THE RELEVANCE OF PRELIMINARY DENERVATION TO THE FREE TRANSPLANTATION OF SKELETAL MUSCLE

A Thesis

by

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SUMMARY

A brief summary on embryology, histology, biochemistry and electrophysiology of the skeletal muscle is first presented. This is followed by a hundred year historical survey on muscle transplantation. The last part of the thesis is about "the relevance of preliminary denervation to the free transplantation of skeletal muscle."

In order to clarify this point the author performed free transplantation of skeletal muscle following different periods of preliminary denervation. From macroscopic, electrophysiologic and microscopic observations, the following statements were made:

1. A transplanted muscle may lose from 0 to 100% of its weight. If muscle is transplanted 6 weeks after denervation this weight loss is decreased.
2. A transplanted muscle is more sensitive to electrical stimulation when it has been denervated for 5, 6 or 7 weeks before transplantation.
3. Preliminary denervation of a transplanted muscle increases slightly the force of the graft.
4. Five to seven weeks of denervation is the ideal length of time that should precede a muscle transplantation.
5. The preliminary denervation does not significantly improve the ability of the muscle to perform work.

Résumé

Un bref résumé d'embryologie, d'histologie, de biochemie et d'électrophysiologie du muscle squelétique est d'abord présenté. Ceci est suivi d'une revue de la littérature se rapportant au muscle squelétique et couvrant une période d'environ cent ans. La dernière partie de la thèse se rapporte à "l'utilité de la dénervation préliminaire dans les cas de transplantation du muscle squelétique".

Afin de clarifier ce point l'auteur a effectué une série de transplantations musculaires. Les muscles transplantés furent préablement dénervés durant des périodes variant de 0 à 7 semaines. Se basant sur les résultats interprétés à l'aide de moyens macroscopiques, microscopiques et électrophysiologiques les conclusions suivantes furent faites:

1. Un muscle transplanté peut perdre de 0 à 100% de son poids. Si le muscle est transplanté 6 semaines après avoir été dénervé la perte de poids est relativement inférieure.
2. Un muscle transplanté est plus sensible aux stimulations électriques quand il a été dénervé durant 5, 6 ou 7 semaines avant d'avoir été transplanté.
3. La dénervation préliminaire d'un muscle transplanté est un moyen d'augmenter légèrement la force de ce muscle une fois greffé.
4. Cinq à sept semaines de dénervation est la période de temps idéal de dénervation qui devrait précéder une transplantation musculaire.

5. La dénervation préliminaire n'améliore pas de façon significative la capacité d'un muscle à fournir un travail.

TABLE OF CONTENTS

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INTRODUCTION

Since Zielonko performed the first transplantation of skeletal muscle in 1874, it has been used in a wide variety of clinical conditions. Muscle has been used to fill cavities such as lung abscesses (Prioleau, 1946), tuberculosis cavities (Coryllos - Ornstein, 1938), bone cavities following surgery for osteomyelitis (Netalon, 1909; Priggs, 1946; Thietmeyer, 1950) and for mastoiditis (Kisch, 1932). It has been used to repair thoracic defects (Robson, 1911; Ramsay, 1963), abdominal wounds (Lesnick, 1953) and for large wounds with exposed bones (Howard, 1871). Muscle flaps have been brought into contact with the heart in coronary heart disease to provide a more adequate heart circulation (Beck, 1935), and it was also used to seal a perforating wound of the heart (Läwen, 1912) and to cover a defect of the pericardium (Robson, 1911). It has been used to close bronchial fistulas (Abrashanoff, 1900; Pool, 1929; Wangensteen, 1929; Garloch, 1934; Carter, 1938; Crafoord, 1940; Mallah, 1948) and to seal other muscle defects (Helferich, 1883). Muscle has been applied to the repair of tracheal and oesaphageal defects (Demers, 1971) and to obtain velopharyngeal competency after cleft palate repair (Chul Song, 1974).

It was successfully used for hemostatic purposes (Unger, 1910; Kocher, 1912; McNealy, 1937; Countryman, 1942) and to relieve lymphedema (Reinhoff, 1937; Treves, 1952).

The use of muscle in reanimation of paralysed areas has been widely reported. Plastic and orthopedic surgeons have

used muscle to improve facial paralysis (Lexer, 1967; Gersuny, 1906; Dawson, 1909; Eden, 1911; Sheehan, 1935; Ashley, 1968; Thompson, 1971; Hakelius, 1974) and for paralyzed extremities (Goldwait, 1895; Deutschlander, 1909; Goebel, 1912; Harmon, 1949-1950; Lacheretz, 1962; Yoshikazu, 1976). Muscle is included in many flaps used for reconstruction such as in nasal reconstruction (Schloffer, 1919).

Other surgeons have used it to obtain sphincter action around the anus (Pickrell, 1954; de Carvalho Pinto, 1955); others have used muscle in an attempt to relieve urinary incontinence (Griffiths, 1968), to correct strabismus and even to repair orbital floor defects (Reese, 1961; Deitsch, 1964).

Despite all the interest that we have had for this tissue since 1874, Neuhof stated in 1923: "The free transplantation of muscle is futile, degeneration and fibrosis of the graft being the end result", and in 1959, following an excellent review, Peer stated: "Muscle cells in free muscle grafts invariably die, are replaced by fibrous tissue and fail to resume their normal function".

However, it was not until a century later that another Russian, Zhenevskaya (1965), reported successful free autogenous muscle transplantation. This was closely followed by Tamai's (1970) first successful transplantation of a completely isolated muscle, using microvascular anastomoses, and by Thompson (1971) who confirmed that free autogenous transplants of skeletal muscle was possible. One may wonder what happened between 1874 and the 1970's that made this

possible?

The major contributions to this success, according to Thompson, have come from the application of three fundamental postulates.

1st postulate, as pointed out by Peer in 1955, states that "muscle must be transplanted as a complete anatomical entity, by transferring the entire muscle belly intact, after subperiosteal stripping of its origin and insertion. This is because muscle fibres are of exceptional length and not infrequently the entire length of the muscle belly, and it is illogical to attempt the transfer of fragments of cells in the transplantation of free tissue grafts".

2nd postulate, as pointed out by Thompson, states that "the free muscle graft must be applied in direct contact with normal innervated skeletal muscle, at the recipient site, so that "muscular neurotization" of the transplant by the ingrowth of axons from the normal host muscle can occur. The outgrowing motor axons spread most rapidly where they enter the endoneurial tubes of the degenerated nerves in the transplant but can also spread along denervated muscle fibres which they can then penetrate to form motor end plates".

3rd postulate, as pointed out by Thompson, states that "the muscle must have been denervated two weeks prior to transfer, in order to increase its vascularity and to establish an economical metabolic level enabling the transplant to survive the initial days of ischaemia and total anoxia following transplantation, while direct vascular anastomoses are established between graft and host at the recipient site".

It is on this last postulate that we have based our research. We have tried to determine the relevance of preliminary denervation to free transplantation of skeletal muscle, and to study the optimal interval between denervation and subsequent grafting.

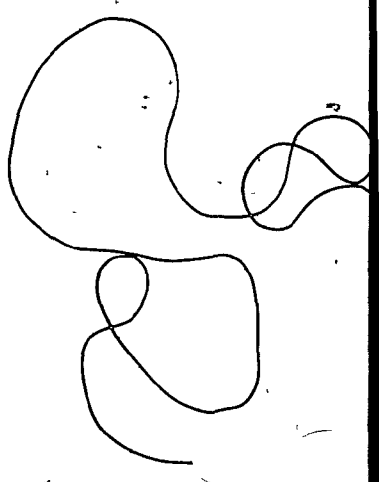
The first part of this paper gives general information about muscle. This constitutes the basic knowledge that one should have in order to work with muscle tissue. It is a brief outline of the embryology, histology, biochemistry and electrophysiology that is necessary in any muscle experiment.

The second part summarizes the large number of articles in literature related to skeletal muscle transplantation.

The third through the sixth segments of the thesis cover preliminary denervation leading to free transplantation of skeletal muscle. The results obtained with the histological, electrophysiological and functional assessment of these free grafts are summarized and the contribution to original medical knowledge is included in this Summary.

Chapter I

THE SKELETAL MUSCLE



A. Embryology

The muscular system develops almost entirely from the mesoderm (Langman, 1975; Moore, 1973). This mesodermal layer lies on each side of the midline or notochord. At 17 days the cells are close to the midline and proliferate forming the paraxial mesoderm. This thickening of the mesodermal layer now lying on each side of the neural tube will divide at 21 days into segments of epithelioid cells, called the somites. The epithelioid cells of the somites proliferate and at 28 days they lose their epithelial shape and start their processes of differentiation.

Each somite located on each side of the notochord will form the sclerotome. The sclerotome is the agglomeration of the mesenchymal cells that will give the fibroblasts, the chondroblasts, and the osteoblasts cells. The remaining somite cells are now called dermatomes, which give rise to a layer of cells that fail to divide and are called the myotomes. The dermatomes left will form the dermis and subcutaneous tissue of the skin. The myotome cells have a spindle shape and are called myoblasts. The myoblasts fuse to form the multinucleated muscle fibres. This layer of myoblast cells now extends toward the coelomic cavity and divides into two portions known as the epimere dorsally and the hypomere ventrally. These portions are innervated by the dorsal primary rami for the epimere, and ventral primary rami for the hypomere. Both segments will then divide in multiple layers to form the various muscles of the body. The total skeletal musculature of the body is derived from the somites, the

branchial arches, and the somatic mesoderm that undergo similar metamorphoses.

Mesoderm layer

↓

Paraxial mesoderm

↓

Somite

↙ ↓ ↘

Sclerotome	Myotome	Dermatome
1. Fibroblast	1. Myoblast	1. Dermis
2. Chondroblast	↓	↓
3. Osteoblast		
↓		
a. Reticular fibres	a. Muscle	a. Dermis
Collagen fibres		
Elastic fibres		
b. Cartilage		
c. Bone		

B. Histology-Ultrastructure

The electron microscope has introduced a new dimension in the exploration of the muscle cell. The skeletal muscle ultrastructure can now be described with more precision.

I. The Muscle Cell

1. The Sarcolemma Membrane: In order to describe the cell organelles and inclusions present in the sarcoplasm, we have to look through a very thin membrane. This membrane is formed by two layers, the plasma membrane and the basement membrane. The latter is thicker and stronger than the plasma membrane and, according to Pease (1969), helps "to maintain the shape and stability of individual cells". This membrane, in the same way as the endoneurium in the nerve cell, forms the tube that guides the growing fibres in regenerating skeletal muscle (Allbrook, 1962). While the basement membrane seems to have a structural function, the plasma membrane has a physiologic one. This membrane is involved in the excitation process by conducting the electrical stimulus from the nerve to the myofibrils through the T-tube system.

2. The Sarcoplasmic Structures: These structures are dispersed in an aqueous fluid containing soluble proteins (myogen and myoglobin), glycogen particles, lipid and lipofuscin droplets (Price, 1969). Among the sarcoplasmic structures are:

a. The nucleus enclosed in its porous envelope, chromatin material, chromatin granules and nucleoli. These nuclei are

located in the periphery of the cell and their numbers are proportional to the size of the cells as demonstrated by Moss in 1968.

b. The mitochondria or sarcosome: The first name came from their thread-like appearance (mitos - thread). Those organelles present in different parts of the muscle cell are more numerous in the red fibres. The mitochondria contains the enzymes of oxidative phosphorylation, the cytochrome, the deshydrogenase and other enzymes which catalyse the reaction of the aerobic metabolism for the production of ATP.

c. The ribosome: These are small particles that are free in the cytoplasm or attached to the membrane of the reticulum and are usually grouped in clusters called polyribosomes and linked together by mRNA. The function of the ribosomes is to synthesize the proteins of the sarcoplasm (Ham, 1969; Anderson, 1967).

d. Golgi complex: discovered by Camillo Golgi in 1898, has the function of concentration and secretion of the granules containing proteins and carbohydrates.

e. Sarcoplasmic reticulum and transverse tubules: These two systems of tubules and vesicles are closely related but independent. The first one forms a complex tubular network, surrounding the myofibrils without evidence of connection to the extracellular system. The transverse tubule is in close relation to the sarcolemma membrane, and to the terminal cisternae of the sarcoplasmic reticulum. The wave of depolarization passes from the fibre surface to the terminal cisternae of the sarcoplasmic reticulum through the transverse

tubule (Ham, 1970).

f. Satellite cells: In 1961 Mauro described these cells located between the two layers of the carcolemma membrane. The same author states that these cells might be involved in the regeneration of new muscle cells. Allbrook (1975) states that the nuclei of these cells are able to undergo mitosis to increase the number of available muscle nuclei. These satellite cells account for 30-35% of all muscle nuclei in the newborn and fall to 5% in the adult. They are present in the glycocalyx and reserved for regeneration under certain conditions. The mechanism of regeneration is still controversial, but we know that muscle regeneration occurs.

g. Myofilaments: These are the most characteristic structure of skeletal muscle. Formed of actin, myosin and tropomyosin protein, they form the myofibril which is the basic contractile material. The myofilaments of actin and myosin interdigitate to form the well known sarcomeres present in a myofibril. Each sarcomere is divided into the socalled A, M, H, I and Z bands. The Z bands correspond to the limits between two sarcomeres -

The anisocoric A bands are formed of parallel filaments of myosin with a transverse density in the middle recognized as the M line. The H bands correspond to the space between two actin filaments of the same sarcomere, and the isocoric I band is the space representing the thin actin filaments that are situated on each side of the sarcomere and which are not superposed on the myosin A band.

II. The Connective Tissue Framework

Each of the muscle fibres described above is surrounded by a thin layer of connective tissue, called endomysium. This connective tissue stroma is in continuity with the perimysium, which surrounds a group of cells. All these groups of muscle fibres are finally surrounded by the most external layer of connective tissue, called epimysium. This layer is continuous with the fascia, tendon, periosteum or other structure, to which the muscle is attached. These three layers of connective tissue contain branches of nerves and blood vessels. According to Peer (1955), "the fibroblast cell in the stroma of free autogenous muscle graft survives the transplantation and it is active in the replacement of degenerating muscle cells in the graft."

III. Veins and Lymphatics

The arteries extend their branches through the epimysium and perimysium toward the endomysium which surrounds the muscle cell. Here they form a rich capillary pattern that is able, because of its tortuosity, to accommodate to the different lengths of extension or contraction (Bloom, Fawcett, 1975). The capillary wall is in close contact with the sarcolemma in order to permit interchange of nutrients.

The veins follow the same pathway as the arteries. The lymphatics in skeletal muscle are not as prominent as the lymphatics in cardiac muscle. Different opinions concerning the lymphatics still persist. They may lie between muscle and the muscle group (Peer, 1955) or they may be confined to

the thicker connective components (Ham, 1955) while Maximow (1948) asserts that they lie in the perimysium.

IV. The Red and White Fibres

Muscle fibres differ in color, size, enzymes and protein contents. They also differ in their ultrastructure and function (Padykula-Gauthier, 1966; Price, 1969; Ham, 1969; Bloom & Fawcett, 1975). The red fibres or type I fibres are characterized by the abundance of mitochondria and its associated enzymes. They have a low phosphorylase activity, a rich blood supply, a small diameter and are rich in myoglobin and fat droplets. The muscles with a predominance of red fibres are the tonic or slow type of muscle and they are involved in a long activity and in posture, where slow and prolonged contraction are necessary.

The white fibres or type II fibres are characterized by fewer numbers of mitochondria, by their different enzyme profile, and their larger size. Their myoglobin and fat droplets content is low. The muscles with a predominance of white fibres are the phasic or fast type of muscle and are involved in short and fast activity.

There is a third group of muscle fibres with intermediate features. Skeletal muscle is composed of a mixture of the three types of fibres, but one type usually predominates, depending on its functional activity.

C. Biochemistry

In 1874 Ranvier noted the difference between the red and white muscle fibres. The former is characterized by a higher quantity of myoglobin, and a high level of mitochondria and oxydative enzyme (Slater, 1960). The red muscle fibres have an aerobic metabolism, while the white muscle fibres have an anaerobic one. This explains the higher content of phosphorylase enzyme in white muscle (Dubovitz, 1960; Pearse, 1960).

In the white fibres - the anaerobic metabolism implicates the stored glycogen which is broken down to form lactic acid by anaerobic glycolysis. This is the most current pathway followed by glycogen in fast contracting muscle where sudden demands of energy may arise. The lactic acid formed by glycolysis may be broken down in water and CO_2 with the participation of oxygen or it might be resynthesized in glycogen by the liver (Cori, 1941). The enzymes involved in the anaerobic glycolysis are mainly the phosphorylase, aldolase, enolase and deshydrogenase (White-Handler-Smith, 1968; Kleiner & Orton, 1966). The phosphorylase, as shown by Corini (1956), is activated during muscle activity and is inactive in fatigued muscle. The glycolysis of glycogen in order to obtain 3ATP (high energy phosphate) implicates also ions such as magnesium, potassium and zinc.

In the red fibres - the mitochondria are present in greater quantity. These complex structures contain the enzyme involved in the oxydative pathway of pyruvate which is a product of the anaerobic glycolysis. The pyruvate is broken down in the

Krebs cycle in a complex sequence reaction. The fatty acid oxydation which is also an important source of energy in muscle is in interrelation with the citric acid cycle or Krebs cycle. Acetyl CoA product of the fatty acid cycle is entering the Krebs cycle in the same way as acetyl CoA formed from the oxydation of pyruvic acid is doing it.

The muscle proteins: As mentioned by Gergely (1969), "the proteins in muscle are in a dynamic state of equilibrium." Equilibrium between synthesis and catabolism, equilibrium between hypertrophy and atrophy.

Protein plays an important role in muscle, it constitutes 75 to 80% of the dry weight muscle. Since the work of Hoagland in 1958 on enzymatic reaction between amino acids and ribonucleic acids as intermediate steps in protein synthesis, a great number of details concerning the role of RNA, ribosomes and DNA in protein synthesis are now known. However the process of biosynthesis of muscle protein is still not clear.

The protein of the myofibrils: Three types of protein are involved in the contraction of muscle, they are the actin, myosin and tropomyosin. The first two constitute distinct filaments that are present in the myofibrils.

Myosin - is a high molecular weight protein with ATPase activity, forming filaments where 25 to 30 molecules of myosin are present. The ATPase activity of this protein is influenced by pH, ionic strength, K, NH_4, Ca, Mg. While Ca^{+} has a stimulating effect on ATPase, Mg^{++} has an inhibiting one. The presence of SH group plays also an important role in the ATPase activity.

D. The Motor Unit: Anatomy - Electrophysiology

Anatomy

"The motor unit consists of: a single motoneuron, its axon, and the group of muscle fibres innervated by this single axon," (Woodbury et al., 1960). It was the work of Sherrington, Liddell, Eccles, Creed and Denny Brown (Sissons, 1969), that introduced the physiological concept of the motor unit.

Located in the anterior horn of the spinal cord, the body of the motoneuron formed by a large cell, extends its large myelinated motor nerve fibre toward the muscle fibres. After penetrating the muscle fibres (3 to 150 according to Woodbury), the nerve ramifies in numerous smaller nerve fibres at the level of a node of Ranvier.

The terminal ramification ends on a single motor end plate located in the muscle fibres. At this myoneural function, the terminal axon loses its myelin sheath a few microns from the end plates. The endomysium of the muscle fibres is in continuity with the schwan cells of the penetrating nerve.

These nerves end in synaptic gutters which are depressions in the muscle fibres. These depressions are formed by a series of infoldings called the junctional folds. The folds (Price, 1969) contain in their sarcoplasm: small vesicles, fibronucleoprotein particles, mitochondria and glycogen granules. The terminal axon lying in the junctional fold contains mitochondria and synaptic vesicles which may possibly contain acetylcholine. High levels of cholinesterase are found in the "subneural apparatus" of the end plate.

Electrophysiology

The depolarization of the motor neuron spreads down to the terminal ramification provoking liberation of minute amounts of acetylcholine. This chemical substance, a methylated quaternary ammonium salt, diffuses in the microscopic space that separates the nerve and the end plate of the muscle. Acetyl choline in the end plate combines with a "receptor", that will provoke depolarization, the depolarization being the result of an increase permeability of the end plate membrane to Na^+ and K^+. This depolarization, also called "end plate potential" or "synaptic potential", spreads passively along each side of the muscle membrane at a velocity of 3 to 5 meters per second (Buller, 1969; Woodbury, 1969). A. choline, which is secreted in higher quantity in the presence of Ca^+, while Mg^+ antagonises its liberation, will be rapidly destroyed by A. cholinesterase. The mechanism of propagation of the muscle action potential from the membrane toward the myofilament is still obscure. However it has been suggested that the transverse tubule and the sarcoplasmic reticulum are the pathway followed by the action potential to reach the contractile substance.

In the sarcoplasmic reticulum the depolarization will cause the release of calcium which is involved in the control of the contractile activity. The mechanism of contraction is still speculative. According to Huxley (1960), the sliding filament theory can be explained by the formation of bridges between the filaments of actin and myosin. The positive and negative interactions of ATP, Ca^{++}, ATPase and Mg are the

bases of the sliding filament theory. Although a large amount of information is available now for the explanation of the excitation-contraction coupling, these theories are still conjectural.

Chapter II

HISTORICAL SURVEY OF THE LITERATURE RELATED TO SKELETAL MUSCLE TRANSPLANTATION 1864-1977

This historical survey starts in 1864 when Zenker reported one of the first observations on muscle regeneration, and ends up with the most recent free muscle transplant done with microvascular anastomose. This extensive review has been divided into four parts:

Part I : review of the basic studies done on muscle anatomy and physiology

Part II : review of the publications on muscle pedicle flap

Part III : review of the publications on autologous muscle transplantation

Part IV : review of the publications on homologous muscle transplantation

Part I

Muscle Anatomy and Physiology

1864 Zenker: The author reported muscle fibre regeneration by nuclear division in patients with typhoid fever.

1873 Ranvier: He described the white and red muscle from a physiological and structural point of view. Differences in the speed of contraction and decontraction of these two muscles are explained. He also described the difference in striations and in the number of nuclei in the white and red muscle.

1908 Schminke: He observed new formation of muscle fibres from elements of old fibres in the

transverse muscle of ichthyosida and sauropsida.

1909 Dawson: The author worked with rat and rabbit muscle. Following injury to specific muscle he observed the process of regeneration and described the "muscle cell tube" formation and the "budding" phenomenon.

1914 Heineke: He did the first demonstration of direct neurotization. He implanted a peripheral motor nerve into a paralyzed muscle. This resulted in the restoration of muscular contraction on Faradic stimulation of the implanted nerve.

1916 Steindler: He did further studies on the question of direct neurotization. He possibly found regeneration after implantation of motor nerves into paralyzed muscles.

1926 Forbus: After damaging rabbit and rat muscle with injection of irritant solutions, the author observed the process of regeneration. He stated that regenerating cell originated from nuclei and the sarcoplasm of preserved muscle fibres.

1934 Von Seeman: He performed denervation of the rectus abdominis in the rabbit and concluded from his observations that the muscle was replaced by fatty and fibrotic tissue

and that 3 years after surgery no neurotization could be observed.

1934 Tiegs: He described the "helicoidal" arrangement of the muscle fibres.

1934 Millar: This author damaged the rectus femoris muscle of the rabbit and observed the process of regeneration. He stated that the damaged end of normal cells were the source of regeneration of normal cells.

1936 Schminke: He studied the regenerative process of damaged muscle cell. He stated that most of the fibres originate from the formation of terminal buds.

1937 Reinhoff: He used pedicled muscle flaps of latissimus dorsi to relief lymphedema of the arm, following radical mastectomy.

1939 Chevremont: He did in vitro observations using tissue cultures of chicken embryo muscle tissue. He described the bud formation from old muscle fibres.

1941 Chouke: Wound healing by proliferation of the different layers of connective tissue was observed in damaged striated muscle.

1942 Naffziger: He performed denervation of latissimus dorsi muscle and reanastomosed it in a second stage. Degeneration of nerve endings and muscle fibres were observed and no frank regeneration was present

after anastomosis of the nerve.

1942 Gutmann: After denervating rabbit muscle, galvanic stimulation was used to prevent atrophy, using this technique atrophy could not be avoided but it was delayed for a few weeks only. The final result after reinnervation was not better in rabbits which had Galvanic stimulation.

1944 Muirhead: The author produced shock in the dog by injecting intraperitoneally a segment of frozen dessicated autogenous muscle. He stated that the muscle contains a substance capable of inducing shock.

1944 Bowden: He described biopsy of long term denervated muscle. Twenty-six years after denervation the muscle tissue was still identifiable.

1944 Hines: He denervated cat and rat muscle and did comparative studies between groups kept inactive and groups with Galvanic stimulation and exercise. He concluded that following peripheral nerve injury, best results were obtained in active muscle.

1944 Gutmann: The author studied muscle reinnervation in rabbits. He observed growth of axons in nerve tubes at a rate of 4.4 mm per day when the nerve was crushed.

Reinnervation of old end plates and new end plate formation was observed. In cases when epineurium and peuneurium were cut, the wrong connection prejudiced the reinnervation.

1945 Milch: He stated on the inaccuracy of ergographic methods to measure muscle strength, and concluded that no amount of objective measurement of work capacity could be substituted for subjective estimate of surgeons.

1945 Levander: He injected alcohol into muscle and observed new muscle growth. From his observations he concluded that regeneration of muscle tissue was a repetition of the embryonal development.

1946 Pogogeff: Amitotic multiplication of rat and human muscle fibres were observed in vitro. Different forms of cells, spontaneous contraction without constant presence of striations were also described.

1950 Sanders: Nerve homografts were transplanted into rabbit muscle. After Schwann cells degenerated the empty Schwann tubes were invaded by regenerating muscle fibres. These tubes acted as endomysial tubes. In autografts the presence of Schwann cells in the tube prevented muscle growth

into it.

1962 Zhenevskaya: The author studied the regeneration process of transplanted skeletal muscle in situations where different types of trauma were inflicted to the muscle prior to transfer. According to Zhenevskaya such traumatized muscle reached a "plastic state" during which the regenerative capacity of muscle tissue was pronounced. This "plastic state" was obtained by mincing the muscle by mechanical trauma, immobilization, tenotomy or denervation. During this "plastic state" the regenerative process increased, the oxygen uptake of the muscle dropped, there was an increased resistance of the muscle to radiation.

1965 Romanul-Hogan: Using denervated gastrocnemius, plantaris and soleus muscle in rats the authors studied the enzyme activity at different periods of denervation (1 to 8 weeks). The differences between the energy metabolism of the individual skeletal muscle fibres corresponded to differences between their characteristics of contraction and innervation. After studying the following enzymes: phosphorylase, esterase, cytochrome, oxydase, dehydrogenase and phosphatase,

they found that following denervation the metabolic differences between the various types of muscles were no longer maintained. Following denervation there was marked decrease activity of enzymes which had been normally high. The fibres with high glycolytic capacity lost the activity of glycolytic enzymes more rapidly and the fibres with high lipid and oxidative metabolism lost the activity of lipid and oxidative enzymes faster. Two months after denervation the activity of all enzymes was very low and little or no difference was present between the enzymic activity of the various types of fibres.

1967 Drachman: In order to investigate the trophic action of acetyl choline the author used injection of the botulinum toxine (long lasting inhibitor of A. choline) and curare to denervate the skeletal muscle of the chick embryo. He also performed surgical denervation in the chick by teasing out the spinal cord at the lumbosacral region. In both situations the skeletal muscle showed degenerative and atrophic change after 7 to 12 days of treatment. Tenotomy (of the muscle

in the chick embryo) that conducts to disuse of muscle did not produce the same degree of atrophy and muscle change as seen with surgical or pharmacological denervation. The author concluded that acetylcholine must be the substance which transmits the trophic influence from motor nerves to muscle.

1967 Romanul: The author used the soleus, flexor digitorum longus and flexor hallucis longus in the cat and the rat to show the effect of cross innervation and reinnervation on this fast and slow contracting muscle. He demonstrated in his previous work (Romanul-Hogan, 1964) that the energy metabolism of the muscle fibres was dependent upon the nerve supply. In this study by cross innervating the slow and fast muscle they proved that the energy metabolism was also determined by the nerve supply and not only dependent upon it. They used histochemical and physiological smears for their demonstration. The fast contracting muscle fibres contracted slowly after being innervated by the nerve of a slow contracting muscle and the high glycolytic enzymatic profile

specific to the fast contracting muscle changed toward low glycolytic and high oxidative enzymatic profile. Reverse change was found in slow contracting muscle.

PART II

Muscle as a Pedicle Flap

1867 Lexer: The masseter muscle was used to reanimate a paralyzed mouth.

1895 Goldwait: In patients with paralysis involving the thigh muscles except the sartorius, this muscle was transplanted as a muscle flap to the quadriceps extensor. Three of his 5 cases were successful in terms of walking and standing.

1897 Rydygier: He experimented with pedicled muscle transplantation in two dogs and based on histological preparation he stated that the results were successful.

1900 Abrashanoff: He was the pioneer in the use of pedicled muscle flaps in bronchial fistula.

1906 Hildebrandt: Unsuccessful transplantation of skeletal muscle with intact nerve supply was performed, however the author emphasized

the importance of intact nerve supply in muscle transplantation.

1909 Deutschlander: He successfully replaced a paralyzed gluteus medius and minimus by a gluteus maximus muscle flap in a paralyzed child.

1909 Dawson: He used a masseter muscle flap to reanimate a paralyzed mouth.

1911 Eden: He used a masseter muscle flap to reanimate a paralyzed mouth.

1911 Robson: Pectoral flap muscle was used to cover a pericardial wall defect following surgery for cancer of the thoracic cage.

1915 Erlacher: He obtained successful muscular neurotization of a paralyzed muscle in guinea pigs by application of a healthy pedicle flap. New end plate formation was observed as well as nerve growth in older pathways.

1919 Schloffer: This surgeon used a composite pedicle flap, including skin and muscle for a nose reconstruction.

1922 Wullstein: He gave the following indications for muscle flap: paralysis after apoplexy, multiple sclerosis, spinal paralysis, spastic paralysis, progressive muscle atrophy, traumatic changes, congenital deformities of the foot, scoliosis, ischemic contracture.

1925 Wangensteen: He used intercostal muscle to plug a bronchopleural fistula.

1929 Pool: Pedunculated muscle flap was used to obstruct a bronchial fistula. The muscle was viable and not completely replaced by fibrous tissue. Clinically this method was used with success.

1932 Kisch: The author successfully used muscle flaps following mastoidectomy in order to fill cavities. He also used muscle flaps for hemostatic purpose.

1934 Garlock: Pedicled muscle flap from the chest was successfully used to repair bronchial fistula.

1935 Beck: He used a flap of pectoral muscle, which he inserted on the heart to improve the circulation of the myocardium.

1935 Scheehan: He used temporal muscle from the unparalyzed side of the face to reanimate the opposite paralyzed side.

1937 McNealy: Successful repair of an arterial wall defect was obtained with the use of pedicled muscle graft. Hemostasis and healing was good.

1938 Carter: Multiple flaps of trapezius, latissimus dorsi, sacrospinalis and intercostal muscles were used to seal chest cavity and bronchial fistulas after surgery.

1938 Coryllos: Tuberculosis cavities were filled with paravertebral pedicled muscle flap. The muscle was replaced by fibrous tissue and the therapeutic effect had a 50% success rate.

1940 Craafoord: Pedicled muscle grafts of latissimus dorsi were successfully used to seal bronchial fistula which resulted from pulmonary abscess.

1940 Sperry: Using rats the author transposed the insertion of autogenous pedicled grafts of tibialis anterior and extensor digitorum longus into the achilles tendon. In some cases the foot movement was successfully restored, but adequate reeducation was not feasible.

1942 Countryman: In order to obtain hemostases in liver wounds, free grafts of rectus femoris were successfully used for tamponade.

1950 Thiemeyer: He used pedicled muscle flaps to fill bone defects in chronic osteomyelitis. Three cases are described.

1953 Lesnick: The author reported a case where he used the external oblique muscle and aponeurosis as a pedicled flap to repair a low abdominal wall defect. Details on this surgical procedure is given.

1962 Lacheretz: The author performed transplantation of

latissimus dorsi and teres major in 28 cases of obstetrical palsy. The results are considered good.

1963 Ramsay: The author described a method of transplanting the rectus abdominis muscle on each side of a pectus carinatum in order to fill the parasternal depression. Procedure was successful.

1966 Termet: The author transplanted the latissimus dorsi muscle, keeping neurovascular bundles intact, around the heart in a series of dogs. He observed by stimulating the transplanted muscle with a peace-maker that latissimus dorsi could massage the heart ventricule in order to obtain a blood pressure of 80 mm Hg for 15 to 20 minutes. In two of seven cases muscle underwent fibrosis degeneration. The others survived and no adhesion was present between host and grafts.

1968 Ashley: He described a one-stage procedure to reanimate a facial paralysis, this procedure being a composite of several procedures, including those of Lexer (1908), Gellies (1934), Bunnel (1937) and Ragnell (1958). In his technique masseter and temporalis muscles were used. Surgery was performed in 17 cases,

and the results were from satisfactory to excellent.

PART III

Free Autologous Muscle Transplant

1871 Howard: The author used autogenous biceps muscle to cover a large would of exposed bone in a leg. Skin was applied over the successful healing graft.

1874 Zielonko: This Russian pathologist working in France did the first transplantation of striated muscle. He transferred free muscle graft into the lymph sac of a frog. This was followed by rapid necroses.

1881 Gluck: He performed autogenous free grafts of pieces of skeletal muscle into defects in hen musculature. This was followed by successful healing and regeneration.

1891 Askanazy: He transplanted muscle into the brain of animals. Regeneration was noted after 1 week. Atrophy started after 3 weeks, and after 2 months only a few fragments of muscle showed fibrillar structure.

1893 Volkman: He performed autogenous skeletal muscle transplantation in rabbits, and concluded from his observations that the transplanted

tissues degenerate and were absorbed. The end result being scar formation.

1900 Saltykow: Working on mice and rats on which he did autogenous transplantation of skeletal muscle, the author observed first degeneration followed by regeneration. The regenerating muscle cell shows an increase in number and size of nuclei and the formation of spindle elements. Because of disuse, the muscles were atrophic at the end.

1905 Kroh: He studied the behavior of muscle transplants when faradic stimulation was applied. He observed a delay in the degeneration of such muscle but fibroses and fatty infiltration were the end results.

1906 Gersuny: He successfully reanimated a paralyzed mouth and shoulder by inserting normal muscle in the paralyzed one. Orbicularis oris and trapezius muscle were used respectively.

1908 Caminiti: He performed free transplants of small pieces of skeletal muscle. The specimen was freed of nerve vessels and aponeuroses. Successful results were obtained.

1909 Netalon: Following bone resection for

osteomyelitis, muscle was successfully transplanted into the cavity to fill the gap.

1909 Schmid: He used Faradic stimulation on muscle transplants. He concluded from his observations that the stimulated muscle regenerated while such regeneration was not observed in the non-stimulated one.

1909 Jores: He performed autogenous transplants of skeletal muscle in the rabbit and did a comparative study between transplants stimulated with an electric current and unstimulated transplants. He concluded from his observations that Faradic stimulation helps the transplanted muscle to survive and regenerate.

1910 Unger: This surgeon used muscle transplants in the cranial cavity for hemostatic purpose.

1912 Askenazy: The author performed autogenous skeletal muscle transplantation. He observed necroses in the center of the transplant with some degree of regeneration at the periphery.

1912 Goebel: The author performed successful free muscle transplants using sartorius and external oblique muscle to replace the flexor muscle of the forearm in a 5 year old boy with ischemic contracture.

1912 Lawen: Pectoralis muscle was used as a free transplant to seal a perforating wound of the heart. One of the two cases showed a well healed muscle transplant. Both cases died.

1912 Kocher: A free muscle transplant was used to obtain hemostasis in an intracranial haemorrhage.

1913 Landois: He obtained negative results in autogenous skeletal muscle transplants even with the use of electric stimulation.

1913 Haberland: He performed autogenous skeletal muscle transplant. Following microscopic observation he stated that most of the muscles were necrotic with the exception of cells at the periphery.

1918 Schulz: Writing a thesis on muscle transplantation, this author stated that muscle was not suitable for free transplantation. This tissue necroses secondary to the lack of functional and nervous influences.

1918 Eden: Free muscle transplants of extensor digitorum communis and anterior tibialis muscle were unsuccessful in three of Eden's patients. Connective tissue was the main component of the transplanted muscle.

1933 Leriche-Fontaine: They used pectoralis muscle graft in dogs

with infarcts of the myocardium and aneurysm. The fraft healed and survived.

1934 Vinke: To help in the choice of muscle for transplantation, Vinke designed the myokinesismeter. This instrument gave information on the time and extent of muscular contraction of different muscles. It found some application in cases of spastic and infantile paralysis.

1937 Von Gehlen: He obtained successful results with autotransplants of skeletal muscle in rabbit. Regeneration and survival were satisfactory.

1939 Livermore: Transplantation of muscle and fat in kidney, damage this organ, the transplanted degenerate and was replaced by fibrotic tissue.

1946 Clark: He observed necroses in the center of autogenous gracilis muscle transplant. Some fibres at the periphery survived. At a later stage regeneration was present in different portions of muscle.

1950 Harmon: The author described 5 types of muscle transplantation he performed on patients with poliomyelitis of the shoulders.

1951 Peer: He performed multiple muscle transplantation in humans. From his experiments he concluded that "the

muscle fibres in the free muscle graft degenerated very rapidly regardless of the host site", and that "muscle cells in free grafts will die in a matter of hours when deprived of their blood supply".

1954 Pickrell: The author described the surgical procedure for rectal reconstruction in 12 cases of anal incontinence. The operated children had spina bifida, meningocele or neuogenic malformation of perineum and rectum. The author concluded that the use of gracilis muscle to reconstruct the anus was most gratifying.

1955 deCarvalho Pinto: The author reported a case of successful surgical correction of anal incontinence using the above-mentioned Pickrell procedure.

1960 Smith: "This paper reports on a study of 15 cases in which Abbe or Estlander flaps were used in surgical reconstruction of the lips. This transplanted tissue has been evaluated for the return of sweating and of sensitivity to pain, touch, and temperature. In addition the reinnervation of muscle has been studied electromyographically. Complete return of function was conclusively demonstrated."

1961 Thompson: "Following histological and histochemical

investigations of muscle biopy taken from the Abbe flaps and the normal lateral lip elements of six patients, evidence is submitted to support the concept of motor reinnervation occurring in muscle flap. Such evidence is based chiefly on the demonstration in the flaps of

1.- normally striated skeletal muscle elements

2.- motor end plates exhibiting cholinesterase activity of normal intensity

3.- nerve axons exhibiting some of the characteristics of motor nerve fibres."

1961 Reese: The author presented 20 cases of orbital exenteration with temporalis muscle transplant. The result is better than exenteration with split thickness skin graft. Advantages and disadvantages of the technique are discussed.

1964 Studisky: Studisky gave this presentation at the Sixth International Transplantation Conference held by the New York Academy of Sciences on February 6, 7, 8, 1964. The author explained that skeletal tissues did not survive free transplantation

because of its high sensitivity to oxygen deficiency. He found that the survival of some muscle was made possible by mincing it with scissors to a "semi-liquid proridge state". Then he stated that the tissue that was damaged had a tendency to compensate for the suffered loss by a reconstructive process. The special state of this tissue at this time was called the "plastic state". In connection with this discovery, he suggested creation of this plastic state in order to give to the muscle the property to survive in conditions of free grafting. Mechanical trauma, denervation may be used as a way to create this plastic state before transplantation. He also remarked that "the transition in plastic state is accompanied by a deep change of energy regime: transformation of aerobic into anaerobic metabolism". (Zhenevskaya worked in the laboratory of Studisky.)

1964 Deitsch: The author reported a case of congenital hypoplasia of the left orbit on which he successfully used temporalis muscle transplant.

1965 Laird: The author stated from his own experiments

on transplantation of autogenous skeletal muscle that degeneration was followed by regeneration and formation of muscle with normal appearance. The same experiments done by transplanting muscle of distrophic mice in normal histocompatible hosts "suggest that the host has no effect on the idsease process."

1965 Zhenevskaya: The authors briefly reported successful free autogenous transplantation of whole muscle bellies on young rats. They stated that denervation of the transplant about 2 weeks prior to grafting may be of benefit for the survival of the graft.

1966 Roy: Roy performed muscle transplantation in the dog. Autotransplants of dorsal musculature were grafted into the tight muscle. From his observations the author concluded "that the autogenous free muscle transplant severed from its nerve and blood supplies, does not survive; it undergoes disintegration and is entirely replaced by fibrotic tissue". Foreign body reaction was surrounding the muscle graft.

1968 Griffiths: The author reported 25 cases of gracilis muscle transplantation for usinary incontinence in women. This incontinence

was secondary to radiotherapy, urethro-vaginal fistula, abdomino-perineal resection of rectum and post-gynaecological procedure. The average results were from slight improvement to excellent.

1970 Tamai: "This is the first report of successful transplantation of completely isolated muscle, using microsurgical technique in dogs." Forty free transplantations of rectus femoris muscle were done with microvascular anastomose nerve repair; 70% succeeded, 30% failed because of thrombosis at the site of anastomoses. There was a period of degeneration, then muscles recovered after 3 months. Light microscopy, electron microscopy and electromyography were used for muscle evaluation.

1970 Allbrook: Tibialis anterior muscle in adult rats was divided in small fragments and implanted in the contralateral side, 28 days later regeneration was present and the author concluded that "following the implantation of small fragments of striated fibres, rather complete reorganization and reconstitution of the muscle belly results".

1971 Demers: Free muscle graft was recommended by

the author in difficult vascular anastomose, and in repair of trachea and esophagus.

1971 Thompson: Fifteen cases of facial paralysis had palmaris longus and extensor digitorum brevis muscle free grafts in order to obtain reanimation. After 3 months he obtained 13 successes based on clinical, electromyographic, histologic and histochemical investigations. Thompson stated that three fundamental postulates had to be respected:

1. Muscle must be transplanted as a complete anatomical entity. Origin and insertion should be included.
2. Muscle must have been denervated two weeks prior to transfer.
3. The free muscle must be applied in direct contact with normal innervated muscle to obtain "muscular neurotization" by ingrowth of axon.

1971 Thompson: This author transferred complete muscle bellie preconditioned by preliminary denervation in the dog. He also did similar procedures in the man for facial paralysis. He used palmaris longus and extensor digitorum brevis. In each case muscles were denervated 2 to 3

weeks before transfer to paralyzed oral sphyncters and eyelids. Evaluation of the transferred muscle was done 5 to 6 months later. Only the denervated muscle showed significant survival of histology and distinct contraction on faradic stimulation. Similar results were found in the 8 operated patients.

1971 Thompson: Using dogs, Thompson did free autogenous muscle graft in orthotopic sites. He used pronator teres, supinator and flexor carpi radialis on dogs' forelegs. Care was taken to preserve the entire muscle bellie and denervation was done 14 to 21 days prior to transplant in order to alter the muscle metabolism. Three quarters of the surviving grafts were normal in 6 of his 8 dogs. Over 6 months most of the muscle was reinnervated. In cases where denervation was not done prior to transfer, only 5-10% of the muscle bellie was normal.

1973 Salafsky: During a symposium on 'Normal and Diseased Muscle', the author stated that, "previously denervated muscle, when subsequently minced and autotransplanted in the presence of a peripheral nerve, will regenerate".

1973 Hironaka: Based on his experience of free transplantation of extensor digitorum longus in the rat, the author from histologic observation stated that "in contrast with the results of other investigations, the tissue reconstitution of our transplant took place, keeping its internal architecture apparent intact".

1974 Hakelius: Twenty-six free autogenous muscle transplants were performed in 21 patients with facial paralysis. Two to three weeks before transplantation of extensor digitorum brevis, palmaris longus and plantaris muscle to the paralyzed face, the muscles were denervated. The facia surrounding the transplant was removed at the time of the second operation. The pattern of reinnervation was studied by means of electromyography. After 2-3 months, all examined grafts showed signs of reinnervation. The author concluded that free muscle transplants in man survived and became reinnervated.

1974 Hakelius: Using extensor digitorum, palmaris longus, plantaris and the superficial flexor of the fourth finger, the author performed 30 free autogenous muscle transplantations for facial paralysis. The transplanted

muscle was denervated, 2-3 weeks prior to transplant. In 23 of the 28 graftings the articulation difficulties, dribbling and irritation of the unprotected eyes were improved.

1974 Carlson: The author "compares the reaction of free autografting of normal and previously denervated extensor digitorum longus and soleus muslce in young rats". In the denervated group, the denervation was performed 14 days prior to transplantation. In the two groups the histological results 2-3 weeks after transplantation were similar. While radial gradient of regeneration was present in normal transplanted muscle, the denervated muscle showed a uniform reaction throughout the graft. From an electrophysiology point of view "except for the ability to contact throughout the early postoperative days, denervated soleus transplants did not differ from normal grafts".

In cross transplanted grafts the author observed a change in the contractile property from slow to fast. He concluded that free grafts of both normal and previously denervated muscles are successful in young rats.

1974 Chul Song: In order to obtain velopharyngeal competency after cleft palate repair, the author performed free transplantation of palmaris longus muscle to the posterior pharyngeal wall. Based on the work of Thompson in 1971, the author denervated the muscle 2-4 weeks before transplantation. In this procedure where the survival of the entire muscle was not indispensable since the bulk effect of transplant was useful, the author performed 3 cases with good results.

1974 Ward: The atuhor performed orthotopic and heterotopic free flap transfer of gracilis muscle in the dog, using microvascular anastomosis, 65% of the muscle survived. "Histologic changes following successful replantation were primarily those resulting from temporary ischemia and denervation."

1974 Gutmann: The author reviewed the factors affecting the success of skeletal muscle transplantation. Concerning the neuronal influences he stated the relatively greater success of denervated free grafts may be related "to the increase of satellite cells in the denervated muscles. The later cells being apparently of great

importance in the regeneration process."

Concerning the hormonal influences the author mentioned the effect of androgen on "target" muscle such as the levator ani of the rat. This muscle when transplanted caused atrophy after castration and hypertrophy after testosterone administration. The effect of hormones on the regeneration process of muscle is still not clear. The influence of the vascularization in the regenerating graft was in close relation with the type of metabolic activity.

"Active glycolytic pathways during early myoblast development may maintain metabolic activity in the early stages of regeneration, but with the ingrowth of capillaries a considerable increase in activity of oxidative enzymes is observed."

The author stated that tension was an important factor in the regeneration process.

Electrophysiologic study showed a better recovery in transplanted muscle performed on young animals in comparison with old ones.

1975 Schaffino: The author studied the process of survival of denervated and free auto-transplanted skeletal muscle. He used

peroneus longus muscle on adults, cats, that he transplanted 3 weeks after denervation. From electron microscopic observation he concluded that three zones could be distinguished in the graft. "The outer zone of the transplant consists of surviving atrophic fibres. The middle zone was characterized both by muscle atrophy, and by fibre regeneration. The inner zone were muscle fibres, all clearly necrotic."

1975 Hakelius: The author studied the vascular supply and the connective tissue in autotransplants of free muscle graft that were denervated prior to the transfer. He found that the capillary network increased slightly during the denervation period of 2 weeks. Two to three months after the transplantation there was a pronounced increase in the vascular supply. After 10 months the capillary pattern appeared to be fairly normal while the graft started to be reinnervated.

Concerning connective tissue there was a marked increase in early stages but as reinnervation occurred, there was a gradual disappearance of endomysium.

1975 Hakelius: Using denervated peroneus longus muscles

that have been transplanted into intercostal spaces, the author studied the end plates formation with histochemical technique for cholinesterase. After 9 weeks all the original end plates were degenerated and could not be seen. Five weeks after the transplantation new end plates were formed and increased in number during 3 months.

1975 Hakelius: Histochemical and electromyographical methods were used to study free autogenous muscle transplant in the cats. Peroneus muscle, which is a white fast twitch muscle, was transplanted in intercostal space; 2 weeks after denervation, similar transplants without denervation were also performed. The author concluded that denervated muscles survived transplantation and became structurally and enzymatically mature whereas muscles transplanted without previous denervation underwent extensive fibrotic changes.

1975 Carlson: The author performed free transplantation of intact soleus and extensor digitorum longus in the rat. Some of the muscles were denervated 14 days prior to transfer. The major difference between normal and denervated muscles were observed during

the first week after transplantation. The normal graft had a centripetal gradient of degeneration and regeneration while these processes were uniform in the denervated muscle. In both cases there was a severe ischemia in the center and more surviving cells in the periphery.

1975 Allbrook: During a lecture on transplantation and regeneration of striated muscle the author stated that there was a competition between "fibroblasts - collagen" and "myoblasts and muscle fibres", the rapid growth of collagen being an obstacle to muscle fibre elongation and growth.

He stated that the revascularization of muscle secondary to devascularization was done through opening up of intravascular anastomoses and new growth of capillaries through the overlying muscle fascia. Concerning the effect of denervation he observed a "sudden small but definite mitotic response in muscle fibres and their containing fibrous tissue matrix and a reduction in nuclear: cytoplasmic ratio." In his study on transplantation of denervated and normal muscle he observed that regeneration was present in both transplants but the

"percentage of necrotic tissue remained much larger in the control muscles."

In the 4 week denervated transplants the regenerated muscle fibres completely reconsitiuted the belly. He concluded that "it could not be confirmed that the speed and completeness of muscle regeneration is actually increased in the prior denervated transplant."

1975 Mastaglia: Using mice the author studied the morphological changes in subcutaneously implanted muscle homografts. From light and electron microscopic observation, the author concluded that "regenerative changes were prominent at the periphery of grafts by 48-72 hours before evidence of graft revascularization could be demonstrated by India ink perfusions." He also stated that regeneration was retarded or completely inhibited by exposure of the donor animal to radiation. Electron microscopic observations in 48-72 hour grafts suggested that primitive mononucleated myogenic cells may form within degenerating muscle fibres by a process of myonuclear sequestation".

1976 Watson: Free transplantation of peroneus longus muscle was performed in dogs 2-3 weeks

after denervation. At the time of transplantation epimysium was stripped from the muscle. From histology and electrophysiology studies the author concluded that "the total failure of all these muscle grafts is in marked contrast to other published results in spite of strict adherence to the principles outlined by Thompson". An attempt to explain this failure follows the study.

1976 Yoshikazu-Ikuta: The author reported one case of successful free muscle graft using microvascular anastomose. The pectoralis major was used to obtain flexion of a thumb and 4 fingers on a 6 year old boy with Volkmann's contacture. The author stated that muscle with one artery and one vein was the best for transfers, and that a certain excursion of the graft was necessary to obtain good results.

1976 Takashi-Kubo: The author performed 55 cases of orthotopic and heterotopic free muscle transplantations in dogs using micro-neurovascular anastomoses. Biceps brachii and rectus femoris have been used with an average success rate of 75%. The orthotopic rectus femoris transplant had a 85% success rate. The muscles were

evaluated with histologic, electron microscopic and electromyographic techniques. The accent was put on selection of muscle for transplantation, conditions of the transplanted bed and surgical techniques.

1976 Kiyonori-Harii: The author successfully experimented the transfer of a compound gracilis flap to a hypogastric recipient site in the dogs using microvascular anastomose. He then performed 3 of such transplants in different patients: one on the face, one on the head and one on the lower limb. He obtained good results.

1977 Terzis-Williams: Using physiological techniques, normal and replanted muscle functional capabilities were studied. The authors stated that following muscle transplantation with microneurovascular anastomose "the muscles survive but never achieve the functional capabilities of normal muscle". They also demonstrated the importance of muscle tension for the good function of this organ.

PART IV

Free Homologous Muscle Transplant

1883 Magnus: Using rabbits, he did homografts and autografts of quadriceps femoris and tibialis anticus in contralateral limbs. After 7 to 60 days he observed fibrosis and replacement with muscle cell degeneration.

1883 Helferich: He used heterograft muscle from a dog to seal a defect in the biceps of a man. He stated that normal function returned after 3 months. Evaluation was done with electrical stimulation.

1914 Shinya: He did comparative study between auto and homo transplanted muscle in dogs. The muscle was inserted in a sciatic nerve after perimysiotomy. He observed new formation of muscle fibres, but at 35 days post-operatively the autogenous muscle had disappeared entirely, and had been replaced by connective tissue.

1929 Elson: He performed homo and auto transplantation of striated muscle in the rat. In both cases he observed degeneration. The homo transplanted muscle disappeared more quickly. Regenerative process from the sarcoplasm and nuclei was observed secondarily.

1930 Bartoli: Implantation of pedicled graft of muscle was performed from one rabbit to another one. The graft was adherent to the host muscle but underwent progressive degeneration associated with change in color and vitality.

1932 Dainelli: Pedicled autoplastic and free homoplastic grafts were performed on the rabbit quadriceps muscle. Some of the homografts were preceded and followed by injection of donor's muscle extract. The three types of procedure show complete replacement by connective and fibrous tissue.

1944 Fowler: He produced shock with implantation of homogenous skeletal muscle into the peritoneal cavity. The autoclaved muscle had a lesser propensity to induce shock.

1952 Andresen: They performed autologous and homologous musculofascial transplants in the rabbit. They observed predominance of lymphatic reaction in homologous transplants and angitis in autologous transplants. The author stated that when "successive homologous transplants were made from the same donor to the same recipient, acute angitis with thrombosis supervened and the lymphocytic reaction failed to

develop or persist".

1970 Jasmin: Free grafts of skeletal muscle were done between normal hamsters of the same strain, between normal and dystrophic animals, and between normal and healthy carriers. Based on histologic studies the author concluded that normal muscle and dystrophic muscle were well tolerated by normal and healthy carrier animals. By contrast muscle transplanted in dystrophic hosts was not tolerated and was transformed into scar tissue.

Chapter III

PRELIMINARY DENERVATION IN THE FREE TRANSPLANTATION OF THE SKELETAL MUSCLE

A review of clinical and experimental works on muscle transplantation reveals a very confusing picture.

Since Zielonko made the first attempt at transplanting muscle, many contradictory publications have been produced. Keeping in mind that different techniques, animals, muscles and methods have been used, positive results have been reported by: Howard (1971), Gluck (1881), Helferich (1883), Salvia (1883), Caminiti (1908), Askenazy (1912), Goebel (1912), Von Gehlen (1937), Clark (1946), Laird (1965), Zehnevskaya (1965), Tamai (1970), Allbrook (1970), Thompson (1971), Salafsky (1973), Hironaka (1973), Hakelius (1974), Carlson (1974), and Takashi Kubo (1976); while unsuccessful results have been reported by: Zielonko (1974), Magnus (1883), Askenazy (1891), Volkman (1893), Saltykow (1900), Capurro (1900), Kroh (1905), Hildebrant (1906), Landois (1913), Haberland (1913), Shinya (1914), Eden (1918), Elson (1929), Dainelli (1932), Peer (1951), Roy (1966) and Watson (1976).

Muscle transplantation is still an enigma.

A few years after Peer (1955) stated that muscle transplantation was still unsuccessful but that the future was promising, Studisky and Zhenevskaya (1960-1964) introduced the concept of the "plastic state". This concept needs more extensive investigation.

It has always been considered that striated skeletal muscle has a poor capacity of regeneration following the post embryo period. However, the Russian workers observed a regeneration reaction during the "plastic state". Zhenevskaya

described proliferation of the protoplasm, amitotic division of the nuclei, emergence of myoblasts followed by a development of typical muscle fibres. "It would therefore appear that a muscle in the plastic state is more suitable for reparative surgery" (Zhenevskaya, 1962). In order to create this "plastic state" during which a high regenerative capacity is present, the muscle should be minced, tenotomized, immobilized, traumatized or denervated.

This last technique is now popular, however the function obtained with transplanted muscle following preliminary denervation is still unsatisfactory (Watson, 1976).

It is now well accepted that skeletal muscle survival depends on blood supply and innervation (Ribbert, 1898; Saltykow, 1900; Hildebrandt, 1906; Schulz, 1918; Peer, 1951; Zhenevskaya, 1962; Roy, 1966; Thompson, 1971 & Hakelius, 1974).

Muscle fibre tension does not seem to have such a vital importance (Watson, 1976), but tension is of great value when function of muscle is considered (Williams, Terzis, 1977). The "complete anatomical entity" concept proposed by Peer in 1955 and Thompson in 1971, is, according to these authors, an important concept in the transplantation of skeletal muscle. They stated that "it is illogical to attempt the transfer of fragments of cells in the transplantation of free tissue grafts". However the successful results obtained by Zhenevskaya and Studisky by transplanting minced muscle does not support this postulate.

We should now consider the importance of blood and nerve supply to a transplanted muscle. Peer stated that

"muscle transplants degenerate and lose contractile power almost immediately, as a result of loss of blood supply; even where vascularity is maintained, gradual but progressive atrophy results from loss of the motor nerve."

So in order to increase the success rate in muscle transplantation it is necessary to provide oxygen and metabolic substrata through adequate blood supply and also to insure innervation.

How can we achieve these two requirements?

Innervation:

Innervation of the transplanted muscle can be obtained using different methods:

1. Heineke in 1914 and Steindley in 1915 demonstrated that direct neurotization was possible by implanting a peripheral motor nerve in a paralyzed muscle.
2. Erlacher in 1915 suggested placing paralyzed muscle in close contact with normal innervated muscle. Nerve fibres will spread directly from the adjacent muscle.
3. There is also the well known perineural or epineural repair that can be performed between the host and graft nerve. But "even upon complete reinnervation the working capacity of replanted muscle is only one-fourth normal" (Williams and Terzis, 1977).

Blood Supply:

We must direct our investigations to find the various

ways to diminish muscle demand of oxygen and metabolic substrates. We also must find a way to increase the blood supply to the transplanted muscle.

In order to decrease the oxygen consumption Studisky and Zhenevskaya (1965) suggested induction of the "plastic state" by the different ways mentioned above. Zhenevskaya stated that "under certain conditions (a strong trauma), the oxygen uptake by the muscle drops to such a low level that free plasticity of muscles may be possible".

In 1971 Thompson suggested denervation of the muscle 2 to 3 weeks before transplantation. Although the author does not make any allusion to the "plastic state", which according to Studisky is induced by denervation, Thompson suggested denervation in order to create a more efficient metabolism in the transplanted muscle.

The white muscle that has been denervated undergoes a shift of its anaerobic glycolytic metabolism toward a more efficient aerobic lipolytic metabolism. These changes in the energy production system, causing more efficiency, whould increase the survival of the transplanted skeletal muscle (Thompson, 1971; Hakelius, 1974, 1975).

Hogan and Romanul in 1965 demonstrated the effect of denervation on the metabolism of skeletal muscle. The enzymes implicated in the anaerobic metabolism of the white muscle decrease in favor of the aerobic metabolism enzymes. This change is progressive and is more pronounced at 8 weeks that at 12 weeks (Hogan & Romanul, 1965; Hogenlius & Engel, 1963). While this change in the metabolism is occurring there is also

a change in the blood supply of the skeletal muscle. The vascular network increases progressively after denervation. Two months after denervation this vascular network is greater than at 2 weeks after denervation (Hakelius, 1975).

According to the advocates of graft preconditioning by preliminary denervation 2 to 3 weeks before transplantation, the chance of survival will be increased, "since denervation in skeletal muscle not only introduced a more economic form of lipolytic metabolism, but also resulted in an increased vascularization to subserve the increased oxydative demands" (Thompson, 1971).

However two questions arise. The first being due to the fact that at 8 weeks more metabolic changes have occurred and vascular supply is greater than at two weeks. Therefore it might be possible that 7 or 8 weeks of denervation might be better than 2 weeks of denervation before transplantation. The second question concerns the amount of time needed for formation of anastomoses between the vessels of the host and graft. This question is still unanswered and one wonders if during this hypoxie period the muscle's chances of survival are not decreased due to a high oxygen demand (aerobic metabolism) and a poor blood supply (incomplete anastomose between host and graft).

In cases where microvascular anastomoses are used at the time of surgery such questions do not arise and in this case we can anticipate good results with preliminary denervation.

In order to assess the relevance of preliminary denervation to the transplantation of skeletal muscle the

following protocol has been designed. In order to assess the different methods of innervation another project is in progress.

Chapter IV

MATERIALS AND METHODS USED TO ASSESS THE RELEVANCE OF PRELIMINARY DENERVATION

Twenty-one white male rabbits (2.5-3.0 kg each) had their right and left rectus femoris muscle investigated. Each group was composed of at least 2 animals, or 4 muscles. Excluding those which became infected, a total of 32 muscles were studied.

Group M: normal rabbit - normal muscle, not transplanted

Group O: no preliminary denervation before transplantation (control)

Group 1: Muscle was denervated 1 week before transplantation

Group 2: Muscle was denervated 2 weeks before transplantation

Group 3: Muscle was denervated 3 weeks before transplantation

Group 4: Muscle was denervated 4 weeks before transplantation

Group 5: Muscle was denervated 5 weeks before transplantation

Group 6: Muscle was denervated 6 weeks before transplantation

Group 7: Muscle was denervated 7 weeks before transplantation

All transplanted muscles were orthotopic from right to left leg and vice versa. All transplantations had their fascia intact. None of them had neuro-vascular anastomoses.

A. Anatomy

The medial portion of the rectus femoris has been used for this study. This muscle originates from the infero-anterior iliac spine and extends its fibres along the anterior aspect of the femur. The insertion of rectus femoris is common with the quadriceps femoris, it is attached to the anterior tibial tuberosity through the patella and the patellar ligament. The femoral nerve, through two of these branches, innervates the muscle. One small branch and a bifascicular nerve enter the muscle in the proximal third.

The arterial blood comes via a small branch of the femoral artery and the venous drainage is done by tiny vena comitante that come out from the medial aspect of rectus femoris. A large vein arises from the surface of the muscle and communicates with the femoral vein.

B. Surgical Procedure

General anesthesia was induced with a facial mask containing ether, then blind peroral endotracheal intubation was performed. A stainless steel tubing connected to a small container, where a 50% air-ether mixture was present, was used in order to control the long standing anesthesia (Mersereau, 1976). Then the animals were shaved from the knees to the mid abdomen and the surgical field was prepped with bethadine solution.

First operation

All sixteen animals had a first operation. Using a ventro-medial incision the right and left rectus femoris were exposed. The nerves were identified and 0.8 to 1 cm of nerve was resected. Denervation of the rectus femoris was confirmed by stimulation of the femoral nerve at a more proximal level. The vascular pedicle was left intact.

Second operation (0 to 7 weeks after first operation)

This operation consisted in transfering the denervated right rectus femoris to the left side (orthotopic transplantation) and the left rectus femoris to the right side.

Before resecting the muscle, electro-stimulation of the femoral nerve was done for the second time to confirm that no neurotization occurred between the time of denervation (first operation) and the time of transplantation (second

operation).

The entire muscle belly was mobilised from its origin to its insertion using blunt dissection. The fascia was left intact. The vascular pedicle was severed. Each muscle was transferred to the contralateral side. Origin and insertion tendons of the transferred muscle were tied with 4-0 silk to the cut ends of origin and insertion of the recipient side. Muscle graft was applied with proper tension. No neurovascular anastomose was performed.

Skin closure was achieved with interruption 5-0 nylon and antibiotic ointment was used on the wound without dressing. The average time of muscle ischemia was 15 to 20 minutes. Before transfer to the contralateral side, the muscle was measured and weighed. Details of the colour, ischemic time, duration of anesthesia and any complication were carefully recorded.

Timing for the Second Operation

Group 0: The muscle was transferred at the time of denervation. This group had only 1 operation performed.

Group 1: The muscle was transferred 1 week after denervation

Group 2: The muscle was transferred 2 weeks after denervation

Group 3: The muscle was transferred 3 weeks after denervation

Group 4: The muscle was transferred 4 weeks after denervation

Group 5: The muscle was transferred 5 weeks after denervation

Group 6: The muscle was transferred 6 weeks after denervation

Group 7: The muscle was transferred 7 weeks after denervation

From a period of 18 to 22 weeks after the second operation, the muscles were assessed.

C. Method of Assessing Recovery

1. Measurement of muscle weight, width and length.
2. Physiological assessment
   a. Measurement of the necessary threshold to elicit a visual contraction
   b. Measurements of the necessary threshold to elicit a measurable contraction
   c. Measurement of the muscle force
      - twitch contraction strength
      - rise time
      - tetanic tension
      - fusion frequency
      - fatigue rate
   d. Needle E.M.G. recording

1. Measurement of muscle weight, width and length

At the time of the second operation, when the muscle was mobilized to be transferred on the contralateral side, the muscle was weighed and its maximum width and its length from origin to insertion were measured. At the time of assessment 18 to 22 weeks after transplantation when the physiological assessment was finished, the weight, width and length were again measured in the same manner.

2. Physiological assessment

When appropriate time for assessment came, the rabbits had a general anesthesia induced with ether mask. Then a 25% solution of urethane was injected intravenously 3 ml per

kg of body weight, this solution was injected slowly until corneal reflex disappeared. A peroral endoracheal intubation was performed in order to ensure proper ventilation. Secretions were suctioned regularly without disturbing the recording area. The animal was then prepped and shaved and the rectus femoris were exposed through a ventromedial incision. A femoral catheter was required in certain situations when the ear veins were blocked. The animal was immobilized on his back in a custom-designed rack; the head was rigidly immobilized in a holding device. The right and left hind limbs were rigidly immobilized by two metal clamps; one clamp was inserted on the femoral bone using two metallic hooks, the second clamp was fixed on the iliac bone and both devices were fixed on the heavy metal rack. With this complete immobilization of the coxofemoral articulation, the iliac bone and the femur, no artefact could be produced by movement in the recording area.

The rectus femoris was mobilized from its insertion. The origin was left intact. Through the patellar ligament a wire suture was passed and connected to a grass force transducer. The transducer was connected to a grass polygraph model 7P15. This polygraph was calibrated with a series of standard weights before each physiological assessment.

The mechanical force generated by the muscle was converted into electric signal that was recorded on the paper of the grass polygraph. Using the calibration, the electric signals were translated into grams of force. For isometric recording, the bony origin was fixed and the tendon of the

distal part was attached to the strain gauge. While the muscle was hung on the transducer from its insertion, a constant irrigation of warm saline was provided to the muscle. A head lamp was also used to keep the muscle warm. At the beginning of each evaluation of the transplanted muscle, the resting tension was measured and recorded in the polygraph.

a. Measurement of the necessary threshold to elicit a visual contraction

With direct stimulation to the muscle, the threshold stimulus that was capable of eliciting a muscle response that could be seen by at least two observers was called "threshold for visual contraction". The stimulus was applied in the first two centimeters of the muscle, then in the middle and in the distal part.

b. Measurement of the necessary threshold to elicit a measurable contraction

With direct stimulation to the muscle, the threshold stimulus that was capable of eliciting measurable muscle response was called "threshold for measurable contraction".

c. Measurement of the muscle force (Terzis, Sweet, Dykes, Williams, 1976)

- Twitch contraction: The twitch contraction is the brief contraction obtained by the application of an electrical stimulus to the muscle. The stimulus was applied at threshold voltage, at 2 and 10 times threshold and at maximal stimulation c.a.d. 100 volts. The frequency was 0.5 stimuli

per second. The stimulus was not applied directly to the nerve since this one was severed at the time of transfer and no anastomose was performed.

- Rise time: This was the time required for the muscle to reach maximum contraction strength. The stimulus applied was equal to threshold, 2 and 10 times threshold and at maximum 100 volts stimulus.

- Tetanic tension: This corresponds to the muscle's maximum contraction force. The tetanic tension was measured at 2 and 10 times threshold at the following frequencies: 1, 3, 6, 9, 12, 15, 18, 21, 30, 40, 60, 80 and 100 Hz.

- Fusion frequency: This was the stimulus frequency at which the contractions began to fuse.

- Fatigue rate: This was the rate of decay of the tetanic tension curves.

d. Needle E.M.G. recordings

Since no nerve anastomose was performed needle E.M.G. recording was produced by stimulating the needle fibres proximally and using a fine copper wire with a sharpened uninsulated tip the muscle response was recorded distally to the site of stimulation. The reference electrode was clipped to the edge of the incision. Stimulation was done at 2T, 10T and at 100 volts.

The response was displayed on an oscilloscope and photographed by an oscilloscope camera.

D. Light and Electromicroscopic Histological Sections of Muscle

When all physiological testings were done, the muscle was fixed on a cork and left in a formaldehyde solution. Transverse sections of proximal and distal muscle, and longitudinal sections of the middle part were done and H & E coloration was performed. Typical areas of each slide were photographed and the following criteria were compared:

- fatty infiltration
- fibre atrophy
- hyaline degeneration
- focus of necroses and of floccular change
- relative increase of nuclei
- hyperchromatic and picnotic nucleus
- fibre loss of contour
- basophilic fibres.

Two specimens of each muscle were also processed for electron microscopy. Methods and results of electronmicroscopy will be discussed under a separate publication.

Chapter V

RESULTS

A. Macroscopic Observation

At the time of the second operation, after various periods of denervation, all the muscles had a pink color and looked slightly atrophic. In some cases the capillary network was increased around the connective tissue. Five months after transplantation all muscle had a more obvious atrophy. Scar tissue was present around the muscle but rectus femoris could be separated without too much trauma to the muscle fibres. Two muscles of group 0, two of group 1 and one of group 3 were completely necrotic and atrophic.

Studied Muscles

Group M: Normal muscle not transplanted

6 muscles recorded (typical response reported in Table 1a)

Group Q: Muscles transplanted without preliminary denervation

5 muscles recorded

1 muscle infected

Group 1: Muscles transplanted after 1 week of denervation

4 muscles recorded

Group 2: Muscles transplanted after 2 weeks of denervation

3 muscles recorded

3 muscles infected

Group 3: Muscles transplanted after 3 weeks of denervation

3 muscles recorded

1 muscle infected

Group 4: Muscles transplanted after 4 weeks of denervation

3 muscles recorded

1 muscle infected

Group 5: Muscles transplanted after 5 weeks of denervation

4 muscles recorded

Group 6: Muscles transplanted after 6 weeks of denervation

2 muscles recorded

2 muscles infected

Group 7: Muscles transplanted after 7 weeks of denervation

2 muscles recorded

2 muscles infected

***A total of 32 muscles were recorded and 10 muscles were infected. 10 rabbits died from respiratory infection and were replaced. 31 rabbits were operated on in total (62 muscles).**

B. Assessment of Functional Recovery

1. Measurement of muscle weight
2. Physiological assessment
   a. Measurement of the necessary threshold to elicit a visual contraction
   b. Measurement of the necessary threshold to elicit a measurable contraction
   c. Measurement of the muscle force
      - twitch contraction strength
      - rise time
      - tetanic tension
      - fusion frequency
      - fatigue rate
   d. Needle E.M.G. recording.

1. Measurement of muscle weight

The average muscle weight after different periods of denervation is shown in graph 1. The weight varies from 6 gm 0 weeks after denervation to 4 gm 6 weeks after denervation. The graft shows a progressive atrophy after 1 week of denervation. Five months after the transplantation the muscle shows an increased weight loss (graphs 2 and 3). The weight varies from 3 gm in the 0 week denervated group to 4 gm in the 6 week denervated group. The maximal weight loss is present in the 0 week group and the minimal in the 6 week groups. If we compare the 2 week group to the 6 week group, the first one has lost more weight than the second one. The longer the period of denervation the minimum was the weight

loss, with the exception of the 7 week group whose weight loss is similar to the 5 week group.

2. Physiological assessment

a&b. Measurement of the necessary threshold to elicit a visual and a measurable contraction

Graphs 4 and 5 show the threshold of different muscles. The threshold to obtain a contraction that could be seen was highest in the 0 and 1 week denervated group and was lowest in the 2 week and 6 week denervated groups. The threshold to obtain a contraction that could be recorded in the polygraph was highest in the 0, 1, 2, 3, and 4 week denervated groups and lowest in the 5, 6 and 7 week denervated groups. Graph 5 shows that there is a poor correlation between these two thresholds.

c. Measurement of the muscle force - Twitch contraction strength

At a stimulation equal to the threshold* the average muscle force of the muscle varies from 0 gm to 4.5 gm (Graph 6). In the 0, 1 and 4 week denervation period groups the muscle force was minimal. In the 3, 5, 6 and 7 week denervation period groups the force is maximal. The stronger muscle belonged to the 6 week denervation period. The 5, 6 and 7 week group had also the highest muscular force at 2T and at 100 volt stimulation (Graphs 7 & 8). If we compare

---

*threshold = minimum amount of electric current necessary to elicit a contraction that cen be recorded

the average muscle force at 2T with the average muscle weight in Graph 8 we do not see any clear correlation.

c. Tetanic tension, fusion frequency, fatigue rate and rise time

All these physiological parameters were measured in each muscle (Table 1a & 1b). However, only 8 muscles over the 27 had measurable value. For this reason these parameters were not used for final comparison or conclusion.

d. Needle E.M.G. recording

This was also tried on all transplanted muscle, but in none of them were we able to record a response.

C. Light Microscopy

0 Week Denervation

Amongst the 5 muscles that were transplanted without previous denervation (muscles of group 0), three of them showed complete necroses, few pieces of muscle cell were present, no nucleus and no transverse striation could be seen in the debris of the muscle cell. In the 2 other muscles some cells were present in the periphery. The size of most of them was inferior to the normal. Longitudinal and transverse sections demonstrate fatty infiltration and necroses as well as fibroses in the middle. The 2 muscles that had some normal muscle cells at the periphery showed a small contraction at 10 and 1 volt stimulation, but no contraction could be recorded, they were so weak.

One Week Denervation

Amongst all 4 muscles that were denervated for 1 week before transplantation, none showed any normal muscle cell but a large amount of fatty infiltration was present. The residual muscle cells had a pale cytoplasm and a pycnotic and hyperchromatic nucleus. Fibroses were also prominent. None of these muscles responded to maximal electrical stimulation.

Two Week Denervation

One of these muscles was completely necrotic and infected. The other one showed the persistance of some normal muscle cell, but necroses and fibroses were also abundant. The muscle which had the best phsyiological response in this group did

not show any striking difference in the number or the size of the persistent muscle cell, when we compared it with the other muscle of the same group, but the difference of these features was significant when the 2 week muscle group was compared with the 0 and 1 week muscle group.

Three Week Denervation

This muscle had 30% of its mass showing healthy muscle cell. Twenty percent of the muscle cells were atrophic and the rest of the tissue was fat and fibrotic tissue. The other muscles of this group showed similar distribution but the atrophic cells were more numerous.

Four Week Denervation

In this group most of the tissue was fatty and necrotic. A small number of muscle cells were present in proximal area. In electrophysiology study, these muscles contracted at a stimulation going from 3 to 50 volts, but none of the contractions could be recorded by the polygraph, the strength of the muscle being too small.

Five-Six-Seven Week Denervation

This group of muscles denervated 5-6-7 weeks before transplantation demonstrated the same cell distribution as normal muscle, most of them being at the periphery. The size of the cells was variable. Atrophy, hyaline degeneration, fatty infiltration and necroses were present in a quantity comparable in the 3 groups.

Chapter VI

DISCUSSION

A. Muscle Weight

In Figures 1 and 3 we observe that muscle weight of rabbits of the same age and weight and performing the same activity is smaller when the denervation period is longer. This progressive atrophy of denervated muscle is not surprising and reconfirms previous work on this subject (Gutman, 1942). However when we compare the weight of these same muscles 18 to 20 weeks after transplantation (Figs. 1 & 3) we observe that muscle weight is higher when the denervation period is longer - which is the contrary to what has been observed before transplantation.

Considering that all muscles lose weight when they are transplanted (Fig. 3) but since this average weight loss is inferior in muscles that have been denervated, we might be tempted to conclude that preliminary denervation before transplantation prevents muscle atrophy, mainly when muscles are denervated for 6 weeks (Fig. 2). However, if we compare muscles of the same group we can observe important variation. For example, in the group of muscles denervated for 3 weeks, 2 transplanted muscles lost 0 gm while one lost 7 gm.

From these observations we can state that: a transplanted muscle may lose from 0 to 100% of its weight; denervation seems to lessen this weight loss mainly if the muscle is denervated for 6 weeks. But studies with a larger number of subjects is necessary to confirm or to refute these statements.

B. Threshold for Contraction

In order to assess the sensitivity of the transplanted muscle, that has been denervated for various periods before transplantation, we measured the minimum amount of electric current necessary to elicit a contraction. This amount of electric current is called threshold. So as to have a base for further discussion we compared the necessary threshold to elicit a visual contraction, with the necessary threshold to obtain a measurable contraction with a grass polygraph 7P15. Figure 5 shows that there is no correlation between these two thresholds.

The threshold to elicit a measurable contraction being more objective, we have chosen this one for comparative purposes. Figure 4 shows that very high electrical stimulation was necessary to record a muscle contraction in the groups of muscles denervated for 0, 1, 2, 3 and 4 weeks; while the muscles denervated for 5, 6 and 7 weeks needed a stimulation that varied form 30 to 40 volts in order to elicit a contraction.

From this observation we can conclude that: a transplanted muscle is more sensitive to electrical stimulation when it has been denervated 5, 6 or 7 weeks before transplantation.

One should notice that if the threshold to elicit a visual contraction is to be considered, it will bring about a different conclusion, the most sensitive muscle being at 2 and 6 weeks (Fig. 5). However the contraction of only a few fibres could be seen without producing a contraction

capable of reducing the length of the muscle and being recorded by the graph transducer. However this type of focal contraction is useless considering that a muscle in order to achieve normal function should reduce its total length when the muscle fibres are stimulated. We can conclude that: the necessary threshold to elicit a visual contraction of a muscle should not be considered as a criteria for assessment of the functional capabilities of a muscle.

C. Measurement of the Muscle Force

Figures 6 and 7 show that in the muscle transplanted without previous denervation, at a threshold stimulation or at 2 or 10 times this threshold, the muscle force recorded was nil. The same result was obtained when muscle was denervated for 1 or 4 weeks before transplantation. A preliminary denervation of 3, 5, 6 or 7 weeks increased the muscle force of the transplanted muscle. This increase is not significant if we compare it with the force of a normal muscle.

The rectus femoris of the rabbit will produce an average twitch contraction of 500 gm (Table 1a) at 100 volts stimulation. Figure 8 shows that the strongest muscle could produce a 10 gm twitch contraction while the others produced an average of 5 gm. This muscle force being so low, we were unable to obtain sufficient numbers of rise times, and fusion frequency values for discussion.

From these observations we can conclude that: preliminary denervation of a transplanted muscle increased slightly the force of the graft. The strongest denervated muscle was 10 times stronger than a non-denervated one, but still 60 times weaker than a normal muscle.

D. Histology

We found a close correlation between our electrophysiological results and histologic observations.

In the muscle where poor contraction was noted histological sections showed large amounts of necroses, fatty infiltration and atrophy.

In more responsive muscles we observed a greater percentage of normal muscle cell.

Common to all muscle was the presence of larger numbers of normal cells at the periphery with more necroses and fat in the center.

CONCLUSION

Figure 9, which shows the muscle force, the weight loss and the threshold for contraction of the muscles after different periods of preliminary denervation (0 to 7 weeks), allowed us to conclude that: it is only after 5, 6 or 7 weeks of preliminary denervation that transplanted muscle shows an increase muscle force associated with a low threshold of contraction and a minimal weight loss.

One should consider this period of 5 to 7 weeks as the ideal period of denervation that should precede muscle transplantation. However, in skeletal muscle transplantation where microneurovascular anastomoses are not performed, the preliminary denervation does not significantly improve the ability of the muscle to perform work.

FIGURE LEGENDS

Figure 1: Weight before and after transplantation of muscle denervated for various periods of time.

Figure 2: Weight loss in transplanted muscles after different periods of preliminary denervation.

Figure 3: Projection of Figure 2 and Figure 3.

Figure 4: Threshold for visual and recorded contraction of
Figure 5: transplanted muscle that underwent various periods of preliminary denervation.

Figure 6: Muscle force at threshold in transplanted muscle.

Figure 7: Muscle force at 2T and muscle weight of transplanted muscle.

Figure 8: Muscle force at 100 volts of transplanted muscle.

Figure 9: Projection of Figures 2-5 and 6.

Tables 1a & 1b: Physiological parameters of transplanted muscles which were denervated for various periods of time before transplantation.

Figures 10 to 18: Histologic sections of normal and transplanted muscle which underwent various periods of preliminary denervation.

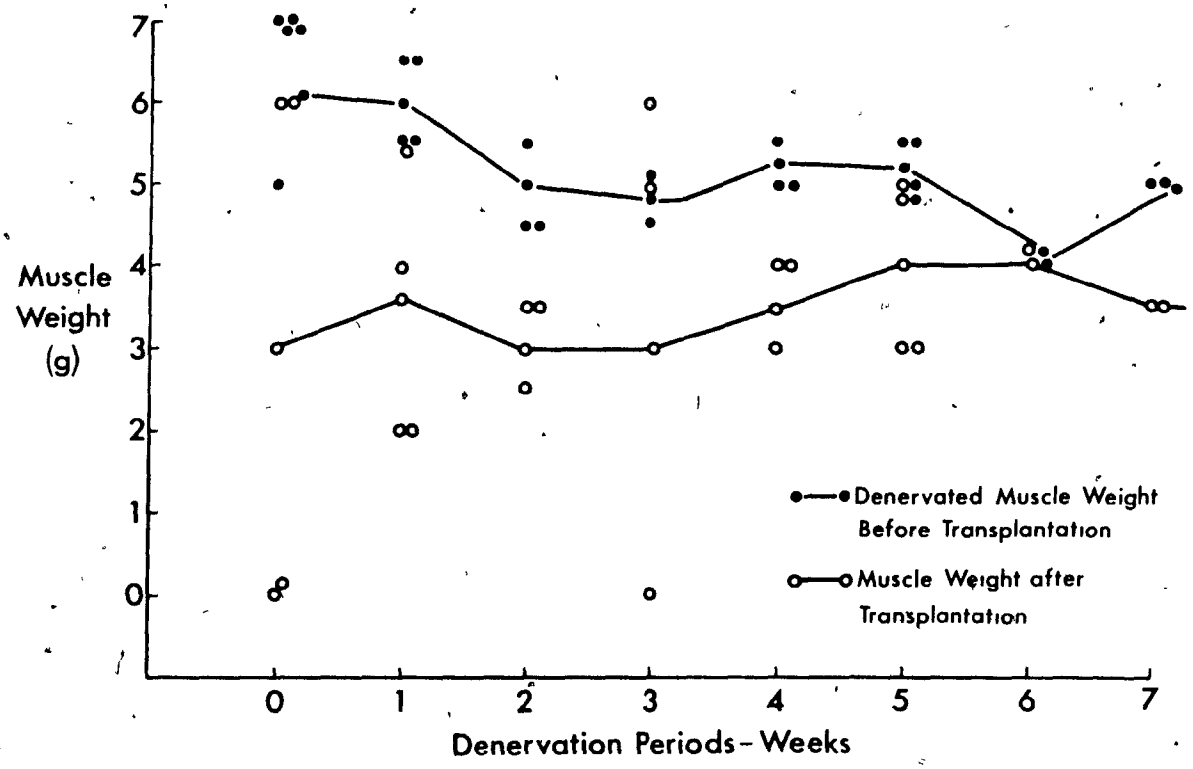


Figure 1

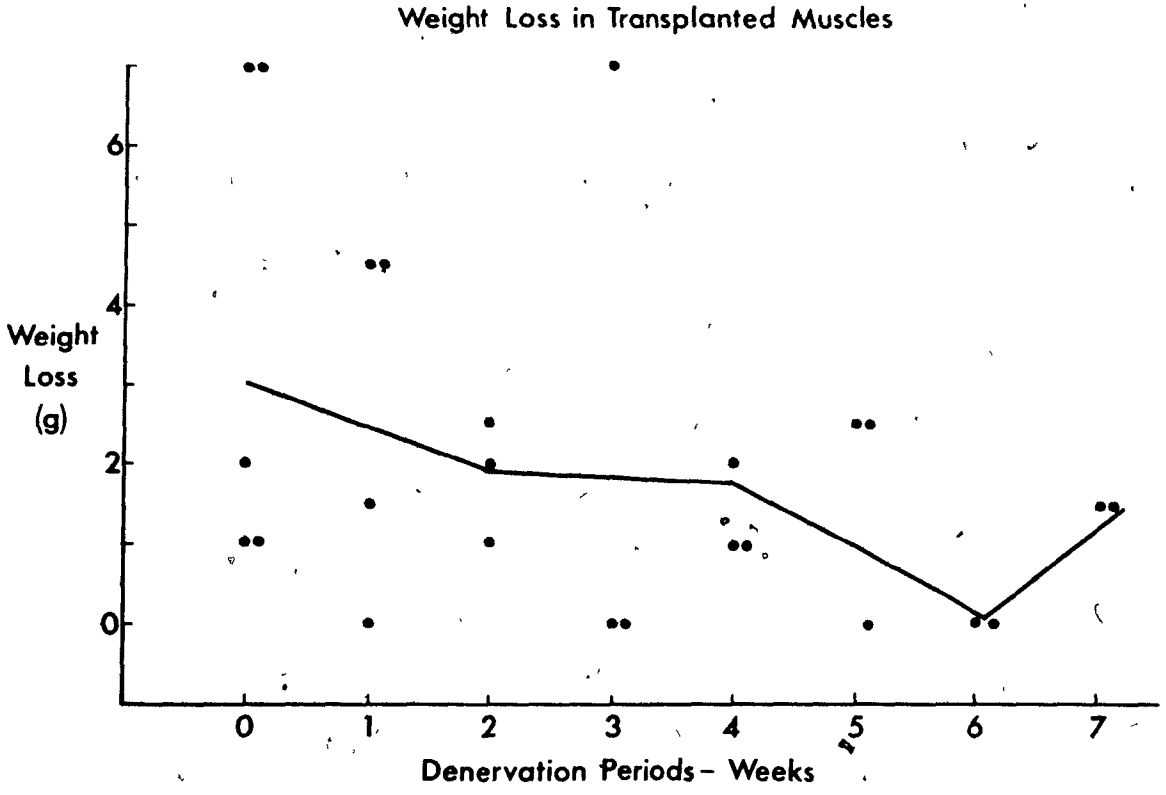


Figure 2

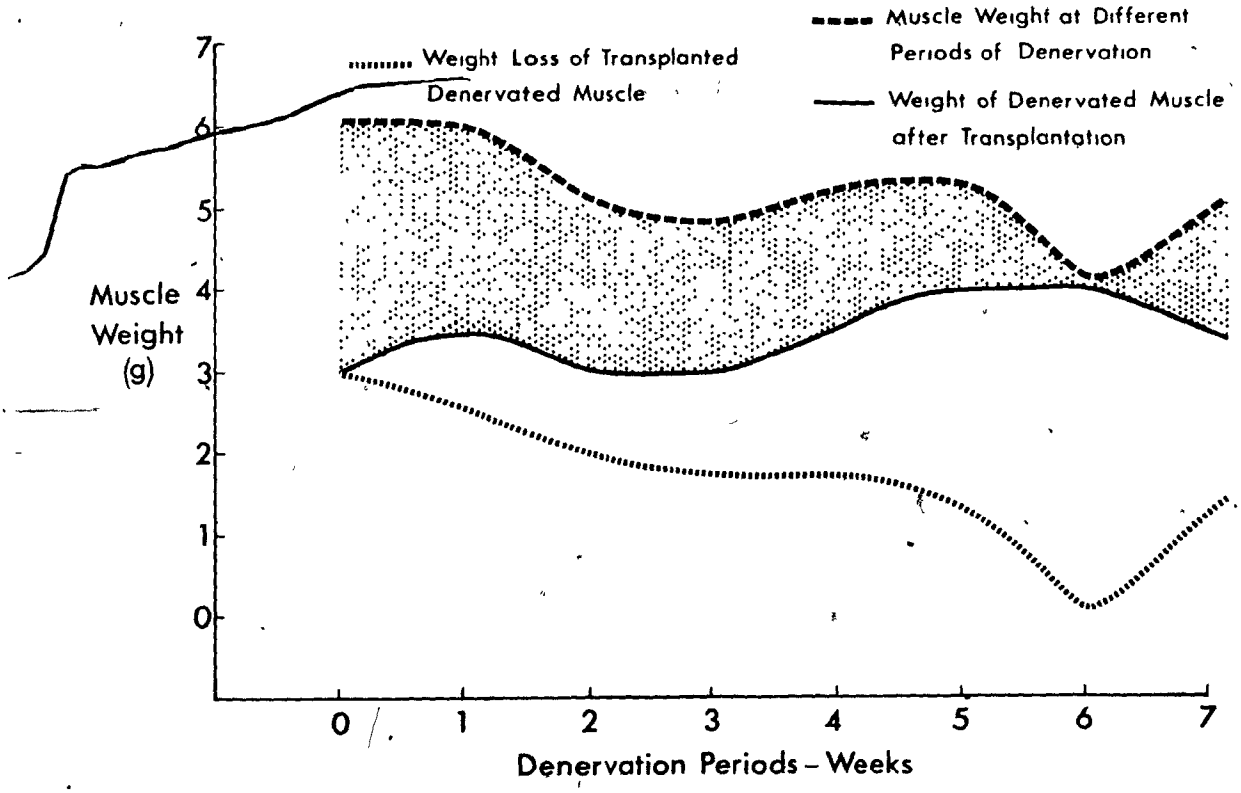

Muscle Weight at Different Periods of Denervation
Weight Loss of Transplanted Denervated Muscle
Weight of Denervated Muscle after Transplantation
Muscle Weight (g)
0
1
2
3
4
5
6
7
Denervation Periods – Weeks

Figure 3

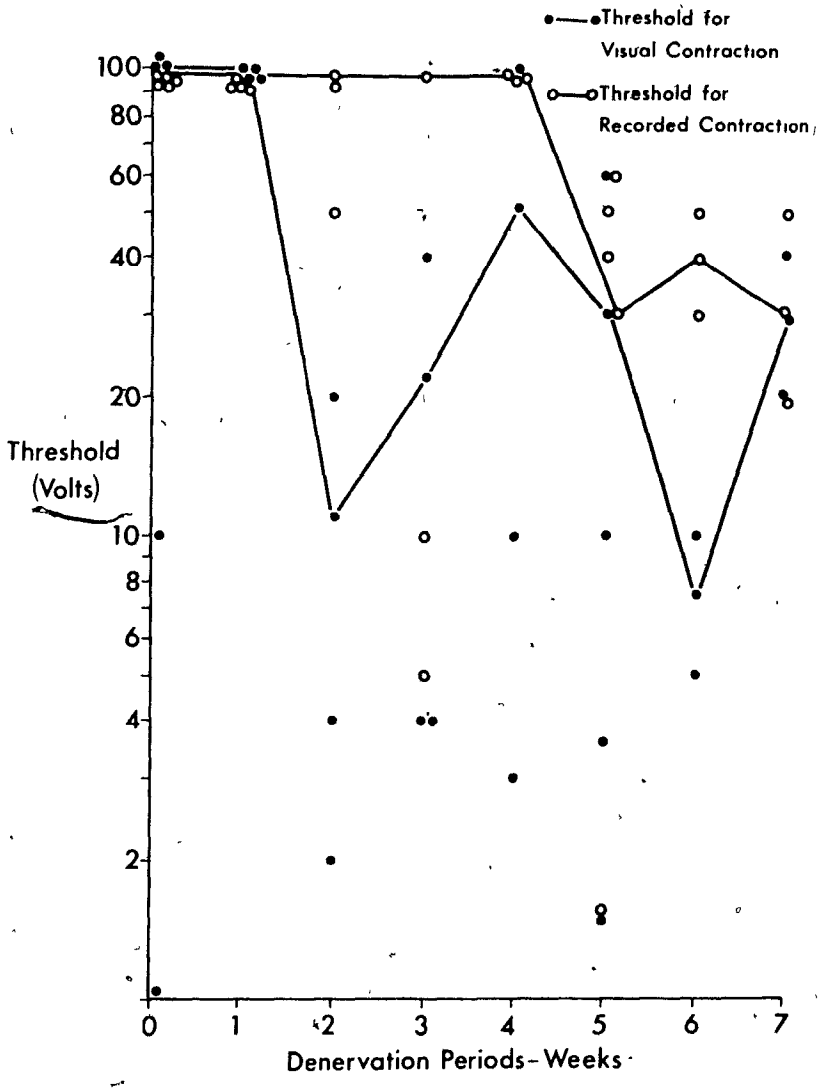

Threshold for
Visual Contraction
Threshold for
Recorded Contraction
100
80
60
40
20
10
8
6
4
2
Threshold
(Volts)
0
1
2
3
4
5
6
7
Denervation Periods-Weeks

Figure 4

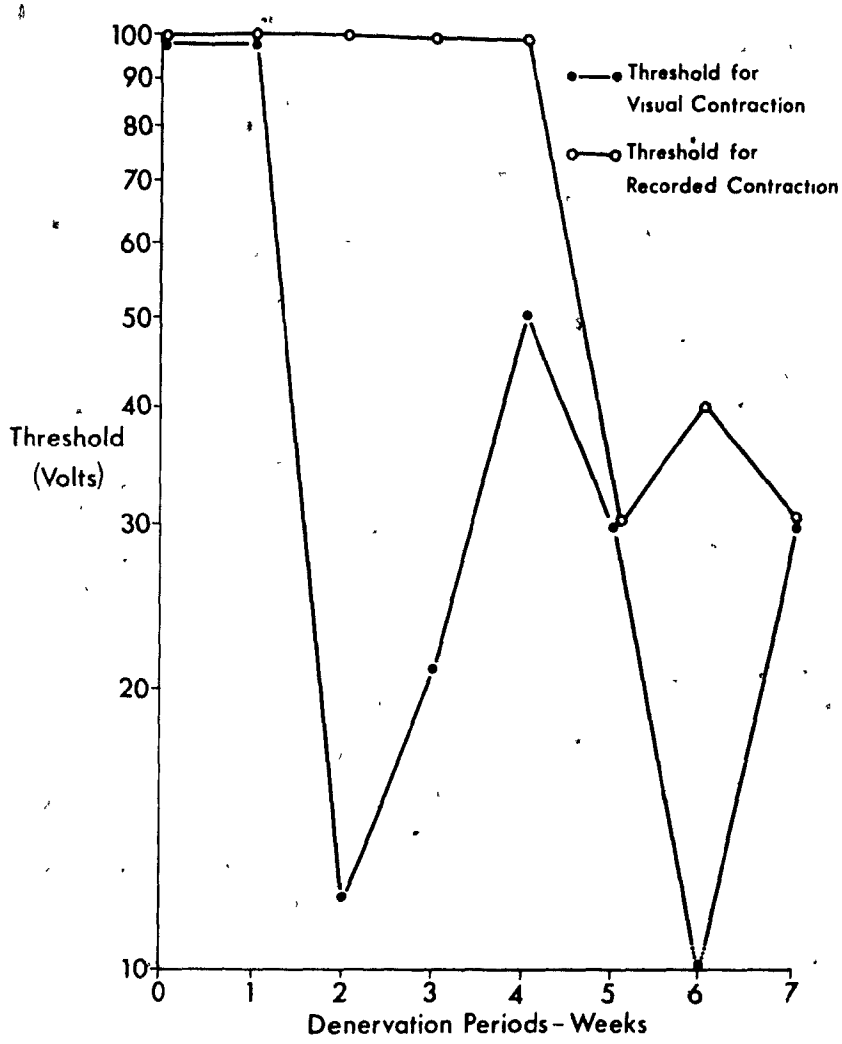

100
90
80
70
60
50
40
30
20
10
Threshold
(Volts)
Threshold for
Visual Contraction
Threshold for
Recorded Contraction
0
1
2
3
4
5
6
7
Denervation Periods - Weeks

Figure 5

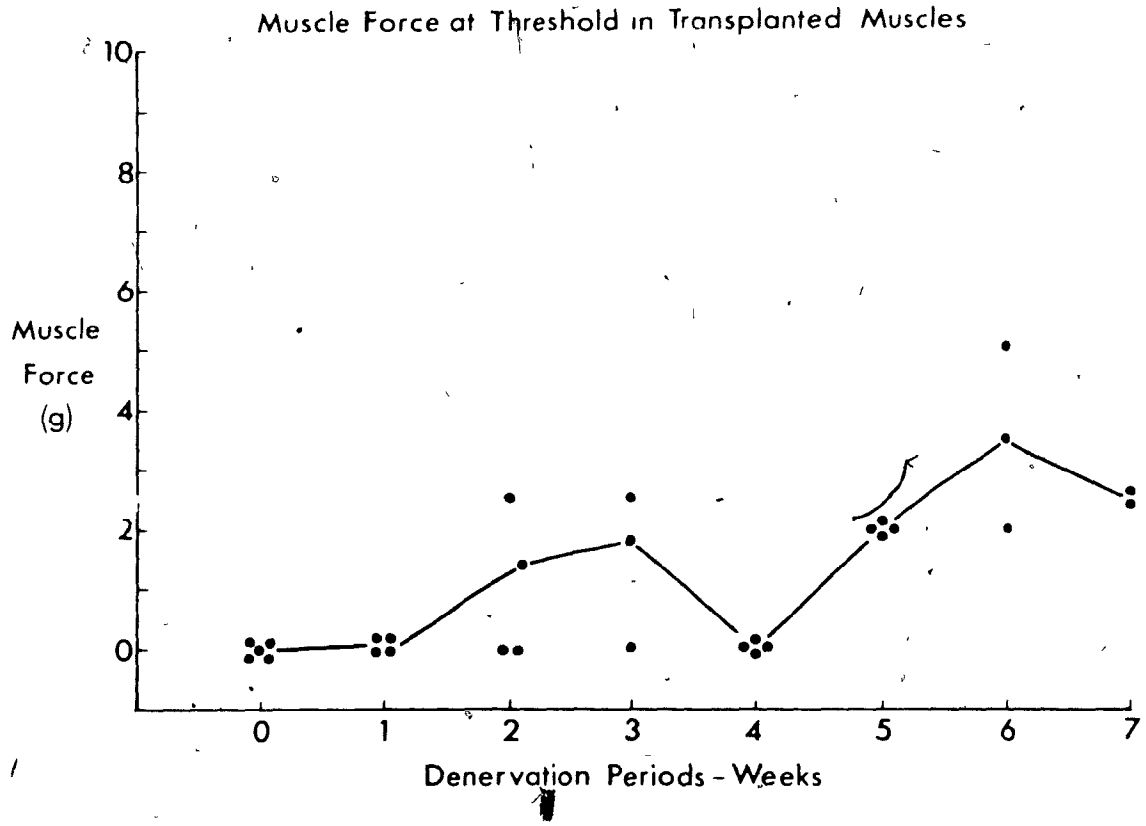

Muscle Force at Threshold in Transplanted Muscles
Muscle Force (g)
10
8
6
4
2
0
0
1
2
3
4
5
6
7
Denervation Periods - Weeks

Figure 6

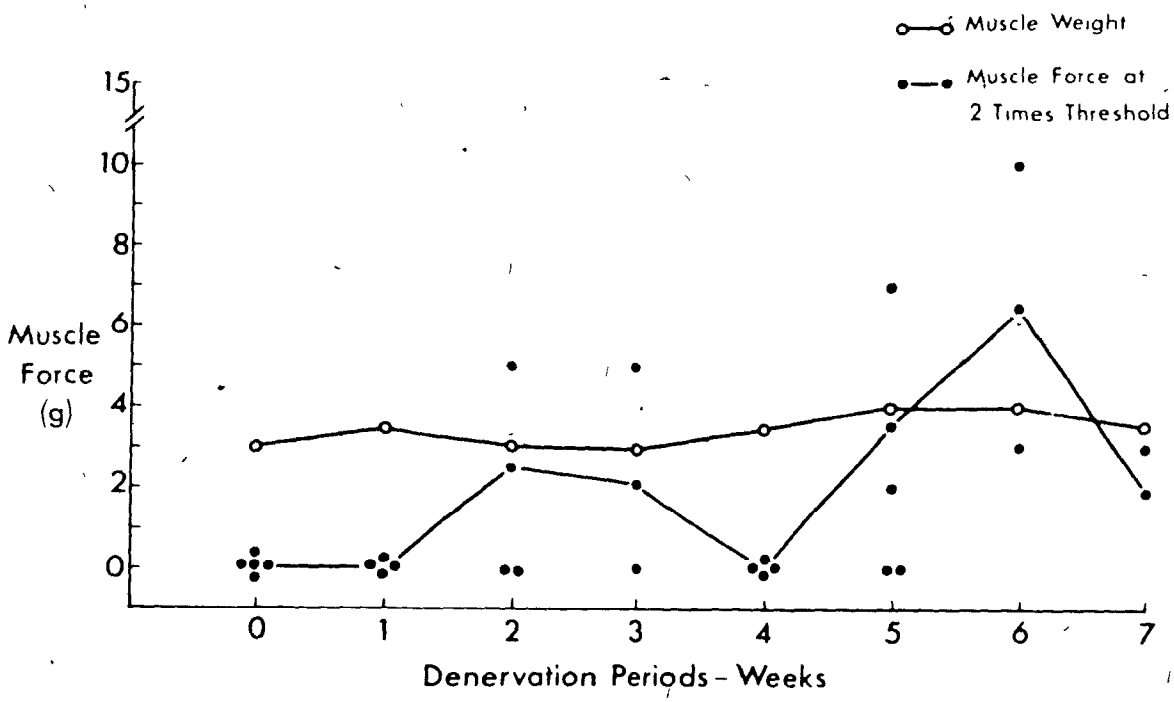


Figure 7

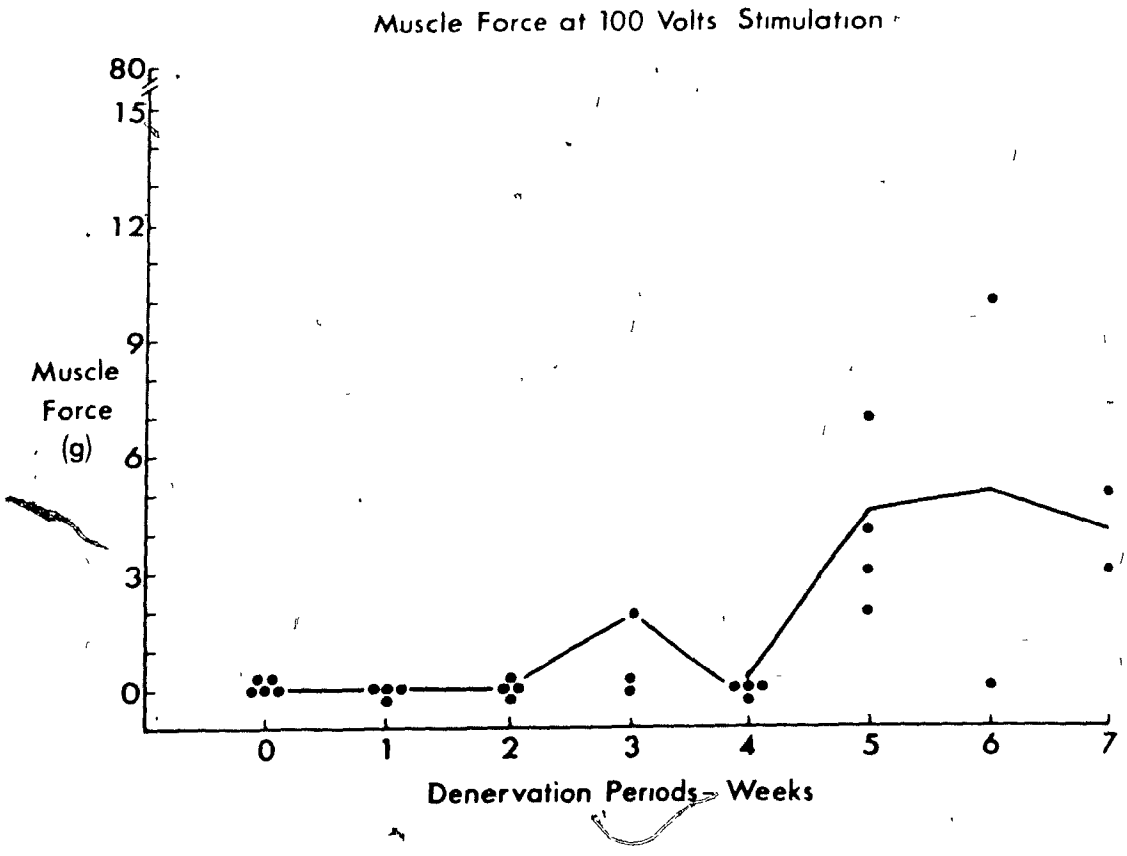


Figure 8

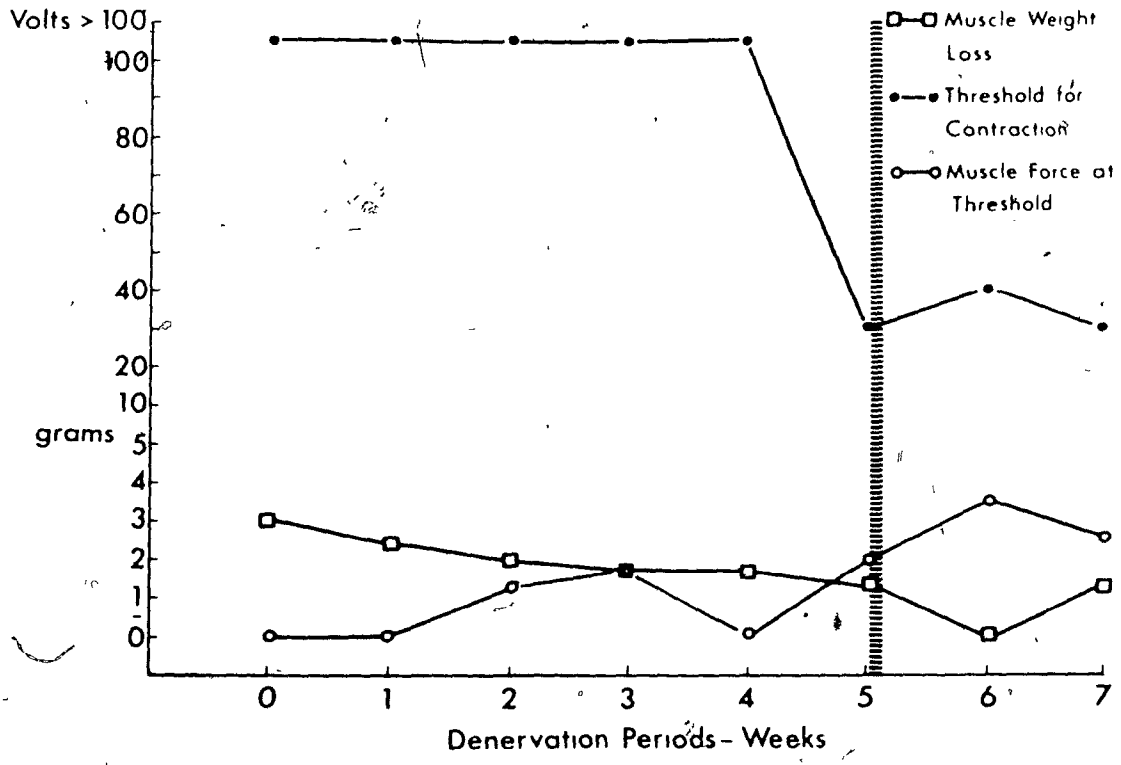

Volts > 100
100
80
60
40
20
10
grams 5
4
3
2
1
0
0
1
2
3
4
5
6
7
Denervation Periods - Weeks
Muscle Weight Loss
Threshold for Contraction
Muscle Force at Threshold

Figure 9

Denervation periods (weeks)	Normal Rabbit Muscle	O^{R}*	O^{L}**	O^{R}	O^{L}	O^{R}	I^{R}	I^{L}	I^{R}	I^{L}	2^{R}	2^{L}
Threshold (volt)	4	^^O	O	O	O	O	O	O	O	O	O	O
Twitch Tension (grams) 2T+	-580	"	"	"	"	"	"	"	"	"	"	"
Twitch Tension 10T	-600											
Rise Time 2T	0.025	"	"	"	"	"	"	"	"	"	"	"
Rise Time 10T (sec)	0.025											
Fusion Frequency (n/sec)	40	"	"	"	"	"	"	"	"	"	"	"

*R - Right
**L - Left
^^O - No response to maximal stimulation
T^{+} - Threshold

Table 1a: Physiological Parameters Versus Denervation Periods

Denervation periods (weeks)	2^{R*}	3^{R}	3^{R}	3^{L*}	4^{R}	4^{L}	4^{R}	5^{R}	5^{L}	5^{R}	5^{L}	6^{L}	6^{R}	7^{R}	7^{L}
Threshold (volts)	50	0^^	10	5	0	0	0	1.5	60	50	40	50	30	20	50
Twitch Tension (grams)															
$2T^{+}$	5	0	5	2	0	0	0	0	0	7	2	10	3	1	3
10T	0	0	2	2	0	0	0	0	0	0	0	0	0	5	0
100T	0	0	2	0	0	0	0	4	3	7	2	10	0	5	3
Rise 2T	0.03		0.08	0.08										0.05	0.03
Time 10T	0	0	0.10	0.08	0	0	0	0	0	0	0	0		0	0
Fusion Frequency (n/sec)	6	0	6	6	0	0	0	0	0	10	0	18	21	6	6

R^{*} - Right

L^{*} - Left

0^^ - No response to maximal stimulation

T^{+} - Threshold

Table Ib: Physiological Parameters Versus Denervation Periods

Figure 10 Normal Muscle

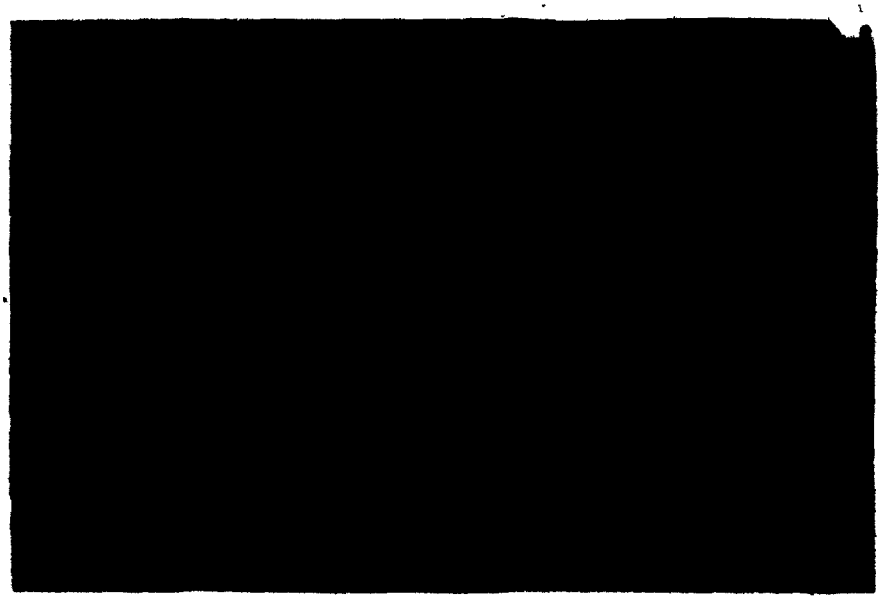
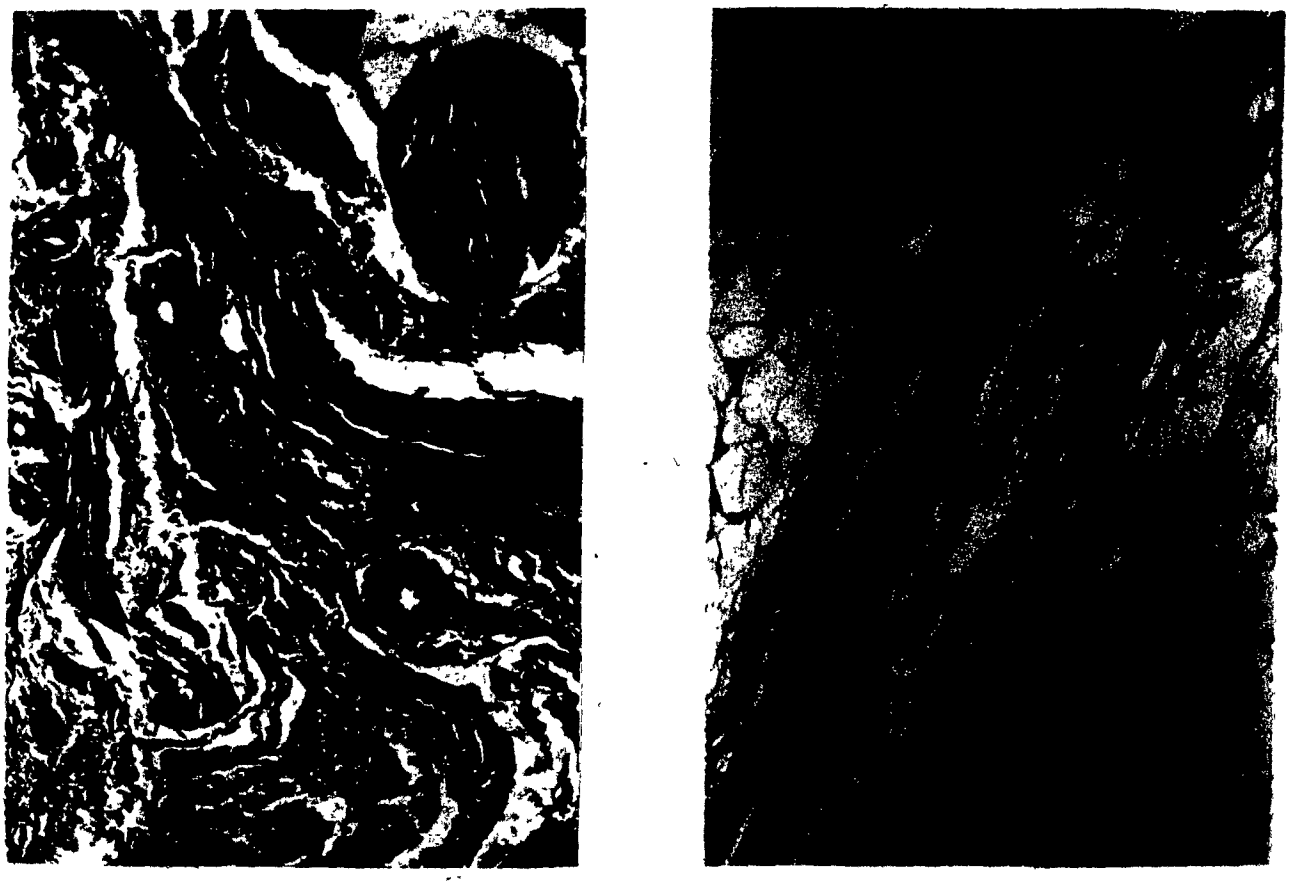


Figure 11: O week denervated Muscles



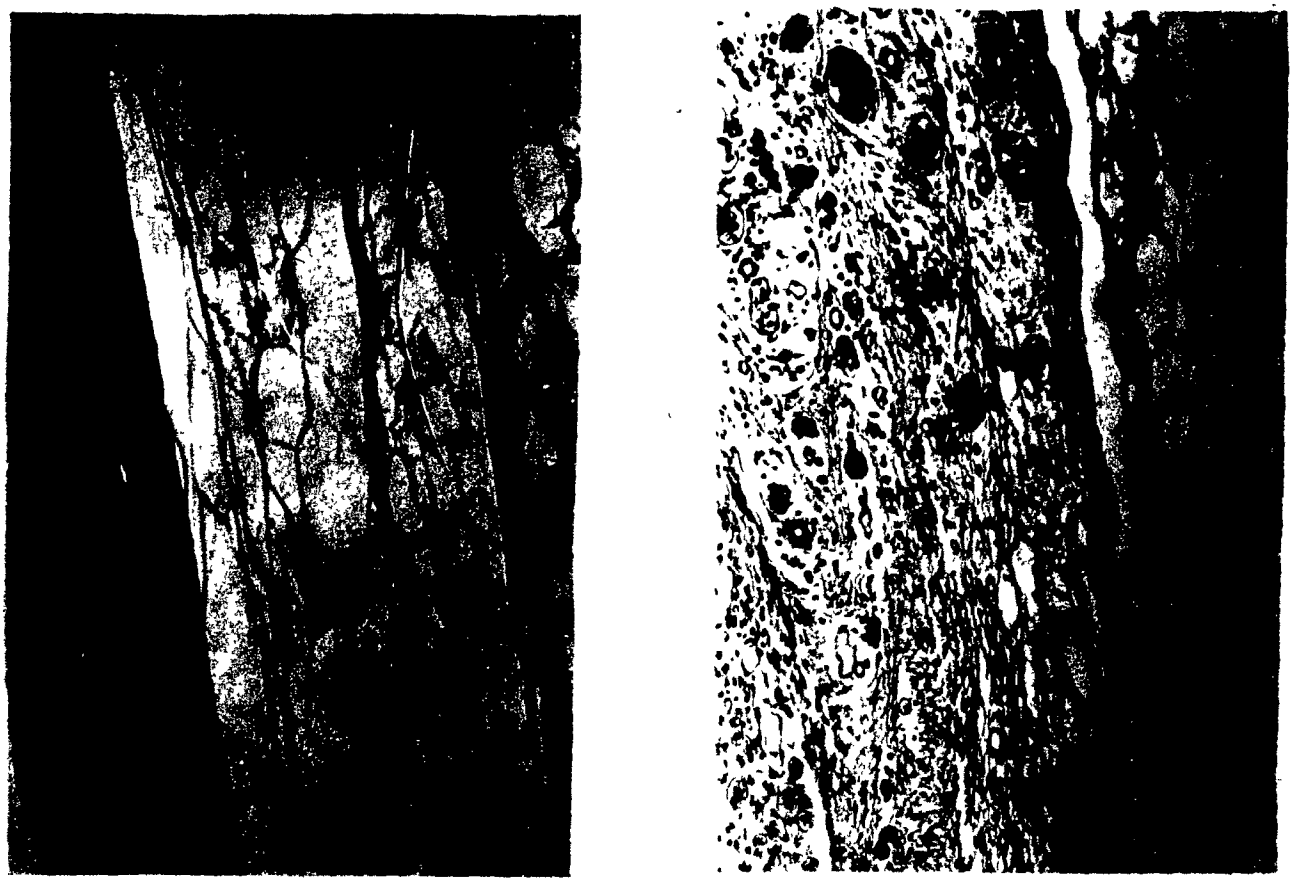
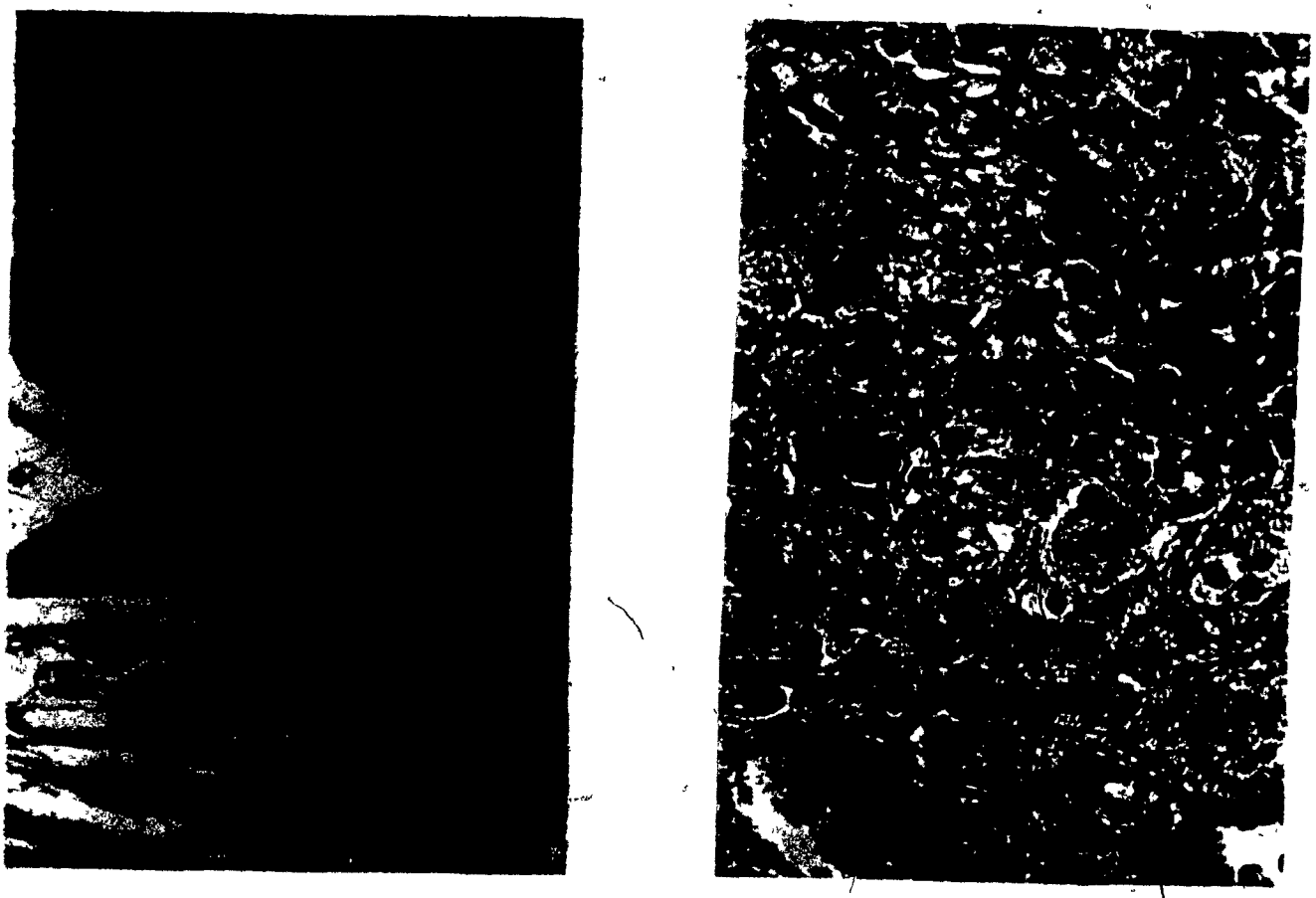


Figure 12 1 week denervated muscles



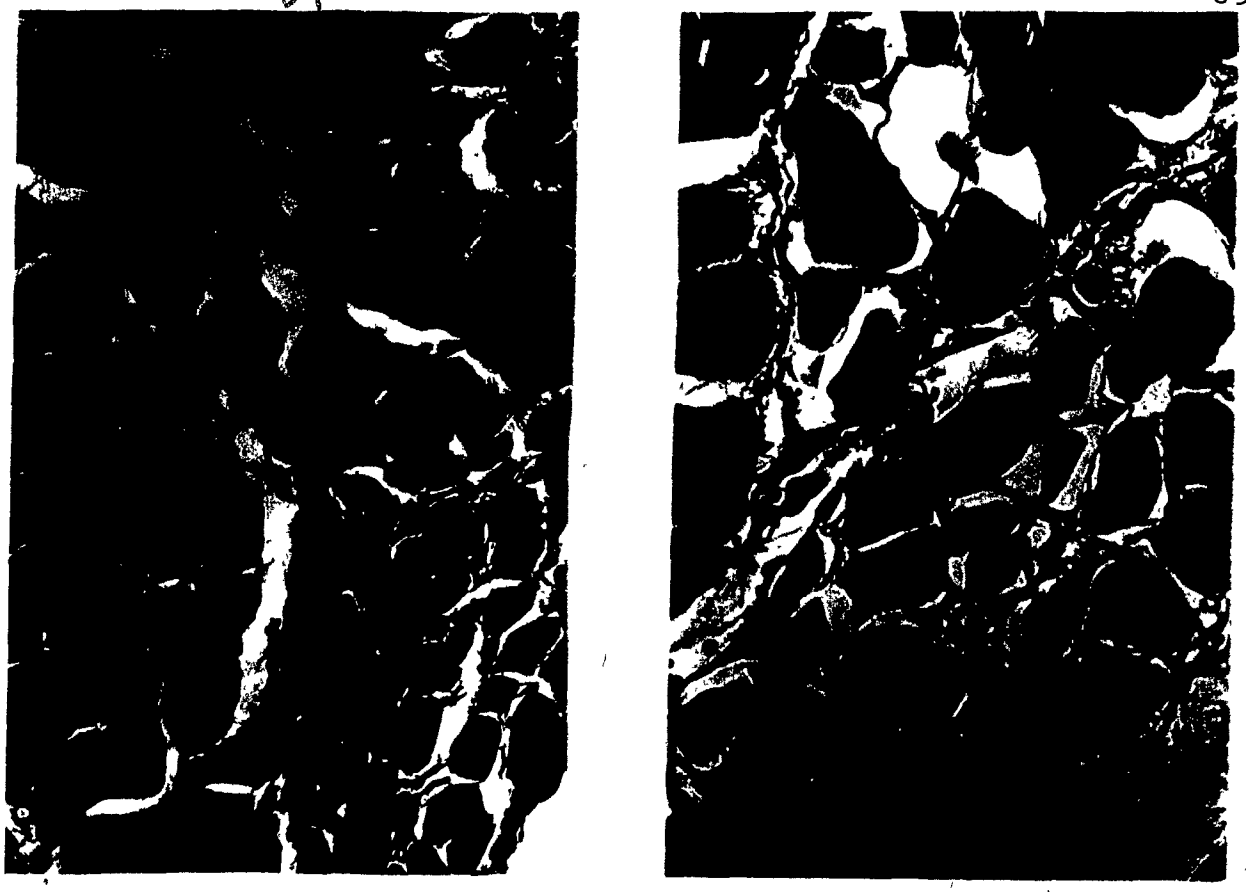


Figure 13: 2 week denervated muscles

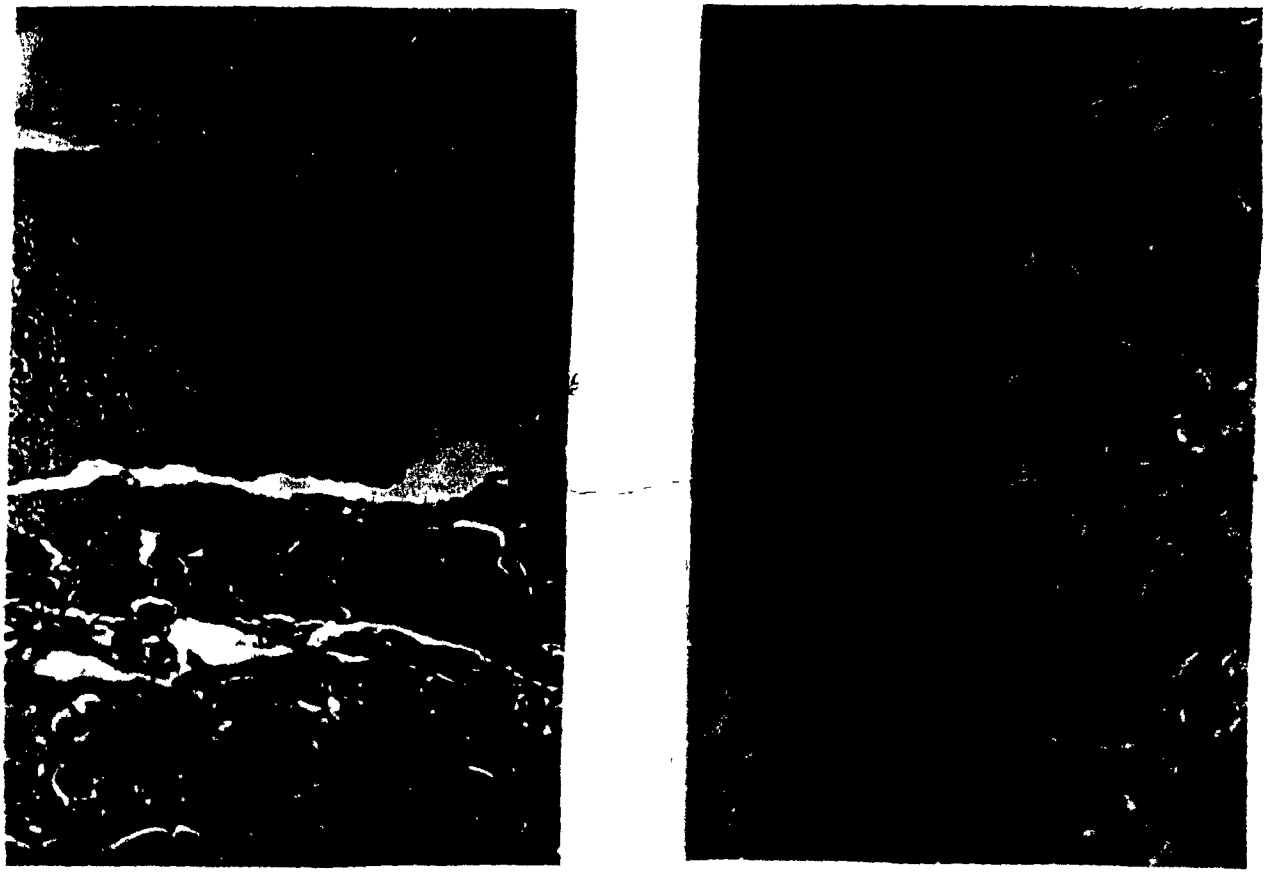
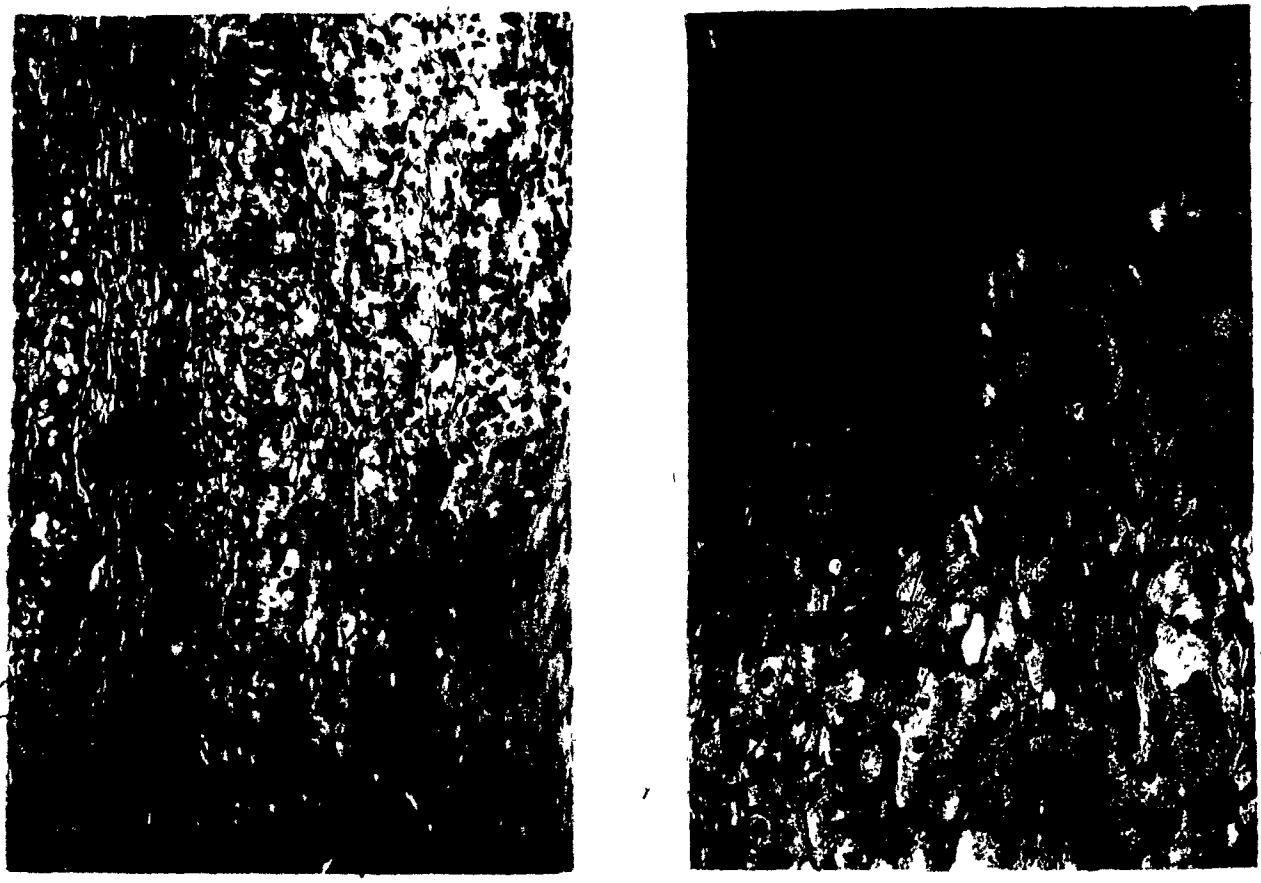


Figure 14. 3 week denervated muscles



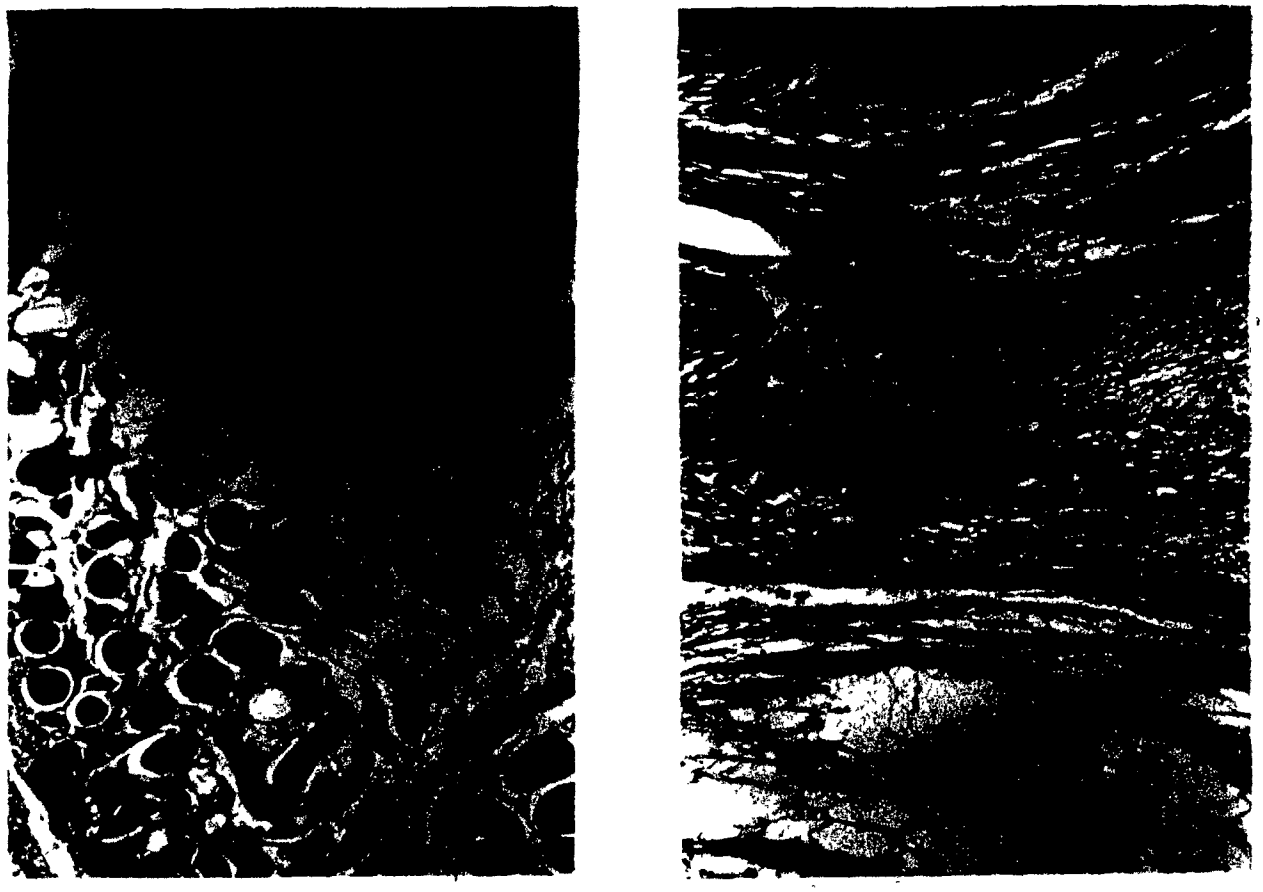
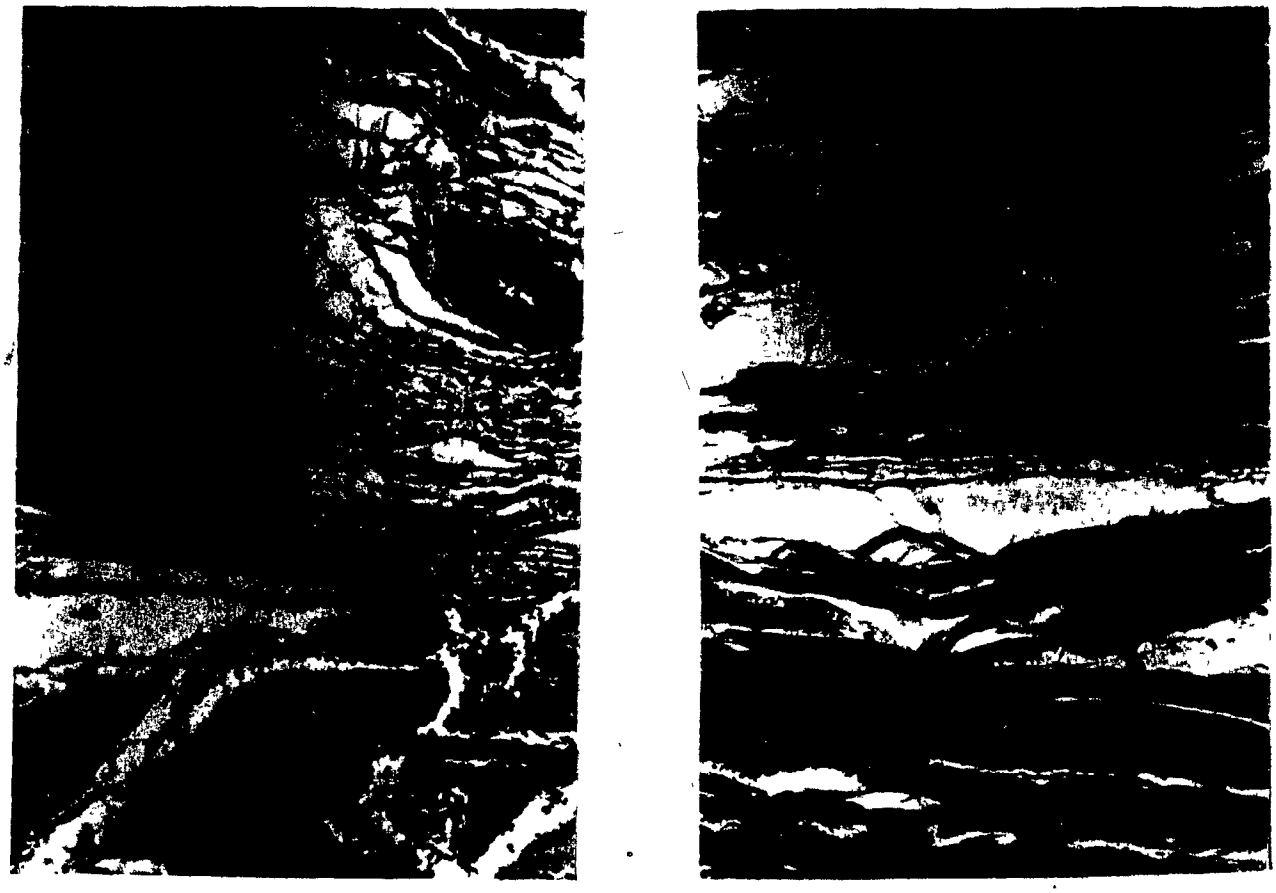


Figure 15 · 4 week denervated muscles



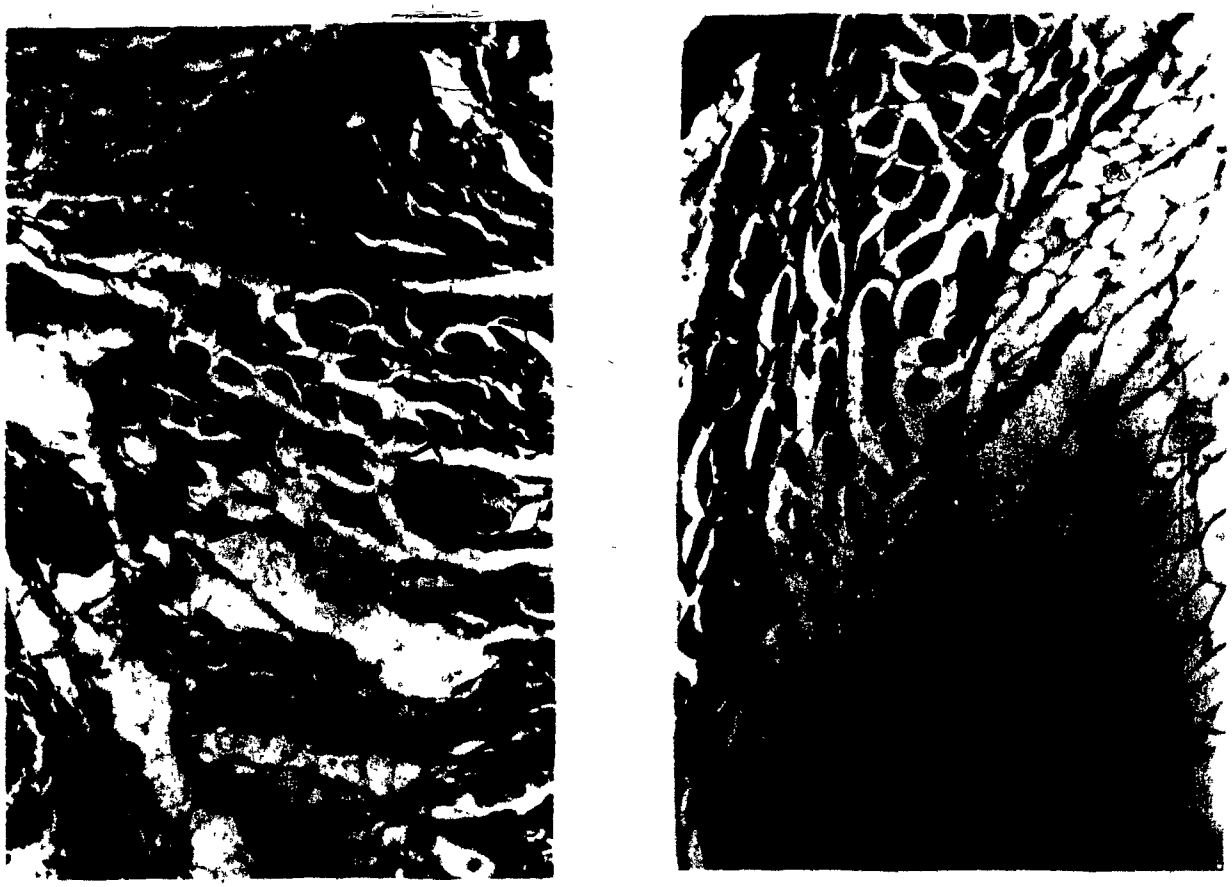


Figure 16A 5 week denervated muscles

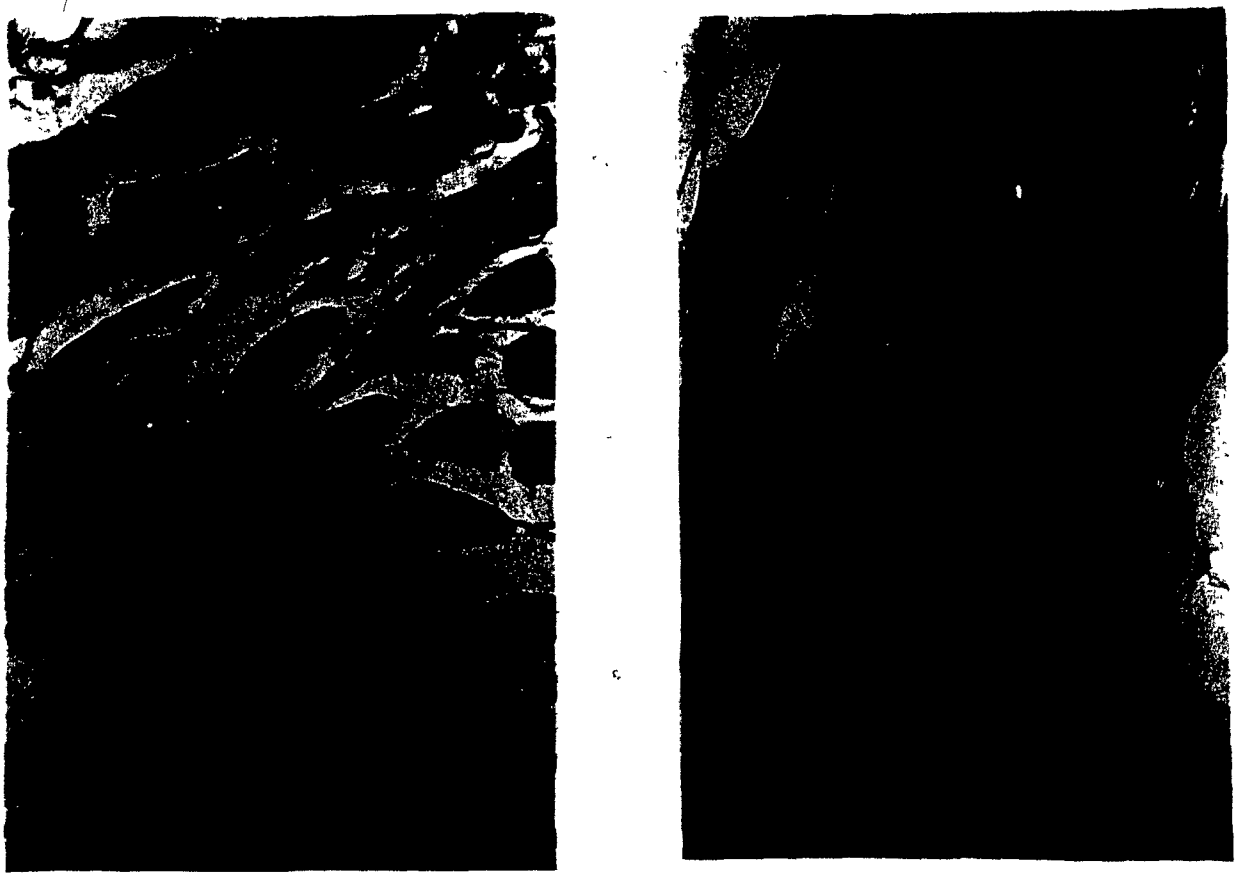


Figure 16B : 5 week denervated muscles

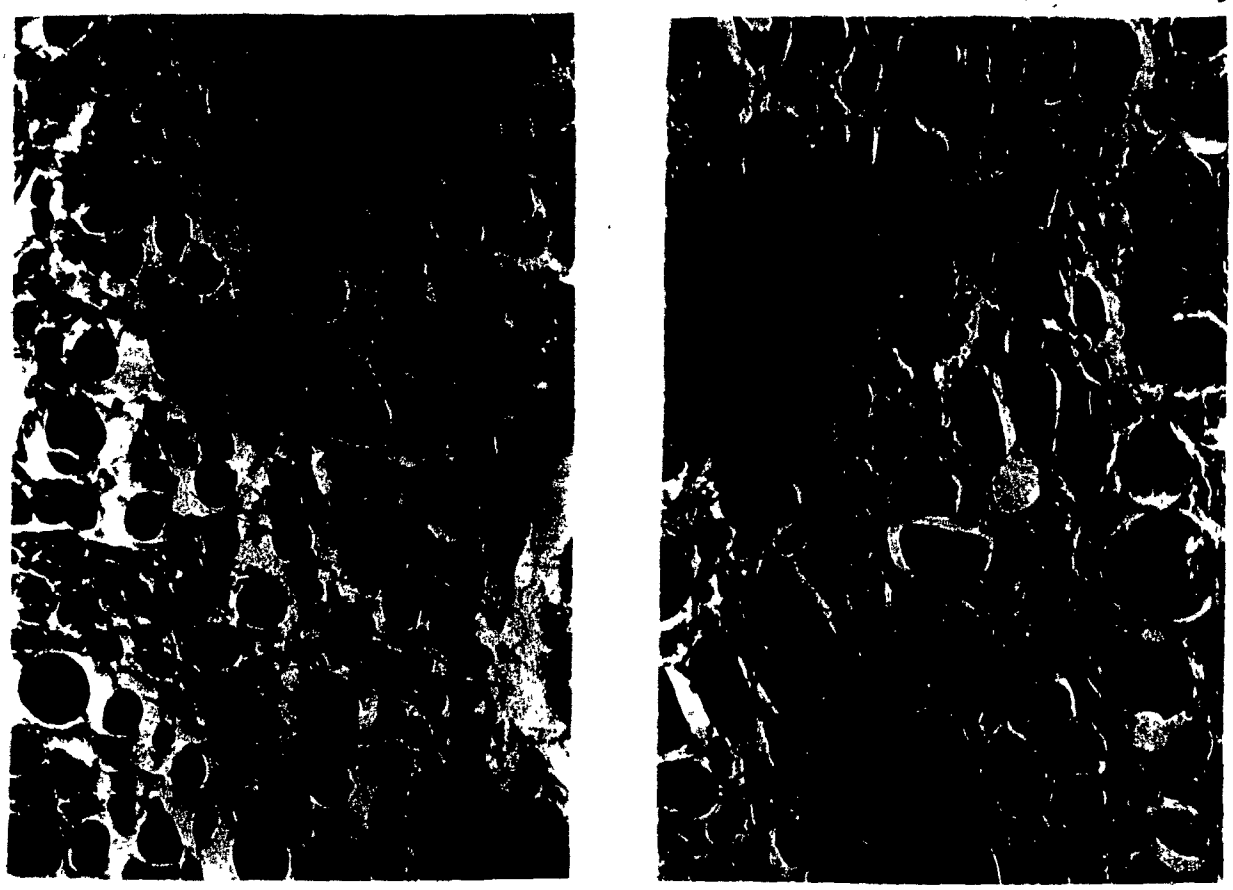
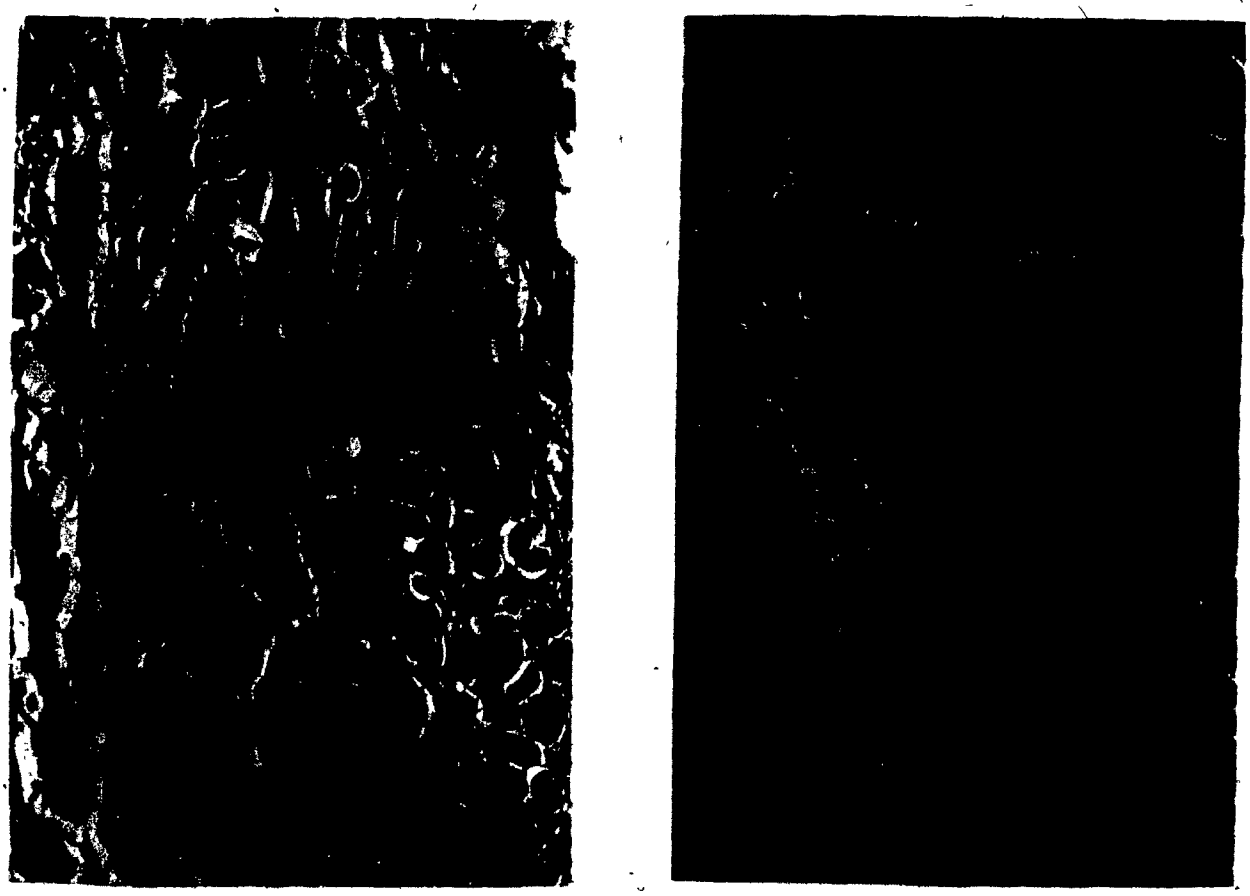


Figure 17. 6 week denervated muscles



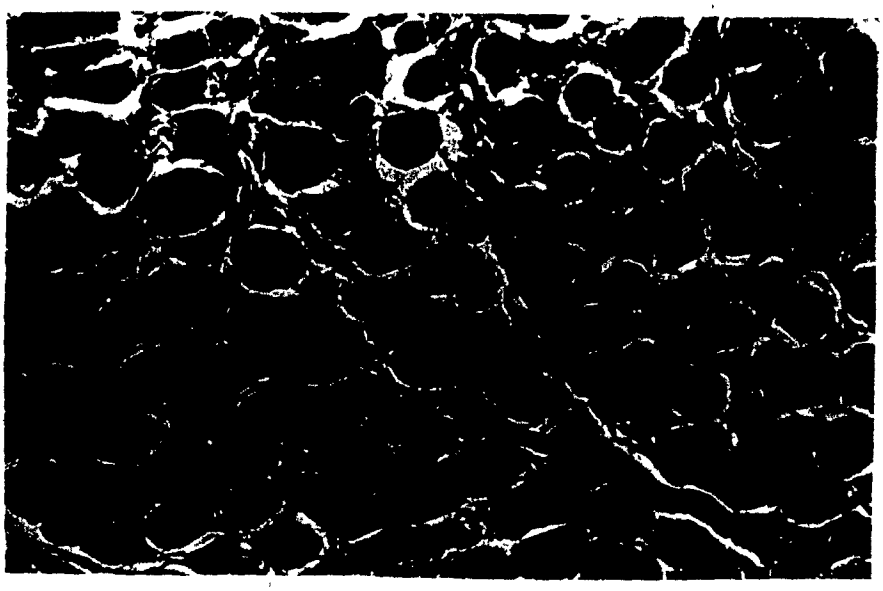


Figure 18 7 week denervated muscles

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