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REINNERVATION OF ALLOGRAFTED PRIMATE UPPER-EXTREMITY TISSUES IN THE PRESENCE OF CYCLOSPORINE IMMUNOSUPPRESSION

by

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To my parents, Yvette and Stephen,
for pointing me in the right direction.
To my teachers for showing me the way.
And, to my wife Janette
for her willingness to understand
and to encourage my love for science.

ABSTRACT

The capability of axons to grow into a peripheral histoincompatible environment, to locate and to functionally innervate their target structures, was examined through documentation of form and function in nonrejected and rejected upper-extremity composite tissue allografts in adult baboons; *Papio h. anubis*. Immunosuppression with cyclosporine (radioimmunoassay levels in serum ranging from 1000 to 1200 ng/ml, 12 h after intramuscular injection), supplemented with low doses of methylprednisolone (4.4 mg/day) was sufficient to achieve long-term survival of seven neurovascular free flaps and two hand transplants.

Electrophysiological recordings of more than 600 single axons, combined with light microscopy of the target tissues, revealed that muscle and most classes of skin sensory mechanoreceptors within the allografted tissues became reinnervated. Axons which served allografted tissues that had undergone repeated episodes of rejection showed a significant decrease in conduction velocity, and smaller receptive fields of irregular distribution. Processes of allograft rejection, more than any other factor, led to altered axonal response characteristics.

RÉSUMÉ

La capacité des axones à se régénérer dans un environnement histoincompatible, à localiser et à réinnerver de façon fonctionelle leurs structures cibles a été examinée du point de vue anatomique et fonctionnel chez des babouins adultes (*Papio h. anubis*) ayant subi des allogreffes composites des extrémités supérieures rejetés ou non-rejetés L'administration de cyclosporine utilisée comme agent immunosuppresseur (dont les niveaux sériques mesurés 12 h après administration varient de 1000 à 1200 ng/ml), supplémentée de faibles doses de methylprednisolone (4.4 mg/jour) fut adéquate dans l'obtention de la survie à long-terme de sept lambeaux neurovasculaires et de deux transplants de main.

L'étude électrophysiologique d'environ 600 fibres nerveuses, combinée à l'examen microscopique du tissus greffé, a revélé que les muscles et que la plupart des types de méchanorecepteurs sensoriels cutanés dans les tissus allogreffés furent réinnervés. De plus, les axones regénérés dans le tissus ayant subi des épisodes répétés de rejet démontrent une baisse significative de leur vélocité de conduction, et des champs récepteurs de taille réduite et de distribution irrégulière. Le processus du rejet des tissus allogreffés est à la source, plus que tout autre facteur, des modifications observées dans les caractéristiques physiologiques des axones.

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LIST OF ABBREVIATIONS

AP ..... action potential
BabLA ..... baboon leukocyte antigen
CNVFF ..... control neurovascular free flap
Co ..... company
Corp ..... corporation
Cs ..... cyclosporine, cyclosporin (generic compound)
CsA ..... Cyclosporin A (Sandimmune®, Sandoz Ltd.)
CV ..... conduction velocity
D1-D5 ..... first digit (through to the) fifth digit
Ed ..... editor(s)
Edn ..... edition
EMG ..... electromyogram(graph)
Fig ..... figure
GF ..... grams force
H&E ..... hematoxylin and eosin
HT ..... hand transplant
HLA ..... human leukocyte antigen
HPLC ..... high performance liquid chromatography
IL-2 ..... interleukin 2
IM ..... intramu cular
Inc ..... incorporated
IV ..... intravenous
Lond ..... London
Ltd ..... limited
M ..... manufacturer's marking
MeBmt ..... N-methyl-(4R)-4-butenyl-4-methyl-(L)-threonine
MHC ..... major histocompatibility complex
Mo ..... month
MP ..... methylprednisolone (Solu-Medrol®, Upjohn)
mRNA ..... messenger ribonucleic acid
N, n ..... sample size
No, # ..... number
NVFF ..... neurovascular free flap

PAS ..... periodic acid-Schiff
PO ..... *per oris*, oral
pp ..... pages
RA ..... rapidly adapting
RF ..... receptive field
RIA ..... radioimmunoassay
SA ..... slowly adapting
SC ..... subcutaneous
SFBR ..... Southwest Foundation for Biomedical Research
SOL ..... statistics of location
S-R ..... stimulus-response
TNVFF ..... transplanted neurovascular free flap
Transl ..... translator
Wk ..... week
W-S ..... Weinstein-Semmes

PREFACE

Statement of Originality

This thesis describes the experiments which led to the first reported long-term survival and innervation of allografted upper-extremity composite tissues in an adult primate. In the context of a multidisciplinary approach, this thesis deals specifically with the survival, morphology and functional capabilities of the allografted sensory mechanoreceptors. This thesis illustrates that host axons can innervate foreign sensory mechanoreceptors in a histoincompatible tissue environment. More importantly, it provides new electrophysiological evidence to support the view that with the selective removal of specific mechanoreceptive structures through the processes of tissue rejection, sensory axons can still present reasonable thresholds of activation and submodality response characteristics. Anatomical and histological documentation is provided to depict the tissue disruption which took place as a result of the rejection of allografted skin, and the structural integrity of the sensory mechanoreceptors which survived. The documentation and descriptive analysis of each of these aspects within the thesis constitutes original contributions to knowledge. Short preliminary reports covering certain aspects of this study have been presented elsewhere [Daniel *et al.*, 1986; Egerszegi *et al.*, 1984; Samulack *et al.*, 1985, 1986, 1988, 1989; Skanes *et al.*, 1986].

Assistance by Others

Any effort of this magnitude is by its very nature and necessity a team effort. The success of these studies was very much attributable to the dedication of many individuals. Most notably were the surgical contributions of Patricia Egerszegi and Susan Skanes who successively, in their year of experimental research during their residency training, performed the microsurgery required for the tissue transplants. The primary motivator for the design and execution of the surgical procedures used in the allograft models was Rollin Daniel. Assistants involved in the surgical transplantation procedures in addition to myself, were Paul Ballard, Craig Howard,

Carolyn Kerrigan, William Rennie and Ronald Zelt. All medical decisions with respect to immunosuppressive and other medical protocols were made jointly between myself and either Patricia Egerszegi or Susan Skanes, under the supervision of Rollin Daniel. The technical aspects of the radioimmunoassay used to determine serum cyclosporine concentrations were performed primarily by Susan Skanes and Susie Spurdens. Support staff to care for the animals and to administer medications included Cory Dykes, Janette Green, Doris Gulbrandsen, Robert Jones, Joseph Kalash, Malia McAuliffe, Lisa Skanes, Saliesh Thaker, and Ronald Zelt.

In addition to my direct involvement as co-investigator throughout the long-term maintenance of the animals and their allografts, I remained responsible as primary investigator for all aspects of the neurophysiological and histological analysis of these tissues. Consultation, direction and assistance was offered by my doctoral supervisor, Robert Dykes. Additional assistance during the lengthy electrophysiological recording sessions was donated by Nick Calamatas, Janette Green, Noor Jehan Kabani, Heidi Kruitwagen, Serge Leclerc and Stephen Schertzer. Debbie Hinton and Brian Tucker were involved in the histological processing of the tissues. Valuable consultation was sought from Bryce Munger with regard to the interpretation of the histological findings. Angel Alonso, Robert Dykes, Janette Green and Robert Waters offered many helpful suggestions and proof-read earlier versions of this report. I was directly involved in all aspects of the data collection for this thesis, unless otherwise indicated. All of the data analysis and thesis preparation, with the exclusion of the fabrication of the final photographic plates, was performed by myself.

INTRODUCTION

I. Historical Perspective

Prior to the turn of the 20th century, anatomists like Meissner, Merkel, Pacini, Vater and others, either with the unaided eye, or with the use of hand held lenses, had already described many of the basic morphological characteristics of sensory nerve endings. Aided with the development of microscopy and the use of silver staining techniques, Ramón y Cajal [1928] published two volumes of scientific observations that still stand today as an authoritative effort. He described the basic structure of nerves and processes of nerve growth, with a detailed description of sprouting, the growth cone, and axon elongation across the site of nerve repair. As well, he discussed theories of trophic influences and guidance mechanisms that are still relevant today.

The question of function in regenerating nerves has evolved more recently Not until many nerve injuries were available during the First and Second World Wars, was a systematic effort made to improve microvascular anastomoses and the repair of peripheral nerves. These studies have been reviewed eloquently by several authors [Daniel and Terzis, 1977; Dellon, 1988; Woodhall and Beebe, 1956]. With the development of the vacuum tube amplifier and the oscilloscope, the electrophysiological study of nerve function had become the focus of basic research programs in which axon response properties were correlated with anatomical data in the same preparation.

In parallel with this progress, development in the field of immunology and the introduction of tissue transplant techniques gave hope that foreign tissues could be used to functionally replace a defective tissue or a tissue deficit. It was not until the discovery of the immunosuppressive properties of Cyclosporin A in 1972 and its subsequent use in experimental transplants, that chemically induced immunosuppression has been specific enough to prevent the rejection of allografted skin in adult animals.

These latter developments served to underline the extent of our ignorance with respect to the capacity of host axons to grow into and function in a foreign tissue environment, and to revitalize interest in processes of innervation and target tissue recognition. The present report documents the first comprehensive study involving parallel observations on the electrophysiology and anatomy of axon growth, survival and function in a histoincompatible extremity tissue environment of a primate species. It was only with the current understanding of the anatomical and electrophysiological characteristics of mechanoreceptor function, the development of successful immunosuppressive protocols, and the mastering of microsurgical technique, that these experiments were possible.

II. Mechanoreceptors and their Response Characteristics

Tissue structures that transduce mechanical energy from the environment into axonal responses have been studied in many species. Most relevant to the topic of this report are those mechanoreceptive structures which have been studied in the skin and underlying tissues of mammals; particularly rat, cat and primate. Investigators have succeeded in associating certain patterns of action potentials in primary afferent fibers with morphologically distinct cutaneous or subcutaneous structures. Although not all identifiable axonal responses have been correlated with a morphologically distinct mechanoreceptor [Horch *et al.*, 1977], and while there is still some discussion as to exactly how many structures there are, or how to best identify and classify them, it is now generally believed that relatively few classes of mechanoreceptors exist [Dykes, 1977, 1983; Mountcastle, 1980; Vallbo and Johansson, 1984]. In this regard, the literature reviewed in this report is not comprehensive, but rather it is intended to provide the reader with the necessary background concerning the tissue structures and response characteristics identified in this study.

Since the studies of Adrian and Zotterman [1926a, b] describing the nerve impulses produced by naturally stimulating sensory nerve endings in skin and muscle, the responses of primary afferent nerve fibers have been divided into two categories based upon their discharge patterns to continuous unvarying mechanical stimulation: rapidly adapting (**RA**) and slowly adapting (**SA**). Fibers characterized as RA detect transient mechanical stimuli, discharging a few action potentials (**AP**) upon the application (and in some cases the removal) of a stimulus, but not during steady application. Afferents characterized as SA respond to the onset of a stimulus with a high-frequency discharge determined by the rate of stimulus application as well as its final magnitude, and they continue to discharge for the duration of the stimulus [Mountcastle, 1980].

Munger [1971a, b] divided sensory mechanoreceptors into five groups on the basis of anatomical characteristics: corpuscular endings, epithelial cell-neurite complexes, fibers serving hair, dermal neural networks, and sense organs in muscle.

Corpuscular receptors consist of one or a cluster of sensory nerve fibers closely associated with specialized lamellar cells more or less segregated from the general connective tissue compartment by capsular elements [Munger, 1977]. For the purposes of this report, the Meissner and the Pacinian corpuscles are the two morphologically distinct receptor structures found in this group. In primate glabrous skin, the Meissner corpuscle is located at the top of the dermal papillae, close to the skin surface [Cauna and Ross, 1960; Hashimoto, 1973]. Through the correlation of electrophysiological studies of the response patterns of sensory afferents and the identification of corpuscles in morphological studies on the same tissues, it is thought that the Meissner corpuscle represents an RA mechanoreceptive unit [Lindblom, 1965; Merzenich and Harrington, 1969; Talbot *et al.*, 1968].

The other RA mechanoreceptive structure in primate glabrous skin (also in hairy skin) is the Pacinian corpuscle It is located deep in the dermis near the subcutaneous structures and has a large lamellated onion-like shape. The early electrophysiological characterization of these corpuscles was done in cat mesentery, and showed that although the AP is set up at the first node of Ranvier [Diamond *et al.*, 1956], the generator potential and hence the basic adaptational properties are characteristic of the axon's unmyelinated terminal segment within the core of the corpuscle [Loewenstein and Mendelson, 1965]. Although the terminal segment is capable of mechanosensory transduction, the rate of rapid adaptation and the mechanoreceptors sensitivity to decompression in addition to compression are related to the interaction between the nerve terminal and the lamellated capsule [Loewenstein and Mendelson, 1965]. The corpuscular lamellae of this receptor have been modeled as a high-pass mechanical filter, allowing only the rapid mechanical transients to travel to the core of the structure [Loewenstein and Skalak, 1966]. The extreme sensitivity of the mechanoreceptive unit to low amplitude (> 1 μm), high frequency (250-400 Hz) vibration, causes it to have an exceptionally

large receptive field (RF) [Munger and Ide, 1987]. The Pacinian corpuscle is the only mechanoreceptive structure in skin which is capable of following high frequency vibration [Gray and Matthews, 1951; Hunt, 1961].

Together, the Meissner and Pacinian corpuscles are believed to provide neural signals sufficient to account for the human subjective capacity to detect vibrations and thus constitute the physiological basis for the dual sensory modality of flutter-vibration in glabrous skin. The psychophysical studies of Talbot *et al.* [1968] were instrumental in these regards. Through the iontophoretic application of cocaine to the superficial layers of epidermis, it was shown that the sensory afferents terminating in superficial glabrous skin (at the location of the Meissner corpuscles) were involved in the mediation of the sense of flutter (a light-weight mechanical stimulus of 5-40 Hz), while afferents terminating in the deep tissues (at the location of Pacinian corpuscles) were involved in mediating the sense of vibration (60-300 Hz).

Although epithelial cell-neurite complexes form highly organized receptor structures, they lack an enveloping capsule and so cannot properly be classified as encapsulated receptors. A similar structure to the Merkel cell-neurite complex identified in the hairy skin of the cat by Iggo and Muir [1969] also resides in the glabrous skin of the cat [Jänig, 1971] and in raccoon glabrous skin [Munger *et al.*, 1971]. Through the correlation of electrophysiologic response properties and the proximity of morphologic structures, the Merkel cell-neurite complex has been characterized as an SA mechanoreceptor. In primate glabrous skin, Merkel cells are found in the epidermis at the dermal-epidermal interface on the tip of the papillae formed by the intermediate epidermal ridge [Cauna, 1954]. The Merkel cell, which is thought to be of epithelial origin [English *et al.*, 1980] and to differentiate in the periphery at a time which is very early in development [Breathnach, 1971; Breathnach and Robins, 1970; English *et al.*, 1980], is characterized by an irregular shaped nucleus and numerous electron-opaque secretory granules in the cytoplasm which are polarized towards the site of contact by the axon [Munger, 1977]. It is thought that they may guide appropriate axons to their location in the extremity tissues through trophic influences [Munger, 1971b]. Merkel cell-neurite complexes have also

been identified in primate hairy skin, primarily associated with sinus hairs, but have not yet been identified in association with monkey guard or vellus hairs which cover most of the body surface [Munger and Halata, 1983].

The Ruffini ending, a second type of non-encapsulated SA mechanoreceptive structure, was characterized both electrophysiologically and histologically in hairy skin of the cat by Chambers *et al.* [1972], and described electrophysiologically in primate glabrous skin by Vallbo and Johansson [1984]. This receptor consists of a group of neurites branching from a myelinated axon. No specialized transducer cells, other than the nerve terminal, have been seen in electron microscopic studies of the Ruffini ending. It is hypothesized, similar to the case of the Pacinian corpuscle, that the neuron itself must function as the mechanoelectric transducer [Iggo, 1974].

The electrophysiological criteria needed to distinguish between the Merkel and Ruffini endings depends upon details of the discharge characteristics [Chambers *et al.*, 1972; Horch *et al.*, 1977]. Although both respond in an SA fashion, axons serving Merkel cells are described as having little or no resting activity, an irregular discharge pattern and being relatively insensitive to lateral skin stretch. In contrast, Ruffini endings have a resting discharge usually between 15-30 AP/s in a nearly regular discharge pattern. These SA receptors are presumed to function in a similar manner in man, and as a result of their adaptational characteristics, are the only ones capable of providing the neural signals for the sensory experience of pressure, or constant touch.

There are at least three distinct morphologies for nerve fibers serving hair follicles in the majority of primate hairy skin [Munger, 1982; Munger and Halata, 1983]. Free nerve endings, thought to be associated with nociceptive function on the basis of physiological parameters, have been found to end blindly in the connective tissue capsule in the angle between the hair shaft and sebaceous gland [Kruger *et al.*, 1981]. In addition, lanceolate terminals like those first described by Andres [1966] are arranged in a parallel palisade with respect to the axis of the hair shaft, like the tines of a fork. They abut the basal lamina and usually encircle the entire

external root sheath. A third distinct nerve ending associated with most primate hair follicles is the pilo-Ruffini complex [Biemesderfer *et al.*, 1978]. Morphologically, the pilo-Ruffini complex is composed of terminal nerve fibers which branch repeatedly and, in association with a connective tissue matrix, forms a collar of axons around the hair shaft. The lanceolate terminals are possibly associated with RA responses from hair follicles [Gottshaldt *et al*., 1973; Iggo, 1974], while the pilo-Ruffini complex was shown by Biemesderfer *et al.* [1978] to represent the morphological correlate to the SA response on the basis of similarities in morphology and discharge characteristics to that of the Ruffini endings in hairy skin of the cat as described by Chambers *et al.* [1972] and the Ruffini corpuscle identified by Halata [1977] in cat knee joint capsule.

The network of neurons which course through the dermis and end in poorly defined manners or simply as free nerve terminals are assumed to be involved in coding for environmental stimuli that will be interpreted as cold, warmth, heat, pain, itch, and tickle (for review, see Besson and Chaouch [1987], Dykes [1983], Tuckett and Wei [1987a, b] and Spray [1986]). As indicated by Dykes [1977], if these assumptions are correct, the apparent homogeneity of free nerve endings must be due to limitations of our investigative techniques. In the present report, there was no attempt to study this class of sensory axons in detail, other than to identify their presence in some of the experimental tissues.

The last group of mechanoreceptors which are relevant to the present study are the sense organs associated with muscle and other deep structures. Annulospiral (group Ia) and flower spray (group II) afferents, as well as those of the Golgi tendon organs and joint capsules are well-established in the literature (for review, see Mendell and Henneman [1980]). For the purposes of this study which is primarily concerned with cutaneous mechanoreceptors, any afferent fiber which was suspected of serving one of these types of end organs was grouped together and labelled as the composite modality muscle/joint/tendon.

In summary, within primate glabrous skin there is thought to be four physiologically distinct axon response characteristics, each associated with a morphologically distinct

mechanoreceptive structure: Meissner corpuscles (RA-I), Pacinian corpuscles (RA-II), Merkel cell-neurite complexes (SA-I), and Ruffini endings (SA-II) [Vallbo and Johansson, 1984]. Thermoreceptors and nociceptors which are thought to be bare nerve endings displaying SA response characteristics. In hairy skin of the primate forearm, the hair follicle apparatus with its lanceolate terminals (RA) and pilo-Ruffini complex (SA) constitutes a very intricate mechanoreceptive structure. Pacinian corpuscles, thermoreceptors and nociceptors are also identifiable in hairy skin. The muscle spindles (SA), Golgi tendon organs (SA) and potentially other receptor structures located in the capsule and ligaments around the joints, provide information regarding mechanical deformation of the deep tissues.

III. Tissue Transplantation

Extremity tissues can be transferred between two locations on the same individual, or transplanted from one individual to another. Although these two concepts are usually mentioned separately, confusion sometimes arises when they are both labelled as tissue transplantation. It may be useful, therefore, to define certain terms which are commonly used in reconstructive surgery [Gingrass *et al.*, 1979] and transplantation biology [Porter and Lance, 1974; Bellanti, 1985], so that there is no misunderstanding as to the intention of their meaning throughout this report.

The term, extremity tissues, refers to those tissues (skin, muscle, nerve, bone, *et cetera*) which are not normally considered as internal organs, or organs of the special senses, but which make up the tissues of the arms and/or legs of an animal. Skin grafts can be referred to as split-thickness, full-thickness, flaps or free flaps, depending upon the depth at which the tissues were excised and the means of their reattachment. Split-thickness grafts include the epidermis and only part of the superficial dermis (depending upon the depth of removal), while a full-thickness skin graft includes all of the dermal structures and leaves behind only the subcutaneous tissues. The site from where the tissues were removed, or where they are to be placed, is referred to as the tissue bed. Flaps and free flaps do not necessarily contain only cutaneous tissues, but may also contain a composite of other extremity tissues. The flap may be peninsular and have one edge attached, or it may be an island flap having an intact pedicle (a vascular or neurovascular attachment which has not been severed). A flap may be replanted to its original bed, or transferred to an adjacent tissue bed. It may also be removed entirely as a free flap with a defined pedicle, and be transplanted to an alternate tissue bed which is far removed.

The term, transplanted, implies a procedure which includes anastomosis of the vessels of the transferred composite tissue to those of the recipient tissue bed. Implantation, in contrast, does not imply immediate revascularization of the graft. Therefore, a skin graft is said to be

transferred or implanted (but not transplanted), while a free flap is always referred to as having been transplanted.

A tissue which is transplanted from one site to another (the same, a similar, or a different site) on the same individual, is referred to as an autograft (autogenous graft). An isograft (isogeneic or syngeneic graft) refers to tissue which has been transplanted between two genetically identical individuals such as monozygotic twins. Tissues which are transplanted between two genetically dissimilar members of the same species are called allografts (allogeneic grafts or homografts). Finally, when tissues are transplanted between members of different species, the grafts are referred to as xenografts (xenogeneic grafts or heterografts).

A. Immunological Aspects

1. Definition of Histocompatibility

A grafted tissue is said to be histocompatible if the graft is capable of being accepted and remaining functional in a tissue bed of an immunologically competent host. This relationship is determined by cell surface glycoprotein molecules referred to as histocompatibility (or transplantation) antigens. In the case of incompatibility, these glycoprotein molecules are responsible for the induction of an immune response by the host, and ultimately graft rejection (see below).

The major histocompatibility complex (**MHC**) is a chromosomal region consisting of a series of genes that code for the cell surface expression of strong transplantation antigens. Two well-characterized systems of classification for these antigens are the human leukocyte antigen (**HLA**) system and the H-2 system in the mouse. A similar system of classification for the baboon leukocyte antigens (**BabLA**) is presently being developed [Templeton *et al.*, 1981].

The genes of the MHC in all mammals thus far studied, code for two general types of transplantation antigens: class I and class II. The human MHC is the best-characterized of the primate MHCs. On human chromosome 6, the structural gene loci HLA-A, -B, and -C, code

for the class I antigens, while the HLA-DR, -DC, and -SB loci code for class II antigens [Woody *et al.*, 1985]. These genes are inherited in a systematic fashion such that an individual receives genes from both parents. Each gene locus contains two alleles (alternate forms of the gene) that represent small variations in the nucleotide sequence of the gene. The small changes result in cell surface glycoproteins with small variations in structure, perhaps a change of one or two amino acids, leading to a small difference in three-dimensional structure. Within a population of individuals, there are many such alleles which can be detected through serological methods (where antiserums are used to detect cell-surface antigens), or by studying mixed lymphocyte reactivity in mixed leukocyte cultures [Bach and van Rood, 1976]. The HLA-B gene has the largest number of detectable alleles with at least 32 serum specificities, and is said to be the most polymorphic of the HLA gene loci.

Histocompatibility antigens are present on the surface of most nucleated cells, but the degree of expression and the variety of different antigens varies between cell and tissue types. Skin has been found not only to be more immunogenic than kidney [Sakai *et al.*, 1980], for example, but also to undergo rejection more readily than other tissues like muscle or bone [Lance *et al.*, 1971].

2. Mechanisms of Solid Tissue Allograft Rejection

Allograft rejection may be defined as the process by which the immune system of the host recognizes, becomes sensitized against, and attempts to eliminate the antigenic differences of the donor tissue. There are three main patterns of solid tissue allograft rejection which have been identified: (1) hyperacute rejection, leading to extremely rapid onset and severity of rejection, (2) acute rejection, and (3) chronic rejection. A detailed account of the cascade of cellular events which are involved in each type of rejection process is not within the intended scope of this report, rather, a short synopsis of the mechanisms of immune responsiveness in these regards is presented in order for the reader to understand the basis for the destruction of

foreign tissues by the host. The reader is directed to any good textbook on immunology, or to one of many review articles available, for a detailed description of the immunological repertoire.

The white blood cells or leukocytes continually enter a vascularized allograft *via* the blood circulation and exit through the lymphatic system. Each of the leukocytes play a direct or mediatory role in the inflammatory and cytotoxic processes involved in allograft rejection and/or the clean-up process after tissue necrosis. Acute rejection has been attributed primarily to the cytotoxic (cell-mediated) mechanisms of T-lymphocytes (T-cells) in association with mononuclear phagocytes (monocytes or macrophages). Both hyperacute and chronic rejection have been attributed to the antigen-antibody recognition (humoral) mechanism of allograft rejection which involves primarily the B-lymphocytes (B-cells). In reality, it is likely that both the cellular and humoral mechanisms contribute to each of the three rejection patterns [Häyry *et al.*, 1989].

The macrophage has been shown to be an essential cell that interacts with the T- and B-lymphocytes and is necessary for the induction of T-cell responses as well as T cell-dependent B-cell responses to antigen. Moreover, these cellular interactions of macrophages, T-cells, and B-cells appear to be genetically controlled (restricted) by the transplantation antigens (Class I and Class II molecules). It is through the recognition of these antigens during cell surface interactions and/or by the presentation of foreign antigens in association with these molecules (the role of macrophages), that 'self' is differentiated from 'non-self'. The exact molecular mechanisms of receptor specificity involved in this 'associative recognition' is not entirely understood.

In the realm of allograft rejection, the initiation of cell-mediated cytotoxicity requires the presentation of foreign antigens to T-helper lymphocytes (a subset of T-cells) by antigen-presenting cells such as the macrophage. Other specialized cells, such as dendritic cells or cutaneous Langerhans cells, that are part of, or related to, the monocyte-macrophage lineage may also serve this function. In part this explains why skin is so immunogenic, in that it

contains its own immunocompetent cells which directly present their cell surface antigens to the host's immune system upon transplantation. Macrophages also release interleukin-1 which is required for T-helper cell activation. In turn, T-cells release other lymphokines including migration inhibitory factor which immobilizes macrophages and tends to localize them in the vicinity of the immune reaction. As well, T-helper cells release interleukin-2 (IL-2) which acts on a further subpopulation of T-cells (cytotoxic T-cells) to activate and to stimulate their proliferation. Cytotoxic T-cells are then directly involved in the destruction of the foreign target tissues.

In the strictest sense, the humoral response involves the clonal selection of B-cells through the sensitization and stimulation of those cells which are predisposed to react with a foreign antigen. Proliferation and maturation of stimulated B-cells results in the secretion of specific immunoglobulin antibody molecules which then bind with the foreign antigen. This mechanism is primarily effective when the antigen has been shed from the allograft and is circulating in the blood. The complex which is formed allows other cells of the host's defense system to recognize and further process the foreign antigen; a function which is predominantly the role of the macrophages.

In both types of immune response, there are memory cells which are produced after a primary challenge and which remain dormant in the host. Although a primary response to an antigenic challenge may take 5-7 d to mount, after sensitization, a future presentation of the same antigenic stimulus may require only 1-2 d to reactivate the immune response as a result of the presence of these memory cells. This is the basis for the rapid onset of tissue necrosis associated with the hyperacute rejection phenomenon. The host can mount a rapid response to the allograft upon first presentation if the host had become sensitized to antigens contained within the allograft through prior exposure to the graft, or to the same histocompatibility antigens contained within the graft, even though they were presented as part of another tissue source. Rapid rejection can also occur to an allograft which has been carried for a prolonged period of time by an immunosuppressed host, if the level of immunosuppression becomes

inadequate, since there was a prior period of sensitization to the alloantigens even though an immunological response could not be mounted.

3. Immunosuppressive Agents

The agents available for immunosuppression fall into three general categories: anti-inflammatory, antimetabolic and cytotoxic agents. Classically, for the purposes of transplantation of organs or other composite tissues, the conventional immunosuppressive agents of choice have been the adrenocortical steroids (prednisone, prednisolone and methylprednisolone), and the antimetabolic agents 6-mercaptopurine and azathioprine (Imuran®, Burroughs Wellcome Co.). As well, X-irradiation has been used clinically, under controlled circumstances. All of these agents or therapies are relatively nonspecific in their mechanism of immune modulation, and many of them induce side-effects and/or a degree of immunosuppression in the host which can be undesirable or lead to conditions which are life-threatening. The cyclosporine (**Cs**) family of fungal metabolites, together with such agents as FK506® (Fujisawa Pharmaceutical Co.), the monoclonal antibody OKT3 (Orthoclone OKT3®, Ortho Pharmaceutical Corp.) and antilymphocyte globulins, have formed a new generation of 'specific' immunosuppressive agents. By far, the best-characterized of these later agents is the fungal metabolite cyclosporin A (**CsA**; Sandimmune®, Sandoz Ltd.).

The use of the immunosuppressive agent CsA in combination with methylprednisolone (**MP**; Solu-Medrol®, Upjohn), theoretically offers a complementary therapeutic effect. The corticosteroid exerts an anti-inflammatory effect in primate tissues through the generalized restriction of leukocyte movement [Fauci, 1985], while CsA selectively disrupts clonal expansion of the cytotoxic T-cells directly involved in solid tissue allograft rejection (see Appendix II).

Corticosteroids administered in pharmacological dosages cause a decrease in the migration and accumulation of neutrophils in inflammatory sites by impeding their adherence to

vascular endothelium; a step which is necessary for their migration across the vascular wall. In addition, they exert a marked but transient depletion of lymphocytes (T-lymphocytes preferentially to B-lymphocytes) and monocytes from the circulation The lymphocytes remaining in the circulation, however, show very little suppression of their functional capabilities Monocytes are more sensitive to the effects of corticosteroids and become poorly responsive to the macrophage migration inhibitory factor produced by the sensitized lymphocytes [Fauci, 1985].

CsA, on the other hand, selectively inhibits the capacity of T-cells to synthesize and release IL-2 and possibly other lymphokines. Although sensitization of cytotoxic T-cells does not appear to be hampered, mechanisms which would lead to clonal expansion are interrupted and the capacity for immune responsiveness through the recruitment of other leukocytes is dramatically reduced.

B. Extremity Tissue Allografts

With the current level of understanding of the mechanisms of immunosuppression, it has been possible to design successful immunosuppressive protocols for the transplantation of internal organs, to the extent that it is almost routine practice. The transplantation of extremity tissues, however, has only just recently come of age. In 1983, at the initiation of the experiments described, effective techniques and protocols for the transplantation of limited quantities of skin, nerve, muscle, vessel and bone were just being established This report describes extremity soft tissue allografts (skin, muscle and nerve) in a model of potential long-term survival and functional recovery, therefore the literature reviewed in this section will be restricted to such soft tissue allografts. Studies which used skin allografts as indices of immune responsiveness of the host will only be considered if relevant. As well, the review will deal primarily with aspects of antigenic challenge of adult allografted peripheral tissues in the

immunocompetent adult host, rather than of antigenic challenge of embryonic allograft models, or of the transplantation of fetal or adult allografted tissue into the central nervous system.

Allografted extremity tissues might have been 'successfully' transplanted as early as the third century. Legend has it that the Saints Cosmas and Damian, martyred twin physicians who were beheaded for their Christian practices, reappeared posthumously to replace the gangrenous, cancerous leg of an aged church sacristan (while he slept) with that of a recently deceased Ethiopian Moor [da Varagine, 1952; Kahan, 1983]. The limb was attached at the thigh and ointments applied to the site. When the sacristan awoke, he no longer experienced the pain nor felt the sores, and discovered that his leg had been replaced with a perfectly healthy black leg. There have been other reports such as this from time to time throughout the literature, many having been created by 'fanciful' writers of the day, but none have been substantiated [Furnas *et al.*, 1983]. Generally, the use of allografted or xenografted extremity tissues was (and still is today) restricted to the use of skin as a short-term biological dressing.

In recent history, it was not until the technical success of vascular anastomoses by Carrel in 1902 that modern scientific practice could hope to emulate the Miracle of the Black Leg. After accounts of successful replantation of extremity tissues, the transplantation of a leg from one fox terrier to another was reported to "heal normally" [Carrel, 1902, 1908], and that scar was not discernible 22 d after surgery. The excellent results of this experiment, although possibly overstated, represent the first documentation of an allografted whole limb. It was not clear whether this limb achieved long-term survival, nor whether the terriers were related.

In the late 1930's, using young rats (8 d to 3 wk old), the survival of allografted limbs with some gross functional capabilities was accomplished through the process of parabiosis (the crossing of circulation between two individuals through vascular union) before the age of 12 d [Lapchinsky, 1940a, b; Schwind, 1936, 1938]. The rationale behind the necessity for allografts to be transplanted before the onset of the host animal's immunologic maturity in order to have survival [Cannon and Longmire, 1952], was not fully realized until the

experimental definition of 'actively acquired tolerance' by Billingham *et al.* [1953]. The need to induce specific immune tolerance at an early age using preconditioning (preferably with donor spleen cells) and/or parabiosis, was further verified for skin allografts by several persons [Gifford, 1955; Mariani *et al.*, 1959; Martinez *et al.*, 1960, and others] During the same period of time, it was also observed that tolerance could be achieved in the immune-compromised host; whether induced [Billingham *et al.*, 1951; Main and Prehn, 1955], or through mechanisms of disease [Damman *et al.*, 1957; Snyderman *et al.*, 1960].

These concepts were used to study further the processes of extremity allograft survival between young littermates, non-littermates of similar age, or from adult donors. Schwind [1962a, b, c], using a rat hindlimb transplant model similar to his early studies, achieved more efficient long-term survival and further described the ability of the rats to use their allografted limbs during walking and to elicit a withdrawal of the appendage in response to pinching. The allografted tissues were said to appear normal when studied histologically; a photomicrograph of allografted skeletal muscle was given as evidence [Schwind, 1962c]. Microscopic sections of nerve were said to contain "a good percentage of normal-appearing nerve cells", however, no photomicrographs were presented.

Lapchinsky [1966], after having had success in the establishment of long-term survival of allografted rat limbs through the use of temporary parabiosis, was able to create tolerance in dogs through the subtotal exchange of blood in newborn pups with donor animal's blood Then, after 1-2 years, the leg of the donor animal (differing in sex, age and breed) was substituted for that of the recipient. In at least two of the cases, indefinite survival was achieved, however an immunologic crisis occurred at approximately 60 d post-transplant, resulting in the lysis of all or a substantial percentage of the epidermis and its derivatives [Lapchinsky *et al.*, 1973]. The crisis was managed with 'antiallergic' drugs (not specified, but probably steroids) and repeated blood transfusions. After recovery, the epidermis regenerated to a thickness of 8-10 layers of cells. Innervation of skin and muscle was said to have

occurred, however, histological evidence was only presented for the latter, in the form of a low magnification photomicrograph showing motor end-plates [Timofeeva and Lapchinsky, 1969].

With the initial optimism generated by these experiments and the introduction of the use of x-irradiation, steroids and antimetabolic drugs into the therapeutic regime, it was inevitable that human limb transplantation was to be attempted. In 1964, a human hand and forearm was transplanted from a cadaver onto that of a patient who had been severely injured in an explosion [Medical Tribune, 1964a] (Peter Bent Brigham Hospital, Boston; Case No. 4-79-99, [Goldwyn *et al.*, 1966]). Despite the use of prednisone, azathioprine and local radiation to the graft, the allografted hand only remained viable for about 14 d and then succumbed to coagulation necrosis and was surgically reamputated on day 23 [Goldwyn *et al.*, 1966; Medical Tribune, 1964b].

Although unsuccessful, this latter event ushered in a new era of attempts to allograft extremity composite tissues using immunologically mature adult animals. Canine hindlimb transplants were attempted by Goldwyn *et al.* [1966], with the use of azathioprine immunosuppression. Although survival in excess of 150 d was achieved, no mention was made of the functional state of the transplant. Lance *et al.* [1971], also using canine hindlimbs and a variety of immunosuppressive protocols, achieved survival times in excess of 300 d and reported protective sensation and muscle function by 100 d, however, no quantification or further details were given.

Prior to the initiation of the present experiments in 1983, several other surgical studies of rat hindlimb allograft models were published [Black *et al.*, 1982; Doi, 1979; Fritz *et al.*, 1983; Lipson *et al.*, 1981, 1983; Poole *et al.*, 1976; Press *et al* , 1983 a, b], however, each of these studies was concerned primarily with prolonged allograft survival and no indications were given as to whether any innervation occurred.

Since the initiation of the present experiments, several more surgical studies were published [Fritz *et al.*, 1984; Kim *et al.*, 1984; Lee *et al.*, 1988] briefly describing hindlimb allograft survival in rats, without any mention of innervation. Other additional reports of rat

hindlimb allografts [Black *et al.*, 1983, 1985, 1988, Furnas *et al.*, 1983; Guzman-Stein and Shons, 1987, Hewitt *et al.*, 1985] and rabbit forelimb allografts [Dorrler *et al* , 1986] briefly mention the return of protective sensation (withdrawal to pin-prick) and/or the detection of motor function (in response to electrical stimulation or as a functional index of quadruped locomotion), without further quantification of these phenomena.

More recent reports from other laboratories (in parallel to the present study) have provided a limited amount of detailed data describing neural function in allografted extremity tissues. In addition to statements describing the results of subjective indexes such as protective sensation and utilization of allografted hindlimbs in the rat, Press *et al* [1986] presented a figure showing multi-unit electromyographic (EMG) data which confirmed axonal elongation and muscle reinnervation in the allografted limb. Unfortunately, there was no data presented from control animals, nor was there a vertical axis provided in the figure in order to make comparisons to normal values.

For allografted extremity tissues of the baboon, the only electrophysiological data published in parallel to that of the present studies [Daniel *et al.*, 1986; Egerszegi *et al.*, 1984, Samulack *et al.*, 1985, 1986, 1988, 1989] were those of Narayanan and Stark [Narayanan *et al.*, 1988; Stark *et al.*, 1987] whereby axonal elongation into an allografted hand, and subsequent innervation of allografted muscle was confirmed. In the former study Narayanan *et al.* [1988] presented oscilloscope traces depicting evoked potentials recorded from the dorsal column nucleus (on the transplanted side) after electrical stimulation of the nerves within the allograft. In the latter study, Stark *et al.* [1987] showed an oscilloscope trace of an EMG response recorded from allografted thenar muscle In both of these reports there are electrophysiologic indications of poor conduction velocities in nerves serving the allografts In addition, brief statements were made regarding the histologic integrity of the tissues within the allografted hand, but no further data or photomicrographs were presented.

In summary, with the exception of preliminary reports related to the present study, description of the innervation of allografted extremity tissues has been restricted to generalized

statements, the analysis of basic functional indexes, or the electrophysiological identification of compound waveforms. None of these analyses have provided a detailed account of the capacity of host axons to innervate allografted extremity tissues, nor have they quantitatively described the quality of this innervation.

IV. Rationale

The fact that CsA can impart specific immunosuppression across major histocompatibility barriers has greatly enhanced the ability to transplant allogeneic tissues and obtain long-term survival. Obviously this has important clinical implications, but for transplantation of these tissues to become a routine clinical procedure, the basic physiological principles underlying this phenomenon have to be understood in detail. Most importantly, if allografted skin and/or other extremity composite tissues are to have a broad therapeutic role in the surgical treatment of patients with congenital malformations, amputations or extensive thermal injuries, it must be demonstrated that the foreign tissue can become innervated sufficiently by the host to provide useful tactile sensation and/or motor function. At the initiation of this study, detailed data concerning the degree to which host axons can grow into an adult peripheral histoincompatible tissue, locate and functionally innervate foreign sensory and motor target structures, was virtually non-existent.

The objectives of this study were: (1) to obtain long-term survival of allografted upper-extremity skin and composite tissues in an adult primate, (2) to characterize and document any differences between the electrophysiological capabilities of host axons serving allografted tissues and those serving autografted tissues which had undergone similar manipulations, and (3) to characterize and document the histological processes which may have contributed to any functional differences in the reinnervation of these tissues.

METHODS

I. Surgical Model

A. Experimental Animal

The animal model used for these studies was the adult female olive baboon (*Papio hamadryas anubis**; 10-14 kg). The decision to select this non-human primate for the study was based upon several criteria: (1) the anatomical similarity of the hand to that of man, including comparable sensory mechanoreceptors, receptor density, glabrous skin surface and nerve pathways, (2) the presence of neurovascular structures of sufficient size to permit microsurgical repair and neurophysiological recording, (3) the ability to surgically manipulate and splint a non-weight bearing graft of composite tissues including bone and isolated muscles, and (4) a previous history of experiments using this species for neurophysiological testing of reinnervation following nerve repair.

The twenty-one animals used for the study (Table 1) were obtained either from unknown sources through Charles River Canada, Inc., or from the Southwest Foundation for Biomedical Research (**SFBR**) in San Antonio, Texas. The animals from the former supplier were caught in the wild, while the animals from the latter supplier were selected from a population of approximately 2000 animals at the SFBR breeding facility. Baboons #16 through 21 were blood typed and serologically tested for class I antigens (BabLA) by the SFBR. Only those animals which, through microcytotoxicity testing using defined antisera (NIH Lymphocyte Microcytotoxicity Technique), showed a high probability of differing in at least two alleles at BabLA loci, were selected as donor-recipient pairs. Although the olive baboon is thought to exhibit a low degree of genetic polymorphism in comparison to man [Coppenhaver

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* On the basis of genetic and reproductive criteria, the olive baboon (formerly thought to be a distinct species, *Papio anubis*) is presently considered to be one of five subspecies of the common long-tailed baboon *Papio hamadryas* Linnaeus 1758 [Groves, 1972; VandeBerg and Cheng, 1986].

et al., 1981; VandeBerg and Cheng, 1986], the major histocompatibility complex for the baboon has only been resolved to a rudimentary degree. Taking these factors into account, pairing randomly acquired animals, or those tissue typed and shown to be genetically dissimilar at one or two class I antigen sites, was judged to be sufficient to generate a strong histoincompatible match.

The animals were cared for at either of two McGill University primate care facilities. Individual animals were housed separately in squeeze-back cages that conform to the guidelines set by the Canadian Council on Animal Care [1980]. Alimentation consisted of water *ad libitum* and a standard protein rich diet (Purina Monkey Chow®) supplemented with vitamins, fruit and vegetables.

B. Surgical Manipulations

In preparation for surgery, the animals were first sedated with an intramuscular (**IM**) injection of a mixture of ketamine hydrochloride (Rogarsetic®, Rogar/STB Inc.) and xylazine (Rompun®, Haver-Lockhart) (5.25 mg/kg and 0.45 mg/kg respectively). After a suitable induction time, the animal (or pair of animals) was transported from the animal care facility to the laboratory. The baboons were then intubated and allowed to respire a mixture of oxygen and/or room air. A temperature probe was inserted into the rectum and a urinary catheter was used to collect urine. Electrocardiographic electrodes were placed on the skin of the left and right sides of the upper chest and in the area of the pubis of the lower limb girdle, in order to monitor the heart rate and electrocardiogram. Then, sedation was slowly converted to deep anesthesia with the intravenous (**IV**) administration of sodium pentobarbital (Somnotol®, M.T.C. Pharmaceuticals; approximately 5 mg/kg/h, or as necessary). A surgical incision was used to secure an IV catheter. Fluids in the form of 5% dextrose in 0.45% NaCl were administered intravenously throughout the surgery. Body temperature, as measured *via* a rectal thermoprobe, was controlled by a circulating water blanket and/or a heat lamp. Hemostasis at

the site of surgical manipulation was controlled during critical intervals with a cuff tourniquet or Esmarch bandage. The above mentioned physiological parameters were monitored along with periodic blood gas and electrolyte determinations (compliments of the Royal Victoria Hospital biochemistry laboratory, Montreal, Quebec). Corrective steps were taken to maintain physiological homeostasis when parameters deviated from normal values.

In most cases the total preparatory and surgical period lasted from 18 to 24 h with ischemia times less than 2 h. Two upper-extremity composite tissue surgical models were studied: the digit soft tissue coverage neurovascular free flap and the total hand transplant.

1. Neurovascular Free Flaps

The term neurovascular free flap (**NVFF**) refers to a surgically isolated island of skin which has portions of its neural and vascular components intact [Daniel *et al.*, 1975]. For the present study, the NVFF of choice was the digital NVFF (a modification of the clinically useful digital island flap). The entire skin coverage of the digit was used in this design, offering the advantages of: (1) an almost pure sensory innervation (digital nerves), (2) a consistent vascular supply (digital artery and dorsal vein), and (3) an adequate pedicle length and vessel diameter, allowing microsurgical anastomosis at the wrist. The NVFF represents a highly successful means to graft skin and to ensure its subsequent viability, since upon surgical anastomosis of its vascular pedicles, the graft becomes immediately perfused.

2 Transplanted Neurovascular Free Flap

Transplanted NVFFs (**TNVFFs**) used in these studies involved the surgical excision of the total soft tissue coverage from either the right or the left index finger of the donor animal and unidirectionally transferring it to a similarly prepared surgical site on the recipient animal. The tissues were isolated by first making an incision down the lateral length of the digit, from the nail bed to the metacarpophalangeal joint, using the border where glabrous meets hairy skin

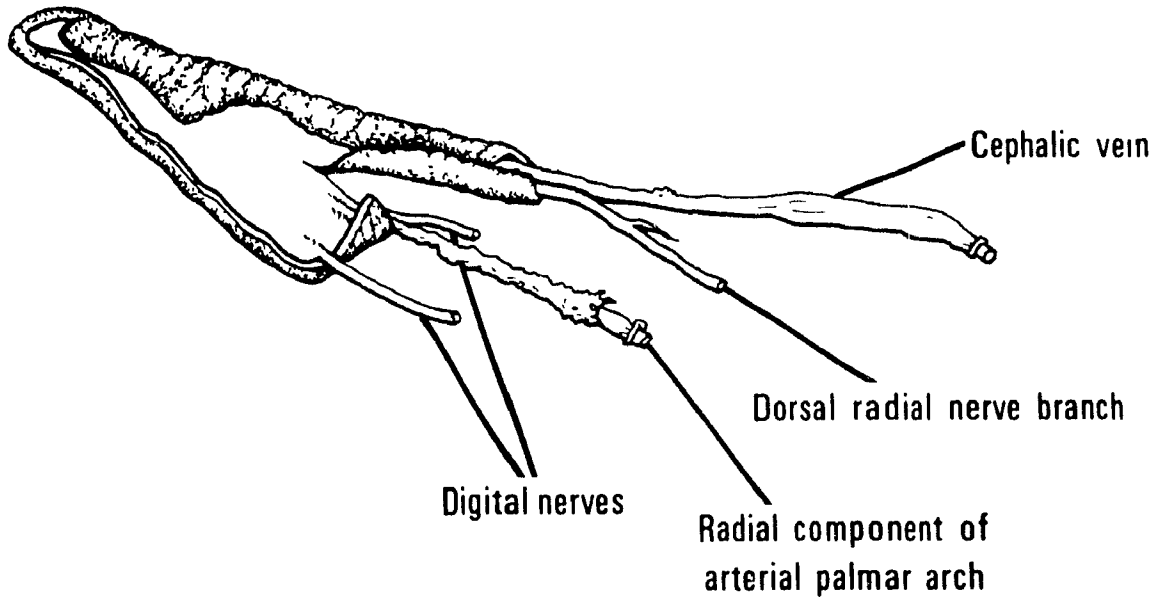
as a guideline. The most distal extent of the incision was then continued around the circumferential aspect of the tip of the finger at a level just proximal to the nail. Another circumferential incision was initiated in the second web space and drawn ventrally to encompass the palmar digital pad of the index finger as part of the graft. This incision was continued laterally to meet the first incision in the first web space between the thumb (**D1**) and the second digit (**D2**), then brought dorsally to encompass the hairy skin overlying the knuckle as part of the graft and then terminated where it was begun, in the second web space. A third incision was started just proximal to the palmar pad of D2 and terminated at the volar wrist.

The digital nerves and dorsal branches of the radial nerve to D2 were then separated from adjacent structures. Those nerves which were of sufficient size for attachment to the recipient animal were isolated and tagged for identification. The digital arteries were dissected back to the radial component of the palmar arch and the dorsal vein was traced back to the cephalic vein. Upon isolation of all the necessary structures, the digital nerves were transected at the mid-palm and mid-dorsum of the hand, while the two major vessels were clamped and transected at the level of the wrist. The index finger was then entirely degloved of its soft tissues and their neurovascular pedicles, in a fashion resembling a bun being removed from a hot-dog. The remaining structures of the digit of the donor animal (phalangeal bones, joint capsules and tendons) were amputated at the shaft of the metacarpal bone and discarded. The donor animal's wound was then sutured with 4-0 polyglycolic acid, using simple interrupted and continuous stitches.

The isolated NVFF (Fig. 1) was then either stored temporarily in a cold (4 °C) isotonic saline solution (if the surgical preparations of the two animals were not done in parallel), or transplanted immediately to a similarly prepared tissue bed on the recipient animal. The vascular anastomoses and neural repair were done with the aid of an operating microscope and 10-0 nylon suture on a 75 μm needle using standard microsurgical technique. Nine to ten simple interrupted stitches were required for each of the vessels. Once the tourniquet was released, the flap was perfused and hemostasis was ensured by electrocautery. The appropriate

Figure 1

The allografted and control NVFF surgical models were similar in design. The total soft tissue coverage of the finger was removed from the digit, while maintaining its nerves and vessels intact. The dorsal digital vein was dissected proximally to include a portion of the cephalic vein. The ventral digital artery was exposed in a manner so that it included a portion of the arterial palmar arch. All digital nerves which were large enough to be anastomosed using microsurgical techniques, were isolated. The vessels were clamped and the nerves were sharply transected. All of the necessary soft tissues, excluding the finger tip and nail bed, were excised as one unit. The NVFF was then either transplanted or replanted to its respective tissue bed after a short period of ischemia (less than 2 h). Blood flow was reinstated after the microsurgical anastomosis of the respective vessels. Digital nerve anastomosis was performed while ensuring a 'best fit' orientation of the fascicles. (Reproduced, with the permission of The C.V. Mosby Co., from Daniel *et al.* [1986]. Copyright © 1986 by the American Society for Surgery of the Hand)


Cephalic vein
Dorsal radial nerve branch
Digital nerves
Radial component of
arterial palmar arch

nerve bundles were trimmed, aligned and then repaired with 3 to 5 simple interrupted stitches. There was often a size discrepancy between the donor and recipient digital nerves. Since each usually had a minimal number of small fascicles, alignment and repair of respective donor and recipient nerve stumps was performed while ensuring a 'best fit' orientation. Donor skin and subcutaneous (SC) tissues were then aligned with those of the recipient animal. Any excess skin was trimmed and the wound was closed with 4 0 polyglycolic acid sutures. Only one-way NVFF transplants of this nature were possible since it was necessary that an area of donor tissue needing to be excised (and then trimmed) was always larger than the area of skin removed from the recipient site. In order to ensure that the finger nail did not produce any post-surgical complications, the nail, its nail bed and the finger tip were excluded from the NVFF. As well, in each case except for animals #2, 3 and 5, the nail, nail bed, germinative zone and finger tip were removed from the distal phalanx of the recipient animal, for the same reasons.

b) Control Neurovascular Free Flap

The control NVFF (**CNVFF**) was similar in design to that of the TNVFF, except that the fourth digit (**D4**) was used instead of D2. All of the surgical incisions were modified in order to be able to isolate the corresponding neurovascular structures of D4. The most important difference between the CNVFFs and the TNVFFs was that the tissues which were isolated as a CNVFF were replanted to the same surgical site from which they were excised. Therefore, these autografted tissues underwent similar surgical manipulations to those of the TNVFFs without the introduction of a histocompatibility challenge to the immune system. The CNVFFs were performed during the same surgical period and on the same hand as the TNVFFs. The CNVFFs served as controls to the surgical manipulation and any events which took place as a result of the histocompatibility differences of the allografted tissues.

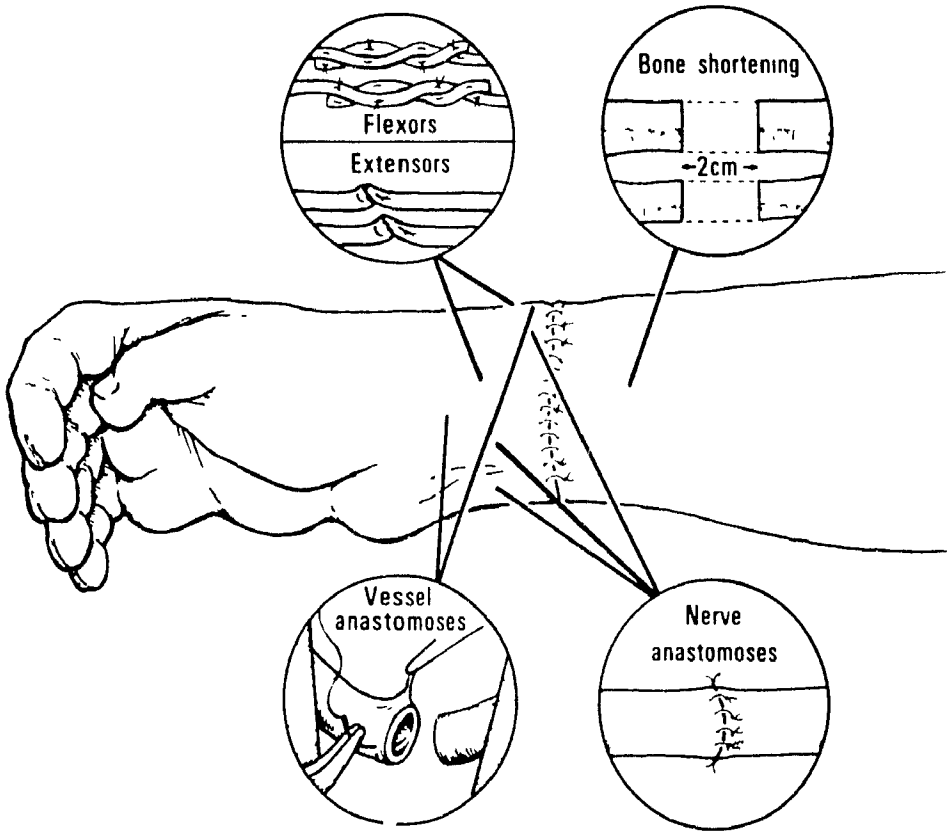
2. Hand Transplant

The second model, the complete hand transplant (HT), was designed to permit assessment of the long-term survival and reinnervation of a comprehensive allografted composite tissue containing skin, muscle, nerve, bone and vascular structures. The surgical aspects of this transplant model involved the reattachment of nerve, vessel, tendon and bone (Fig. 2) in a similar fashion to that which is used in clinical replantation of severed hands. In contrast to the TNVFFs in which all of the transplants were unidirectional, some HTs were attempted in a bidirectional manner (animals #12 and 13) with each animal in the donor-recipient pair receiving an allografted hand in exchange for its own.

Donor-recipient pairs of animals were chosen so that their hand sizes were similar. Under pentobarbital anesthesia, each baboon was placed in the supine position. The proposed forearm skin incisions were marked in a circumferential manner at a location 4 cm proximal to the junction of the wrist and palmar skin. After inflation of a pneumatic tourniquet around the upper arm to stop blood flow, the skin was incised to the level of the fascial plane surrounding the muscles. This allowed the skin and subcutaneous tissues to be raised as a unit The proximal and distal skin flaps were resected and all tendons were tagged for easy identification and then marked at distances of 2 and 4 cm from the wrist. The median, ulnar and radial nerves were sharply transected at a level approximately 2 cm from the wrist The radial artery and cephalic vein were also identified at the same level. They were clipped with small hemoclips and transected Dissection was then carried deeper to expose the radius and ulna In the case of the unidirectional transplants, the bones were transected 4 cm above the wrist with an electric saw. Approximately 2 cm of bone was excised from the donor extremity before transplantation. For bidirectional transplants, a 2 cm segment of bone (between 3 and 5 cm proximal to the wrist) was removed from each animal's forearm. These excisions of bone were necessary to allow tendon overlap upon transplantation and to perform neural and vascular anastomoses with no longitudinal tension.

Figure 2

The hand allograft model involved the transplantation of all the distal tissues of the forearm from a point just proximal to the wrist. Skin, muscle, nerve, vessel, tendon, bone and all of the connective tissues of the hand were incorporated into one graft. Upon transplantation, the radius and ulna were shortened to allow for repair of the nerves, vessels and tendons. The bone union was ensured and strengthened with the use of compression plates. Extensor and flexor tendons were repaired while ensuring proper tension. The radial artery and cephalic vein were microsurgically anastomosed to the respective vessels of the host. As well, the nerves were aligned on a 'best fit' basis and anastomosed with epineurial sutures. (Reproduced, with the permission of The C.V. Mosby Co., from Daniel *et al.* [1986]. Copyright © 1986 by the American Society for Surgery of the Hand)


Flexors
Extensors
Bone shortening
2cm
Vessel anastomoses
Nerve anastomoses

Attachment of the donor tissues to the host animal was begun by attaching the radius and ulna to their respective shafts with the use of bone compression plates. Next, all deep tendons were repaired utilizing mattress sutures in the extensor compartment and the Pulvertaft technique for the flexors (see insert of Fig. 2). Tension was adjusted by lining up the 2 cm mark of the donor tendon with the 4 cm mark of the recipient tendon prior to repair. The vascular anastomoses were undertaken using standard microsurgical technique with the aid of an operating microscope Following the excision of overlapping vessels, 10 to 12 simple interrupted stitches of 10-0 nylon on a 75 μm needle were required. With the release of the tourniquet, the hand was revascularized and hemostasis was ensured with bipolar electrocautery and hemoclips. The appropriate nerves were trimmed and repaired in such a fashion that their was suitable fascicular alignment. Depending upon the size of the nerve, four or more simple interrupted epineurial stitches of 10-0 nylon suture material was used for the repair. Excess skin was removed and the wound was sutured with simple interrupted and continuous stitches using 4-0 polyglycolic acid suture material. These operative procedures took approximately 24 h, during which the ischemia time of the tissues was kept as short as possible (about 1-2 h).

C. Postoperative Animal Maintenance

For the purposes of these experiments, the 'surgical success' of the replantation or transplantation of the tissues was defined as the potential for long-term survival, judged by the quality of recovery within the first week following surgery. The general criteria used to determine viability of the extremity tissues was colour, temperature (upon touch), and the patency of the vessels which served the tissues. The latter parameter was determined by the colour of the fresh blood which was brought to the skin surface as a result of a punctate stab wound with a sterile scalpel blade.

1. Splinting and Dressing Changes

Immediately following surgery, the surgical site and the forearm were washed with sterile saline and dried with gauze pads. Excess coagulated blood on the skin surface was removed with 3% hydrogen peroxide. All incisions were covered with chlorhexidine acetate gauze (Bactigras®, Smith & Nephew) and several dry sterile gauze pads and bandages were used to wrap the entire forearm loosely.

A custom-made lightweight splint, fabricated from thermoplastic (San-splint®, Smith & Nephew) and rivet-like 'binding' screws, was used to protect the surgical site and forearm of the animal. The splint, a modified version of the rigid upper limb splint [Rose *et al.*, 1983], was spacious and designed to allow space for bandages and some freedom in movement of the forearm and extremity. A right angle flexion of the splint at the elbow prevented the animal from removing it. The unfastening of the screws along the entire peripheral edge of the splint allowed quick removal of the splint and easy access to the arm during changes of the surgical dressing.

Twice a week for the first three weeks and then weekly thereafter (or as necessary), each animal was tranquilized with ketamine and xylazine (see Methods, Section I.B.), in order to inspect the grafts. Upon removal of the splint and old dressings, detailed photographic and written records were obtained to document the progress of the grafts. Then the forearms were washed with either a 0.5% alcoholic, or a 0.05% aqueous mixture of Hibitane® (Ayerst). After drying with sterile gauze pads, topical antibiotics (0.2% nitrofurazone; Furacin®, Austin Laboratories, or polymyxin sulfate; Polysporin®, Burroughs Wellcome Inc.) and/or chlorhexidine acetate gauze was applied if necessary. The forearms were once again wrapped loosely with sterile bulky gauze dressings. The thermoplastic splints were washed with soap or other disinfectant, and upon drying, were used again for the same animal. Under normal circumstances, the entire procedure for the change of dressings took no more than 45 minutes.

Some of the animals which were designated to be recipients of allografts, had their forearms splinted three to five days prior to surgery, in order to allow them time to become comfortable with the splint. Most of the animals had their forearms in a splint for the duration of the study. The splints for animals #20 and 21 were no longer used after days 120 and 123, respectively, following surgery.

2. Immunosuppression

The host animals which received allografts, were induced into an immunosuppressed state through the daily administration of CsA and MP. This was done, in the face of inevitable sensitization to the foreign tissue antigens, in an attempt to control the immune responsiveness of the host towards the allograft. In the case of HTs, this precaution was also necessary to control any graft-*versus*-host reactions which were theoretically possible. The CsA was considered as the primary immunosuppressive drug administered in the study, since it was the most immunologically specific agent used to prevent allograft rejection (see Appendix II), and it was used for prolonged periods of time at therapeutic levels far in excess of MP.

a) Cyclosporine

The circular peptide CsA is not water soluble, and hence, its administration and the maintenance of adequate therapeutic blood levels in a large experimental animal were formidable tasks which required many hours of technical support. Inert oil-based compounds were used as the primary vehicles in which to dispense the drug (see Appendix III). Several routes of delivery were explored: oral (**PO**) administration, IM and SC injection. By far, the IM preparation using Miglyol 812® was the most practical and dependable route of delivery. The IM preparation was stable with a high concentration of CsA, and the volume administered was able to be precisely controlled.

Throughout the duration of the CsA therapy for each animal, the blood serum levels of Cs were monitored by radioimmunoassay†(**RIA**; Cyclosporine RIA-Kit, Sandoz Ltd.). Since CsA was administered twice daily (approximately every 12 h; see below), the RIA blood serum 12 h trough levels of Cs were used as an indication of the amount of CsA which was being absorbed and then circulated through the animal. These RIA values, as well as the clinical state of the allografts, were used as indications of the level of immunosuppression induced. Since CsA has many toxic side-effects (see Appendix II), blood chemistries such as urea nitrogen and creatinine levels were routinely monitored, as was the appetite and general well-being of the animal. Each of these values and observations were taken into consideration during the management of the CsA therapy.

b) Methylprednisolone

The anti-inflammatory steroid, methylprednisolone is a short acting glucocorticoid. As in many clinical organ transplants, it was used in conjunction with CsA as part of a dual-therapy in an attempt to reduce the rejection phenomenon. Two preparations of the drug were used: 40 mg/ml and 62.5 mg/ml, IM injectable. Throughout most of the immunosuppressive therapy of the animals, it was used in small doses (4.4 mg/d; 40 mg/ml preparation) with a minimal immunosuppressive effect. During the immediate postoperative period, or at the onset of indications suggesting allograft rejection, it was administered in large doses (125 mg/d, 62.5 mg/ml preparation) in an attempt to curtail the progression of the immunological response. This approach offered a means of immediately increasing the level of immunosuppression in a

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† Due to the polyvalent character of the sheep antiserum utilized in the kit, it was presumed that there was cross-reactivity with many Cs metabolites, including some of which are not immunologically active. The RIA values were used only as an estimate of the amount of parent CsA which was present in the sample (see Appendix II).

rejection crisis, an option not possible with the use of IM-injectable CsA which had slower absorption characteristics. Once rejection was under control, and effective CsA blood levels were obtained, a gradual reduction (tapering) of the MP therapy over a period of 14-21 d was effective in safely returning the MP levels back to baseline (see below).

c) Immunosuppression Protocol

The approach towards an effective immunotherapy in these experiments evolved as experience was gained in the management of the allografts. In the early stages of the experiment (animals #3-11), a conservative regimen of drug administration was only marginally effective in achieving prolonged survival of some of the allografts. Initial pretreatment of the recipient animals for 1-3 d prior to surgery consisted of the administration of CsA at 20-40 mg/kg/d in one or two (12 h apart) doses. During surgery and the first postoperative week, CsA was administered at 30-40 mg/kg/d in two equal doses spaced 12 h apart. As the Cs circulating levels (determined by RIA) increased, the CsA therapy was adjusted to maintain the desired level. The RIA serum 12 h trough levels of Cs targeted during these early stages of the experiment were under 1000 ng/ml (measured at room temperature; approximately 20-22 °C). MP therapy was started 3-15 d after surgery and consisted of 3 d at 125 mg/d (single dose), followed by a tapering of the regimen over a 14-21 d period (two doses per day, amounting to 25.6, 21.2, 16.8, 12.8, and then 8.8 mg/d) to a baseline single dose of 4.4 mg/d; prednisone tablets were given in equivalent dosages for short periods of time if MP was in short supply.

In the latter stages of the experiment (animals #12-21) experience determined that a greater degree of immunosuppression was necessary in order to ensure extremity allograft survival in the nonrejected condition. Pretreatment of the recipient animal was initiated 4-9 d prior to surgery. Perioperative CsA dosages were increased to approximately 45 mg/kg/d and was administered in two equal doses on a 12 h schedule. This pretreatment schedule, along

with the initiation of the MP regimen on the day of surgery, ensured that the host was adequately immunosuppressed by the time that the allograft was introduced. The Cs RIA levels were allowed to rise in excess of 1200 ng/ml as long as the animals showed satisfactory blood biochemistry, appetite and well-being; the CsA administration was reduced or stopped temporarily if the conditions were otherwise.

3. Prophylactic Medications

In addition to immunosuppressive agents, other medications were also administered to the animals during the perioperative period, as well as during rejection episodes or whenever warranted (see Appendix I). The most important of these medications were the analgesics and antibiotics.

During the first week after surgery, analgesics such as levorphanol tartrate (Levo-Dromoran®, 0.6-1.0 mg twice a day; Hoffmann-La Roche Ltd.) or meperidine hydrochloride (Demerol®, 50 mg three times a day; Winthrop Laboratories) were administered in order to control any discomfort experienced by the animals. In addition, small amounts of acetylsalicylic acid (100-200 mg twice a day) were given to the animals during the recovery period, not only for its analgesic properties, but also to minimize the risk of blood clotting within the allografts as a result of surgery.

Antibiotics in the form of Penlong S® (150,000 IU benzathine penicillin G, 150,000 IU procaine penicillin G, and 375 mg dihydrostreptomycin sulfate, Rogar/STB Inc.) once a day, and netilmicin sulfate (Netromycin®, 35-50 mg twice a day; Schering Canada Inc.), were administered during surgery, as well as during the 7-14 d postoperative recovery period. In cases of allograft rejection, these antibiotics were given during the course of active rejection and recovery. If the allograft sustained significant damage as a result of immunological processes and remained structurally unstable, one or both of these antibiotics were administered over a prolonged period of time, as was warranted (see Appendix I). Analgesics

and antibiotics were also given to animals that donated tissue and which were allowed to recover from surgery.

II. Electrophysiology

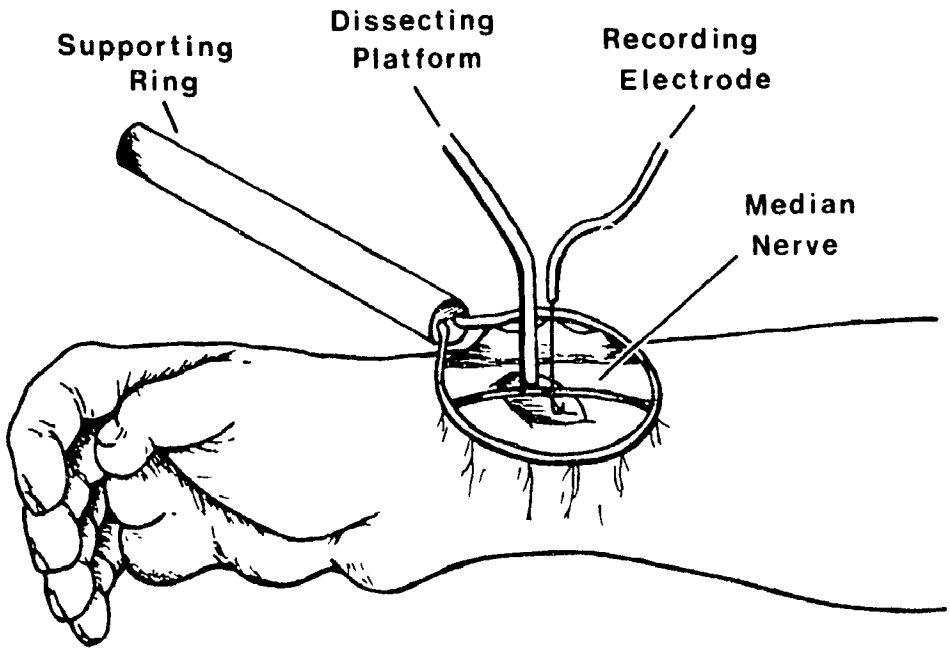
A. Preparation of the Animal

In preparation for electrophysiological testing of the flaps, each animal was sedated with ketamine and xylazine (see Methods, Section I.B.) and transported to the laboratory. The hair on the experimental arm was shaved and detailed photographs were obtained of the hand. The animal was intubated and allowed to breath room air. It was placed on its back in a position, such that its head and chest were slightly elevated with respect to the pelvis and lower limbs. An IV catheter was inserted into the femoral vein and the anesthetic agent was slowly converted to sodium pentobarbital. As previously described, IV fluids and prophylactic antibiotics were administered. Body temperature, electrocardiogram, blood gases and electrolytes were monitored, while taking measures to ensure that the parameters were maintained within the normal physiological range for baboons.

Using clean surgical technique, an 8 cm incision in the skin of the lower ventral or dorsal forearm allowed isolation of the median, ulnar or radial nerves proximal to the site of surgical repair. As shown in Fig. 3, the edge of the skin incision was sutured to a brass supporting ring using continuous stitches with 00-silk suture material. During the construction of this 'recording well', the nerves were kept moist with warmed (37 °C) saline (0.9% NaCl). The recording well, once complete, was filled with saline. The entire forearm was placed into a mold fabricated from plasticine and the upper arm was either immobilized with a clamp on the humerus, or prevented from moving significantly with the use of a brass bar overlying the arm at the elbow. Two thick brass pins were also used to hold the radius and ulna fixed in position. A small plastic platform was placed under the nerve to be studied in order to provide a working surface for microdissection. The saline in the pool was replaced with warmed silicon fluid to prevent desiccation while providing electrical isolation of the axons to be recorded. A thin silver wire recording electrode was positioned near the nerve with the use of a

Figure 3

To record electrophysiologic signals from axons which served a graft, or normal skin, a recording well was constructed by suturing the skin of the lower forearm to a brass supporting ring. Warmed silicon fluid was used to fill the well, prevent desiccation and to provide electrical isolation of the axons being recorded. Upon microdissection of either the median, radial or ulnar nerve, fascicles were carefully cut away and teased into small bundles and then into groups of axons with the aid of a dissecting microscope. Each group included approximately 1 to 4 axons that, when placed upon a silver wire recording electrode, could be differentiated by action potential size and shape when the amplified signal was viewed on an oscilloscope.


Supporting
Ring
Dissecting
Platform
Recording
Electrode
Median
Nerve

micromanipulator (Narshige). The reference electrode was attached to the edge of the skin incision and to the grounded brass supporting ring.

B. Microdissection and Recording Techniques

Microdissection of the nerve was performed with the visual aid of a surgical dissecting microscope (Zeiss). After the connective tissue sheaths of the nerve were opened along a distance of approximately 1 to 2 cm, a bundle of nerve fibers was cut free (using microscissors) and stripped away from the nerve in a proximal-to-distal direction with the use of sharpened No. 5 forceps. This freed bundle was then lowered onto the dissection plate, where it was physically teased into smaller bundles. These smaller bundles were teased further until they contained between 1 and 4 healthy axons, that, when placed upon the recording electrode, could be differentiated on the basis of their respective AP size and shape as viewed with an oscilloscope. Great care was taken during the teasing of the nerve bundles to avoid unnecessary tension or stresses to the nerve or teased bundles.

The recorded axonal signals were fed through a preamplifier (WP Instruments) to an amplifier (Princeton Applied Research). The signals were then sent in parallel to a storage oscilloscope (Tektronix), an audio monitor (Radio Shack), and a computer assisted data collection system. Single APs could be identified and counted with the use of a custom built voltage discriminator and a rate meter.

C. Data Collection

Once a single afferent fiber was identified, based upon its ability to produce an AP in response to peripheral tactile manipulation, attempts were made to identify its peripheral target structure, define an RF, measure its threshold of activation, study its response characteristics as described by its adaptation rate, and to measure its conduction velocity (CV).

The axons serving Normal skin were sampled from forearms of baboons having no history of immunosuppression‡. The fibers which served Normal (Experimental), Surgical Control, Allograft (Nonrejected) and Allograft (Rejected) tissues were sampled from forearms of baboons which were immunosuppressed with CsA and MP. The majority of axons from the first three of the four experimental groups (excluding those from rejected allografted tissues) were sampled in the presence of very high levels of CsA (animals #20 and 21, see Appendix I).

In order to maintain clarity throughout this report with respect to how axons (and hence, data) were handled, several words are used consistently and with intent throughout the text. Not every axon which was 'dissected' was considered to have been 'identified' Only those fibers from which a distinct individual AP could be amplified and recorded on an oscilloscope were considered to have been identified At that point, priority was given to those axons which served allografted tissue (primarily skin; towards which the majority of the experimental time was allotted). Second in priority were axons which served autografted skin, and then those which served Normal (Experimental) skin. At no time was one submodality of axons given priority over another. With these priorities in mind, attempts were made to ensure a reasonable sample of axons from Surgical Control and Normal (Experimental) tissues During the recording sessions, many more axons were identified than those for which data was recorded For a number of reasons, including the quality of the amplified signal, the number of axons within the teased bundle, experimental priority, and time constraints, many identified axons were passed over. Of all the identified axons during the experimental period, only those for which any data was recorded (submodality, RF, CV, threshold of activation, spontaneity of firing, *et cetera*), were considered as having been 'sampled' Those fibers for which several,

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‡ The data presented with respect to this group of axons was derived with permission from the original data of Dykes *et al.*, 1984. These data are included for comparative purposes only, and are neither counted as animals studied in Table 1, nor fibers studied in Table 2.

or all, of the sampling parameters were recorded were considered to have been 'studied', however, distinction between the latter two levels of data acquisition is less important.

1. Identification of Receptive Fields

Classically, axons serving somatic receptors have been divided into two modalities: skin or deep. Various workers have also identified different submodalities or receptor types (for review, see Dykes [1983] and Horch *et al.* [1977]). For the purposes of these experiments, the deep modality was subdivided into the submodalities muscle, joint and tendon. Fibers that could neither be identified as serving muscle, joint or tendon, nor activated through squeezing or otherwise compressing overlying folds of skin with smooth-tipped forceps, but nevertheless appeared to serve subcutaneous structures, were simply called Tap. Generally, axons classified as Tap gave one or two impulses in response to taps on the skin surface with a blunt probe.

The axons which served skin were subdivided into two submodalities which reflected the response properties of receptors found in the skin: rapidly adapting (RA) and slowly adapting (SA). As well, axons which served thermoreceptors were recognized by their characteristic spontaneous activity and their response to warmed or cooled metal objects placed on or near the skin surface. Nociceptive axons were identified as those fibers responding to potentially damaging (usually punctate) stimuli to the skin or to an extremely hot item (soldering iron) held approximately 1 mm or more above the skin surface for a few seconds. Stimuli comparable to those used to identify nociceptive fibers produced the feeling of tolerable pain when applied to oneself. If no RF was identified, the fiber was left unclassified, or was logged as unknown.

When a single fiber serving skin was isolated, it was characterized as to its submodality either by tactile manipulation or with the aid of hand held blunt glass or metal probes If there was still a question as to whether the response was RA or SA, a small lead weight or similar object capable of exerting a steady pressure was placed upon the RF. A sustained train of APs

lasting longer than several seconds after the object had stabilized was considered an SA response. If the submodality was still uncertain, the axon was logged as questionable (?).

The outline of the cutaneous RF of the axon was defined carefully with the use of a commercially available Weinstein-Semmes (**W-S**) pressure esthesiometer (a modern nylon monofilament substitute for the classical Von Frey hair, Stoelting) designed to apply a punctate tactile stimulus of approximately 5 g (see below). Since the apparent RF size changes with the applied force used to map the RF [Johansson, 1976], normalization of the field size was attempted through the use of this standard stimulus. This ensured that freshly innervated RFs, or those in skin of differing textures or structure were mapped in a manner comparable to the rest.

2. Sampling Thresholds of Axonal Activation

The sensory threshold of a nerve fiber, for the purposes of these experiments, was defined by the least forceful W-S monofilament that could elicit one AP per stimulus when the free end of the monofilament was delivered to the most sensitive point of the RF. The probes used to test this 'threshold of activation' consisted of W-S monofilaments of different calibers; each attached to rigid plastic handles. An array of twelve of these probes were independently calibrated on four occasions by three persons over the period of 7 mo, using a sensitive mechanical balance (Mettler). The probes were calibrated in turn, by applying each monofilament to the pan of the balance, as described by others [Levin *et al.*, 1978, Werner and Omer, 1970]. The plastic handle was held horizontal to the balance and the monofilament was maintained perpendicular to the pan surface with its free end resting on a piece of rubber glove placed in the center of the pan in order to avoid slippage. It is important to note, that the probes were held in a similar manner when testing each RF, since the force exerted by the probe depends largely upon its angle of application (see below). When slowly applying the probe, the mass in grams which produced sufficient force to initiate a bow (first-order buckling) in the

monofilament was recorded as the grams force (GF) for that monofilament. The average GF obtained for each probe was then the empirical value assigned to that monofilament. The monofilaments delivered GF values ranging from $6x10^{-3}$ g to approximately 6.9 g. The assigned GF values for each of the twelve probes are shown at the bottom of Figs. 13 and 14.

Although the manufacturer's marking (M) on each probe records the logarithm of 10 times the force in milligrams required to bow the monofilament at time of fabrication [Levin *et al.*, 1978], it was preferred to use the measured GF value since the probes were not recently purchased and showed signs of wear. By measuring the GF in this fashion it was apparent that M values were no longer a valid representation of the probe's characteristics and that the ordered sequence of the probes based upon their GF values had changed; probe 3.61 came before probe 2.83 on an increasing scale of GF (see Figs. 13 and 14). In the presentation of these data, the values for the probes were linearly arranged along the abscissa and labelled 1 to 12. The GF and M values were also stated for each probe. It is important to note that limitations exist in this presentation since probes 1-3 were very similar in value, as were probes 4-6. Whenever possible, the RF was sampled using the array of W-S monofilaments in an ordered fashion from 12 to 1 or *vice versa*, until the probe which elicited approximately one AP per application was chosen. The value of this probe was taken as the threshold for that RF. In cases where the threshold seemed to lay between probes, the probe of larger GF value was chosen.

The W-S monofilaments work by buckling in an elastic manner at a critical force [Levin *et al.*, 1978]. Although the force approaches a plateau as the nylon monofilament begins to bow (first-order buckling mode), it continues to grow with further bending such that approximately three times the force would be required to enter the S-shaped second-order buckling mode [Levin *et al.*, 1978]. The force is also dependent upon the angle of contact with respect to the skin surface. Thus the calibrated GF values which were empirically assigned, represent only a value around which the actual threshold values for the axons might have fluctuated. The GF values for the probes were considered to be ordered noncontinuous

variables. The increment in GF values for the probes was nonlinear and approximated a power function ($y = be^{mx}$ where b = -5.0649 and m = 0.6024; correlation of 0.9621) In addition, a large range of mechanical properties in the tissues tested may have contributed to the wide range of thresholds observed. For example, rejected allografted glabrous skin contained surfaces ranging from soft and moist to dry and encrusted (sometimes within the same RF) Hence, the histogram bins in Figs. 13 and 14 represent only an approximate threshold for the receptive fields tested. For all of these reasons, and in some cases because of small sample size, the data obtained for thresholds of axonal activation was not optimally amenable to the application of parametric statistical tests. The Mann-Whitney *U*-test, which does not depend upon assumptions about a normal distribution, was considered appropriate to determine the statistical significance of the data with the recognition that the GF values were ordered categories [Moses *et al.*, 1984].

3. Studying Axonal Response Characteristics

The responses of some cutaneous mechanosensitive afferent fibers to precisely controlled tactile stimuli were viewed on an oscilloscope and recorded by a computer. Stimuli were applied to the most sensitive spot within the RF using a plastic probe with a tip diameter of 0.8 mm, controlled by an electromechanical transducer [Chubbuck, 1966] capable of delivering displacements of up to 2.5 mm with an accuracy of 0.5 μm The maximal response of the stimulator was independent of frequency up to 40 Hz, but fell at -40 dB/decade at higher frequencies [Dykes and Terzis, 1979].

In the case of RA fibers, data were obtained from responses to sinusoidal skin displacements using up to 10 frequencies spaced approximately equidistant on a logarithmic scale; between 2 and 200 Hz. In order to mechanically stimulate these fibers, the stimulator probe was positioned just above the skin surface. A step of 1 s duration and approximately 125 μm amplitude brought the stimulus probe onto the skin. A sine wave was initiated 300 ms later

and lasted for 500 ms. The amplitude of the step indentation and the superimposed sine wave were adjusted from fiber to fiber, to that value which gave an optimal response across a variety of frequencies The tuning point at each frequency was defined as the minimum peak-to-trough amplitude of the sine wave that caused the fiber to fire one AP for each cycle of the sinusoidal stimulus. The collection of these points plotted on a frequency *versus* amplitude graph were referred to as the tuning curve for that fiber. The parameters (entrainment frequency and stimulus amplitude) defining the lowest point of the tuning curve gave an indication of the level of mechanical sensitivity for the RF; sometimes referred to as the vibratory threshold.

For SA fibers, the controlled stimulus paradigm consisted of sets of up to ten identical skin displacements (trapezoid waveform, 30 ms rise time, 1.5 s duration) of a given amplitude. One stimulus was delivered every 7-12 s, allowing time for mechanoreceptor recovery. At rest, the probe lightly touched the skin surface with no visible deformation or alteration in spontaneous act vity (when present). For each axon characterized, a range of stimulus amplitudes (the experimental variable) from 0 to about 1 mm was usually covered in 5-10 steps; each step represented by a data set of 5-10 responses to the same stimulus. A typical stimulus-response (**S-R**) curve was constructed by plotting the arithmetic mean of the number of APs which were elicited during the first 1 s of stimulation for each data set, and then connecting these points to form a line. When plotting both the RA and SA curves, the error bars were excluded to simplify the presentation.

4. Sampling Conduction Velocities

Attempts were made to determine the CV of each identified axon which served skin. Just-threshold electrical stimulation was applied under the skin surface in the center of the RF (if one was identified) using electrodes constructed from insect pins. The CV of the axon in meters per second was calculated from measurements of the latency and distance travelled by the AP from the peripheral site of stimulation to the proximal site of recording. The CVs for

Normal skin (from the original data of Dykes *et al.* [1984]) were sampled in a manner similar to that for each of the other groups, except that the recording well was located more proximally on the forearm.

For the sake of clarity in presentation, CV values were classified on the basis of whether the RF(s) was in glabrous or hairy skin. Previously published data suggested that this was a more meaningful division than dividing them on the basis of the nerve in which they travelled, or whether the RF and/or nerve was found dorsally or ventrally. Secondarily, the values were grouped on the basis of the 'type' of tissue they served: Normal, Normal (Experimental), Surgical Control, Allograft (Nonrejected), or Allograft (Rejected) On a tertiary level, the values were subdivided into categories of RA or SA fibers on the basis of their rate of adaptation to natural stimuli applied to the center of their RFs

In order to simplify the presentation of the data, and to make the numbers more coherent, the mean and median values for the conduction velocity samples are placed in the figure legend on the page facing the figure. It is believed that this is a more efficient presentation of the data, rather than having additional tables which would be set apart from the figures.

5. Identifying Muscle Innervation

The innervation of the intrinsic musculature of the allografted hands was verified through the study of motor neuron activation by means of electrical stimulation of the median or ulnar nerves. Large bundles of axons were separated from the intact nerve at a location distal to the site of sensory testing. Each bundle was mounted, in turn, onto a silver wire stimulating electrode and received a train of stimulating pulses (0.1 ms duration, 0.5 Hz).

EMG responses were recorded differentially from a pair of 25 μm teflon-insulated silver wires. The first 1 mm of insulation was removed from each end of the short wires, and one end of each wire was inserted through the skin of the palm of the hand into the belly of a muscle, using a hypodermic needle as a guide. The reference electrode was inserted into the

tissues of the volar aspect of the wrist. Other electrodes were inserted into the thenar and hypothenar eminences as well as into each of the lumbrical muscles. The free end of the EMG electrode was connected to a differential amplifier and filtered signals were monitored on a storage oscilloscope.

The intensity of transfascicular stimulation was slowly increased from 0.1 V until the first EMG was observed (defined as threshold; typically around 1-5 V). The intensity of stimulation was further increased to activate more fibers within the nerve bundle. The stepwise increase in amplitude of the elicited EMG was interpreted as 'motor unit' recruitment. Observations of gross movements of muscles and/or body parts were also documented.

III. Histology

To prepare the experimental tissues for light microscopy, the animal was deeply anesthetized with sodium pentobarbital and then euthanasia was performed through intravascular perfusion with 0.9% saline, followed by 10% neutral buffered formalin. The forearm was excised and immersed in more buffered formalin for storage. After adequate fixation, skin and nerve samples were removed for histological examination.

Specimens of skin were cut into cubes which measured approximately 1 cm^3 and contained a profile of epidermal and dermal tissues. Some blocks were embedded in paraffin, while others were further processed in Winkelmann's fixative [Winkelmann, 1960]. The paraffin embedded tissues were blocked such that 6-8 μm sections could be cut perpendicular to both the skin surface and the epidermal ridging pattern. Serial sections were mounted on glass slides and stained with hematoxylin and eosin (**H&E**) or were silver impregnated with the method of Sevier and Munger [1965]. Alternate sections of a series were also prepared using H&E and the periodic acid-Schiff (**PAS**) reaction, or using PAS and the Sevier-Munger silver technique. The Winkelmann-processed tissues were frozen, cut into 100 μm sections using a sliding microtome, and then impregnated with the silver method of Winkelmann.

Specimens of nerve were excised and embedded in epon plastic and cut into serial semithin sections (approximately 1 μm) with the aid of glass knives and an ultramicrotome (DuPont). The sections were mounted on glass slides and stained with methylene blue.

IV. Analysis of the Significance of Data

In the presentation of the electrophysiological data, the figures are consistently structured with the experimental groups organized to depict the transition from Normal to Normal (Experimental), Surgical Control, Allograft (Nonrejected), and finally, to Allograft (Rejected). This arrangement reflects the underlying hypothesis that consecutive steps from one experimental group to the next – from Normal through to Allograft (Rejected) – involves an increased degree of tissue manipulation, Normal skin was not surgically manipulated, Normal (Experimental) skin supported some fibers which were close to surgical incisions, Surgical Control skin consisted of NVFFs which were replanted (using microvascular anastomoses and nerve repair) to their original histocompatible tissue bed, while allografted NVFFs were transplanted to an alternate histoincompatible tissue bed and withstood various degrees of tissue necrosis as a result of immunological processes mounted by the host animal.

Statistical analysis of the data was performed using these experimental groups. Samples were compared in a pairwise fashion between groups or between categories within a group. To avoid problems related to the fact that the observations were sampled from populations which may not have been normally distributed, or that the observations were not independent, nonparametric analysis were used whenever possible.

Two samples were determined to be derived from populations with significantly different statistics of location (**SOL**) through the use of the Mann-Whitney *U*-test (as described by Sokal and Rohlf [1981], and performed with the software package StatView 512+™ from BrainPower, Inc.); the term 'location', describing the position of a sample along the abscissa representing a variable. The Mann-Whitney *U*-test is based upon the sum of ranked unpaired observations and is the nonparametric version of the two groups unpaired t-test or analysis of variance, however, the null hypothesis is not concerned with specific parameters (such as the mean in the case of analysis of variance). Instead, descriptive statistics such as the location and

distribution are emphasized. Arithmetic mean, median[§], and mode of the samples will also be discussed and compared when appropriate.

Tested in this manner, two samples were determined to be derived from populations with significantly different SOL when the probability of similarity of location, or the null hypothesis, was rejected (not accepted) with a significance level of 5% or less ($P \leq 0.05$) in a two-tailed test. That is to say that the two samples were determined to be significantly different, when, as a result of chance alone, there was a probability of only 5 (or less) out of 100 that they came from populations having the same SOL. The word 'moderate' was reserved to describe differences in sample SOLs which were not shown to be statistically significant at 5% in a two-tailed test, but for which there was a significant shift ($P < 0.05$) in SOL to higher or lower values as determined by a one-tailed test.

In order to be consistent with the literature, some statistical comparisons were made between combined (Total) samples of axons, even though the Total sample inherently served a mixed population of mechanoreceptors. For example, data from sampled axons which served glabrous skin were combined with data from axons which served hairy skin in order to derive a Total sample in a given experimental group (or skin condition), for comparison with Total samples from other experimental groups.

In the case of the thresholds of axonal activation, the observations were sampled with the use of monofilaments for which empirical values were assigned Even though the sampled variable was noncontinuous and its increment was nonlinear, the fact that it consisted of ordered categories rendered the data amenable to nonparametric analysis with the use of the Mann-Whitney *U*-test [Moses *et al.*, 1984]. Another advantage of the use of this test is that,

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§ In nonparametric distributions, sometimes the median is a more representative measure of location than the arithmetic mean, since the mean will tend to be normally distributed even if the original data is not (a consequence of the central limit theorem), and the mean is markedly affected by outlying observations while the median is not [Sokal and Rohlf, 1981].

through its design, it is capable of rendering comparisons between samples with very small sample sizes; tabled critical values are cited for tests where $n_1 = 3$ and $n_2 = 4$, and significant differences ($P = 0.05$; two-tailed test) can be found between two samples with each sample having $n = 4$ [Rohlf and Sokal, 1981]. Throughout this report, although statistical significance was stated for comparisons between samples of small sample sizes, conservative judgement was exercised when making comparisons between experimental groups on the basis of these tests.

On a cautionary note, it is important to realize that the Mann-Whitney test statistic, although sensitive to location, is not very sensitive to sample distribution. Therefore, in order to differentiate samples derived from populations having similar SOLs but different distributions, the nonparametric Kolmogorov-Smirnov two-tailed test was used (as described by Sokal and Rohlf [1981], for small samples, or, as described by Siegel [1956], and performed with the software package StatView 512+™ from BrainPower, Inc., for large samples). The Kolmogorov-Smirnov test is less powerful than the Mann-Whitney *U*-test with respect to differences in sample location, however, the Kolmogorov-Smirnov tests differences in both shape and location of the distribution and is therefore a more comprehensive test [Sokal and Rohlf, 1981].

Unless indicated otherwise, the probability values stated throughout the text are those for the Mann-Whitney two-tailed test when comparing SOL, or for the Kolmogorov-Smirnov two-tailed test when comparing sample distributions; as derived from the statistical tables of Rohlf and Sokal [1981].

RESULTS

I. Allograft Survival

After experience in managing the allografted tissues was acquired, and with adequate immunosuppression protocols in place, long-term survival of allografted upper-extremity primate tissue was achieved (see also Egerszegi [1990] and Skanes [1990]). A summary of the outcome of these efforts is presented in Table 1. Twelve allografts were attempted (8 TNVFFs and 4 HTs). The longest surviving allograft was the TNVFF carried by animal #11, which survived until 413 d after surgery; at which time the tissues were surgically removed from the host. As well, three autografted NVFFs were performed as surgical controls to the TNVFFs. A detailed synopsis of the course of survival for each of the allografted and autografted tissues is outlined in Appendix I.

Of the eight TNVFFs attempted, seven survived for a period longer than 3 mo. Three of these allografts (carried by animals #17, 20 and 21) remained in excellent condition for a period greater than 4 mo. The TNVFF on D2 of animal #21 is depicted in Fig. 4B, as it was seen at 193 d after surgery. The TNVFFs of animals #20 and 21 were used at approximately 6 mo for electrophysiological and histological studies. At the time of electrophysiological recording, these latter two allografts showed little outward sign of having undergone processes of rejection throughout the duration of their survival. It is for this reason, that the data obtained from these tissues was considered as having been derived from 'nonrejected' tissues, even though later histological analysis revealed that even these tissues experienced some degree of epidermal erosion presumably as a result of immunological processes (see Fig. 7C). The nonrejected allografts, although harvested at approximately 6 mo, presumably could have survived for prolonged periods of time.

For the purposes of this report, allografted tissues which underwent one or more substantial episodes of rejection which led to widespread tissue necrosis, will be referred to as 'rejected' tissues, whether or not the tissues were rescued and maintained thereafter in a stable

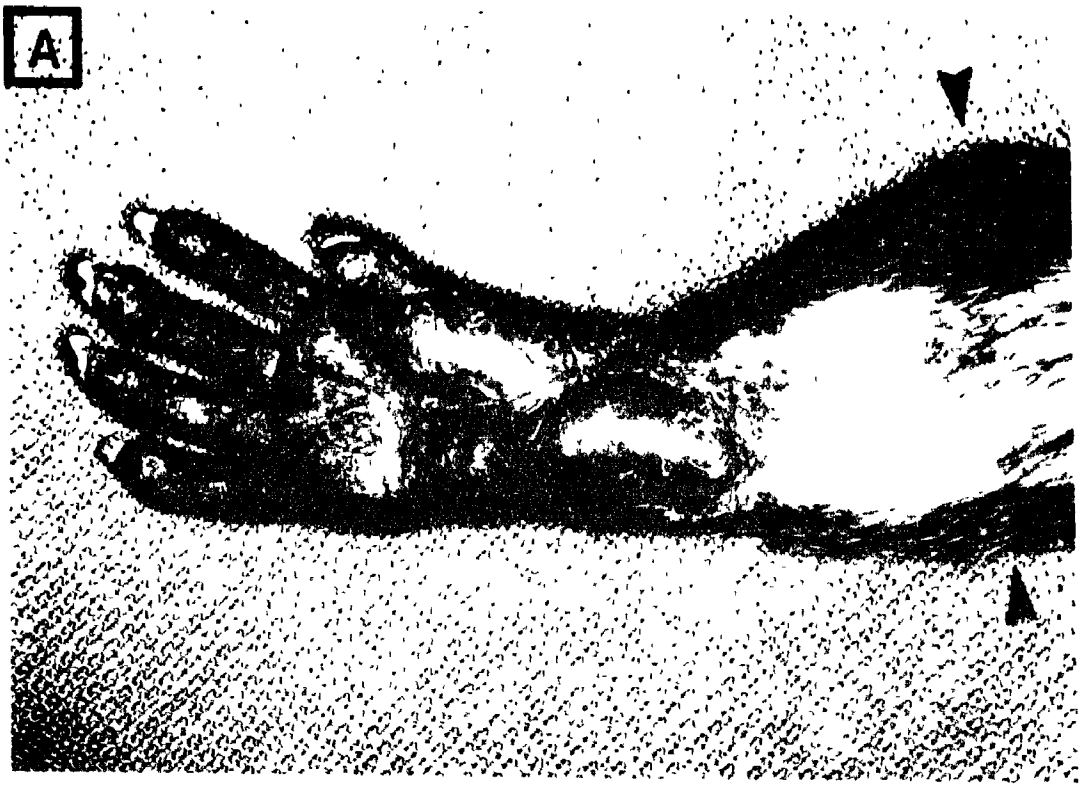
Table 1

In this summary of the transplantation results, each animal which was utilized is represented as a number from 1 to 21. Each animal's genetic compatibility is identified as either being unknown (randomly acquired through Charles River Canada, Inc.), or tissue typed (selectively obtained from the Southwest Foundation for Biomedical Research). The tissues which were utilized from each animal is stated under the heading Surgical Procedures; donor and recipient animals are identified. Brief descriptions of the course of survival of the allografted tissues are listed in the column labelled Evidence of Rejection. A descriptive and more detailed account of each case history can be found in Appendix I. Those tissues for which there was no visible indications of rejection at the time of electrophysiological testing were considered nonrejected, even though later histological examination showed some epidermal erosion by infiltrating inflammatory cells. The column entitled Experimental Status gives an account of the length of survival of each of the allografts (each graft representing an experiment), as well as the electrophysiological tests (if any) which were performed on the tissues prior to harvesting. Abbreviation: NA, not applicable.

Animal Number	Genetic Compatibility	Surgical Procedures	Evidence of Rejection	Experimental Status
1	Unknown	Pilot study, design	NA	NA
2	Unknown	Replant and design, donor to #3	NA	Terminated 6 d after surgery, determined a surgical success
3	Unknown	TNVFF recipient from #2	Early, extremely rapid	Terminated 20 d after surgery
4	Unknown	Donor to #5	NA	NA
5	Unknown	TNVFF recipient from #4	Early, 4 wk duration	Terminated 161 d after surgery, sensory testing
6	Unknown	Donor to #7	NA	NA
7	Unknown	HT recipient from #6	Intermittent, mild, final episode involved acute vascular complications	Terminated 311 d after surgery, sensory and motor testing
8	Unknown	Donor to #9	NA	NA
9	Unknown	HT recipient from #8	Early, extremely rapid	Terminated 26 d after surgery
10	Unknown	Donor to #11	NA	NA
11	Unknown	TNVFF recipient from # 10	At approximately 3 5 mo for 6 wk duration and reoccurrence at 8 mo	Terminated 413 d after surgery, sensory testing at 211 d
12	Unknown	HT donor to #13, recipient from #13	At approximately 1 5 mo, irreversible	Terminated 71 d after surgery
13	Unknown	HT donor to #12, recipient from #12	At approximately 2 mo, 4 wk duration	Terminated 188 d after surgery, sensory and motor testing
14	Unknown	Donor to #15	NA	NA
15	Unknown	TNVFF recipient from #14	At approximately 2 mo, chronic	Terminated 147 d after surgery, sensory testing
16	Typed	Donor to #17, donor to #20	NA	NA
17	Typed	TNVFF recipient from #16	Minimal visible indications	Terminated 141 d after surgery
18	Typed	Donor to #19, donor to #21	NA	NA
19	Typed	TNVFF recipient from #18	At approximately 1 5 mo, 4 wk duration	Died 123 d after surgery
20	Typed	TNVFF recipient from #16, CNVFF	Minimal visible indications	Terminated 193 d after surgery, sensory testing
21	Typed	TNVFF recipient from #18, CNVFF	Minimal visible indications	Terminated 196 d after surgery sensory testing

Figure 4

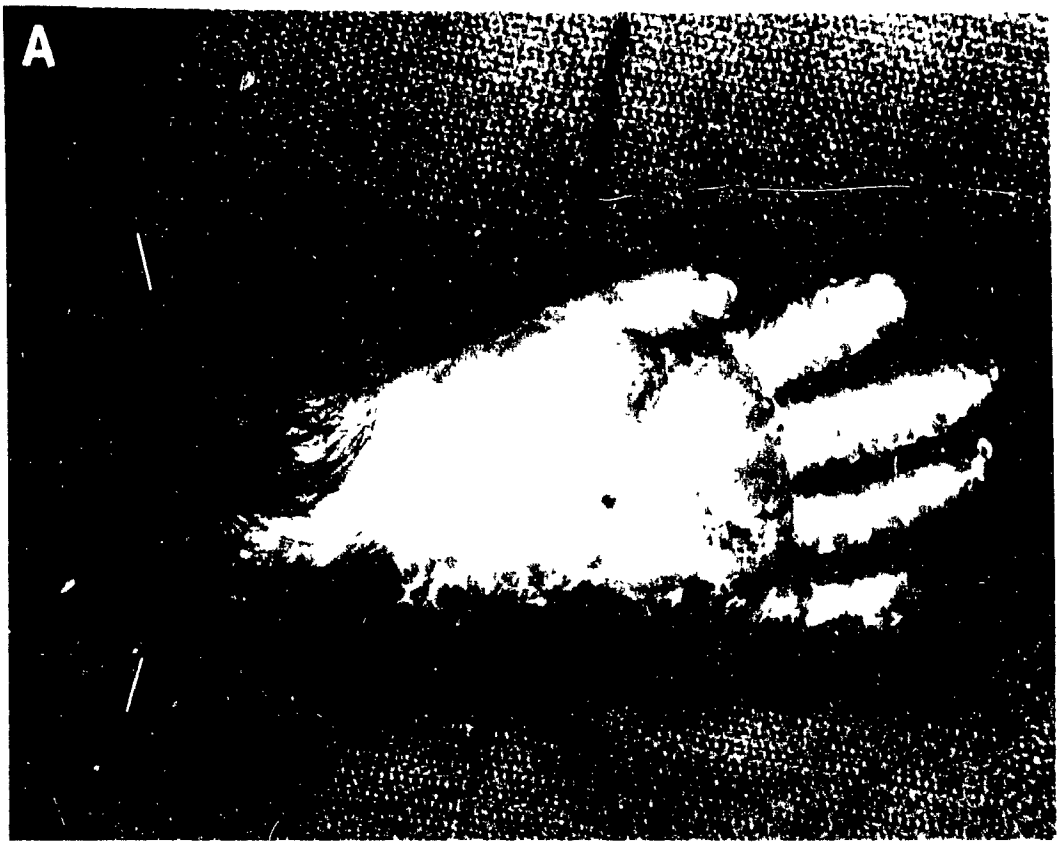
Long-term survival of nonrejected primate allografted composite tissues. (**A**) A complete HT (animal #7; right hand), as seen at 296 days after surgery. The arrows indicate the zone of transition between allografted and host tissues. The size discrepancy seen between the allografted hand and the host's forearm was in part a result of the surgical shortening of the bones of the forearm at the time of surgery to allow for proper tendon repair, as well as muscle atrophy in the hand. (**B**) TNVFF of the index finger along side an autografted fourth finger CNVFF (animal #21; right hand) as seen at 193 days after surgery. The index finger pigmentation is representative of the donor animal. The line of transition between the allografted skin of the TNVFF and host skin can be seen running perpendicularly between the two white marks superimposed upon the photograph. Relative scale is similar to that in Fig. 6 and 12. (Reproduced, with permission, from Samulack *et al.* [1988].)


A


B

Figure 5

Long-term survival of rejected primate allografted composite tissues. **(A)** A complete HT (animal #13; left hand) as seen at 132 days after surgery. The arrows indicate the zone of transition between allografted and host tissues. **(B)** TNVFF on the index finger (animal #15; right hand) as seen at 103 days after surgery. In both cases, note the striking lack of pigmentation as a result of the sloughing of glabrous epidermis which occurred during the rejection episode(s). As well, note the swelling in the tissues which was still present several weeks following the reversal of a severe rejection episode. Relative scale is similar to that in Fig. 6 and 12. (Reproduced, with permission, from Samulack *et al.* [1988].)


A


B

state for a prolonged period of time. On the basis of overall tissue disruption, two allografts (carried by animals #5 and 7; TNVFF and HT respectively) were considered to have undergone minor episodes of rejection which led to a mild degree of tissue necrosis. The other allografts (carried by animals #3, 9, 11, 12, 15 and 19) which experienced substantial tissue disruption, were considered as severely rejected tissues. The TNVFF shown in Fig. 5B (animal #15; as seen at 103 d after surgery) was representative of allografted tissues which had undergone a major episode of rejection and survived long-term in a stable condition.

Of the four HTs attempted, each experienced some degree of tissue rejection, however, two survived for 6 mo or longer. The longest surviving allografted hand (Fig. 4A; carried by animal #7, as seen at 296 d after surgery) underwent a series of minor rejection episodes. Initially these episodes produced only mild tissue disruption, however, the allograft finally succumbed to vascular compromise and was removed on postoperative day 311; after sensory and motor testing. The second HT which survived long-term (Fig. 5A; carried by animal #13, as seen at 132 d after surgery) underwent a major rejection episode and sustained severe tissue disruption. In this latter case, and in other cases where severely rejected tissues survived long-term, the duration of the rejection period was curtailed by increasing the level of immunosuppression (see Methods, Section I.C.2.). The allografted tissues carried by animal #13 survived and were maintained in a stable state until day 188; at which time sensory and motor testing were performed and the tissues were removed from the host.

Two of the allografts (carried by animals #3 and 9; TNVFF and HT respectively) underwent major rejection episodes within the first 2-3 wk after surgery. The circumstances which led to a surge in the immunological response and severe necrosis of the tissues were likely the result of insufficient perioperative immunosuppression of the host. Each of these latter allografts were removed within 1 mo after surgery, either in response to the irreversible nature of the necrosis, or because of persistent infections acquired subsequent to the tissue damage.

A. Synopsis of Skin Transformations

1. Alterations Related to Surgical Intervention

On the basis of patent circulation, all of the autografts and allografts which were performed were judged to be surgical successes. This is not to indicate that certain aspects of surgery or the surgical design did not contribute to the demise of any of the allografts, associated tissues or components. For example, the nail and nail bed were not removed in the early TNVFFs (animals #3 and 5) and this may have led to the demise of these host tissues as a result of necrosis due to poor blood circulation.

Other tissue transformations were also observed to take place during the first week after surgery. For example, reddish undertones in the proximal volar hairy tissues of the HTs were a common occurrence. In one of these instances (animal #9) the tissues of the volar wrist transformed from their normal beige or light brown appearance (often with a blue undertone, characteristic of many nonhuman primates) to become orangy-red and covered with clear fluid-filled vesicles, which later merged and eventually subsided. In other circumstances, single fluid-filled blisters of variable size occurred on the allografted hairy skin in the web-space between some of the digits of animals #9, 12, 13 and 17. In each of these cases the transformations were only observed during the early postoperative period, and once healed, they were not observed to reoccur throughout the course of the allograft's survival.

Some of these observations were paralleled by similar events which took place in host tissues of the experimental forearm. In animals #7, 9 and 12, the hairy tissues of the host's forearm, adjacent to the attachment site of the allografted tissues, also became reddened, although the extent and duration of the condition was less than for the allografted tissues. As well, the ulnar aspect of the host's forearm of animal #13 developed several clear fluid-filled blisters within the first three days of postoperative recovery, while it too was very swollen

In the recognition of the fact that several days are required to mount an immunological response to allografted tissues if antigenic sensitization had not previously occurred (see

Introduction, Section III.A.2.), it was thought that these latter transformations were the result of surgical manipulation. The redness and associated tissue transformations which occurred to the hairy skin of the HTs and the host's tissues, in the days following surgery, were likely related to ischemia as a result of interrupted blood supplies and/or tension applied to the tissues during and following surgery. In the postoperative period, swelling of the manipulated tissues was common for the TNVFFs, HTs and host tissues. The single blisters found in the web spaces and on the host's forearm may have been the consequence of pressures exerted by gauze bandages under tension as a result of swollen tissues during the immediate postoperative period.

2. Alterations Related to Immunologic Processes

At various times throughout their survival, nearly all of the allografts (except three; carried by animals #17, 20 and 21)) underwent obvious processes of rejection. At the histological level, 'all' of the foreign tissues underwent some degree of structural modification as compared to autografted control tissues. Hence, each of the allografts presented a suitable antigenic challenge to the host (see Discussion, Section I.). In return, the allografts showed signs that the host's immune system responded to the immunological challenge in each case. The degree of response mounted by the host was curtailed in most of the animals by the presence of the immunosuppressive drugs administered throughout the study. The amount of tissue destruction, and in some respects the type of tissues which were identifiable as the targets of rejection processes, were a reflection of both the level of immunosuppression as well as the degree of host sensitization. A comparison of host responses and a synopsis of the patterns of rejection processes observed are summarized in this section. The specific details of the timing and extent of the rejection episodes for each individual allograft are outlined in Appendix I.

The diversity of behaviors among the allografts of the same model, with respect to the patterns of rejection processes and subsequent tissue necrosis, were not much different than the diversity observed between the two models (TNVFF *versus* HT). In most cases, the onset of a primary rejection episode in an allograft could be identified by increased swelling and colour changes in the undertones of hairy skin. This increased swelling and tendency towards redness of hairy tissues as a result of rejection was differentiated from that seen postoperatively by their timing and diffuse nature.

The initial stages of rejection in hairy skin involved mild tissue disruption which resulted in the loss of hair shafts from one or more isolated regions of the allograft. Events which led to further structural changes, or changes in the concentration of melanin pigment of the tissues usually occurred in parallel with ongoing rejection processes in glabrous skin (to be described later). However, in at least one instance (TNVFF carried by animal #20), changes in pigmentation occurred over an extended period of time in allografted hairy skin without visible evidence of a rejection episode.

The emergence of structural changes in allografted hairy skin, as a consequence of rejection, revealed itself in many forms. In most cases (TNVFFs carried by animals #11, 15 and 19; HT carried by animal #13), restructuring of the epidermis occurred over a varied period of time with little or no breakage of the tissues. The transformation was relatively quick in onset, with loss of hair and a shift in pigment density from the normal light brown (with greyish-blue undertones) to greyish-pink. As well, the texture changed from its normal wrinkled appearance displaying widely spaced shallow grooves, to one which was relatively smooth. In some of these allografts (animals #13 and 19), where small isolated islands of pigmentation remained, the density of pigment increased such that the colour of the isolated areas of skin were dark brown (almost black); the rest of the tissues remaining greyish-pink

The visual signs of onset of rejection in hairy skin of other allografts were more pronounced. In one instance (HT carried by animal #9), in addition to the loss of hair, the most superficial layers of epidermis were shed in patches on the dorsum of the allografted hand. In

another case, isolated areas of the TNVFF (dorsum of D2) carried by animal #5 underwent diffuse breakdown as a result of mild shredding of the epidermis. Moist orangy-red fragile tissues were revealed initially, which soon healed to become dry and greyish-pink. Subsequently, the structure of the hairy skin component of the graft was gradually modified over a period of time by a continued breakage and healing of the epidermis.

The most rapid onset of rejection seen in hairy skin was expressed in the grafts carried by animals #3 and 7. Shredding and sloughing of isolated areas of epidermis revealed orangy-red underlying tissues which were not wet and in some cases had a surface which was jelly-like in texture. The hairy tissue most susceptible to rejection of this nature was the transitional skin on the lateral margins of the fingers and in the web-spaces between the fingers. It was of interest to note that in two allografts (HTs carried by animals #9 and 12) these same areas of tissue underwent similar processes of rejection in parallel with extreme glabrous skin rejection, while little or no hairy skin on the dorsum of the graft was affected during the same time frame; other than that which included swelling, redness, and loss of hair. This latter point suggested that transitional skin on the lateral margins of the fingers and in the web spaces between the fingers represented a tissue which was not only structurally different than either hairy or glabrous skin, but may have been immunologically different as well.

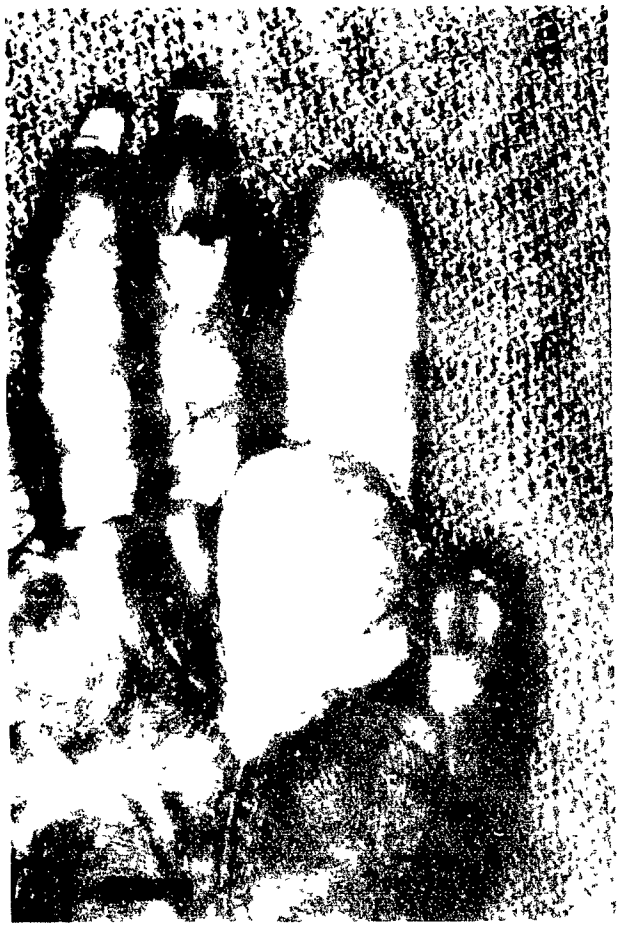
Perhaps due to the nature of the tissues, rejection episodes in glabrous skin led to more dramatic tissue disruption than that for hairy skin. The thickness of the keratinized layers of glabrous skin made it difficult to recognize any redness in the deeper strata of the tissues. Next to the indirect signs of generalized swelling of the tissues and possibly redness in the transitional skin at the edges of the palm, mild maceration of the keratinized tissues (especially in the major skin folds) and related dampness of the gauze dressings were the first signs of ongoing rejection in glabrous skin. If identified early, increased administration of immunosuppressive drugs quite often helped curtail the extent of the damage.

In the case of minor rejection episodes, the first sign of recovery consisted of the drying of the layer of keratinized glabrous skin. If the layer of keratinized tissue was thin to begin with, the dried tissues would crack, break-up and be able to be peeled off during subsequent weeks like pieces of a jig-saw puzzle (animal #11, TNVFF finger tip) If the keratinized layer was thick, the dried tissues would take on a smooth leathery appearance After a couple of weeks, the latter tissues were able to be delicately pried off as a single 'cap' of tissue (animals #5 and 11; TNVFF palmar digital pad) In either case, the removal of the dried tissues revealed relatively healthy tissues underneath. In some cases, ridging patterns were conserved, although the keratinized layer which remained was substantially thinner. The underlying tissues were usually lighter brown than the original healthy glabrous skin and often had unpigmented areas The pigmentation was usually variable in density and sometimes was patchy in location Over the course of the following weeks, the mosaic of light pink and beige pigmented tissues turned to a more uniform light-brown colour. Continued thinning of keratinized glabrous epidermis of the allografts carried by animals #11 and 13 soon left the glabrous surface raw, even though no single event of sloughing of tissue took place

If the rejection processes in glabrous skin were not identified early, or if the rate of onset was rapid, a major rejection of glabrous skin followed As depicted in Fig 6A (TNVFF carried by animal #15, similar events occurred in HTs), although there was swelling and mild maceration of the edges of the allografted glabrous skin and some of the surrounding host tissues, these signs were only partially indicative of the actual tissue damage which had occurred. Immediately upon soaking for a short period of time using sterile aqueous Hibitane® (not necessary in all cases) the necrotic glabrous tissues were easily peeled from the allograft in one piece (Fig. 6B). When the slough of tissue was thick, the lateral extent of tissue which could be peeled usually only included the tissues classically referred to as glabrous; leaving the transitional skin intact but inflamed. If the level at which sloughing occurred was deep, the raw and inflamed glabrous tissues which were revealed were often brilliant red, soft, wet, smooth but pitted in texture, and devoid of any papillary ridging patterns. If sloughing occurred at a

Figure 6

The allografted NVFF on the index finger of the right hand of animal #15, as viewed in a series of photographs spanning a 43 day period. The sequence depicts the tissue destruction and partial recovery which occurs during a severe episode of glabrous skin rejection. **(A)** At postoperative day 61, the index finger was swollen. The glabrous skin surface had a lifeless, shiny appearance. The edges of the flap and surrounding tissues showed maceration. **(B)** Minutes later, after a short period of soaking the hand in aqueous Hibitane®, the sloughed epidermis could be easily removed as a single piece, to reveal the raw inflamed tissues of the TNVFF underneath. The remaining epidermis and dermis of the TNVFF were seen to be wet, soft and pitted in texture, and brilliant red in color. **(C)** With increased immunosuppression (Appendix I), the rejection episode was brought under control. The TNVFF, 22 days after the tissue sloughed, had a tough smooth surface which was a mosaic of light pink and reddish brown colours. **(D)** At a time of 43 days after the tissue sloughed, the TNVFF was still swollen and without pigmentation. It became mottled and scaly. It was fragile and susceptible to mechanical damage. The allograft remained in this unstable condition with further episodes of rejection, until postoperative day 147 at which time sensory nerve recording was performed and the experiment was terminated by removal of the graft. Scale bar = 1 cm.



more superficial level, the tissues which were revealed were pinkish-red in colour and often showed some residual papillary ridges.

If immunosuppression of the host remained insufficient, the rejection processes continued Allografts which were exposed to prolonged periods of either mild or severe rejection processes succumbed to either infection or vascular compromise leading to mummification of the digit(s); the latter only occurred in HTs. At the point of time that either of these two events were understood to be out of control, the allografts were removed and/or euthanasia of the animal was performed.

In cases where increased immunosuppression was able to curtail the progression of the rejection episode, further tissue necrosis was minimized and recovery of the allograft ensued. For glabrous skin which had undergone sloughing of epidermis, the remaining tissues reverted from their raw-red condition and soon became dry, pink, and smooth, but encrusted with white flakes of thin keratinized tissues (Fig. 6C and D). Hairy skin which underwent shredding or sloughing also assumed a similar condition.

This state was maintained for prolonged periods of time for allografts carried by animals #11, 13 and 15, as long as sufficient immunosuppression was maintained. If rejection re-occurred (animal #15), the allograft shed its encrusted exterior and once again exhibited inflamed reddened tissues. With further immunosuppression, the tissues reverted to their encrusted state. When adequate immunosuppression was obtained, whether or not secondary rejection of this sort occurred, both the glabrous and hairy tissues of the allograft eventually became milky-white on the surface with greyish-pink undertones. After prolonged survival in this condition, the tissues became extremely fragile and susceptible to puncture. As well, the allografts soon lost much of their form, and as a result of their swollen nature, only major skin creases were identifiable.

B. Functional Capabilities of Allografted Tissues

In the majority of cases, to minimize the potential for self-mutilation, the protective thermoplastic splint was only removed from the experimental forearm while the animal was either tranquilized or anesthetized. Due to the curious nature and dexterity of the baboons, it was considered unwise to expose tissues which were potentially denervated (during the first three months of postoperative recovery), undergoing processes of active rejection, or which were unstable (in the recovery phase after rejection), since it would increase the risk of trauma and/or infection of the allograft. The functional capabilities of one HT (animal #7) and two TNVFFs (animals #20 and 21), however, were studied extensively.

1. Hand Transplants

The allografted right hand of animal #7 was observed for a period of several hours on postoperative day 251, while the animal was allowed to partially recover from ketamine/ xylazine anesthesia. Although hand movements were weak, there was no doubt that flexion, abduction and adduction of the wrist were possible. A small degree of flexion and extension of the fingers was also observed. Of most importance was the fact that the animal was capable of spreading the fingers. The latter observation was the first indication that there was voluntary control over at least some of the intrinsic musculature of the allografted hand, and that allografted muscle had indeed reinnervated.

2. Neurovascular Free Flaps

The forearm splints on animals #20 and 21 were removed at approximately 4 mo after surgery (animal #20 at 120 d, animal #21 at 123 d). The arms remained unsplinted for the remainder of the course of survival for each of the allografts (about 2.5 mo). Both the CNVFFs and TNVFFs remained intact and in excellent condition during this period of time.

Upon splint removal, apparent curiosity was expressed towards the uncovered extremity (sniffing and licking) by the animals After a short period of time very little further attention was paid to the experimental site, and the allografted tissues seemed to be accepted as autologous tissues. The TNVFF (D2) and CNVFF (D4) were used in a comfortable manner throughout all the daily activities (cage swinging, food manipulation, grooming and foraging). On occasion, while eating, the animals would use the finger with the TNVFF in order to scoop out food (yogurt) from plastic containers.

In short, there was no apparent loss of function encountered in the use of the CNVFF or TNVFF after a suitable recovery period had elapsed and while the allograft remained in the nonrejected state.

II. Histology

A. Integument Structure

For the purposes of histology, the forearm integument of the experimental animals was considered as two major tissue types: glabrous skin and hairy skin. No particular emphasis was placed on the transitional zone of skin at the border between glabrous and hairy skin Comparisons were made between samples of glabrous or hairy skin of each of the experimental skin conditions: normal, autograft, nonrejected and rejected allograft.

1. Normal Skin

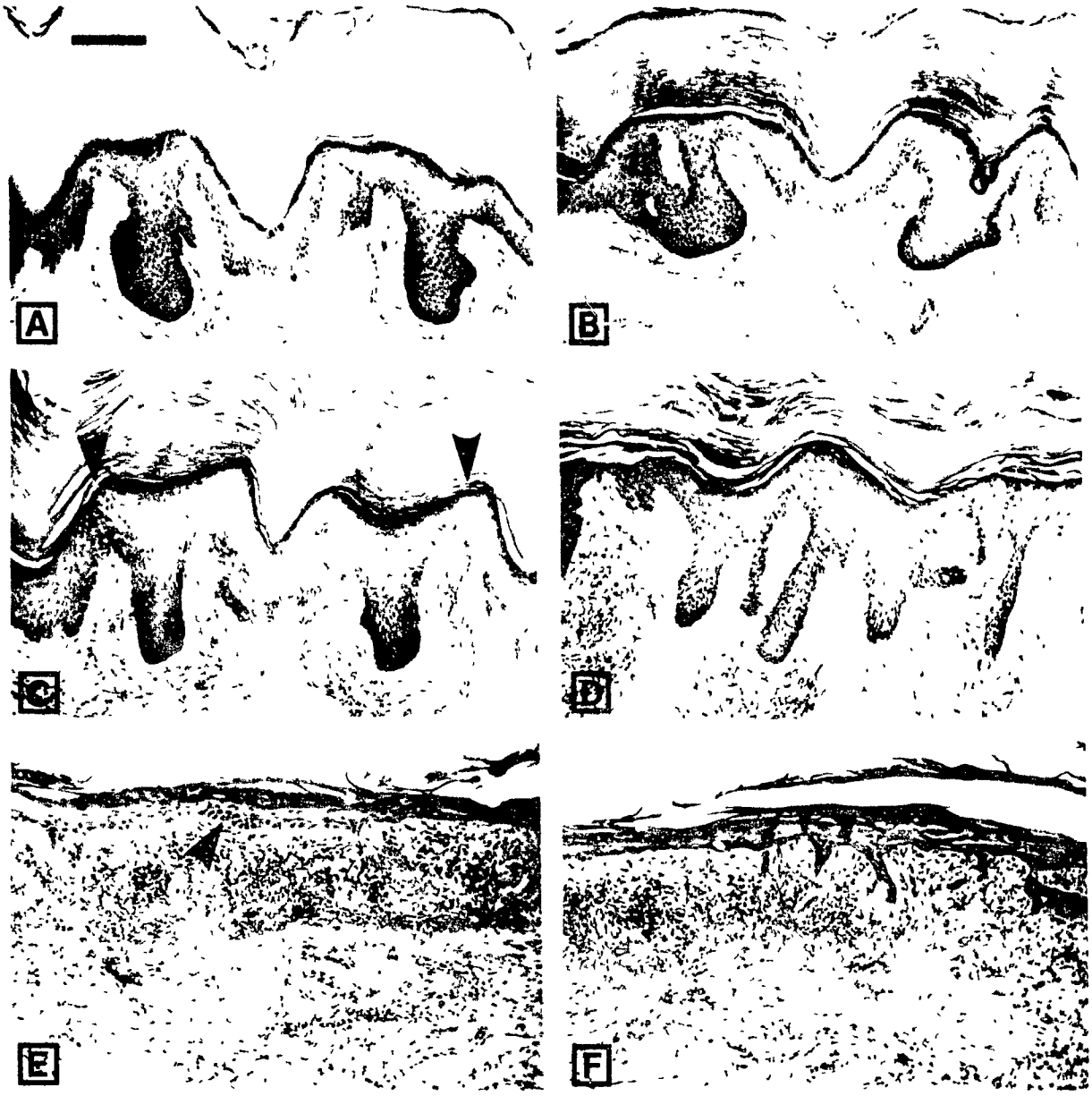
a) Glabrous Skin

The glabrous skin of the olive baboon, like that of many mammalian species, was characterized by a thick superficial layer of keratinized cells, a distinct epidermal ridging pattern and a lack of hair follicles. The epidermal ridging pattern was apparent, upon gross inspection, as whorls or patterns of ridges on the surface of the skin, commonly referred to as papillary ridges or dermatoglyphic patterns. When parallel papillary ridges were sectioned perpendicular to their axis of orientation, the physical nature of the deep structural elements was revealed Each papillary ridge had deep to it a vertically oriented epidermal thickening (the intermediate ridge) and was subtended at either edge by what is commonly referred to as the limiting ridge In correlation to (but not a mirror image of) the superficial papillary ridges, these deep epidermal ridges were of uniform spacing, orientation and size (Fig. 7A).

The cellular layers of the epidermis were identified on the basis of keratinocyte cell morphology (after Ham and Cormack [1979]). The most superficial layer of normal glabrous epidermis contained a highly keratinized zone, the stratum corneum, which was often thicker than all of the rest of the epidermis combined. Next, a layer of granulated epithelium 1 to 4 cells thick, the stratum granulosum, was composed of roughly spindle shaped cells aligned such that the long axis of each cell was parallel to the overlying surface (as seen in histological

Figure 7

Cross-sections of baboon glabrous skin depicting the structural changes which occurred as a result of autografting or allografting. The light microscopic sections were cut approximately perpendicular to the skin surface and at a right angle to the epidermal ridging pattern. Each frame is represented at the same magnification (scale bar = 150 μm) and spans the distance of approximately two epidermal ridges: **(A)** Normal (Experimental), **(B)** autograft (CNVFF), **(C)** nonrejected allograft (TNVFF), the large arrowheads indicate areas where inflammatory cell infiltrate caused erosion of the epidermis, **(D)** mildly rejected allograft (HT), showing thinning and irregularity of the epidermal ridging pattern, **(E)** severely rejected allograft (TNVFF), the large arrowhead indicates an area of cell proliferation containing large cuboidal epidermal cells, **(F)** severely rejected allograft (TNVFF), the small arrowheads point to Meissner corpuscles. The survival and location of these mechanoreceptors indicates that the loss of glabrous tissue, as a result of sloughing in cases of acute severe rejection, was primarily epidermis. The structural separation of the layers of keratinized epidermis in frames B and C were due in part to an artifact of histological preparation (H&E stain).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section). The stratum spinosum involved the bulk of the epidermis, and was composed of pleomorphic shaped cells whose configuration seemed to depend upon their location in the epidermal ridging pattern. In general, the cells of the stratum spinosum were almost circular in shape in regions deep to the papillary ridge, vertically aligned and spindle shaped in the intermediate ridge, and spindle shaped (the long axis of which was parallel to the overlying surface) in the limiting ridge The stratum spinosum ranged from approximately 3 to 10 cells in thickness in the limiting ridges, to approximately 25 cells of vertical thickness in the center of the intermediate ridge. The stratum germinativum formed the innermost layer of epidermis, abutting the basement membrane (basal lamina) at the border where the epidermis met the dermis. This layer was approximately 1 to 2 cells thick and was composed of cells which were quite tightly packed in a pseudocolumnar fashion; the long axis of the cells being perpendicular to the basal lamina. Melanocytes within the stratum germinativum of the intermediate ridges contained a dense packing of melanosomes. These, in addition to the relatively sparse distribution of melanin granules in melanocytes within the rest of the basal stratum, contributed to the dark brown pigmentation of this skin.

The basal lamina appeared to follow the base of the stratum germinativum throughout its whole course. The basal lamina of the intermediate ridges appeared thinner and less prominent than that of the limiting ridges. In the latter case, the basal lamina was often observed to penetrate into the dermis in the form of short pegs or 'tongues'.

Deep to the epidermis of the glabrous skin, was found the dermis. The thin outer (papillary) layer was composed of loose connective tissue which was highly vascularized with capillaries. The papillary layer followed the contour of the underside of the epidermis, sending dermal papillae upwards to surround the intermediate ridges. The inner (reticular) layer of the dermis was relatively avascular and was composed of dense irregularly arranged collagen bundles. The collagen bundles often formed cords which could be seen to extend upwards to the underside of the limiting ridges in the form of pillars. Within the dermis, some inflammatory cell infiltrate was always present, either closely associated with the capillary

loops in the dermal papillae or at the base of the intermediate ridges (especially near sweat ducts) within the papillary layer. This presence of immunocompetent cells represented the baseline of the natural scavenging and defense system of the host. Very little, if any, inflammatory cell infiltrate was observed within the collagen matrix of the reticular layer.

Eccrine sweat glands were readily identifiable throughout the glabrous skin. Their secretory structure was situated immediately below the dermis in the subcutaneous tissue In each case, the duct of the gland traversed upwards from a fat pocket of the subcutaneous tissues, through the dermis, and into the core of an intermediate ridge where it pursued a spiral course through the epidermis. Upon reaching the stratum corneum, the spiral became tighter, often reaching 5 to 6 full turns before penetrating the surface of the skin at the crown of the papillary ridge.

The observed pattern of epidermal ridging was very uniform for normal glabrous skin if the tissue was sectioned in the proper plane. If a section happened to pass through a sweat duct traversing upward through the epidermis, or if the angle of the cut was not vertical and perpendicular to the papillary ridges, the formation of the intermediate and limiting ridges appeared irregular in shape. As well, in cases where the papillary ridges were merging, the histological epidermal ridging pattern was hard to interpret Nevertheless, the fact that the stratum germinativum of the intermediate ridges contained a substantially higher concentration of melanin granules than it did in the limiting ridges, helped in defining the orientation of the tissue and subsequently the identity of the surrounding structures.

b) Hairy Skin

In contrast to glabrous skin, the epidermis of hairy skin lacked a uniform pattern of epidermal ridges. The overall skin surface was fairly smooth with no obvious papillary ridges, however, widely spaced shallow grooves were present which traversed the surface in all

directions. As well, at the point of exit of the hair shaft from each of the follicles was a steep depression of the epidermal surface, called the follicular orifice.

Although the epidermal surface was relatively uniform, the underside of the epidermis was irregular in contour. There was no apparent correlation of the ridging patterns of the basal epidermis to the superficial grooves. Instead, short dermal papillae, which appeared to project randomly into the epidermis, were the major contributing factors to the contour of the basal epidermal strata.

The stratum corneum was thin and often became detached from the epidermal surface during the histological processing and sectioning of the tissue. In contrast to glabrous skin, the stratum granulosum was not apparent as a distinct continuous cellular layer. However, granule-containing cells typical of this layer could be identified. The stratum spinosum was composed of a layer of rounded or spindle-shaped cells stacked 2 to 6 cells thick. The long axis of these cells was usually aligned parallel to the skin surface. Cells of the stratum germinativum were not as tightly packed as those for glabrous skin, although they were still sufficiently packed to become cuboidal in nature and to be stacked 1 to 2 cells thick. The melanocytes within the stratum germinativum showed variability in their distribution as well as in the density and number of their melanosomes. As a result, the apparent concentration of melanin pigment in normal baboon hairy skin was less than that of normal glabrous skin, but the distribution of pigmentation was relatively uniform throughout the stratum germinativum. Melanin granules were not only visible in the depths of the epidermis, but were also spread throughout the stratum germinativum of the external root sheaths of the hair follicles. The keratinocytes of the epidermis, and the external root sheath of the hair follicles, lay in continuity with each other.

The dermis of hairy skin was thinner than that of glabrous skin, primarily due to a thinner inner layer The outer layer was still predominantly more vascular than glabrous skin; having numerous capillaries. Irregularity in the appearance of the collagen fibers of the inner layer was accentuated in hairy skin, in comparison to glabrous skin, due to the disruption of

their course by the hair follicles. Arrector pili muscles of the hair follicles could be identified in the dermis, but they could not be found in all tissue sections. As well, sebaceous glands were easily identifiable, although they were sparse in number and the point at which their ducts opened to the skin surface were hard to find in histological section.

2. Autografted Skin

The integument structure of the autografted tissues of the CNVFFs was not very different than that seen in normal skin. The papillary ridges of autografted glabrous skin formed dermatoglyphic patterns which were well defined. The layer of keratinized epidermis was thick and contained spiral sweat ducts similar to those identified in normal skin. As depicted in Fig 7B, the thickness and periodicity of the epidermal ridging pattern were comparable to those of normal tissues. The structural detail of the basal lamina was preserved, including the fine tongues which penetrated the dermis at the base of the limiting ridges. The density and distribution of melanin granules within the stratum germinativum was similar to that seen in normal glabrous skin. In addition, the dermis was well-structured and contained only the baseline level of inflammatory cell infiltrate which was seen to occur in normal glabrous skin The distribution of this cellular infiltrate in a circumferential manner around the base of the intermediate epidermal ridges was also characteristic of normal glabrous skin.

Autografted hairy skin was also similar in structure to normal hairy skin. The surface of the epidermis contained steep follicular orifices at the site of exit of the hair shafts. The underside of the epidermis, as delimited by the stratum germinativum and basal lamina, was similar in contour to normal skin. The distribution and density of melanin pigment in the tissues was also reasonably uniform. The skin contained a dense packing of well-structured hair follicles with intact external root sheaths and hair shafts. The condition of the dermis also resembled that described above for normal hairy skin.

3. Nonrejected Allografts

Upon gross inspection, the nonrejected TNVFFs appeared to maintain glabrous papillary ridging patterns which resembled those found in CNVFFs or normal skin. The periodicity of the epidermal ridging pattern was conserved and the thickness of the epidermis was similar to that seen in autografted or normal tissues. The stratum corneum was intact and supported spiral sweat ducts. As well, the stratum germinativum of the intermediate ridges contained melanosomes which were comparable in location and concentration. In hairy skin, the hair follicles were intact, and at a level of low magnification they appeared healthy.

Closer inspection, however, revealed that some structural alterations of the nonrejected allografts had occurred (Fig. 7C). The most noticeable difference was the increased number of inflammatory cells present in the papillary layer of the dermis. The infiltration was most apparent in the dermal tissues which were circumferential to the intermediate ridges, and extended upward into the dermal papilla. It was predominantly at the top of the dermal papilla, above the capillary loops, where inflammatory cell invasion of the epidermis led to lysis of the surrounding cells. As a result of these isolated patches of epidermal erosion, the intermediate ridges acquired a triangular appearance. There was surprisingly little inflammatory cell invasion of the dermal tissues underneath the limiting ridges, however, this tissue was relatively avascular and contained a high density of collagen. Although epidermal pegs remained at the base of some limiting ridges, in general, there was an apparent thinning of the basal lamina in these locations and very few tongues of basal lamina penetrating into the dermis were observed.

Although similar immunological events to those which were happening in nonrejected allografted glabrous skin were also present in hairy skin of the same TNVFF, evidence of epidermal erosion was less evident. For the most part, the structure of nonrejected allografted hairy skin resembled that seen for similar autografted or normal tissue. General thickness and irregular contour of the epidermis, steep follicular orifices, confluent pigmentation in the

stratum germinativum, and healthy follicular structure, were all characteristics of nonrejected allografted hairy skin.

4. Rejected Allografts

a) Structural Changes as a Result of Mild Rejection

Allografted glabrous skin which sustained numerous episodes of mild rejection without significant sloughing of tissues, was characterized by loss of definition in the papillary ridges and loss of dermatoglyphic patterns. As can be seen in Fig. 7D, there was a lack of correlation between the presumed papillary ridges and the epidermal ridging pattern. The stratum corneum was thin and unstable, and there was no continuous well-defined stratum granulosum. The intercellular spacing within the strata spinosum and germinativum of the intermediate ridges was increased, perhaps as a result of retraction of the finger-like cytoplasmic spines of the keratinocytes, cell lysis and edema. At the base of the intermediate ridges, the presence of melanin granules was greatly decreased. As well thinning and tilting of the intermediate ridges obscured the normal periodicity seen in the epidermal ridging pattern when glabrous skin was cut in cross-section. The papillary layer of the dermis retained the predominance of the inflammatory cell infiltration, however, the reticular layer of the dermis also contained considerable infiltration.

Even though the epidermal structure in mildly rejected glabrous skin remained relatively intact, rejected hairy skin of the same allograft underwent considerable transformation. The most striking feature was the lack of intact hair follicles. Hair shafts were shed and the majority of the external root sheaths were destroyed. All that remained of the hair follicle was a bulbous epidermal thickening (5-10 cells thick and similar to, but more spherical than, that seen in Fig. 10C) which was cup-shaped and formed a wide and deep follicular orifice (or in this case a depression) which was usually filled with a plug of dead keratinized cells and other debris. The majority of pigmentation in the basal layers of epidermis was lost, however, isolated patches of

melanin granules remained. The areas of epidermis between the hair follicle remnants remained relatively intact, but the irregular folding of the basal layers seen under normal conditions was less prominent. The dermis of hairy skin which had undergone mild rejection contained pockets of inflammatory cell infiltrate primarily located under remnants of the hair follicles, around sweat glands and in highly vascularized areas.

b) Structural Changes as a Result of Severe Rejection

Allografted skin which underwent episodes of severe rejection sustained the most dramatic alteration in integument structure. The histological profile of allografted glabrous skin which underwent severe rejection is presented in Figs. 7E and F. From these photomicrographs it can be appreciated that no papillary ridges remained. The stratum corneum which was originally very thick, was now represented by only a very thin layer of fragile keratinized epidermis. No stratum granulosum was identified and the stratum spinosum was only one or two cells thick in some places. The stratum germinativum remained as a single layer which was aligned by either spindle shaped or simple cuboidal cells, and there was little indication of melanin granules. In the epidermis which remained, areas of active cell proliferation could be identified (Fig. 7E; large arrowheads). The regular epidermal ridging patterns which were seen previously, however, were nonexistent, and only remnants of the epidermal ridges (most likely intermediate ridges) remained. The survival and location of Meissner corpuscles in severely rejected allografted glabrous skin (Figs. 7F, 8C and G), indicated that the sloughing of tissue took place predominantly at the dermal/epidermal interface, at a level which was primarily superficial to the tops of the dermal papillae.

There were a large number of inflammatory cells within the papillary layer of the dermis which, through processes of cell lysis, contributed to considerable tissue edema. The papillary layer of the dermis appeared thicker than usual, in part because of edema but, primarily because of the thinning and/or loss of the intermediate ridges. Much of the basal lamina of the

intermediate ridges remained *in situ* and marked their original location (Fig. 8C). There was also an increase in the inflammatory cell infiltration of the reticular layer of the dermis, but it existed predominantly around blood vessels.

In hairy skin there was a further reduction in tissue structure from that described for grafts with mild rejection. The epidermis was reduced in most places to a uniform coverage of cells layered 3-7 cells thick. The spindle shaped cells of the stratum spinosum were very thin and elongated in an axis parallel to the skin surface. The stratum germinativum was composed of circular or cuboidal cells stacked 1-2 cells thick. There was very little evidence of epidermal ridging at the interface with the dermis, and pigmentation was isolated to small patches. The widely spaced remnants of the external root sheath resembled inverted cone-shaped structures with the vertex pointing towards the dermis. Using PAS staining techniques, the remnants of the basal lamina of the external root sheath could be identified in the dermis. In some cases, the basal lamina still surrounded clumps of epidermal cells which were discontinuous with the epidermis covering the skin surface. In most cases, however it circumscribed fibrous remnants of tissue and could be described as an empty envelope (Fig. 10C). The dermis contained patches of inflammatory cells which were larger in size and number as compared to hairy skin which had only undergone mild episodes of rejection, but did not parallel that of glabrous tissues of the same allograft.

B. Mechanoreceptor Survival

Upon analysis of the transplanted tissues using light microscopy, it was clear that there was variability in the survival of the several classes of sensory mechanoreceptors. Differences in morphology, physical orientation, reinnervation quality, and number were also noted for certain receptors when grafts were compared on the basis of histocompatibility (autograft *versus* allograft) and/or the severity of rejection they underwent. In almost all cases, the sensory receptor structures which survived in allografted tissues showed abnormal

morphological characteristics as compared to those of either the autografted or normal tissues. These differences were most pronounced for tissues which underwent severe rejection and were not specific to either the HT or TNVFF model.

1. Meissner Corpuscles

The Meissner corpuscles in allografted glabrous skin showed two very prominent features: a decrease in number (as estimated by the number of identifiable corpuscles per section) and a wide variation in the degree to which individual receptors were innervated by host axons (as evidenced by axonal contact). Although both of these features were observed in the autografts, they were manifested in allografted tissues to a greater extent.

Allografted glabrous skin, which had undergone minor episodes of tissue rejection, showed marked alterations of the normal epidermal ridging patterns (see Results, Section II.A.1.a.) and in the location and orientation of the Meissner corpuscles (Fig. 8A and B). Many corpuscles assumed an acute angle with respect to the skin surface (Fig 8B), as compared to their normal (almost perpendicular) axis In most cases, the corpuscular structures were shifted downward slightly from their normal position high in the apex of the dermal papilla (Fig. 8A; asterisk). Since the latter phenomenon was also observed in autografted tissues, the mechanism probably involved factors such as the swelling of tissues after surgery (with or without the presence of rejection processes) and the atrophy of the mechanoreceptor structure after nerve transection.

Related to these findings was the observation (using PAS and silver stains on allografted tissues) that in some instances the corpuscular structure maintained compacted lamellae on the more superficial aspect of the corpuscle. This compacted disk of basal lamina was thought to represent a remnant of the atrophied state of the corpuscle during the time period after nerve transection and before reinnervation. Although only observed infrequently for Meissner corpuscles in allografted glabrous skin (Fig. 8A; between asterisk and arrowhead), it was

Figure 8

Survival and reinnervation of Meissner corpuscles in primate allografted skin **(A)** A poorly reinnervated corpuscle in mildly rejected skin. Note the single axonal loop (arrow) within the lamellar structure, as well as the receptor's downward displacement with respect to the apex of the dermal papilla (asterisk). (Silver stain, animal #7 at 311 d after surgery, scale bar = 30 μm). **(B)** A well-reinnervated corpuscle. The unusual angle of the mechanoreceptor with respect to the skin surface is most probably due to the thinning and irregularities of the adjacent epidermal ridge, induced by minor episodes of rejection (Silver stain, animal #7 at 311 d after surgery, scale bar = 30 μm). **(C)** An intact Meissner corpuscle (arrow) surrounded by perfuse inflammatory cell infiltration and extreme glabrous tissue rejection. Note that it remains near the top of what was once a dermal papilla, as indicated by the faint outline of epidermal basal lamina remnants (coursing downward to the right; from the top of the arrow, to the corner of the insert). The very large homogeneously stained cells just below the tissue surface are thought to be engorged macrophages (PAS stain, animal #13 at 188 d after surgery, scale bar = 50 μm). **(D)** A higher magnification view of the same corpuscle (PAS stain, animal #13 at 188 d after surgery, scale bar = 15 μm). **(E)** A glabrous tissue section illustrating the regenerative capability of host axons (arrow) in a severely rejected histoincompatible tissue environment. Note the structure which is thought to be a growth cone at the tip of the most superficial axon (small arrows). (Silver stain, animal #15 at 147 d after surgery, scale bar = 50 μm). **(F)** A higher magnification view of the proposed host axon's growth cone in near the allografted glabrous skin surface (Silver stain, animal #15 at 147 d after surgery, scale bar = 10 μm). **(G)** Reinnervation of an allografted Meissner corpuscle by host axons, despite prolonged episodes of severe tissue rejection Note the absence of any epidermal ridging of the glabrous skin (Silver stain, animal #15 at 147 d after surgery, scale bar = 30 μm). (Reproduced, with permission, from Samulack *et al.* [1986].

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presumed also to occur in autografted tissues for which regeneration and reinnervation occurred.

The variation in the degree to which host axons were able to re-establish the normal innervation pattern in the Meissner corpuscles during the process of reinnervation of the grafts (autografts and allografts) is also best exemplified by Fig. 8. In contrast to normal Meissner corpuscles which were often found to be innervated by multiple axons or axon branches which coursed back and forth many times through the lamellar structure, reinnervated corpuscles quite often had only a single axon loop (Fig. 8A), circumferential axon contact (Fig. 8G), or had highly convoluted axonal penetration (Fig. 8B). These observations argued strongly that very simple axonal contact by one axon was sufficient to maintain the corpuscular structure which is thought to be lost lost in denervated corpuscles. Yet, as late as 6 mo after surgery, in allografts as well as autografts, Meissner corpuscles could be identified which had obvious lamellar structure, despite the fact that no obvious axonal contact could be identified (as determined by light microscopic methods of silver staining axons).

Observations specific to allografted tissues were derived from grafts which underwent episodes of severe tissue rejection. Mechanoreceptor loss was most pronounced in extreme cases of rejection, however, even in cases of severe rejection, some corpuscles survived. As depicted in Fig. 8C and D, despite the sloughing of the glabrous epidermis and cellular destruction as a result of inflammatory cell infiltration into the tissues, a Meissner corpuscle was able to survive and maintain its general location near the apex of what appeared previously to have been a dermal papilla (as outlined by remnants of epidermal basal lamina). The fact that the corpuscle was able to survive in the presence of such cellular destruction and maintain a moderately healthy lamellar appearance (Fig. 8D; PAS stain) with relatively little inflammatory cell invasion of its structure, suggested the possibility of an immunologically privileged status for this class of mechanoreceptors.

In the face of gross tissue destruction, host axons grew into a severely rejected (but still viable) histoincompatible tissue environment even when potential target mechanoreceptors or a

structured epidermis no longer existed (Fig. 8E). An enlarged view of a host axon's growth cone can be seen in Fig. 8F as it approached the surface of what remained of the allografted glabrous skin It is noteworthy that the host axon apparently left the remnants of its fascicle and coursed 25-30 μm into the rejected tissues without Schwann cell support (as determined through light microscopic observations of silver-stained axons in formalin-fixed paraffin-mounted tissues).

In allografted tissues which had survived episodes of severe rejection, Meissner corpuscles were often found which were innervated In most cases of allografted glabrous skin, where the destruction of epidermis was extensive, the long axis of the corpuscles still retained a relatively vertical orientation with respect to the skin surface even though little if any remnants of epidermal ridging patterns remained (Fig. 8G).

2. Pacinian Corpuscles

The location of Pacinian corpuscles within the deep (relatively nonvascularized) reticular layer of the dermis may have spared this class of mechanoreceptors from the destructive effects of allograft rejection. There were few differences encountered in the structure of the corpuscles identified in allografted tissues as compared to those seen in autografted tissues In general, those corpuscles which were not reinnervated by host axons showed irregularly spaced lamellae, a dense fibrous core, and in some cases, collapse of the normal onion bulb appearance Pacinian corpuscles which were reinnervated, usually had distinct lamellae and a single axon which coursed to the core of the structure (Fig. 9A) Those Pacinian corpuscles which were not reinnervated, seldom had free axons coursing in their vicinity In the allografted glabrous skin of animal #20, a Pacinian corpuscle was identified with multiple innervation. As can be seen in Fig. 9B, two axons (or two branches of the same axon) penetrated into the core of the corpuscle. In the photomicrograph, one axon can be seen as it terminates in a bulb formation (within the plane of focus), while the second axon penetrated

Figure 9

Reinnervation of Pacinian corpuscles in allografted glabrous skin (TNVFF, animal #20 at 193 d after surgery). Upon histological examination, those allografted corpuscles which were seen to be innervated by host axons usually had a single central axon, however, a corpuscle with multiple innervation was also identified. **(A)** A healthy reinnervated Pacinian corpuscle. Notice the distinct lamellar structure with a characteristic single axon (cut in tangential section) coursing through the core of the corpuscle. (Silver stain, scale bar = 50 μm). (Reproduced, with permission, from Samulack *et al.* [1986]. Copyright © 1986 by Elsevier Scientific Publishers Ireland Ltd.) **(B)** An allografted Pacinian corpuscle innervated by two axons (or branches of the same axon). The resolution of the photomicrograph is not optimal due to the intended thickness of the section (100 μm), however, two axons can be seen as they penetrated the core of the corpuscle. One axon ends as a bulb formation within the plane of focus, while the second axon extends further into the corpuscle to end in a similar fashion. (Silver stain, scale bar = 50 μm.)

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deeper into the section (approximately 20 μm) and ended in the same fashion.

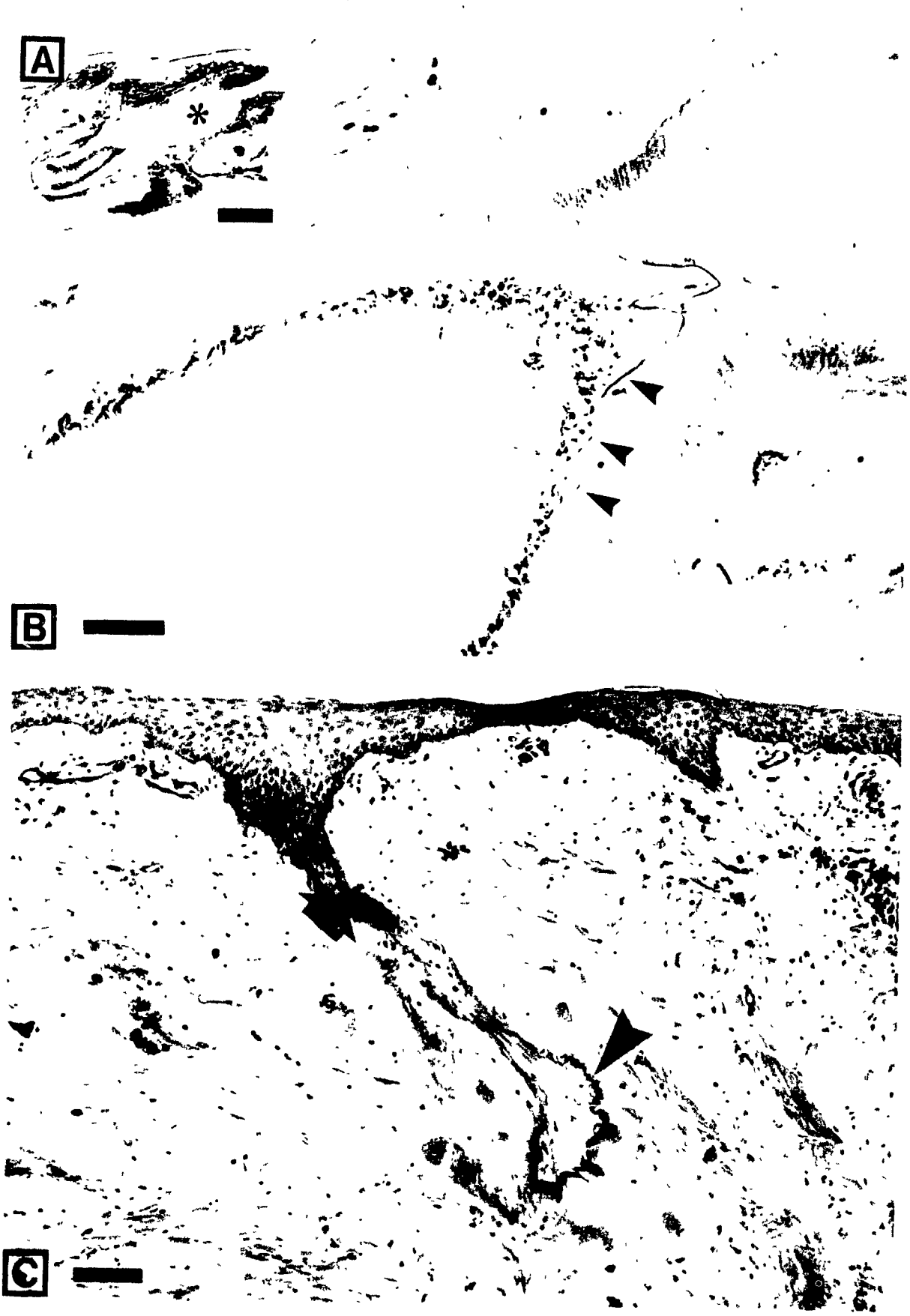
3. Hair Follicles

The survival of hair follicles and their associated structures in allografted hairy skin depended upon the degree to which the tissues had undergone alteration due to the rejection processes. In TNVFFs of animals #20 and 21, where the signs of allograft rejection were minimal, the morphology of the follicles was well conserved (Fig. 10A) and resembled that of follicles in autografted tissues. In both the autografts and the allografts, the follicles were poorly reinnervated, although the quality of innervation was generally better for follicles in autografted rather than allografted hairy skin. Axons which characteristically envelop the entire neck region of the external root sheath of normal autologous hair follicles with lanceolate, Ruffini and free nerve terminals, appeared to reinnervate the grafted follicles only to a rudimentary degree, if at all. This fact is exemplified in Fig. 10B, where an allografted follicle is shown to be innervated only by sparse lanceolate terminals probably being derived from one axon. In this case, as well as for most other hair follicles in allografted skin, the normal palisade of lanceolate terminals and the enveloping Ruffini complex are not present.

In the other allografts in which rejection took place, structural damage to the hair follicles occurred. The period of time over which the damage evolved was proportional to the overall intensity of the rejection processes. However, in the case where an allograft underwent rejection, the eventual result was that the hair shafts were shed and only remnants of the external root sheaths survived (Fig. 10C). In the less extreme cases, the basal lamina of the external root sheath remained as an empty envelope circumscribing the area where the intact hair follicle once stood. In more severe cases, the only trace of the follicle was a pit-like depression on the epidermal surface and/or a thickening of the remaining epidermal cells in the form of a cone.

Figure 10

Reinnervation and possible fate of hair follicles in allografted primate hairy skin. **(A)** A low magnification view of an allografted hair follicle in non-rejected hairy skin, indicating the approximate location (asterisk) of the host axon terminals shown in B (Silver stain, animal #21 at 196 d after surgery, scale bar = 500 μm). **(B)** Lanceolate terminals (arrowheads) at the neck region of an allografted hair follicle (Silver stain, animal #21 at 196 d after surgery, scale bar = 30 μm). **(C)** The remains of a hair follicle following rejection episodes in allografted hairy skin. Note the empty envelope of basal lamina (arrowhead) which outlines the remnants of the external root sheath (bold arrow) of the remaining follicle (PAS stain, animal #13 at 188 d after surgery, scale bar = 100 μm). (Reproduced, with permission, from Samulack *et al.* [1986].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Although single host axons were observed to course through the dermis near the remnants of the external root sheath, there was no evidence of axon differentiation into a dimensional structure like lanceolate terminals, or other structures normally associated with hair follicles. No evidence was found to indicate that an allografted hair follicle which was disrupted as a result of severe rejection processes could be reinnervated or could reconstruct any normal semblance of its prior self. The remnants of the external root sheath remained simply as an epidermal thickening without innervation.

4. Merkel Cell - Neurite Complex

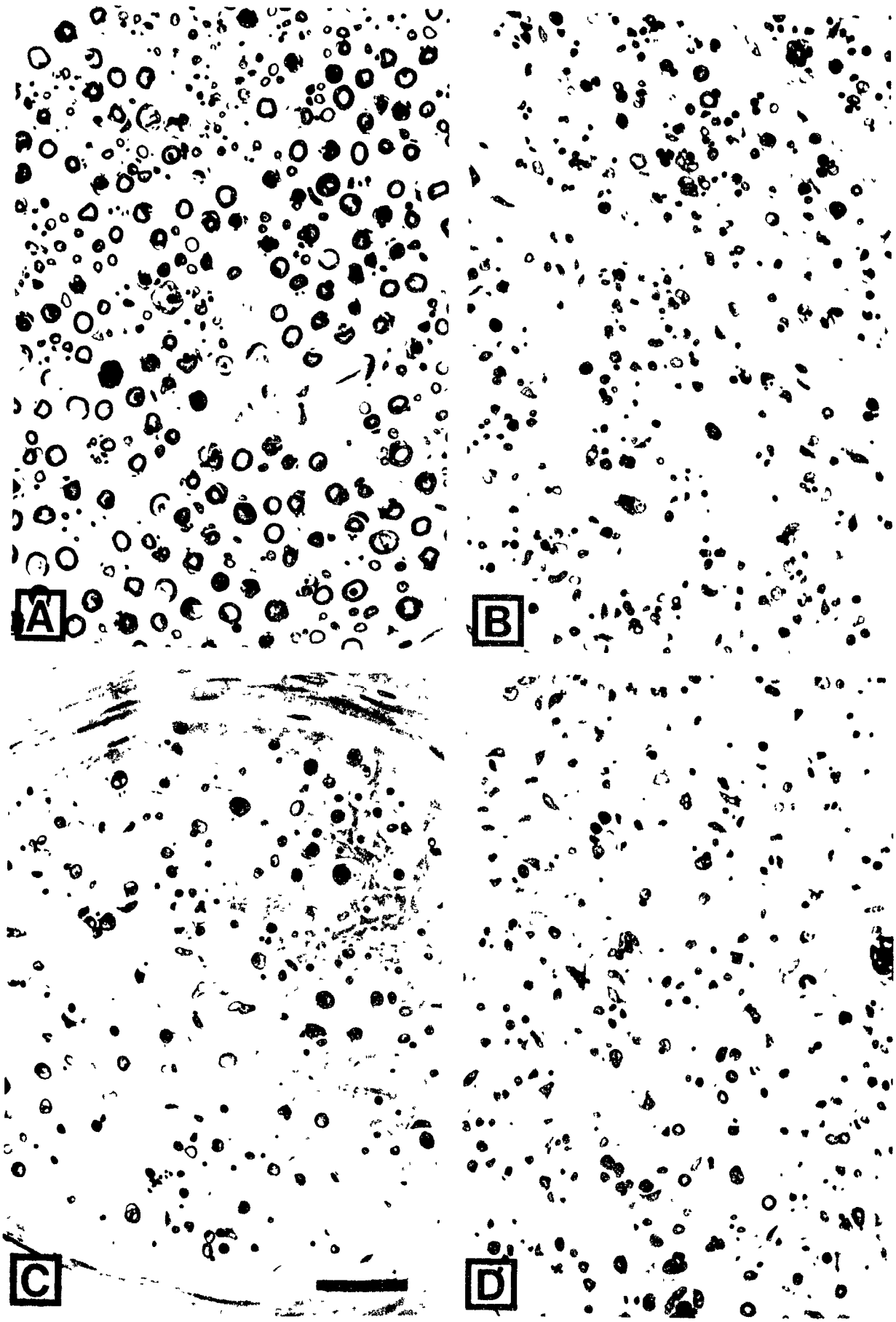
Merkel cells were not observed in any specimen of allografted glabrous skin. The concentration of melanin granules within the basal layers of the healthy allografted glabrous skin of the baboon may have obscured their positive identification under light microscopy, but axons were only rarely observed near the base of the intermediate ridges; the normal site of Merkel cell - neurite complexes. The preliminary electron microscopic studies to date have shown no evidence of Merkel cell survival in the allografted skin. These observations suggest that allografted Merkel cells (epidermal origin), in contrast to Meissner corpuscles (dermal origin), are very susceptible to the destructive processes of tissue rejection (even in cases where the tissue disruption was minimal), since they are located in the epidermis at the primary site of allograft rejection.

C. Quality of Graft Reinnervation

At the time of surgery there were few animals in which there was a major size discrepancy between the nerve stumps of the host and the graft, and the nerve repairs yielded apparently good results. Despite this acceptable appearance of the nerve at the time of repair, innervation densities varied between, as well as within, the grafts.

Figure 11

Low magnification cross-sections of peripheral nerves at the level of the proximal digit: **(A)** normal (D2 from the contralateral hand to the experimental site, animal #7), **(B)** autografted (CNVFF; carried by animal #21 at 196 d after surgery), **(C)** nonrejected allograft (TNVFF; carried by animal #20 at 193 d after surgery), **(D)** rejected allograft (TNVFF; carried by animal #15 at 147 d after surgery). Regenerated axons travelling through autografted or allografted tissues were fewer in number and generally were of smaller axon diameter than those found in normal nerves serving adjacent tissues. Axons in autografted nerves showed a broader spectrum of axon calibers than did those in allografted tissues. Inflammatory cells were more prominent in extrafascicular rather than intrafascicular allografted tissues, and were present in nerves serving nonrejected as well as rejected allograft tissues. Each frame is shown at the same magnification; frame C, scale bar = 30 μm. The tissues were formalin-fixed *in situ*, embedded in epon plastic and cut as semithin sections. (Methylene blue stain)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In Fig. 11, it can be seen that the nerve segments from the autografted CNVFF (Fig. 11B; carried by animal #21) and the allografted TNVFF (Fig. 11C and D; carried by animals #20 and 15, respectively) contained fewer axons than was found in normal nerve (Fig. 11A; left hand of animal #7) at the same anatomical level. As well, the diameter of the host axons distal to the site of nerve repair and within the grafts was generally smaller than the diameter in normal nerve of corresponding sites. The number of axons contained in allografted nerve segments which were part of TNVFFs was similar to that of CNVFFs, however, electron microscopic views showed that there were substantially fewer small diameter myelinated and unmyelinated axons in the allografted nerves.

The photomicrographs of Fig. 11 represent cross-sections through nerve fascicles of formalin-fixed tissues. Although each of the tissues showed histological artifacts associated with the method of fixation (the extent of which is best appreciated in the section of normal digital nerve represented in Fig. 11A), the nerve segments within the graft components of the CNVFF and the TNVFFs contained axons which also showed evidence of Wallerian degeneration as indicated by disintegration of the myelin sheaths. The anatomical processes which led to the latter observation may have been (in part) a consequence of the nerve transection associated with the electrophysiological nerve recording session which took place during the days prior to tissue fixation (see Methods, Section II.B.).

It is noteworthy that although allografted nerve segments within rejected TNVFFs (Fig. 11D) contained a similar number of host axons as those nerves which served nonrejected tissues (Fig. 11C), the rejected TNVFFs contained host axons for which very few identifiable RFs could be found (see Methods, Section II.C.1. and Fig. 12).

III. Electrophysiology

A. Synopsis of Fibers Sampled

Electrophysiological studies were performed on seven of the animals which received allografts (animals #5, 7, 11, 13, 15, 20 and 21; 5 TNVFFs, 2 HTs, and 2 CNVFFs). As was detailed in Table 1, testing of sensory innervation was carried out in all seven of these animals Testing of motor innervation in HTs was done on two grafts (carried by animals #7 and 13).

In total, data was recorded for five hundred and ninety-seven axons serving either skin or subcutaneous tissues of the forearm and hand of immunosuppressed baboons A summary of the distribution of the fibers which were studied is presented in Tables 2 and 3 The animals from which the fibers were sampled are listed at the top of each table, and are segregated on the basis of allografted skin condition at the time of testing. The total number of axons studied in each animal, or for each condition, are stated at the bottom of the columns. On the left margin of the tables, the axons are grouped with reference to the type of tissue they served The total number of axons studied in each group of fibers is located in the far right hand margin. For fibers which served skin, special groups were provided for axons which served RFs which were primarily confined to scar tissue (Incisional Scar), and for those axons for which an RF was either not identified, or for which documentation was neglected (Unclassified) As well, a special group was reserved for the identification of Pacinian corpuscles, since in some cases, due to the sensitivity of the mechanoreceptor, one could not be sure of the exact location of the corpuscle. The fibers from the latter three groups were not included in any analysis of thresholds of activation, or axon conduction velocities, when comparing glabrous and hairy skin.

Of special interest is the observation that the sampled fibers which served tissues of animals #20 and 21 had a significantly higher incidence of spontaneous activity in comparison to axons sampled in the other animals. For skin, this was reflected by a 6-fold increase in occurrence when animals #20 plus 21 were compared to the rest of the animals studied (31.6%

Fibers Studied Serving Skin

		Animal Specimen: Rejected, Mild, #5	Rejected, Mild, #7	Rejected, Severe, #11	Rejected, Severe, #13	Rejected, Severe, #15	Nonrejected, #20	Nonrejected, #21	Total
Normal (Experimental)									
Glabrous	SA	14/0	-	-	-	2/0	18/4	7/5	41/9
	RA	19/0	-	-	-	-	11/0	5/0	35/0
	?	5/0	-	-	-	1/0	-	-	6/0
Hairy	SA	-	-	-	-	-	2/0	6/3	8/3
	RA	-	-	-	-	-	1/0	11/3	12/3
	?	-	1/0	-	-	-	-	-	1/0
Surgical Control									
Glabrous	SA	-	-	-	-	-	6/1	16/14	22/15
	RA	-	-	-	-	-	5/1	7/0	12/1
	?	-	-	-	-	-	-	1/0	1/0
Hairy	SA	-	-	-	-	-	-	3/1	3/1
	RA	-	-	-	-	-	-	4/1	4/1
Allograft									
Glabrous	SA	9/1	10/1	-	26/2	6/1	31/13	25/17	107/35
	RA	5/0	12/0	-	32/0	9/0	19/0	8/3	85/3
	?	-	-	-	-	2/0	-	-	2/0
Hairy	SA	1/0	17/4	3/0	-	4/0	15/3	13/2	53/9
	RA	7/0	31/3	8/0	7/0	3/0	10/0	12/2	78/5
	?	1/0	1/0	-	-	-	-	-	2/0
Incisional Scar	SA	4/1	-	-	-	-	-	5/3	9/4
	RA	2/0	-	-	-	-	-	-	2/0
	?	2/0	-	-	-	-	1/0	-	3/0
Pacinian	RA	2/0	-	-	-	-	-	1/1	3/1
Unclassified	SA	-	-	-	-	-	-	2/1	2/1
	RA	-	-	-	-	-	1/0	1/0	2/0
		71/2	72/8	11/0	65/2	27/1	120/22	127/56	493/91
		246/13					247/78		

Table 2

A summary of the axons sampled which served cutaneous tissues in immunosuppressed baboons. The animals from which the fibers were sampled are listed at the top of the table and are segregated on the basis of the condition of the allografted skin at the time of testing. Animals #20 and 21 had been receiving very high levels of CsA.

The respective groups into which the sampled axons were classified are represented on the left margin. Normal (Experimental) fibers are those which served normal tissues on the experimental hand which did not undergo direct (intended) surgical manipulation. Surgical Control fibers served skin of the CNVFF. Allograft fibers served skin of the TNVFF or HT. Incisional Scar fibers had RFs which were primarily associated with scarred tissue. Fibers for which RF location was identified, but which had adaptation characteristics that were unclear or not documented, were considered as fibers of questionable (?) submodality. Axons which had Pacinian-like responses are grouped by themselves. Axons which were partially characterized during the experiment, but for which documentation as to the location of the RF was neglected, are referred to as Unclassified.

Numbers in bold type refer to the quantity of axons sampled. The numbers to their immediate lower right, represent the quantity of axons within that sample which were observed to be spontaneously active.

Table 3

A summary of axons sampled which served deep, unresponsive or unidentifiable tissues in the immunosuppressed baboon. The animals from which the fibers were sampled are listed at the top of the table, and are segregated on the basis of the condition of the allografted skin at the time of testing. Animals #20 and 21 had been receiving very high levels of CsA.

The respective groups into which the sampled axons were classified are represented on the left margin. Normal (Experimental) fibers are those which served normal tissue deep within the experimental hand or wrist, which did not undergo direct (intended) surgical manipulation. Allograft fibers served deep tissues which were part of the HT model. The group Unknown is reserved for those fibers which were identifiable electrophysiologically on the basis of their spontaneity, or through retrograde electrical stimulation, but for which a RF could not be determined.

The submodality Muscle/Joint/Tendon refers to those fibers which could be activated through natural manipulation of the musculoskeletal axis. The submodality Tap refers to those axons whose response properties were not easily defined and not considered to be of cutaneous origin, but which could be activated by very intense mechanical stimulation.

Numbers in bold type refer to the quantity of axons sampled. The numbers to their immediate lower right, represent the quantity of axons within that sample which were observed to be spontaneously active.

Fibers Studied Serving Deep Tissues

	Animal Specimen							
	Rejected					Nonrejected		
	Mild		Severe					
	#5	#7	#11	#13	#15	#20	#21	Total
Normal (Experimental)								
Muscle / Joint / Tendon	10/0	2/0	-	-	-	7/5	3/0	22/5
Tap	5/0	-	-	-	3/0	8/0	10/4	26/4
Allograft								
Muscle / Joint / Tendon	-	9/0	-	7/1	-	-	-	16/1
Tap	-	15/0	-	3/0	-	-	-	18/0
Unknown	4/1	9/5	-	-	1/0	4/4	4/3	22/13
	19/1	35/5	-	10/1	4/0	19/9	17/7	104/23
	68/7					36/16		

versus 5.3%). For axons which served deep tissues, the difference was also significant in a similar comparison, yielding a 4-fold difference (44.4% *versus* 10.3%).

In cutaneous tissues it was primarily the SA fibers which served glabrous skin of the CNVFFs and TNVFFs which had the highest percentage of spontaneously active fibers (68.2% for CNVFFs and 53.6% for TNVFFs). These axons, having undergone nerve transection and repair, had regenerated and reinnervated their target tissues. The fact that the TNVFFs of animals #20 and 21 showed few signs of rejection upon gross visual inspection and resembled the CNVFFs in this regard, coupled with the fact that the axons which served the Normal (Experimental) tissues of the same animals also had a very higher percentage of spontaneously active fibers (36 0% for Glabrous SA fibers), suggests that the abnormally high incidence of spontaneous activity was not directly related to processes of regeneration alone. This observation is further supported by the fact that tissues from animals #5, 7, 11, 13 and 15, which had undergone denervation and reinnervation, as well as various degrees of rejection (which possibly led to further denervation and reinnervation processes), were not served by axons which had an excessively high incidence of spontaneous activity. Within the sampled axons for animals #20 and 21, the difference in the percentage of spontaneously active SA neurons found in glabrous skin (36%) as compared to those found in hairy skin (37.5%) was not significant, however, a similar comparison within the allograft group showed a 3-fold difference (53 6% for Glabrous SA *versus* 17.8% for Hairy SA) In both cases, the proportion of spontaneously active fibers was greater than that sampled in other animals.

Not only did the sampled SA fibers which served glabrous skin of nonrejected TNVFFs show a higher percentage of spontaneously active axons in comparison to those of other groups of axons (or animals), but of the fibers sampled, there was consistently a higher percentage of SA *versus* RA fibers in the glabrous skin of animals which received high levels of CsA (animals #20 and 21) as compared to the other animals. The SA fibers sampled which served Normal (Experimental) glabrous skin of animals #20 and 21 were approximately 1.5 times more numerous than the RA fibers sampled in the same tissue, and in allografted

glabrous skin of these animals the SA fibers sampled represented over 2 times the number of RA fibers sampled. This is in contrast to the collective group of animals which carried rejected allografts and which generally maintained lower levels of CsA, where the reverse was true (a greater number of RA *versus* SA fibers were sampled). The consistency of this observation (although not all comparisons were statistically significant) is interesting to note, since there was no special priority given to the SA submodality when sampling the axons (see Methods, Section II.C.).

The identification of axons which served muscle, joint and tendon sensory organs in the deep tissues of the allografted hands was an important indication that the afferent limb of the motor circuitry to allografted muscle had reinnervated and was at least partially intact. It is also noteworthy that the reinnervated fibers were still functional in the aftermath of processes of severe rejection of the cutaneous tissues of the allograft carried by animal #13. In addition, the electrophysiological identification of axons which served severely rejected allografted tissues is one indication that the disruptive force of the rejection processes, at the level of immunosuppression induced, was confined to the cutaneous tissues and was not necessarily disruptive to the underlying neural tissues.

B. Identification of Receptive Fields

Despite the differences in the outward appearance of the rejected allografted tissues as compared to those of nonrejected allografts or control tissues (Figs. 4 and 5), host axons were still able to grow into, locate and functionally innervate their target tissues. An extreme example of this capability was observed in animal #13. At the time of sensory testing, even though the HT had undergone major episodes of severe rejection (starting at day 60 of postoperative survival), several RFs of reasonable size (up to 1 cm) and of both the RA and SA submodalities were identified on the volar aspect of the fingertips (15 cm distal to the site of nerve repair). Not all axons innervating the grafts reached target tissues, however, and there

were important differences in the quality and quantity of reinnervation observed in the rejected allografted tissues as compared to that observed in nonrejected allografts or control tissues.

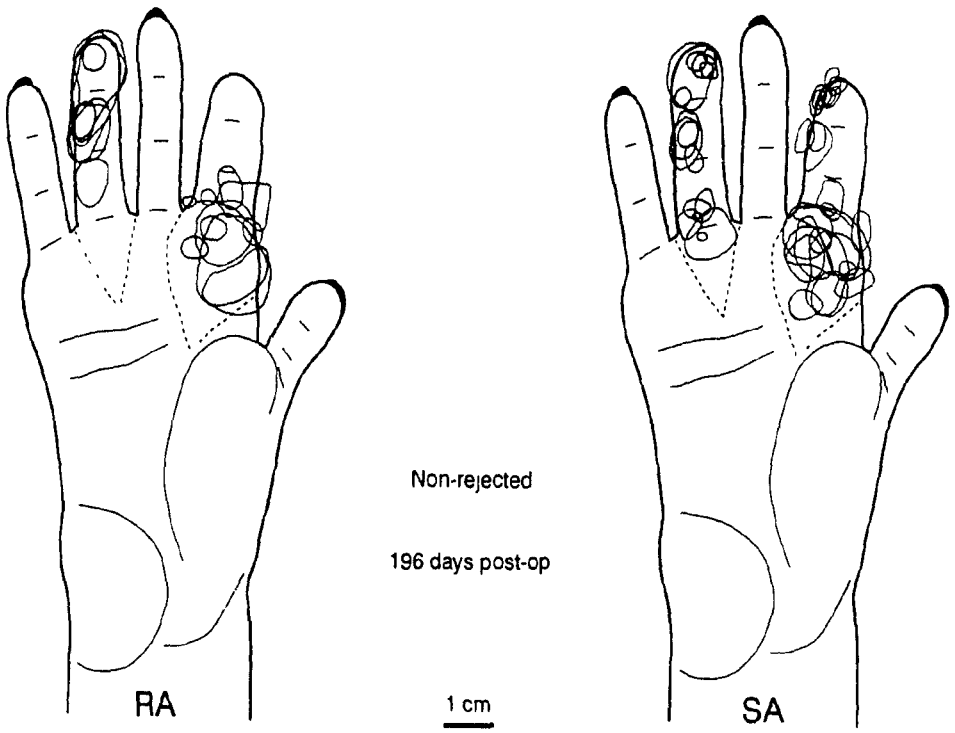
Figure 12 illustrates the approximate shape and location of the cutaneous RFs studied in glabrous skin of the TNVFFs and CNVFF of animals #15 and 21. The radial extent of the RFs were determined using the W-S monofilament which elicited a GF value of approximately 5 g (M = 4.56; see Methods, Section II.C.1 and 2.). The two diagrams in the upper panel represent the right hand of animal #21 (seen previously in Fig. 4B). The upper left diagram depicts the RFs for the RA fibers identified which served the CNVFF (D4) and the TNVFF (D2). The diagram on the upper right, depicts similar information for SA axon RFs. The diagrams in the lower panel are drawn to represent the right hand of animal #15 (seen previously in Figs. 5B and 6), and show the outlines of the RFs for the RA and SA fibers that were identified on its TNVFF.

It is easily appreciated from Fig. 12 that the location of the identified RFs for the CNVFF and TNVFF of animal #21, tended to be patchy; although the RA and SA RFs were roughly isolated to the same innervation territories. The location of the identified RFs for the TNVFF of animal #20 (not shown) were more evenly dispersed, and covered the whole glabrous surface of the flap. In any of the animals, if the location of the identified RFs was patchy, the distribution reflected the innervation territories of the lateral or medial components of the digital nerves, a cutaneous branch of the median nerve (which served the D2 palmar digital pad), or a cutaneous branch of the radial nerve (which served the hairy skin overlying the proximal knuckle of D2). This territorial distribution of innervation was best displayed by the distribution of the RFs found in the grafts of animal #21 (Fig. 12, upper panel), where RFs resided primarily along the ulnar margin of the CNVFF and TNVFF glabrous tissues, and on the D2 palmar digital pad, even though all of the nerve reattachment were between nerve stumps with minimal size discrepancies and met the criteria for a good microsurgical repair at the time of surgery. The fact that the sampled RFs along the ulnar margin of the TNVFF of animal #21 was only represented by SA characteristics was purely by chance.

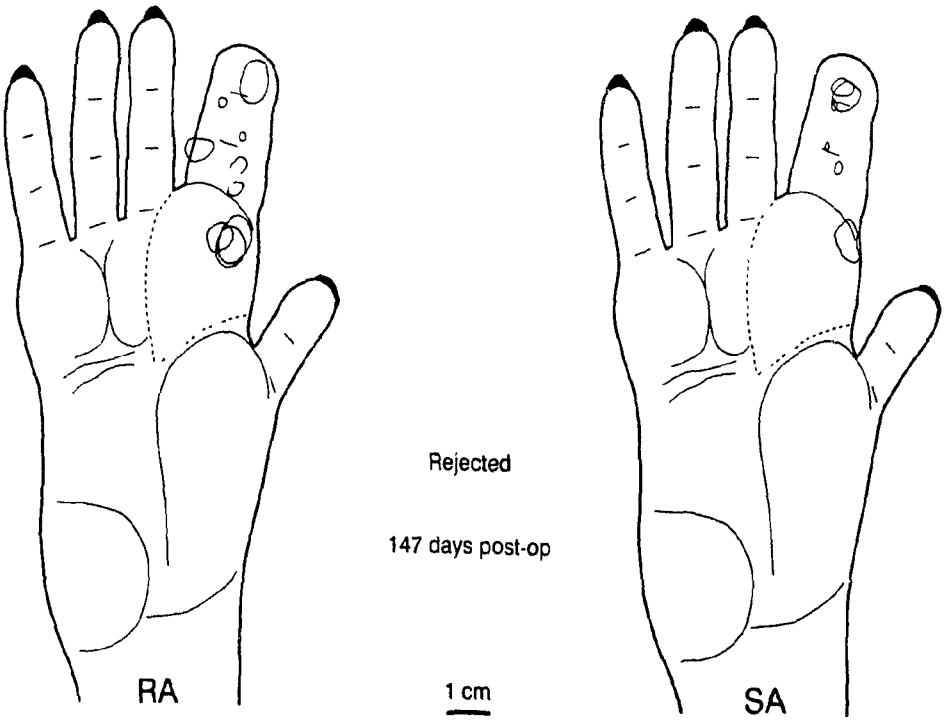
Figure 12

The approximate shape and location of cutaneous RFs for RA and SA fibers which served allografted (TNVFF; D2) and autografted (CNVFF; D4) tissues. Note the lower distribution density of identifiable axon RFs serving rejected allograft tissues (animal #15; lower panel) compared to those serving nonrejected and control tissues (animal #21; upper panel) for a similar number of sampled axons. The dashed lines on the palmar aspect of each hand identifies the extent of the flap as determined by the scars of the surgical incisions. The number of days of postoperative recovery refers to the time at which the electrophysiological recording was finished. (Reproduced, with permission, from Samulack *et al.* [1988]. Copyright © 1988 by Grune & Stratton, Inc.)

Baboon #21



Baboon #15



In Fig. 12 (upper panel), after taking into consideration the patchy nature of the innervation, it can be appreciated that the RF diameter and distribution density (as defined by the number of identified RFs per area of tissue, based upon a similar number of dissected fibers) for sampled axons in nonrejected glabrous skin of the TNVFF, were similar to those of axons which served the autografted glabrous tissues of the CNVFF. Yet, the identifiable RFs of a similar number of dissected axons that served rejected allografted glabrous skin (animal #15; lower panel) were noticeably reduced in diameter and distribution density when compared to either nonrejected TNVFFs or CNVFFs. Although animals #15, 20 and 21 carried TNVFFs with allografted nerve segments which supported what appeared to be a similar number of host axons, in animal #20 the identified RFs covered virtually the whole glabrous surface of the flap, while animals #15 and 21 showed very patchy RF distributions. Despite the fact that nerve segments from the TNVFF of animal #15 supported many host axons (see Fig. 11D; digital nerve from the ulnar side of D2), there were vast areas on the flap within the innervation territory for which RFs could not be identified. A reduced mechanoreceptor population in rejected allografted tissues, or the fact that rejection processes may have disconnected axons from their target mechanoreceptors in the superficial cutaneous tissues, may account for these latter observations.

The majority of the RFs studied which served cutaneous tissues of the two HTs were similar to each other in size and distribution density (data not shown), despite the fact that one HT (animal #7) had undergone repeated mild rejection processes, while the other (animal #13) had undergone severe rejection processes. The HT of animal #7 experienced circulatory problems in the digits which led to their partial mummification (see Appendix I). The fact that all of the cutaneous RFs observed for this latter HT were proximal to the digits, with the vast majority of them being located proximal to the mid-palmar creases, was probably a consequence of the poor circulation the graft was experiencing. The size of the RFs for the HTs were generally larger than those shown for animal #15, with some fields being comparable in size to the larger RFs of the nonrejected TNVFFs and CNVFFs.

For all of the grafts studied, the RFs in hairy skin followed similar patterns of shape and distribution density as those for the glabrous tissues of the same graft, with a tendency for the RFs to appear slightly larger in diameter.

C. Thresholds of Activation

Four hundred RFs sampled in glabrous or hairy skin were tested for their threshold of activation in response to punctate cutaneous stimuli applied to their most sensitive spot. The threshold values obtained are represented in Figs. 13 and 14. Within each experimental group, the data is divided into two histograms on the basis of the axon's adaptation characteristics. It is important to remember when comparing these data, that the abscissa is nonlinear, and that bins 1-3 (as do bins 4-6) have very similar GF values in comparison to bins 7-12 (see Methods, Section II.C.2.). The data is presented in this way, because many of the RFs were able to clearly differentiate between probes with M values from 1.65 to 3.22 (bins 1-6).

1. Fibers which Served Glabrous Skin

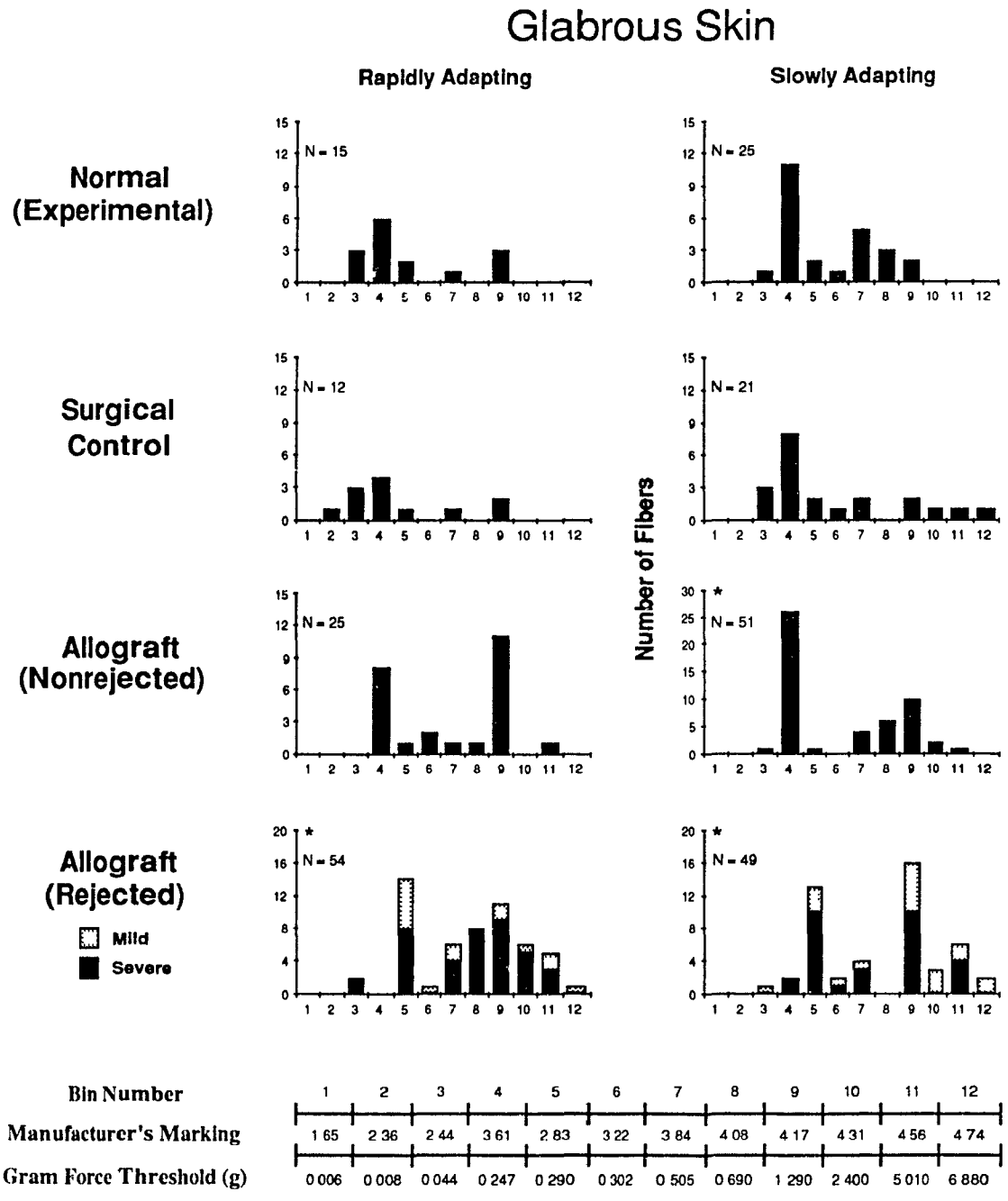
For glabrous skin, the statistics of location (SOL) and distribution characteristics for RF thresholds were very similar between RA and SA samples for each experimental group (Fig. 13). Only in the Allograft (Nonrejected) group was there a moderate difference (although not statistically significant; $P < 0.1$) between the RA and SA fibers with respect to SOL. In this latter case, the SA submodality had a greater proportion of lower threshold values.

The distribution of sampled RF thresholds for the RA fibers in each of the experimental groups was bimodal, with the modes of the distributions being approximately 2.5×10^{-1} g and 1.3 g. The peaks of the bimodal distribution were more pronounced for the allografted tissues, with the Allograft (Rejected) distribution showing a wider peak in the higher threshold values. The SOL and distribution of thresholds for RA fibers were similar in the Normal (Experimental) and Surgical Control groups, but the SOL for these two groups differed

Figure 13

Histograms representing the thresholds of activation of axons sampled from four experimental groups of glabrous skin in immunosuppressed baboons; Normal (Experimental), Surgical Control, Allograft (Nonrejected) and Allograft (Rejected). The threshold (in grams) was measured for axons serving glabrous skin, using an array of twelve nylon monofilaments of various calibers. The average GF value for each probe is shown on the longitudinal scale at the bottom of the figure; note the nonlinearity in the increment of the values The histogram bins numbered 1 to 12 correspond to the 12 probes utilized. The total sample from each category is divided on the basis of the adaptation characteristics; rapidly or slowly adapting. In addition, the histograms for the category Allograft (Rejected) indicate which portion of the sample came from tissues which had undergone mild or severe rejection. The value N for each histogram represents the respective sample size. Note the change in the ordinate scale for the histograms designated by an asterisk (*).

Receptive Field Thresholds



significantly from the SOL of both allograft groups. The sampled RF thresholds for RA fibers which served nonrejected allografted glabrous skin did not have significantly different SOL from those of the Allograft (Rejected) group; even if the sampled fibers which served mildly rejected or severely rejected tissues were compared separately. There was a definite tendency towards higher threshold values for RFs in rejected allografted tissues, as compared to Normal (Experimental) or Surgical Control tissues, since more than 50% of the fibers which served rejected glabrous skin had thresholds of activation equal to or greater than 0.7 g.

For SA fibers which served glabrous skin, the sampled thresholds of RFs in the Normal (Experimental), Surgical Control, and Allograft (Nonrejected) experimental groups, all had similar SOL. The SOL for SA fibers which served RFs in rejected allografted glabrous skin were significantly different ($P < 0.001$) from any of the SA fiber samples for the other experimental conditions. The latter was true even when SA fibers which served mildly, or severely rejected allografted glabrous tissues were considered independently. As was the case for sampled RA fibers which served glabrous skin, more than 50% of the sampled RFs in rejected allografts served by SA fibers had a GF value equal to, or greater than 0.7 g.

Similar to that of the RA fibers, the distributions of the thresholds for the sampled RFs of the SA submodality was bimodal in nature, except for the Surgical Control group where the mode of the distribution in the upper threshold values was not well-defined. Although the mode values for the region of the distribution in the lower thresholds (2.5×10^{-1} g) was similar to that of RA fibers in each of the experimental groups, the mode in the region of higher thresholds for SA fibers changed from approximately 0.5 g in Normal (Experimental) conditions, to 1.3 g in the Allograft (Rejected) group, in contrast to a stable 1.3 g for RA fibers in each experimental group.

The SOLs for the sampled fibers which served mildly rejected or severely rejected allografted glabrous tissues were not significantly different, whether they were compared to each other within their submodality, or between submodalities (RA *versus* SA).

2. Fibers which Served Hairy Skin

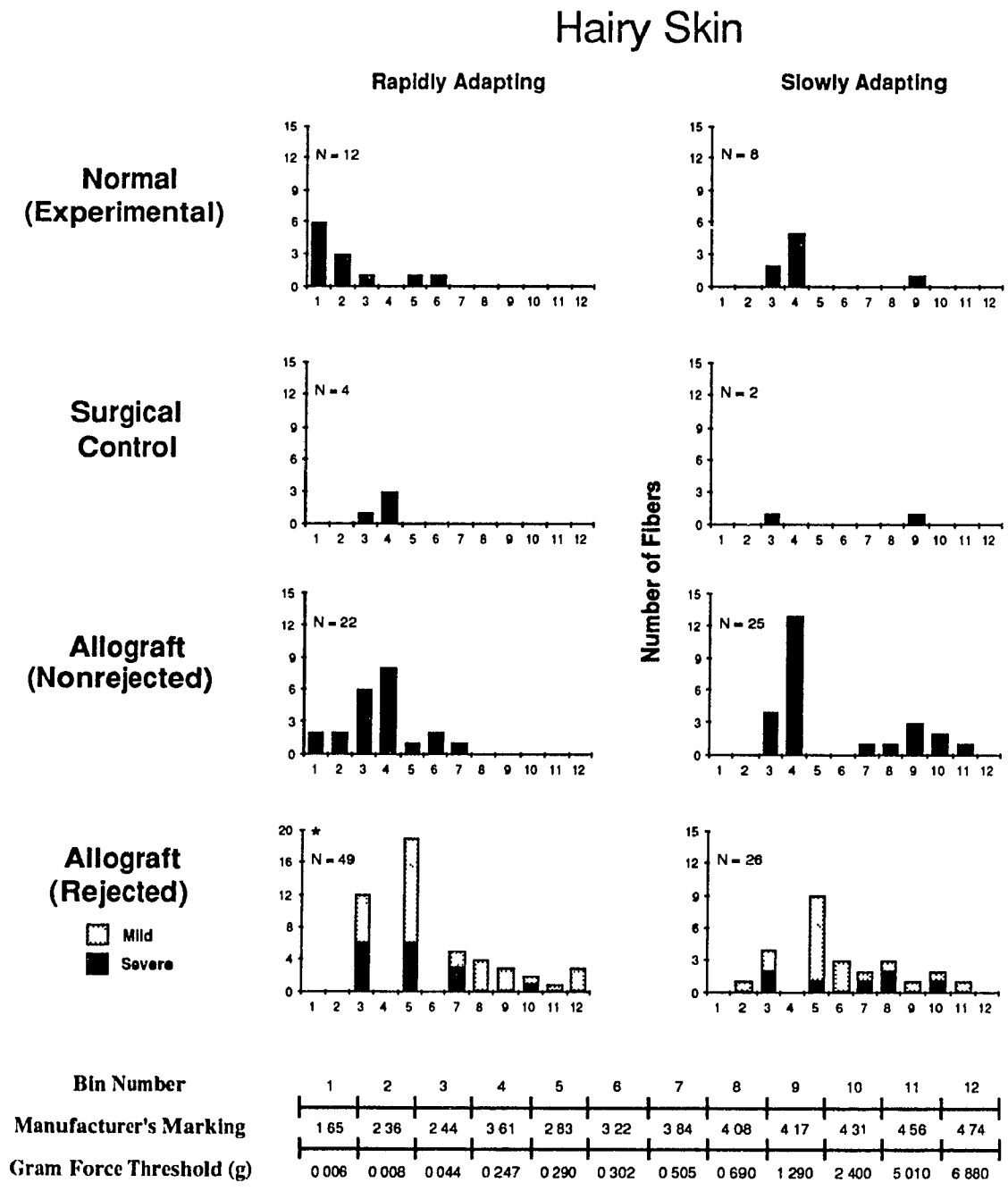
Thresholds of activation for axons which served cutaneous RFs in hairy skin were determined for one hundred and forty-eight fibers; the majority of which were sampled from allografted tissues. As seen in Fig. 14, the distribution of thresholds for RA fibers which served hairy skin tended to be unimodal in character with the GF values generally being quite low. This contrasts with the tendency towards a bimodal distribution of thresholds for the SA fibers, where both the lower and higher GF values were always represented. Statistical comparisons between the sampled RA and SA fibers within each of the Normal (Experimental) and Allograft (Nonrejected) groups for hairy skin showed SOLs which were significantly different ($P < 0.02$). Although for the SA fibers of the Surgical Control group, there was representation in the lower as well as the higher threshold ranges, and the sampled RA fibers had values only in the lower threshold ranges, judgement is reserved as to the significance, since the sample sizes were small. Based upon the Mann-Whitney *U*-test which compares ranked sum differences and has tabled critical values for tests among such small sample sizes (see Methods, Section IV.), the Surgical Control samples did not have significantly different SOL in comparison to any of the other experimental groups. For the Allograft (Rejected) experimental group, the SOLs were not significantly different between the RA and SA fiber samples; even when fibers which served either mildly rejected or severely rejected tissues were tested separately.

The sensory thresholds for RA axons which served RFs in Normal (Experimental) hairy skin were extremely sensitive, showing a significant number of RFs with GF thresholds of activation less than 10 mg. A comparison of these fibers to those which served reinnervated tissues (CNVFFs, TNVFFs, or HTs) revealed a tendency towards elevated thresholds for the fibers which had undergone nerve transection. Although the RA fibers sampled which served Surgical Control tissues did not have significantly different SOL when compared to Normal (Experimental) tissues, the sample for RA fibers which served the CNVFFs showed larger

Figure 14

Histograms representing the thresholds of activation of axons sampled from four experimental groups of hairy skin in immunosuppressed baboons; Normal (Experimental), Surgical Control, Allograft (Nonrejected) and Allograft (Rejected). The threshold (in grams) was measured for axons serving hairy skin, using an array of twelve nylon monofilaments of various calibers. The average GF value for each probe is shown on the longitudinal scale at the bottom of the figure (note the nonlinearity in the increment of the values). The histogram bins numbered 1 to 12 correspond to the 12 probes utilized. The total sample from each category is divided upon the basis of the response characteristics of the axons studied: RA or SA. In addition, the histograms for the category Allograft (Rejected) indicate which portion of the sample came from tissues which had undergone mild or severe rejection. The value N for each histogram represents the respective sample size. Note the change in the ordinate scale for the histogram designated by an asterisk (*).

Receptive Field Thresholds



mean, median and mode values and had distribution characteristics which were statistically different ($P < 0.025$; based upon the table values for the Kolmogorov-Smirnov test) than that for the Normal (Experimental) sample. The SOL for sampled RA fibers which served nonrejected allografted tissues were significantly different (higher) than those for the Normal (Experimental) group, but were similar to those for control tissues.

The RA fibers which served RFs in rejected allografted hairy skin, however, had noticeably higher GF values than any of the other groups. The SOL for the total Allograft (Rejected) sample for RA fibers was significantly different than those for nonrejected allografted tissues, as well as for Normal (Experimental) tissues. Although the SOL for the Allograft (Rejected) group were not significantly different than that for the Surgical Control group ($P < 0.1$), the distribution was significantly different ($P < 0.03$), with an obvious tendency towards higher GF threshold values (Fig. 14). It was interesting to note that the GF values of sampled RA fibers which served RFs in severely rejected hairy skin, showed similar SOL ($P < 0.1$), as compared to those which served skin which had only undergone minor rejection episodes.

SA fibers which served hairy skin showed similar SOL for threshold values when each of the experimental groups was compared (Fig. 14; right column). There was no significant difference in the SOLs between Normal (Experimental), Surgical Control, Allograft (Nonrejected) and Allograft (Rejected) groups. For the first three groups (taking into consideration sample size), each of the samples indicated a bimodal distribution of GF values with modes at approximately 0.2 g and 1.3 g. For the Allograft (Rejected) sample, however, there was an apparent shift in the distribution of the GF values, in the lower threshold range (values in bin 4 may have shifted towards bins 5 and 6), showing a tendency for a unimodal distribution of the sample. In fact, the distribution of the total sample for SA fibers which served rejected allografted tissues was significantly different than that for Allograft (Nonrejected) tissues ($P < 0.005$) as well as the others.

Like that for RA fibers which served rejected allografted hairy skin, the SA fibers which served mildly rejected tissues showed similar SOL in comparison to SA fibers which served severely rejected tissues.

3. Comparisons between Glabrous and Hairy Skin

In general, the RFs (RA and SA) in hairy skin were served by a larger percentage of axons which had very low thresholds of activation as compared to those for glabrous skin (GF < 50 mg; 30.4% for glabrous *versus* 6.0% for hairy skin). As well, the SOL for each sample of RA fibers which served hairy skin were significantly lower than each respective sample which served glabrous tissues, except for a comparison between the RA fibers of the Surgical Control samples; the small sample size within the RA Surgical Control group of Hairy skin may have contributed to the latter finding. In contrast, only the sampled SA fibers which served hairy skin in the Allograft (Rejected) tissues showed significantly different SOL when comparisons were made between glabrous and hairy skin.

For both glabrous and hairy skin, sampled axons which served rejected allografted tissues showed a tendency towards higher threshold values (as indicated by single bin shifts) when comparisons were made to nonrejected or control tissues; although SOL were not significantly different in each case.

D. Submodality Response Characteristics

The response characteristics of several RA or SA fibers which served either glabrous or hairy skin were studied with the use of a computer controlled stimulus paradigm (described in the Methods, Section II.C.3.). The data are presented in Figs. 15-18. While the data represented by 'thick' curves in graphs A and B of each figure were derived from immunosuppressed animals in the present study, the thinly drawn curves were constructed from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed),

and are included with permission for comparative purposes. The autografts from the present study were NVFFs, whereas the autografts from the study of Dykes *et al.* [1984] were full-thickness skin grafts.

1. Rapidly Adapting Fibers

a) RA Response Characteristics in Glabrous Skin

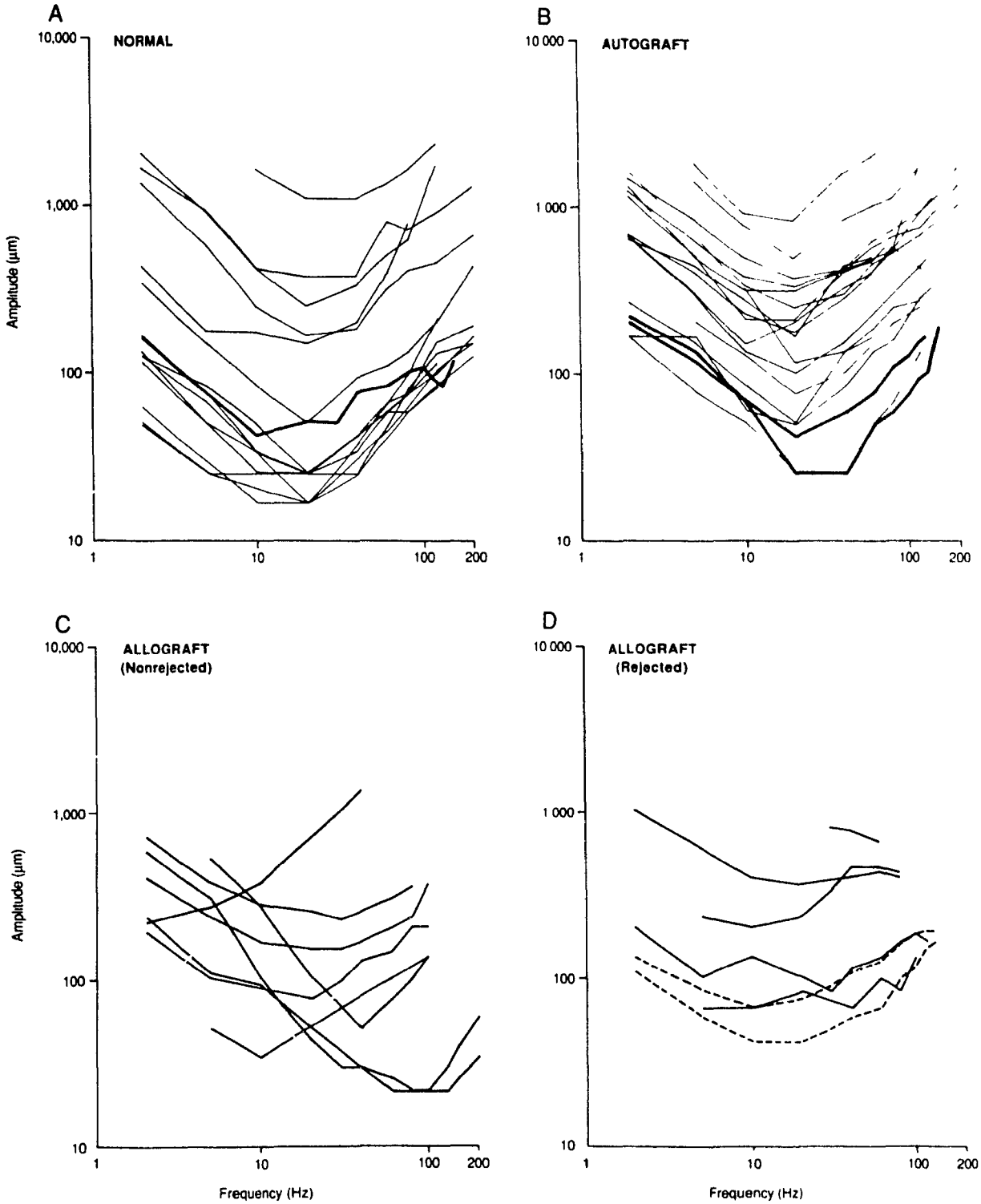
Normal RA fibers with their RFs in glabrous skin (Fig. 15A, thin curves) showed a wide range of sensitivity to repetitive stimuli. The most sensitive of the fibers entrained to frequencies between 10 and 40 Hz at stimulus amplitudes which ranged from 17 to 40 μm. In comparison, a fiber which served Normal (Experimental) glabrous skin (thick curve) had its lowest tuning points between 8 and 30 Hz at stimulus amplitudes of 42-50 μm. Taking into consideration that the less sensitive fibers which served Normal skin had curves with minimum tuning points ranging in stimulus amplitude from 0.2 to 1.1 mm (one order of magnitude higher than the sensitive fibers), the RF in Normal (Experimental) skin displayed a range of sensitivity comparable to the moderately sensitive RA fibers which served Normal skin.

The tuning curves for RA fibers which served autografted tissues displayed a range of sensitivities similar to those for Normal skin. As well, the shape of the tuning curves were similar, with the minimum tuning points being located at frequencies between 10 and 40 Hz. In contrast to fibers which served Normal tissues, however, those axons which served RFs in autografted full-thickness glabrous skin grafts (Fig. 15B, thin curves) showed a larger percentage of sampled axons with tuning curves having minimum tuning points between 100-1000 μm (75% for full-thickness grafts *versus* 35.7% for normal tissues). At all of the frequencies tested, axons which served glabrous skin of the CNVFFs (Fig. 15B, thick curves) had RFs with tuning curves showing lower vibratory thresholds than the majority of the fibers which served full-thickness skin grafts. As well, the curves for RA fibers which served

Figure 15

Tuning curves for RA fibers which served glabrous skin. As described in detail in the Methods section, tuning points (the minimum amplitude in micrometers of a mechanically induced sinusoidal vibration that would elicit one AP for every cycle of a sine wave for 0.5 s) were obtained for a series of frequencies between 2 and 200 Hz and connected by a line to form the tuning curve. The curves were plotted on a log-log axis. The fibers which were most sensitive to vibration had the lowest tuning point. (A) An axon which served Normal (Experimental) skin (thick curve) is compared to axons which served Normal skin (thin curves). (B) Axons which served autografted CNVFFs (thick curves) are compared to those which served autografted full-thickness skin grafts (thin curves; one-half of the fibers were chosen to represent the range of thresholds found). (C) Axons which served nonrejected, and (D) rejected allograft tissues are also presented. Those axons which served allografted tissues which had undergone minor episodes of rejection are depicted by dashed lines, while axons which served severely rejected allografted tissues are represented by solid lines. The curves represented by thin lines in (A) and (B) were constructed from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.

RA TUNING CURVES — GLABROUS SKIN



glabrous skin of the CNVFFs had tuning points comparable to those of the most sensitive fibers which served glabrous skin in Normal and Normal (Experimental) tissues.

The RA fibers which served RFs in allografted glabrous skin had tuning curves with a range of sensitivities comparable to that for normal or autografted tissues, however, the curves were more shallow for fibers which served allografted glabrous skin (Fig. 15C and D), indicating that they had a more uniform sensitivity across the range of stimulus frequencies. This tendency was most pronounced for RA fibers which served rejected allografted glabrous skin (Fig. 15D). It is interesting to note that the curves which were derived from axons which served mildly rejected glabrous skin (dashed lines) had minimum tuning points which were comparable to the more sensitive of the fibers which served normal or autografted tissues. As well, the tuning curves were more sensitive for the majority of the RA fibers which served allografted glabrous skin of the TNVFFs than for fibers sampled which served full-thickness glabrous skin grafts. This latter observation indicated that the physical nature of the full-thickness skin grafts may have contributed to the relatively poor sensitivity of their sensory units to vibratory stimuli.

For nonrejected tissues, two very sensitive axons were studied which showed minimum tuning points in the 60 to 130 Hz range (Fig. 15C), although these two curves resemble closely those which might have been derived from axons which served Pacinian corpuscles, the fibers had well-defined RFs (5-8 mm in diameter, as measured with monofilament M = 4.56) on the finger of the TNVFF (animal #20), and axonal threshold of activations of approximately 0.3 g. The possibility that these fibers represent immature reinnervation of Pacinian corpuscles is discussed later in this report (see Discussion, Section III).

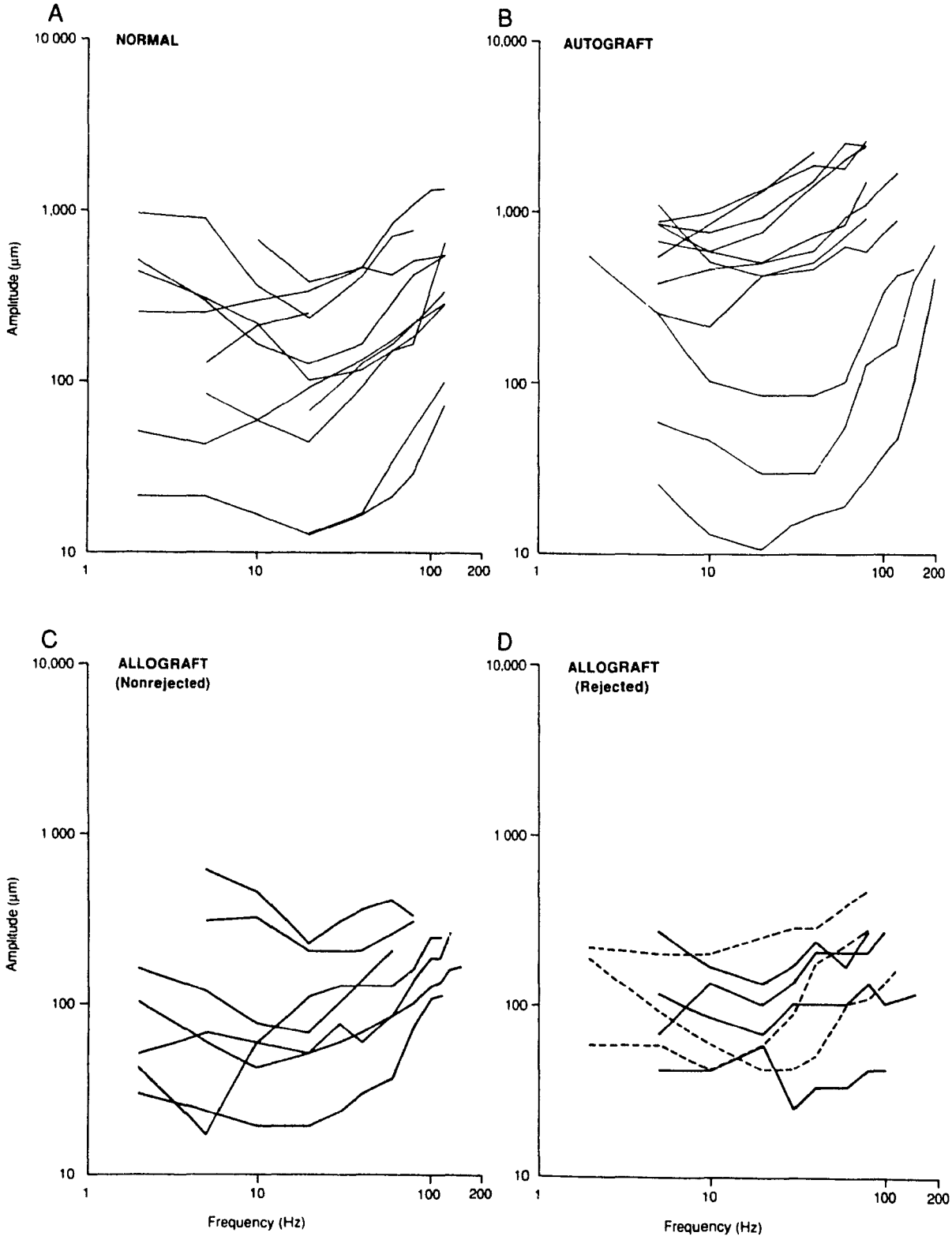
b) RA Response Characteristics in Hairy Skin

The tuning curves of sampled axons which served RFs in hairy skin (Fig. 16) generally had shapes which were less uniform and less U-shaped than those derived from glabrous skin.

Figure 16

Tuning curves for RA fibers which served hairy skin. Plotted on a log-log axis in a similar manner to those of Fig. 15, the fibers most sensitive to vibration had entrainment thresholds at the lowest amplitudes. The graphs represent axons which served: **(A)** normal skin, **(B)** autografted full-thickness skin grafts, **(C)** nonrejected allografted tissues, and **(D)** rejected allografted tissues. Dashed lines depict axons which served allografted tissues that had undergone minor episodes of rejection. Solid line depict axons which served severely rejected allografted tissues. The curves represented by thin lines in (A) and (B) were constructed from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.

RA TUNING CURVES — HAIRY SKIN



For each experimental group from hairy skin there were fibers with tuning curves which had a nearly horizontal limb in the lower frequencies and/or minimum tuning points below 10 Hz. The majority of the sampled RA fibers which served hairy skin were clearly more sensitive to low frequency stimulation (2-10 Hz) than were fibers which served glabrous skin in the same experimental position.

For the Normal and both Allograft experimental groups (Fig. 16A, C, and D), there were some fibers which were very sensitive (requiring stimulus amplitudes of only 10-30 μm), but the majority of the fibers had minimum tuning points with amplitudes between 40 and 400 μm. The sample which served full-thickness skin grafts (from the original data of Dykes *et al.* [1984]), although having some very sensitive fibers, generally had tuning curves with minimum tuning points that required more than 400 μm stimulus amplitude for entrainment. Therefore, the fibers which served autografted hairy tissues were significantly less sensitive than fibers which served normal or allografted hairy skin.

There was very little difference, between the sensitivity of the sampled fibers which served rejected or nonrejected allografted hairy skin. As well, of the axons studied in rejected tissues, those fibers serving RFs in mildly rejected skin were similar in sensitivity to those serving RFs in severely rejected skin.

2. Slowly Adapting Fibers

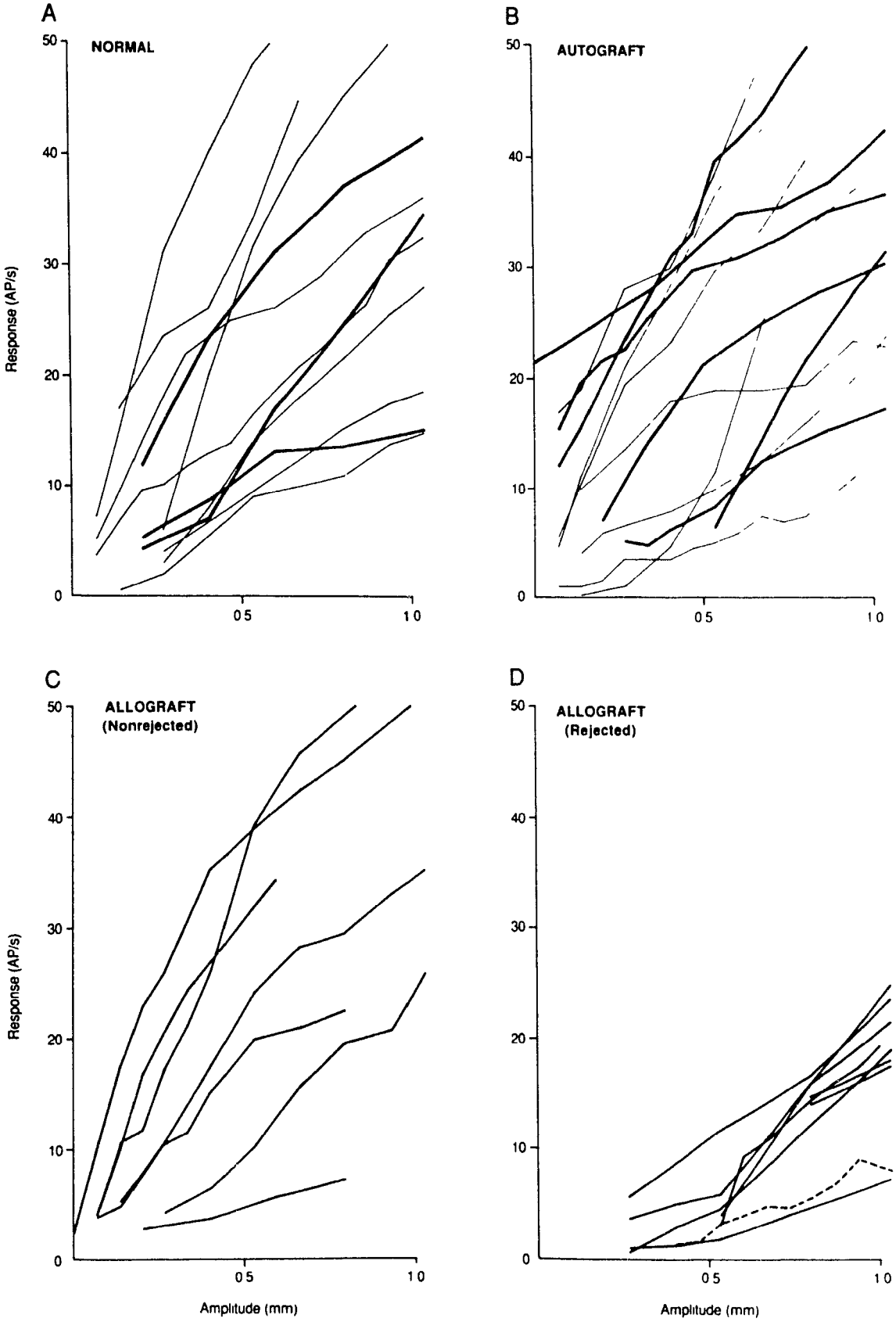
a) SA Response Characteristics in Glabrous Skin

Stimulus-response curves are presented for SA fibers which served RFs in glabrous skin (Fig. 17). Axons in each of the experimental skin conditions displayed a wide range of responsiveness to the mechanical induction of a step indentation (see Methods, Section II.C.3.), except for those axons which served rejected allografted glabrous skin. The sampled fibers which served Normal (Experimental), and Surgical Control skin (Fig. 17A and B; thick curves), as well as the sampled fibers which served Allograft (Nonrejected) tissues (Fig. 17C)

Figure 17

Stimulus-response curves for SA fibers serving glabrous skin. The points on each curve represent an axon's mean firing rate (AP/s) during the first 1 s of a step indentation of the skin (amplitude in mm) induced by a mechanical stimulator. **(A)** Axons which served Normal (Experimental) skin (thick curves) are compared to those serving Normal skin (thin curves). **(B)** Axons which served autografted CNVFFs (thick curves) are compared to those which served autografted full-thickness skin grafts (thin curves; one-third of the fibers were chosen to illustrate the range of thresholds found). **(C)** Axons which served nonrejected, and **(D)** rejected allograft tissues. An axon which served allografted tissues that had undergone minor episodes of rejection is depicted by a dashed line, while axons which served severely rejected allografted tissues are represented by solid lines. Curves represented by thin lines in (A) and (B) were constructed from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.

SA STIMULUS-RESPONSE CURVES — GLABROUS SKIN



displayed S-R curves which were comparable in shape and slope, to those curves which were derived from normal glabrous skin or full-thickness skin grafts (from the original data of Dykes *et al.* [1984]).

Axons which served glabrous skin of the rejected TNVFFs (Fig. 17D) had S-R curves which were considerably less sensitive (stimulus necessary to initiate a response) and less responsive (decreased slope of curve) than the majority of the fibers in each of the other experimental skin conditions. Even though one of the axons (Fig. 17D, dashed line) served glabrous skin which only underwent minor episodes of rejection (TNVFF, animal #5), it also had S-R characteristics which were less sensitive and/or responsive than the majority of SA fibers which served glabrous skin of nonrejected allografts.

b) SA Response Characteristics in Hairy Skin

The SA fibers which served RFs in normal or autografted hairy skin (Fig. 18A and B, both sets of curves constructed from the original data of Dykes *et al.* [1984]) were generally more responsive than those which served glabrous skin (Fig. 17). Some of the fibers were extremely responsive, as indicated by the very steep slope of their S-R curves, and by the fact that they exhibited onset responses in the order of 20-40 AP during the first 1 s of a step indentation of only 100-300 μm.

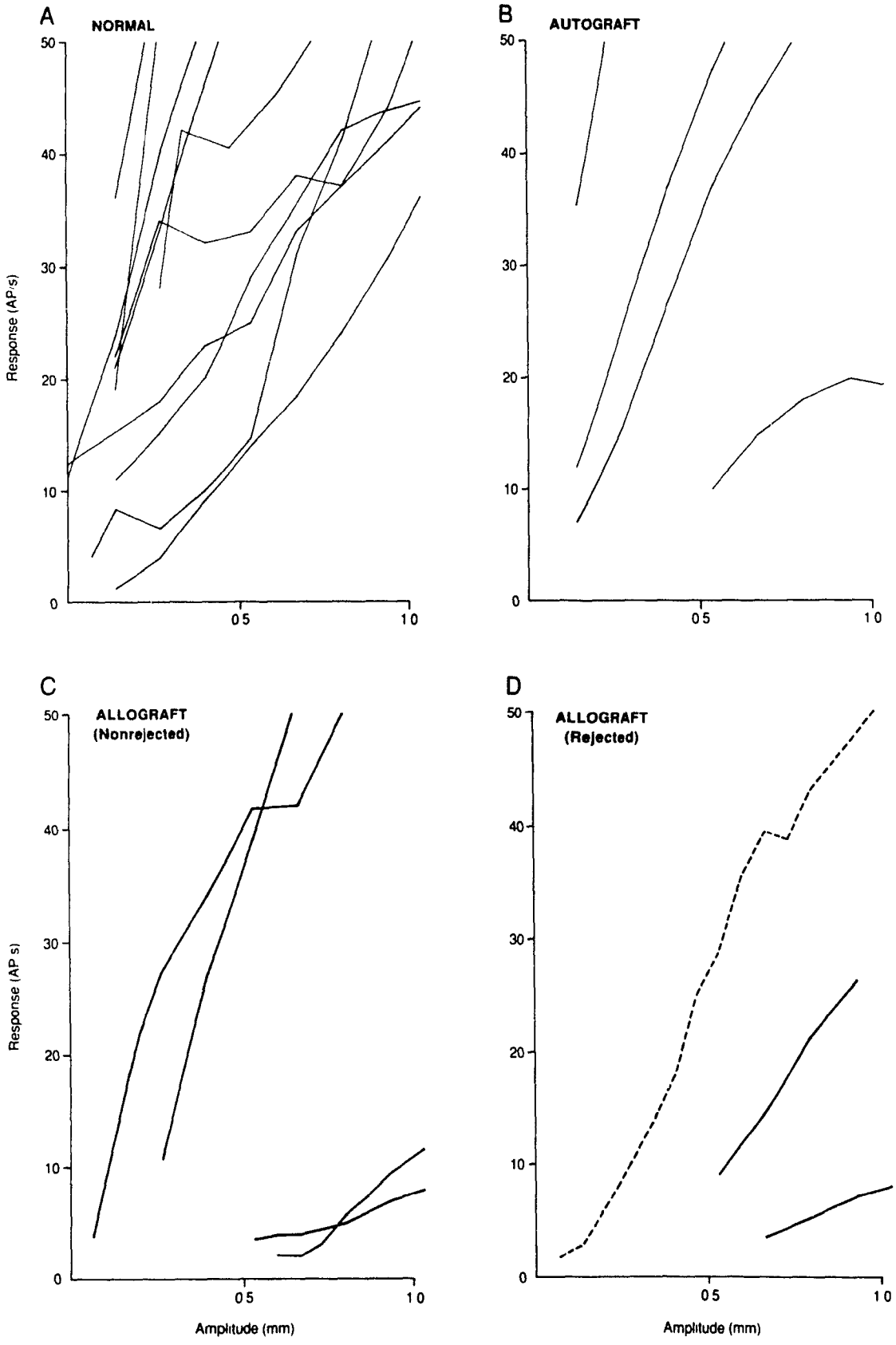
The S-R characteristics of SA axons which served allografted hairy skin were mixed; some fibers showed very responsive S-R curves with steep slopes, while others were quite insensitive to step indentation and responded by giving only 2-10 AP during the first 1 s of the stimulus. None of the fibers sampled in allografted tissues showed S-R curves with extremely steep slopes, as did some of the highly responsive fibers in normal and autografted skin.

Similar findings as those for nonrejected allografted hairy skin were seen for rejected allografted hairy skin. The fiber which served an RF in mildly rejected skin (Fig. 18D, dashed

Figure 18

Stimulus-response curves for SA fibers which served hairy skin. The points on each curve represent an axon's mean firing rate (AP/s) during the first 1 s of a step indentation of the skin (amplitude in mm) produced by a mechanical stimulator. The curves are divided into those axons which served **(A)** Normal skin, **(B)** autografted full-thickness skin grafts, **(C)** nonrejected and **(D)** rejected allografted tissues. One curve representing an axon which served allografted tissues that had undergone minor episodes of rejection is depicted by a dashed line. Axons which served severely rejected allografted tissues are represented by solid lines. Curves represented by thin lines in (A) and (B) were constructed from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.

SA STIMULUS-RESPONSE CURVES — HAIRY SKIN



line), had a curve which showed S-R characteristics similar to those for the average responding fibers of Normal hairy skin, as well as those for the sensitive fibers which served nonrejected allografted hairy skin.

In nonrejected and rejected tissues, the axons with S-R curves resembling those from Normal hairy skin, had medium to large well-rounded RFs (6-10 mm in diameter). The curves in Figs. 18C and D, which showed poor sensitivity and responsiveness to step indentations, were derived from axons whose RFs were small (< 5 mm in diameter) or very small (punctate, 1-2 mm in diameter), but for which SA response characteristics were clearly defined.

E. Conduction Velocities

The CVs of three hundred and forty-nine axons which served either Normal (Experimental) skin, CNVFFs, or allografted skin were studied and compared. The data is presented in histogram form in Figs. 19-23. In addition, histograms describing the CVs of axons which served Normal skin were constructed from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes in parallel with the data derived from axons which served Normal (Experimental) tissues.

1. Normal (Experimental) as a Special Group

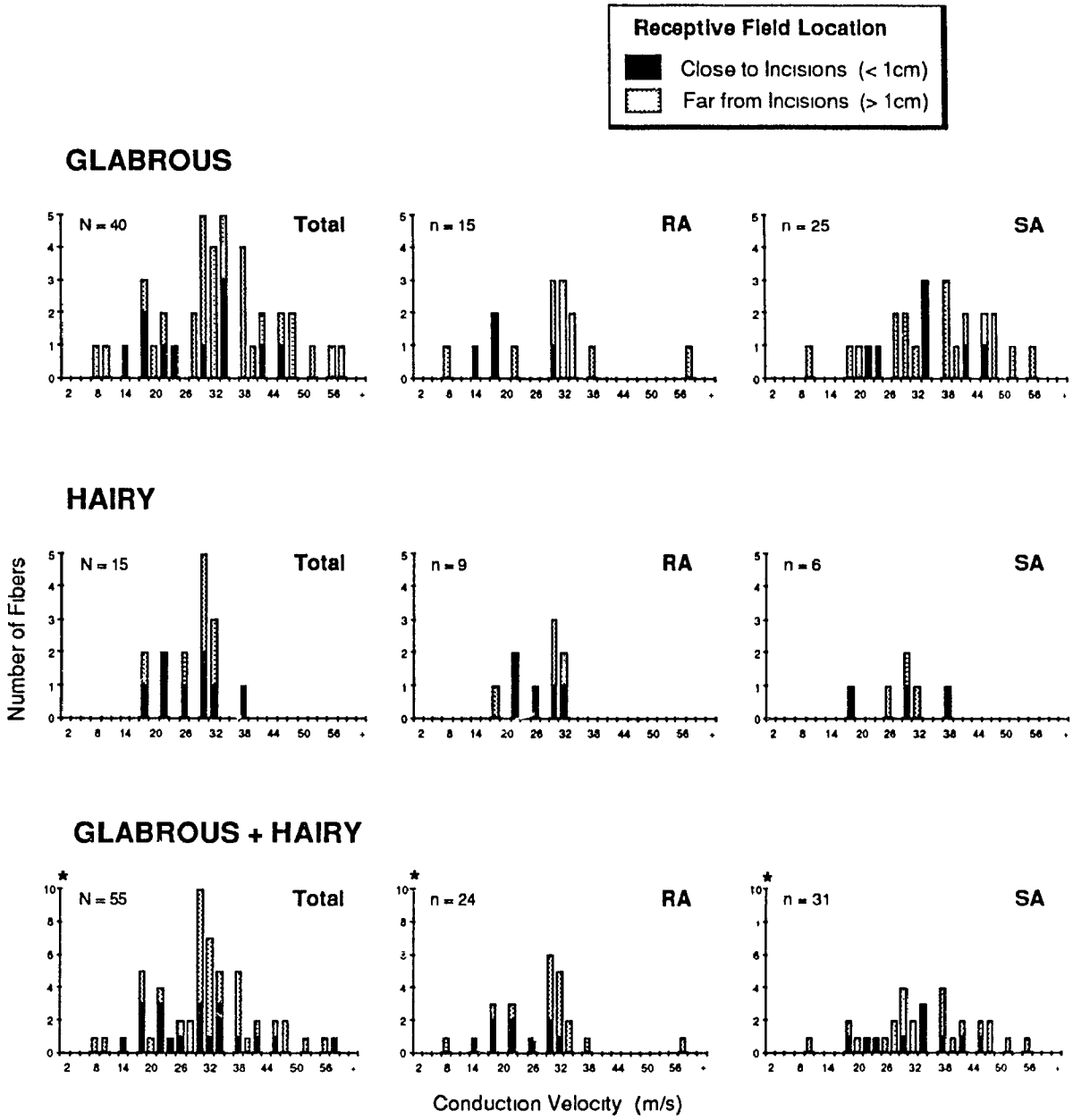
The axons sampled from Normal (Experimental) tissues had RFs in areas of skin which had not intentionally been surgically manipulated, but which were on the same hand as the TNVFF and CNVFF. There was a chance that some of the axons which served RFs on normal skin near the sites of surgical intervention might have been interrupted inadvertently. Data presented in Fig. 19 are an attempt to clarify whether there was any effect of this surgical procedure on axons in normal tissue, by asking if there was a significant difference in the SOL and distribution between the CV values obtained from axons with RFs which were within 1 cm

Figure 19

Frequency histograms illustrating the sampled values of CV for axons serving Normal (Experimental) skin. In each histogram, the data is divided into two subsets; those axons serving RFs in 'close' proximity to surgical incisions and those serving RFs 'far' removed from incisions. The total sample for the Normal (Experimental) group is segregated into the categories (rows) Glabrous, Hairy, or Glabrous + Hairy, based upon the type of skin in which the RFs were located. Each category Total is then further subdivided into samples depicting those axons which were characterized as RA or SA (columns) based upon their rate of adaptation to a sustained stimulus. Note the change in scale of the ordinate axes marked by an asterisk (*).

In only one histogram (Glabrous + Hairy, RA) were the CV subsets (close *versus* far) determined to be significantly different (P = 0.05) and to come from populations having different SOL (Mann-Whitney *U*-test; see text for details).

NORMAL (EXPERIMENTAL)



(close) of a surgical incision and those fibers whose RFs were further than 1 cm (far) from an incision. To determine whether the group of sampled axons truly represented a homogeneous sample, the two parts of the sample were compared with the Mann-Whitney *U*-test.

In most comparisons, there was no significant difference in SOL or distribution between the CVs of axons located close or far from surgical incisions. For SA fibers, both sample subsets in each of the histograms showed similar SOLs and distributions. In contrast, the sample for RA fibers which served RFs in glabrous skin close to incisions, showed a moderate shift in SOL ($P < 0.1$) towards lower CV values, as compared to those which were located far from surgical incisions, however, there was no significant difference in the distribution of the two samples ($P = 0.1$). In only one histogram (Glabrous + Hairy, RA) was the CV subsets (close and far) determined to come from populations having significantly different SOL ($P = 0.05$), however, the sample distributions of the two subsets were similar ($P > 0.1$).

The statistically significant differences between the SOLs of the two subsets of RA fibers which served the combined sample of glabrous plus hairy skin probably arose from four factors: (1) the right-hand tail (larger CV values), within the distribution of the combined sample for glabrous skin, was predominantly represented by axons with RFs which were far from surgical incisions, (2) the distribution of sampled CVs for RA fibers which served glabrous skin consistently had larger mean and median values, as compared to those for hairy skin of the Normal (Experimental) group as well as for each of the other experimental groups studied (see Fig. 22), (3) the SOL and distribution of CVs for the two subsets of RA fibers which served hairy skin, although similar, showed a tendency for CV values of 'close' fibers to be lower than those for 'far' fibers, and most importantly, (4) the pooling of data from axons which served glabrous skin with that of axons which served hairy skin, is done so, knowing that these axons serve different types of mechanoreceptors, and that this alone may account for heterogeneity in the sample.

As is shown below in Fig. 20, the sample of RA fibers which served Normal (Experimental) glabrous skin had SOL which were significantly different (lower) than those

for the Normal group ($P < 0.01$). This was true, as well, for the SA fibers which served hairy skin. The fact that three of four samples (Glabrous SA, Hairy RA and SA) showed no statistically significant differences in the SOL or distribution of CVs derived from fibers which served RFs in close or far proximity to surgical incisions, indicates that location alone was unlikely to be the reason for the differences seen.

It was therefore concluded for the purposes of further comparisons in this study, that although proximity to surgical manipulation may have had an effect on the SOL for RA fibers which served glabrous skin (although not statistically significant), that for all intents and purposes, the axons which served RFs in Normal (Experimental) skin of the immunosuppressed baboons composed a homogeneous group of fibers similar in nature to those which served Normal skin of baboons which were not immunosuppressed.

2. Comparisons among Glabrous Skin Samples

When the sampled axons which served glabrous skin in each experimental group were compared on the basis of their CVs (Fig. 20), several patterns were apparent. The arithmetic mean and median of each sample decreased in a regular progression from Normal to Allograft (Rejected) within each category (Total, RA, or SA). As well, when these same parameters were compared between RA and SA fibers, the values for RA fibers were consistently smaller than those for the SA fibers in each experimental group. Although these patterns were consistent, not all of the samples showed significantly different SOL when adjacent histograms were considered in pairs using the Mann-Whitney *U*-test.

When the CV values for the RA fibers in each experimental group were compared to the neighboring group (vertically in the center column of Fig. 20), only the comparison between Normal and Normal (Experimental) groups showed a statistically significant difference in SOL ($P < 0.01$). Each RA sample, however, did have a significantly different SOL when compared to the sample at least one histogram removed (vertically) from it. For example, differences

Figure 20

Frequency histograms representing the CV of single axons serving cutaneous RFs in glabrous skin. The total number of axons sampled for each experimental group (N; left column) is subdivided into categories on the basis of their response properties to cutaneous stimulation: rapidly adapting (RA; center column), slowly adapting (SA; right column). The bin heights in each histogram are expressed as a percentage of the total sample size (N) for each experimental group. The value N represents the total number of axons for both the RA plus the SA samples. Note that N for Surgical Control and Allograft (Rejected) is larger than the sum of the values of n for the RA and SA samples. The difference represents fibers within the category for which submodalities were not defined, but for which CVs were obtained. Note the change in scale of the ordinate axes marked by an asterisk (*).

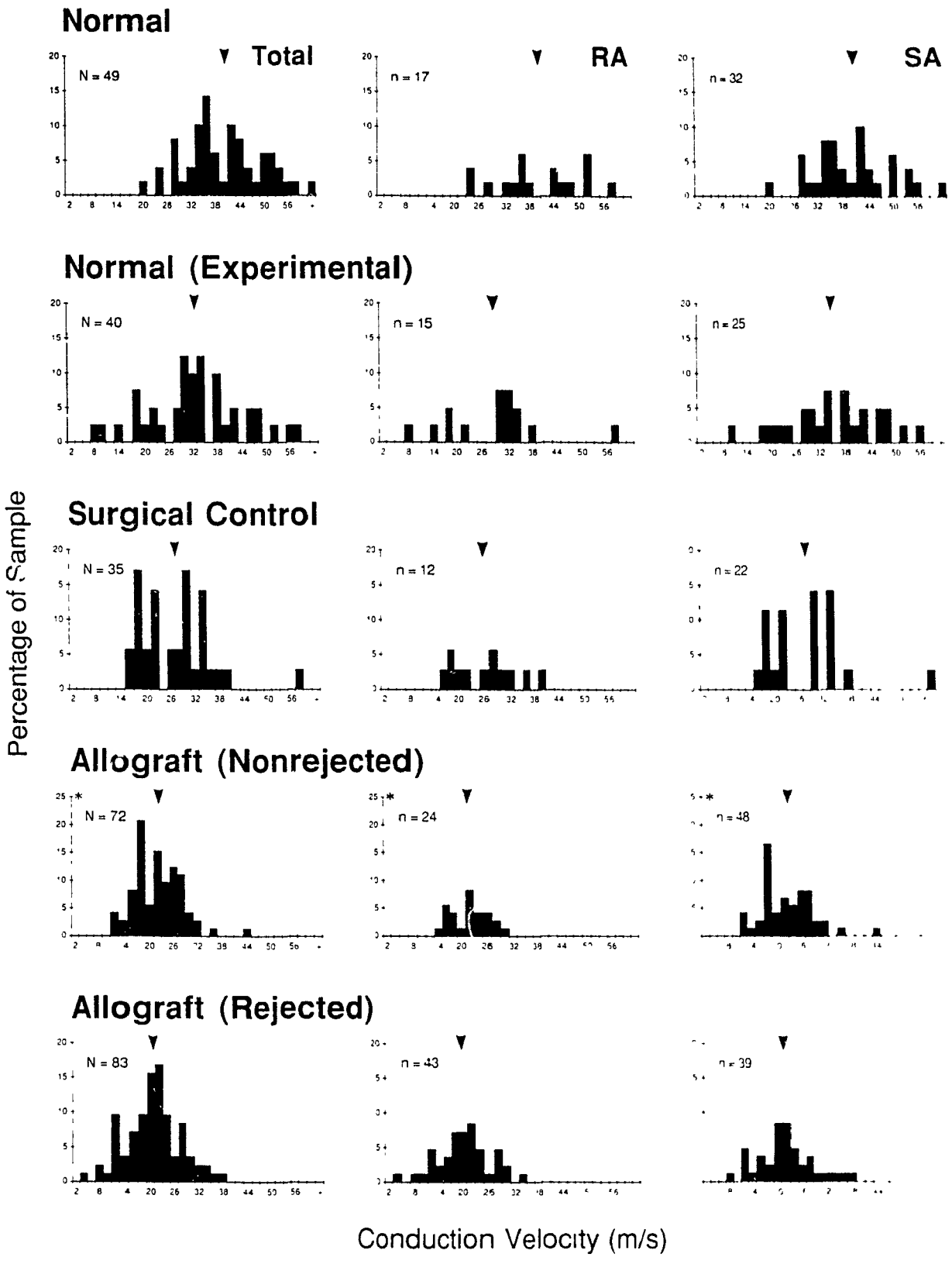
The location of the arithmetic mean of each distribution is indicated by an arrowhead. The values of the mean and median for each distribution are presented in the following chart.

Glabrous Skin

Experimental Condition	Conduction Velocity (m/s)		
	Total	RA	SA
Normal	39.4 ± 10.2 37.8	39.2 ± 10.1 36.8	39.5 ± 10.4 38.7
Normal (Experimental)	31.5 ± 11.8 31.5	27.6 ± 12.0 28.9	33.9 ± 11.3 32.6
Surgical Control	26.0 ± 8.9 27.1	25.0 ± 7.7 25.6	26.7 ± 9.8 28.6
Allograft (Nonrejected)	21.3 ± 5.8 21.0	20.6 ± 4.4 21.0	21.7 ± 6.4 21.0
Allograft (Rejected)	19.9 ± 6.5 19.8	19.0 ± 6.2 19.4	20.6 ± 6.6 20.2
			mean ± 1 S.D. median

Refer to the body of the text for a description of the differences between the samples based upon their SOL (Mann-Whitney *U*-test). The histograms which describe the CV of axons which served Normal skin were derived from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.

GLABROUS SKIN



between the RA samples of Surgical Control and Allograft (Rejected), or between Normal (Experimental) and either of the allografted tissues were significant.

In contrast to the RA fibers, the CVs of SA fibers in the Normal and Normal (Experimental) groups did not have significantly different SOL ($P < 0.1$), however, the mean and median CV values for the Normal (Experimental) group was lower than that for Normal skin. The SOL for SA fibers which served the Surgical Control group was significantly different than that for the SA fibers of either of the groups of normal glabrous skin, as well as from each Allograft group.

The CVs of fibers of the RA and SA submodalities were similar, in that there were no statistical differences in SOLs between the samples which served rejected or nonrejected allografted glabrous skin. In addition, although samples of RA fibers had means and medians which were consistently lower than those for SA fiber samples in each of the experimental groups, the SOLs were not significantly different between the RA and SA samples. Only in the Normal (Experimental) group was there a moderate shift in SOL (towards lower values) for the RA sample as compared to the SA sample ($P < 0.1$).

When Total samples were compared between each of the experimental groups, each vertical pairwise comparison in SOL for the samples in adjacent histograms was significantly different, except for a comparison between the two groups of allografted skin This may have been a reflection of the fact that the same results were true for the RA and SA fiber samples. The Total samples for the experimental groups which involved allografted tissues were significantly different than control or normal values based upon SOL.

3. Comparisons among Hairy Skin Samples

When the mean and median values were compared for each of the CV samples for axons which served hairy skin, patterns were noticeable which were similar to those seen for axons which served glabrous skin. The mean and median values of samples in each category (Total,

Figure 21

Frequency histograms representing the CV of single axons serving cutaneous RFs in hairy skin. The total number of axons sampled for each experimental group (N; left column) is subdivided into categories on the basis of their response properties to cutaneous stimulation: rapidly adapting (RA; center column), slowly adapting (SA; right column). The bin heights in each histogram are expressed as a percentage of the total sample size (N) for each experimental group. The value N represents the total number of axons for both the RA plus the SA samples. Note the change in scale of the ordinate axes marked by an asterisk (*).

The location of the arithmetic mean of each distribution is indicated by an arrowhead. The values of the mean and median for each distribution are presented in the following chart.

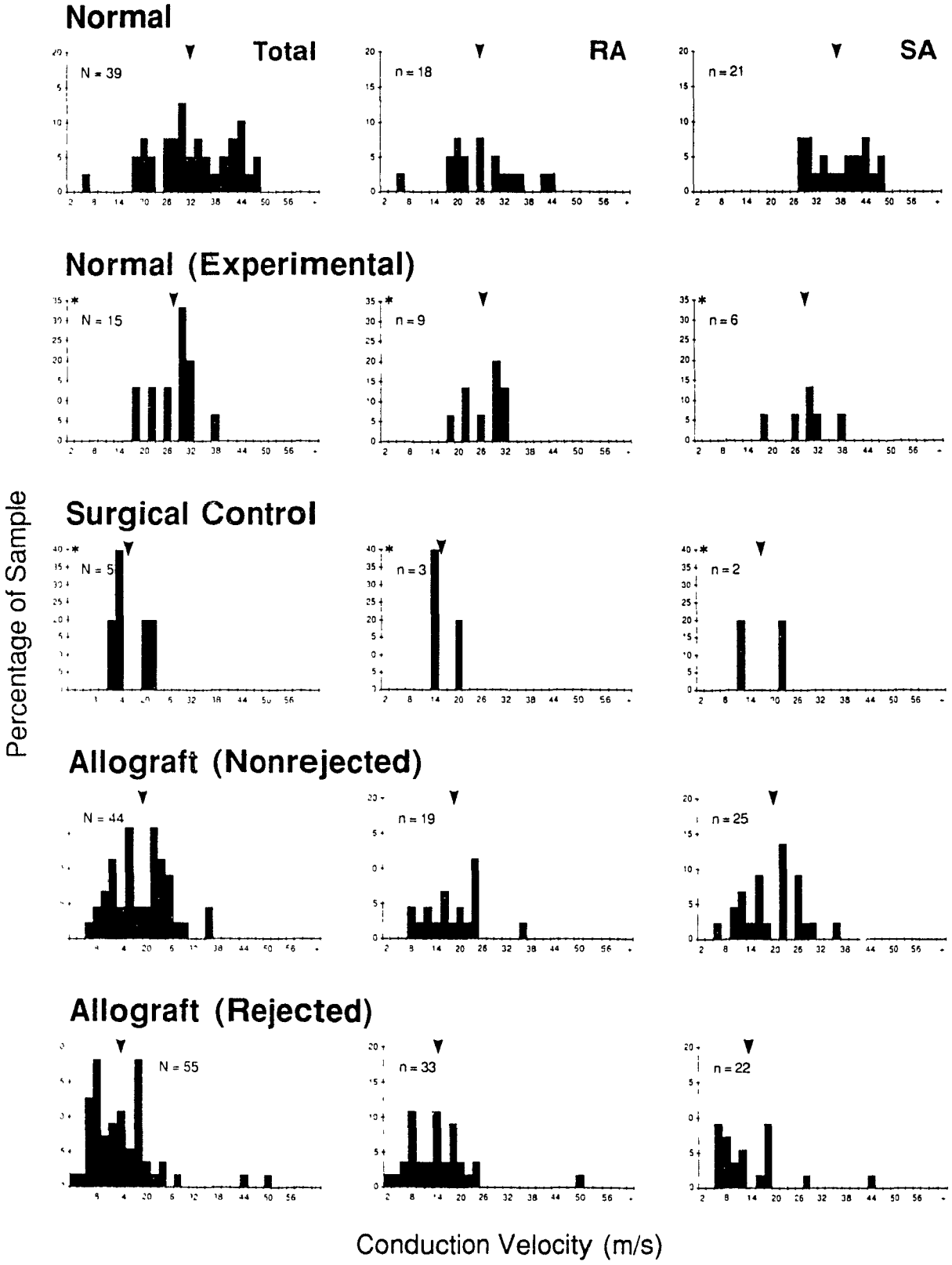
Hairy Skin

Experimental Condition	Conduction Velocity (m/s)		
	Total	RA	SA
Normal	31.0 ± 9.8 30.6	25.0 ± 9.3 24.5	36.1 ± 7.0 36.8
Normal (Experimental)	26.7 ± 5.8 28.2	25.8 ± 5.0 28.2	28.0 ± 7.1 29.0
Surgical Control	15.0 ± 4.1 12.5	14.5 ± 3.9 12.5	15.8 ± 6.0 15.8
Allograft (Nonrejected)	18.1 ± 7.1 17.7	17.5 ± 7.1 17.0	18.5 ± 7.3 20.0
Allograft (Rejected)	13.1 ± 8.6 10.8	13.5 ± 8.5 13.4	12.5 ± 9.0 10.1

mean ± 1 S.D.
median

Refer to the body of the text for a description of the differences between the samples based upon their SOL (Mann-Whitney *U*-test). The histograms which describe the CV of axons which served Normal skin were derived from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.

HAIRY SKIN


Normal
Total
RA
SA
N = 39
n = 18
n = 21
Normal (Experimental)
N = 15
n = 9
n = 6
Surgical Control
N = 5
n = 3
n = 2
Allograft (Nonrejected)
N = 44
n = 19
n = 25
Allograft (Rejected)
N = 55
n = 33
n = 22
Percentage of Sample
Conduction Velocity (m/s)

RA, and SA) showed a progressive decrease when compared from Normal to Allograft (Rejected) in Fig. 21 (top to bottom), with the exception of the Surgical Control sample. The fact that the mean and median values for the Surgical Control group were out of sequence and smaller than those for nonrejected allografts, may have been a result of small sample sizes.

When the mean and median values were compared between the RA and SA categories, the values for the RA samples were generally less than those for the SA samples, as they had been for fibers which served glabrous skin, except for the comparison within the Allograft (Rejected) experimental group, where the values for the SA sample were less than those for the RA sample. There was variability, however, in the statistical significance of these patterns. A comparison between RA and SA submodalities in hairy skin showed that for Normal skin, the sampled RA fibers had CV values with SOL which were significantly different than those for SA fibers ($P < 0.001$), however, for the rest of the experimental groups, the differences in SOL between RA and SA samples were not statistically significant. In the cases of Normal (Experimental) and Surgical Control groups, the small sample size likely influences the significance of the differences between the SOL for the samples.

For the RA fibers which served hairy skin, the SOL of the sampled CVs for Normal and Normal (Experimental) groups were similar, and each was significantly different than that of fibers which served Surgical Control tissues. Although the SOL for axons which served CNVFFs were similar to those for either the Allograft (Nonrejected) or Allograft (Rejected) groups, the two samples which served allografted tissues were significantly different than each other ($P < 0.05$) and were also significantly different than either normal group. Similar patterns were seen in comparisons among Total samples.

Comparisons among samples in the SA submodality paralleled those of the RA and Total samples in most cases. The only two exceptions were that the SOL for the CVs of SA fibers which served Normal hairy skin were significantly different than that for Normal (Experimental) skin, and that the SOL for the Surgical Control sample was not significantly different than those for the Normal (Experimental) group. In both of these latter cases, sample

size was a contributing factor. Of most interest was the fact that, although the SOL for the Surgical Control sample was not significantly different than those for either of the Allograft samples, the sampled CV values for the Allograft (Nonrejected) and the Aliograft (Rejected) experimental groups had SOLs which were significantly different than each other ($P < 0.01$), as well as those of the normal groups.

4. Comparisons between Glabrous and Hairy Skin Samples

As can be seen in Fig. 22, for sampled CVs of both the RA and SA submodalities, the arithmetic mean and median of the samples which served glabrous skin were consistently higher than those for the respective samples in hairy skin, however, these differences were not all statistically significant.

For RA fibers, the sampled CVs of Normal, Surgical Control, and Allograft (Rejected) groups had SOLs which were significantly different when fibers which served glabrous skin were compared to those which served hairy skin. In contrast, the RA fibers which served glabrous skin of the Normal (Experimental) group did not have SOL which were significantly different than those for similar fibers which served hairy skin. Within the Allograft (Nonrejected) group, hairy skin was served by RA fibers with CVs which were moderately lower in value than those for glabrous skin, however, the difference in SOL was not significant ($P < 0.1$).

When SA fibers were compared in a similar fashion, only the samples of the Allograft (Rejected) group showed a significant difference in SOL, while samples in each of the other experimental groups had similar SOLs. The CVs of SA fibers which served nonrejected allografted hairy skin showed SOL which were moderately lower than those for similar fibers which served glabrous skin, however, the difference was not significant ($P < 0.1$).

It is interesting to note that for both the RA and SA fibers, sample comparisons between glabrous and hairy skin of nonrejected allografted tissues showed CVs of axons which served

Figure 22

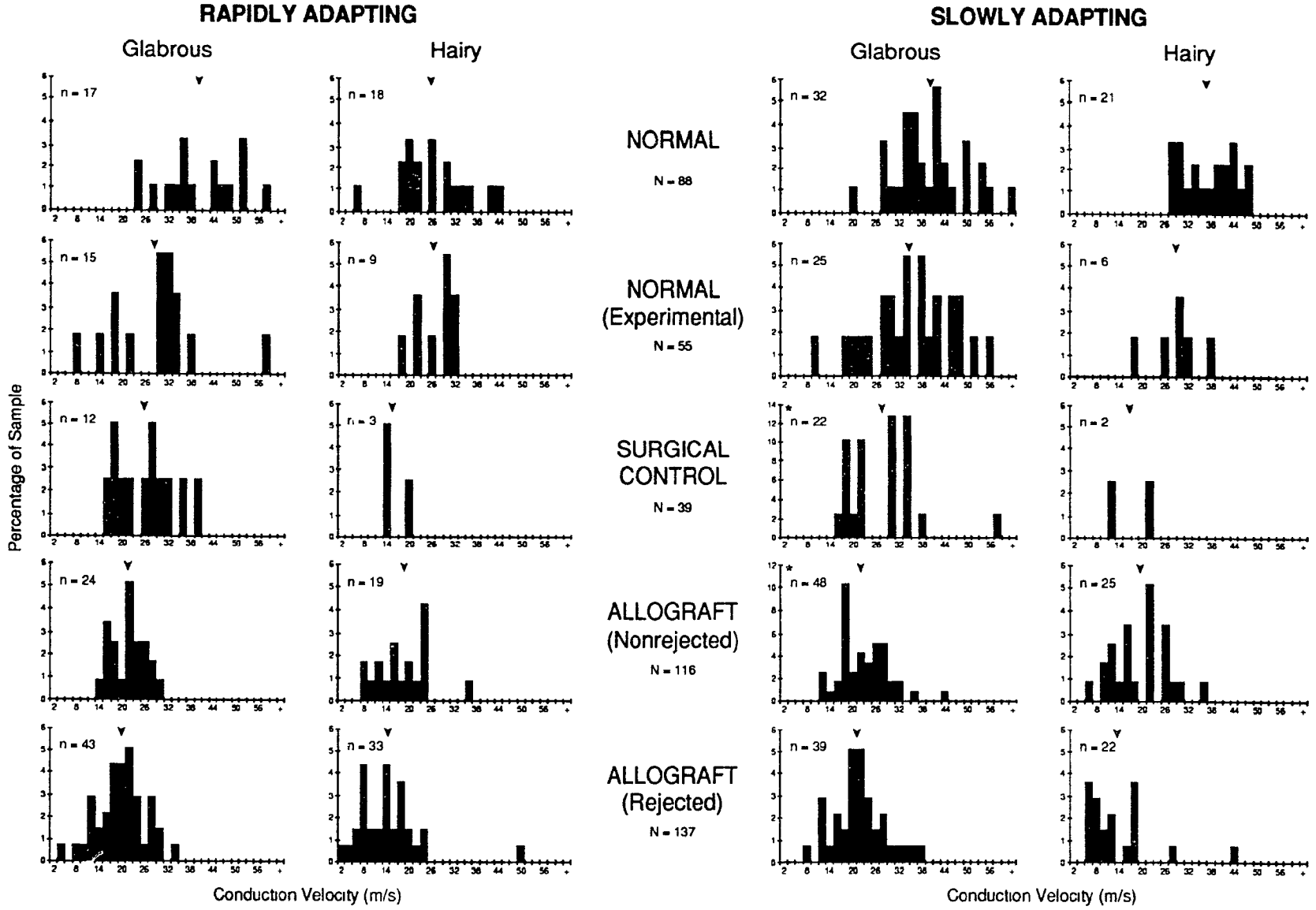
Frequency histograms, similar in nature to those presented in Figs. 20 and 21, are represented for ease of comparison between sampled axons which served glabrous or hairy skin on the basis of submodality. For each experimental group (rows), the histogram bin heights are expressed as a percentage of the total number of axons sampled (N; center column). Note the change in scale of the ordinate axes marked by an asterisk (*).

The location of the arithmetic mean of each distribution is indicated by an arrowhead. The values of the mean and median for each distribution are presented in the following chart.

Rapidly Adapting		Experimental Condition	Slowly Adapting	
Glabrous	**Hairy**		**Glabrous**	**Hairy**
39.2 ± 10.1 36.8	25.0 ± 9.3 24.5	Normal	39.5 ± 10.4 38.7	36.1 ± 7.0 36.8
27.6 ± 12.0 28.9	25.8 ± 5.0 28.2	Normal (Experimental)	33.9 ± 11.3 32.6	28.0 ± 7.1 29.0
25.0 ± 7.7 25.6	14.5 ± 3.9 12.5	Surgical Control	26.7 ± 9.8 28.6	15.8 ± 6.0 15.8
20.6 ± 4.4 21.0	17.5 ± 7.1 17.0	Allograft (Nonrejected)	21.7 ± 6.4 21.0	18.5 ± 7.3 20.0
19.0 ± 6.2 19.4	13.5 ± 8.5 13.4	Allograft (Rejected)	20.6 ± 6.6 20.2	12.5 ± 9.0 10.1

mean ± 1 S.D.
median

Refer to the body of the text for a description of the differences between the samples based upon their SOL (Mann-Whitney *U*-test). The histograms which describe the CV of axons which served Normal skin were derived from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.


RAPIDLY ADAPTING
SLOWLY ADAPTING
Glabrous
Hairy
Glabrous
Hairy
NORMAL
N = 88
NORMAL
(Experimental)
N = 55
SURGICAL
CONTROL
N = 39
ALLOGRAFT
(Nonrejected)
N = 116
ALLOGRAFT
(Rejected)
N = 137
n = 17
n = 18
n = 32
n = 21
n = 15
n = 9
n = 25
n = 6
n = 12
n = 3
n = 22
n = 2
n = 24
n = 19
n = 48
n = 25
n = 43
n = 33
n = 39
n = 22
Percentage of Sample
Conduction Velocity (m/s)
Conduction Velocity (m/s)

hairy skin to be moderately lower (but not significantly different; $P < 0.1$) than for glabrous skin. In addition, Allograft (Rejected) samples showed similar comparisons to be significantly different ($P < 0.001$) with a high degree of certainty that the CVs for axons which served hairy skin were lower.

5. Comparisons among Sample Totals

All of the axons whose CVs were studied are represented in Fig. 23. For each of the experimental groups, the sampled fibers which served glabrous skin (center column) were combined with those which served hairy skin (right column) to form the category labelled Total (left column). Once again, in each category, the arithmetic mean and median of the respective samples for each experimental group decreased when compared from Normal to Allograft (Rejected), except for the Surgical Control sample in Hairy skin which had values smaller than those for the Allograft (Nonrejected) sample. As well, the mean and medians for the Glabrous samples were larger in value than those for Hairy samples. In every case, the vertical comparisons of Total sample SOLs were statistically significant ($P < 0.001$) As well, almost all the differences in SOL between Glabrous and Hairy samples were significant ($P < 0.001$); the one exception being the comparison between the SOLs for the CVs of axons which served Glabrous skin and those which served Hairy skin in the Normal (Experimental) group ($P < 0.1$).

The SOL for the Glabrous sample which served Normal (Experimental) skin was significantly different than that which served Normal skin, with the Normal (Experimental) group having more axons with slower CVs ($P < 0.01$). The same comparison for the CV of axons which served hairy skin showed no significant difference between the SOLs ($P < 0.1$), although Normal hairy skin was served by some axons with considerably higher CVs.

It is also interesting to note that although for hairy skin, the Total, RA, and SA samples had SOLs which were significantly different when the Allograft (Rejected) group was

Figure 23

Frequency histograms representing the CV of single axons serving cutaneous RFs in glabrous and hairy skin. The total number of axons sampled for each experimental group (Total; left column) is subdivided into categories on the basis of the type of skin that the axons served; Glabrous (glabrous total; center column), or Hairy (hairy total; right column). The bin heights in each histogram are expressed as a percentage of the sample total (N) for each experimental group. The value N represents the total number of axons for both the glabrous plus the hairy skin samples.

The location of the arithmetic mean of each distribution is indicated by an arrowhead. The values of the mean and median for each distribution are presented in the following chart.

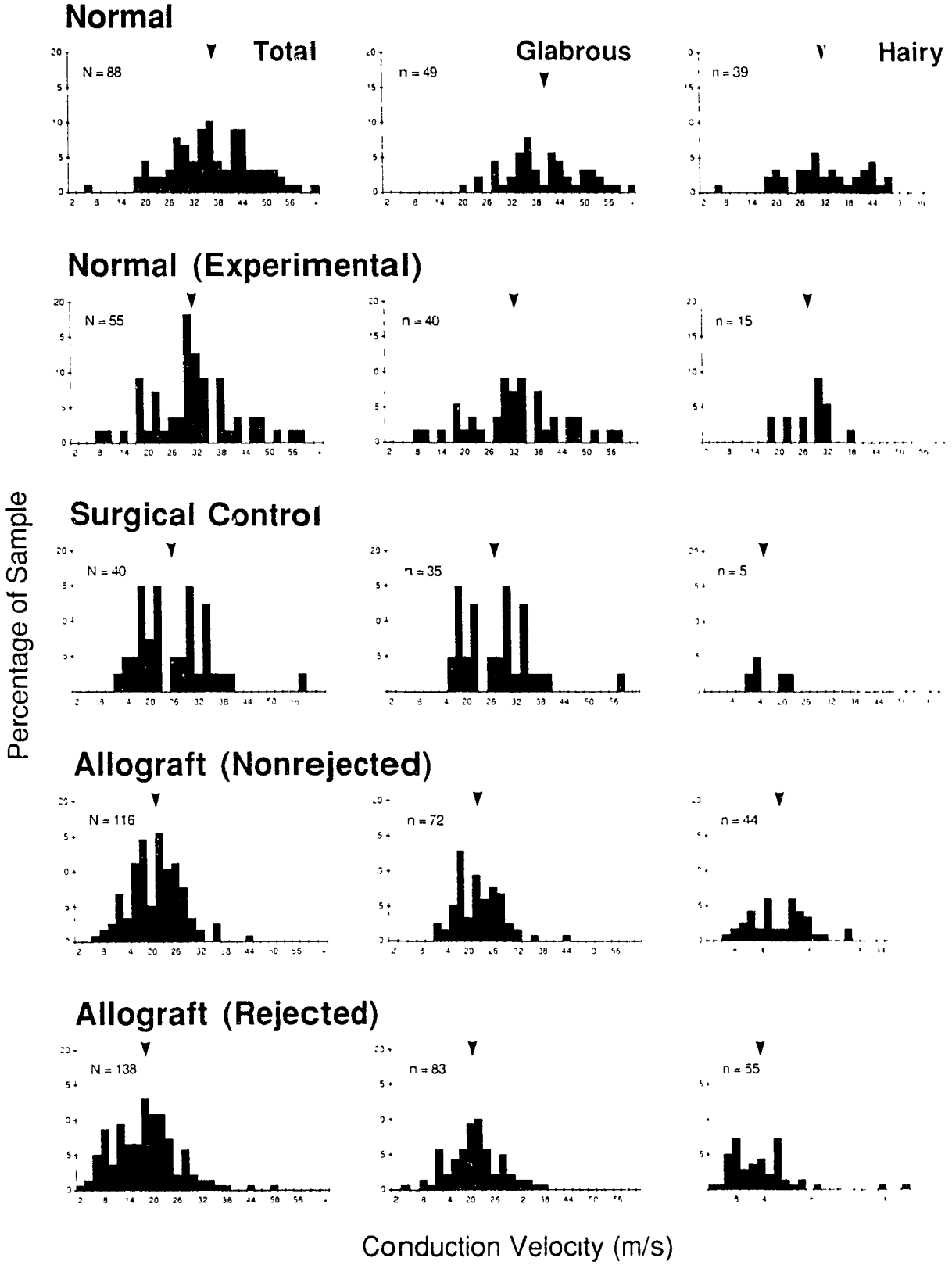
Glabrous + Hairy Skin

Experimental Condition	Conduction Velocity (m/s)		
	Total	Glabrous	Hairy
Normal	35.7 ± 10.8 35.2	39.4 ± 10.2 37.8	31.0 ± 9.8 30.6
Normal (Experimental)	30.2 ± 10.7 29.8	31.5 ± 11.8 31.5	26.7 ± 5.8 28.2
Surgical Control	24.7 ± 9.2 22.8	26.0 ± 8.9 27.1	15.0 ± 4.1 12.5
Allograft (Nonrejected)	20.1 ± 6.5 20.3	21.3 ± 5.8 21.0	18.1 ± 7.1 17.7
Allograft (Rejected)	17.2 ± 8.1 16.9	19.9 ± 6.5 19.8	13.1 ± 8.6 10.8

mean ± 1 S.D.
median

Refer to the body of the text for a description of the differences between the samples based upon their SOL (Mann-Whitney *U*-test). The histograms which describe the CV of axons which served Normal skin were derived from the original data of Dykes *et al.* [1984] (baboons which were not immunosuppressed), and are included with permission for comparative purposes.

GLABROUS + HAIRY SKIN



compared to the Allograft (Nonrejected) group (Fig. 21), the exact opposite was true for glabrous skin (Fig. 20), where the SOLs were similar in each case. This latter point illustrates that tissue necrosis as a result of rejection processes in hairy skin significantly reduces the conduction velocity of axons which served that tissue, while the same processes do not reduce axon CV to a significant degree in glabrous skin.

F. Motor Unit Characterization

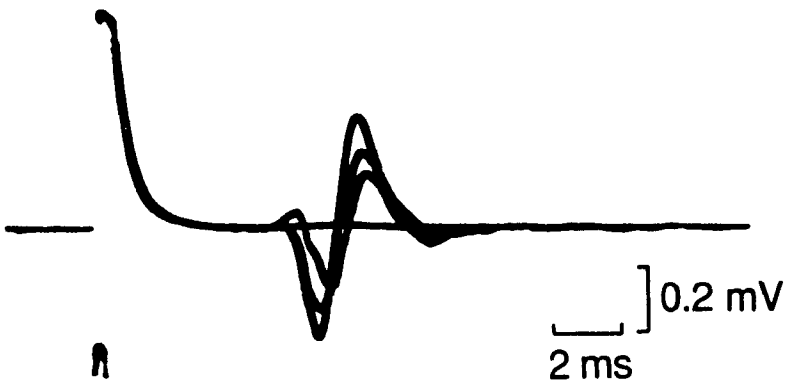
Although the primary emphasis in the electrophysiological characterization of the allografts was on observations related to the sensory innervation of skin, the return of motor function was examined in the allografted hands. HTs carried by animals #7 and 13 were tested for motor innervation by stimulating the median or ulnar nerve and recording EMG responses from the intrinsic muscles of the allografted hand. Examples of the EMGs recorded from the abductor pollicis brevis muscle in the thenar eminence of the HT carried by animal #13 is shown in Fig. 24. EMGs were also recorded from the abductor digiti minimi, adductor pollicis and each of the lumbricalis muscles. At each recording site, several motor units were identified.

In Fig. 24, the EMG traces elicited by three different stimulus intensities are superimposed. The stimulus artifact on the left side of the trace marks the onset of stimulation. There was generally a 4-5 ms delay between the onset of nerve stimulation and the onset of the EMG. The trace with the lowest amplitude waveform represents the first EMG elicited at threshold stimulation. The waveform of medium amplitude represents a summation of two motor unit responses; the unit elicited at threshold, plus an additional unit which was recruited when the stimulus intensity was increased by 18 mV. The waveform of largest amplitude represents the recruitment of all five of the motor units identified at this recording site.

Throughout the stimulating and recording session, movements of the hand which were noted included flexion of D2-D4, flexion and opposition of D1 and D5, and the adduction of

Figure 24

The innervation of allografted muscle was verified electrophysiologically through the identification of motor units which contributed to the EMG response of the intrinsic musculature of the HTs. Electric stimulation of a bundle of axons in the median nerve elicited an EMG response and contraction of the abductor pollicis muscle in the allograft carried by animal #13. The oscilloscope trace shows three superimposed EMGs recorded at three different levels of stimulus intensity. The waveform with the lowest amplitude represents the threshold activation of a single motor unit. With a 15 mV increase in the stimulus intensity over threshold, a second motor unit was recruited and the summation of the two motor units gave rise to the EMG of medium amplitude. The waveform with the largest amplitude represents the recruitment of all five motor units which were identified at the location of the recording electrode. The large stimulus artifact at the left of the trace indicates the time of stimulation and shows that there was a 5 ms delay between the onset of nerve stimulation and the initiation of the muscle contraction.


0.2 mV
2 ms

D2. As a result of the nature of the nerve stimulation, synergistic and antagonistic muscle groups were stimulated simultaneously, hence, the finger movements were transitory and with no coordination. They did, however, correspond to the types of finger movements observed for the HT of animal #7, while the animal was allowed to partially recover from anesthesia.

DISCUSSION

This report represents a study of physiology in its most traditional sense, in that it describes the form and function of a biological phenomenon. As part of a multidisciplinary study, it required the presentation of anatomical, histological and electrophysiological data in order to adequately describe the survival and reinnervation of sensory mechanoreceptors in allografted tissues. In addition, the success of the study required working knowledge of immunology and pharmacology. The primary emphasis in this report, however, is on the documentation of form and function of the afferent fibers which served nonrejected and rejected allografted skin and the morphological characteristics of the sensory mechanoreceptive structures contained therein.

This study showed that host axons will innervate allografted adult primate extremity tissues and that this innervation will take place, in some cases, despite the lack of integrity of normal mechanoreceptive target structures. As well, the activity of axons innervating allografted tissues have similar submodality response characteristics to those of normal or autografted tissues, however, thresholds of activation increased for the majority of fibers serving rejected allografted tissues. The SA fibers serving rejected glabrous skin of the allografted tissues showed the most significant change in S-R characteristics and experienced both an increase in threshold as well as a decrease in responsiveness. As well, glabrous allografted tissues which had undergone processes of rejection supported axons with significantly slower conduction velocities as compared to normal or control tissues

There was a significantly higher proportion of SA *versus* RA fibers sampled from animals receiving high levels of CsA In addition, a higher than normal percentage of these fibers were spontaneously active It is suspected that CsA therapy may have played a role in altering the axonal response characteristics in these animals, however, the mechanism through which this alteration took place is unknown. The influence of Cs at the axon membrane or in axon-Schwann cell interaction is suspected.

I. Experimental Design

With adequate immunosuppressive protocols, long-term survival of allografted upper-extremity composite tissues was achieved. Two TNVFFs remained in excellent condition for more than 6 mo and showed every indication that survival could have been extended indefinitely; had they not been used for sensory nerve testing and histology. The longest surviving allograft (TNVFF, animal #11) was harvested from the host after 413 d. This transplant survived even though it had undergone an episode of severe rejection after 4 mo of postoperative recovery. Two HTs survived long-term, and although both experienced differing degrees of rejection, one of the allografted hands (animal #7) survived for 311 d after transplantation.

Allograft rejection presented itself in one of four modes: acute rejection during early postoperative recovery, sev ·re rejection involving tissue sloughing, mild rejection involving epidermal erosion, and vascular compromise as a result of persistent but mild rejection processes. Minor differences between rejection processes which resulted in epidermal erosion or slough, observed in HTs and/or TNVFFs, were more likely a function of the level of immunosuppression that the host was experiencing, rather than immunologically different approaches to tissue destruction. There were, however, obvious differences between glabrous, hairy and transitional skin with respect to their durability in the face of rejection processes. Two of the problems which masked the onset of rejection in baboon glabrous skin, and hence delayed diagnosis, were the thickness of the keratinized layer of epidermis and the darkness of the pigmentation. Hairy skin did not express either of these characteristics, and therefore, monitoring rejection processes in this tissue was easier. Transitional skin in the webspaces between the fingers, and glabrous skin of the volar aspect of the hand, were both more susceptible to rejection, and although redness in hairy skin was usually the first sign of the onset of tissue rejection, these two zones of tissue were usually the first to show dramatic tissue disruption. Although the experiment was not designed to specifically address this

question, one possibility exists that this may have been a reflection of different antigenic phenotypes in the three cutaneous tissues.

The severity of tissue necrosis during a first-time major rejection episode in glabrous skin was probably accelerated as a consequence of serous fluid leakage from ruptured sweat ducts in the intermediate ridges, maceration of the epidermis underlying the keratinized layer, and tearing of the epidermis along the epidermal/dermal border. Glabrous skin which underwent epidermal thinning prior to the onset of major rejection, experienced patchy tissue disruption, but did not slough a large expanse of superficial epidermis. In retrospect, steps taken to reduce the production or presence of sweat within the rejecting allografts might have limited the extent of tissue disruption. Once glabrous or transitional skin had sloughed, the brilliant redness of the underlying tissues was a reflection of the depth of tissue disruption, but also, it signified the proximity of dense capillary plexi to the underside of the epidermis. This latter characteristic was less prominent in hairy skin.

All of the tissues transplanted among random (unknown) source animals underwent some degree of physical change related to allograft rejection. There is no certainty as to whether the increased percentage of survival for those allografts transplanted among animals which were tissue-typed is a reflection of the fact that they were obtained from a single breeding colony, or whether it was a result of improved monitoring and immunosuppressive protocols. It is important to note, in this regard, that the animals supplied by the SFBR were designated as histoincompatible donor pairs with the most sophisticated serological testing methods for baboons which were available at the time. In addition, of the four allografts attempted using these animals, one TNVFF (carried by animal #19) underwent severe tissue rejection at approximately 1.5 mo after a 5-10 d period of inadequate immunosuppression (Cs 12 h serum trough levels < 1000 ng/ml, RIA) This was more than enough time to mount an immunological response in a sensitized host. As well, histological assessment of the tissues carried by animals #20 and 21, indicated that epidermal erosion had occurred, even at very high circulating levels of Cs. Furthermore, all of the allografts among random source animals

underwent rejection while their Cs-RIA levels were below the estimated therapeutic level for these animals (1000-1200 ng/ml, RIA 12 h serum trough levels at room temperature). Of these tissues, once adequate immunosuppression was re-established, rejection processes were curtailed and the allografts remained viable in a stable condition for prolonged periods of time. Therefore, it is the interpretation of the author, that all of the allografts provided sufficient immunological challenge to classify them as having been histoincompatible.

The antigenic characteristics of the skin component of the allografts appeared to be a greater stimulus to the host's immune system than any of the other components of the composite tissues. This may account for the very high levels of CsA which were necessary to control the immune response as compared to conventional therapy in humans for internal organ transplants. The administration of large amount of CsA (*circa* 40-50 mg/kg/d), although tolerated reasonably well by the baboons in this study, could not be used in a clinical setting for prolonged immunosuppressive therapy. For internal organ transplants in humans, prolonged CsA therapy at blood levels in excess of 250 ng/ml (RIA, serum trough) may result in nephrotoxic side-effects, which although reversible, cannot be tolerated for extended periods of time [Kahan, 1985a; Kahan *et al.*, 1982]. Although there were signs of nephrotoxicity and other side-effects associated with extremely high Cs levels (> 1500 ng/ml, serum 12 h trough; RIA) in the baboons utilized in this study, they were controllable by adjusting the CsA therapy and prolonged immunosuppression was possible with minimal metabolic consequences.

II. Histology

Histologic changes were observed in all the grafted tissues in this study as a consequence of transplantation and/or nerve transection. Changes in the autografted NVFFs were limited to the quality of nerve regeneration and the reinnervation of target tissues, including the sensory mechanoreceptors. In contrast, the allografted NVFFs and HTs experienced additional changes involving the epidermal structure of the tissues and its constituents. The most dramatic alterations took place in allografted glabrous skin, where structural modification to the epidermis occurred involving the thinning of the keratinized layer, thinning and tilting of the epidermal ridges, and/or depigmentation and the reduction of the epidermis to a uniform thickness of only a few cells. These structural changes appeared permanent; once a characteristic was altered, there was little evidence found to indicate that it would revert to its former structure.

Two events in allografted glabrous skin which were not predicted by previous nerve transection and regeneration models were (1) the structural modification and poor innervation experienced by the hair follicles in severely rejected allografted hairy skin, and (2) the reduction in number (and possibly the selective destruction) of identifiable Merkel cells in allografted glabrous skin. Since both of these mechanoreceptor structures are thought to be of epithelial cell origin, it is conceivable that their composition and location were factors which predisposed them to irreparable damage. With respect to the lack of identifiable Merkel cells in allografted glabrous skin, a negative result is not easily illustrated, and hence, judgement is reserved as to their fate until further electron microscopic analysis of the tissues is completed. No evidence was seen in light microscopic sections to verify their presence, however, the concentration of melanin pigment in baboon glabrous skin may have prevented their identification.

The fact that host axons were seen to innervate severely rejected glabrous skin of a HT (carried by animal #13) as far as 15 cm distal to the anastomotic site of the allograft (as

indicated by the identification of its cutaneous RF) argues strongly that while serious tissue disruption occurred in epidermis, the presumed target tissue, the immunological response was not as disruptive to the supporting elements of the allografted nerve located deep to the rejecting tissues, during the same time frame.

No evidence exists in these studies to state unequivocally that axon-mechanoreceptor continuity can be maintained throughout the processes of active rejection. It is hypothesized that if the processes of tissue rejection are severe enough at the site of the target tissue, that host axons would undergo some degree of degeneration and would retract as a result of the loss of the supporting tissues (target mechanoreceptor and/or Schwann cells, both of which would be foreign tissues). In cases where mechanoreceptors survived a major episode of tissue rejection in allografted skin and were seen later to be innervated by host axons, it is proposed that the innervation which was observed took place following the rejection episode. In support of this latter hypothesis, is the fact that within severely rejected allografted glabrous skin, the focus of inflammatory cell infiltrate was primarily located in the most superficial 200 μm of dermis. Since others have shown in the rat that axons can penetrate as far as 1 cm into a once successful but later severely rejected allografted nerve segment containing very few support cells [Zalewski *et al.*, 1982], it seems likely that axons could easily reach the epidermal surface even after their retraction in the face of active rejection processes. Indeed, axons were identified as they grew freely through areas of inflammatory cell infiltrate within grossly rejected but stabilized allografted glabrous skin (see Fig. 8F). It is consistent with the histological data that because the axons are autologous tissue and tolerated by the host's immune system, they continue to search for, identify, and innervate surviving mechanoreceptors (or other target structures), or survive in the remaining tissues as free nerve endings. Although in rejected allografted nerve segments, axons which have penetrated into the tissues have limited survival, this survival is primarily dependent upon the establishment of contact to target tissues on the other side of the nerve segment [Aguayo *et al.*, 1977, Mackinnon *et al.*, 1987]. Once axons in composite tissue allografts have elongated across the

short expanse of rejected tissues, trophic influences as a result of their contact to a target structure could conceivably help to maintain their union, despite the temporary lack of Schwann cell support in the area of rejection.

III. Electrophysiology

The growth of an axon across the site of nerve repair and its elongation through remnants of pre-existing nerve fascicles which determines its innervation territory, is an observation reported many times in the literature, dating back to the studies of Ramón y Cajal [1928]. The present study is unique, in showing that host axons were capable of growing up to 15 cm into histoincompatible nerve tissue to locate and functionally innervate their target structures

Although cutaneous RFs of the nonrejected TNVFFs were similar in distribution, shape and size to those of the CNVFFs, the RFs of axons which served rejected TNVFFs had sizes and shapes resembling the immature innervation described by Terzis and Dykes [1980]. They showed that axons which recently arrived at the skin surface had small, irregularly shaped RFs. Immaturity of the innervation in rejected allografted skin is the simplest explanation of the small or punctate RFs seen in rejected tissues. These observations are also consistent with the idea that intense rejection processes surrounding the axon tip temporarily prevent host axons from innervating mechanoreceptors and disconnect those axons which had innervated mechanoreceptors previous to rejection.

Due to the extent of tissue disruption in rejected allografted tissues, however, other considerations also have to be taken into account in order to explain phenomena observed for these tissues. In rejected allografted skin, for example, some SA fibers had large RFs that were similar in shape and diameter as those for CNVFFs, yet they had elevated thresholds. One possible explanation is related to the physical character of the tissues. The lysis of epidermal cells and destruction of the epidermal ridging pattern led to a dramatic alteration of the structural milieux, and hence to changes in the mechanics of the tissue. It is possible that the RA mechanoreceptors in glabrous skin (primarily Meissner corpuscles in this study) are less sensitive to the structural alteration of its physical surroundings than Merkel (SA-I) or Ruffini (SA-II) mechanoreceptive structures, since there was no significant difference in the SOL for RF thresholds of RA fibers, while there was for SA fibers which served rejected glabrous

skin. Dynamic mechanical properties in rejected allografted tissues have not yet been studied in this context and therefore the answer to this question is presently unknown, however, it has been shown that subtle changes to the physical character of a normal epidermal tissue can dramatically alter mechanoreceptor response properties in raccoon glabrous skin [Pubols and Pubols, 1983; Rasmusson and Turnbull, 1986].

Another possible explanation of the increased threshold of activation and the reduced responsiveness of some of the SA fibers identified in rejected allografted glabrous skin is that, as a result of the physical disruption of the tissues, free nerve endings continue to elicit SA-type responses, and that the quality of these responses depends upon the degree of Schwann cell myelination and the ability of the host axons to efficiently propagate a high frequency train of APs. Although there is no conclusive evidence in this study to indicate that the generation of SA-type responses is possible once an axon has become disconnected from its mechanoreceptive structure, it was shown that free nerve endings do exist in rejected allografted tissues and that SA-I and SA-II type responses are identifiable (as defined by the criteria for mechanoreceptor classification by Horch *et al.* [1977]) for axons which served rejected or nonrejected allografted tissues. In addition, these attributes existed even though Merkel cells were not able to be identified in the allografted tissues during preliminary electron microscopic studies [Samulack *et al.*, 1989]. These findings support the view suggested in the literature that Merkel cells may not be necessary for the transduction of the mechanical stimulus into electrical energies within the axon [Diamond *et al.*, 1986; Gottschaldt and Vahle-Hinz, 1981; Munger, 1977]. The poor responsiveness and increased threshold of the SA fibers in rejected glabrous skin *versus* nonrejected tissues, may reflect the impact that tissue structure has on SA response characteristics. Poor SA responsiveness may also be a result of the disruption of the electrical insulation properties of the supporting tissues (Schwann cells) and hence, poor propagation or integration of generator potentials and/or APs elicited near the tip of the axon. This issue of the quality of myelination and/or foreign Schwann cell contact to host

axons is a central theme in the discussion of CVs and the levels of spontaneous activity exhibited by axons which served allografted tissues (see below).

The quality of reinnervation in nonrejected allografted hairy skin, as determined by the threshold of axonal activation and characterization of axonal responses to controlled stimuli, was very similar to that of autografted NVFF control tissues which underwent nerve transection and regeneration under comparable circumstances It was remarkable, that despite the loss of hair shafts and almost total destruction of the morphology of the external root sheath in rejected allografted hairy skin, both RA and SA responses as well as clearly defined RFs were sampled in these tissues. Only the thresholds of activation for RA fibers which served rejected hairy skin was significantly higher than those for similar nonrejected tissues In addition, the RA tuning curves for axons which served rejected allografted hairy skin, as well as those for nonrejected hairy skin, resembled those of the majority of axons which served normal hairy skin. Yet, there was no histological evidence that the normal receptor structures were present, nor that axon's were innervating their remnants The most reasonable interpretation, is that even without the mechanical apparatus, these axons are still capable of transducing localized mechanical stimuli in a fashion which resembles that of normal neural-epithelial sensory mechanoreceptor complexes.

The role of the mechanoreceptor structure in adult tissues and during embryogenesis may be to guide axons to their location in the skin, to ensure morphological specialization of the growing axon tip when contact is made, and to nurture the axon through trophic factors to maintain the connection. This question has also arisen in another context. Diamond *et al* [1986] has shown that the Merkel cell of the salamander is the target for cutaneous axons that are rapidly adapting. Yet the same workers have confirmed the observation of Iggo and Muir [1969] and others, that the Merkel cells in mammals serve axons that are slowly adapting. It is possible that once maturation of the mechanoreceptor is complete, the structural elements of the receptor may serve only to lower the threshold of activation for the axon, and the membrane of the axon may be capable of transducing mechanical energies into a generator potential on its

own. These concepts are not new; Loewenstein [1960] showed, in an elegant set of experiments, that the electrical characteristics of the generator potential and AP of an axon innervating a Pacinian corpuscle remained virtually unchanged after removal of all the outer lamellae of the corpuscle and even after portions of the inner core of the corpuscle had been removed. He was able to show that the axon terminal was itself rapidly adapting and produced only a transient generator potential in response to a sustained pressure.

For Pacinian corpuscles identified morphologically in this study, it was established that multiple innervation of the corpuscle can occur in allografted tissues as a result of host axon regeneration. Multiple innervation has been carefully characterized by Zelená [1984], through histological studies of the crural interosseous membrane in autologous hindlimb tissues of the rat. Also, in the present studies, Pacinian-like responses were identified electrophysiologically in nonrejected allografted glabrous skin on the basis of tuning curves having minimum tuning points at frequencies greater than 100 Hz. They had medium sized well-defined RFs on the finger (5-8 mm in diameter). Thresholds of activation were approximately 0.3 g. These are not like normal Pacinian responses, which are usually extremely sensitive (vibratory threshold at 100-400 Hz, amplitude > 1 μm, very large RF [Dykes, 1983]), yet it remains that the two curves are clearly different in tuning characteristics from the others collected in this study. Unfortunately, tuning curves derived from 'identified' Pacinian corpuscles which have been reinnervated after nerve regeneration, have not been described in the literature. Although similar responses to the ones described herein, have been recorded from axons serving mechanoreceptors of unknown origin in primate full-thickness skin grafts [Dykes *et al.*, 1984] or after nerve crush in rat glabrous skin [Sanders and Zimmermann, 1986]. It is reasonable to suspect, and consistent with the observations of Sanders and Zimmermann [1986], that on the basis of the response characteristics of these axons, and on the experimental conditions in which they were observed, that they may represent the functional characteristics of reinnervated Pacinian corpuscles.

The CVs of axons studied in this report were indicative of the amount of tissue manipulation experienced by the tissue that they served. Although not necessarily statistically significant in each case when one mean was compared to another, when the experimental groups were ranked in order of severity of tissue manipulation and/or rejection from Normal to Allograft (Rejected), there was a consistent trend for both the mean and median of each sample to be progressively smaller in value. Exceptions to this pattern occurred primarily in samples for which there was less than nine axons.

In most cases, except for when the sample size was small, the CVs of samples of axons which served the CNVFFs or either of the allografted tissues, had SOLs which were significantly lower than those which served either of the normal tissues. The small diameter of the fibers which served the grafts, as a result of nerve transection and regeneration, is believed to be the main reason for these differences. Comparisons of sample SOLs between nonrejected allografts and Surgical Control autografted tissues showed no consistent statistically significant differences. Small sample sizes of axons which served CNVFFs may have contributed to the inconsistencies, but even when RA and SA samples were grouped together, only the Glabrous total showed significantly different SOLs between Surgical Control and Allograft (Nonrejected) tissues; primarily as a result of the significant difference observed for the SA fibers in this tissue. This suggests that under nonrejected conditions, allografted Schwann cells provide the myelination and trophic support necessary for host axons to provide saltatory conduction in a similar manner as they would in reinnervated autologous tissues.

The CV of host axons in allografted hairy skin which had undergone rejection was significantly different than for axons which served nonrejected hairy skin For both the RA and SA axons, the SOL of sampled CVs was significantly less in rejected hairy skin than the SOLs of a comparable sample was in nonrejected hairy skin. This was not the case for allografted glabrous skin, where the exact opposite was true; samples of RA and SA fibers which served nonrejected and rejected skin were similar in SOL. It is consistent with the electrophysiological data, that as a result of the loss of structure in the target tissues of hairy skin, the axons

although retaining the submodality specific functional properties, require the further support available from healthy target tissues in order to provide efficient propagation of the electrical signal. The necessary tissue support may be in the form of trophic influences from intact target mechanoreceptors, however, mechanoreceptor support alone is not necessary to attract axons into adult skin, nor is it necessary for these axons to generate submodality specific mechanoelectric transduction of cutaneous stimuli.

The poor performance of axons innervating hairy skin grafts have been previously reported by Dykes *et al* [1984] and by Ridley [1970], for full-thickness skin grafts. It is apparent from the present study that the poor performance of the RA fibers in the full-thickness hairy skin grafts reported by Dykes *et al.* [1984], with respect to their frequency tuning curves, may have been due to physical parameters involved in the design of the graft and/or how it became attached to the underlying tissues, rather than aspects of reinnervation, since axons from TNVFFs in this study responded as well as the majority of the RA fibers which served normal skin.

In addition to a trend towards a reduction of CV in allografted tissues, another observation suggesting that neural function was compromised was the significantly increased incidence of spontaneously active fibers, and the increased proportion of SA fibers sampled in animals which received very high levels of CsA for prolonged periods of time. Clinically, and in an increasing number of animal studies, neurotoxic side-effects of CsA have been reported; tremor, psychologic disturbances, seizures, paralysis, paresthesia and temperature sensitivity [Ang *et al.*, 1989; Atkinson *et al.*, 1984; Krupp *et al.*, 1986; Najarian *et al* , 1985]. Whether the neurotoxic side-effects in the clinical setting are the result of CsA therapy alone [Najarian *et al.*, 1985], or as a result of dual therapy including MP [Durant *et al.*, 1982], has yet to be resolved. Some neurophysiological effects have been noted in experimental animals when large doses of MP (30-60 mg/kg) are administered intravenously at the time of nerve recording [Hall, 1982], but no reports have been found to indicate that the IM injection of low doses of MP distant to the experimental site would elicit the abnormal electrophysiological effects

reported here. There is however, a report which suggests that CsA significantly increases the frequency of spontaneous contraction of the vascular smooth muscle in portal veins [Lamb and Webb, 1987]. In the latter report, it was hypothesized that CsA toxicity altered vascular adrenergic neurotransmission. The data presented in the present report is consistent with such a hypothesis, and suggests that the neurological symptoms seen during prolonged high dose CsA therapy may be related to wide-spread alteration of the conduction and signalling characteristics at the single axon level.

IV. Concluding Remarks

These experiments indicate that long-term survival of upper-extremity allografted tissues (skin, muscle, nerve and bone) is possible in a primate species. Anatomical data showed that host axons are able to grow into a histoincompatible tissue environment, locate and innervate target structures in the adult animal. Electrophysiological data showed that the quality of innervation, although potentially similar to that of autografted tissues with the conservation of submodality specific mechanoelectric transduction of cutaneous stimuli, was related to the structural characteristics of the allografted tissue. These studies indicate that the quality of innervation in allografted glabrous skin (whether nonrejected or rejected) surpasses that of comparable hairy skin, however, hairy skin allografts had a greater likelihood of long-term survival and experienced less disruptive structural alterations during rejection. It is uncertain at this time, what sort of sensation the reinnervated allografted glabrous or hairy skin would impart to the host, since even in nonrejected allografts there is the possibility of increased spontaneous firing rates for the innervating axons, as well as an alteration in the spectrum of sensory mechanoreceptors. For rejected allografted skin, the loss of mechanoreceptors below that which provides adequate tactile acuity would be crucial, however, the innervating axons will still be able to provide some degree of mechanosensory transduction of environmental stimuli.

At the present time, extremity composite tissue allografts do not appear feasible as a surgical alternative in reconstructive surgery, since clinical indications suggest that the levels of immunosuppression which were required to maintain the grafts in the nonrejected state in this study would not be well-tolerated by humans. With the possibility of revised therapeutic protocols using new and more specific immunosuppressive drugs or techniques, the future of the transplantation of extremity composite tissues looks encouraging. These studies indicate that sensory and motor innervation and the potential for functional recovery of allografted extremity tissues will occur. In addition, these studies predict that areas of skin which have

survived extensive thermal injury and have been surgically covered with artificially cultured epidermal cell allografts (which have been rendered histocompatible) will reinnervate and that the innervating axons may provide useful tactile sensation despite the lack of superficial mechanoreceptive structures.

APPENDIX I

Animal Maintenance Records

Animal #1

Utilization: Acute surgical exploration of baboon neurovascular structures; design of the index finger NVFF. After euthanasia, the carcass was perfused with neutral-buffered formalin and used as a reference specimen.

Animal #2

NVFF: Replantation of the total soft tissue coverage of the right D2; excluding the fingertip, nail and nail bed.

Autograft Survival: 6 d; no surgical complications.

Case History:

Recovery from surgery and autograft survival was uneventful. The NVFF was determined to be a surgical success and potential long-term survival was predicted. At 6 d of postoperative survival the tissues of the left D2 were utilized as donor tissues for animal #3, and euthanasia of the animal was performed.

Animal #3

TNVFF: Transplantation of the total soft tissue coverage of the left D2; excluding the fingertip, nail and nail bed.

Donor: Animal #2; unknown source.

Allograft Survival: 20 d; underwent severe rejection.

Nerve Recording: Not possible due to insufficient time for mature reinnervation.

Mean Measured Weight During Study: 11.3 kg.

CsA Dosing: 18-21 mg/kg/d; most commonly *circa* 20 mg/kg/d (IM). Temporary regimens involving PO administration were also utilized.

Mean Sampled Cs Blood Levels (RIA, serum 12 h trough): 225 ± 91 ng/ml; approximately 200 ng/ml at onset of primary rejection, most commonly *circa* 250 ng/ml.

MP Dosing: None.

Other Drugs Administered:

Penlong S® (regular; > 50% of the time).

Acetylsalicylic acid (frequent, < 50% of the time).

Meperidine hydrochloride, netilmicin (intermittent; < 25% of the time).

Case History:

At the time of the first change of dressings, 6 d after surgery, the allografted tissues were slightly swollen, but reasonably healthy. The edge pf the flap was dry and secure, with only a couple of loosened sutures. Of concern, was a reddish undertone in the normal light brown colour of the allografted hairy skin overlying the proximal phalanx and its interphalangeal knuckle. By day 13, the hairy skin overlying the proximal phalanx was overcome by severe rejection processes which led to massive tissue necrosis. The structure of the affected tissues changed from that of healthy hairy skin, to orangy-red unpigmented tissues, with no evidence of hair follicles. The damage to the allografted tissues was most apparent on the dorsal and radial aspects. Mild maceration and general loosening of the glabrous tissues on the volar aspect of the affected area was also apparent. Healthy circulation was still intact as evidenced by the colour of the blood which was brought immediately to the skin surface via a punctate stab wound to the volar aspect of the mid-finger. The rejection processes continued through to day 15, by which time the glabrous skin of the whole volar aspect of the index finger, was whitened and macerated. Most of the allografted hairy skin succumbed to necrosis except for the most proximal 1 cm^2 area. The autologous tissues which comprised the most distal aspects of the digit (glabrous fingertip, nail and nail bed) were still intact and reasonably healthy. By day 20, fluids which leaked as a result of the rejection processes and tissue necrosis, caused the bandages to become wet. Subsequently, all of the glabrous tissues of the allograft (as well as those of D3 and D4) were macerated. The glabrous tissues of the allograft were loose and wrinkled. The tissues on the dorsum of the finger remained much as they were on day 13. No indication was apparent that the allograft would recover, so euthanasia of the animal was performed.

Animal #5

NVFF: Transplantation of the total soft tissue coverage of the right D2; excluding fingertip, nail and nail bed.

Donor: Animal #4; unknown source.

Allograft Survival: 161 d; underwent mild rejection.

Nerve Recording: During postoperative days 160 to 161.

Mean Measured Weight During Study: 10.8 kg.

CsA Dosing: 19-46 mg/kg/d; most commonly *circa* 32 mg/kg/d (IM). Temporary regimens involving SC administration were also utilized.

Mean Sampled Cs Blood Levels (RIA, serum 12 h trough): 808 ± 315 ng/ml; approximately 350 ng/ml at onset of primary rejection, most commonly *circa* 900 ng/ml.

MP Dosing: Postoperative; starting at day 15

125 mg/d for 3 d, tapered to a maintenance dose of about 4.4 mg/d by day 26.

Other Drugs Administered:

Netilmicin, Penlong S® (regular; > 50% of the time).

Dimenhydrinate (frequent; < 50% of the time).

Acetylsalicylic acid (intermittent; < 25% of the time).

Furosemide, levorphanol tartrate, prednisone (when needed; < 10% of the time).

Case History:

The quantity of tissues comprising the proximal portion of the TNVFF was slightly in excess of those removed from the host to prepare the bed for the allograft. Hence, at the completion of surgery, the palmar pad and the hairy tissues overlying the knuckle were bulbous. As in the previous cases, the tissues of the host's fingertip (the nail and nail bed) were left intact. During postoperative days 6 through 15, in addition to swelling, there was a moderate maceration of the tissues of the flap as well as the surrounding host's tissues. By day 15, it was obvious that rejection processes were most likely the cause. The hairy skin overlying the proximal interphalangeal joint were macerated, broken and reddened. The incision line along the radial aspect of the graft opened at several sites. Whether as a result of surgery, or a result of inflammation due to local rejection processes, the proximal interphalangeal joint became fixed at a 90° angle. At day 18, although the rejection processes had slowed, much of the hairy tissues along the length of the finger were now disrupted. The hair of the affected area had fallen out and the tissues left behind were a broken mosaic of orange, yellow and reddened tissues. The hairy portion of the flap overlying the knuckle and dorsum of the hand appeared relatively unaffected. The same was true for the allografted glabrous tissues, despite the whitened appearance due to maceration. The tissues of the allograft, as well as those of the host, began to dry around day 20. For the most part, rejection had stopped. The hairy skin on the dorsum of the hand remained intact. The broken, relatively unpigmented and scarred hairy tissues of the finger were dry and the fingernail was easily removed on day 23. All of the glabrous tissues of the flap remained pigmented, but were dry and leathery in both appearance and texture. By day 39, the surface of the allografted glabrous skin of the palmar pad was smooth and hardened. The skin overlying the dorsal surface of the proximal phalangeal joint was not healed but remained fleshy and granulated. All of the dried keratinized superficial glabrous tissue was removed in the form of a single hardened 'cap' on day 42. The underlying glabrous skin had a heterogeneous appearance and was composed of both pink and light brown tissues. The skin which was revealed was otherwise healthy, and

had well-defined epidermal ridges with no lesions. At this point in time, the original site of autologous glabrous tissues at the tip of the finger remained intact. However, one could not be sure that the autologous tissues were in fact still present, since the hairy component of these tissues appeared to undergo similar, if not the same rejection processes as those of the allografted hairy tissues adjacent to them. Evidenced by the loss of the nail and scarring of the autologous tissues, it was likely that the maceration and possible compromised blood supply led to their demise. By day 49, the granulating tissues on the dorsum of the proximal interphalangeal joint had not healed and a split-thickness autograft from the proximal forearm of the animal was used to cover the site. On day 74, most of the lesions on the index finger were healed. The index finger's glabrous palmar pad was a mixture of light pink, beige and dark brown tissues; the darkened pigment now covered approximately 70% of the area. Most of the allografted hairy skin on the dorsum of the hand remained unaffected and supported luxuriant hair growth. The glabrous and hairy tissues of the finger (but not the palmar pad) had regressed to the point of tough whitish-pink scarred tissue with small patches of dark pigmentation. The tissues on the tip of the finger had become pointed and showed little evidence that autologous tissues once occupied that area. The allograft remained in this condition until day 96, when processes of rejection once again took their toll on the glabrous and hairy portions of the distal finger. The tissues became fragile and covered in small reddened ' sions. There was some bleeding; primarily where gauze bandages stuck to the wounds. By day 110, the tissues healed much like they did around day 74. The pigment of the allografted glabrous skin on the palmar pad had become uniformly light brown in colour. The tissues remained intact, with few changes, until nerve testing and removal of the graft 161 d after surgery. At that point in time the palmar glabrous skin of the allograft was not as well keratinized as that of the host, but it had well-developed epidermal ridging patterns, light pigmentation and was pliable. The hairy skin on the dorsal surface of the hand had normal uniform pigmentation and supported good hair growth. The tissues of the finger, however, were heavily scarred, relatively unpigmented and tough in texture.

Animal #7

HT: Transplantation of the total right hand; including wrist.
Donor: Animal #6; unknown source.
Allograft Survival: 311 d; underwent mild rejection.
Nerve Recording: During postoperative days 309 to 311.
Mean Measured Weight During Study: 12.7 kg.

CsA Dosing: 26-60 mg/kg/d; most commonly *circa* 28 mg/kg/d (IM). Temporary regimens of IM, SC or mixed routes of administration were also utilized.

Mean Sampled Cs Blood Levels (RIA, serum 12 h trough): 1202 ± 581 ng/ml; approximately 800 ng/ml at onset of primary rejection, more commonly *circa* 900 or 1700 ng/ml.

MP Dosing: Postoperative; starting at day 6

125 mg/d for 3 d, tapered to a maintenance dose of about 4.4 mg/d by day 27.

Rejection; starting at day 140

125 mg/d for 3 d, tapered to a maintenance dose of about 4.4 mg/d by day 147.

Rejection; starting at day 186

125 mg/d for 4 d, tapered to a maintenance dose of about 4.4 mg/d by day 235.

Prophylactic; starting at day 255

125 mg/d for 3 d, tapered to a maintenance dose of about 4.4 mg/d by day 284.

Other Drugs Administered:

Dimenhydrinate, Penlong S® (intermittent, < 25% of the time).

Acetylsalicylic acid, levorphanol tartrate, netilmicin, prednisone, ticarcillin disodium (when needed, < 10% of the time).

Case History:

The allografted hand was slightly smaller than the hand which it replaced, however, surgical attachment was without major problems. On day 5, there was mild redness in the volar hairy skin of both the allograft as well as the host's tissues adjacent to the attachment site. There was considerable swelling in the hand but circulation was fine. By day 20, some of the swelling had subsided. The colour of the host's forearm had returned to normal. The allografted hairy skin of the volar aspect of the wrist was healthy, but maintained a light pink chafed appearance. The hair growth on the graft was less than that on the recipient's forearm. As time passed, the survival of the transplanted extremity was relatively uneventful. The swelling of the tissues reduced significantly by day 50 and evidence of muscular atrophy became apparent. Pinkness of the volar wrist soon faded and left little evidence of its occurrence. The hair growth on the hand was reasonable at day 70, but was still considerably less than that of the host's forearm. The darkly pigmented keratinized tissues of the glabrous palm had thickened to the point of cracking. Crevasses were formed along the major crease lines of the palm and the general trends in epidermal ridging patterns were defined by thin cracks. There was no gross evidence of any ongoing rejection processes until around day 140. At that point in time, there was a general fragility of the superficial epidermal layers in the

allografted hairy skin. The dorsal and lateral margins of the fingers, including the skin overlying the knuckles, were the areas most affected. A full sloughing of the tissues did not occur. Instead, the superficial layers underwent a process of shredding and shedding; revealing raw reddened tissues underneath. During the initial onset of rejection, hair loss seemed primarily constrained to the affected areas of the fingers. The dorsum of the hand was relatively unaffected. As well, the allografted glabrous tissues showed little or no outward sign of involvement. As time progressed towards day 170, the lesions caused by the initial rejection processes healed, however, the tissues did not remain stable. There was a further loss of hair on the dorsum of the hand and the keratinized layers of glabrous skin thinned. Of interest was the fact that these latter events occurred even though there was little or no overt undertones of redness in the tissues. Around day 186, rejection started once again. This time the glabrous tissues of the distal palm and fingers were the main (if not the only) site of rejection. Like in the previous episode, there was no sloughing of tissues. The thinned glabrous skin became fragile and opened as small reddened lesions. Within a few days the lesion sites healed and left behind shiny (but still pigmented) areas of tissue with little excess keratinization. By day 215, the allografted hand had stabilized. Both the glabrous and hairy tissues showed near normal pigmentation and texture. The hand appeared thin and elongated because of muscle atrophy. While splinted, there was little opportunity for useful physiotherapy. During the change of dressings on day 308, it was noticed that the distal tips of D2 and D3 were starting to become mummified. In confirmation of further ongoing rejection processes, the hairy skin overlying the distal joints of the first three digits were easily removed; revealing small patches of raw reddened tissues. Evaluation of nerve reinnervation was completed and the allograft was removed, 311 d after surgery.

Animal #9

HT: Transplantation of the total left hand; including wrist.

Donor: Animal #8; unknown source.

Allograft Survival: 26 d; underwent severe rejection.

Nerve Recording: Not possible due to insufficient time for mature reinnervation.

Mean Measured Weight During Study: 13.3 kg.

CsA Dosing: 33-45 mg/kg/d; most commonly *circa* 38 mg/kg/d (IM).

Mean Sampled Cs Blood Levels (RIA, serum 12 h trough): 932 ± 700 ng/ml; approximately 600 ng/ml at onset of primary rejection, most commonly *circa* 550 ng/ml.

MP Dosing: Postoperative: starting at day 4

125 mg/d for 3 d, tapered to a maintenance dose of about 8.8 mg/d by day 22.

Other Drugs Administered:

Acetylsalicylic acid, dimenhydrinate, netilmicin sulfate, Penlong S® (regular, > 50% of the time).

Levorphanol tartrate (intermittent, < 25% of the time).

Case History:

Immediately postoperative, the allografted hand showed reddened hairy skin on the volar and radial aspects of the wrist. The surgical closure was awkward and there may have been tension in the flaps of skin at the attachment site. On day 4, the hand was observed to be severely swollen. The host's hairy skin, just proximal to the attachment site, had pinkish-red tones, but for the most part, appeared healthy. The allografted hairy skin on the volar and radial aspect of the hand was far from healthy. The tissues were orangy-red in colour and covered with small fluid-filled vesicles. The glabrous skin bordering these tissues appeared unaffected. The hairy skin on the dorsum of the hand, although darker brown (normal pigment for the donor), had no obvious abnormalities. There were two blisters (one large, one small) in the web space between D1 and D2. These latter blisters, unlike those of the wrist, were more than likely pressure sores caused by increased tension in the bandages as a result of the swelling of the hand. By day 7, many of the vesicles on the volar aspect of the wrist had merged; giving the tissues of the volar wrist a translucent jelly-like appearance. As well, the colour of the tissues had turned more of an orangy-grey. The host's hairy skin adjacent to the allograft site was no different than that seen at day 4. There was still no apparent damage to the surface of the glabrous tissues or to the hairy skin on the dorsum of the hand. The large blisters between D1 and D2 increased in size to about 1 cm^2. From day 11 through to day 19, the area of hairy skin on the volar wrist dried and took on a reddened chafed appearance. The blisters in the first web space started to dry and collapse. Yet, the center of the palm and much of the dorsal hairy tissues began to develop red undertones. On day 23, the hand, still very swollen, did not (upon first visual inspection) appear to be any worse than on day 19. However, the true story on the state of the tissues was quite different. After a short period of soaking, roughly 85% of the keratinized glabrous epidermis was easily removed; leaving raw reddened tissues underneath. This sloughing of tissues extended circumferentially around the fingers. Only small patches of superficial epidermis could be easily removed from the hairy tissues on the dorsum of the hand or in the volar wrist area. There was only a loss of approximately 50% of the hair. By day 26, mummification of the digits had begun and euthanasia was performed.

Animal #11

TNVFF: Transplantation of the total soft tissue coverage of the right D2.

Donor: Animal #10; unknown source.

Allograft Survival: 413 d; underwent severe rejection.

Nerve Recording: During postoperative days 210 to 211.

Mean Measured Weight During Study: 10.6 kg.

CsA Dosing: 25-43 mg/kg/d; more commonly *circa* 28 mg/kg/d or 40 mg/kg/d (IM). Temporary regimens of IM, oral, SC or mixed routes of administration were also utilized.

Mean Sampled Cs Blood Levels (RIA, serum 12 h trough): 1342 ± 938 ng/ml; approximately 900 ng/ml at onset of primary rejection, more commonly *circa* 850 or 1900 ng/ml.

MP Dosing: Postoperative, starting at day 3

250 mg/d for 1 d, 125 mg/d for 2 subsequent days, tapered to a maintenance dose of about 4.4 mg/d by day 16.

Rejection; starting at day 122

125 mg/d for 3 days, followed by 1 d at 25.6 mg.

Rejection; starting at day 126

125 mg/d for 3 d, tapered slowly to a maintenance dose of about 4.4 mg/d by day 185.

Rejection; starting at day 228

125 mg/d for 3 d, tapered slowly to a maintenance dose of about 4.4 mg/d by day 285.

Other Drugs Administered:

Penlong S® (intermittent, < 25% of the time).

Acetylsalicylic acid, cimetidine, dimenhydrinate, levorphanol tartrate, netilmicin sulfate, prednisone, ticarcillin disodium (when needed, < 10% of the time).

Case History:

At the time of the first splint and dressings removal on postoperative day 3, there was considerable swelling of the TNVFF and minor bleeding along the palmar incision lines. Towards day 6, the hairy portion of the allograft showed pinkish-red undertones in comparison to the surrounding host tissues. Due to further swelling, much of the allografted tissues appeared to be under considerable tension (especially along the radial edge). By day 14, some of the swelling in the flap, as well as the redness of hairy skin, had subsided. The keratinized glabrous surface of the allograft took on a glossed leathery appearance. This trend towards thick keratinization of glabrous tissues continued for both the allograft as well as the

host tissues. In addition, the allografted hairy skin surface became more dry and wrinkled; giving the surface appearance of a sausage. By day 26, because of the thickened keratinized layers, there was very little epidermal ridging patterns seen on the surface of the host's palm. The keratinization of the allografted glabrous skin did not reach levels as marked as those for the host tissues. However, on day 33, a 'cap' of hardened dry keratinized epidermis could easily be peeled away from the radial edge of the tip of the finger. Healthy dry pink, unpigmented, nonridged tissues were revealed from underneath; an area of skin measuring approximately 1 cm^2. The reason for this event was unknown, but may have been related to the reddened tissues observed around day 6. During days 63 through 69, the dark brown keratinized superficial tissues of the allograft's glabrous skin cracked and was able to be picked off the finger surface like pieces of a jig-saw puzzle. The underlying tissues, although healthy and lightly pigmented, had pink undertones. The finger was slender and still considered healthy. The area on the tip of the finger was still pink and relatively unchanged since day 33. From this point onward, towards day 104, there was a progressive and generalized thinning of the keratinized layer of allografted glabrous epidermis, while the keratinized of the host's glabrous tissues was still very thick. The whole flap had very light pink undertones, but showed no lesions. Allografted hairy skin supported luxuriant hair growth. On day 106, the glabrous surface was about 50% light pink instead of light brown, and there was slight surface fragility and bleeding where gauze bandages stuck to the surface. This thinning of glabrous tissue continued further until day 118, when although there were still no lesions or major sloughing of skin, the graft was now essentially 70% pink in colour and had a dry glossy appearance. The sheen was due to the fact that there was little if any glabrous epidermal ridging patterns. Gross rejection, as determined by breakage of the glabrous epidermal surface in areas which were either raw or fragile, started around day 122 During the early stages, hairy skin was spared. It was interesting to note that with immunosuppressive intervention, at any one time, only about 25% of the glabrous skin surface was raw. The affected area changed every couple of days. Some raw areas would heal and return to the pink condition, while other areas became raw. By day 128, much of the hair shafts had fallen out and there were minor lesions in hairy skin. Towards day 134, most of the active rejection processes had subsided, although there were still a few punctate lesions. Approximately 80% of the allografted glabrous skin surface was at some point in time actively undergoing processes of rejection. At day 160, the allograft was stable. Most of the volar surface was now pink in colour. The remainder had dark brown (almost black) pigmentation. Almost all of the hair was absent from the flap. Areas of hairy skin on the ulnar and radial curvatures of the flap were unpigmented, while the dorsum of the flap was darkly pigmented like that of the glabrous skin surfaces described above The texture of both hairy and glabrous skin was smooth and tough; much like its

condition at day 118. The finger was slender, but the digital palmar pad for D2 was bulbous The allograft remained stable in this condition throughout the period of nerve recording and onwards towards day 228; when similar rejection processes started again. At that time, the fragile areas were much smaller and more diffuse. By day 245, the areas of dark pigmentation had faded. The finger gradually lost much of its slender form, to the point that only joint creases remained easily identifiable. On day 277, the glabrous skin surface was no longer smooth, and was very fragile. Thin white flakes of dried keratinized epidermis encrusted the surface of allografted glabrous tissues. The hairy skin of the TNVFF became milky-white in appearance and had very little form. The flap survived in this condition, with periodic mild episodes of surface breakdown, until day 413: when it was removed.

Animal #12

HT: Transplantation of the total right hand; including wrist.

Donor: Animal #13; unknown source.

Allograft Survival: 71 d; underwent severe rejection.

Nerve Recording: Not possible due to insufficient time for mature reinnervation.

Mean Measured Weight During Study: 11.7 kg.

CsA Dosing: 17-51 mg/kg/d (highest doses were given during perioperative days); most commonly *circa* 17 mg/kg/d because of the animal's poor clinical tolerance to the medications administered. Temporary regimens of IM, SC or mixed routes of administration were also utilized.

Mean Sampled Cs Blood Levels (RIA, serum 12 h trough): 1260 ± 778 ng/ml; approximately 900 ng/ml at onset of primary rejection, most commonly *circa* 1050 ng/ml.

MP Dosing: Postoperatively; starting at day 0

125 mg/d for 3 d, tapered to a maintenance dose of about 4.4. mg/d by day 24.

Rejection; starting at day 55

250 mg/d for 1 d, 125 mg/d for 2 subsequent days, tapered to a maintenance dose of about 8.8 mg/d by day 64.

Other Drugs Administered:

Netilmicin sulfate, Penlong S® (regular. > 50% of the time).

Acetylsalicylic acid, dimenhydrinate (frequent, < 50% of the time).

Levorphanol tartrate, prednisone (when needed, < 10% of the time).