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Histological Study of Reattachment of Masseter Muscle After Its Surgical Detachment from Its Bony Attachment

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HISTOLOGICAL STUDY OF REATTACHMENT
OF MASSETER MUSCLE AFTER ITS
SURGICAL DETACHMENT FROM
ITS BONY ATTACHMENT

by
Virendra K. Seth

A Thesis Submitted to the Faculty of the Graduate School of
Loyola University in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

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Virendra Kumar Seth was born in Layallpur, India, on August 23, 1937. He finished his high school studies in Queens College, Banaras, India in 1952. He graduated with a degree of Bachelor of Science (B. Sc.) from Banaras Hindu University, Banaras, India in 1956. He enrolled at Dental College and Hospital, Lucknow University, Lucknow, India, in June 1956 and completed his studies for the degree of Bachelor of Dental Surgery (B.D.S.) in June 1960. He worked as House Surgeon in the Dental College and Hospital, Lucknow, India from June 1960 to July 1961. During the period of August 1961 until July 1962 he was appointed as a Clinical Fellow in the Murray and Leonie Guggenheim Dental Clinic, New York, New York. In September 1962, he began a two year graduate program at Loyola University leading to a Master of Science Degree in Oral Biology.

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INTRODUCTION

In connection with the fact that recent research on regeneration processes is not confined to the tissue aspect only, the study of conditions contributing not solely to tissue but also to the organs, regeneration of muscles is of importance.

Experimental histology is used for studying correlation between tissues and between the latter and the organisms for, the development of the organism and the carrying out of its normal and pathologic reactions to external stimuli. There are several works devoted to analysis of the behavior of striated muscle tissue in vitro. The data obtained and their analysis enabled us to plan further research.

In our laboratory, an attempt is made to elucidate the role played by the muscle and the periosteum from which the muscle is detached during the post-surgical healing period.
REVIEW OF LITERATURE

The regenerative capacity of striated muscle as reported in some early observations of Forbus (1926), Miller (1934), Levander (1945), and Speidel (1937) was concerned mainly with the initial stages of regeneration. In general they dealt with material in which the regeneration process was abortive.

As early as 1865 Waldeyer pointed out that regeneration of skeletal muscle could occur after injury. Since then others have shown that muscle regeneration may follow various types of damage including trauma, (Weber 1867, Newman 1868), ischaemia, (Clark, and Blomfield 1945), and toxic degeneration (Hoffman 1867, Forbes 1926). More recently the details of the histological processes involved in muscle regeneration were described by Clark (1926). It has been established that some degree of muscle repair can take place even in the complete absence of innervation as in the case of transplanted muscle tissue Jones (1949), Clark (1946).

The knowledge about muscle tissue by experimental methods is inadequate. This is coupled with the fact that the majority of biologists hold a concept of limited plastic capacity of the muscle tissue. Many investigators believe that the regeneration processes are effected in the muscles by some specific method through the formation of muscular buds, prominences of muscle fibers without the development of any cellular elements of the type
of myoblasts responsible for the histogenesis of skeletal muscles (Clark, L. G., Bloomfield, L. B., 1945, 1946, and 1947).

Studitsky (1949), demonstrated the high regenerative capacity of muscle tissue in birds, which ensures the restoration of muscles after removal of a segment measuring one third its length. It was further demonstrated that muscle tissue of rats has similar plastic property by Ignatieva (1949). These facts are in complete accord with the biologic theory of regeneration, Studitsky (1952) according to which the level of the regeneration activity of tissues should be in proportion to the level of metabolism, noted for its high intensity in birds as well as in such mammals as rats.

The traumatic factor has always been a factor of importance during the investigation on the regeneration of muscle. Various workers have discovered characteristic reactions of muscle to traumatic lesions. These include swelling of the impaired muscle fibers; amitotic division of the nuclei, which leads to the formation of long chains of nuclei; the appearance of muscle buds on the muscle fiber stumps; and finally appearance of myoblasts. On the basis of numerous experiments which he conducted and of published material, Studitsky (1958 and 1960) came to the conclusion that all of these changes comprise the reaction of muscles to impairments of their activity. He suggested that the complex of all these changes be called the plastic state of the muscles. He demonstrated such changes in state can be brought about by any impairment of their
vital activity: mechanical trauma, denervation, tenotomy, immobilization. Plastic processes are specially obvious during mechanical traumas. The plastic state insures the development of the myogenic tissue necessary for the restoration of the muscular functions and structures impaired by a harmful agent. In a special investigation Studitsky (1960) demonstrated the influence of a preliminary trauma on the restorative reaction of muscle tissue: the gastrocnemius muscle of rats was damaged by making two or three transverse cuts, while the biceps muscle of young cocks was cut in two. Several days later a second operation was performed that is, excision of the entire middle portion of the traumatized muscle. The result obtained showed that the rate of completeness of the regenerative process following a preliminary trauma increases abruptly as compared with the controls (excision of the middle portion of the muscle without preliminary trauma). It would therefore, appear that a muscle in the plastic state is more suitable for reparative surgery.

The data cited testifies to the fact that the traumatic factor is a powerful mechanism involved in bringing muscles to the plastic state and ensuring the intensive regenerative process of the muscle tissue.

**Histogenesis of Muscle Regeneration:**

According to W. E. Le Gros Clark (1946) no regeneration was observed until the third day after the local crush injury. The histological changes observed during the first two days were hyaline degeneration and
fragmentation of injured muscle fibers, endomysial oedema, and the invasion of the damaged area by a large number of polymorphonuclear leucocytes and histiocytes. He further states that in the case of fibers which have been crushed the sarcolemmal tubes of the damaged segments of the muscle fibers becomes rapidly filled with histiocytes, and by the second day they are tightly packed with these cells. In this manner the reaction clots are formed on the crushing of muscle.

The first sign of regeneration is a plasmodial outgrowth of granular sarcoplasm which protrudes from the stump of the uninjured part of the fiber. Where the sarcolemmal sheath has preserved its integrity, the outgrowth extends along it in an even course forming a cone shaped mass with a blunt tip. Normally, the tip of the outgrowth is extremely fine and pointed, but if it meets with obstruction in its continuous outgrowth, the terminal part may spread out to form large multi-nucleated masses. The nuclei within the outgrowth tend to be arranged in parallel rows. The sarcoplasm often shows a longitudinal fibrillation. Where the sarcolemmal sheath is disturbed or damaged, the outgrowth is correspondingly irregular.

Based on extensive histological material of regenerating muscle Clark (1946) states that the new muscle fibers are probably entirely formed as direct and continuous outgrowths derived from old muscle fibers. It has been suggested by Levander (1941 and 1945) that myoblasts are formed from disintegrated and isolated fragments of damaged fibers so that they are
not formed in direct continuity with old fibers. Levander (1945), further states that in the course of regeneration of mammalian muscle new myoblastic elements arise by the differentiation of generalized connective tissue cells, which he supposes results from a process of induction. In support of his contention, he gives a microphotograph showing isolated myoblasts. According to Clark (1946) this is due to the oblique sectioning of long continuous strands which can be ultimately traced without interruption to the stump of preexisting fibers.

It has been noted and figured by Forbus (1926) that as the young regenerating fibers grow down into the necrotic zone of the devascularized muscle it is not uncommon for them to divide so that two or three new fibers are found to be formed from the stumps of a single old fiber. He further states that the reconstituted tissue in the necrotic zone of a muscle may contain many more fibers than the normal muscle. In one experiment, in which the tibialis anterior of one side had been devascularized for twenty-one days, transverse sections through the regeneration of the lower part of the muscle were compared with equivalent sections of normal muscle of the other side. Counts of the muscle fibers were made by Clark (1946), who found that the regenerated fibers on the operated side were approximately 50 per cent in excess of those of the normal side. On the other hand in the experiment in which regeneration was allowed to proceed for four months, the tibialis anterior was of approximately normal bulk and the sections showed the regenerated portion to contain muscle fibers of
approximately normal diameter. His interpretations of these findings are that at least some of the excess fibers which are first formed do not gain maturity. On the other hand, in the four months material bifurcating fibers are still occasionally to be seen.

In a careful study of all the histological material of regenerating muscle, Clark (1946) states that nuclear proliferation of growing fibers takes place normally by amitosis. Occasional mitotic figures are seen in the sarcolemma sheath, but it appears probable that in many instances these belong to histiocytes which still remain within the sheaths. In one experiment in which the gracilis muscle of a rat had been crushed four days previously, colchicine was administered to the animal ten hours before death. As a result the sections showed many mitosis in the connective tissue elements at the site of injury, but no mitosis could be definitely assigned to the sarcoplastic outgrowth of regenerating fibers. It may be inferred, therefore, that if mitosis does occur in the regenerating fibers, it is an unusual phenomenon.

According to Speidel (1938) after muscle fibers have been injured leucocytes may be attracted to the site and become active in dispersing of debris and in expediting other adjustments. He has cited a case in which the remains of an injured fiber several days after the injury including a portion of the sarcolemma and an enclosed myoblast which underwent mitosis. A leucocyte had invaded the space within the
sarcolemma. He also mentioned the presence of an active macrophage within the sarcolemma of an injured but recovering fiber.

In discussing the recovery of many muscle fibers after gross injuries Speidel (1938) states that the regenerated new muscle fibers are not arranged in an orderly myotonic or segmental fashion, but display quite an irregular arrangement.

Reaction of Soft Tissue to Injury:

A problem of regeneration of muscle tissue has attracted the attention of a number of authors Chlopin (1946), Nikitin (1955), Volkman (1893), Zenker (1964), Zavarzin (1938). Some of them Volkman (1893) and Zavarzin (1938) admitted that the course of regeneration may depend upon the character of the damage. In 1955 and 1961 Nikitin undertook studies on traumatized muscle tissue regeneration. His observations showed that under conditions of regeneration, the skeletal muscle tissue in mice and rats possessed a reactivity and plasticity.

The type of damage applied affects the microstructure of the wound focus and thus determines, not only the course of regeneration process, but its final result as well. In a case when the muscle fiber and the stroma structures in the focus of trauma are completely severed, the neoformation of muscle fibers occurs through continuity between the damaged and regenerating fibers.
When a large muscle is subjected to trauma, a striking sequence of events ensues. Clark (1946), Forbus (1926), Johnson (1952), Gilmer and Anderson (1959) have presented their findings as follows:

The damaged area is roughly spherical with maximum damage at the center. The associated hemorrhage also centers in the area of the greatest damage. Initially there is degeneration and necrosis of the tissue and retraction of the disrupted muscle fibers. Within two days histiocytes invade the injured tissue region and the necrotic debris is removed. Dystrophic calcification of damaged muscle fibers may be marked. This is a transient phenomenon and does not appear to be related to subsequent bone formation. Within three or four days fibroblasts from the endomysium extend into the damaged area and rapidly form broad sheets of immature fibroblastic cells. At the same time primitive mesenchymal cells proliferate within injured fascia and other connective tissue. Simultaneously, sarcolemma nuclei at the ends of damaged muscle fibers begin to proliferate. First, chains and columns of plump polyhedral cells appear. Within a very short time the production of sarcoplasm becomes evident in buds, straps, or club like projections which are in continuity with preexisting fibers, and the sarcolemma nuclei clusters in the form of a clot, granulation tissue, or proliferating endomysium, they are blunt, turned aside, or made to branch.
Circumscribed Bone Formation:

The term "Myositis Ossificans" is used here in a sense related to the formation of new bone or cartilage, following trauma to the soft tissue. In this we implicate the process as well as the product.

Gilmer Jr. and Anderson (1959) have described Myositis Ossificans as the localized formation of heterotopic, non neoplastic bone or cartilage that results from physical trauma and usually occurs in or adjacent to the muscle and in proximity to the bone. The lesion may appear at several anatomic sites dependent upon circumstances.

It is generally agreed that injury plays an important etiological role in the heterotopic ossification of skeletal muscle and that the formation of reparative fibroblastic tissue, with or without preceding hemorrhage, is a frequent antecedent to the bone development. The subject has been extensively studied by Geschickter and Maseritz (1938), and Constance (1954), but the origin of bony tissue is still a matter of speculation.

In some cases of traumatic myositis ossificans the precipitating factor may not be clearly evident, however, a careful study will usually uncover some previously undetected or ignored local trauma. A simple blow from a striking object or from a fall, recurrent minor athletic injuries, gunshot wounds, fractures, sprains, dislocations, joint damage, and severe muscle strain have all been cited as obvious causes. Daley (1961).
Johnson (1957) has reported three such cases when extra-articular ossification, ankylosis, and associated soft tissue contractures occurred during long periods of hospitalization for burns. Postoperative scar tissue may also afford a media for ossification. Bony tumors have been observed to develop in scars following suprapubic prostatectomy, cholecystectomy and gastrectomy. McGinnis and Leavitt (1958), Abeshause (1948), and Sanders (1955).

A possible clue to the pathogenesis of osseous tissue formation seen within surgical scars of the abdomen has been suggested by McGinnis and Leavitt (1958). Both the linea alba and linea transversa (which are divided by midline abdominal incision) may contain osteogenic rests of rudimentary sternum and rib. McGinnis and Leavitt (1958) postulated that either these rest cells or normal fibroblasts can be stimulated by muscle injury or ossification. Mesimian's (1952) experimental studies on guinea pigs support his belief that heterotopic bone growth depends upon the presence of osteoblasts from pieces of living bone and periosteum. This view is strengthened by the evidence that the strain of muscle fibers at the point of insertion on bone may dislodge periosteal or osseous fragments which act as foci for extra-osseous bone growth, Sanders (1955) and Johnson (1957). Sanders (1955) goes further, however, to point out that muscle, tendon and connective tissue, because of their mesenchymal origin, contain bone forming elements which may be triggered by any agent or injury sufficient to
cause proliferative repair. Helmen and associates (1949), on the other hand, have offered evidence in rabbits which suggested that calcification can occur in the absence of any osteogenic substance.

Greschickter and Maseritz (1938) state that it is probably direct periosteal trauma occurring through muscle tissue, which most commonly precipitates osseous tumor growth within tissue continuous to a bony surface.

Periosteal thickening and new bone formation have been known to accompany circulatory insufficiency, venous stasis, or edema from various causes, Johnson (1957). Metabolic endocrine, and electrolyte disturbances have been generally mentioned in the literature in relation to extra-skeletal ossification Johnson (1957).
MATERIAL AND METHOD

Eighteen Albino female rats were used for this study. The average weight of the rats was 100 gms. The animals were anesthetized by 0.02 c.c. of 5 per cent solution of Sodium Pentobarbital (Nembutal, Abbot.) intraperitoneally. The surgery was carried out in sterile conditions.

Method:

An incision was made in the skin from the middle of the external ear extending up to 1 c.m. before the angle of the mouth, using a number fifteen blade mounted on a Bard Parker scalpel. After incision in the skin and its subsequent reflection, followed by superficial and deep fascia, the masseter muscle on the lateral surface of the mandible was exposed.

Starting from its inferior border, the muscle was reflected from its bony attachment by a perlosteal elevator (Dr. Ohl's Perlosteal elevator No. 2879, Silverman's Dental Supply House). Approximately the lower two-thirds of the muscle was reflected superiorly from the bony attachment.

The masseter muscle was reflected from the mandible and was placed on the bony surface into its normal position and left loose. The skin flap was repositioned over it and was sutured with 000 black silk using interrupted sutures.
After surgery, the animals were carefully laid down in a position which would facilitate breathing, until the animals recovered from anesthesia. Animals were kept on a regular diet (Wayne Laboratory Blox) for mice and rats, and water. The animals were observed every day until the time of sacrifice.

Preparation of Specimens:
The animals were sacrificed by the lethal dose of Ether (Ethylether Concentrated, Fisher). The interval of sacrifice of the animals was as follows:

1. Forty-eight hours.
2. Ninety-six hours.
3. Seven days.
4. Ten days.
5. Fourteen days.
6. Twenty-one days.
7. Twenty-eight days.
8. Forty-two days.
9. Fifty-six days.

The head of each rat was removed and fixed in 10 per cent neutral formalin solution. The solution was changed in twenty-four hours and the specimens remained in it until fixation was completed. They were then subjected to decalcification in the solution of 90 per cent Formic Acid
with Sodium Citrate solution prepared as follows:

Solution A: 50 grams of Sodium Citrate and 250 c.c. of distilled water.

Solution B: 90 per cent Formic Acid 125 c.c. and distilled water 125 c.c.

Equal parts of solutions A and B were used. The solution was changed every three to four days. Roentgenograms were taken to detect the decalcification of the tissue. Following decalcification, a block of the specimen containing the ramus and the body of the mandible covered by the masseter muscle was cut away from the head. Block sections were cut through the ramus and the masseter muscle.

Tissues were then washed and dehydrated in 75 per cent alcohol, followed by 95 per cent and finally in absolute alcohol.

The tissue was then embedded in paraffin (tissue mat, Fisher Company). The embedding was done in vacuo, at a melting point of 56.6°, under a pressure of 15 pounds per square inch for a period of 15 minutes.

Serial sections were then cut at a thickness of 6 to 7 microns. The mounted sections were then deparaffinized in the following manner. Immersion for five minutes in absolute alcohol, followed by three minutes in 95 per cent alcohol, and subsequently in 75 per cent alcohol for three minutes. Finally, sections were washed in distilled water for five minutes. The sections were stained by Hematoxylin, Eosin
method and used for histopathological study.

The sequence of repair was observed, recorded and photographed at each of the sacrifice periods.
When any tissue is destroyed or injured in a healthy vertebrate, an immediate defensive reaction occurs in the surrounding connective tissues known as inflammation. The reaction is characterized by vascular dilatation, fluid exudate and the migration of white blood cells. All of these begin more or less simultaneously. The basic character of this reaction is almost always the same, regardless of the nature of the injury. The vascular changes are seen first, since they develop most rapidly. From this stage, the inflammatory response may progress or resolve.

Accompanying and following the acute inflammatory response there is the proliferative stage in which there is evident, a cell multiplication rather than exudative response, with a predominantly mononuclear cell infiltration (macrophages and lymphocytes). Polymorphonuclear leucocytes are also present in the initial inflammatory reaction and closely accompanies the exudative response. However, the proliferation stage is chiefly composed of fibroblasts and new capillaries. As the blood clot forms the wound becomes populated by this highly vascularized, actively growing connective tissue. This accompanied by a component of acute inflammatory exudate is known as granulation tissue.
The stage of differentiation is overlapped by the proliferation stage characterized by the formation of new tissue which, however, is immature at this time. The removal of necrotic debris and the concomitant gradual decrease in the inflammatory cells improves the picture of differentiation and in the final stages the newly formed tissue attains maturity.

On the external surface of the ramus of the mandible in its lower half, the field of insertion of the masseter muscle towards the inferior border of the mandible, shows ridges to which the muscle is attached by tendons and grooves between the ridges to which the fleshy fibers of the muscle are attached through intervening periosteum. Because of this mode of attachment, the muscle when it was reflected from the mandible showed incomplete separation from these bony ridges and complete separation with the periosteum as it tears away from the bony surface during the process of the reflection of the muscle.

Degeneration of the muscle fibers and the formation of the clot are the earliest findings. However, as early as the fourth day after the injury, myotubes grow into the area between the detached muscle and bony surface. The simultaneous organization of the blood clot and the formation of myotubes fills the gap between the bone and the muscle completely and by the end of the third week the muscle fibers terminate through the periosteum and through the intervening fibrous tendons.
HISTOLOGICAL APPEARANCE AT VARIOUS INTERVALS:

1. Forty-eight hours:

**Muscle:**
The muscle shows areas of degeneration in segments, single or in small bundles. The cut muscle fiber bundles show some shrunken nuclei. Most of the nuclei of the muscle fibers are at the periphery of the fibers. Sarcolemmal tubes are present in the area of injury with centrally located nuclei. The most conspicuous appearance of sections is the hemorrhage, edema and lacy fibrinous net work. Between muscle and bone and within the muscle bundles, fragments of muscle fibers are surrounded by moderate infiltration of polymorphonuclear leucocytes and a few lymphocytes. Lack of the cross striations of the muscle tissue is evident at the junction of tissue injury. Cross sections of the muscle show fragmentation of muscle fibers and bundles. The increase in number of capillaries and the dilatation of intermuscular capillaries is of significance at the ends of the cut muscle fibers. Most of the new capillaries are directly in front of the cut muscle fiber and this is also the area of concentrated perivascular mitosis. Multiple numbers of mitotic figures are seen in a perivascular position. Capillaries running transversely to the longitudinal sectioned muscle tissue also show an abundant number of mitotic figures.

**Periosteum:**
Near the site of injury there is an apparent loss of the osteogenic cell compartment of the periosteum as the edema, clot and muscle debris appear
to be directly contacting bone. Just beneath the site of injury, towards
the lower border of the mandible the periosteum shows a gradual thickening
due to an increase in the number of cell layers. The fibrogenetic zone
of the periosteum is also thicker, and contains an increased vascular
compartment, and shows active cell differentiation.

Bone:
A band of osteoid tissue which stains basophillic is seen along the
surface of the bone thus indicating rapid cellular formation of this
material. The parenchymal cell of the bone, the osteocyte, appears to
be vital in most areas. However, small zones of osteocyte absent lacunae
are observed. These areas of vacuolated lacunae are indicative of severe
bone injury or are artifact caused by histological preparation.

The osteoid appears to be thicker at the injury site. Macrophages are
active at the periphery of the periosteum and around the injured muscle
stubs thus indicating possible phagocytosis of muscle debri.

2. Four days (ninety-six hours)

Muscle:
In the injured muscle some nuclei appear to be just leaving the
sarcolemmal site while others are centrally located in the cell. In some
tubules more rows of nuclei can be observed. Areas of microvacuolization
of muscle cells can be seen more distinctly and nuclear degeneration also
is present at the stubs of cut muscle fibers. These stubs of muscle are
swollen and fields of Cohnheim become more prominent between fibers. The spaces of Cohnheim have multiple numbers of mononuclear cells present. Loss of striation is complete near the site of injury. The muscle nuclei are oval instead of flattened. Perivascular dividing cells are observed in a longitudinal section of the tissue. Histocytes are swollen with engorged material in the sites of degenerating muscle fibers. At the junction of connective tissue and cut muscle stumps a rich capillary supply is observable, myotubes which are highly nucleated are seen to grow along the rows of necrotic tissue:

*Periosteum:*

Howships lacunae are present on the bony surface filled with mononuclear cells, and some empty lacunae are also present. In another area the osteoblasts are observed and a very cellular type of bone is being formed. Connective tissue adjoining to the periosteum is abundant and cannot be distinguished from the fibrogenic zone of the periosteum.

*Bone:*

The bone at ninety-six hours shows two distinctly different processes taking place. In the ramus parallel to the muscle fibers we see empty Howships lacunae with resorption taking place. At the inferior border of the mandible we see cellular coarse fibrillar bone being laid down called as osteophytic bone. These plate-like trabeculate of bone show reversal line and is very cellular with large lacunae. The bone appears to thicken at a very rapid rate.
3. One week:

**Muscle:**
Muscle shows many outgrowths of stubs of cut muscle with a granular appearance. Nuclei are enlarged but no intranuclear mitosis is seen. Multinucleated myotubes are very conspicuous. Muscle fibers show a wavy irregular outline with no visible striations near the damaged tips. The histiocytes are not as obvious in this section as much of the degenerating muscle fibers have been removed. Any remaining vacuolated muscle tissue which is hyalinized is being engulfed by residual macrophages. The cellular granulation tissue is more mature as collagenous fibers are becoming prominent with the reduction in round cells and fibroblasts. The outline of the capillaries has also matured. Very few extra-vascular red blood cells are apparent.

**Periosteum:**
Osteophytic bone is being deposited over areas showing reversal lines. The bone being laid down is very cellular. The fibrous layer peripheral to the osteogenic layer of periosteum is much thicker than that previously seen and has a mature vascular component.

**Bone:**
The spicules of osteophytic bone are very cellular being deposited on a reversal line seen in old compact bone. Osteocytes within the new bone are present and all lacunae appear to be occupied by vital cells.
4. Ten days:

Muscle:
The regenerating muscle tissue shows organization with an increase in myotubes invading the connective tissue and showing a very close relation to the osteoblasts and bone. The muscle fibers are becoming more densely compacted together with a reduced clear intercellular area. The young muscle fibers near the bone have centrally positioned nuclei and do not appear to be striated. Microvacuolization of muscle myofibrils is evident but it is not a prominent feature. The connective tissue has reduced in thickness and is not vascularized to the degree seen at 4 and 7 days. Inflammatory cells are less conspicuous between muscle and bone and intramuscularly.

Periosteum:
The periosteum compartment is considerably reduced in thickness with the major decrease coming in the fibroblastic layer.

Bone:
Bone is being laid down in the peripheral side of the ramus with most of it being osteophytic spiculated immature bone. The trapped osteocytes appear normal with all the lacunae having osteocytes. The endosteum side of the bone shows Howship's lacunae signifying undermining resorption and possible repair of immature bone previously deposited. Some area of the bone shows close approximation of muscle fibers to bone.
5. Fourteen days:

**Muscle:**

This section is a continuation of previously described section with more of the muscle approaching the immature bone. Muscle fibers are seen to be interdispersed with osteoblasts suggesting an insertion into this mass. Myotubes are present. The young muscle tissue is more basophillic staining than the remains of older cut muscle stumps but as yet, young muscle is not striated.

**Bone:**

The bone is of an immature variety with many trapped cellular components from the periphery. The bone is being eroded by tendinous like material associated with the approximating muscle tissue.

6. Twenty-one days:

**Muscle:**

The muscle shows complete peripheral displacement of nucleus with few multinucleated myotubes present adjacent to bone only. Most muscle observed has normal striation for the first time since injury. The vascular component of the muscle can also be considered within normal range. The swollen stubs of cut muscle are also absent. The muscle appears to be continuous with the osteoblastic layer and the bone thus suggesting a beginning of tendinous insertion of regenerating muscle. Few of the phagocytes and mononuclear cells are apparent, suggesting reduction of chronic inflammatory action.
Periosteum:
Osteoblasts are functionally producing osteoid tissue with the osteogenic compartment 4 to 6 cell layers thick. The marrow spaces show a few osteoblasts Howships lacunae. The periosteum is preparing a pathway for tendinous insertion of regenerating muscle.

Bone:
The bone and muscle appear to be connected to each other in many areas as the muscle is lying on the surface of bone in many areas. Modeling resorption is evident in the marrow spaces. Osteoblastic proliferation is continuing at a rapid rate forming osteophytic bone.

7. Twenty-eight and forty-two days:

Muscle:
The muscle tissue shows no evidence of previous injury other than at the junction of the bone and muscle where areas of peripheral attachment of muscle to bone is still in progress. The injury site is inconspicuous. Almost all of the muscle fibers are mature, with peripheral nuclei and normal myofibrils apparent.

Periosteum:
The periosteum is a 3 to 5 cell layer thick compartment with the normal appearance of proliferating cells and there is a close adaptation and attachment of muscle to bone by the periendothelial cells. At the inferior
Bone:
The bone shows an increase in total thickness when compared to normal control sections. The inferior border of the mandible specifically shows an enlargement of the bone mass. The osteocytes appear normal with all lacunae filled with cells. At the later time (six weeks) the new bone attaching to the mandible is of a mature variety.

8. Fifty-six days:

Muscle:
At eight weeks the muscle is observed as attaching to the bone via a tendinous insertion at the inferior border of the mandible. Striations and myofibrils all appear to be within the range of normal. The field of Cohnheim are reduced in size. The size and strain of the sarcoplasm, myofibrils and muscle appears to be similar to the control groups. The new cut muscle fibers are new inserted into the new compact type of bone. The nuclei of the muscle cells are all peripheral to the respective myofibrils and in contact with individual sarcolemmas. The once irregular wavey bundles of muscle are now similar to the control groups. The granulation tissue is inconspicuous and the site of injury is unobservable. Some histiocytes with yellow appearing debris in their cytoplasm is all that remains of the degenerated muscle debris.
Periosteum:
The periosteal compartment appears normal with a thickness of 3 to 5 cell layers which is similar to control. The tendinous like continuation of muscle fibers into the bone is complete because of periosteal proliferation.

Bone:
The bone mass appears to be considerably thicker than the control sections used in our study. A reversal line stained basophilic is present which shows the junction of the old and new bone formed after the injury. The inferior border of the mandible is especially thickened by the apposition of bone that has taken place. The muscle insertion into the inferior border of the mandible is complete and the only evidence for this repair is the thickened bone mass at the inferior border of the mandible.
DISCUSSION:

The main purpose of this study is to describe the regeneration of injured muscle tissue and its reattachment to bone. The present series of observations shows the regenerated masseter muscle fibers to be of normal structure and of normal insertion both in the periosteum and the bone of the mandibular ramus in the albino rat following surgical separation.

The reflection of the masseter muscle from the inferior border of the ramus of the mandible is difficult as it has a tendinous insertion into the bone in this region. On the external surface of the ramus the muscle is inserted only to the periosteum. The muscle and the attached periosteum are easily stripped from the ramus once the muscle is separated from the ramus at its inferior border.

The blood clot that appears following injury seals the wound and cements together the detached muscle, periosteum and bone. The infiltration of polymorphonuclear leucocytes at the site of injury is an immediate reaction to surgery and together with the blood clot serves as a temporary defensive and reparative reaction to the injury. The temporary repair and defense is maintained during the period of cell proliferation.
The histological changes as reported by Le Gros Clark (1946) during the first two days after injury were haywire degeneration of muscle and the invasion of the damaged area by a large number of polymorphonuclear leucocytes and histiocytes. Our findings at forty-eight hours agree with those as reported by Le Gros Clark (1946) and Pfuhl (1937).

The degenerating muscle fibers are phagocytized by macrophages. This is in agreement with the findings as reported by Spedel (1938) and Forbus (1936). The macrophages differentiate from the proliferating perivascular loose connective tissue cells found in the muscle. There are many mitotic figures found in such loose connective tissue. The presence of mitosis and macrophages around degenerating muscle demonstrates that proliferation overlaps the inflammatory reaction and muscle degeneration. Moreover as macrophages are functionally differentiated cells, differentiation also occurs.

The proliferation of the periosteum characterized by mitotic figures and increased cellularity is also associated with differentiations. This is evident as seams of new osteoid are formed on the inner osteogenic layers of the thickened periosteum.

The blood clot becomes infiltrated by many large stellate and fusiform cells and new capillaries. Within the blood clot some mitosis is seen in the undifferentiated connective tissue cells.
There are evidently overlapping phases of acute inflammation, proliferation and differentiation of perivascular cells in the muscle and periosteum. Such cells also contribute to the organization of the clot. The fibrin is removed by macrophages differentiation within the organizing blood clot. The trypsin like enzymes liberated by the polymorphonuclear leucocytes digest any denatured protein in the degenerating and necrotic muscle.

The organizing blood clot intervenes between the ramus of the mandible and the detached muscle fibers. As the blood clot becomes organized there is a loss of fibrin, polymorphonuclear leucocytes and macrophages. Where periosteum was detached from the bone, the newly organized granulation tissue differentiates into a new periosteum.

The surviving stumps of muscle fibers serve as a "nidus" for the differentiation of perivascular cells into solid cords or syncitium of cells. As such maturation of muscle occurs, the nuclei become separated as the newly formed bundles of fibrils increase in volume.

Miller (1934), LaGros Clark (1946) and Hess (1945), insisted that the single cells play no part in the regeneration of muscle fibers. Forbus (1926), Speidel (1937) and Adams (1953), believed that regeneration of the muscle fibers proceeds from isolated muscle cells (myoblasts) through their proliferation. Regeneration of this type has been called discontinuous or embryonal because it follows the sequence of events in
ontogeny, in contrast to the budding of continuous sarcoblastic ribbons connected with their parent fibers. The most significant mode of muscle regeneration in our material is through terminal budding from intact fiber stumps. The myotubes are seen on the surface of the surviving muscle fibers. Therefore, myotubes seem to arise from connective tissue surrounding the muscle fibers. Our findings support the view expressed by Le Gros Clark (1946), Studitsky (1958) and Maximow and Bloom (1960).

The inflammatory exudate, macrophages and fibrin are largely absent after one week. At such time, the temporary repair afforded by the blood clot now is replaced by granulation tissue. Such tissue serves as a new periosteum, when in contact with bone previously separated from its periosteum. Also, it serves as an attachment for the newly formed muscle fibers. At the inferior border of the mandible, the periosteum apparently forms both new bone and the fibrous tendinous attachments joining the newly regenerated muscle to bone. This dual function of bone and tendon formation is in agreement with those of Mosiman (1952), Sanders (1955) and Johnson (1957).

There is evidence for a maturation of all tissue two weeks following injury. The osteophytic bone on the ramus appears less cellular. There is little evident mitotic activity in the periosteum. Furthermore, the periosteum appears to be only as thick as the normal control. The muscle fibers all terminate in the periosteum except at the inferior border where
insertion in the bone is seen. The bone tissue at the inferior border is considerably thickened but shows less cellularity indicating maturation.

For the first time three weeks after injury, the inflammatory cells are seen to disappear. The nuclei are seen to migrate or be displaced to the periphery in myotubes which show striations. The osteogenic compartment of the periosteum is 4 to 6 cell layers thick and the muscle appears to be connected to the bone in many areas. All these findings substantiate the view that maturation of the wound is well in progress.

We further observe that at the end of eight weeks the muscle is attached to the bone at the inferior border via a tendinous insertion. The slight irregularity of newly formed muscle agrees with the view of Speidel (1938).

The bone on the inferior border of the mandible shows osteocytes in the lacunae which appear to be normal. The Halversson system contains no inflammatory cells. These findings further indicate the complete cell maturation and the complete repair of the injury site.

Provided with an orderly stromal framework delineating the anatomical arrangement of the injured part, and given an adequate circulation, the regeneration of skeletal muscle may lead to restitution. The intrinsic potency of mature striated muscle for proliferation and regeneration, expressed in conditions which do not limit growth, is considerable from the point of view of Oral Surgery.
SUMMARY AND CONCLUSION:

Eighteen albino female rats with an average weight of 100 grams, were used for a histological study of the reattachment of the masseter muscle after its surgical separation from the external surface of the ramus of the mandible.

The rats were sacrificed at intervals of two, four, seven, ten, fourteen, twenty-one, twenty-eight, forty-two, and fifty-six days. The head of each rat was removed and fixed in formalin. Following decalcification, a block of the specimen containing the ramus and body of the mandible covered by the masseter muscle was cut away from the head, washed, dehydrated, embedded in paraffin and sectioned at six to seven microns. The sections were stained by hematoxylin and eosin.

The sequence of repair was observed at each of the sacrifice periods and was recorded and photographed.

Our findings lead us to the following conclusion:

Skeletal muscle has the potential for regeneration. The regeneration is through the terminal budding from intact fiber stumps, which seem to arise from connective tissue surrounding the muscle fibers.
The newly organized granulation tissue differentiates into new periosteum. The periosteum performs a dual function in that it forms both new bone and the fibrous tendinous attachment joining the newly regenerated muscle to the bone. A new mature bone is laid down in the area of new attachment of the muscle at the inferior border of the mandible, while the rest of the renewed attachment is through the periosteum.

The muscle injury thus undergoes the inflammatory stages of degeneration, proliferation and eventually maturation. These stages are seen to overlap considerably.
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Figure 1. Photomicrograph X 25 of a 48 hours specimen showing the bony surface of ramus with edema in the adjoining muscle showing inflammatory cells (polymorphonuclear leucocytes).

Figure 2. Photomicrograph X 400 of a 48 hours specimen showing hemorrhage and edema in the muscle with perivascular mitotic figures.
Figure 3. Photomicrograph X 400 of a 48 hours specimen showing the inflammatory cells in the muscle.

Figure 4. Photomicrograph X 400 of a 48 hour specimen showing mitotic figures around the blood vessel in the muscle.
Figure 5. Photomicrograph X 400 of a 48 hours specimen showing increased mononuclear cells in between the muscle fibers.

Figure 6. Photomicrograph X400 of a 48 hours specimen showing degeneration of detached muscle fibers, inflammatory cells are also seen.
Figure 7. Photomicrograph x 400 of a 48 hour specimen showing inflammatory cells and a portion of blood clot in the injured muscle.

Figure 8. Photomicrograph x 100 of a 96 hours specimen showing thickened periosteum and highly inflamed injured muscle.
Figure 9.  Photomicrograph X 100 of a 96 hours specimen showing proliferating periosteum and new bone deposition at the inferior border of the mandible.

Figure 10.  Photomicrograph X 100 of a 96 hours specimen showing bone with Howship's lacunae without osteoclasts, thickened periosteum and myotubes.
Figure 11. Photomicrograph X 400 of a 96 hours specimen showing multinucleated myotubes with mitotic figures.

Figure 12. Photomicrograph X 400 of a 96 hours specimen showing mitotic figures.
Figure 13. Photomicrograph X 400 of a 96 hours specimen showing cell proliferation, surviving muscle bundles, capillaries and myotubes.

Figure 14. Photomicrograph X 400 of a 96 hours specimen showing Howships lacunae on the bony surface filled with mononuclear cells.
Figure 15. Photomicrograph X 100 of one week specimen showing new osteophytic bone, thickened periosteum and muscle approaching periosteum with myotubes.

Figure 16. Photomicrograph X 400 of one week specimen showing thickened periosteum and new osteophytic bone.
Figure 17. Photomicrograph X 400 of one week specimen showing osteophytic bone, organizing blood clot and muscle with some fibers growing to the bone.

Figure 18. Photomicrograph X 400 of ten days specimen showing bone, thickened peristeum and multinucleated myotube.
Figure 19. Photomicrograph in oil immersion of ten days specimen showing myotubes.

Figure 20. Photomicrograph X 400 of a ten days specimen showing bone, periosteum and muscle.
Figure 21. Photomicrograph X 100 of a two week specimen showing osteophytic bone, periosteum and the muscle.

Figure 22. Photomicrograph X 100 of four weeks specimen showing muscle attached via periosteum.
Figure 23. Photomicrograph X 100 of six weeks specimen showing muscle with the tendinous attachment to the bone at the lower border of mandible.

Figure 24. Photomicrograph X 25 of a controlled specimen showing normal arrangement of muscle, periosteum and bone.
Figure 25. Photomicrograph X 100 of a controlled specimen showing normal arrangement of muscle, periosteum and bone.

Figure 26. Photomicrograph X 400 of a controlled specimen showing normal arrangement of muscle, periosteum and bone.
APPROVAL SHEET

The thesis submitted by Dr. Virendra K. Seth has been read and approved by four members of the Department of Oral Biology.

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval with reference to content, form and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

5/14/64  
Date

[Signature of Advisor]  
5/14/64  
Date