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Regeneration of Denervated Striated Muscle in the White Rat

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REGENERATION OF DENERVATED STRIATED
MUSCLE IN THE WHITE RAT

by

William Constantine Avgerin

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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LIFE

William Constantine Avgerin was born in Chicago, Illinois, on January 22, 1933.

He was graduated from Sullivan High School, Chicago, Illinois, in June of 1950 and entered Quincy College, Quincy, Illinois, in September of that year. He received his Degree of Bachelor of Science from Quincy College in June of 1954.

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CHAPTER I

INTRODUCTION

Zenker was the first investigator to study the degenerative pattern in striated muscle (Adams, Denny-Brown and Pearson, 1954). His tissues were obtained from fatal cases of typhoid which occurred during the epidemic in Dresden between 1859 and 1862. Zenker studied 120 cases and found that degenerative lesions of the rectus abdominis were not uncommon. Many other muscles were also prone to this type of lesion, but the rectus abdominis was so frequently involved that this lesion became a significant manifestation of the disease. Zenker attributes the original description of hyaline degeneration to Louis who discovered this phenomenon in 1830.

The lesion described by Zenker consisted of hyalinization of the contractile substance of the muscle fibers ultimately ending in complete or partial destruction of the muscle substance. Under gross observation the muscle had marked pallor and was quite brittle. Microscopically the lesion showed loss of striations of the fibers with a peculiar clumping of the hyaline contractile substance. This combination of events led to the complete breakdown of the fibers.

In the few years following the discovery of "Zenker's Degeneration" numerous persons undertook the study of degeneration and regeneration.

Forbus (1926) points out that notable among these persons were Waldeyer (1865), Weber (1867), Hoffmann (1869), Neumann (1868), and Tschainski (1868). Hoffmann was the only investigator who based his work on lesions resulting from disease processes. The others produced lesions experimentally and described the results.

In 1918, United States Army investigators studied degeneration of muscle in fatal cases of pneumonia associated with the influenza epidemic (Forbus, 1926).

Forbus (1926) produced degeneration of striated muscle by introducing powerful irritants such as alcohol, phenol and boiling water, or by depressing the blood supply to the muscle for a period of three hours. He described degeneration as seen by Zenker. His chief concern, however, was to study the character and origin of the cells which partake in these processes. Waldeyer, Weber, and Zenker had all studied these cells and disagreed on their origin. Zenker said that all cells within the sarcolemma, both phagocytic and regenerative, were of connective tissue origin. Waldeyer believed that all cells originated from the nuclei of muscle fibers. Weber said that muscle cells might be transformed into "inflammatory cells". He also thought that all cells were derived from muscle cells.

Forbus used vitally stained animals and found that phagocytic cells were of extramuscular origin and the regenerating muscle fibers arose from cells which developed from the nuclei and sarcoplasm of the old preserved

fibers. Forbus had an opportunity to study degeneration of muscle in twenty-five cases of pneumonia secondary to influenza or measles. His description paralleled that of Zenker.

Millar (1934) worked on regeneration in rabbits and gave a classical picture of the events. He noticed that during the first three days there was a blood clot present at the site of injury with a marked infiltration of leucocytes. In the next couple of days the most striking feature was the invasion of the injured zone by large numbers of histiocytes. Degenerating fibers showed fragmentation and their nuclei showed no signs of activity, while the nuclei in the injured but living fibers were lining up in rows towards the middle of the fiber. At six to eight days after trauma, regenerating sprouts were seen arising from injured fibers. These fibers increased in size and number until the area was barely discernible from the uninjured portions of the muscle.

Millar was the first to notice the rhombic shape of the muscle nucleoli after three to five days and called them "Rhombosomes". He thought that the rhombosomes were associated in some way with the laying down of new myofibrils. He described the giant cells and held that they were not phagocytic. He thought that any debris found within a giant cell was merely not extruded or absorbed as yet.

Speidel (1938) after injuring muscles by numerous methods, found that "retraction caps of injury" formed at the site of injury. These caps formed

as a result of retraction of the contractile material. A muscle fiber subjected to hot water might have many "retraction clots" throughout its length. These were similar to the "caps" although the "clots" occurred throughout a fiber and the "caps" occurred only at the site of injury. Speidel correlated the retraction caps with the cross striations. The caps could not be induced in myoblasts or muscle plasmodia even though these were on the verge of developing cross striae. They could not be induced in fibers which had lost their striae through injury. They could, however, be induced in young fibers immediately after regaining cross striations. According to Speidel, these caps were advantageous in that they tended to localise an injury and prevented further retraction of the fibers.

Speidel did his work on tadpoles. He was the first to observe individual muscle fibers in a living vertebrate over a prolonged period of time. He was unique in producing degeneration in some of his animals by electric shocks. One of the outstanding features of Speidel's work was the fact that he took cine-photomicrographic pictures of a myoblast dividing by mitosis.

Levander (1945) made some interesting observations concerning regeneration. He noticed an abundant development of mesenchymatous tissue around degenerated muscle fibers after injecting alcohol into striated muscle. He believed that new muscle fibers were induced to form from the mesenchymatous source and not from old degenerating fibers.

LeGros Clark and Blomfield (1945) described regeneration after blocking

the blood supply to the muscle of rabbits for various lengths of time. Their main interest was to trace the anastomoses of vessels within muscles and to indicate their importance in regeneration. They found that necrotic areas in devascularized muscles were rapidly replaced by regenerating muscle fibers. This led to a partial reconstitution of the muscle. They also studied the effects of gunshot wounds on the vascularity of muscle. They pointed out that a gunshot, four to five millimeters in diameter, may produce a zone of devascularization extending over large areas up to fourteen by eight millimeters. This was shown by injecting the animal in the ear vein with Bromo-Phenol blue, a dye which penetrates tissues rapidly, but does not color devascularized areas. They said that the effects were evidently due to lack of blood supply to the area because the unstained portion was too extensive to be ascribed to the direct traumatic injury to the muscle created by the bullet.

LeGros Clark (1946), also using rabbits, studied regeneration of striated muscle in excised portions of muscle which were immediately replaced. Regeneration took place in the normal way. He also replaced excised pieces of muscle at right angles to their original position. He found that as muscle fibers from the normal muscle entered the graft they oriented themselves in the direction of the grafted fibers. This indicated the importance of the sarcolemmal tubes and the role they played in directing the new fibers.

Sissons and Hadfield (1953) studied the effects of cortisone on regeneration of muscle in rabbits. They found that regeneration occurred, but that cortisone retarded the process. Regenerating sprouts, instead of appear-

ing about four days after injury, were seen seven to eight days after crushing. Unphagocytized necrotic fibers remained for some time after regeneration commenced. Some of the necrotic fibers showed calcification, and focal areas of necrosis were found in muscle remote from the injured area similar to that found in Zenker's degeneration.

Saunders and Bissons (1953) are the only men who studied regeneration of striated muscle after denervation. They found that muscle in the rat does regenerate when injured three weeks after denervation even though atrophy was found throughout the muscle. Their procedure included excising one centimeter of the sciatic nerve. Three weeks later the denervated muscle was crushed.

Gay and Hunt (1954) studied striated muscle of rats at various intervals after transection. They macerated and inspected the teased fibers under the phase microscope. They wanted to see whether individual fibers from one side of the incision united, with direct continuity, with fibers from the other side. This was shown to occur. In the early stages, the two fibers are connected by an amorphous cytoplasm. At the end of thirty days cross and longitudinal striations appeared in many anastomoses. The nuclei were reduced in number and had moved to the periphery of the fibers. The fibers were nearly parallel to one another and in line with the long axis of the muscle so that the entire muscle mass appeared close to the normal.

Constance (1955) found that skeletal muscle of guinea-pigs had remarkable powers of regeneration, but the degree to which regeneration occurred depended on the amount of proliferating fibroblastic tissue and the type of

trauma. In a simple crush injury, regeneration might be complete. If the cut ends of a muscle were widely separated, regeneration might be halted. Three days after trauma produced by excision or incision, Constance found what appeared to be "cellular tubes" resulting from the invasion of dead fibers by histiocytes. There was proliferation of the fibroblasts in the endomysium between these fibers. Constance also reported giant cells. He saw no evidence of mitotic activity in the regenerating sprouts.

Ellis (1955) treated rabbits with cortisone for various lengths of time and produced lesions, in muscles, which became more extensive with the passage of time. The degenerative changes grossly were similar to those seen by Zenker. Microscopically, the earliest signs of degeneration were swelling of fibers and loss of myofibrillae. The nuclei were aligned in parallel rows in the center of the fiber. Regenerative changes were first noted in the form of protoplasmic tips growing out from uninjured portions of fibers. After twenty-one days, some forty to eighty percent of the muscle fibers in any given field displayed all the stages of degeneration and regeneration mentioned above. Five rabbits were treated for twenty-one days with cortisone. From six to twenty-two weeks after discontinuing the treatment, muscles were histologically normal in every instance.

Godman (1957) described regeneration as seen by previous workers. He reported giant cells present in the six-day stage and also "Hydropic" vacuoles in degenerating fibers. He said that true regenerative elements have been observed to arise only from preexisting muscle segments. One mitotic figure

was reported in a sarcoblast ribbon. Godman also presented some figures of elongated nucleoli similar to those first described by Millar.

CHAPTER II

MATERIALS AND METHODS

Sixty-six Sprague-Dolly white rats were used. Ether anesthesia was difficult to control so was abandoned in favor of pentobarbital. This anesthetic caused convulsions making it impossible to do detailed work on the animal. This was also discarded in place of using a combination of the two. Sometimes breathing would stop and artificial respiration had to be performed. Finally ether was successfully employed. Losses during operations all occurred in preliminary experiments.

The rectus abdominis muscle was used in all operations. This was chosen because of its accessibility and also because it is frequently incised in surgical procedures. The rectus is innervated by thoracic nerves five through thirteen which enter the muscle from its lateral border.

A vertical incision was made in the skin from the xyphoid process to within one centimeter of the pubic symphysis. The skin was reflected by cutting the subcutaneous tissue thus exposing the rectus muscle on the left side. Midway between the superior and inferior borders of the rectus muscle, the anterior rectus sheath was exposed and a transverse incision was made through the entire depth of the muscle down to the posterior rectus sheath. The anterior rectus sheath in the upper one half is closely adherent to the

underlying muscle. The fact that this sheath is so closely adherent to the muscle facilitates excellent approximation of the cut ends. After many preliminary operations the technique improved and the inferior epigastric artery, which runs along the lateral border of the rectus muscle was avoided. Two sutures were placed across the gap. Deknatel, triple 0 braided silk surgical suture was found to be most advantageous in this procedure because this type of suture does not swell when dampened, it ties easier, and when cut, the ends do not fray. Number 20 stainless tissue needles were used.

The second incision was made along the entire length of the lateral border of the rectus muscle from the inferior border of the thoracic cage to within one centimeter from the pubis. This incision extended at least one centimeter distal to the transverse incision and penetrated the entire depth of the muscle into the peritoneal cavity. Four to five stitches were used to close the incision. This procedure insured the transection of all the thoracic nerves leading to the rectus muscle.

The transverse incision was sutured before beginning the lateral incision in order to approximate the cut ends better. If this is not done in this sequence, the closing of all incisions becomes difficult and sloppy.

Experience proved that it was not necessary to shave hair from the site of the incision. In suturing the skin flap, four sutures were used. The animals were isolated for one day postoperatively.

Electromyographic recordings were taken on a few animals in order to make sure that the muscle was denervated. The animals submitted to this

procedure were from the two-day, four-day, and six-day stages. None of the later stages were checked as this procedure was not adopted in the early stages of the work.

The skin was reflected on the animals under ether anesthetic and bipolar pickup electrodes were placed on the rectus muscle superior to the transverse incision. After a few minutes the anesthetic mask was removed and the animal was aroused. Spontaneous activity was recorded. The anesthetic was then replaced and the electrodes were moved to a new site lateral to the lateral border of the rectus muscle. The animal was once more allowed to recover and then placed under anesthetic again. Stimulation sufficient to activate the intercostal nerves was also applied lateral to the muscle. This whole procedure was repeated on the opposite unoperated side.

The animals were sacrificed at two, four, six, seven, nine, fourteen, twenty-one, twenty-eight, and fifty-six days. For each stage, eight animals were operated on except for the two and seven day stages. Four animals for each of these stages were used.

The tissues were fixed in Calcium Formal and sectioned at eight micra. Some serial sections were cut in order to trace individual fibers. The stains used were Hematoxylin and Eosin and HCL-Biebrich Scarlet-Methyl Blue Variant. The latter is a connective tissue stain.

CHAPTER III

RESULTS

Two-Day Stage.

The zone of injury is recognizable, under low power, by the interruption in continuity of the muscle fibers and the abundance of basophilic staining cellular elements. Coagulated blood can be observed within the gap, but does not cover the entire zone.

Under high power the muscle is seen to have been infiltrated with a great number of histiocytes, fibroblasts and polymorphonuclear leucocytes and some lymphocytes.

Degenerating fibers may be noticed by the vacuolation and fragmentation present in them.

The sarcolemmal tubes are not recognizable in the center of the zone of injury. On either side of the zone, the walls of the tubes persist but their contents have been phagocytized except for a small number of fragments which have remained. The sarcolemmal tubes at the periphery of the zone, contain histiocytes which are tightly packed against the muscle tissue.

Mitotic figures were noticed in this stage. Some appeared to be within fragmenting fibers.

When staining with HCL-Biebrich Scarlet-Methyl Blue Variant, it is

found that the nuclei within muscle fibers are sharply defined along with their contents, while the nuclei of fibroblastic cells are ill-defined and their contents appear as a black mass.

At this stage there was no evidence of regeneration.

Four-Day Stage.

Degenerating fibers may still be defined by the numerous vacuoles they contain. They have maintained their cross striations and muscle cell nuclei have moved toward the center of the fibers. Myofibrillae are seen to be separating from one another and the muscle fibers have an irregular, more or less wavy appearance (Fig. 1).

At this stage the first signs of regeneration appear in the form of slender strap-like protrusions which have entered the zone of injury (Fig. 2). These fibers appear to be originating from unphagocytized portions of fibers at the periphery.

These strap-like protrusions grow for the most part in parallel with the preexisting fibers (Fig. 1). They are irregular in shape, have smaller diameters than normal fibers and contain no cross striations. They do, however, possess faint longitudinal striations and stain a violet hue with Hematoxylin and Eosin in contrast to the pink of the normal muscle sarcoplasm. The strap-like protrusions stain deep blue with HCL-Diebrich Scarlet-Methyl Blue Variant, while the fibers from which they originate stain scarlet.

In some tissues, the transection was made close to a tendinous inscrip-

tion. In these specimens, the fibers have degenerated to the tendinous inscription. Regenerating fibers extend from the inscription out to the zone of injury. These fibers appear longer than the sprouts coming from the opposite side of the zone of injury. Fibers beyond the area of inflammation were not subject to degeneration (Fig. 2).

The nuclei in regenerating sprouts may be found lined up in chains of twenty or more (Fig. 1). Many regenerating fragments exist within the zone of injury.

Histiocytes and fibroblasts are still numerous in the center of the injured zone and even between fibers distal to the injury. No histiocytes were found within the sarcolemmal tubes; indeed, no sarcolemmal tubes are recognizable in the zone of injury. The histiocytes are most often recognized by the debris which they have engulfed.

Six-Day Stage.

Regeneration has progressed at a rapid rate. The healing area appears smaller and many more regenerating sprouts are seen. Regenerating fragments are found from one end of the healing area to the other, although no single fiber is seen in the microscopic field extending across the injured zone.

The fibers near the periphery of the injured zone have undergone changes which include vacuolation, separation of myofibrillae and the nuclei are lined up in chains. The fibers vary greatly in their characteristics. These fibers are striated except near the junction with the new plasmodial

outgrowths. They are acidophilic whereas the regenerating fibers are basophilic (Fig. 3).

Regenerating sprouts are longer than in the four-day stage. The diameter of the sprouts has not markedly increased. Longitudinal striations have become slightly more distinct. The nuclei are often found in rows and sometimes in clumps (Fig. 4).

Histiocytes and fibroblasts are still present in large quantities in the zone of injury and between nearby fibers. Fibroblasts surround a few regenerating sprouts. The fibroblasts appear in long chains and seem to be connected.

Regenerating sprouts were found passing through the interrupted portions of the anterior rectus sheath (Fig. 4).

Seven-Day Stage.

The quantity of histiocytes and fibroblasts has not diminished. Adjacent fibers continue to show vacuolation and cross striations. Again the cross striations are everywhere except at the origin of the regenerating sprouts, at which point the staining is lighter and there seems to be a more granular appearance. The nuclei of these fibers are very large and well defined. They are not all found in chains but are also scattered throughout the fibers. These fibers are acidophilic when using Hematoxylin and Eosin stain.

The regenerating sprouts are quite numerous. The nuclei are very

large and in most cases are as much as twice the size of nuclei of the surrounding fibroblastic tissue (Fig. 5). Myofibrillae are apparent in these basophilic sprouts.

Nine-Day Stage.

In this stage the regenerating fibers have definitely crossed the zone of injury (Fig. 6). They are more acidophilic in color and are still quite irregular in shape. The diameter of the fibers has increased and the nuclei are more distinct. No cross striations are present.

For the most part, the adjacent fibers have lost their vacuoles. Cross striations are present and the myofibrillae are still separated from one another. The nuclei appear very large, are more heavily stained and are more distinct. They are found in the center of the fibers as well as towards the periphery.

At the periphery of the zone, there is a mixture of scarlet and deep blue fibers, when staining with HCL-Biebrich Scarlet-Methyl Blue Variant.

In some animals sutures were placed deeper into the mass of the muscle. The fibroblastic material completely surrounded the suture and all fibers were diverted around it (Fig. 7).

In some areas regenerating sprouts have advanced through a gap in the anterior rectus sheath (Fig. 6). Histiocytes and fibroblasts have decreased in number.

Fourteen-Day Stage.

In general, the gap is considerably smaller and completely filled with regenerating sprouts. Vacuoles have disappeared, and the nuclei are still large and more are located near the periphery of the fibers. Myofibrillae are separated and cross striations are evident. For some distance beyond the injured zone, the nuclei may still be lined up in chains. However the chains are not as numerous and they do not contain as many nuclei as in previous stages.

The histiocytes and fibroblasts have decreased in number and those found between fibers are only seen near the zone of injury.

The regenerating sprouts are not yet equal in diameter to normal fibers. They appear light violet and have faint cross striations except in the area of the fiber which is in the center of the zone of injury. This is shown with Hematoxylin and Eosin stain. Individual fibers are found which stain scarlet, violet, or blue, when using HCL-Biebrich Scarlet-Methyl Blue Variant stain. The scarlet colored fibers are normal and their cross striations are very striking. The violet fibers contain cross striations which are barely visible, while the blue fibers have no cross striations.

The nuclei of the regenerating sprouts are not generally clumped at this stage.

Later Stages.

In the later stages, the zone of injury is recognizable by the irregu-

larity of the fibers. Some fibers appear to have been cut at an oblique angle resulting in what appears to be fragments (Figs. 8, 9, 10). These stain blue with HCL-Diebrich Scarlet-Methyl Blue Variant, as opposed to the scarlet hue of the normal fibers.

A few chains are still found with three or four nuclei but these are steadily diminishing until the fifty-six day specimens where they have disappeared completely; indeed, the zone of injury is no longer distinguishable in the fifty-six day stages.

Faint cross striations show up within new fibers in the zone of injury in the four-week stage.

The diameter of the regenerating fibers has apparently increased considerably between twenty-eight and fifty-six-day stages because no difference in diameter can be seen in any regenerating fibers of the fifty-six-day stage.

The normal fibers beyond the zone of injury have become atrophic. In the two-day stage, the diameters of one hundred fibers were measured. The average was 55.5 micra. In the four-week stage the average diameter of one hundred fibers was 27.0 micra; in the fifty-six-day specimens, 21.0 micra.

Very little fibroblastic tissue is left in the three-week stage and disappears completely by the fifty-six-day stage.

The results of the electromyographic studies proved interesting in connection with denervated muscle.

In the anesthetized animal, fibrillation potentials of fifty to three

hundred micro-volts amplitude and one to two milliseconds duration were recorded from bipolar electrodes placed in the rectus muscle superior to the transverse incision. When the animal was allowed to recover with the electrodes still in place, the same fibrillation potentials were recorded. When the animal was again subjected to the anesthetic and the electrodes placed in the rectus muscle of the opposite side, no activity was found. Upon recovery, motor unit activity of increasing amplitude and number was recorded as the anesthetic wore off.

In the anesthetized animal, sufficient stimulation to activate the intercostal nerves was applied to the lateral abdominal wall. No reaction to this stimulation was recorded from the rectus muscle. A normal twitch reaction was recorded on the normal side when the same procedure was utilized.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Skeletal muscle denervated at the time of transection regenerates as shown.

Degeneration of the ends of the cut fibers precedes the regeneration. In the two-day stage, it is well on its way. Portions of fibers are being invaded by histiocytes and the fibers are breaking down and fragmenting. The fibers are stained red with Hematoxylin and Eosin, and their cross striations are apparent. The nuclei have enlarged somewhat and not all nuclei are found at the periphery of the fibers. The most advanced degenerating fibers appear as a light pink granular mass or meshwork. Using HCL-Biebrich Scarlet-Methyl Blue Variant, fibers which are beginning to undergo degeneration appear scarlet.

As degeneration progresses, the scarlet gives way to a blue. Before a fiber breaks down completely, it loses its cross striations. Where tendinous inscriptions are near the transection, degeneration progresses through the sarcolemmal tubes by histiocytic invasion until reaching the inscription. Here it is halted. On the other side of the inscription, normal fibers are present.

In the four-day stage, degenerating fibers are still present but regenerating sprouts are found scattered amongst them. The sprouts are stained

deep blue with Hematoxylin and Eosin and their nuclei are lined up in long chains. Where a tendinous inscription is found, the regenerating muscle extends to the inscription (Fig. 1). An interesting finding about the regenerating fibers on the tendinous side of the injured zone is that they are much longer than those on the opposite side of the zone.

As stated before in the results, mitotic figures were evident in the two-day stage. These were seen only near the periphery of the zone of injury. The mitotic figures were found within degenerating fibers and also scattered loosely within the connective tissue. Mitosis was also seen by Lash, Swift and Holtzer (1956) prior to the alignment of nuclei.

Since the regenerating fibers between a tendinous inscription and the transected zone are not in continuity with any normal muscle, it seems evident that they are being formed by the muscle nuclei remaining in the sarcolemmal tube of the degenerated fiber.

Hematoxylin and Eosin was very effective in exposing, with clarity, most elements except for the reticular and collagenous fibers of the connective tissue. Muscle nuclei stained violet. The nuclei had an oblong appearance except in later stages where they enlarged and became more oval. The nucleus contained one and often two nucleoli. The karyoplasm appeared as a network of fine fibers.

The fibroblasts appeared as irregular, cigar shaped cells. Their nuclei stained a more intense violet. The fibroblasts contained a network within the karyoplasm which was coarser and more deeply stained than that found

in the muscle nuclei.

Histiocytes were most often recognized by the yellow debris which they contained. The nuclei were round and often indented with a very clear deep violet stained border. The nuclei contained granules which stained violet. An abundance of cytoplasm surrounded the nucleus which was irregular in form, stained light pink or very light blue, and appeared to have a network of light violet fibers within it.

Polymorphonuclear leucocytes appeared as cells with pink cytoplasm and small, multi-lobed violet nuclei.

Degenerating fibers have a light pink granular consistency with many vacuoles scattered throughout the fiber. Regenerating fibers stain violet with light violet stained myofibrillae present throughout them. Normal fibers appear pink or red with light blue cross striations.

The connective tissue fibers all appear deep pink.

HCL-Biebrich Scarlet-Methyl Blue Variant is primarily a connective tissue stain. However, it became quite useful in determining the extent of loose muscle cells within the fibroblastic tissue and also in illustrating differences between regenerating and degenerating fibers.

Normal muscle fibers were stained a deep scarlet. Cross striations were exposed as vivid, black lines. Collagenous and reticular fibers were stained deep blue. The degenerating fibers ranged from scarlet to blue, depending on the extent of degeneration. The most advanced degenerating fibers were stained blue.

Muscle cells stained blue or scarlet with bright red nucleoli. Histocytes and fibroblasts were so deeply stained blue, that they appeared black. Loose muscle cells could be found across the entire zone of injury.

An interesting point brought out by this stain is that as a fiber gets older, it changes from blue to scarlet. A regenerating sprout will appear blue while the parent fiber from which it originates maintains a scarlet color. This indicates that the two fibers have a different consistency.

This stain exposed the contents of muscle cells much better than Hematoxylin and Eosin. There can be no mistake that the karyoplasm of the muscle cells is made up of a network of fine fibers and that the nucleus contains from one to four nucleoli.

The Electromyograph was useful when applied to the muscle in order to check on denervation. Fibrillation potentials were recorded, in animals under ether anesthesia, with bipolar electrodes placed in the rectus muscle rostral to the transection. These fibrillation potentials were of fifty to three hundred micro-volts amplitude and one to two milliseconds duration. Electrical potentials of this type indicate denervated muscle (Licht, 1956, and Marinacci, 1955). The fibrillation potentials were recorded even after the animal recovered from the anesthetic. When sufficient stimulation to activate the intercostal nerves was placed lateral to the injured rectus muscle, no reaction was recorded from that muscle. As a control, similar tests were run on the opposite normal rectus muscle. Under anesthesia, no action potentials were recorded. As the animal recovered, motor unit activity resulted. When stimu-

lation was applied to the intercostal nerves, a simple muscle twitch was recorded. These findings show the injured muscle to be denervated.

Saunders and Sissons (1953) showed that denervated muscle regenerates. There are a number of differences between their work and ours. They used a crush injury on the gastrocnemius muscle of rats. Three weeks before crushing the muscle, one centimeter of the sciatic nerve was removed. The muscle on the opposite leg was crushed and used as a control. Saunders and Sissons found that regeneration is not prevented by lack of innervation of the muscle, but that atrophy does occur. Denervation of the muscle was permanent.

Atrophy also occurred in the specimens in this study. Although Saunders and Sissons did not report the diameters of fibers, the figures published indicate a greater degree of atrophy than observed here. This can be explained because they limited movement of the animal by crushing the muscles on both legs, after denervating the muscle three weeks previously.

The degenerating fragments seen by some workers (LeGros Clark, 1946 and Constance, 1946) in the zone of injury, are not, to any great degree, seen in the present study. This is probably due to the nature of the trauma on the muscle. An injury by crushing, by chemical means, or by a rifle bullet (LeGros Clark, 1945), does an extensive amount of damage to parts of a muscle distant to the original site of trauma. A simple transection may do little injury to adjacent muscle tissue.

It does not appear that the fibers in a transection injury degenerate much past the site of transection. Exactly what stops degeneration at any

given point is not known. Speidel (1938) thought that the retraction caps halted degeneration. These caps did not appear in our sections. The degree to which fibers degenerate is fairly constant. Where tendinous inscriptions were found, degeneration did not proceed beyond the inscriptions. However, if the inscription was near the transection, degeneration took place the whole distance from the transection to the inscription even though this distance was greater than the usual extent of degeneration. Regeneration of these fibers cannot be as sprouts from adjacent muscle fibers. It seems logical to assume that the regeneration here is carried out by the muscle cell nuclei.

Godman (1957) saw what he calls "Hydropic Vacuoles". It is not known whether or not the vacuoles described in our present study contained any fluid. The stains used rendered the vacuoles colorless.

The sarcolemmal tubes are believed to be advantageous in orienting the fibers in the proper direction; however, they do not seem as important as might be expected. The tubes may also help in reducing the time it takes for the muscle to regenerate, providing the histiocytes are not too closely packed within the tubes. In our sections, the sarcolemmal tubes were completely broken down and yet regeneration progressed at a rate equivalent to that found by other workers whose sections still contained intact tubes.

The majority of fibers, in later stages, contain cross striations and yet there are still some fibers, very few, which have no cross striations. These are thought to be continuations of newly formed fibers which have not advanced to the stage where they bear cross striations.

CHAPTER V

SUMMARY

1. A transverse incision in the rectus abdominis muscle was made in sixty-six Sprague Dolly white rats. Another incision was made along the lateral border of the rectus muscle in order to denervate that muscle. All incisions were sutured and the animals were sacrificed two, four, six, seven, nine, fourteen, twenty-one, twenty-eight, and fifty-six days following surgery. Tissues were fixed in calcium formal and stained with Hematoxylin and Eosin and HCL-Biebrich Scarlet-Methyl Blue-Variant.

2. Some animals were subjected to the Electromyograph, the results of which proved that the muscles were denervated.

3. A summary of the histological findings are as follows:

- a. Two-day stage. The zone of injury shows a mass infiltration of histiocytes, fibroblasts and polymorphonuclear leucocytes. Vacuolation and fragmentation of degenerating fibers occurs and mitotic divisions were found in some cells.
- b. Four-day stage. Regenerating sprouts were seen for the first time and muscle nuclei have moved towards the center of fibers and formed long chains. Where tendinous inscriptions are found, degeneration progresses to the inscription but

goes no further. Regenerating fibers between the inscription and the healing zone may be much longer than sprouts found on the opposite side of the zone of injury.

- c. Six-day stage. The healing zone is smaller.
- d. Seven-day stage. Nuclei of the regenerating sprouts are twice the size of the nuclei of the connective tissue.
- e. Nine-day stage. Regenerating fibers have definitely crossed the zone of injury. Histiocytes and fibroblasts have decreased in number and the adjacent fibers are losing their vacuoles.
- f. Fourteen-day stage. The injured zone is completely filled with regenerating sprouts. Vacuoles have disappeared. More nuclei have moved towards the periphery of the fibers.
- g. Later stages. The zone of injury is recognizable by the irregularity of the fibers. Normal muscle fibers have atrophied to an average diameter of 21.0 micra. Fibroblastic tissue disappears completely by the fifty-six-day stage.

4. The results show that two methods of regeneration may take place in an incised striated muscle. The first is by plasmodial outgrowths which originate from adjacent portions of fibers at the periphery of the zone of injury. The second is by a resynthesis of muscle within sarcolemmal tubes which contain no myofibrils. This resynthesis is presumably done by the nuclei of the degenerated muscle fiber.

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Figure 1

Four-day stage. The figure consists of many fine regenerating sprouts with nuclei lined up in long chains (A). The fibers at the lower right corner (B) are normal. They are shown terminating at a tendinous inscription (C). The wavy, vacuolated fiber (D) is degenerating. Hematoxylin and Eosin, 200x.

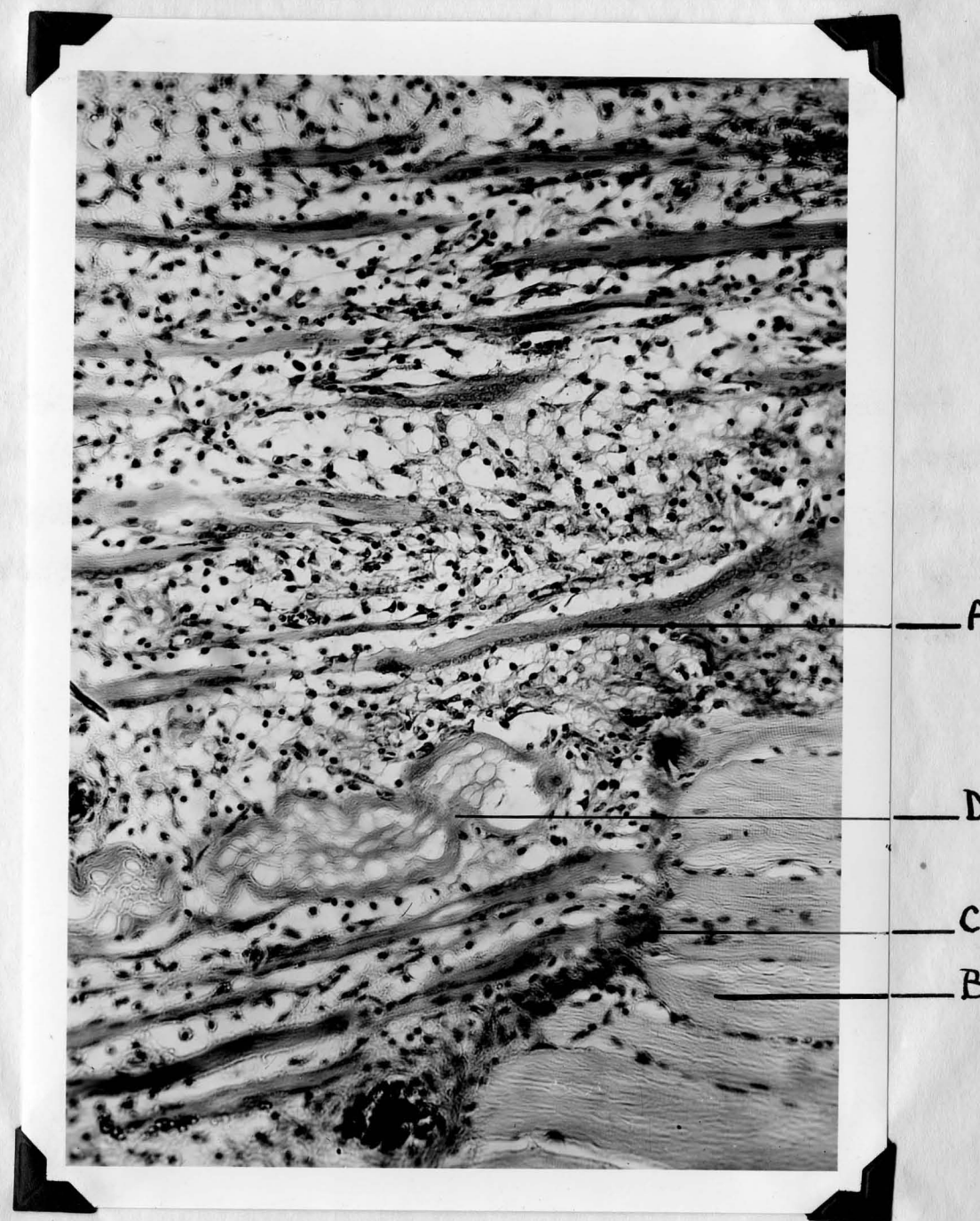


Figure 1

Figure 2

Four-day stage. The zone of injury is invaded by large numbers of histiocytes, fibroblasts and polymorphonuclear leucocytes. At the periphery of the zone of injury, fibers are shown with their nuclei in the center of the fiber (A). Fragments of regenerating sprouts are seen throughout the healing area (B). Hematoxylin and Eosin, 110x.

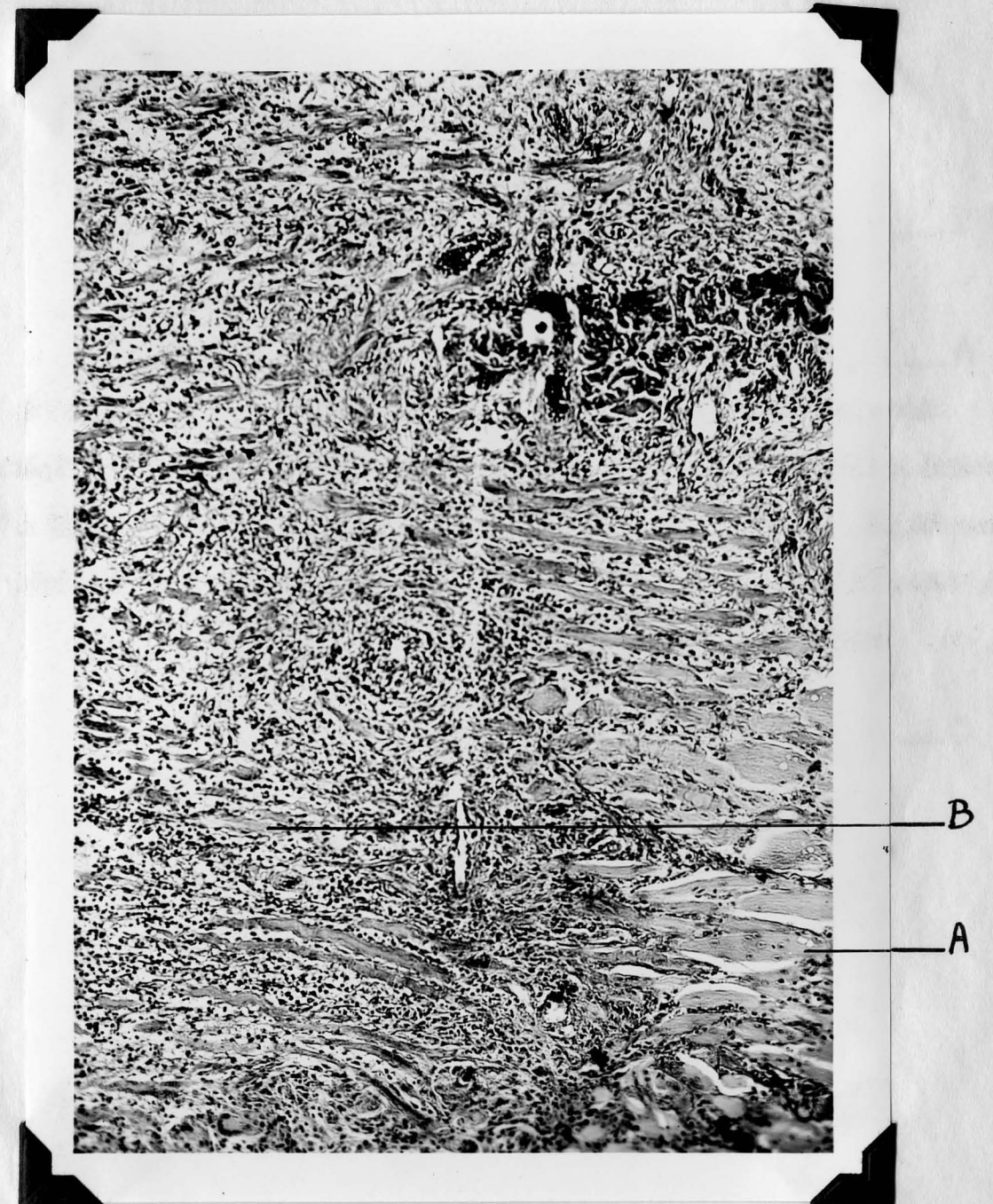


Figure 2

Figure 3

Six-day stage. The figure illustrates the vacuolated fibers (A). Plasmodial outgrowths (B and C) are seen originating from these fibers. The regenerating fibers show the presence of myofibrillae. Hematoxylin and Eosin, 200x.

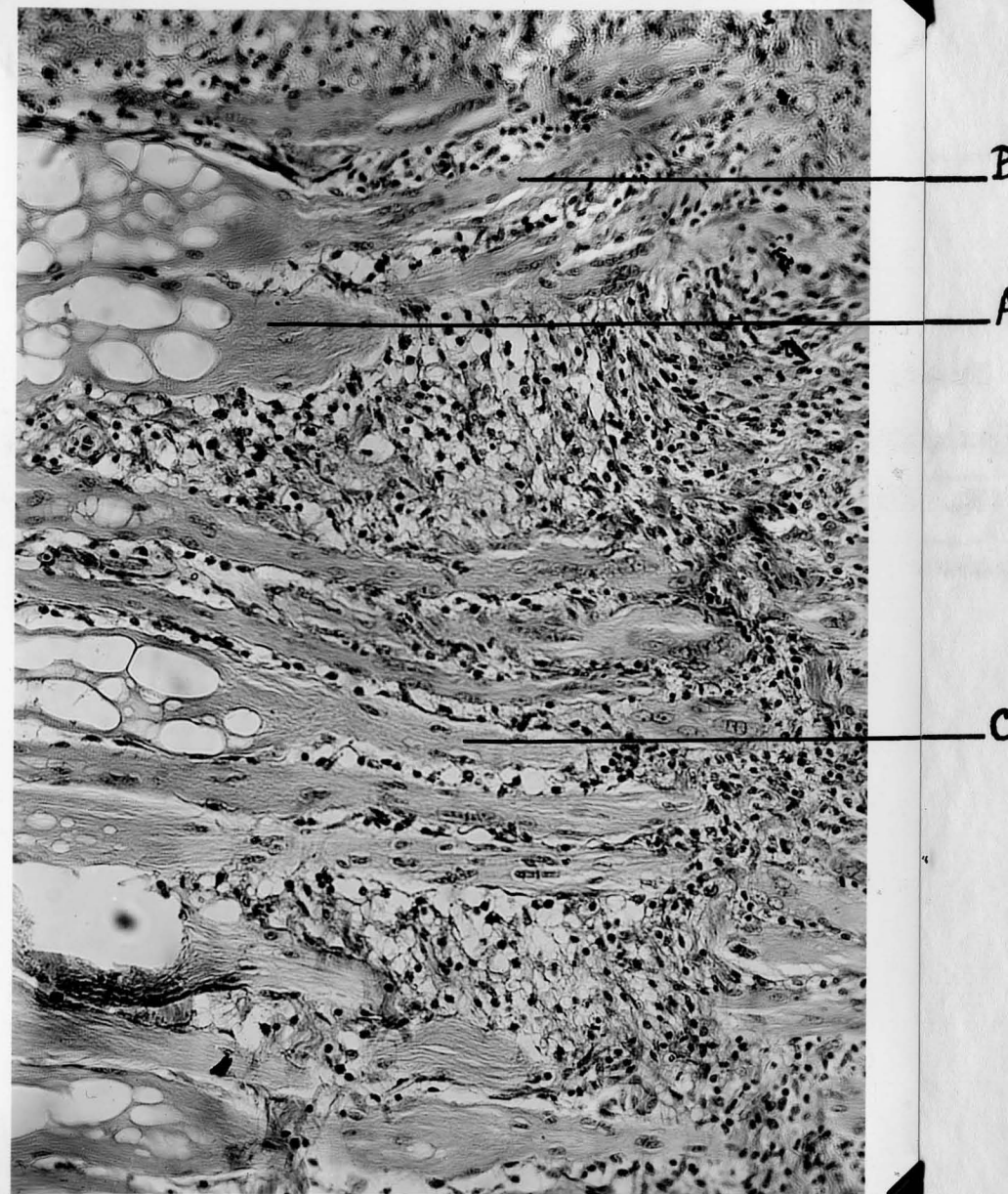


Figure 3

Figure 4

Six-day stage. This figure clearly shows the anterior rectus sheath (A) with regenerating sprouts passing through the interrupted portions of the sheath (B). The regenerating sprouts show nuclei clumped (C) and also lined up in chains (D). Hematoxylin and Eosin, 110x.

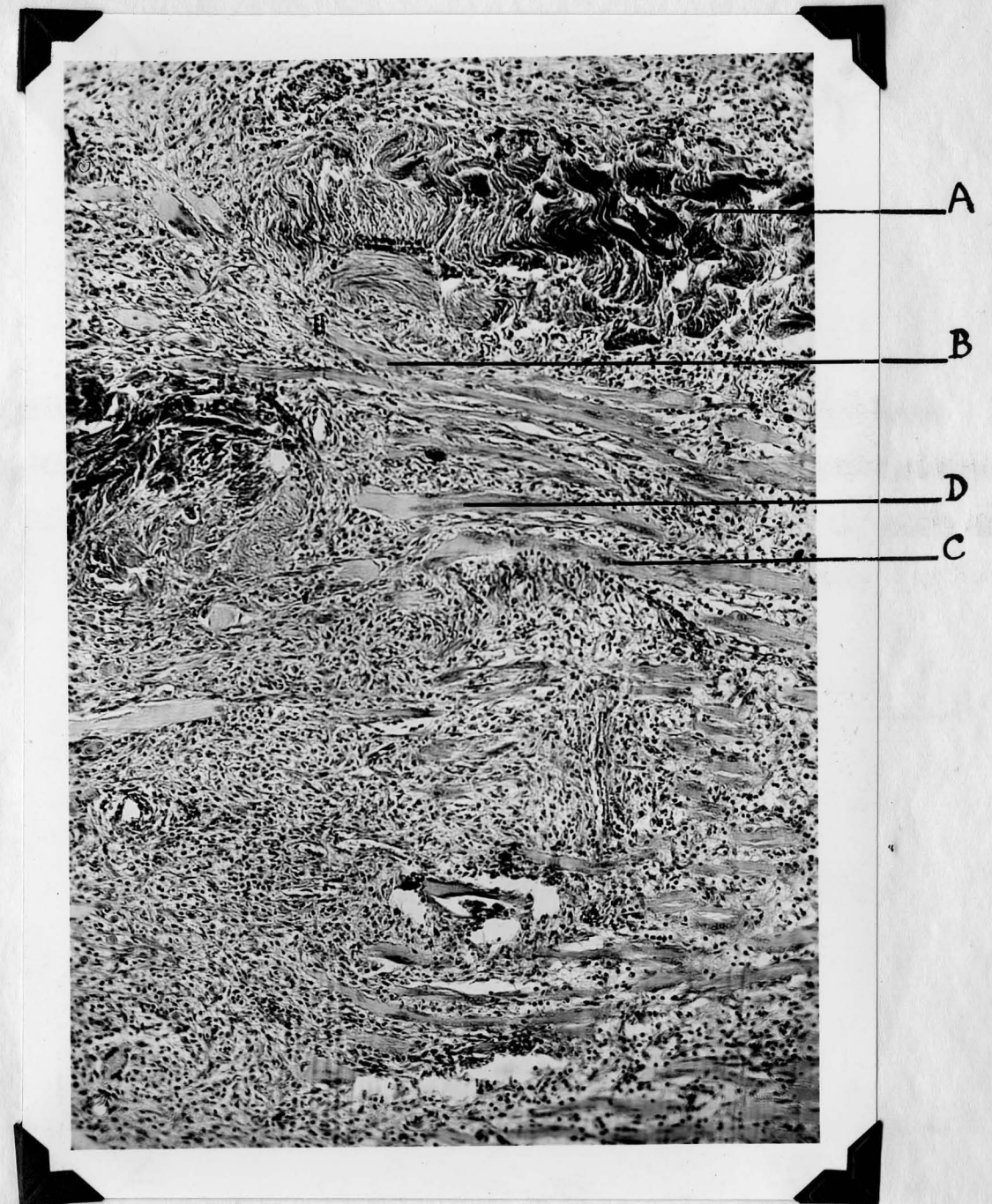


Figure 4

Figure 5

Seven-day stage. Regenerating sprouts are seen which contain nuclei in long continuous chains (A). The nuclei (B) are twice the size of nuclei of the fibroblastic tissue. Hematoxylin and Eosin, 400x.

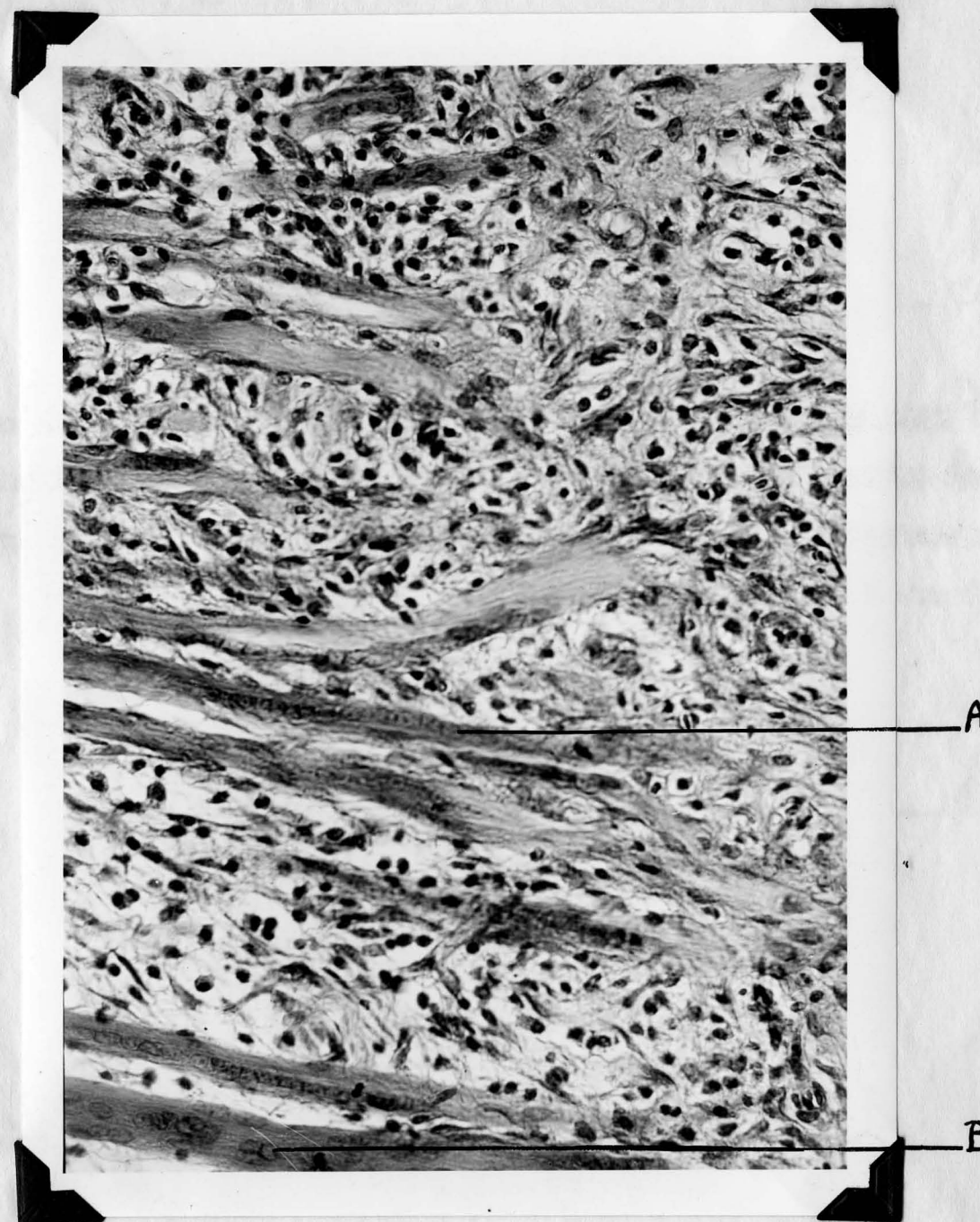


Figure 5

Figure 6

Nine-day stage. Regenerating fibers have definitely crossed the zone of injury (A). Vacuolation is nearly absent. This figure shows regenerating sprouts passing through the anterior rectus sheath (B) into an inflammatory zone (C) anterior to the rectus sheath (D). Hematoxylin and Eosin, 110x.

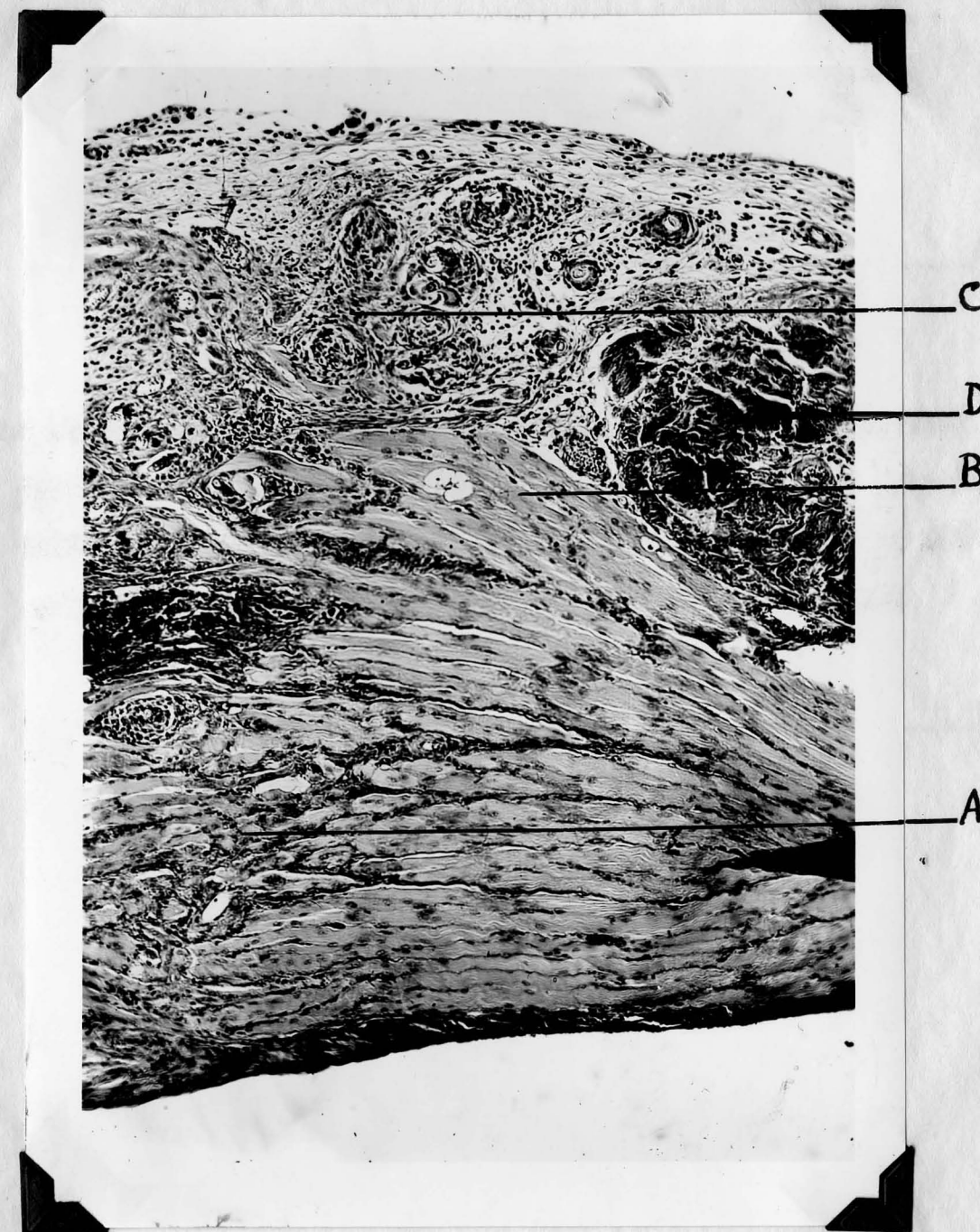


Figure 6

Figure 7

Nine-day stage. This figure shows a suture (A) impairing the growth of regenerating fibers (B). Fibroblastic tissue has grown around the suture (C). Hematoxylin and Eosin, 110x.

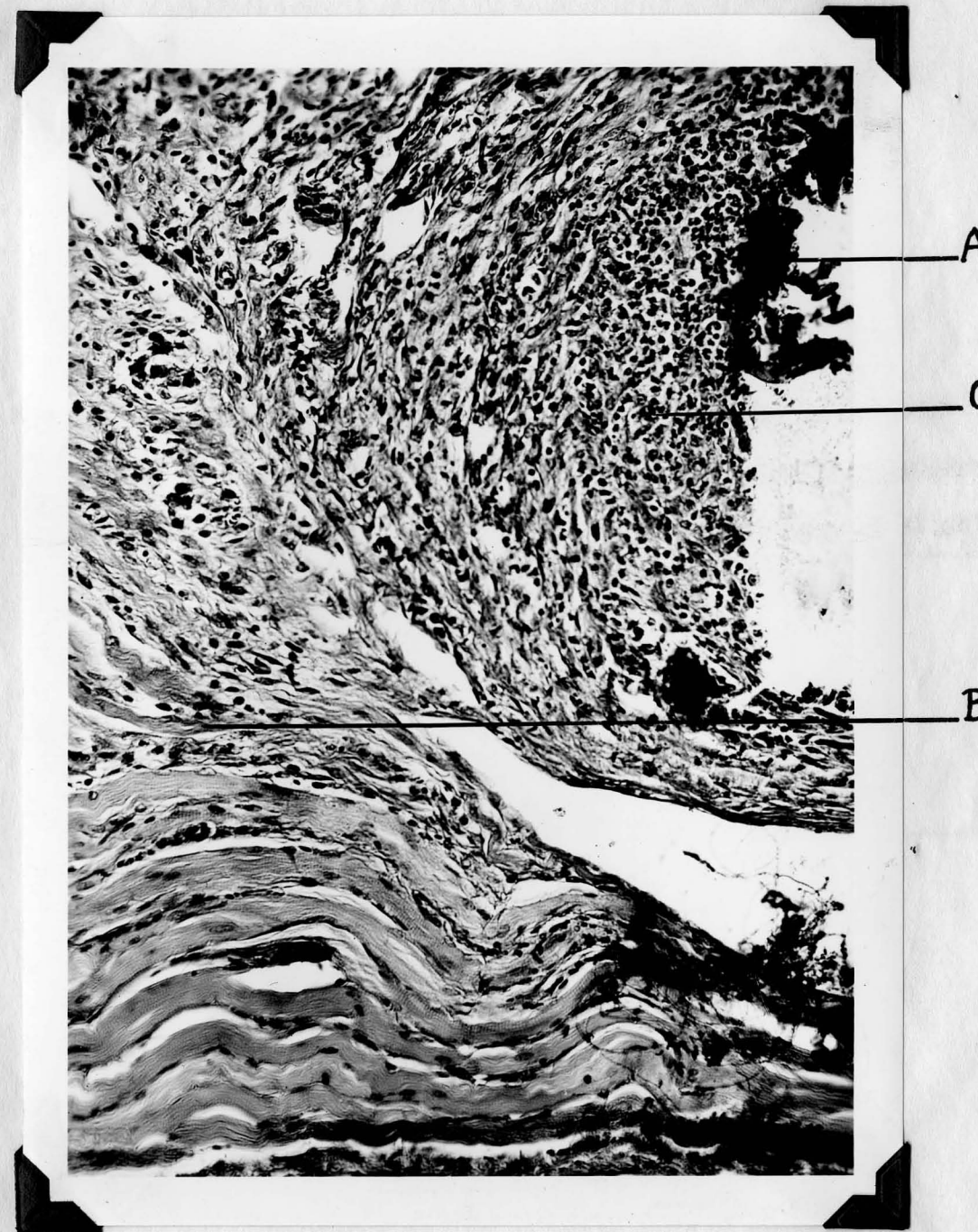


Figure 7

Figure 8

Three-week stage. The figure illustrates the irregularity of the regenerating fibers (A). Some fibers were sectioned at an oblique angle resulting in fragments (B). Hematoxylin and Eosin, 110x.

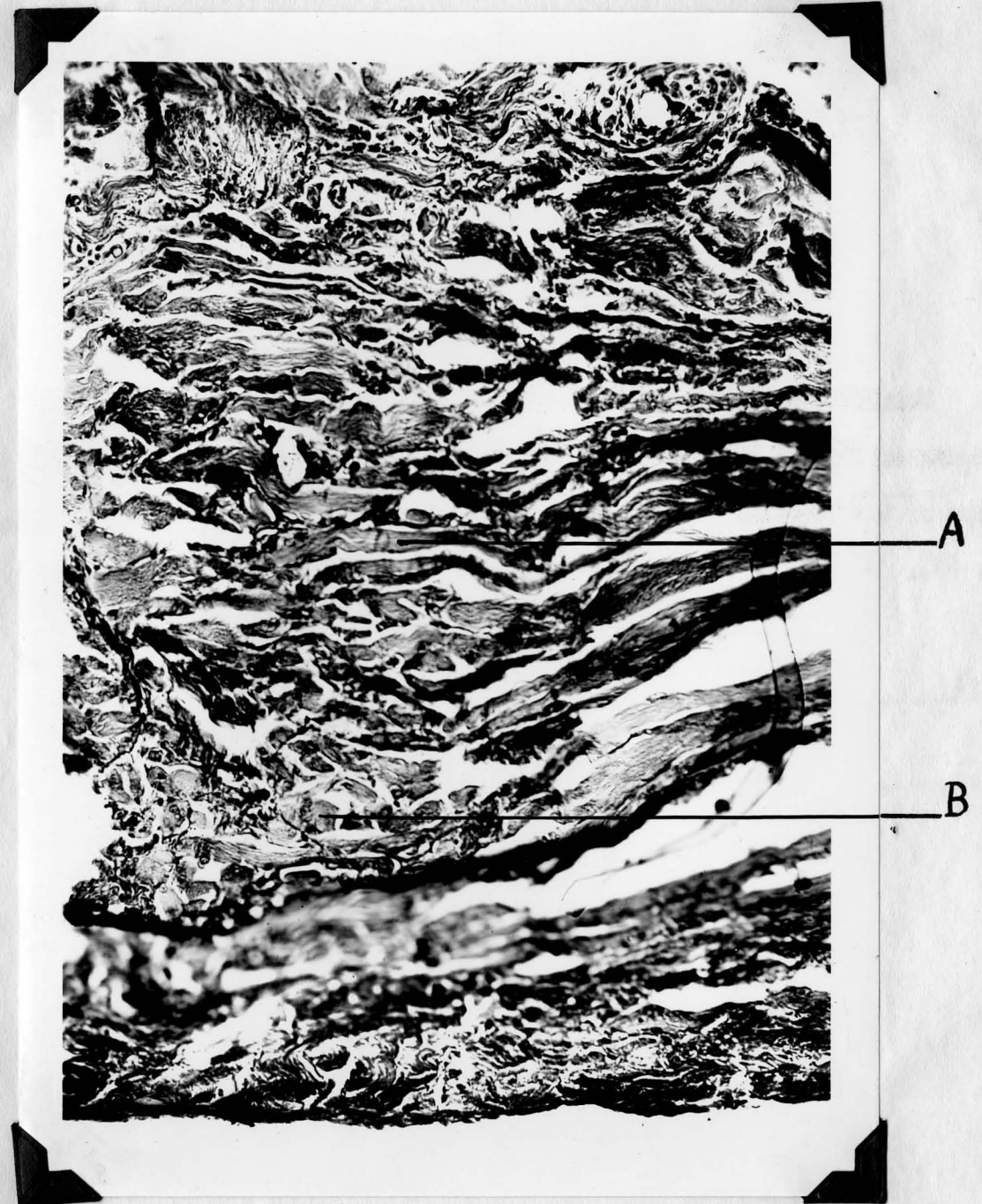


Figure 8

Figure 9

Four-week stage. This figure is similar to Figure 8, in showing fragments of fibers (A). The dark line in the center (B) is due to the folding of the tissue. The most recent sprouts do not contain cross striations (C). Hematoxylin and Eosin, 110x.

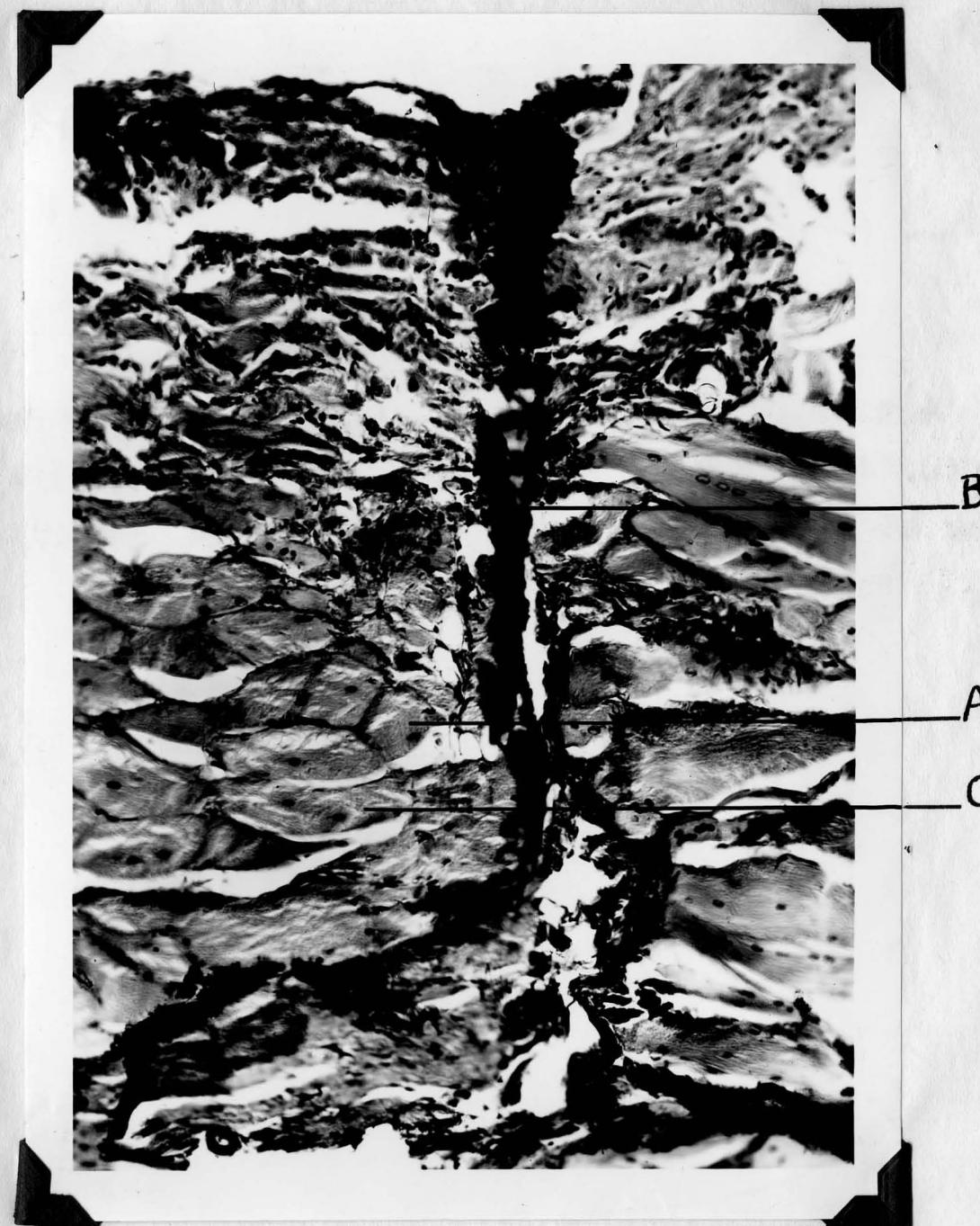


Figure 9

Figure 10

Four-week stage. The figure shows a suture in the anterior rectus sheath (A). The fibroblastic tissue in the healed area has diminished to a minimum. Hematoxylin and Eosin, 110x.

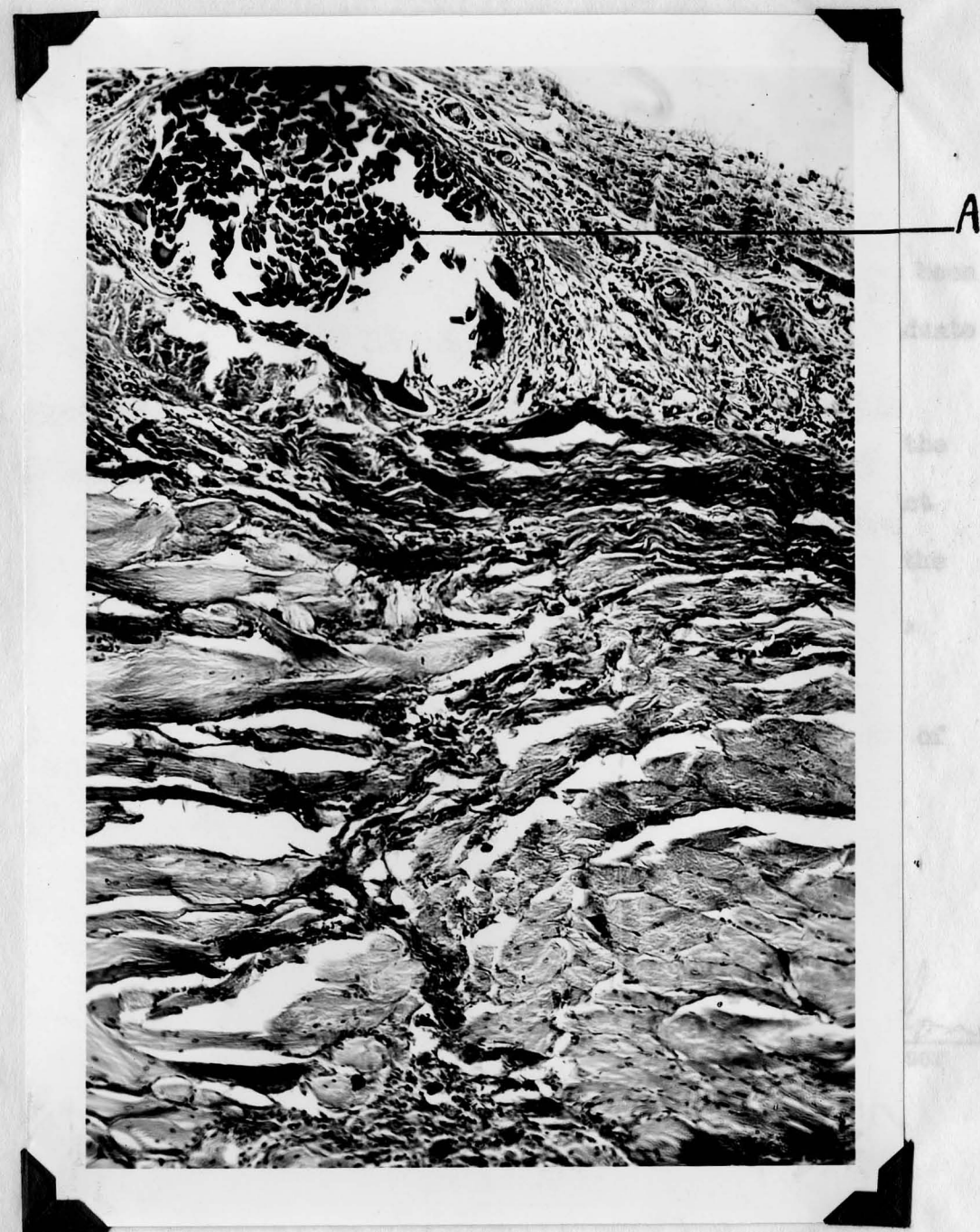


Figure 10

APPROVAL SHEET

The thesis submitted by William Constantine Avgerin has been read and approved by three members of the faculty of the Graduate School.

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.

1 - 22 - '58
Date

David S. Jones
Signature of Adviser