half of K on a weight basis. It obviously was not 3 times as effectve as postulated above. On an atoms percent basis K was, to the contrary, about 3 times as effective as Rb in the adults and 6 times as effective in the experiments with infant rats. Also since K is essential to growth it would be proportionately more effective than Rb in the growing animal as compared to the adult.

Summary. Increasing the K intake of rats above requirements reduced the half-retention time for ^{134}Cs but at a decreasing rate. Maximum intake was 5% which resulted in $T_{\frac{1}{2}}$ of 6 days. The Na added at 0.3 or 2% to high K diet had no influence on the $T_{\frac{1}{2}}$ for ^{134}Cs. The influence of Rb salts on ^{134}Cs retention would indicate that this ion is less effective than K. The decreased effect of Rb may be due to its failure to adequately substitute for K in normal muscle cell metabolism.

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Transplantation in the Marmoset (*Callithricidae*): A Technique for Orthotopic, Fitted Skin Grafts* (32823)

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The marmosets, among the smallest of the New World (Platyrrhin) primates, have in their reproductive physiology three features that make them unique among mammals: (a) a greater than 80% frequency of twinning occurs (1,2); (b) the twinning is considered to be almost always dizygotic (2); and (c) a consistent placental vascular anastomosis occurs between the fetal twins (2,3). A natural, apparently inclusive, hematopoietic chimerism resulting from the biologic interaction of these three reproductive qualities has been demonstrated by Benirschke *et al.* (4) and Gengozian *et al.* (5). To the transplantation immunologist, therefore, the marmoset offers a unique biologic tool for the study of cellular immunologic tolerance. Thus, natural chimerism, occurring early in embryologic development, suggests that a state of immunologic tolerance must exist between the two genetically determined, different blood-forming systems of such twins. Studies on rare dizygotic humans and in more numerous cattle twins having natural chimerism have shown an almost indefinite allograft tolerance (6,7). To test tolerance to skin allografts between marmoset twins, it was necessary to develop a reproducible autograft technique. This paper sets forth procedures that permit successful skin grafting in the marmoset.

Materials and Methods. Animals. One of the three species of white-lipped tamarins, *Saguinus fuscicollis ssp.*[3] Hershkovitz (8), members of the marmoset family *Callithricidae* was used in this study. These tamarins, with

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[3] Designated as *Tamarinus nigricollis* in our previous publications.

an adult body weight ranging from 250 to 350 gm, typify the advantages and problems of this taxonomically diversified group of subhuman primates imported from the upper basin of the Amazon River (Peru and Colombia). Random members of a large colony that had been in captivity from 2 to 18 months were used. Information concerning handling, dietary, and environmental needs has been published (9).

Surgical anesthesia. For skin surgery, satisfactory results were obtained with local anesthesia. A subcutaneous injection of xylocaine administered to the site sufficed, but only if the animal was tranquilized deeply. A tranquilizing drug, phencyclidine,[4] given intramuscularly at the dosage of 0.2 mg/100 gm of body weight, is quite effective. Nembutal given intravenously, at a dose of 2.5–3.0 mg/100 gm of body weight, gave satisfactory parenteral general anesthesia for surgery. However, for skin grafting, we prefer a local anesthetic in conjunction with a tranquilizer rather than any of the general anesthetics. Open-drop ether or methoxyflurane[5] to a tranquilized tamarin produced an optimal plane of anesthesia, but usually gave rise to respiratory difficulties if the animal was anesthesized for more than 1 hour. Anesthesia was also attempted on a single occasion with an electroanesthesia instrument[6] with and without the use of a tranquilizing drug. Even with a tranquilizing drug a satisfactory surgical plane of anesthesia was not obtained. Although analgesia was complete, relaxation was poor and the body trembled so much that the animal would have been a poor subject for surgery.

Selection of graft site. Three sites were investigated: the chest, back, and tail. The ventral aspect of the rib cage proved to be the most favorable. The advantages of this area are (a) it has good subgraft mechanical support; (b) it is readily bandaged to prevent early mechanical removal during the important primary union of the donor skin to the graft bed; (c) the pelage there is considerably less dense than on any other part of the body; and (d) the site is convenient for easy observation when the animal perches in its cage. The other sites investigated were the dorsal rib cage which was occasionally used with success and the surface of the tail. With the latter site, problems arose in securing the bandage around a tail graft and in standardizing the graft area; more important, when the animal leaps about the tail strikes the side of the cage, traumatizing the area repeatedly. Thus, tail skin grafts are not recommended.

Size of skin graft. A reasonable graft size technically in the tamarin is a circle 9–10 mm in diameter. For the larger adults four such grafts on each side of the chest is the maximum if succeeding pairs of grafts are transplanted after the preceding pair has healed or been rejected. Larger (15–20 mm diameter) grafts survived as well as the intermediate size graft (9 mm diameter), but valuable space over the rib cage is wasted if additional grafts are anticipated. Smaller transplants (5 mm diameter) were difficult to suture and offered only a few hair follicles as markers for assessment of survival. The sites for these smaller-sized grafts also tended to contract more than those of the intermediate and large-sized grafts.

Hair growth as a criterion for selection of graft site. Waxing and waning of all physiologic activities in skin, most conspicuously reflected by variations in hair growth, has been reported to affect survival time of skin grafts (10–12). The prolonged resting or *telogen* phase of hair growth in rodents, for example, affords grafted skin a better chance of survival than the rapidly proliferating or *anagen* stage. Therefore an attempt was made to learn whether such phases could be discerned in marmosets. A number of prospective graft animals were closely clipped of their body hair biweekly for several months. In some animals regrowth was virtually absent for as long as 3 months and animals having the *telogen* phase over the prospective site could be easily chosen from the colony as needed.

Grafting technique. A tamarin, selected on the basis of hair growth (*telogen* phase) at

[4] Sernylan (phencyclidine hydrochloride), Parke Davis & Co., Detroit, Michigan.

[5] Penthrane, Abbott Laboratories, North Chicago, Illinois.

[6] Hewlett-Packard model #2380B, Palo Alto, California.

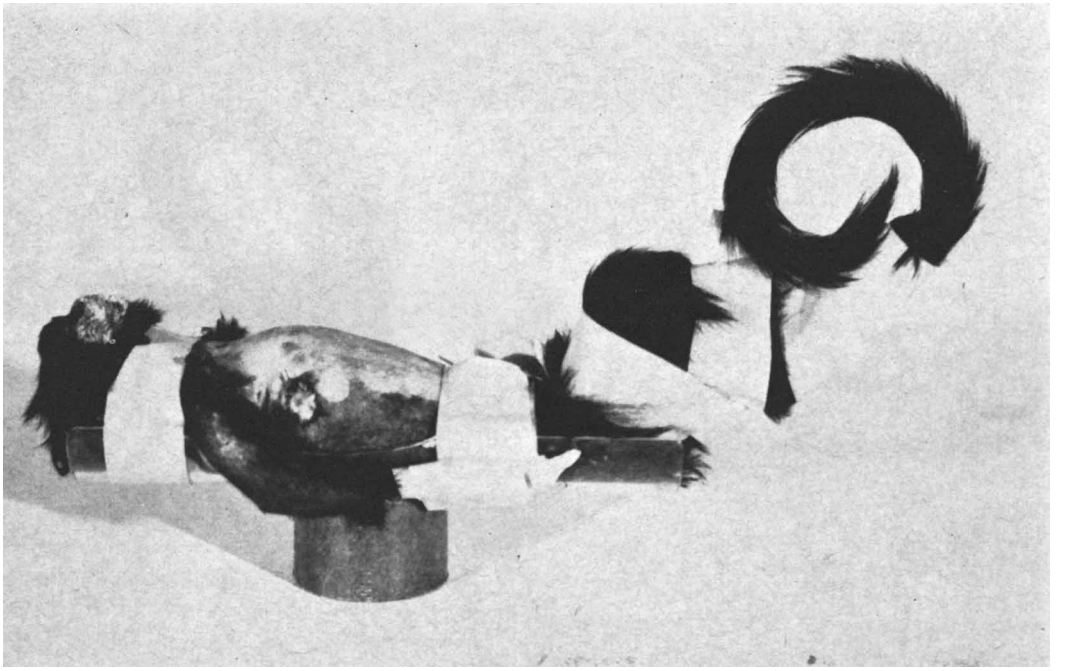

FIG. 1. Tamarin fastened to a holding device for skin grafting procedure. The surgical drape has been removed, revealing a recently sutured graft.

the intended graft site, is captured with a net and tranquilized with phencyclidine. The tranquil animal is closely clipped of its circumferential body hair from the lower abdomen upward to the shoulders. The medial aspects of the arms, the axilla, and any protruding long hairs from the neck and shoulders are also clipped. The tamarin is then washed from the neck down in a warm surgical-soap[7] bath. Depilatory cream[8] is applied generously over the upper trunk to the axillas. After 2 min the animal is washed with fresh warm surgical-soap bath, towelled dry, and kept warm on an electric heating pad.

A holding device, shown in Fig. 1, made from a split lucite tube 5 cm in diameter and a lead weight, is used to restrain the animal's movements and allows free rotation of the animal for a right- and left-side procedure. Masking tape, 2.5 cm wide, is used to fasten the animal's waist and neck to the holder. Its arms are held by its sides by taping the hand to the lucite. Its legs and tail are restrained in a stretched-out position by spiralling tape from foot pads to thigh. The chest is covered with a surgical drape having a 6.2 cm adhesive-backed aperture.[9] A dry gauze square is placed between the body and arms to absorb blood from the surgery. The face is left uncovered. Stray loose hairs over the prepared surgical area are removed with adhesive surface of a cellophane tape. With freshly prepared antiseptic solution, thimerosal (1:1000), the exposed draped site is further cleaned and then dried rapidly by applying 70% ethyl alcohol.

Surgery is performed with boiled instruments, sterilized materials, and no-touch sterile technique. The site is injected subcutaneously with 0.5 ml of 0.5% Xylocaine. A circular mark is made on the center of the taut skin surface with a dye-dipped 9-mm diameter cork bore. The tautness of the skin allows a consistently uniform area of skin to be marked. A very light incision with a scalpel defines the shape outlined by the dye. A

[7] Phisohex, Winthrop Laboratories, New York, New York.

[8] Surgex, Crookes-Barnes Laboratories, Inc., Wayne, New Jersey.

[9] Steri-Drape, Minnesota Mining & Manufacturing Company.

sheet of epidermis and some of the dermis within the boundary incision is dissected from the superficial fascia with a No. 15 scalpel and fine "mouse tooth" forceps. The strokes of the blade are directed nearly perpendicular to the surface so that the larger blood vessels parallel to and supplying the dermis are undisturbed. If the blade inadvertently perforates the superficial subdermal fascia a new edge and direction of strokes must be started because the cut layer of fascia adheres to the dermis and the plane of separation between the two is not easily regained. Sometimes, removing this small piece of skin properly requires 10 min and more than one scalpel blade. The removed skin is not processed further. If it is not to be immediately grafted, it is placed on sterile saline-moistened gauze in a covered dish; the wound is covered with the surgical drape. The graft bed need not be processed further.

To clearly identify transplanted skin, a graft is always planted so that hair protruding from it is opposite to the direction of the hair in the implant area; hair color and skin pigment are also used as markers. The graft is first positioned with four interrupted tie-down sutures, and then completely sutured in place with as many uninterrupted stitches (usually 10) as necessary. The uninterrupted stitches serve as a "purse string" and keep the edge of the defect from expanding. The surgical site is then cleaned with aqueous thimerosal (1:1000).

Graft sites are circumscribed with a water-soluble antibacterial ointment[10] and covered with sterile cloth impregnated with sterile petroleum jelly before applying the final bandage. Two opened dry 5-cm gauze squares are placed over this lubricated dressing. The dressing is covered with a cast consisting of a piece of wet plaster of paris bandage, 7.5 cm wide and long enough to overlap slightly after surrounding the upper trunk. To prevent this cylindrical body cast from slipping down and exposing the graft sites, cross-harness straps of rolled 4-cm wide plaster of paris bandage are crossed over the shoulders. The harness straps are tied with twine, front and back, to form a round collar around the neck. This is necessary to prevent the animal from catching its long lower canines in the fibers of the cast material. Figure 2 shows a tamarin with a completed protective cast with its waist strapped to the holding device. The entire procedure for two grafts is complete in about 1 hour.

Graft inspection. After day 6, grafts are well united to the bed; the casts and sutures can be removed on days 6 to 8. To capture the tamarin with minimal excitement, it is coaxed into a removable side-cage box. A tight fitting lucite slide door closes the box that serves as an anesthetic chamber. Methoxyflurane is introduced into the chamber and the animal is removed when quiet (3–5 minutes). The cast is cut away with scissors. The sutures are removed, the sites cleansed with thimerosal (1:1000) and coated lightly with an antibacterial ointment.[10] The procedure requires about 5–6 minutes. The tamarin is released into a clean cage by itself for 1 week. After the tenth postgraft day the animal may usually be captured in a net and placed with other animals without fear of damage to the graft.

Results. Of 50 successive autografts attempted by the technique described, only two failed. One 7-day-old graft that appeared to be in good condition was scratched out by the animal soon after the sutures had been removed. In the other instance, one half of the graft was necrotic and dry when first examined at 7 days. The other half survived. The area of necrosis corresponded exactly to an underlying defect in the superficial fascia that had been produced inadvertently when the skin was being removed from that graft bed.

In general, during the observable course of primary union (first inspection onward) autografts were slightly edematous, with a supple but dry and sometimes scaly surface, until about day 20. Slight erythema was usually noticed that lasted until onset of rapid hair growth. The pigment of the grafted skin remained the same; there was no evidence of pigment spread. The dead cast of graft epithelium, i.e., "ghost-graft" of Billingham and Medawar (13) became noticeable only when grafted skin was deeply pigmented. A slight transient eschar was usually found at the

[10] Furacin soluble dressing, Eaton Laboratories, Norwich, New York.

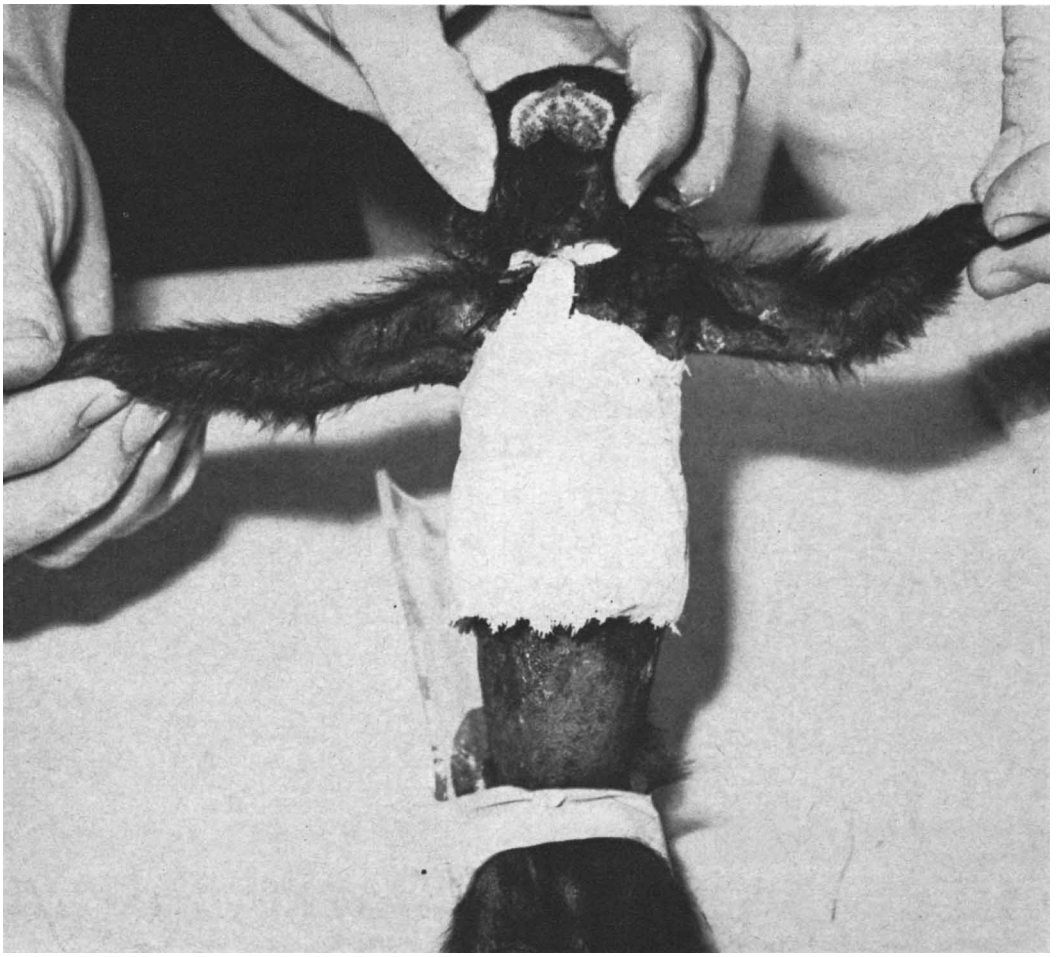

FIG. 2. Tamarin with a freshly applied protective plaster of paris cast covering chest skin grafts. Cross-harness straps are tied in front and back to form a collar to prevent animal's teeth from being caught in fibers.

edge of the graft during the early periods of observation. Infections were not evident; however, local foreign-body inflammation was usually observed around the sutures.

Some animals died after the grafting procedure. The percentage dying postgrafting (up to 30 days) was about double the percentage for the remainder of the colony during the same period (12% vs 5%). Of the animals dying at 4, 7, 7, 19, 20, and 25 days after grafting, 4 had symptoms of pneumonitis; 2 had had evidence of a slight respiratory infection even before surgery. In the remaining 2 animals cause of death was not ascertained. The grafts on dead animals always appeared to be in as good condition as grafts of the same age on living members, and were considered surviving.

Autografted skin tissues from the dead animals were fixed and prepared for histologic studies. Tissue from autografts transplanted for more than 30 days was also obtained from the dead animals because some of them had been used for more than one transplant. In the 4- and 7-day grafts, the epidermis was hypotrophic. New hair follicles and other appendages of the dermis were growing in from a hypertrophic epithelium in the 19-day graft. Sections from a 70-day dorsal and a 32-day ventral graft (Fig. 3) revealed well-healed graft skin with regrown hair. The ventral skin section still showed cytologic evidence of a

low-grade traumatic inflammation that was observed grossly during the first few weeks after grafting, as expected at this stage of healing (13). The sebaceous glands were hypertrophic.

Position and appearance of three consecutive fitted skin autografts to the chest are shown in Fig. 4. This photograph illustrates the graft-marker techniques of skin pigment and hair direction used throughout this study.

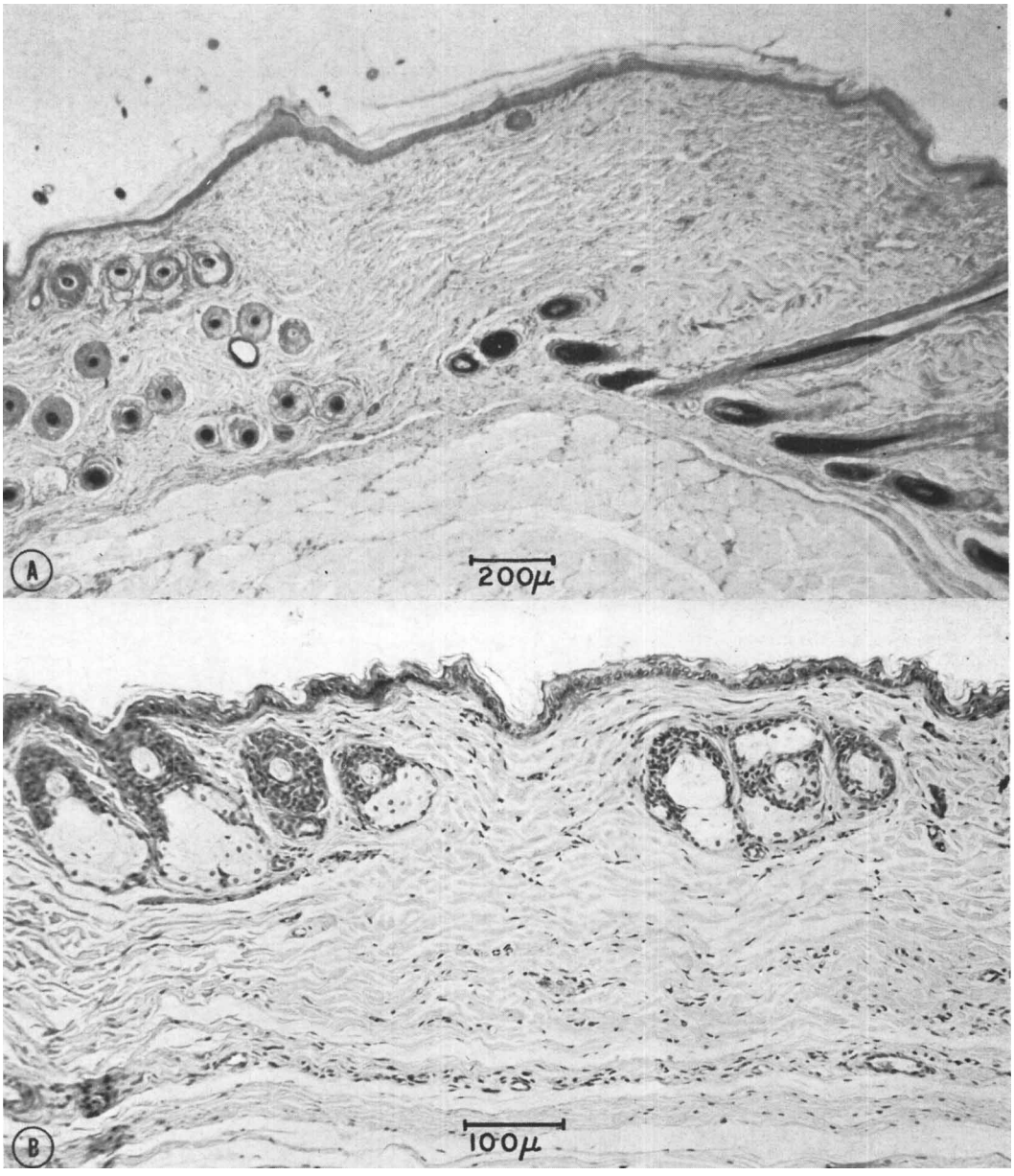

FIG. 3. Autograft skin sections. H & E stain. (a) A day 70 dorsal thoracic graft showing hair follicle bulbs in cross section and the recipient area follicles in a longitudinal plane indicating 90° rotation of graft skin. (b) A day 32 ventral thoracic graft showing sebaceous glands associated with young hair follicles still hypertrophic at this time. A greater number of follicles are present in the dorsal skin than in the ventral.

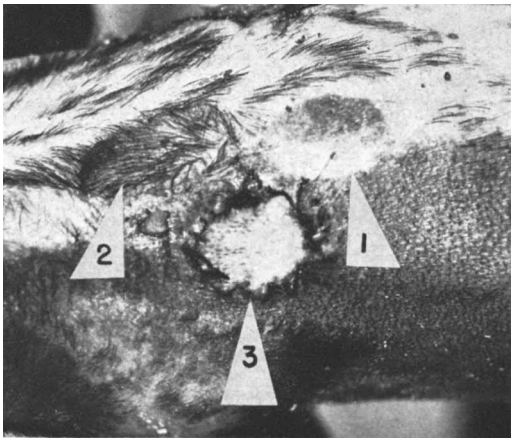

FIG. 4. Position and appearance of three consecutive 9-mm diameter autografts to the right chest wall (photograph taken at time of first inspection of third graft). 1) First graft at day 112; pigmentation of one half of graft serves as a marker; no hair regrowth at day 7; graft in *telogen* phase. 2) Second graft at day 56; 7-day hair regrowth equal to scattered sites observable on center of chest, *anagen* phase; direction of hair opposite to neighboring skin serves as a marker. 3) Third graft at day 7; sutures not removed, pigmented skin area vs a nonpigmented graft skin as a marker.

Competently transplanted full-thickness skins reassumed, with time, the appearance of the original donor area and grew hair. Figure 5 shows a day 60 transplanted full-thickness graft having a full complement of hair growing in the opposite direction to the surrounding area.

Discussion. Autografts must be accepted with regularity before immunologic experiments using allografts can be considered. To assure viability of marmoset skin, and to standardize dose effect of graft antigens by the method described, we believe the following procedures should be followed: (a) use of donor skin that is in the same phase of hair growth consistently, *telogen* phase preferably; (b) size of graft be consistent; and (c) preparation of a graft bed that has only a slightly damaged, or wholly uninterrupted underlying principal blood and lymph vasculature. For survival of donor skin it is essential to keep an intact underlying superficial fascia. Like the chest-wall skin of the guinea pig, mouse, rat, and man (13), the marmoset does not have a *panniculus carnosus,* or underlying muscle layer, associated with it. The absence of this muscle layer and a *panniculus adiposus* (14) makes it difficult to dissect the skin down to the exact level of the areolar connective tissue above the principal vessels of the skin. This loose areolar connective tissue, which allows the integument as a whole to move freely besides supplying the graft with necessary nutriment and cellular components via its blood vessels, may also accomplish the following: (a) Provide the graft a flexible bed over the underlying chest musculature because without this fascial layer the skin would seek vascularization from the muscle; the initial weak blood supply from the muscle surface very likely becomes detached during rigorous body movements and thus has a poor chance of survival. (b) Prevent undue lateral stretching of the edge of the wound, allowing healing of the edges of the graft by primary intention.

The development of a successful technique for orthotopic transplantation of marmoset skin will now permit us to consider the question of immunologic tolerance between fraternal twins. Skin transplant studies using co-twins known to have hematopoietic chimerism are in progress.

Summary. The marmoset, a small New World primate, *Callithricidae,* is known to be naturally endowed with blood-cell chimerism. The high frequency of fraternal twinning with placental vascular anastomosis between young in this family explains this consistent mosa-

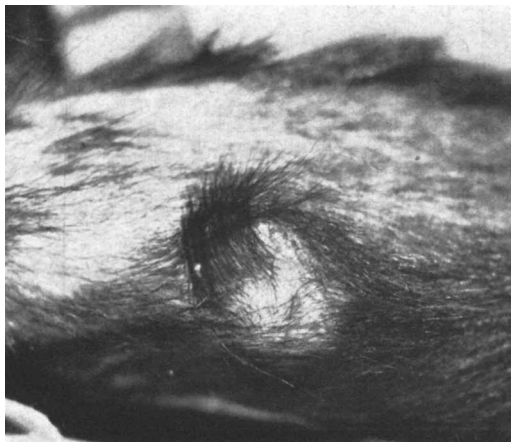

FIG. 5. Day 60 skin autograft to the chest showing complete hair regrowth.

icism. This unique feature suggests hematopoietic tolerance. In anticipation of skin allograft studies to demonstrate immunologic tolerance between twin marmosets, skin autografts were attempted. A successful technique for fitted skin grafts to the chest emerged from the consideration of hair-growth phase, graft size and site, surgical anesthesia, control of infection, and mechanical protection. Of a series of 50 consecutive autografts reported, 48 survived.

We wish to express our thanks to Gretchen Humason for her work in preparing the histologic sections; we are indebted to and thank Ralph Hall, D. V. M., for his assistance with the electroanesthesia instrument. We also wish to thank Tom Smith, Mark Brewer, and James Kline for their technical assistance in handling and maintaining the animals.

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Sudomotor Activity as Affected by the Thermal Status of the Skin in a Warm Environment* (32824)

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Although human eccrine sweating is primarily mediated through an alteration of the intracranial temperature, an influence of skin temperature on sweating activity has been demonstrated by several investigators (1, 3, 10, 13, 14). In an earlier publication (4) from this laboratory it was concluded that the cutaneous thermal status can affect the sweat output in two ways. One of these acts through the reflex pathways involving the peripheral thermal receptors and the central nervous system and the other, a direct effect, acts locally, either on the sweat gland or on the neuroglandular junction. This latter effect is observable within the range of ambient temperatures in which sweating normally occurs; and thus is considered to be different from the direct effect noted by Randall (8) and by Issekutz *et al.* (6) where sweating was initiated at a very high skin temperature in a cooler environment.

Attempts were made to further characterize the influence of the thermal status of the skin on the rate of eccrine sweating acting by two different mechanisms. The results of these experiments are reported herein.

Methods. The experiments were carried out on 6 healthy male subjects, 20–33 years old at different ambient temperatures. Cutaneous

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