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# The Distribution of *Mycobacterium leprae* in the Hair Follicle of the Eyebrow[1]

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Madarosis, or thinning and the eventual loss of the eyebrows, is a well-established clinical feature of lepromatous leprosy. Similarly, the presence of *Mycobacterium leprae* within the hair follicle has long been recognized ([2,9]) and yet little attention has been paid to this, the largest appendage within the skin, which is so often seen as the initial site of the leprosy granuloma.

The hair follicle is derived in fetal life principally from the epidermis. However, its cyclic existence through the stages of anagen (growing), catagen (regressive), and telogen (resting) is controlled by the dermal papilla, a little understood collection of specialized fibroblast-type cells of dermal origin without which the hair follicle ceases to function.

During the anagen phase (Fig. 1) the follicle is composed of a number of concentrically arranged cylinders of specialized cell layers. At the core is the developing hair shaft (cortex and cuticle) around which lies the inner root sheath (cuticle, Huxley layer, and Henle layer). Surrounding this is the outer root sheath, a layer several cells thick, composed of epidermal-like cells, and continuous with the epidermis. The dermal papilla is situated in an involuting pocket at the base of the follicle and is separated from the epithelial portion of the follicle by a basement membrane that is eventually continuous with the basement membrane of the epidermis.

After a comparatively short catagen phase, the follicle enters the telogen or resting phase (Fig. 2). Melanogenesis and production of the hair shaft have ceased and a club root composed of differentiated cortical cells forms at the base of the hair shaft. The lower epithelial portion of the follicle retracts towards the epidermis accompanied, or possibly preceded by, a pleating of the connective tissue sheath around the follicle. The dermal papilla remains within the lower dermis, attached to the retracted follicle by a thread of epithelial cells and connective tissue. The inner root sheath regresses to a single layer of hardened cells.

The complex array of nerve fibers surrounding the follicle during anagen contracts with the follicle during telogen. However, the microvasculature, particularly those blood vessels that are seen within the dermal papilla during anagen, disappear. During the early phases of anagen of the successive hair cycle, the lower epithelial portion of the follicle grows downward to encompass the dermal papilla and re-differentiates to form the new anagen hair shaft and accompanying inner root sheath. The microvasculature similarly undergoes differentiation to supply the dermal papilla with a new vascular plexus.

The hair follicle is of interest in the study of leprosy for at least two reasons: a) There is the characteristic involvement of the eyebrows resulting in madarosis and the concurrent social stigma attached to this clinical manifestation([11]). b) Since the hair follicle "excretes" its final product, the hair and its root sheath onto the skin surface, it may be a site from which the leprosy bacillus is disseminated. Previous workers who have suggested that the hair follicle is a site from which *M. leprae* may be disseminated into the environment ([5,6,9]) have, however, taken a simplistic view of its complex structure.

The aim of this study was to localize the leprosy bacillus within the various specific

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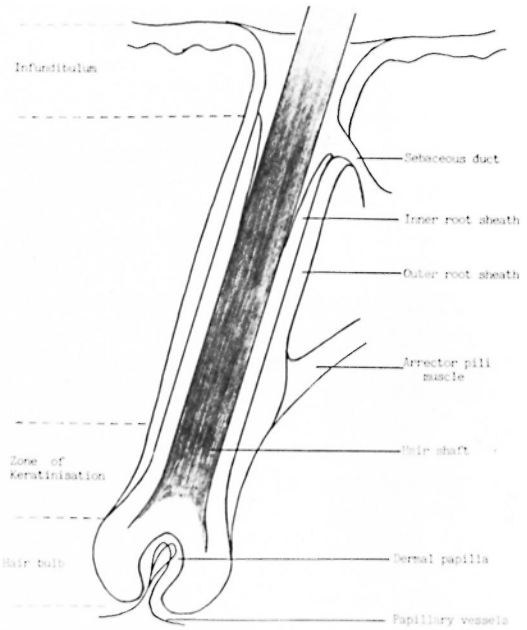


FIG. 1. Schematic diagram of the anagen (growing) hair follicle.

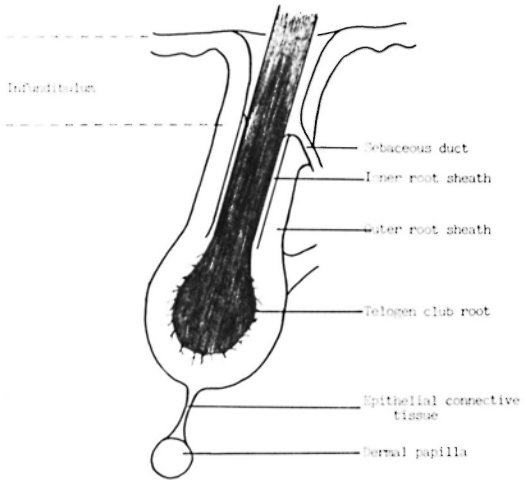


FIG. 2. Schematic diagram of the telogen (resting) hair follicle.

component structures of the follicle with a view to assessing the possible involvement of the hair follicle in the transmission of *M. leprae.*

## MATERIALS AND METHODS

Elliptical biopsies of the lateral aspect of the eyebrow showing diffuse hair loss were taken from five patients with active, untreated, lepromatous leprosy. The tissue was fixed in cold Karnovsky's fixative ([4]). The biopsies were then divided into blocks containing approximately three follicles, post fixed with 2% osmium tetroxide, dehydrated in graded ethanol and propylene oxide, and then embedded in Spurr's resin, a low viscosity resin to facilitate complete infiltration into the follicular canal ([10]).

Semithin (1–2 $\mu$) and ultrathin (60 nm) longitudinal sections of 20 hair follicles were cut on an LKB mk III Ultratome and stained with either toluidine blue or uranyl acetate/lead citrate ([8]), respectively. All ultrathin sections were viewed on an Elmiscop 101D transmission electron microscope.

## RESULTS

**Dermal papilla.** Both solid and fragmented bacilli were found in the dermal papilla at each stage of the hair cycle, i.e., anagen, catagen, and telogen. In those papillae that showed capillary loops, occasional bacilli were seen in the endothelial cells. Numerous bacilli were seen in those cells immediately adjacent to the capillary basal lamina in the papilla. At no point did the vascular basal lamina observed within the papillae show evidence of reduplication.

The papillary cells showed normal nuclei and cell membranes at all stages of the follicular cycle with no apparent increase in cytoplasmic organelles and no clearly identifiable lysosomes. However, many of the cells of the papilla showed large vacuoles of lipid-like material containing numerous bacilli. The vacuoles were not membrane bound but were continuous with the cell cytoplasm (Figs. 3 and 3a). In telogen, the resting phase of the follicle, the lipid-like content of the cells appeared to be greatly reduced although the number of bacilli, both solid and fragmented, was similar to that seen in the anagen papillae (Fig. 4).

The basement membrane surrounding the papilla and separating it from the germinative bulb showed no abnormalities. Some cells, particularly in anagen, showed normal clumps of intracellular pigment granules. Normal collagen and elastic fibers were seen in all of anagen papillae studied, and no neural components were seen.

**Hair bulb** (proliferative zone of the hair shaft and inner root sheath). The sequential

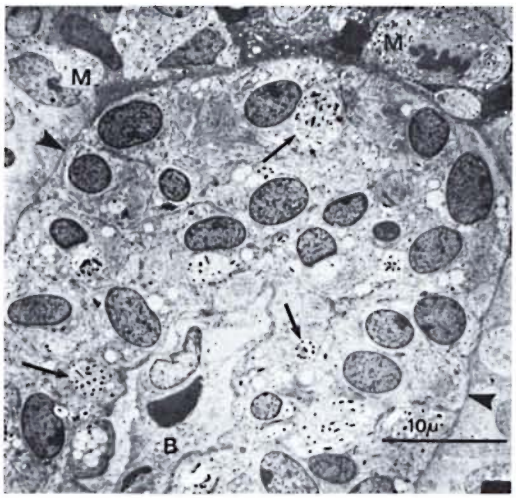


FIG. 3. Anagen dermal papilla. Numerous intracellular solid-staining bacilli (➡) in lipid-like inclusions. Active melanocytes cover the apex of the papilla (M).
B = papillary blood vessel
▶ = basal lamina of the papilla

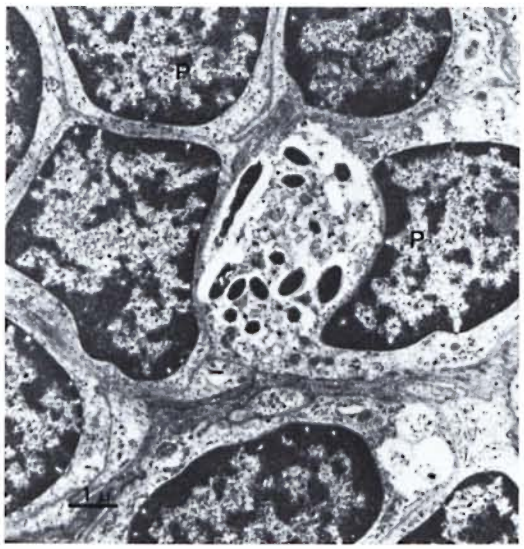


FIG. 3a. Higher power of anagen papillary cells (P) containing *M. leprae.*

differentiation of the cell types in the hair bulb to form either the hair shaft, composed of the hair cuticle and cortex, or the inner root sheath, composed of a cuticle, Huxley layer, and Henle layer, appeared to be unaffected. All of these cell lines were followed through the progressive stages of differentiation, protein deposition, and hardening and/or keratinization. Numerous dendritic melanocytes containing pigment granules in all stages of maturation were seen adjacent to the dermal papilla of anagen follicles. However, follicles in the telogen phase, during which the differentiating bulb is absent, showed only one or two melanocytes, containing only mature pigment granules, adjacent to the dermal papilla. These observations are in keeping with normal hair follicles.

In four of the five biopsies studied no bacilli were found in the bulb region of the follicles or within the various stages of differentiation of the hair shaft and inner root sheath. However, in one biopsy two anagen follicles showed bacilli in the bulbar region of the hair follicle. Bacilli were found in both presumptive inner root sheath cells and cortical cells of the hair shaft, i.e., the keratinocytes. No bacilli were observed in the final stages of differentiation and complete keratinization of either of these cell lines.

**Follicular canal and infundibulum.** No bacilli were found in the follicular canal or the inner root sheath cells that line the canal up to the level of the infundibulum. The infundibulum, lined by cells continuous with

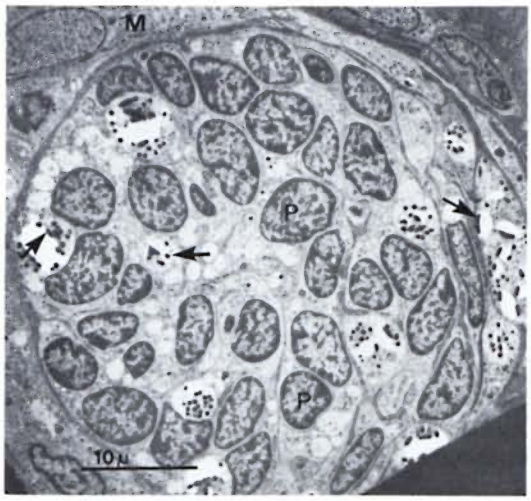


FIG. 4. Telogen dermal papilla. Numerous solid-staining bacilli (➡) within the papilla during the resting phase of the follicle.
P = papillary cell nuclei
M = melanocyte

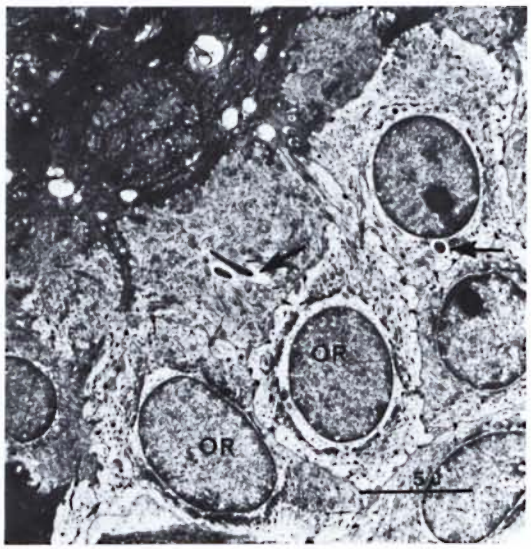


FIG. 5. Individual solid-staining bacilli (➡) within outer root sheath cells (OR).

and of similar cytology to the epidermis, was similarly free of bacilli.

**Outer root sheath.** The number of bacilli found within the outer root sheath varied greatly, both between biopsies and individual follicles in similar stages of the hair cycle. On many occasions the bacilli were single, solid-staining, and intracellular, and no clearly identifiable lysosomes or lipid-like material were seen (Fig. 5). However, globi of intracellular, solid-staining bacilli were also observed surrounded by a lipid-like material.

There appeared to be an increase in the number of bacilli in follicles in the telogen phase of the hair cycle although this was not quantitatively confirmed. No bacilli were found in those cells forming the follicular infundibulum.

**Associated follicular structures.** Fragmented bacilli in small lipid-like vacuoles were occasionally seen within the cytoplasm of the arrector pili muscle cells. Individual solid-staining bacilli without vacuolar formation were rarely to be found. One arrector pilorum muscle showed signs of apparent atrophy although this was unique. All the muscles and their innervation appeared to be normal, and bacilli were not always present.

No bacilli were found in the sebaceous glands of any of the hair follicles studied.

## DISCUSSION

In this study we have shown the distribution of *M. leprae* in the component structures of the hair follicle. Bacilli were found predominantly in the dermal papilla and outer root sheath of both anagen and telogen follicles.

The specialized cells of the dermal papilla are fibroblastic in origin, a cell line which, under specific conditions, has been shown to phagocytose *M. lepraemurium* ([1]). However, the apparent lack of well-defined lysosomes suggests that the papillary cells do not possess, in their normal functional role, the ability to lyse the leprosy bacillus.

Bacilli were not seen to cross the basal lamina surrounding the dermal papilla into the proliferative zone of the hair shaft and inner root sheath which would imply, because of the non-motile nature of the leprosy bacillus, that there is no exchange of part or whole cells across the papillary basal lamina as seen in the microvasculature ([3]). The presence of numerous bacilli within the dermal papilla without the presence of a lepromatous infiltrate is a good indication for the hematogenous spread of the bacillus throughout the dermis.

The presence of several bacilli in the proliferative bulbar region of two of the anagen follicles studied is likely to be the result of bacilli being present in the outer root sheath cells of the previous telogen phase which then, as an epithelial column connecting the dermal papilla to the base of the resting follicle, redifferentiate in the successive anagen phase of the hair cycle to form the new hair bulb. The fate of any bacillus entering the proliferative cells of the hair bulb would be one of incarceration within keratinizing cells where the deposition of globular and/or fibrillar proteins is at the expense of all other cell organelles. Should a bacillus remain viable after such drastic cellular changes it would, in the case of the hair shaft, remain entrapped for perhaps many years, the hair being very resilient to environmental weathering. Bacilli within the inner root sheath are likely to be degraded, along with the keratinized inner root sheath cells, by sebaceous enzymes.

The dermal papilla is only vascularized during the anagen phase of the hair cycle, allowing the bacilli to be carried into and

presumably out of the papilla. However, during the resting telogen phase the papillary vasculature is absent, trapping the bacilli within the quiescent papilla. Therefore, one may postulate an inadvertent, nursery-like micro-environment of phagocytically incompetent cells within the dermis in which the bacilli can multiply until such time as the papilla is revascularized in the successive anagen phase and allows the dispersal of bacilli via the papillary vasculature.

The presence of bacilli in the outer root sheath of both anagen and telogen follicles, although obvious, is difficult to explain. Outer root sheath cells have not been reported to be actively phagocytic, nor do they possess a direct blood supply to facilitate the transport of bacilli. Additionally, this cell line is separated from the surrounding dermis by a complex connective tissue sheath. Therefore the mode of entry of the bacillus into the outer root sheath remains a subject for further study. It was obvious, however, that bacilli were only present in the true outer sheath cells and not in cells of the upper infundibulum which is composed of essentially true epidermal cells. The most striking structural difference between these two zones is the absence of pigment granules in the outer root sheath below the infundibulum. One must therefore conjecture whether there is a direct relation between the presence of pigment granules and the absence of bacilli in the epidermis. This speculation is not new in the discussions of leprosy ([7]).

The formative process of the hair shaft, its root sheaths, and pigmentation do not appear to be affected; hence the actual cause of hair loss is still not understood. However, one may speculate that any disruption of the delicate biochemical balance of the dermal papilla by bacillary invasion over a period of time will be to the detriment of the hair follicle.

It is important when considering the possible role of the hair follicle in lepromatous leprosy not to view the follicle as simply a hole in the skin surface leading directly into the dermis. The hair follicle, as an invagination of the epidermis, remains separated from the dermis by a connective tissue sheath; only the dermal papilla originates from and remains in contact with the dermis. This, too, is separated from the epithelial portion of the follicle by a basement membrane.

The fact that bacilli are found in those cells (the dermal papilla and outer root sheath), which are not in direct contact with the environment, would suggest that bacilli are not extruded into the follicular canal. It is therefore unlikely that the hair follicle is a means whereby significant numbers of *M. leprae* are disseminated into the environment.

## SUMMARY

Longitudinal sections of hair follicles from the eyebrows of patients with active, untreated, lepromatous leprosy were studied. *Mycobacterium leprae* were found in the dermal papilla and outer root sheath of both anagen and telogen hair follicles. Bacilli were rarely found in those cell lines continuous with the environment, i.e., the hair shaft and the inner root sheath. The biochemical fate of these cell lines is such that they would not provide a suitable medium for the survival and transport of bacilli into the environment.

We conclude that it is unlikely that the hair follicle plays a significant role in the dissemination of *M. leprae* but it may be important in providing a suitable site for the incubation of the bacillus within the dermis.

## RESUMEN

Se estudiaron secciones longitudinales de los folículos pilosos de las cejas de pacientes con lepra lepromatosa activa no tratada. Se encontraron bacilos (*Mycobacterium leprae*) en las papilas dérmicas y en la capa exterior de la raíz tanto de los folículos anágenos como de los telógenos. Raras veces se encontraron bacilos en aquellas estructuras celulares contiguas al ambiente como son el tallo piloso y la capa interna de la raíz. El funcionamiento bioquímico en estas estructuras celulares es tal que no proporciona un medio adecuado para la supervivencia o transporte de los bacilos al microambiente.

Concluímos que el folículo piloso no juega un papel significante en la diseminación del *M. leprae*, aunque puede ser importante como un sitio de incubación del bacilo dentro de la dérmis.

## RÉSUMÉ

On a étudié des coupes longitudinales des poils des sourcils chez des malades atteints de lèpre lépromateuse active non traitée. On a mis en évidence *Mycobacterium leprae* dans les papilles dermiques et dans la gaine externe de la racine des poils, tant au niveau

des poils anagènes que telogènes. Des bacilles ont été trouvés, mais rarement, dans la tige des poils et dans la gaine interne. L'évolution biochimique de ces cellules est telle qu'elle ne pourrait pas fournir un milieu approprié pour la survie de transport des bacilles dans l'environnement.

On a conclu que les follicules pileux ne jouent pas un rôle significatif dans la dissémination de *M. leprae*, mais qu'ils pourraient être importants cependant en fournissant un endroit d'élection pour l'incubation du bacille dans le derme.

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