an exceptional instance, considering the highly limited conditions under which the disease develops, and the generally mild nature of the shortlived infection.

The histopathologic studies were not detailed enough to warrant much description of how the various fungi appeared in tissue. Assuming the appearance

in human scalp hair to be prototypical, I rarely found matching pictures; the course is too brief for mature stabilized hair infections to occur and in general the findings were rather nondescript, demanding the most careful search to determine whether the picture was roughly similar to human hair infections. A few hairs infected with each of the species of Microsporum did show an ectothrix pattern with a mosaic of spores and *T. tonsurans* infected hairs did occasionally exhibit the endothrix habit (Figure 3A). The tissue reactions were of the kind expected for the *T. mentagrophytes* infections corresponding in severity. Again, the disparity between the inflammatory response and the sparseness of fungus elements was noted as well as the failure of the great majority of follicles to become invaded. It was assumed that an inflammatory infiltrate signified infection whether or not fungi were actually visualized.

Rat infections with T. mentagrophytes

Approximately 50 young Carworth Farms white rats, weighing 30–40 grams, were utilized to determine whether infections induced by *T. mentagrophytes* resembled the disease in mice. The results conformed so closely to the mouse that a synopsis of the findings will suffice. The susceptible period was between the 12th and 19th days. Inoculations in the latter part of anagen evolved more rapidly. Quite inflammatory eruptions were the rule with the virulent strain used, more consistently so than with mice. Histopathologic examinations exhibited changes similar to the mouse.

It seemed worthwhile to study the responses of older animals and so, a dozen rats weighing 200 grams were inoculated in groups of 3 at different stages. Some differences were present. Again, it was not possible to establish an infection before the 12th day of anagen; however, in these older animals anagen sometimes lasted for a month and in consequence the duration was longer. Even inoculations on the 25th day of anagen (no studies were made beyond this time) resulted in infection. During telogen the rats were completely resistant as expected and telogen, as usual, marked the end of the active phase of infection. The course was more erratic than in young animals. In general, clinical evidence of infection did not become manifest for about 7–9 days when the inoculation was on the 12th day of anagen, but in late anagen scaling appeared in 4–5 days, augmenting the impression that both mice and rats are most susceptible in late anagen.

Studies with dermatophytes other than *T. mentagrophytes* were not undertaken in rats, but it seems likely that rats, like mice, would prove susceptible to many other species provided the inoculations were performed at the right time. At any rate, it is possible to explain a singular observation of Bloch's (30) which has never been confirmed. He easily produced infections in infantile rats with *M. audouini*. By chance, he happened to make the inoculations during anagen. It is inevitable that rats of this age would have been in their first skin cycle.

Infection of hamsters and rabbits with T. mentagrophytes

It seemed worthwhile to take a "quick look" at ringworm infections in rabbits and hamsters since these are also animals which exhibit skin cycles. The peculiari-

ties of rabbit skin which 2–3 times a year enters a diffuse inactive phase with no hair growth anywhere, but at other times shows irregular islands and patterns of growth has been described by Roney *et al.* (31). I could find no information on skin cycles in hamsters; the few adult hamsters which I examined preliminary to infecting them exhibited cyclic waves with anagen lasting about one month. The duration of telogen was not precisely determined but exceeded two months.

Six adult hamsters inoculated with *T. mentagrophytes* on the 14th day of anagen became infected, and developed inflammatory crusted lesions similar to mice and rats. These regressed spontaneously after telogen. In telogen, the animals were resistant. While these results are obviously provisional, the mechanics of infection in these animals seems to correspond to mice and rats.

In contrast to the above animals, adult rabbits were susceptible to *T. mentagrophytes* infections in areas which were in telogen as well as in anagen; however, the infection developed more quickly in anagen areas. The inflammatory response provoked by the fungus in telogen areas, similar to the initiating effects of a variety of irritant chemicals or treatments (31), invariably initiated a new cycle after a few weeks. It was significant, however, that invasion of the hair in telogen inoculated areas did not occur until a new cycle of hair growth was underway. The duration of the infection varied from 2 to 4 months and was not dependent on the onset of telogen. These results should be compared to those of Reiss and Leonard (32) who, while they did not take skin cycles into account, found *M. canis* infections of the rabbit to be rather variable, lasting for months. It is noteworthy that they found infantile rabbits to develop more regular infections. In short, infection in the rabbit is not strictly conditioned by the stage of the life cycle although hair invasion takes place only when the hair is growing.

COMMENT

The principal finding in this investigation is that ringworm infections in mice, rats and hamsters are exquisitely dependent on the stage of the skin cycle. The results in a sense are more informative of the physiologic subtleties of mouse skin than they are about ringworm infections; that is, we learn more of the peculiarities of the host than the qualities of the disease by such a study. One more point of documentation is furnished for the now unassailable law, that no research should be undertaken in skin-cycle animals without clearly relating everything that is done to the stage of the cycle.

Many questions, of course, remain be answered; possibly the foremost of these is why the stratum corneum of telogen mouse skin should be any less suitable for fungus growth than during a certain portion of anagen—this layer is dead in either case but, of course, the substances contained within it may be different at different stages even if we assume the keratin to be the same.

With *T. mentagrophytes*, the "natural" species of mouse ringworm, it was not possible to reproduce mouse favus in its classical form, nor were the alopecias without marked inflammation encountered in some spontaneous laboratory epizootics able to be elicited. The presumption is that these variations in clinical expression are conditioned by host factors and not by the parasite, the various

strains of which can hardly have undergone much change in a few decades. The variables which affect the host in spontaneous mouse infections are numerous and largely unknown: obvious differences might be age, (older animals it will be recalled have longer cycles and a greater chance for chronicity), heredity, nutrition and prior disease.

Even though the mouse seems to be susceptible to infection with a number of dermatophytes, this animal, in my opinion, has many limitations for ringworm studies: 1) the infection is too brief; 2) the period of susceptibility is too short; 3) the conditions for infection are too precarious; 4) the animals must be "prepared" or watched so that they are known to be in the proper stage; 5) fungus elements are scanty and the exuberant tissue reaction greatly out of proportion; and finally, 6) the host structures, such as hairs, are so small and fragile that they are not readily studied with the clarity possible in human hair. It might appear that the mouse would be suitable for studying agents to be used parenterally against ringworm infections since current topical therapy leaves so much to be desired; however, there is a special pitfall to be taken into account if false conclusions are to be avoided. Any ingested or injected agent, whether it were frankly antifungal or had some particular pharmacologic effect on the host, might prevent infection indirectly by shifting or altering the skin cycle so that the skin was in an inhospitable phase. The results of such studies would not be applicable to human skin which is in a "steady" state. Whereas awareness of the rhythms of mouse skin is what makes experimental infections possible, it is precisely this physiologic instability which renders this animal unfit for most studies.

The presence of fungus elements within the corium raises a controversial point. How does the fungus get to this site if its growth is confined to dead keratinized substrates? This question applies also to human infections, exemplified by Majocchi's granuloma and kerion, in which hyphae and spores are found within the tissue itself. I am persuaded that in all such instances the fungus is transported passively into the corium owing to collapse of the follicle and sequestration of fragments of infected hair. The hair undergoes lysis and the fungus then has the appearance of having directly penetrated into the cutis. Wilson (33), viewing the same evidence, takes the contrary stand and believes that the dermatophytes are not strictly limited to non-vital regions. Similarly, it seems to me that Newcomer's *et al.* (34) demonstration of the growth of dermatophytes within the wall of granuloma pouches of the rat, is not as they maintain, a decisive proof that these organs have the potentiality of invading living tissue. What has been demonstrated by an artificial method is an unusual instance of the characteristic *necrophilia* of the dermatophytes for it was only in the necrotic wall of the pouches that the hyphae proliferated, as their pictures brilliantly show. Theoretically, it is possible, if viable organisms happen to become deposited in a necrotic area, such as an abscess, that some growth could take place, and I have not hesitated to call attention to this evidence (Figure 3B). Perhaps the keratinophilia of this group of organisms has been too strictly interpreted but as yet the necrophilia is without proved exception.

Finally, what has been learned about mouse and rat infections has some bearing

on the important question of how dermatophytes cause inflammatory reactions without actually being *inside* the tissue. I have determined that these animals do not become allergic to the products of the fungus, that is, the skin does not react to an intradermal test with trichophytin as does human or guinea pig skin following inflammatory ringworm infection. Because the guinea pig does become sensitized reinfections run a shorter course and have a brief incubation period; in the absence of sensitization in mice and rats, the course of the reinfection does not differ from the primary disease and no "immunity" is apparent. Still, violent inflammatory reactions ensue which require some explanation other than the allergic mechanism. I consider that the elaboration of toxins or irritants by the superficially located fungi is the means by which inflammatory reactions are provoked in the mouse and rat, as well as in trichophytin negative humans with mildly eczematous ringworm lesions. I have elsewhere begun to elaborate the thesis of toxins contributing to the inflammatory reaction (35). The mouse is a pure example of this mechanism.

SUMMARY

(1) In the mouse, rat, and hamster, ringworm infection is conditioned by the stage of the skin cycle. The period of susceptibility is the latter part of anagen.

(2) The mouse is susceptible in varying degrees to many species of dermatophytes.

(3) The findings are discussed in relation to the physiology of mouse skin, the suitability of the mouse for ringworm research and the light thrown on the pathogenesis of the inflammatory reaction.

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