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The Morphologic Changes in the Muscles of Mastication Due to Direct Intramuscular Injection of Lidocaine, Carbocaine, Procaine and Citanest

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The Morphologic Changes in the Muscles of Mastication
Due to Direct Intramuscular Injection of
Lidocaine, Carbocaine, Procaine and Citanest

by

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Dean of the School of Basic Sciences and Graduate Studies

CURRICULUM VITAE

ACKNOWLEDGEMENT

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INTRODUCTION

One of the most commonly experienced symptoms in dentistry and one that is of major concern to the dentist is pain. Since pain is manifested when an enviromental change occurs causing injury to responsive tissues, it is often spoken of as a protective mechanism.

Pain has been described by a variety of terms: sharp, burning, aching, cramping, dull and throbbing! (Monheim, 1969). Therefore, it is a difficult term to define. Monheim (1969) describes pain as an unpleasant sensation created by a noxious stimulus that is mediated along specific nerve pathways into the central nervous system, where it is interpreted as pain.

Local anesthetics are drugs that will temporarily interfere with conduction when absorbed into the nerve. A blockage of the afferent transmission produces analgesia, while interruption results in motor paralysis.

Local anesthetic compounds are the most widely used drugs in dentistry and all are synthetic compounds with the exception of cocaine which no longer is used because of its unusual high toxicity. Structural changes in the molecules of these drugs alter the toxicity, potency, diffusibility, profoundness, and duration of anesthetic activity of these compounds. Frequently, as the length of the anesthetic molecule increases, so does its anesthetic activity. Also, structural changes in the molecule may increase toxicity or irritancy without increasing potency. In most cases toxicity tends to rise as potency increases.

Monheim (1969) has suggested that ideal local anesthetics should possess the following properties:

(1) Its action must be reversible.

(2) It should have a low degree of systemic toxicity.

(3) It should have a rapid onset and be of sufficient duration to be advantageous.

(4) It should have a potency sufficient to give complete anesthesia without using harmful concentrated solutions.

(5) It should have sufficient penetrating properties to be effective as a topical anesthetic.

(6) It should be relatively free from production of allergic reactions.

(7) It should be stable in solution and readily metabolized by the body.

(8) It should be either sterile or capable of being sterilized by heat without deterioration.

(9) It must be nonirritating to the tissues and produce no secondary local reaction.

No local anesthetic in use today fulfills to perfection all of these requirements.

Since local anesthetics are the most commonly used drugs in dentistry for routine procedures when anesthesia is desired, it is of particular interest to study the morphologic changes in the muscles that are situated in close proximity to major sensory nerve fibers to the teeth. It is also of interest to question that local anesthetics are nonirritating to the tissues; in fact, they do produce a definite and precise lesion.

Another point of interest is to use and apply local anesthetics to produce a definite lesion clinically to aid in the treatment of a common dental syndrome, the myofacial pain dysfunction syndrome formerly known as Costens Syndrome.

REVIEW OF THE LITERATURE

The major muscles of mastication are the masseter, medial and
lateral pterygoids, and the temporalis and they are involved in most
movements of the mandible. These muscles are situated anatomically
near the sensory nerve fibers to the teeth. As a consequence, portions
of some of the muscles of mastication may be affected due to the in-
filtration of anesthetic solutions following a mandibular block, and
in infiltrating for superior alveolar nerves, long buccal and
palatine nerves. In addition, intramuscular injection inadvertently
may occur.

Many investigators have studied the effects of chemical injury
due to local anesthetics used commonly in dentistry. (Benoit, 1971;
Margolis, 1953; Tait, 1958; Brun, 1959; Burke, 1972) A previous
study indicated that certain local anesthetics used in dental offices
would produce degenerative lesions in skeletal muscle followed by
regeneration of injured muscle fibers (Benoit, 1971).

Burke (1972) described degenerative changes in striated muscle
of the rat tongue following injection of 2% Lidocaine HCl without
vasoconstrictor. Lidocaine caused degeneration in muscle fibers by
12 hours, and degeneration was severe to the extent that all myofibrils
in some fibers disappeared leaving behind empty sacrolemma sheaths.
Benoit (1971) described the effects of some local anesthetics on the
gracilus muscle of the rat. Following a subcutaneous injection of
2% Lidocaine HCl without vasoconstrictor, the muscle fibers showed
hyalinization and phagocytosis of the necrotic debris. Regeneration
was well established by three days and was complete by two weeks
(Benoit, 1971). Mannheimer, Pizzolato and Adriani (1955) also

described the effects of 2% Lidocaine on rat skeletal muscle, and reported similar results. Similar investigations have been done with 1 to 4% Procaine and Carbocaine (Margolis, 1953; Tait, 1958; Mannheimer, 1954). Brun (1959) showed Carbocaine and Xylocaine regularly produced inflamation and necrosis in rat skeletal muscle. Procaine produced no irritation nor lesion. Burke (1972) showed that Tetracaine and Lidocaine produce degenerative lesions in the rat tongue followed by repair. Procaine produced no lesions other than those noted along the path of injection.

The use of Xylocaine in dentistry has been described as producing reactions. Weidling (1948) and Lunqvist and Löfgren et al (1948) showed that "These reactions were due to metal ions, especially copper, released by acid injection solutions. In these solutions a low pH is necessary to stabilize the adrenaline. The metal ion sometimes leads to hemorrhagic edema, associated with an inflamatory reaction." Gordh (1948) found that intra and subcutaneous injections of 1% Xylocaine without a vasoconstrictor produced no demonstrable reaction in man.

Bjorlin (1954) found that Xylocaine without adrenaline had a much stronger local irritative effect in skeletal muscle of the rat than did Procaine in equal concentrations. During the first days after the injection of Procaine, gross hemorrhages and microscopic degenerative changes were seen in the connective tissue, and vascular damage with thrombosis and hemorrhages as well as inflamation were noted. These alterations subsided on the third day and then disappeared. Xylocaine produced similar, but more pronounced alterations which persisted for one week. Brum (1958) showed that subcutaneous injections

of Xylocaine and Carbocaine in the rat resulted in relatively wide
spread alterations. The lesions were localized and consisted of
infiltrations of histiocytes and fibroblasts between muscle fibers,
which also showed hyalinization and disintergration, as well as a
decrease in fiber diameter.

Opinions thus differ on the effects of local anesthetics on
skeletal muscle. Gross inspection of the reaction of the tissue to
an anesthetic must surely be of some value. Discrepancies have been
found between results of microscopic and macroscopic studies. Such
discrepancies have indicated that variations in reaction to local
anesthetics range from the production of no lesion or irritancy,
to degenerative lesions and ultimately to necrotic lesions.

Local anesthetics have been shown to affect muscle tissue
at different degrees of reaction. Such observations suggest a way of
using certain local anesthetic agents to the advantage of the dental
clinician.

HISTORY OF LOCAL ANESTHETICS

The first local anesthetic agent to be discovered was Cocaine, which had been used for centuries by the natives of the Peruvian Andes in the form of Cocoa leaves. Nieman in 1860 first isolated the substance and noted it made his tongue numb. Von Antrep in 1879 studied its properties and reactions and reported that subcutaneous injection produced insensitivity to a pinprick.

Cocaine was brought into clinical use as a local anesthetic agent in ophthanologic procedures after the studies of Koller and Freud in 1884. It was rapidly brought into use in dentistry later in 1884 by Hall.

Also in 1884, Holstead administered the first inferior alveolar nerve block with Cocaine. By 1892, Einhorn had begun the search for synthetic substitutes, this led to his synthesis of Procaine in 1905.

PURPOSE

The purpose of this experiment is to clarify and describe the
morphologic effects of some local anesthetics on the muscles of
mastication using light microscopy. The information derived from
this study will be used to describe the course of the developing
lesion in muscle caused by local anesthetics and subsequent regenera-
tion of damaged components of the muscle fibers. It will also clarify
that local anesthetics seemingly produce a degenerative lesion and not
a necrotic one. It may also help to explain the observation that
relief is often obtained following injection of local anesthetic
in muscles affected by spasm and trismus so often encountered by the
dental practitioner when he diagnoses the presence of the myofacial
pain dysfunction syndrome.

LOCAL ANESTHETICS USED IN DENTISTRY

Local anesthetics used in dentistry can be divided into two groups: the ester compounds and the amide type compounds.

The ester group was at one time the most commonly used. It is composed of 1) an aromatic, lipophilic group 2) and intermediate chain containing an _ester_ linkage 3) a hydrophilic secondary or tertiary amino group which forms water soluble salts combined with acid.

The chemical pattern of the ester type compounds is as follows:

$$R \Longleftrightarrow COOR' -- N \begin{matrix} R_2 \\ \\ R_1 \end{matrix} \quad or \quad \begin{matrix} H \\ N-- \\ H \end{matrix} \Longleftrightarrow \overset{O}{\underset{}{C}}-O-C-C-N \begin{matrix} H\ H\ C_2H_5 \\ \\ H\ H\ C_2H_5 \end{matrix}$$

Procaine
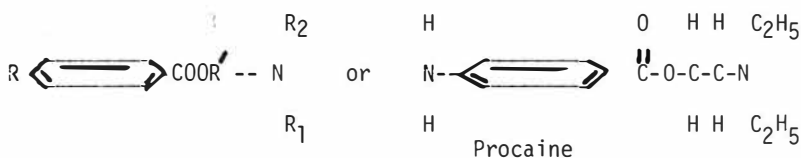
The amide group, which is presently the most popular, is composed of 1) an aromatic, lipophilic group 2) an intermediate chain containing an _amide_ linkage 3) a hydrophilic or tertiary amino group which forms water soluble salts when combined with acids.

The following structure represents the chemical pattern of the amide compounds.

$$R \Longleftrightarrow -NHCO-R_2--N \begin{matrix} R_3 \\ \\ R_4 \end{matrix} \quad or \quad \Longleftrightarrow -NH-CO-CH_2-N \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \begin{matrix} C_2H_5 \\ \\ C_2H_5 \end{matrix}$$

Lidocaine
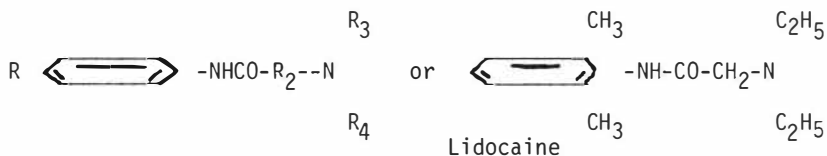
PROPERTIES OF AN IDEAL LOCAL ANESTHETIC

Local anesthetic compounds that are synthetic are weakly basic
in nature and as such are poorly soluble in water. They are, however,
combined with hydrochloric acid to form salts that are soluble in water
and acid in reaction. Their chemical characteristics are so balanced
that they have both lipophilic and hydrophobic properties. If the
hydrophilic group predominates, the free base is not readily precipitated
on injection into the tissues, and the ability to diffuse into the
lipid-rich nerves is diminished. If the molecule is too lipophilic, it
is of little clinical value as an injectable anesthetic, since it is
insoluble in water and unable to diffuse through the interstitial
tissues (Monheim, 1969).

Toxicity, as well as potency, varies with the chemical structure
of local anesthetics. The chemical structure of the ideal local
anesthetic would make possible the combination of high potency and
low toxicity. In many instances changes of the chemical formula may
increase only slightly potency but increase markedly toxicity of the
new compound. Therefore toxicity, potency and local irritancy,
although related, are of extreme importance. The degree of toxicity
does not always coincide with potency (Monheim, 1969). However, as
a general rule, as potency increases, toxicity is likewise increased.
Injectable local anesthetics, while chemically different in many
respects, possess the following common properties (Monheim, 1969):

(1) They are all synthetic compounds.

(2) They all contain amino groups.

(3) They all form salts with strong acids.

(4) Their salts are water soluble.

(5) Alkali will hydrolize the salt to free the alkaloidal base.

(6) The alkaloidal base is soluble in lipids.

(7) The anesthetic salts are acid in reaction and relatively stable.

(8) They are all either hydrolyzed by plasma esterases or detoxified in the liver.

(9) The action of all drugs are reversible.

(10) They are all compatible with epiniphrine or other vaso-constrictors.

(11) They are incompatible with metal salts of mercury, silver etc.

(12) They are all capable of producing toxic systemic effects when sufficiently high plasma concentration is reached.

(13) They all affect nerve conduction in a similar manner.

(14) They all have little or no irritating effects on tissues in anesthetic concentration.

It is the last property which is of question at this time.

Local anesthetics can be grouped according to their chemical structures. This is particularly important from the point of view of metabolism as well as from that of possible allergic reaction.

The chemical groups of the injectable anesthetics used are:

I. Para-amino benzoic acid esters

    A. Procaine (Novocaine)

II. Anilide (nonester type)

    A. Lidocaine (Xylocaine)

    B. Mepivacaine (Carbocaine)

    C. Prilocaine (Citanest)

As a general rule the potency of a local anesthetic depends upon its chemical structure, whereas the duration can be altered by the addition of a vasoconstrictor drug.

Local anesthetic agents differ considerably from most other classes of drugs. For example, local anesthetic agents are applied directly to the region of the body in which they exert their desired pharmocologic action whereas most other drugs are administered parenterally or orally and then transported by the circulatory system to their target organ, which is located at some distance from the site of administration.

Although local anesthesia has been an accepted clinical procedure for many years and the use of local anesthetic agents has been wide-spread in the general population, basic pharmocologic knowledge (Covino, 1972) and the study of adverse affect on muscle has been relatively sparse.

The purpose of this report is to describe the developing lesion in the muscles of mastication. No attempt will be made to describe or discuss the mechanism of action of local anesthetic agents on peripheral nerves, nor will the physiology on nerve excitation and conduction be considered.

CHEMISTRY AND PHARMACOLOGY

Procaine[1] - (Winthrop Labs):

Procaine (diethylaminoethyl ester of para-aminobenzoic acid) was synthesized by Einhorn in 1905, and for over fifty years it was considered the standard of comparison for the local anesthetics (Monheim, 1971). The introduction and wide acceptance of the nonester type local anesthetics have reduced the importance of procaine. However, throughout the period of its extensive use it has had an enviable safety record (Monheim, 1969).

Chemistry:

$$N \quad \langle = = = \rangle \quad \overset{O}{\overset{\|}{C}}-\overset{H}{C}-\overset{H}{C}-N \quad \begin{array}{c} C_2H_5 \\ \\ C_2H_5 \end{array}$$
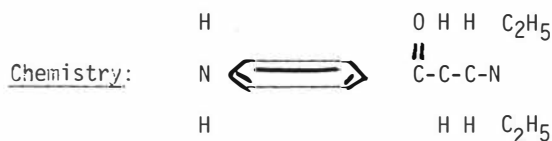
Procaine (Novocaine) is a white crystalline powder having a melting point of about 60° C. The drug itself is only slightly soluble in water and has an alkaline reaction. However, it is most commonly used as the hydrochloric salt, which is freely soluble in water and is acidic in reaction, having a pH of approximately 4.5 and a melting point of 154°C. Solutions of procaine hydrochloride are decomposed rapidly by alkali but withstand boiling and autoclaving. The drug is compatable with epinephrine, phenylephrine, corbasil and levarterenol.

Pharmocology: Procaine is a weak anesthetic agent possessing a low degree of toxicity. It produces analgesia under practically all circumstances.

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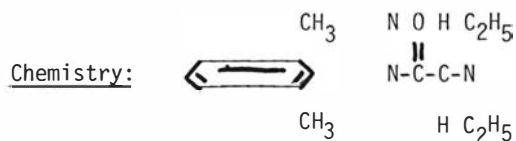[1]Procaine, Winthrop Laboratories, N. Y., N. Y., 1971.

Procaine is readily absorbed following injection into the tissues and is hydrolyzed to para-aminobenzoic acid and diethylaminoethanol. This is believed to be accomplished by the enzyme pseudocholinestesase, which is present in the plasma and the liver. The end products, particularly para-aminobenzoic acid, are excreted by the kidney.

Due to its rapid hydrolysis, Procaine has a low degree of systemic toxicity. It does possess vasodilatating properties that cause it to be more readily absorbed into the systemic circulation. This more rapid absorption and subsequently increased toxicity can be markedly controlled by the addition of a vasoconstrictor to procaine solutions.

Procaine is used in dentistry in 2% solutions. This percentage strength is nonirritating (according to the manufacturer) to tissues and has sufficient anesthetic potency to ensure adequate analgesia. Procaine should not be used in concentrations exceeding 2%, as the vasodilating action shortens the anesthetic time and markedly increases the toxicity.

The onset of analgesia with procaine depends to a degree upon the concentration and the method employed. Procaine has no effect upon the cardiovascular system other than vasodilatation of the microcirculation in the area of injection since it is, depending upon the concentration used, a mild to potent vasodilator (Monheim, 1969; Novocain, 1966).

Lidocaine[1] - (Astra Pharmaceutical):

$$CH_3 \qquad N \ O \ H \ C_2H_5$$
$$\text{Chemistry:} \quad \langle \!=\!\!=\!\!=\!\rangle \qquad \overset{\parallel}{N-C-C-N}$$
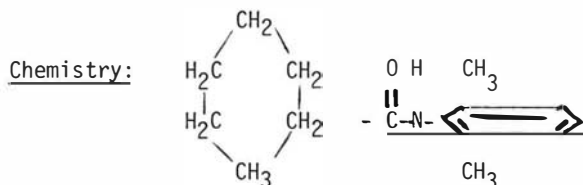$$CH_3 \qquad \quad H \ C_2H_5$$

The structural formula of Lidocaine (diethylamino acet-2, 6-xylide; Xylocaine), represents an important chemical departure from the procaine type (para-aminobenzoic acid ester) local anesthetic, because Lidocaine is the first non-ester type local anesthetic compound to be used in dentistry. It is a white crystalline powder with a melting point of $69^\circ C$ and is used as the hydrochloride salt. The drug is compatable with all vasoconstrictors and withstands boiling and autoclaving. Lidocaine base is only slightly water soluble, but the hydrochloride salt is readily soluble in water (Lidocaine, Astra-Pharmaceutical 1966).

Pharmacology: Lidocaine diffuses readily through interstitial tissues and into the lipid rich nerve, giving a rapid onset of anesthesia (Monheim, 1969). Lidocaine has a pKa of 7.85 and therefore creates a favorable rate of ionization and the production of a conduction block (Astra-Pharmaceutical 1966).

Experimental investigations on man and animals has established the potency of lidocaine to be 2-3 times that of procaine. The type of anesthesia induced by this agent has been found to be rapid, extensive, profound, highly predictable and longer lasting than procaine (Wiedling, 1952; Goldberg 1947). In general, it leaves the site of injection at a slower rate than procaine (Sung, 1954).

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[1]Lidocaine Hydrochloride, Astra Pharmaceutical Products Inc., Worchester, Mass., 1966.

Carbocaine[1] - (Winthrop Laboratories)

Chemistry:

$$H_2C \overset{\displaystyle CH_2}{\underset{\displaystyle CH_3}{\diagup}} \; \underset{CH_2}{\overset{CH_2}{\Big|}} \quad \overset{O\;H}{\underset{}{}} \; \overset{CH_3}{\underset{CH_3}{}}$$

$$-\overset{O}{\overset{\|}{C}}-\overset{H}{N}-\langle\!=\!=\!=\rangle$$

Carbocaine (d, l-N methyl - pipecolic acid - 2, 6 dimethylanilide;
Mepivacine) is a white crystalline, odorless powder that is soluble
in water, and very resistant to both acid and alkaline hydrolysis. It
is a nonester anilide compound with a melting point of $261^{\circ}C$ and a
molecular weight of 285.5. The drug is compatible with all vasoconstric-
tors and withstands boiling and autoclaving (Winthrop Labs, 1971).

Pharmocology: Carbocaine is very similar to lidocaine in its
action within the body. It produces anesthesia of moderately long
rate by stablizing the neuronal membrane and prevents the initiation
and transmission of nerve impulses. "Carbocaine does not ordinarily
produce irritation or tissue damage" (Winthrop Labs, 1971).

Citanest[2]- (Astra Pharmaceuticals)

Chemistry: $\langle\!=\!=\!=\rangle$ - $NH-CO-CH-NH-CH_2-CH2CH_3$

with CH_3 above and CH_3 below.
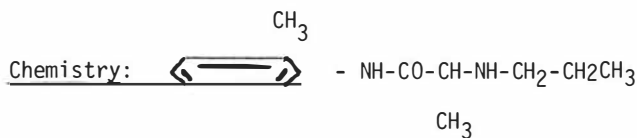
Citanest (a-n-propylamino-2 methyl-propronanilde; Prilocaine)
is a stable amide whose hydrochloride salt is freely soluble in water.
It has a molecular weight of 256.8 and a melting point of 167° to $168^{\circ}C$.

Pharmocology: Citanest is a short acting local anesthetic whose
potency and safety is comparable with lidocaine (Astra Pharmaceutical, 1965).
It is said to have a lesser degree of toxicity to the central nervous

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[1] Carbocaine Hydrochloride, Winthrop Laboratories, N. Y., N. Y., 1971.

[2] Citanest Hydrochloride, Astra Pharmaceutical, Worchester, Mass., 1965.

system than lidocaine and is more rapidly metabolized. The drug is absorbed from the injection site less rapidly than is lidocaine.

Metabolic studies indicate that Citanest is broken down in the liver much more rapidly than lidocaine. Citanest is a derivative of toluidine and therefore one of the metabolites of citanest appears to be O-toluidine, a substance which has been found to produce methemoglobin; and it is consequently contraindicated in those rare patients with congenital or idiopathic methemoglobinemias (Astra Pharmaceutical, 1965).

PROCEDURE

90 Wistar white female rats obtained from Flow Laboratories, Dublin, Virginia, weighing approximately 150 grams each, were divided into five groups, 18 animals to a group. Each group was sub-divided into five subgroups consisting of 3 animals in each subgroup. The animals were anesthetized with Metafane (Methoxyflurane) by placing each animal in a closed jar with a few drops of anesthetic. Once anesthesia was obtained, the animal was placed on a head restraint and its mouth propped open. Both masseter muscles, anterior deep divisions were injected with 0.25 ml alloquots of the agents intraorally, using a 22 guage 1 inch needle. The following local anesthetic agents were used: (1) 2% Lidocaine HCl (Astra Pharmaceutical); (2) 2% Procaine HCl (Winthrop Laboratories); (3) 3% Carbocaine HCl (Winthrop Laboratories); (4) 4% Citanest HCl (Astra Pharmaceutical); (5) Isotonic Saline. All were administered without vasoconstrictor. The injected animals were then sacrificed at days 1, 2, 3, 4, 7, and 15. The heads were removed and fixed for 24 hours in 10% neutral buffered formalin. The anterior deep division of both masseter muscles were than dissected, washed and processed for sectioning at 5-7 micra, and stained with hematoxylin and eosin.

Discussion:

Metafane [R](Methoxyflurane)[1] is a halogenated ethyl methyl ether with the clinical name, 2, 2-dichloro-1, 1-difluroethyl methyl ether. It contains 0.01% Butylated hydroxytoluene as the antioxidant. It is a

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[1]Metafane (Methoxyflurane), Pitman-More Inc., Fort Washington, PA, 1969.

non-explosive type of inhalation anesthetic and exhibits low toxicity.
It maintains anesthesia with ease and possesses marked muscle relaxing
properties. It has a wide margin of safety between surgical levels
and toxic levels. Recovery is smooth and of short duration. It is
compatible with commonly used preanesthetic and other anesthetic agents.

Clinical-pathological tests with methoxyflurane have shown that
minimal hepatic damage occurs only on gross overdosing. The depth of
anesthesia was based on clinical judgement and by the degree of
muscle relaxation and the rate and character of respiration. Loss
of muscular tension of the limbs, operative site, and jaws were reliable
signs of adequate surgical anesthesia (Pitman-Moore Inc., 1969).

MATERIALS AND METHOD

On the day of sacrifice, the animals were placed in a glass jar
with ether until death. The heads were skinned, exposing the injected
muscle, removed and fixed in 10% neutral buffered formalin for 24 hours.
A biopsy of the anterior deep division of the masseter muscle, bilaterally
was obtained. The specimens were identified and prepared for proper
fixation and preparation for paraffin sections as described by Lilly (1965).

Preparation of tissues for Light Microscopy

Fixation	24 to 48 hours
H_2O Washing	1 hour
70% ETOH	12 hours
80% ETOH	1 hour
95% ETOH	1 hour
100% ETOH	1 hour
100% ETOH	1 hour
100% ETOH + Chloroform	1 hour
Chloroform	1 hour
Chloroform + Paraffin	1 hour
Paraffin	1 hour
Paraffin	1 hour

Embedding in paraplast block

Sections of the biopsies were cut at 6 to 10 micra and stained with
hematoxylin and eosin, followed by dehydration and mounting.

Hematoxylin and Eosin Staining of Tissue Sections:

1. Deparaffinization

Xylene	2 minutes
Xylene	2 minutes

	100% ETOH	6 dips
	95% ETOH	6 dips
	80% ETOH	6 dips
2.	H_2O	rinsing
3.	Harris' hematoxylin	15 minutes
4.	H_2O	rinsing
5.	Differentiation and Alcohol 0.50%	2 dips
6.	Rinsing in tap water	1 minute
7.	Bluing with Lithium Carbonate	3 dips
8.	Eosin	15 seconds
9.	Dehydration	
	95% ETOH	2 dips
	95% ETOH	2 dips
	100% ETOH	2 minutes
	100% ETOH	2 minutes
10.	Clearing	
	Xylene	3 minutes
	Xylene	3 minutes
	Xylene	3 minutes
11.	Mount with Permount	
12.	Dry	

Histologic changes in muscle were than observed. Bethlems interpretation of muscle biopsy was chosen as an aid in determining histologic changes in muscle:

INTERPRETATION OF A MUSCLE BIOPSY

1. Changes in fiber diameter

2. Changes in the structure of muscle fibers

    a. Floccular changes

    b. Hyaline Degeneration

    c. Basophillia

    d. Vacuoles

3. Ringed fibers and sarcoplasmic masses.

4. Changes in sarcoplasmic nuclei

5. Changes in the muscle spindle fibers

6. Changes in the intramuscular nerve branches.

7. Changes in the blood vessels.

8. Changes in the interstitial tissues.

9. Cellular infiltration.

10. Pattern of the lesion.

Discussion:

Muscle tissue has a relatively limited range of histologic changes in reaction to disease (Bethlem, 1970). It is therefore necessary to elaborate on what is to be observed in an interpretation of a muscle biopsy.

Changes in muscle fiber diameter can only be judged with any reliability in transerse sections. The diameter of the fibers are dependent on the fixation method, sex and age, the muscle examined and the nutritional state of the subject.

Structural changes consist of floccular collections of eosinophilic material in conjunction with phagocytosis. These changes are often referred to as necrotic changes.

Hyaline degeneration is the term applied to a swollen muscle fiber whose cross striations are no longer recognizable. These fibers often contain a homogenous eosinophilic material. Waxy degeneration, cloudy aspect (Greenfield, Shy, Alvord and Berg, 1957), degenerescence vitreuse (Durante 1902) and Wachsartige degeneration (Zenker 1864) are other terms often encountered to describe this change.

Basophilic fibers represent an increase of ribonucleic acid indicative of repair.

RESULTS

Procaine, Lidocaine, Carbocaine and Citanest exhibited the same
morphologic changes; therefore all four will be discussed together.
Figures 1 through 4, pages 39-42   are self-explanatory.

Day 1 (refer to Figure 5 and 6)

The first noticeable change in the region of deposition of local
anesthetic was the massive infiltration of inflamatory cells (Figure 5,
Lidocaine day 1). The interstial tissues show marked edema with
numerous polymorphonuclear leukocytes and macrophages. Myofibrils
became disrupted with vacuoles, leaving some empty sarcolemma tubes.
Cross striations disappeared. Flocular changes occurred with fibers
showing marked eosinophillia and phagocytosis of the myofibrillar debris.
Hyalin degeneration was evident with fiber diameter increasing in size.
The once peripherally placed elongated sarcolemma nuclei now became
very prominent with migration into the center of the fiber. The
swollen appearing vesicular nuclei continued to exhibit a marked increase
in hyperchromicity. The nuclei which were present with the myofibrils
and in the endomysium were of polymorphonuclear leukocytes and macro-
phages. No evidence of mitosis was observed, nor was there evidence
to suggest how an increase in intrafiber nuclei came about. Many muscle
fibers lost their myofibrils by phagocytosis, but still retained an intact
sarcolemma, leaving an empty sarcolemmal tube with a small peripheral
sheath of sarcoplasm and muscle fiber nuclei.

The cross section photomicrograph (Figure 6) reveals marked edema
in the endomysium. The individual fibers show varying degrees of
degeneration. Point A (Figure 6) represents the peripheral arrangement
of nuclei showing marked hyperchromicity and migration into the center

of the fiber.   Vacuolization is also present with phagocytosis of the
myofibrillar debris.   Point B (Figure 6) represents another stage in
degeneration in which the peripherally boardered nuclei have migrated
centrally, phagocytosing the myofibrillar debris.   The interstitial
cellular infiltrate can be seen clearly in Figure 6 as polymorphonuclear
leukocytes and macrophages.

Day 2 - (Refer to Figures 7 and 8)

The evidence of injury at 48 hours post injection is quite remarkable. As revealed in figure 7, vacuolization became more evident. A marked increase in celluarity is quite apparent within both the individual damaged muscle fibers and interstitium. Highly basophilic multinucleated tubes predominate which represent empty muscle fibers with an intact sacrolemma. Nuclei appear disorderly in appearance in some fibers and are more organized in others. Nuclear movement seems to be directed toward the center of the fiber forming a continuous line of nuclei.

Fiber diameter decreased (Figure 8) but a peripheral rim of basophilic cytoplasm is apparent. Large vesicular cells with pale nuclei and prominent nucleoli with basophilic cytoplasm appear on the periphery of the damaged fibers. These cells resemble myoblasts and will form new skeletal muscle fibers.

Day 3

Tissues which were autopsied at 72 hours post injection revealed minor changes and closely resembles the 48 hour picture. The highly basophilic multinucleated tubes overwhelmingly predominated. Long strains of nuclei were beginning to organize within the center of the damaged muscle fibers. Fiber diameter still remained diminished in size. Polymophonuclear cells became less numerous being replaced by large oval cells with basophilic cytoplasm and prominent multiple nucleoli. These cells typically resembled myoblasts but their origin remains unknown.

Day 4 - (Refer to Figures 9 and 10)

By day 4, myofibrillar regeneration has progressed at a rapid rate. The marked basophilic multinucleated tubes are quite evident (Figures 9 and 10). Large vesicular nuclei with very large prominent nucleoli became very well organized. The nuclei seemed to be attracted to each other and were lined up side by side to form a continuous chain. The sarcoplasm was intensly basophilic, indicative of ribonucleic acid synthesis. Interstial tissues still showed evidence of edema with a highly disorganized cellular infiltrate of large basophilic cells with ovoid nuclei. These cells also appear to be disorganized inmature myoblasts and to have produced myofibrillar substances which coalesced, forming new skeletal muscle fibers.

Day 7 - (Refer to Figure 11)

On day 7, the muscle fibers were losing their basophilic appearance
and showed increases in eosinophillia. Large centrally placed hyper-
chromatic nuclei still were evident. The cells of the interstitium
were progressively becoming organized and synthesizing a homogeneous
basophilic substance. These cells apparently were myoblasts, whose origin
is unknown. They produced a myofibrillar cytoplasm which   replaced
the injured fibers as was evident in the cross section photograph of
figure 11. Nuclear organization was quite remarkable with long
continuous strains of centrally placed nuclei within the regenerating
fibers.

Day 15 - (Refer to Figures 12 and 13)

By day 15 regeneration was almost complete. Figure 12 reveals intact fibers with cross striations. These fibers exhibited the multinucleated centrally placed vesicular nuclei as mentioned previously. Point A (Figure 13) reveals an interesting phenomena in which nuclear fragmentation seemed to be taking place, leaving behind a normal appearing muscle fiber with peripherally placed elongated nuclei. Sarcoplasmic basophillia indicative of protein synthesis and repair, has been replaced by normal eosinophilic cytoplasm. Fiber diameter increased in size and cross striations have reappeared.

<u>Saline Control (Firgure 14)</u>

In control animals, muscle fibers in the region of infiltration exhibited only mild degeneration in the localized region of injection. Degeneration of the muscle fibers was not truly evident until day 3. Morphologically, the cells present were basically polymorphonuclear leukocytes and macrophages. The cellular pattern typically represented an acute inflamatory response in the early stages. By day 4, muscle fibers exhibited a decrease in cross striatións and the sarcoplasm showed marked basophillia with large centrally placed vesicular nuclei. The nuclei exhibited hyperchromatism with prominent nucleoli.

The region of degeneration was so small and localized that it is the author's point of view that this degenerative pattern was due to the injection of the saline solution. By day 15, repair was complete to the extent that no evidence of scar tissue was present and cross striations appeared as in normal striated muscle. Pycnotic nuclei were peripherally located and the sarcoplasm exhibited its normal color.

Discussion

The sequence of morphologic changes in the muscles of mastication following a single injection of either Lidocaine, Carbocaine, Citanest or Procaine clearly indicates a rapid reversable destruction of striated muscle fibers followed quickly by phagocytosis and regeneration. Benoit (1970) observed the irreversible destruction in the gracilis posterius muscle following a single injection of Bupivacaine, long acting local anesthetic, followed by regeneration by 30 days. The fact that regeneration does occur implies that necrosis does not take place. In the region where no local anesthetic agent is deposited, the degenerative tissues are removed rapidly by macrophage activity followed by a regrowth of new cellular material which is derived from either the stump of old unaffected fibers or from new myoblasts. The origin of the myoblasts presently is unknown.

As the affected fibers degenerate, an orderly sequence occurs. A floccular change, characterized by collections of eosisophillic material and phagocytosis, is the first of a series of changes. Hyaline degeneration is second, characterized by the appearance of swollen fibers with a loss of cross striations and a homogeneous eosinophilic sarcoplasm. Basophillia follows shortly, representing a repair process, replacing the myofibrillar sarcoplasm.

Nuclear changes are very drastic. The normally elongated nuclei of the muscle fibers are called sarcolemmal nuclei because they are situated just beneath the sarcolemma. According to Greenfield, Shy, Alord and Berg (1957), an average of four to eight nuclei per fiber is normal in cross sections. Fiber degeneration followed by regeneration shows a marked increase of nuclei with the formation of nuclear chains seen only in longitudinal sections. The interval between the nuclei

in a chain can be so slight that the nuclei cannot be individually identified. These nuclei are large and vesicular in shape and exist for only a short period of time. Subsequently they disperse, leaving behind a skeletal muscle fiber with eccentrically peripherally placed elongated sarcolemma nuclei. The early invasion of polymophonuclear leukocytes and macrophages clearly indicates an acute inflamatory response or a polymyositis. The origin of the myoblasts remains a mystery, although transformation of the macrophage into a myoblast cannot be ruled out until proven otherwise. Mitotic activity was not observed, although the possibility always exists that a specific time in the degeneration of an intact muscle fiber, nuclei of adjacent unaffected myofibrils migrate to the affected fibers and revert to blastic forms.

The Vascular response of the tissues was not of any remarkable significance. No granulomatous tissue appeared.

Clinical Significance

There have been a number of reported cases of patients who complain of discomfort and pain in the preauricular region radiating up to the temporal region. In some of these patients, opening of the mouth causes severe discomfort. This discomfort can become so unbearable that the patient refuses to eat, leading to weight loss and dehydration. There has also been reported cases in which patients have committed suicide because of the unbearable pain associated with this myofacial pain dysfunction syndrome.

Most dental clinicians feel that the majority of such patients are experiencing pain due to malocclusion of the teeth. Other clinicians feel that there are other reasons for this syndrome. The psychological and stress factor cannot be ruled out as a contributing factor, nor can faults developing in the temporomandibular joint be ignored. As one can clearly see, no one cause can be the specific etiologic factor in the myofacial pain dysfunction syndrome. Joint pathology, muscular incoordination and trismus, malocclusion and stress must be considered to.

It is the author's point of view that patients complaining of facial pain when all pathology is ruled out, are experiencing a number of problems. Stress associated with some family or personal problem causes muscle tensions and spasms leading to incoordination of the muscles of mastication. The stress causes bruxing or clenching of the teeth subconsciously. This leads to pain and fatigue. To relieve the patient of pain, a reverisble procedure must be used. The injection of local anesthetics without vasoconstrictors into the origin and insertion of the

affected muscle causes the immediate relief of pain. Muscle fibers degenerate and regenerate by 30 days without evidence of scarring. The patient is also placed on tranquilizers and followed for a period of two weeks. While the patient is free of discomfort, the psychological problem hopefully will have decreased and the trismus and spasms will have subsided.

This method of treatment is a more conservative and reversible one compared to complete occlusal adjustments and surgery.

Summary:

1.   The local anesthetics Lidocaine, Carbocaine, Citanest and
Procaine were administered intramuscularly into the anterior boarder
of the masseter muscle bilateraly of 90 Wister rats.

2.   Degeneration of the muscle fibers progressed followed by
regeneration.

3.   Necrosis of the muscle fibers did not occur.

4.   The lesions produced by all the local anesthetics were
essentially the same for all the local anesthetics tested.

5.   The lesion produced was reversible.

6.   The clinical significance of using local anesthetics in the
treatment of myofacial pain dysfunction syndrome must be considered
as an adjunct to therapy because of its reversibility and conservatism.

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Figure 1. Injection of local anesthetic into the anterior deep division of the masseter muscle.
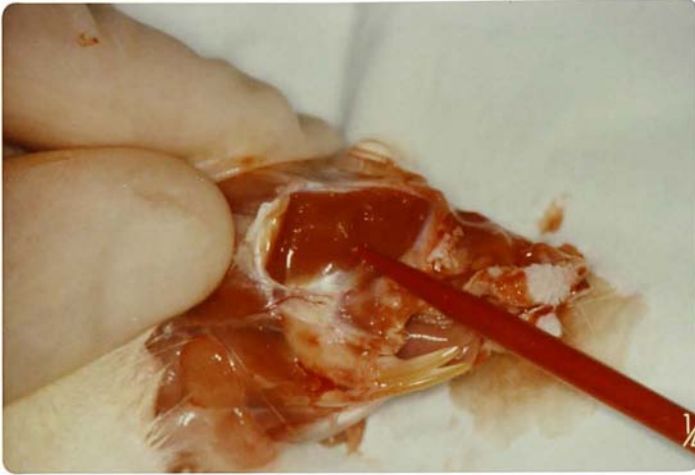
Figure 2. Dissected head revealing the needle pathway and masseter
muscle.

Figure 3. Muscle biopsy of a normal masseter muscle.

Figure 4. Muscle biopsy of an injected masseter muscle. Note change in
          appearance of tissue in the upper left region of the sample
          as compared to Figure 3.
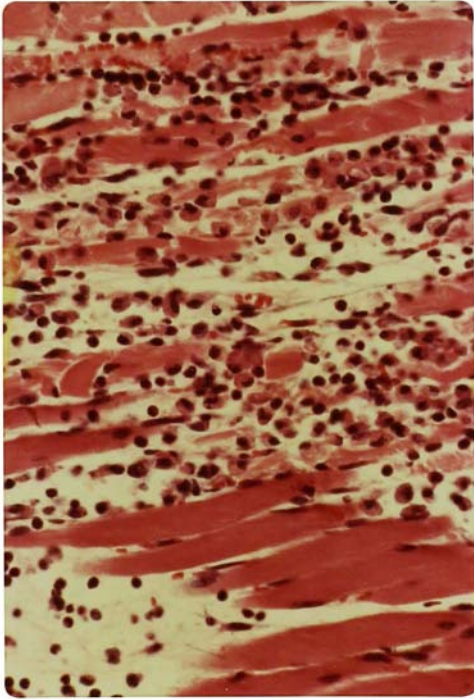
Figure 5. 24 hours post injection: 100X-LS: Interstial tissues show
marked edema with numerous polymorphonuclear leukocytes and
macrophages. Myofibrils are disrupted and cross striations
have disappeared.
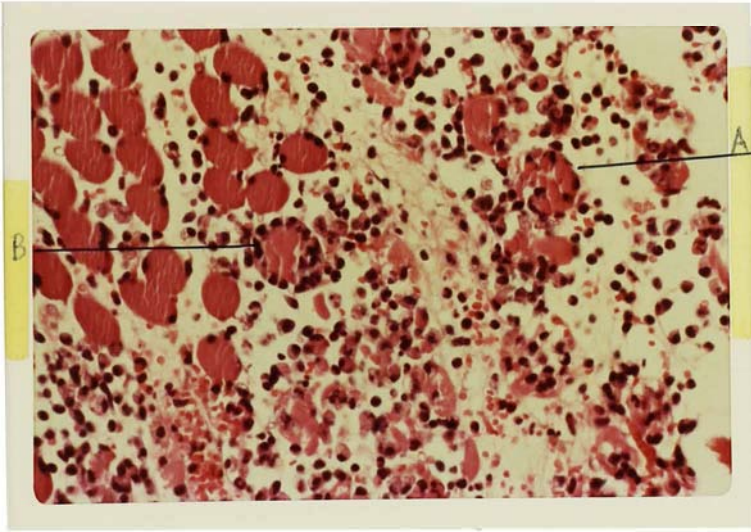
Figure 6. (100X-XS) 24 hours post injection revealing marked cellular
           infiltrate and varying degrees of degeneration.
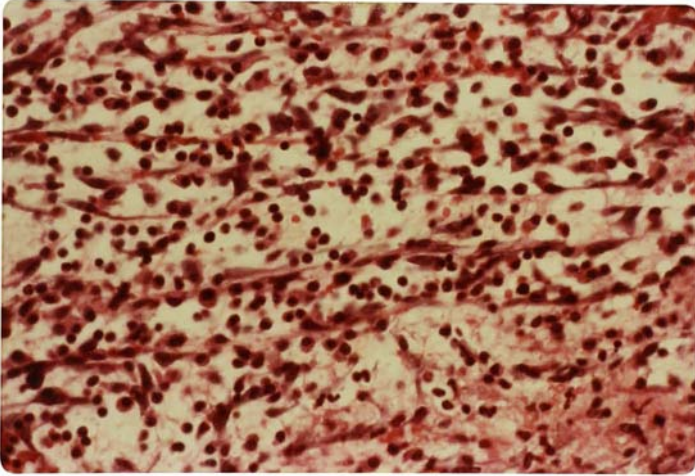
Figure 7. 48 hours post injection (100XLS) Marked vacuolization of fibers leaving multinucleated tubes with an intact sarcolemma.
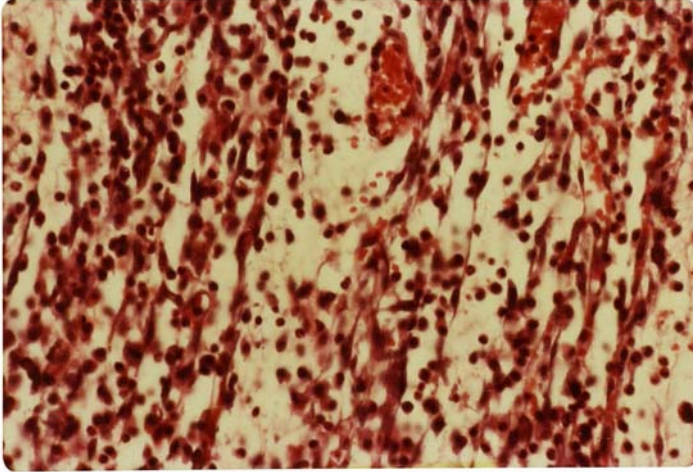
Figure 8. 48 hours post injection (100XLS) revealing basophilic peripherally
           placed sacroplasm and a decrease in fiber diameter. Note
           cellular infiltrate both within the interstitium and fibers.
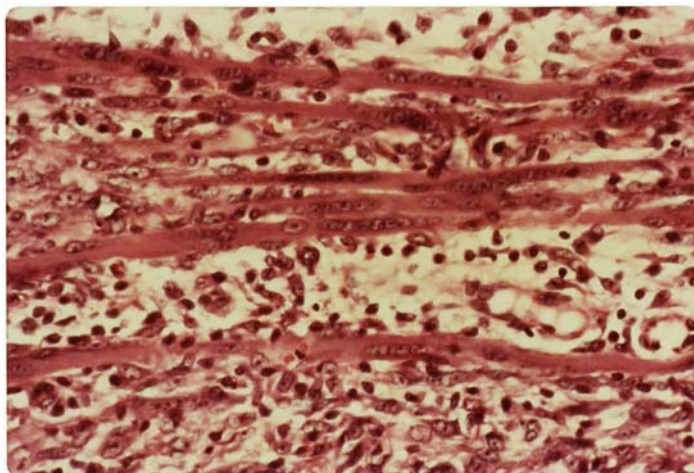
Figure 9.   (100XLS) Marked basophilic multinucleated tubes with large
             vesicular nuclei and prominent nucleoli. Note that the
             nuclei are centrally placed and are lining up side by side
             to form a continuous chain. 4 days post injection.
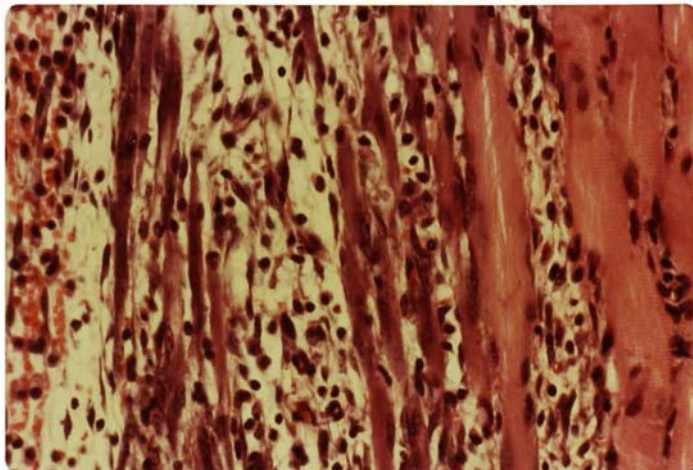
Figure 10.   Day 4 (100XLS) Note relatively unaffected fibers on right
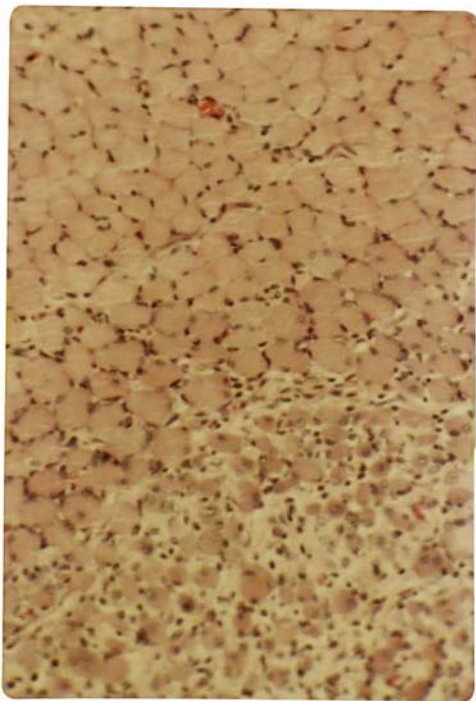and affected fibers on left.

Figure 11. 7 days post injection (20X-XS) large centrally placed nuclei in the damaged region in the lower half represent the repair process as compared to the peripherally placed nuclei of the uninjured fibers in the upper half of the photograph.
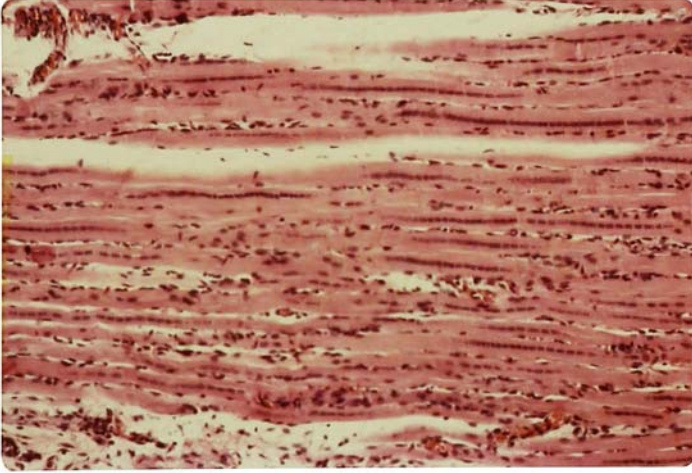
Figure 12.   15 days post injection (40X-LS) note relatively intact
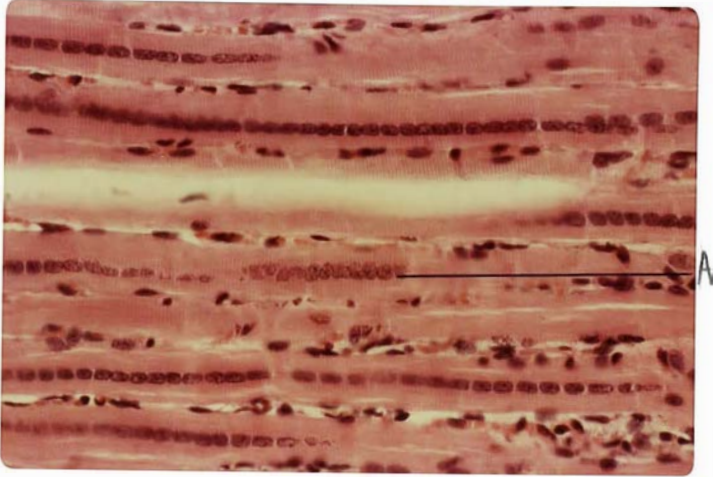   multinucleated fibers.

Figure 13. 15 days post injection (100XLS) Point A reveals nuclear
            fragmentation leaving behind normal appearing nuclear
            fibers with peripherally placed sacrolemma nuclei.
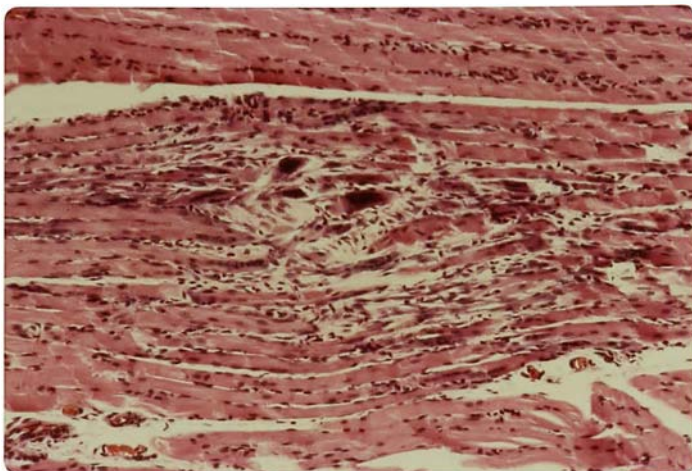
Figure 14.   Day 7 Saline central (40XLS) reveals a localized region of
             degeneration following the same pattern as the lesion
             produced by local anesthetics.