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Reinnervation of Hair Follicle End Organs and Meissner Corpuscles in Skin Grafts of Macaques

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Plugs of occipital hairy scalp and pieces of digital pads were transplanted to the frontal scalp of stump-tailed macaques (*Macaca arctoides*). Both types of grafts grew well and retained their original appearance for several years. We traced the regrowth and reinnervation of hair follicles and Meissner corpuscles in sequential biopsy specimens of these grafts. Two weeks after transplantation, hair follicles in the grafts appeared to have lost all integrity but began to regrow after 4 weeks. The nerve and organs of hair follicles began to reappear at 8 weeks. Thereafter, grafts with large terminal hairs remained viable in the host bald frontal scalp for as long as 8 yr. In the digital skin grafts, the cytoskeleton of the Meissner corpuscles could be distinguished after 4 weeks; after 8 weeks nerves from the host tissue could be traced to the end organs. Perivascular nerve plexuses and nerves to the piloarrector muscles were clearly seen in both types of graft after 8 weeks.

In human skin grafts, modalities of cutaneous sensation are reestablished at varying rates. Return of sensation is better in full-thickness than in split skin grafts and depends on the degree of tissue recovery in the grafts [1, 2]. Kadanoff, Wassilev, and Matev [3] reported that a few months to 14 yr after a skin-graft operation, nerve fibers were partly associated with blood vessels and hair follicles; they found minute arborizations under the epidermis in human skin grafts but Meissner corpuscles only in a few grafts. No one seems to have observed the sequential process of tissue recovery and reinnervation of skin grafts. Reinnervation in grafts cannot simply be compared with nerve regeneration in injured or denervated skin. The recovery of skin structures in graft tissue is a primary step toward full neurotization.

Using the frontal scalp of stump-tailed macaques as a recipient site, we observed the sequential recovery and reinnervation of skin grafts from hairy occipital scalp and glabrous skin from digital pads.

The hairy skin graft allowed us to observe the regrowth of hair follicles and the reinnervation of the end organs around them. In the digital skin grafts we traced the fate of Meissner corpuscles and their eventual reinnervation by the host sensory nerves.

MATERIALS AND METHODS

Three juvenile and 5 adult stump-tailed macaques (*Macaca arctoides*) were used for autologous skin transplantation from the occipital to frontal scalp and from the digital pad to frontal scalp. Full-thickness punch-biopsy specimens (9 to 6 mm) from the occipital hairy scalp and from the digital pads were grafted into punched-out areas in the frontal scalp. The plugs were sutured to the host with thin chromic thread. Before the operation, each animal was anesthetized with ketamine hydrochloride (5 mg/kg). During the first 2 postoperative months the animals were placed in restraining chairs to prevent damage to the grafts. The animals were then transferred to individual cages. Biopsy specimens of each graft, including some surrounding host skin, were taken 1, 2, 4, 8, and 16 weeks after transplantation. For long-term observation of hair growth in the grafts, the occipital skin grafts of three animals were kept intact for 8 yr. Photographs of the scalps of these animals were taken twice a year.

Frozen serial sections 60 to 80 μm thick were cut with a cryostat; 1 out of 5 consecutive sections was cut 10 μm thick. The sections were thawed, stretched on glass slides, and dried at room temperature. After fixation in cold neutral buffered 10% formalin, the thin sections were stained with hematoxylin and eosin, and the thick sections were prepared according to an acetylcholinesterase (AChE) histochemical procedure [4]. Some specimens were cut vertically to the skin surface in 2-mm-thick slices and fixed with gluteraldehyde-formalin solution for embedding in glycol methacrylate resin. Details of these techniques have been described elsewhere [5].

RESULTS

Transplantation of Hairy Occipital Skin to Frontal Scalp

Gross observation: Two months after plugs of occipital skin were transplanted to the frontal scalp areas of juvenile animals, the grafts were completely fused with the surrounding host skin after 2 mo. New hair began to emerge on the surfaces of grafts after 4 mo. Subsequently, graft hairs became thick and dense, i.e., unlike the rather sparse hairs in host frontal scalp (Fig 1). As the animals matured the frontal scalp became bald, but the long, thick hairs in the grafts continued to grow (Fig 2). Hair growth continued unchanged in these plugs for more than 8 yr after transplantation.

Histological observation: One week after transplantation, the graft skin was fused with the surrounding host skin (Fig 3). The graft epidermis was almost completely fused with the host epidermis except for the presence of a crust and epidermal bridging process at the junction of graft and host skin. The dermis of the graft contained the original collagenous bundles; the lateral and basal edges of this tissue were surrounded by vascular, fibroblast-rich host tissue infiltrated with some leukocytes. Hair follicles, sebaceous glands, sweat glands, and piloarrector muscles of the graft skin were in varying degrees of degeneration.

In the frozen graft sections prepared by means of the AChE technique, nerve fibers of the piloarrector muscles reacted positively but the end organs of hair follicles did not.

Two weeks after transplantation, the dermal collagenous bundles in the plugs and all the cutaneous appendages seemed to have disappeared; the dermis of the grafts was replaced by a vascular, fibroblast-rich tissue that proliferated from the underlying host tissues (Fig 4). The epidermis of the graft skin was slightly acanthotic. There were no traces of graft hair follicles or sebaceous glands. Even the piloarrector muscles seemed to have disappeared.

The graft tissue contained no AChE-positive fibers, but in the host, dermal tissue under the graft showed reactive, proliferating fine nerve fibers, mostly around the vessels; some re-

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Abbreviations:
AChE: acetylcholinesterase

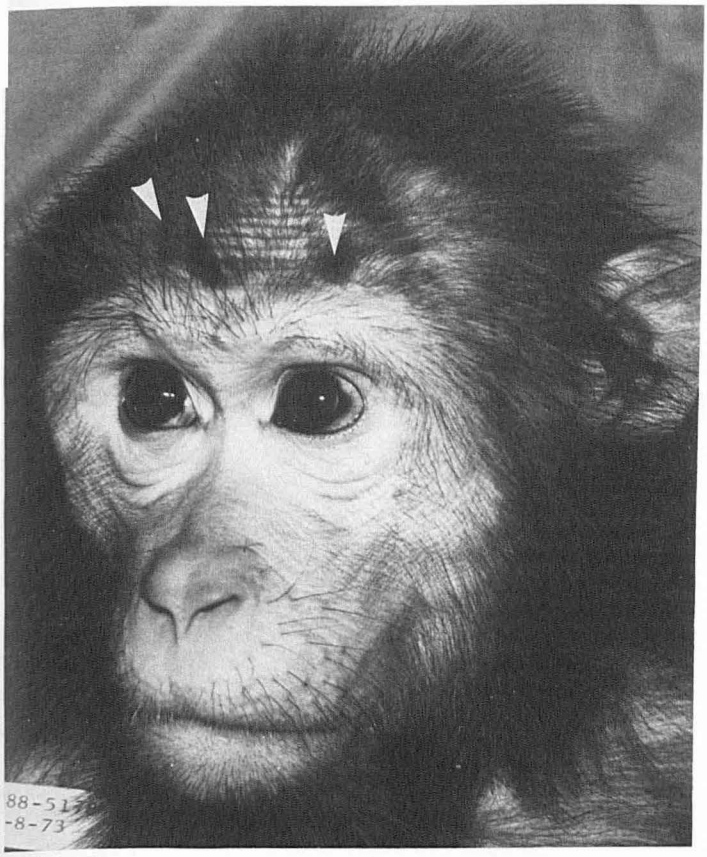

FIG 1. Adolescent stump-tailed macaque with 3 tufts of long occipital hairs (*arrows*) growing from 2-mo grafts on the frontal scalp of an adolescent macaque.

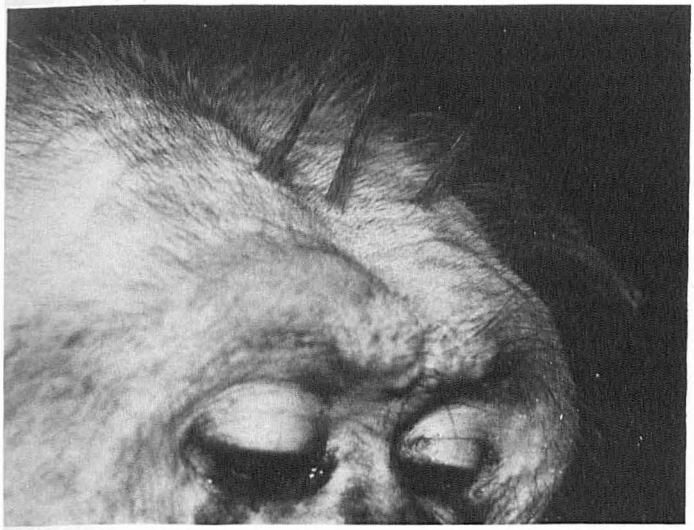

FIG 2. Long tufts of occipital hairs growing from grafts on the bald scalp of a mature male stump-tailed macaque several years after transplantation.

active fibers appeared to be derived from stumps of severed somatosensory nerve bundles.

Four weeks after transplantation, there was no structural difference between epidermis of the graft and host tissues. A few hair follicles in early anagen seemed to be developing from the epidermis of the graft skin. Piloarrector muscles and collagenous bundles are again visible in the fibroblast-rich dermal tissues of the graft (Fig 5). There were no nerves around the new hair follicles and piloarrector muscles.

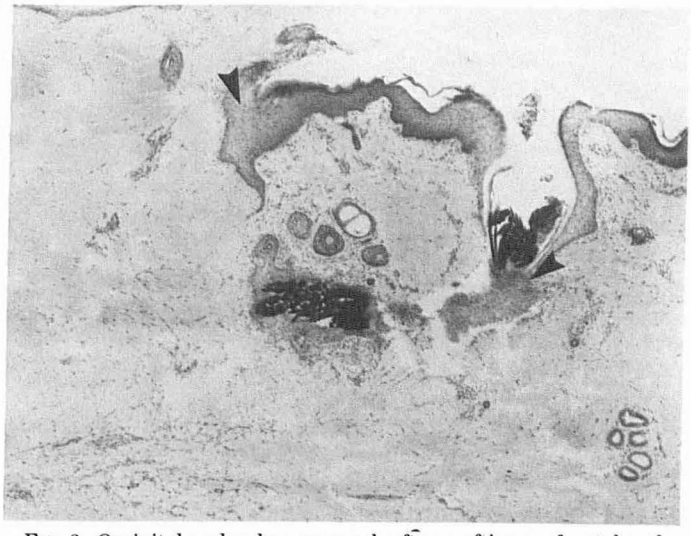

FIG 3. Occipital scalp plug one week after grafting on frontal scalp. The epidermis of the graft (*between the 2 arrows*) is completely fused with that of the host. The hair follicles are becoming disorganized. The 2 black masses are fragments of sutures used to hold the graft. Frozen section (60 μ) (hematoxylin and eosin, Ca, reduced from ×20).

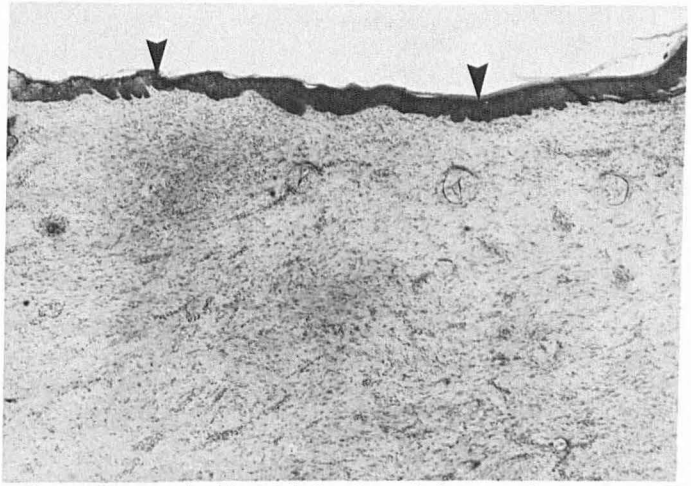

FIG 4. Two-week-old occipital scalp graft, *between the 2 arrows*. All of the graft epidermal appendages have disappeared and the graft dermis has been replaced by fibroblast-rich host connective tissue. The demarcation between graft and host tissue does not exist. The *arrows* indicate where the demarcation existed. Frozen section (60 μ) (hematoxylin and eosin, Ca, reduced from ×20).

Two months after transplantation the epidermis and dermis of the graft skin were similar to those of the neighboring host skin. With the hair follicles were fully developed sebaceous glands and piloarrector muscles. There were AChE-positive nerve fibers in the piloarrector muscles, and proliferating nerve sprouts from the subcutaneous nerve bundles deep in the host dermis.

Four months after transplantation, graft and host tissues were indistinguishable except that the graft had large terminal hair follicles (Fig 6); the surrounding bald frontal scalp of the host had typical small vellus-type follicles. All hair follicles, graft and host, were surrounded by AChE-positive end organs; piloarrector muscles contained dense plexuses of nerve fibers. All of these were connected to the nerve fibers in the underlying host skin (Fig 7).

Seven years after transplantation, the graft skin still had large terminal hair follicles with no sign of regressive changes. All the follicles in the graft skin had intact nerve end organs and the piloarrector muscles were well innervated.

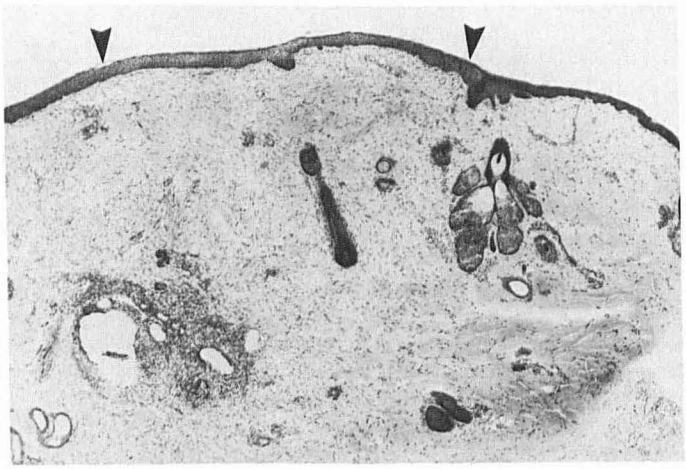

FIG 5. Four-week old occipital graft, *between the 2 arrows*, with a reorganized, growing hair follicle. Frozen section (60 μ) (hematoxylin and eosin, Ca, reduced from ×20).

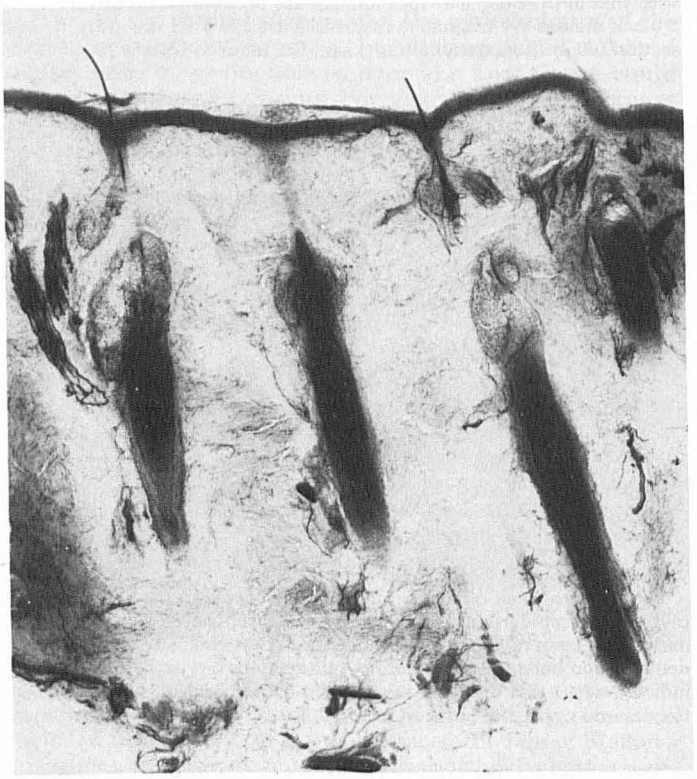

FIG 6. Occipital graft after 2 mo, with large, fully reinnervated hair follicles. The 2 vellus follicles are from the host tissue. Frozen section (80 μ) prepared for AChE (Ca, reduced from ×20).

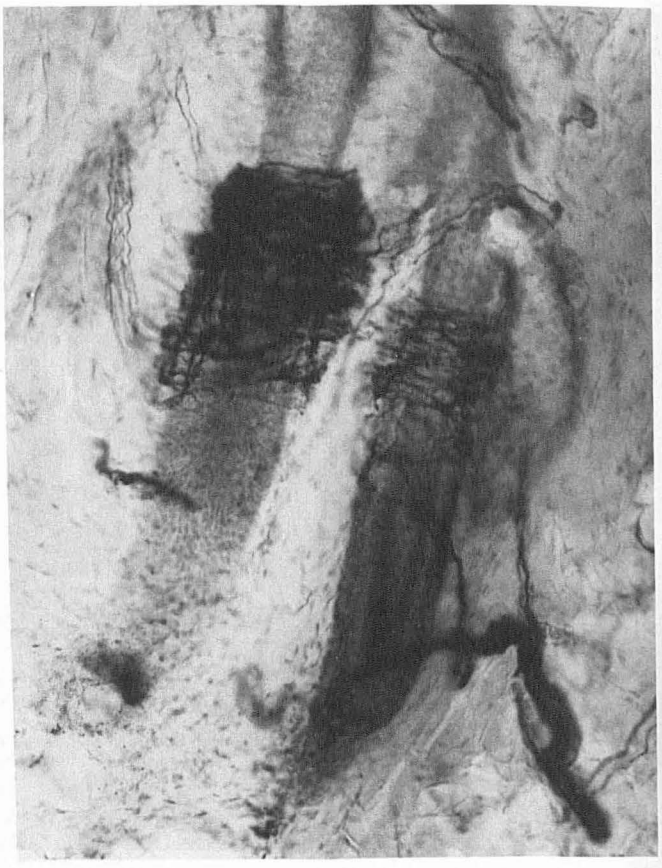

FIG 7. Details of fully restored, normal hair follicle nerve end-organs from Fig 6 (Ca, reduced from ×100).

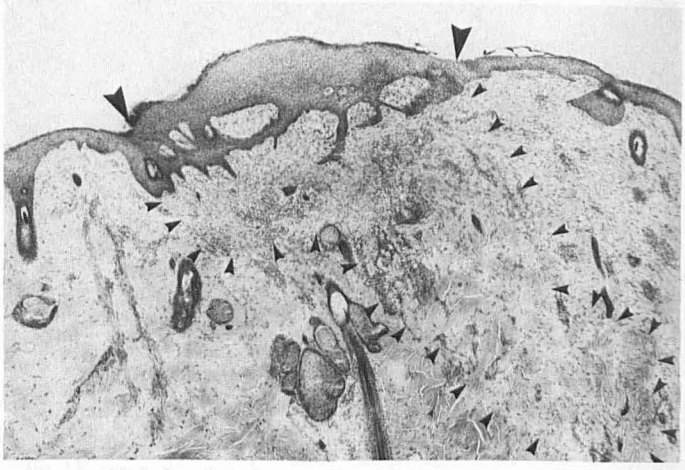

FIG 8. Digital pad graft 1 mo after transplantation with the epidermis fused with the host scalp epidermis. There is no thick horny layer on the epidermis of the graft. This is an oblique section and the graft tissue is indicated by the *arrows*. Frozen section (60 μ) (hematoxylin and eosin Ca, reduced from ×20).

Transplantation of Digital Pad Skin to Frontal Scalp

Gross observation: During the first 2 to 3 weeks after transplantation, the surfaces of the grafts were covered with a dry crust. After about 1 mo the dried tissues were shed from the graft surface and the remaining surface epidermis was pink and soft. This new epidermis gradually became dark brown and ridged. The graft remained clearly distinguishable from the white smooth skin of the host scalp for longer than 4 mo.

Histological observation: One month after transplantation, the graft was completely fused with the host tissue (Fig 8). The epidermal layer of the graft skin was much thicker than the adjoining host epidermis and had gradually elongated rete ridges; there was no thick keratinized layer on the epidermis at this time. The dermis of the graft was replaced by a vascular, fibroplastic tissue infiltrated, in the perivascular area, by some leukocytes. There were no AChE-positive nerve fibers and no Meissner corpuscles.

Two months after transplantation, the epidermis of the graft had elongated rete ridges, a thick keratinized layer, and a conspicuous granular layer (Fig 9). The dermis consisted of

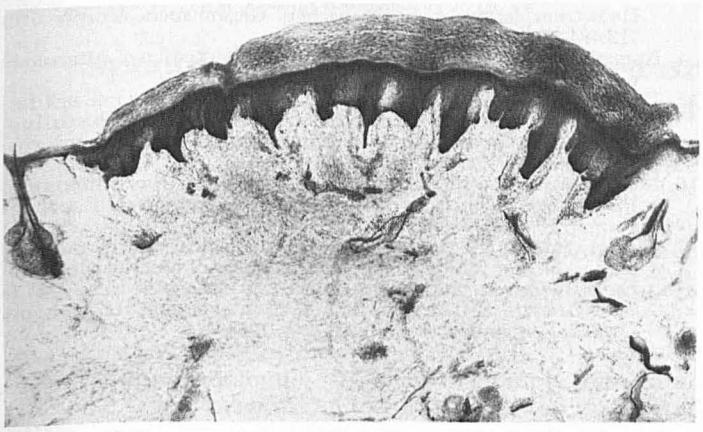

FIG 9. Fully reconstituted digital skin graft, with keratinizing epidermis. Even the ducts of nonexisting sweat glands have regrown. Frozen section (80 μ) (hematoxylin and eosin, Ca, reduced from ×20).

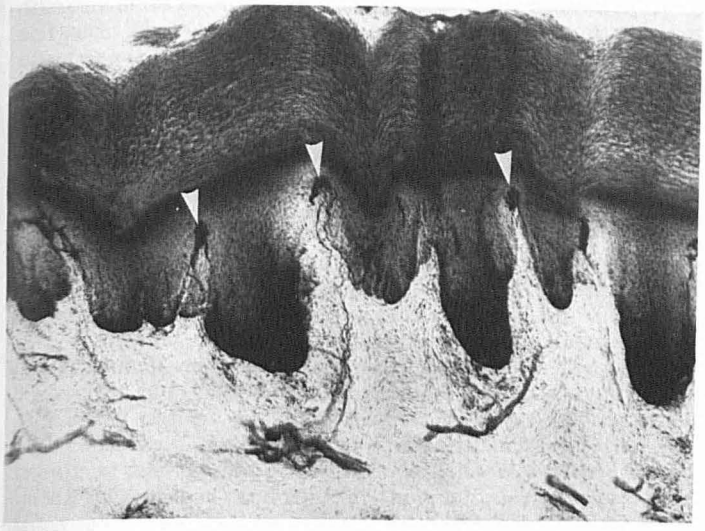

FIG 10. Reconstituted digital skin graft, showing reformed and normal Meissner corpuscles connected with nerves (*arrows*) that arise from the host tissue. AChE preparation (Ca, ×60).

vascular, fibroblast-rich tissue with a mild degree of leukocytic infiltration. Meissner corpuscles and AChE-positive nerve fibers appeared in the dermal papillae of the graft skin. The corpuscles were connected with nerve fibers arising from the underlying host skin. Thick, AChE-positive nerve bundles and perivascular plexuses were also found in the dermis of the graft.

Four months after transplantation, the epidermis of the graft was typical of digital pad skin. On its surface were typical dermatoglyphic patterns. Moreover, the many Meissner bodies in the dermal papillae received nerve fibers from the host dermis (Fig 10). The structure of the Meissner bodies was similar to that in normal digital pad skin; each had an elongated spherical body surrounded by lamellated structures (Fig 11).

DISCUSSION

The bald scalp of adult stump-tailed macaques is a suitable recipient site in which to observe the fate of skin grafts. Baldness in these animals is not a pathological condition but a natural hypoplastic change in the hair follicles that occurs at puberty [6, 7].

Regrowth of the cutaneous structures in the graft appears to be from the epidermal stem cells in the donor skin by means of a genetic blueprint. Occipital skin grafts regrow large follicles

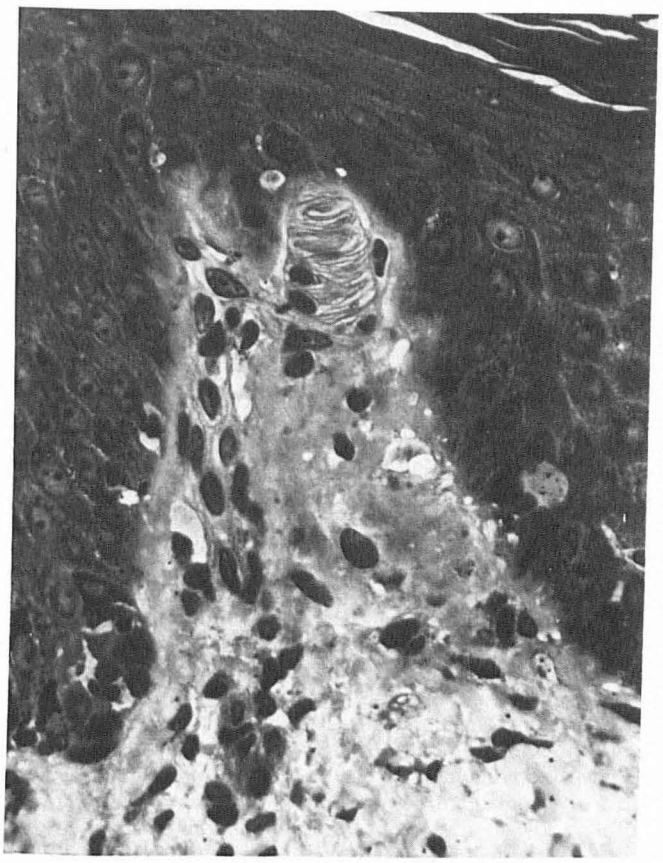

FIG 11. Lamellated Meissner corpuscle in methacrylate-embedded section (1 μ) stained with methylene blue (Ca, ×1,000).

similar to those in the original plugs. Digital pad skin regrows the thick, keratinized epidermal cell layers and dermatoglyphic inscriptions characteristic of friction surfaces. After repair, once the graft tissues have completed their remodeling, they maintain these structural properties for years without showing effects of host skin influence.

We make the above statements with caution lest it be assumed that we claim to have demonstrated a de novo formation of hair follicles from the graft epidermis. We are fully aware that during tissue reorganization existing hair follicles can be reduced to mostly irrecognizable tissue rests. A study of the minutest details of these events is now in progress.

Unlike the epidermal components, the majority of dermal tissues in the graft appear to be replaced with host dermal tissues. Shortly after transplantation, there is vascular, fibroplastic tissue in the dermis of the host-graft border. The original dermal collagenous tissues of the graft dermis are replaced by this vasculofibroplastic tissue; only then does remodeling of the dermal structures occur. During these sequences, proliferation of both perivascular nerves and somatosensory nerves from the subcutaneous nerve bundles are often seen in the underlying host dermal tissue. Meissner corpuscles cannot be seen in the graft about 1 mo after transplantation, but they and their connections with host nerves are seen after 2 mo. End organs of hair follicles in the grafts are also re-formed after 2 mo.

In the study reported here, we showed that Meissner corpuscles in grafts, which are heterotrophic organs in scalp skin, become connected with sensory nerves in host scalp skin. Regenerating axons from the host cutaneous sensory nerves, then, not only connect with or reform hair follicle end organs, but also connect with other types of cutaneous sensory receptor organs in the graft. The graft, then, must determine the fate of the invading nerves from the host.

Although sensory recovery of graft skin can be proved only through physiological examination, the results of these morphological approaches clearly suggest a potential for recovery of the cutaneous sensory receptor-neurite complex in graft skin.

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