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MUSCLE FIBER SIZE AND FIBER TO CAPILLARY RATIO OF
THE GASTROCNEMIUS MUSCLE OF SEDENTARY ADULT RATS

BY

LOUIS R. DELLASANTA



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GASTROCNEMIUS MUSCLE OF SEDENTARY ADULT RATS

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Louis R. Dellasanta

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Anatomy
College of Veterinary Medicine

1964

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12-1-44

Dedicated
to
my parents
and
brother

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Roger E. Brown, my major advisor, and Dr. M. Lois Calhoun, Professor and Chairman of the Department of Anatomy, for the continual assistance and guidance they have given me. Mr. Rexford Carrow's common interests and assistance have been very much appreciated. Gratitude is expressed to all members of the department staff and fellow graduate students for their timely advice and criticisms.

A special word of appreciation is extended to Dr. Wayne VanHuss and his associates from the Michigan State University Human Energy Research Laboratory for their interest, technical assistance and acquisition of experimental animals used in this study.

The inspiration these people have given me will never be forgotten.

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INTRODUCTION

Skeletal muscle is a complex and specialized tissue. A better understanding of its morphology, related functional aspects, and histochemistry can be helpful to all areas of biology.

The purpose of this research was to measure the cross-sectional fiber area of sedentary adult rats and to study the effects of different injection pressures on the open capillary/fiber ratio of the rat gastrocnemius muscle.

From this study of the sedentary adult, comparisons can be made with experimental conditions which involve variations or changes in environment, nutrition or activity.

A wealth of information is present in the literature concerning skeletal muscle and its microcirculation. A careful review of the literature reveals little data concerning the average cross-sectional area of a rat gastrocnemius muscle fiber under sedentary conditions. Stein and Padykula (1962) measured the muscle fiber cross-sectional area of the rat gastrocnemius with polar planimetry but gave no data as to their size except that soleus fibers (red) are larger in cross-sectional area than the red fibers of the mixed gastrocnemius muscle. They also stated that the A fibers (white) are larger than the intermediate sized B fibers (red) or the smallest C fibers (red) in cross-sectional area.

The average fiber diameters of various species and muscles are recorded in the literature. However, this is not an accurate means of measurement because of the irregularity of the fiber periphery. Also,

there was considerable variation in the pressure of injection and perfusion used by various investigators.

It is the hope of the author that this paper will serve as a useful reference for those who will be investigating the microcirculation of skeletal muscle and muscle fiber cross-sectional area.

REVIEW OF LITERATURE

Muscle Fibers

Skeletal muscle is composed of red and white fibers. From their histochemical analysis Stein and Padykula (1962) found the rat gastrocnemius muscle to contain red and white muscle fibers. They also found that white fibers have a more extensive sarcoplasmic reticulum, higher glycogen content, larger cross-sectional area per fiber, and are faster contracting than red muscle fibers. Valdivia (1958) found that the gastrocnemius muscle of the guinea pig is composed of both red and white areas. It was also reported that the white area fibers are more variable in size and shape than fibers of red areas. The red area fibers average eighteen-hundred (1800) square microns per fiber. The largest white area fibers are larger than the red area fibers.

Huber (1916) found that skeletal muscle fibers of the rabbit and rooster have blunt ends at the tendinous attachments, but otherwise have filamentous intrafascicular terminations. Van Linge (1962) stated in his study of the plantaris muscle of the albino rat that skeletal muscle fibers taper and do not extend the entire muscle length. Paff (1930) reported it necessary to average the cross-sectional area of muscle fibers because they taper at the ends.

The diameters of myosin and actin filaments are unchanged during stretch or isometric exercise. However, with a 50% shortening there is a 13% increase in myosin diameter and a 20% greater distance between myosin filaments with a corresponding 44% increase in cross-sectional fiber area. The interfibrillar space is increased 250% with

a 40% contraction (Carlsen et al., 1961). Huxley (1958) reported that during muscle contraction actin filaments meet and thicken.

Capillaries

Valdivia (1958) observed a higher capillary concentration and a more even distribution in red areas than white areas of the vastus lateralis muscle of the guinea pig. Younger guinea pigs have more capillaries per unit area than older animals. Ballantyne (1961) reported that the number of capillaries per muscle fiber varied between muscle groups, muscle within a group, and between thirds of the same muscle in the pig. The lateral head of the gastrocnemius muscle of the pig has less capillaries per muscle fiber than the medial head at proximal, middle, and distal levels. The capillary to fiber ratio increases from proximal to distal end in both lateral and medial heads of the pig gastrocnemius muscle.

The capillary network is denser in warm than cold blooded animals and more concentrated in smaller than larger animals (Krogh 1918 - 1919). Lusk (1928) and Paff (1930) reported that the rat has a higher rate of metabolism than the guinea pig. The rat has more capillaries per unit of area than the guinea pig and this is related to the metabolic rate (Paff 1930).

Pearson (1959), Valdivia (1958), and Zweifach and Metz (1955) reported that the capillary architecture is parallel to the muscle fibers.

Capillaries viewed histologically in a transverse section are regularly distributed. The number of unfilled or inactive capillaries varies greatly depending upon the animal species, the muscle examined,

and the state of rest or activity immediately prior to examination (Pearson 1959).

Capillary Behavior

Krogh (1918-1919) reported that every capillary field contains numbers of capillaries whose cross-section, even in a relaxed state, is so small that they will not take India ink. In exercise created by one electrical stimulus per second, the blood flow increases five to twenty times in the rat gastrocnemius (Abramson 1962). Martin et al. (1932) found that exercise releases capillary constrictor influences and approximately doubles the number of open capillaries.

Cohnstein and Zuntz (1888) observed capillaries in a relaxed state that were too small to receive corpuscles but opened with a high pressure. Hartman et al. (1929) reported that open capillaries respond to increased pressure passively.

According to Zweifach (1961) and Burton (1954) some of the precapillary sphincters open when the feeding arterial pressure and mean capillary pressure begins to rise. Zweifach and Metz (1955) and Zweifach (1961) observed that closure at the precapillary sphincters appears to develop spontaneously as a consequence of changes in blood pressure and possibly in local vessel tone. If the pressure falls below a critical value, determined by the "unstretched" radius of the vessel and the vasomotor tone, complete closure and cessation of flow results (Burton 1951). Abramson (1962) stated that intermittency of capillary blood flow may be due to the activity of vascular sphincters, changes in blood pressure differential in various areas of the micro-circulatory bed, temporary lodging of intravascular aggregations of

formed elements at junctions, slow rhythmic changes in caliber of the walls of small arterioles (vasomotion), or perhaps local changes in permeability which may include stasis.

The morphological pattern of the capillaries in a cadaver or experimental animal cannot be demonstrated by the usual methods of injection of colored substances, because it is impossible to avoid or make allowances for artefact consequent upon the necessary pressure employed (Pickworth 1934).

Martin et al. (1952) reported that extreme arterial pressures do not increase or decrease the capillary count per square millimeter significantly. Six dogs under drastic experimental treatment and under the influence of amytal for at least an hour and a half were used. Two animals were curarized: one animal had the phrenic nerve cut, and the other three animals had vigorous artificially induced exercise of all main muscles for fifteen minutes followed by a recovery period of at least an hour. The gracilis muscle was used for the capillary count.

Smith and Giovacchini (1956) found that capillaries in some areas were not filled with India ink at 400 mm Hg injection pressure in the aorta of the rabbit. When arterioles had little ink, capillaries were not filled at all, but if the smaller vessels were filled so were the capillaries.

Folkow (1952) observed that an increase of blood pressure on denervated vascular smooth muscle cells results in contraction, and lowering the pressure causes relaxation. The transverse sectional area of the muscular bed adapts so that variations of perfusion pressure will not influence the blood supply of the tissue to any appreciable degree. The cat was used as an experimental animal.

Nicoll and Webb (1955) found that a sudden increase in pressure resulted in a stretching of the vessel walls in the bat wing. The vessels responded immediately with a powerful contraction and many vessels closed.

Capillary Count

Many methods for measuring capillary numbers are used. Drummond (1944) compared India ink injection and erythrocyte staining and found both to be of equal value in capillary studies of the brain. He mentions three possible defects in the injection method. (1) The injection agent may produce a reflex effect upon the vasomotor mechanism and walls of the capillaries which may cause them to contract or dilate abnormally. (2) Shrinkage that occurs in the injection mass may yield results that are not representative. (3) The pressure of injection may affect the capillary count.

Hakkila (1955) presented an excellent critique on the methods of counting capillaries. According to him, Campbell and de Langen as well as Roberts and Wearn believed that the weakness of the erythrocyte stain lies in the fact that some capillaries are only partially filled with corpuscles or only plasma is permitted to flow through. However, Krogh (1929) demonstrated that most anesthetics cause dilation of the frog capillaries. Hakkila quoted Hartman et al., Gianotti and Vannotti, Lindgren, Sjostrand, and Engstrand, as reporting the same effect in the mouse, guinea pig and rabbit. Hakkila stated that Sjostrand found the influences of operative procedure to increase the peripheral blood flow in muscles of the guinea pig and mouse during laparotomy. These effects aid in the erythrocyte staining method by increasing the capillary blood volume. Hakkila quoted Linzbach as saying that the nuclei of

capillary endothelia are incontestably demonstrable for counting capillaries. Yet according to Hakkila, von Boros reported that connective tissue nuclei detract from the serviceability of this method of determination.

Fixation

Pickworth (1934) reported that fixation may cause emptying of capillaries of the brain during the first stage of the procedure.

According to Shipley et al. (1937) heart muscle shrinks two and one-half percent after three to ten days in formalin. Formalin fixation also results in considerable weight loss. Paraffin and celloidin methods cause as much as sixty percent shrinkage. Bouin's fixative does not induce shrinkage of muscle tissue (Martin et al., 1932).

Anesthesia

Lutz and Fulton (1954) reported that hamsters, eight to ten weeks old, had a mean blood pressure drop from 116 mm Hg to 90 mm Hg after light anesthesia with sodium pentobarbital. The blood pressure was recorded directly from the carotid artery.

Zweifach (1954) found gaseous anesthesia a more satisfactory means of controlling depth of narcosis. Ether and oxygen, nitrous oxide or oxygen and trichlorethylene create a minimum of interference in functional responsiveness of the terminal vascular bed.

Nash et al. (1956) reported that after sodium pentobarbital anesthesia, cardiac output ~~fell~~ progressively for the first two to three hours. Except for the first ten minutes the mean blood pressure does not deviate markedly from the unanesthetized animal.

Bagwell and Woods (1962), North et al. (1961) and Jones et al. (1962) reported that blood pressure and respiration rate of the dog decrease with an increase in the depth of methoxyflurane. North et al. (1961) stated that three percent is the maximum obtainable concentration of methoxyflurane in air.

Temperature

Krog (1959) reported the need for a constant check on body temperature in most physiological experiments with anesthetized animals because hypothermia or hyperthermia may ensue following anesthesia with sodium pentobarbital.

The critical closing pressure of vessels supplying the capillary loops of the nailfold is lower after body heating than after body cooling (Gaskell and Krisman 1958).

Barcroft et al. (1955), Roddie et al. (1956), and Edholm et al. (1956) found an increase in blood flow in the human forearm with an increase in body temperature. The increase in flow is due to increased skin circulation with increased body temperature.

The rat's normal body temperature is 38.1°C (Adolph 1951).

Isolation

Hatch et al. (1963) observed the effects of isolation stress in rats. Ten days of isolation yielded a lower resistance to stress, lower food consumption and weight gain, and smaller adrenals. Handling of isolated rats five to ten seconds daily for four months, partially succeeded in overcoming isolation stress.

Blood Pressure

Byron and Wilson (1938) measured the blood pressure in the tail of young adult etherized rats with a plethysmograph and found it to be an average of 106 mm Hg. Chanutin and Ferris (1932) found the average carotid artery blood pressure in etherized rats to be 120 mm Hg. Durant (1927) reported that the blood pressure of male rats was slightly higher than the blood pressure of female rats and was correlated with the average differences in weight of the two sexes. The average blood pressure was 119 mm Hg.

METHODS AND MATERIALS

Experimental Animals

Thirty-one Sprague-Dawley* white albino rats were used for India ink injection in this experiment. The animals were 173 days old when killed. Four animals of the same sex and strain were used for measuring the blood pressure but were four days younger.

The animals were housed in metal cages 7.0" by 9.5" by 7.25" high. The cage floor was made of 1/4" mesh screening.

Their diets consisted of Wayne Lab-Blox** which has a minimum of 24.0% crude protein, 4.0% minimum of crude fat and a 4.5% maximum of crude fibers. The rats had free access to water.

The animals were handled about three times a week at feeding time to prevent isolation stress.

Blood Pressure Materials

A twenty gauge needle was inserted into one end of a piece of polyethylene tubing which in turn was connected at the other end to a model 268 A Sanborn pressure transducer.*** The transducer impulses were fed into a model 64-500 B strain Gage**** amplifier and then recorded from a 150 A model oscilloscope screen. This equipment was calibrated in the following manner (Plate 1). A piece of polyethylene

*Sprague-Dawley Co., Chicago, Illinois

**Allied Mills, Inc., Chicago, Illinois

***Sanborn Co., Waltham, Massachusetts

****Sanborn Co., Cambridge, Massachusetts

tubing was slipped over the 20 gauge needle shaft and the other end was connected to a glass tube leading to a jar of water at room temperature. The water jar had two pieces of glass tubing leading from it. One piece of glass tubing was below the waterline and connected to the tubing containing the needle within its lumen. The second piece of glass tubing entered the bottle above the waterline and was connected to a sphygmomanometer. Thus a recorded pressure of water against the needle orifice could be calibrated on the oscilloscope screen. The degree of water pressure was controlled by the sphygmomanometer induced pressure.

Blood Pressure Methods

The experimental animals were anesthetized with ether and placed on a board for surgery. A laparotomy was performed by making a three or four inch mid-sagittal incision. The abdominal aorta was exposed caudal to the renal arteries so that a ligature could be positioned before the abdominal aorta was closed off with a bulldog clamp. The fluid backed 20 gauge needle which was connected to the pressure transducer was inserted cephalad into the aorta so that the needle orifice was about one inch caudal to the left renal artery (Plate 5). The needle was tied in place with the ligature followed by releasing the anteriorly positioned bulldog clamp. Tying the needle was necessary because bleeding was extensive otherwise. The pressures of four animals were recorded when they became stabilized.

The heart rate was not recorded but was very rapid. The difference between systolic and diastolic blood pressures was not large by this method. Systolic pressures were recorded.

Injection Materials

Higgin's* India ink, which was a pH of 9.0, was used as the injection material for studying the effects of pressure on the capillary count per unit area. The ink's alkalinity is due to the ammonium hydroxide concentration which helps keep the carbon particles in a colloidal state (Brown 1964). The India ink was prepared for injection by adding a 2% concentration of commercial formalin to it. The author observed that a concentration of more than 4% of commercial formalin caused the particles of suspended carbon to precipitate. The perfusion fluid used did not cause any precipitation of the carbon particles. The reason for using a 2% concentration of formalin in the injection fluid was to aid in a more rapid and even fixation of the skeletal muscle.

Mammalian Ringer's solution was used to perfuse the rear portion of the animals for four minutes to remove blood prior to injection of India ink. The solution was made by adding 9.0 gm of NaCl, 0.42 gm of KCl, 0.5 gm of Na HCO₃ and 0.24 gm of CaCl₂ to one liter of water at 38°C. The pH of this solution was 7.6.

To the sphygmomanometer pressure lead, a Y glass tube was connected with two pieces of rubber tubing originating from the two branches of the Y (Plate 2). These two pieces of rubber tubing were connected to a flask of India ink and a flask of mammalian Ringer's solution. Thus any pressure from the manometer would be equal in both flasks. The filled flasks were immersed in a water bath of 38°C. so that the injection and perfusion fluids would be at the normal body temperature of the experimental animals. A polyethylene tube, from both the perfusion and injection flasks, was connected with a two-way

*Higgin's Ink Co., Inc., Brooklyn 15, N. Y.

valve forming a single cannulation tube. This way the cannula could be used for both the perfusion and the injection without double cannulating. The length of the tubing outside the water bath was kept as short as possible so there would be a minimum loss of heat.

A pressure of 264 mm Hg on the manometer scale was equal to 200 mm Hg at the tip of the cannula. A manometer pressure of 164 mm Hg equaled 120 mm Hg at the cannula orifice and 64 mm Hg equaled 40 mm Hg at the cannula. These three different pressures were the desired injection pressures for the three groups of animals.

Injection Methods

The animals were anesthetized with methoxyflurane which was developed by the Dow Chemical Company. Methoxyflurane was used for two reasons. The anesthetic has a pleasant non-stimulating smell which prevents the effects of hyperactivity on the circulatory system. The anesthetic has a ~~maximum~~ 3% concentration in air. Thus the depth and effects of anesthesia can be precisely controlled.

A special anesthetic chamber was built for the purpose of conserving the use of methoxyflurane. However, the animal could not be returned to the chamber after surgery was started. Therefore, minimum quantities of ether were used after the initial use of methoxyflurane to maintain anesthesia because the surgery and injection time averaged approximately 25 minutes per animal and the methoxyflurane did not maintain anesthesia for this period.

The abdominal aorta was closed off with a bulldog clamp just caudal to the left renal artery. With the aid of a magnifying glass a small opening was made in the wall of the aorta about one inch caudal

to the bulldog clamp. A polyethylene tube approximately the extended diameter of the aorta was inserted caudad and secured with a ligature.

Mammalian Ringer's solution at 38°C. was perfused for four minutes with a pressure of 120 mm Hg at the orifice of the cannula. The interior vena cava was cut immediately after the start of perfusion to allow unresisted flow. The perfusion time of four minutes was determined by the extreme dilution of blood at the end of this time.

After the perfusion, the valve was immediately turned to the India ink reservoir for the injections at 40 mm Hg, 120 mm Hg, and 200 mm Hg. Injection time was two minutes for the lowest two pressures and one minute for the animals injected at 200 mm Hg pressure.

The entire body below the cannulation point immediately turned black when the India ink began to flow. The left leg of each animal was held in an extended position during the entire injection time.

Preparation of Tissues

The left leg was removed by disarticulating the limb at the coxo-femoral joint. There was very little leakage of India ink from the blood vessels. The skin was carefully removed from the disarticulated limb and the gastrocnemius muscle was exposed. All skeletal muscle was removed anterior to the knee joint and on either side of the gastrocnemius muscle.

A paraffin coated lead weight weighing 6.85 gm was tied to the Achilles tendon. The weights were of equal volume because they each displaced the same amount of water. After the weights were tied, the Achilles tendon was cut distal to the thread supporting the weight. The plantaris, soleus, and gastrocnemius muscles were reflected from

their insertion at the Achilles tendon. The origin of the soleus muscle was cut to permit the weight to be supported by the two heads of the gastrocnemius at their origin. The lead weight gave a constant stretch for all muscles and prevented distortion of the muscle fibers. Because the tendon of origin was cut the weight of the soleus muscle also aided in the amount of tension on the other two muscles.

The three muscles were suspended at right angles to the upper and lower leg in 10% formalin (Plate 3). The femur and tibia supported the lead weight and muscles, by resting on two metal bars placed over the container of fixative. The tissues remained in the fixative for 17 hours before they were removed.

The three muscles were removed from the femur by freeing the two heads of origin of the gastrocnemius muscle. The plantaris and soleus muscles were stripped free from the gastrocnemius muscle.

The tissues were washed for 24 hours to remove the fixative and the whole gastrocnemius muscle was placed in a 10% gelatin solution at 40°C. for a 24 hour infiltration period followed by an equal time in 20% gelatin. The muscles were removed from the 20% gelatin and placed in a refrigerator to harden. After the gelatin solidified the muscle was easily trimmed for making blocks. Each muscle was divided into four pieces of equal length. Tissue samples were blocked in 20% gelatin from the proximal, middle and distal levels of each muscle (Plate 4). The proximal level was one-fourth the gastrocnemius muscle length and the middle level began at one-half the muscle length to be sectioned toward the origin of the muscle. The distal level was three-fourths the muscle length and was sectioned toward the insertion of the muscle.

After the gelatin mold hardened around the tissues the blocks were trimmed to a smaller size and placed in 10% formalin to denature the protein of the gelatin. This made the blocks firmer and easier to handle.

Seven sections, 16 microns thick, were cut from each block with a freezing microtome. Two sections were stained lightly with eosin and five sections were stained for glycogen concentration with the McManus' Periodic Acid-Schiff method (McManus and Mowry, 1960). To prevent confusion of injected capillaries with muscle fiber nuclei the tissues were not counterstained with hematoxylin. The sections were mounted in glycerin jelly because it is miscible with water.

Measurement

The number of fibers per unit area of nine animals was counted at 100 magnifications. The 10x objective and 10x oculars of the same binocular microscope were used throughout the measurement. Fibers half-way or more within the unit area (129,600 sq. microns) were counted as one. Each of the levels (proximal, middle and distal) was divided into mixed and glycogen negative areas and counted. The mixed areas of each section were those areas which contained glycogen positive fibers with some glycogen negative fibers intermingled. The glycogen negative fibers were the second classification for measurement of fiber numbers per unit area.

Thirty values were recorded for the proximal, middle, and distal levels at both lateral and medial heads of the same muscle. Thus a total of 180 counts was taken from each gastrocnemius muscle.

The number of capillaries per unit area was counted in 29 experimental animals. As in the muscle fiber count each muscle was divided into the three levels, lateral and medial heads, and glycogen positive and glycogen negative areas. Twenty counts at each level, head and area were taken which totaled 120 counts for each animal. The areas where counts were taken are shown in Plate 4. The capillaries of all injected animals were counted in an area of 15,625 sq. microns at 396 magnification with the same binocular microscope. This was deemed necessary because the filled capillaries at 40 mm Hg injection pressure were difficult to see at lower magnifications. Vessels nine microns or less were counted as capillaries.

The fibers were classified into four types and their cross-sectional areas were measured with the 95x oil immersion objective. The number of unit areas (25 sq. microns) that was within the fiber boundaries was counted. Fractional areas of squares which were not entirely within the fiber area were estimated as close as possible into the total area of each fiber. The fibers were classified into dark, light, white, and peripheral white fibers. The dark fibers were stained very red indicating high glycogen positive concentration. The light fibers were glycogen positive but intermediate in color with the white appearing fibers showing no glycogen content.

The microscopes used were recalibrated periodically to assure accurate measurement.

RESULTS AND DISCUSSION

General Statement

Before continuing, a brief discussion of terms would be helpful. Red fiber areas are found in the deeper parts of the gastrocnemius muscle and most of these fibers show a high glycogen concentration or dark red color after staining (Plate 6). The white fiber areas are found on the periphery of the muscle and are predominantly glycogen negative or colorless after staining (Plate 6). The difference arises when using Stein and Padykula's (1962) classification of red and white muscle fibers and not areas. From their classification, the slow contracting red fibers are predominantly found in glycogen negative areas and the rapid contracting white fibers are found primarily in glycogen positive areas.

The author uses the terms mixed fibers in reference to the predominantly glycogen positive fibers or red fiber area. The terms mixed fibers are used because both red and white fibers are present in the same area, as reported by Stein and Padykula (1962), and because some of the glycogen negative fibers are found between the greater numbers of glycogen positive fibers after staining. The terms white fibers are used in reference to the white fiber areas of the muscle periphery which predominantly stains negative for glycogen.

Blood Pressure

The blood pressure of four etherized ~~per~~experimental animals was measured to compare with that reported in the literature and to establish

one of the three injection pressures used in these experiments. The pressures were recorded as 133, 140, 140, and 210 millimeters of mercury. All scores were above the average blood pressure reported in the literature and indicate the need for further investigation to establish a revised average blood pressure for this particular strain of rat.

Because the time for further investigation of blood pressure was limited, the value of 120 mm Hg from the literature was accepted as an average. This value was used for all perfusion pressures and for one of the injection pressures in addition to the extreme injection pressures of 40 mm Hg and 200 mm Hg. In this way the effects of pressure variations on the number of open or functional capillaries were studied.

Fiber Area

It was found that the mixed fibers of the deeper portions of the muscle are smaller in cross-sectional area than the peripheral white fibers. Both the mixed and white fibers are progressively larger in cross-sectional area at lower levels of the muscle (Table 6). Graph 4 indicates that as the body weight increases the mixed fibers increase proportionally in cross-sectional area. The average fiber area was found by dividing the value of the unit area by the number of fibers within the area.

Valdivia (1958) used the same histological technique on the gastrocnemius muscle of the guinea pig and found the middle third red area fibers average about 1800 sq. microns. It was found in this study that the fibers at the same level and area in the rat gastrocnemius muscle average 2379.7 sq. microns per fiber. Paff (1930) reported

data on the cross-sectional area of the guinea pig gastrocnemius muscle but the values were very low due to the shrinkage factor caused by the paraffin method used.

Values for fiber cross-sectional area measured on an individual basis are less than those obtained using the previously described method. This procedure eliminates the error caused by measuring the area of connective tissue between fibers and fractions of fibers within the unit area. This was found to be true in those areas where mixed fibers are inversely proportional to the glycogen concentration. Again the white fibers of the periphery are much larger than the other fibers (Table 7). Fibers measured individually show no relationship to body weight (Graph 5).

The larger peripheral fibers are circular in shape while the smaller mixed fibers are polygonal and deep within the muscle (Plate 6). George and Naik (1957) reported similar fiber morphology and location in the pigeon pectoralis major muscle.

The slow contracting red fibers are found in the immediate area of collagenous tissue which is an extension of the Achilles tendon in and around the muscle tissue (Plate 4). This arrangement gives a more direct and greater mechanical efficiency for contraction of these slow muscle fibers. These fibers are smaller than the faster contracting peripheral fibers. The smaller and more numerous fibers result in a greater surface area than the larger peripheral fibers. This facilitates the rapid transport of nutrition and oxygen during periods of exercise because of the increased surface area. A more adequate blood supply would be expected in association with the increased area of the slower

contracting fibers. This relationship will be discussed at a later time.

Some fibers appear to be in an interphase of glycogen storage or release (Plate 6). Some individual fibers are only partially filled with glycogen and demonstrate portions that have no glycogen (Plate 7). These glycogen positive fibers may act as storage areas for immediate use when needed.

Capillary Supply

The injection pressures of 120 mm Hg for two minutes and 200 mm Hg for one minute show little or no difference in the number of open capillaries of the rat gastrocnemius muscle (Tables 2 and 3). Using a T test as described by Guenther (1964) it was found that the number of open capillaries is significantly less at 40 mm Hg injection pressure at the .20 alpha level.

Variations in the number of open capillaries per unit area are evident at different levels and areas of the same muscle for all three injection pressures (Tables 1, 2, 3, and 5). The number of capillaries per unit area gradually decreases in the areas toward the tendon of insertion or lower levels. The lateral head of the muscle has fewer capillaries per unit area than the red fiber areas. Valdivia (1958) also found more capillaries per unit area in the mixed fiber areas than in the peripheral red fibers of the guinea pig.

The combined capillary/fiber ratio of animals injected at 120 mm Hg and 200 mm Hg is the largest at the middle level followed by a lower value for the distal and proximal levels respectively (Table 4). This trend was found to be true for both mixed and white fiber areas.

Ballantyne (1961) found that the capillary/fiber ratio increased from the proximal to distal level in the pig gastrocnemius muscle.

The slightly lower counts for those animals injected at 200 mm Hg may be due to the constrictor reflex mentioned by Folkow (1952), and Nicoll and Webb (1955) which is the result of increased pressure on the vessel walls.

The animals injected at 40 mm Hg definitely have a lower capillary count than those injected at the two higher pressures. Many of the areas were not injected and those capillaries which did contain ink appear smaller because they were filled with less carbon particles than the capillaries injected at higher pressures. This phenomenon can be partially explained by the passive closure of small vessels and capillaries due to interfacial tension between the injection fluid and vessel wall. Madow as quoted by Fulton and Zweifach (1958) stated that interfacial tension between blood and the blood vessel wall causes passive closure below a pressure of 5 - 10 mm Hg.

Non-functional capillaries, which open with increased pressure, as described by Zweifach (1961), are shown by the difference in counts resulting from the injection pressures of 40 and 120 mm Hg. From these comparative counts, the opening of capillaries due to the increase in pressure is not apparent.

The mixed fiber areas have many functional patterns. First the fibers have a greater surface area for absorption as was described previously. Secondly they have a better blood supply than the white peripheral fibers and lastly they have a higher myoglobin concentration which acts as an oxygen reservoir (Lawrie 1952). These factors enhance the rapid metabolism of the muscle fiber when greater demands are

called for as in exercise. With exercise, it is known that the muscle myoglobin concentration increases as a compensatory factor. Lawrie (1953), Reynafarje (1962), and Van Linge (1962). The capillarity also increases with exercise but the cause of this phenomenon is not clearly understood.

The main blood supply to the gastrocnemius muscle is from two branches of the popliteal artery. The external sural artery supplies part of the soleus and plantaris muscles, plus the lateral head of the gastrocnemius muscle, while the internal sural artery supplies the medial head. Both the internal and external sural arteries enter the muscle just below the medial and lateral head bifurcation (Greene 1959). This may be the explanation for the lower capillary concentration of the more distal levels of the muscle. Because of the greater distance to the distal levels of the muscle, the blood pressure within these vessels is reduced due to the increased resistance encountered. Thus, less capillaries are open per unit area.

The greater capillary/fiber ratio of the middle level can supply these cells with more nutrition and oxygen per unit of time. The bulk of the gastrocnemius muscle fibers is found at the middle level (Plate 4) and presumably for this reason more work is done in this region than at other levels. This greater capillary supply per fiber insures a greater capacity for work. The capillaries paralleled the muscle fibers as described by Woodlard and Weddell (1934) and Zweifach and Metz (1955) (Plate 9).

SUMMARY AND CONCLUSIONS

The effects of three different injection pressures on the number of open capillaries of the rat gastrocnemius muscle were studied. The injection pressures of 120 mm Hg and 200 mm Hg for two minutes and one minute respectively showed no significant difference in the number of open capillaries. The injection pressure of 40 mm Hg for two minutes was proven to be statistically different because the capillary counts at this pressure were much lower than the two higher injection pressures.

Each muscle was divided into three levels (proximal, middle, and distal) with mixed and white fiber areas and two heads (lateral and medial). The number of capillaries per unit area was counted at each level in both heads and in two different fiber areas.

Four types of fibers were classified as to location and glycogen concentration. The cross-sectional area of these fibers was measured individually at the proximal level of the medial head only. The number of fibers per unit area was measured at the three levels and two different areas of nine animals. The average of the cross-sectional area of the fibers from the two regions was determined. Comparisons were made between the capillarity and muscle fibers at the three levels and two fiber areas. The following conclusions were made from this study. (1) There is no significant difference in the number of open capillaries resulting from the injection pressures of 120 mm Hg and 200 mm Hg for two and one minute respectively. (2) A reduction in injection pressure from 120 mm Hg to 40 mm Hg results in the closure of

some capillaries as shown by a lower capillary count per unit area.

(3) The medial head has a greater amount of capillarity than the lateral

head. (4) The capillary concentration per unit area decreases from proximal to middle and from middle to distal levels respectively. (5)

Mixed fibers are smaller in cross-sectional area and have a greater

blood supply than the peripheral white fibers. (6) The middle level has a greater capillary/fiber ratio than the distal or proximal level.

(7) Muscle fibers of both the white and mixed areas become progressively larger in cross-sectional area from proximal to distal levels.

TABLE 1

Capillaries Per Unit Area* At 40 MM. Hg Injection Pressure

Animal	Body Wt.	Level**	Mean ¹	Mean ²	Mean ³	Mean ⁴
2	421 Gm.	P	12.80	13.56	7.40	6.66
		M	7.56	11.20	5.94	4.19
		D	10.26	6.64	2.20	2.50
14	446 Gm.	P	10.13	13.73	3.43	3.73
		M	8.93	10.06	3.93	3.66
		D	6.86	9.50	3.43	6.63
15	420 Gm.	P	14.76	16.58	7.80	6.96
		M	12.40	14.61	6.56	5.74
		D	10.38	10.46	5.26	4.61
16	430 Gm.	P	19.30	21.86	9.19	9.83
		M	18.50	19.16	10.03	9.06
		D	14.00	11.66	8.60	7.38
17	440 Gm.	P	24.83	26.23	10.54	10.76
		M	21.93	22.70	9.33	6.43
		D	14.83	18.33	7.50	6.43
18	491 Gm.	P	6.86	8.16	4.33	4.76
		M	---	---	---	---
		D	6.00	5.33	2.36	1.86
20	462 Gm.	P	8.90	10.10	3.03	4.10
		M	7.96	10.36	1.96	3.90
		D	9.50	10.21	3.53	3.93
22	466 Gm.	P	13.46	15.96	4.30	4.00
		M	14.63	18.03	4.06	4.63
		D	12.60	11.63	4.86	3.30
23	336 Gm.	P	19.22	17.89	4.26	4.00
		M	15.00	18.23	4.36	4.06
		D	11.06	16.56	4.40	4.25
24	460 Gm.	P	9.08	13.03	5.33	4.94
		M	8.68	11.00	3.03	2.58
		D	10.62	8.93	1.23	1.13
Mean		P	13.93	15.71	5.96	5.97
		M	12.88	15.03	5.46	5.17
		D	10.61	10.93	4.34	4.20
Standard Deviation		P	5.68	5.35	2.46	2.54
		M	5.04	4.64	2.76	2.27
		D	2.79	4.00	2.33	2.10

*15625.0 Sq. Microns

**P - Proximal Level

M - Middle Level

D - Distal Level

1 - Lateral Head (Mixed Fiber Area)

2 - Medial Head (Mixed Fiber Area)

3 - Lateral Head (White Fiber Area)

4 - Medial Head (White Fiber Area)

TABLE 2

Capillaries Per Unit Area* At 120 MM. Hg Injection Pressure

Animal	Body Wt.	Level**	Mean ¹	Mean ²	Mean ³	Mean ⁴
1	488 Gm.	P	15.40	17.40	4.80	5.60
		M	16.90	19.35	5.35	6.15
		D	19.35	8.95	4.30	4.40
3	420 Gm.	P	18.00	23.25	7.40	6.35
		M	19.25	22.45	5.75	5.10
		D	19.25	20.60	4.95	4.95
4	440 Gm.	P	11.70	17.65	4.30	6.10
		M	11.45	16.55	5.00	5.80
		D	---	6.40	2.90	4.80
5	485 Gm.	P	14.45	17.15	5.25	5.20
		M	15.70	20.10	5.80	5.80
		D	13.75	12.30	3.90	5.85
6	461 Gm.	P	17.85	20.15	5.10	6.60
		M	17.85	17.70	6.00	5.70
		D	12.25	9.65	4.30	4.05
7	492 Gm.	P	19.25	21.30	5.70	6.50
		M	15.55	19.50	5.35	8.05
		D	14.95	16.55	4.55	5.90
9	438 Gm.	P	20.25	22.40	5.90	6.15
		M	23.40	22.60	6.10	6.15
		D	17.50	16.05	4.60	4.36
10	498 Gm.	P	17.70	20.25	5.20	7.45
		M	15.05	18.70	5.60	6.35
		D	18.10	19.15	6.80	5.50
12	499 Gm.	P	16.70	19.55	5.55	6.00
		M	17.25	20.30	5.35	6.05
		D	---	---	2.53	3.30
13	442 Gm.	P	24.65	29.85	6.20	7.95
		M	22.30	24.75	5.20	6.60
		D	21.75	23.80	6.25	6.00
Mean		P	17.60	20.90	5.54	6.39
		M	17.47	20.20	5.50	6.18
		D	17.11	14.82	4.51	4.91
Standard Deviation		P	3.49	3.75	0.84	1.36
		M	3.50	2.46	0.41	0.77
		D	3.19	5.86	1.30	0.90

*15625 Sq. Microns

**P - Proximal Level

M - Middle Level

D - Distal Level

1 - Lateral Head (Mixed Fiber Area)

2 - Medial Head (Mixed Fiber Area)

3 - Lateral Head (White Fiber Area)

4 - Medial Head (White Fiber Area)

TABLE 3

Capillaries Per Unit Area* At 200 MM. Hg Injection Pressure

Animal	Body Wt.	Level***	Mean ¹	Mean ²	Mean ³	Mean ⁴
21	445 Gm.	P	32.70	23.90	8.70	7.45
		M	18.60	24.75	8.10	7.25
		D	16.75	20.90	8.05	7.25
25	492 Gm.	P	12.70	17.60	4.70	6.85
		M	20.55	18.85	5.95	6.65
		D	13.05	16.70	2.70	4.90
27	463 Gm.	P	15.30	16.60	4.30	5.95
		M	13.85	12.70	5.70	3.70
		D	10.70	15.25	6.50	4.25
28	432 Gm.	P	14.40	17.60	33.10	4.60
		M	16.00	23.40	5.50	4.95
		D	16.70	18.95	4.80	4.50
29	450 Gm.	P	17.00	18.45	6.40	4.15
		M	18.90	21.35	5.15	6.15
		D	16.40	19.25	4.20	4.60
30	466 Gm.	P	15.05	15.15	4.80	4.10
		M	15.65	17.40	4.45	4.75
		D	12.70	14.15	4.55	4.40
31	436 Gm.	P	20.55	26.70	5.55	7.05
		M	19.55	23.75	5.35	4.40
		D	12.10	17.75	5.75	5.70
32	494 Gm.	P	20.75	22280	6.00	4.95
		M	19.95	21.10	5.10	6.10
		D	21.30	19.80	5.95	5.00
33	456 Gm.	P	12.15	16.30	3.75	4.80
		M	9.15	13.95	5.10	4.95
		D	8.90	10.75	3.35	3.95
Mean		P	17.44	19.46	5.26	5.55
		M	16.91	19.80	5.60	5.43
		D	14.29	17.05	5.10	4.95
Standard Deviation		P	4.02	3.99	1.66	1.30
		M	3.63	4.30	1.05	1.15
		D	3.74	3.21	1.36	0.99

*15625 Sq. Microns

**P - Proximal Level

M - Medial Level

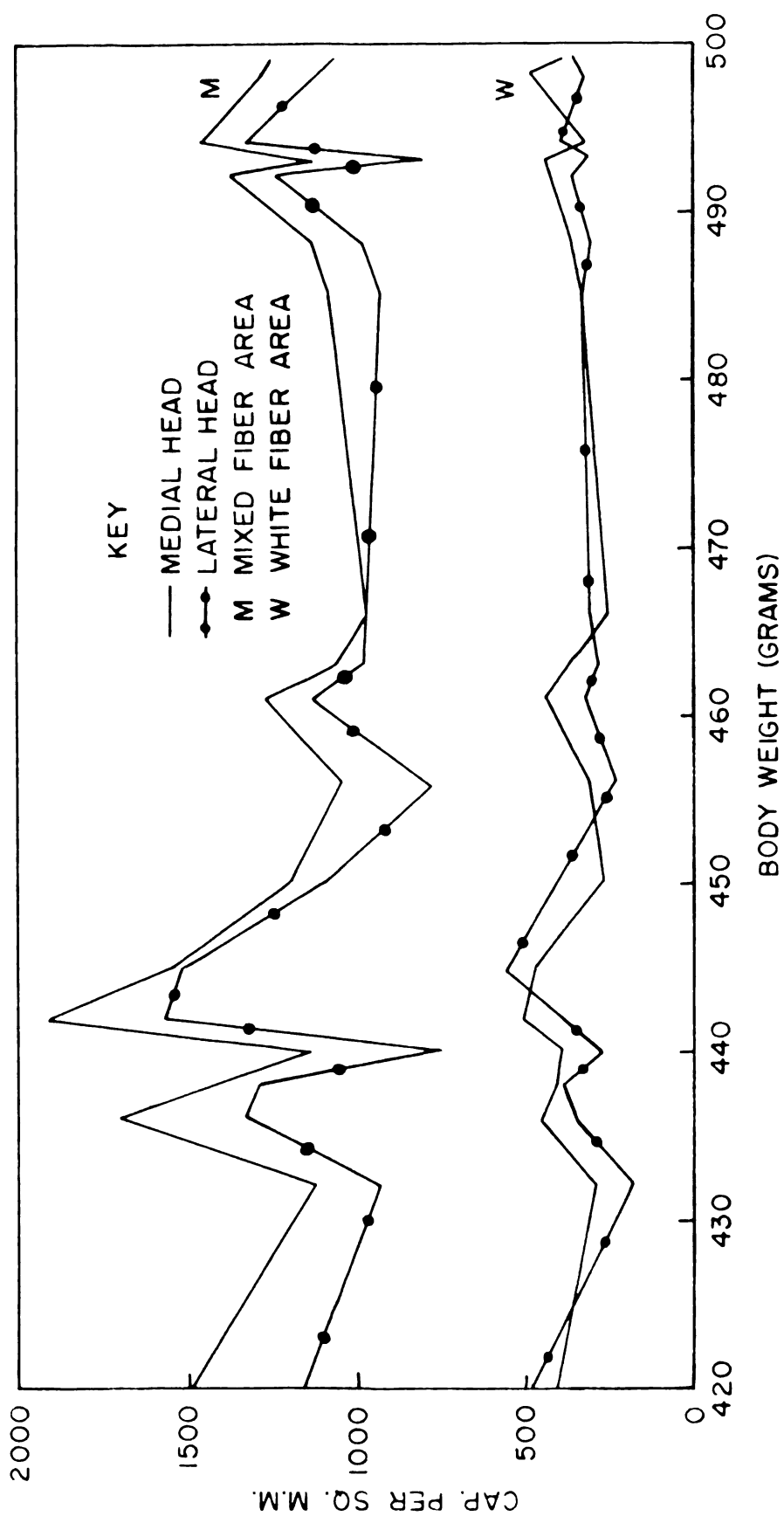
D - Distal Level

1 - Lateral Head (Mixed Fiber Area)

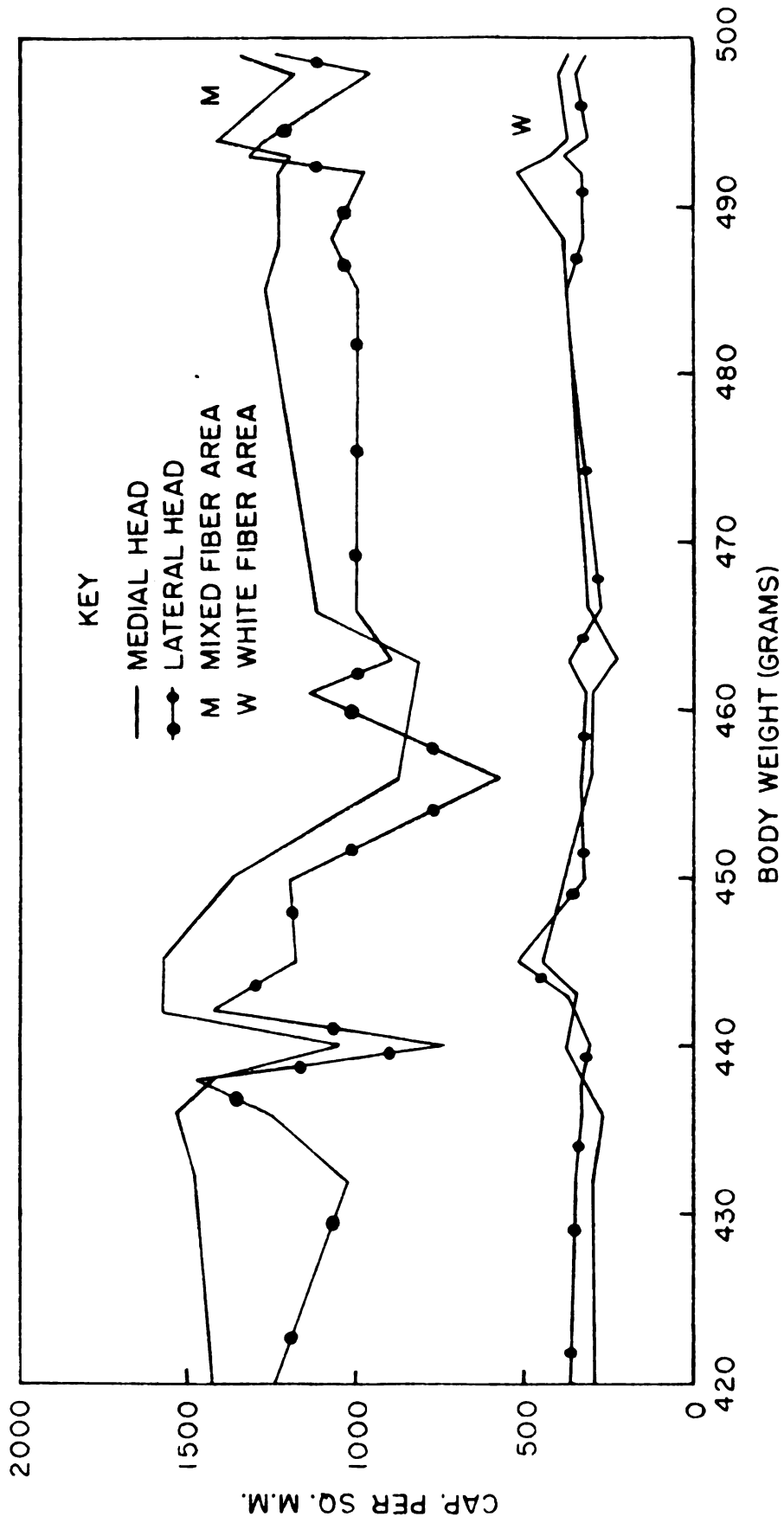
2 - Medial Head (Mixed Fiber Area)

3 - Lateral Head (White Fiber Area)

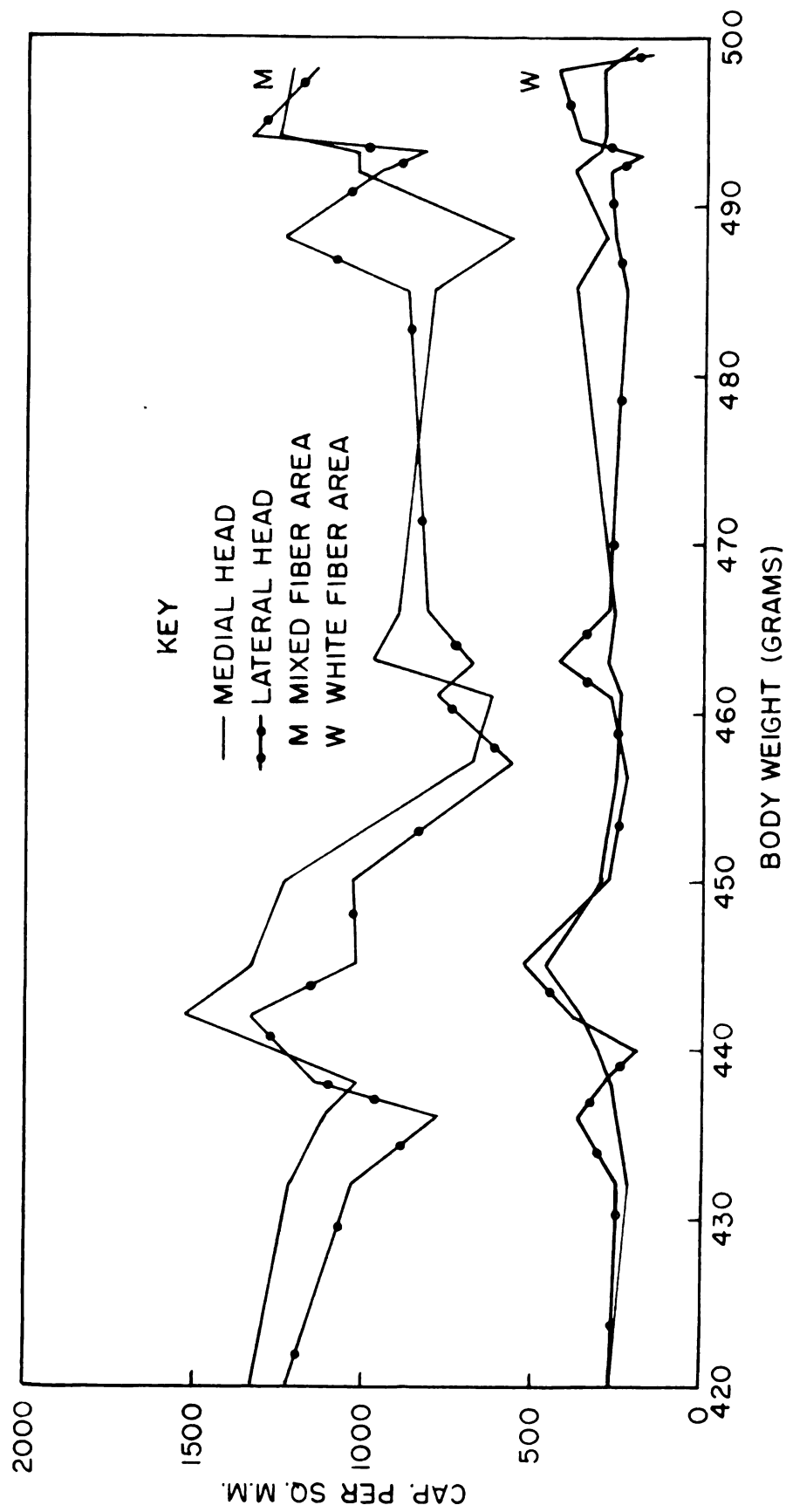
4 - Medial Head (White Fiber Area)



CAPILLARITY OF THE PROXIMAL LEVEL OF THE GASTROCNEMIUS MUSCLE



CAPILLARITY OF THE MIDDLE LEVEL OF THE GASTROCNEMIUS MUSCLE



CAPILLARITY OF THE DISTAL LEVEL OF THE GASTROCNEMIUS MUSCLE

TABLE 4

Comparison of Mean Capillary Counts per Sq. Micron With
Capillary/Fiber Ratio of the Three Muscle Levels

	<u>Cap./Sq. Micron</u>		<u>Cap./Fiber Ratio</u>	
	Mean ¹	Mean ²	Mean ³	Mean ⁴
Proximal	.00120	.0036	2.33	1.18
Middle	.00118	.0037	2.80	1.36
Distal	.00101	.00032	2.68	1.23

- 1 - Mixed Fiber Area
2 - White Fiber Area
3 - Mixed Fiber Area
4 - White Fiber Area

TABLE 5

Capillaries Per Square Millimeter at the Three Injection Pressures

Pressure mm Hg		Level*	Mean ¹	Mean ²	Mean ³	Mean ⁴
40	Mean	P	891.5	1005.4	381.4	383.1
		M	824.3	961.9	349.4	330.9
		D	679.0	699.5	277.8	268.8
120	Mean	P	1126.4	1337.6	354.6	409.0
		M	1158.1	949.4	352.0	395.5
		D	1095.0	948.5	288.6	314.2
200	Mean	P	1116.2	1245.4	336.6	355.2
		M	1082.2	1267.2	358.4	347.5
		D	914.6	1091.2	326.4	316.8

- | | |
|---------------------|-------------------------------------|
| *P - Proximal Level | 1 - Lateral Head (Mixed Fiber Area) |
| M - Middle Level | 2 - Medial Head (Mixed Fiber Area) |
| D - Distal Level | 3 - Lateral Head (White Fiber Area) |
| | 4 - Medial Head (White Fiber Area) |

TABLE 6

Fibers Per Unit Area* With The Mean Cross-Sectional
Area in Sq. Microns

Animal	Body Wt.	Level**	Mean ¹	Mean ²	Mean ³	Mean ⁴
25	492 Gm.	P	39.9	3248.1	26.0	4984.6
		M	38.3	3383.8	24.9	5204.8
		D	34.5	3756.5	26.7	4853.9
28	432 Gm.	P	43.9	2952.1	30.9	4194.1
		M	40.6	3192.1	28.6	4531.4
		D	40.6	3192.1	27.6	4699.2
22	466 Gm.	P	67.5	1920.0	37.9	3419.5
		M	57.4	2257.8	41.1	3153.2
		D	37.9	3419.5	40.5	3203.1
14	446 Gm.	P	72.5	1787.5	42.3	3063.9
		M	58.3	2222.9	35.1	3692.3
		D	52.2	2482.7	33.1	3915.4
26	490 Gm.	P	69.8	1856.7	40.0	3240.0
		M	55.6	2330.9	35.6	3640.4
		D	50.1	2586.8	31.8	4075.4
23	336 Gm.	P	86.3	1501.7	50.1	2586.8
		M	65.4	1981.6	40.4	3207.9
		D	55.5	1974.7	37.4	3465.2
31	436 Gm.	P	91.3	1419.4	44.8	2892.8
		M	62.0	2090.3	39.9	3251.3
		D	59.1	2194.3	39.2	3306.1
32	494 Gm.	P	57.1	2271.2	34.7	3739.1
		M	52.0	2494.2	32.0	4050.0
		D	59.1	2191.7	36.1	3587.0
21	445 Gm.	P	70.7	1834.1	49.7	2609.7
		M	60.6	2132.6	40.4	3205.5
		D	50.0	2592.0	31.7	4093.4
Mean		P	66.5	1947.7	39.6	3273.5
		M	54.5	2379.7	35.3	3668.6
		D	48.8	2657.4	33.8	3836.6
Standard Deviation		P	17.2		8.2	
		M	9.3		5.9	
		D	9.1		4.9	

*129,600 Sq. Microns

**P - Proximal Level

D - Distal Level

M - Middle Level

1 - Mixed Fibers Per Unit Area

2 - Fiber Area (Sq. Microns)

3 - White Fibers Per Unit Area

4 - Fiber Area Sq. Microns

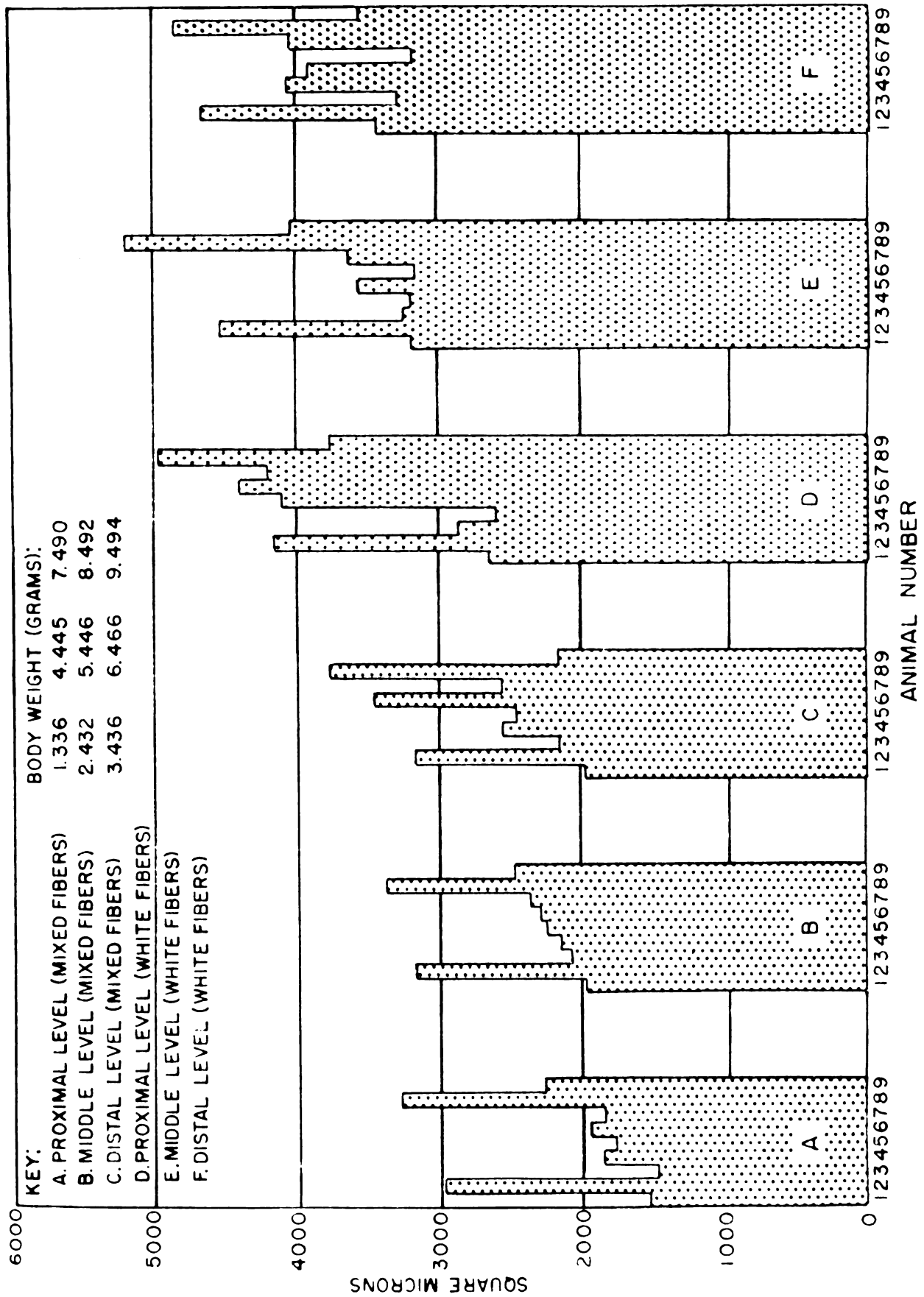
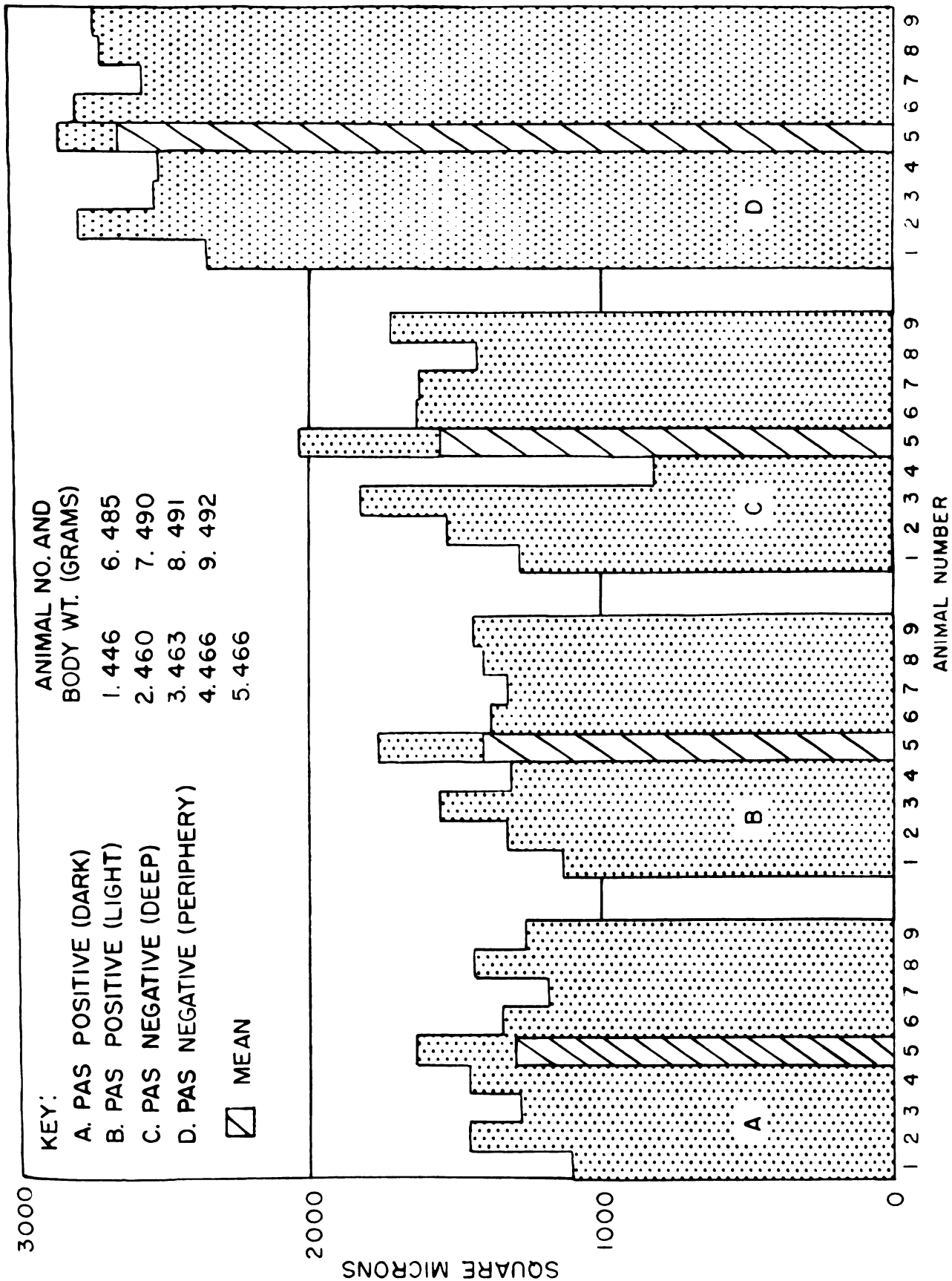


TABLE 7

Measurement of Individual Fiber Cross- Sectional Area
in Sq. Microns At the Proximal Level of the Medial
Head According to Color or Concentration of
Glycogen and Fiber Location

Animal #	Body Wt. Grams	Dark	Light	White	Peripheral White	
8	492	1264.0	1425.0	1703.3	2724.0	
27	463	1278.0	1554.8	1820.0	2533.0	
14	446	1100.8	1141.3	1296.3	2358.5	
22	466	1454.3	1301.3	816.3	2827.5	
26	490	1196.3	1328.8	1639.3	2593.8	
18	491	1431.3	1431.3	1415.0	2730.0	
5	485	1346.3	1389.3	1638.0	2788.0	
30	466	1646.5	1768.0	2047.5	2831.8	
24	460	1462.5	1308.3	1526.0	2789.3	
Mean		Mean	1353.3	1405.8	1544.5	2652.8
		Standard Deviation	32.9	35.5	71.8	31.6



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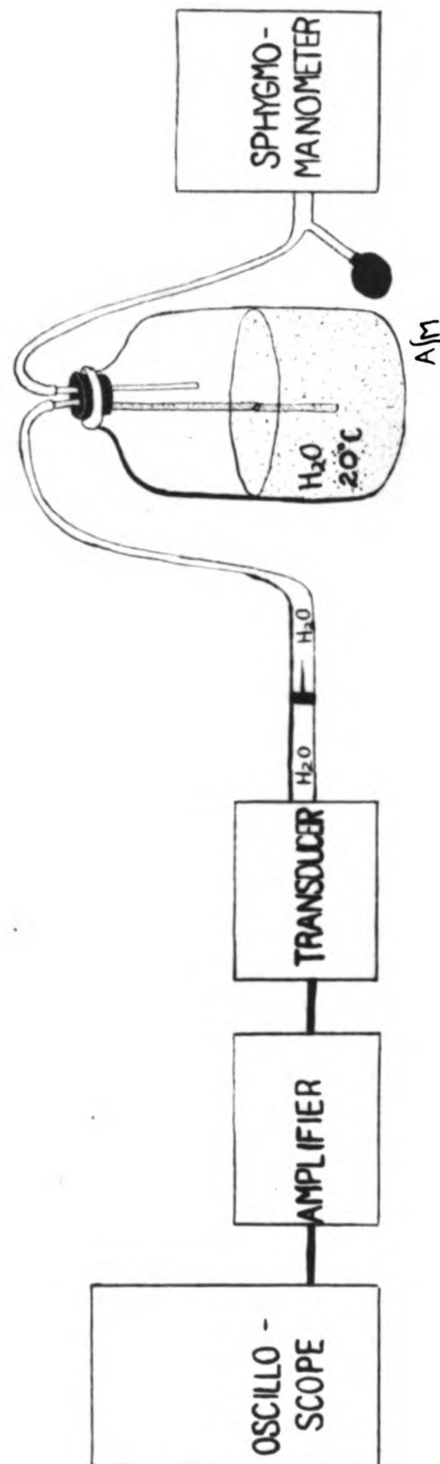


Plate 1. Apparatus for needle calibration.

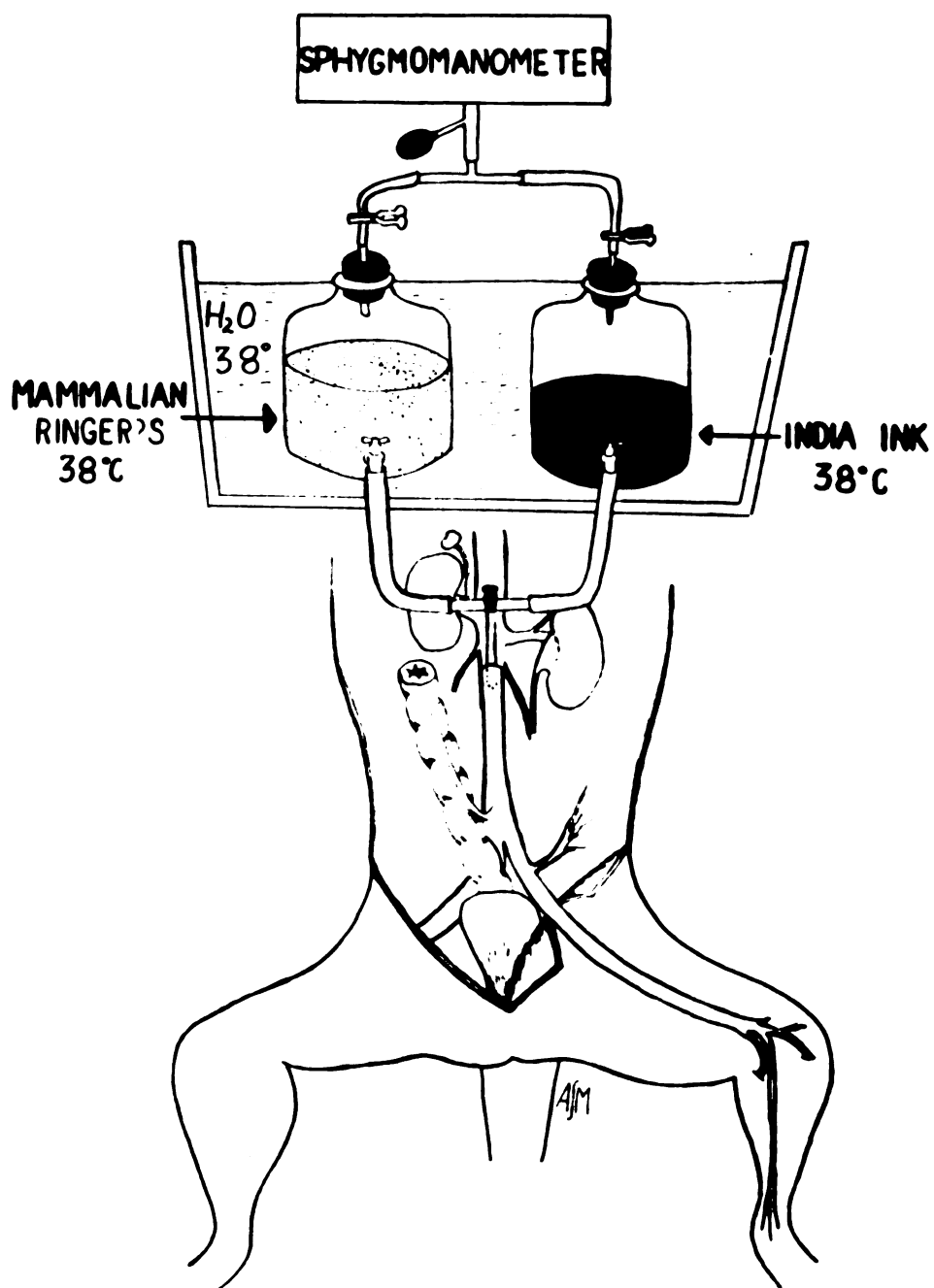


Plate 2. Injection apparatus.

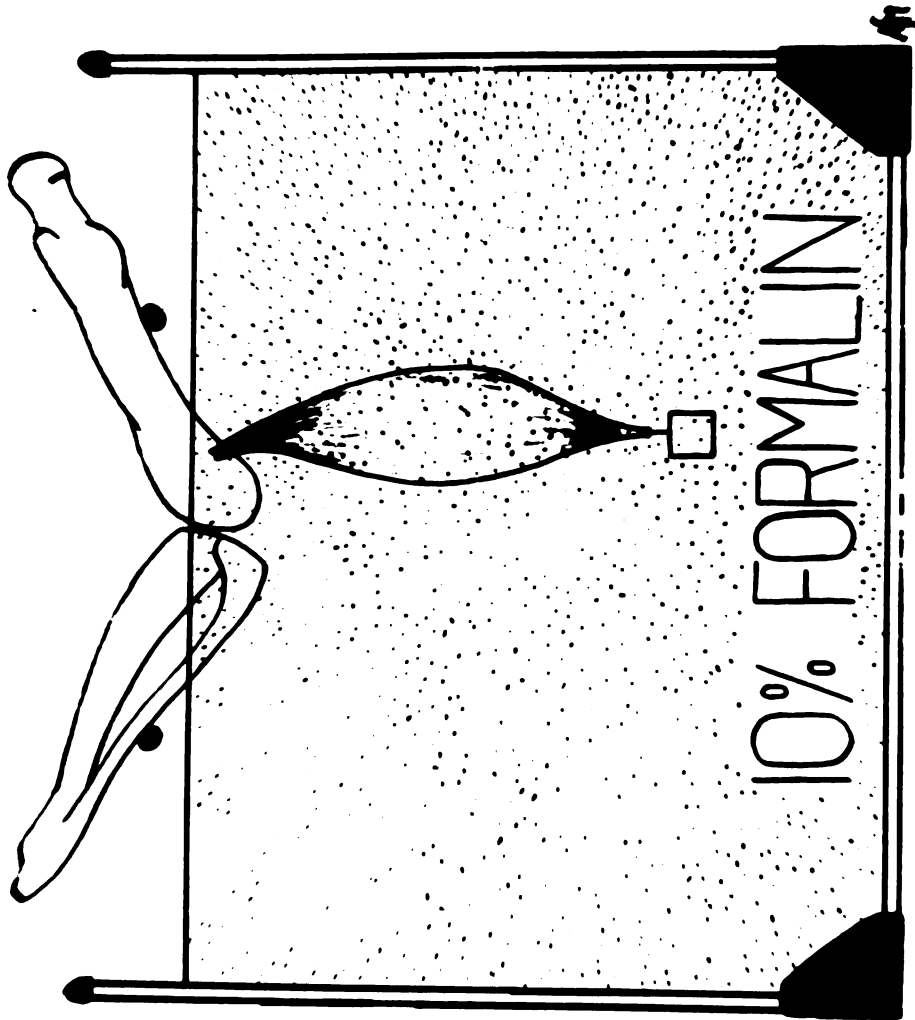


Plate 3. Apparatus for tissue fixation.

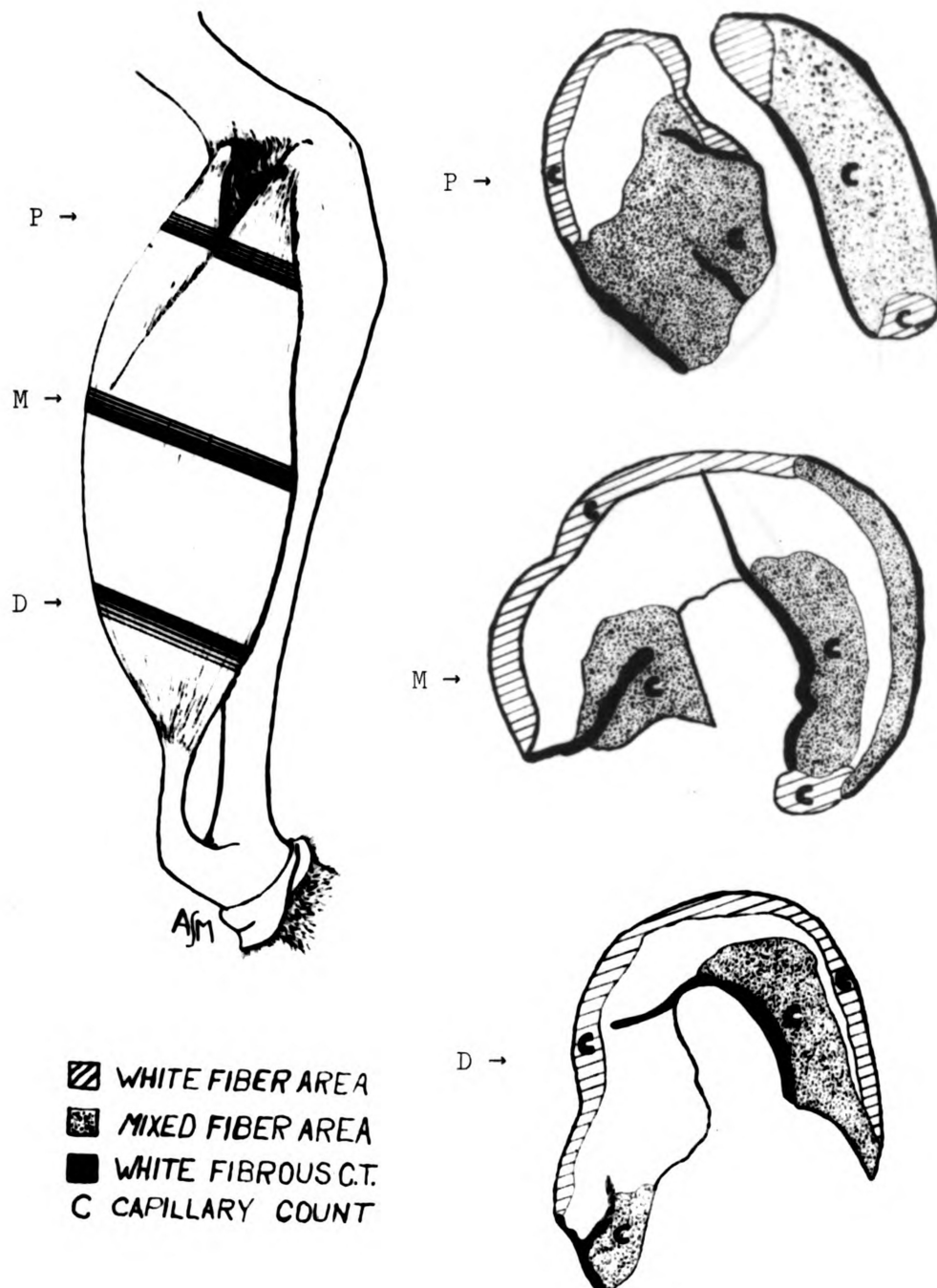


Plate 4. Left gastrocnemius muscle with mappings of the cross-sections from three levels.

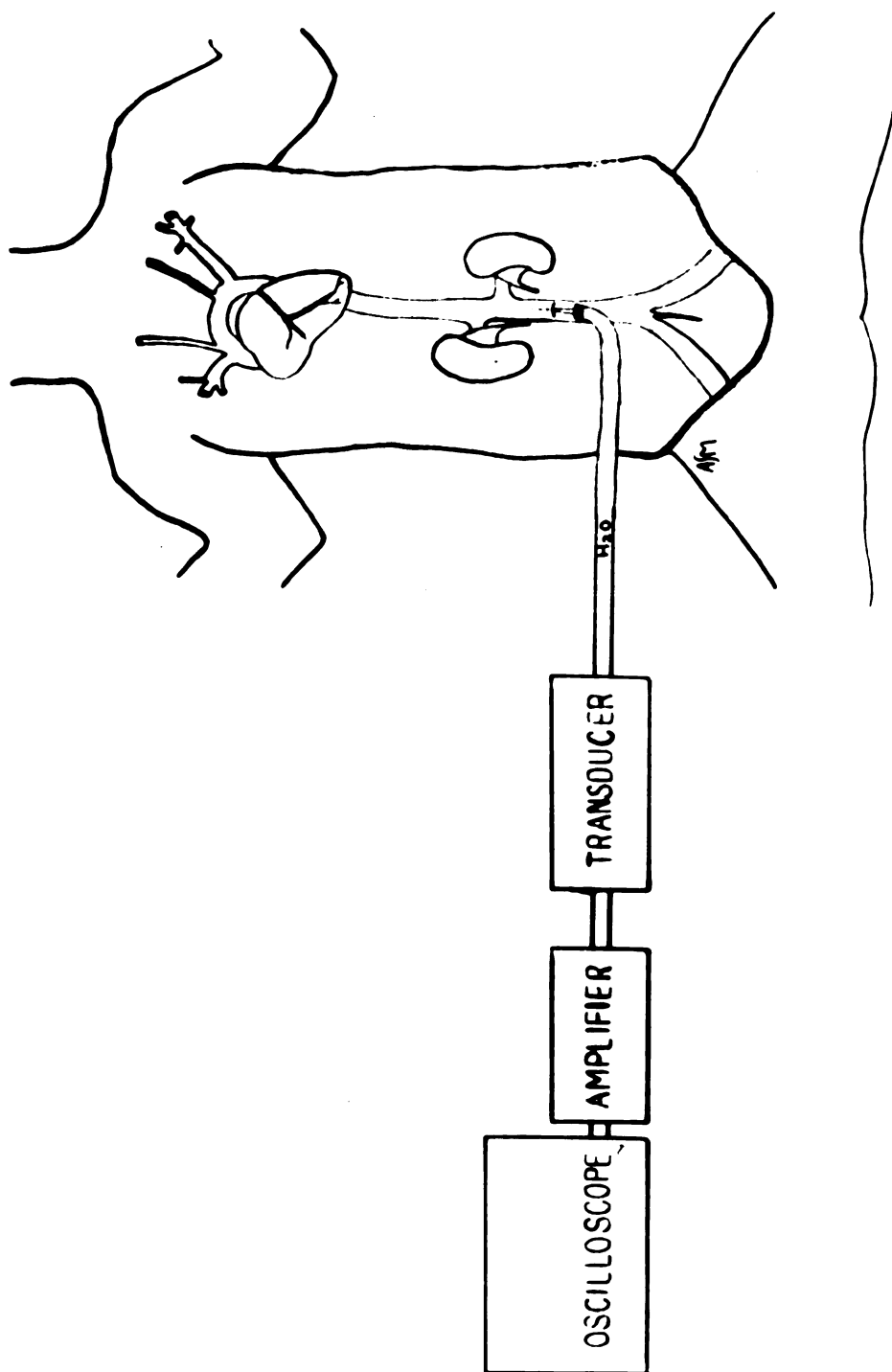


Plate 5. Apparatus for recording blood pressure.

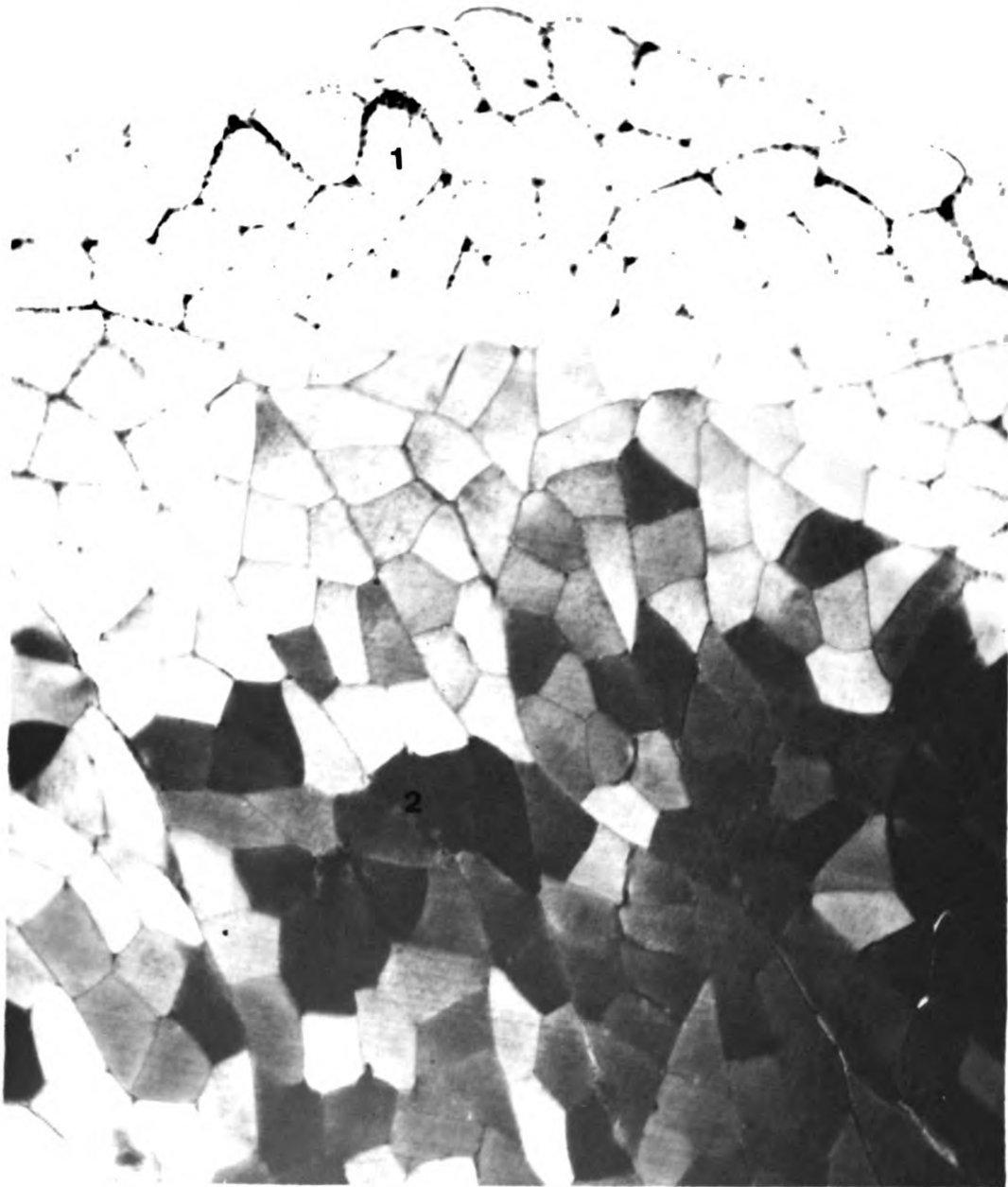


Plate 6. Cross-sectional view of pronounced peripheral white fibers and deeper glycogen positive fibers with varied concentrations. Injected at 40 mm Hg. 1, peripheral red fibers; 2, deep glycogen positive fibers. PAS stain; x 245.

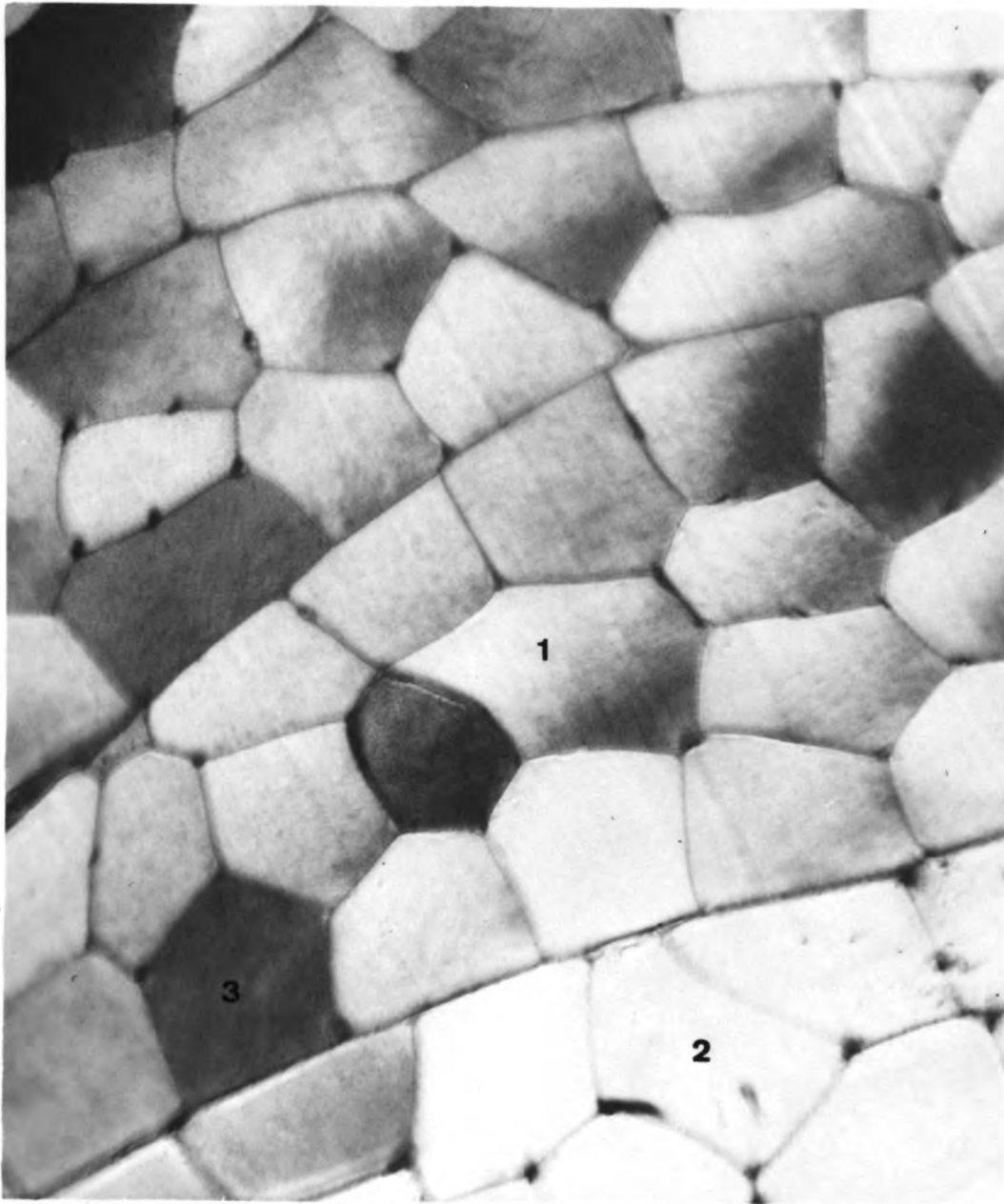


Plate 7. Cross-sectional view of muscle fibers showing uneven glycogen concentrations. 1. "phasic" fiber; 2, peripheral ^{white} red fiber; 3, glycogen positive fiber (dark). PAS stain; x 612.

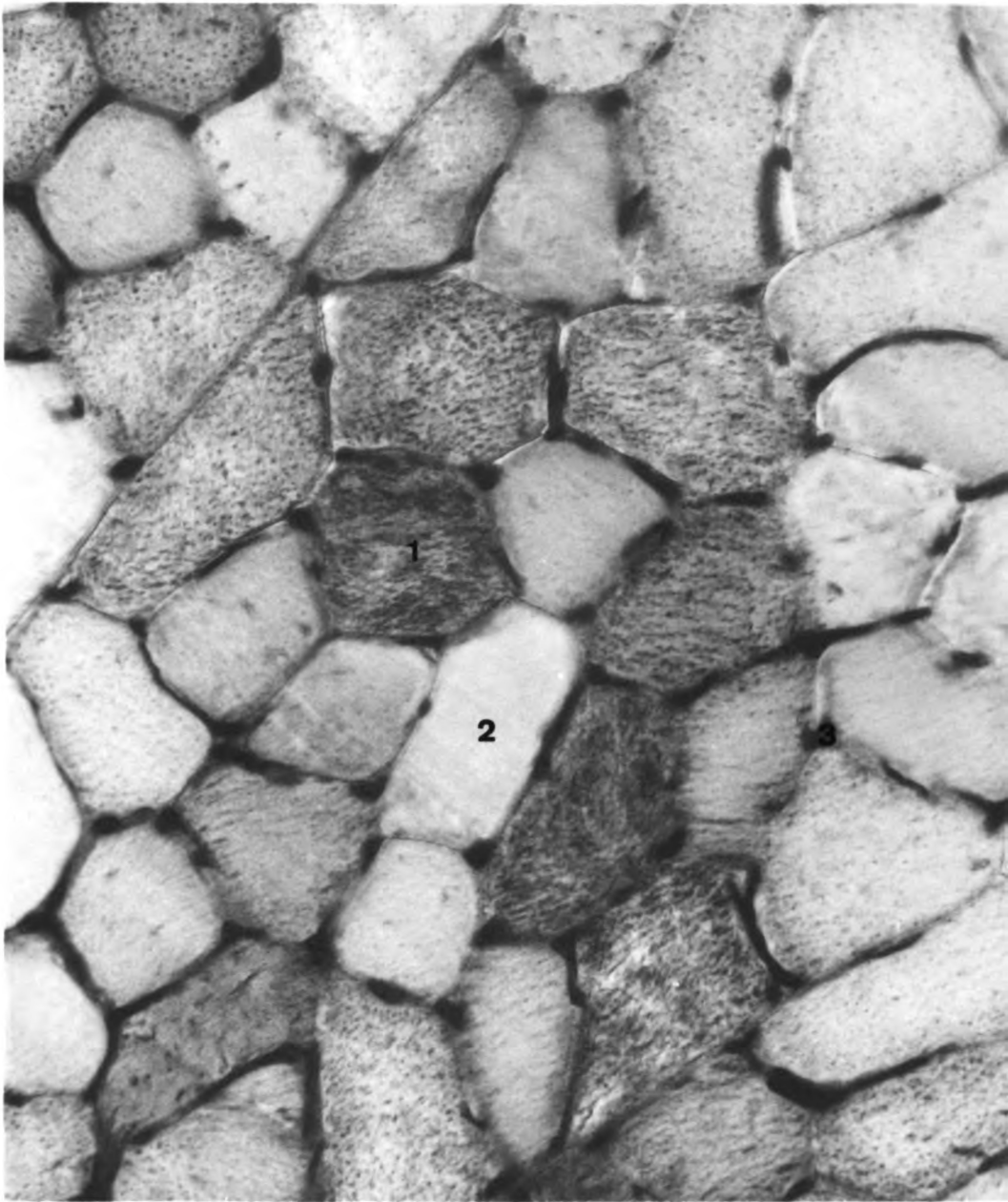


Plate 8. Cross-sectional view of muscle fibers demonstrating different patterns of glycogen positive granules. Injected at 200 mm. Hg. 1, glycogen positive fiber; 2, glycogen negative fiber; 3, capillary. PAS stain; x 582.

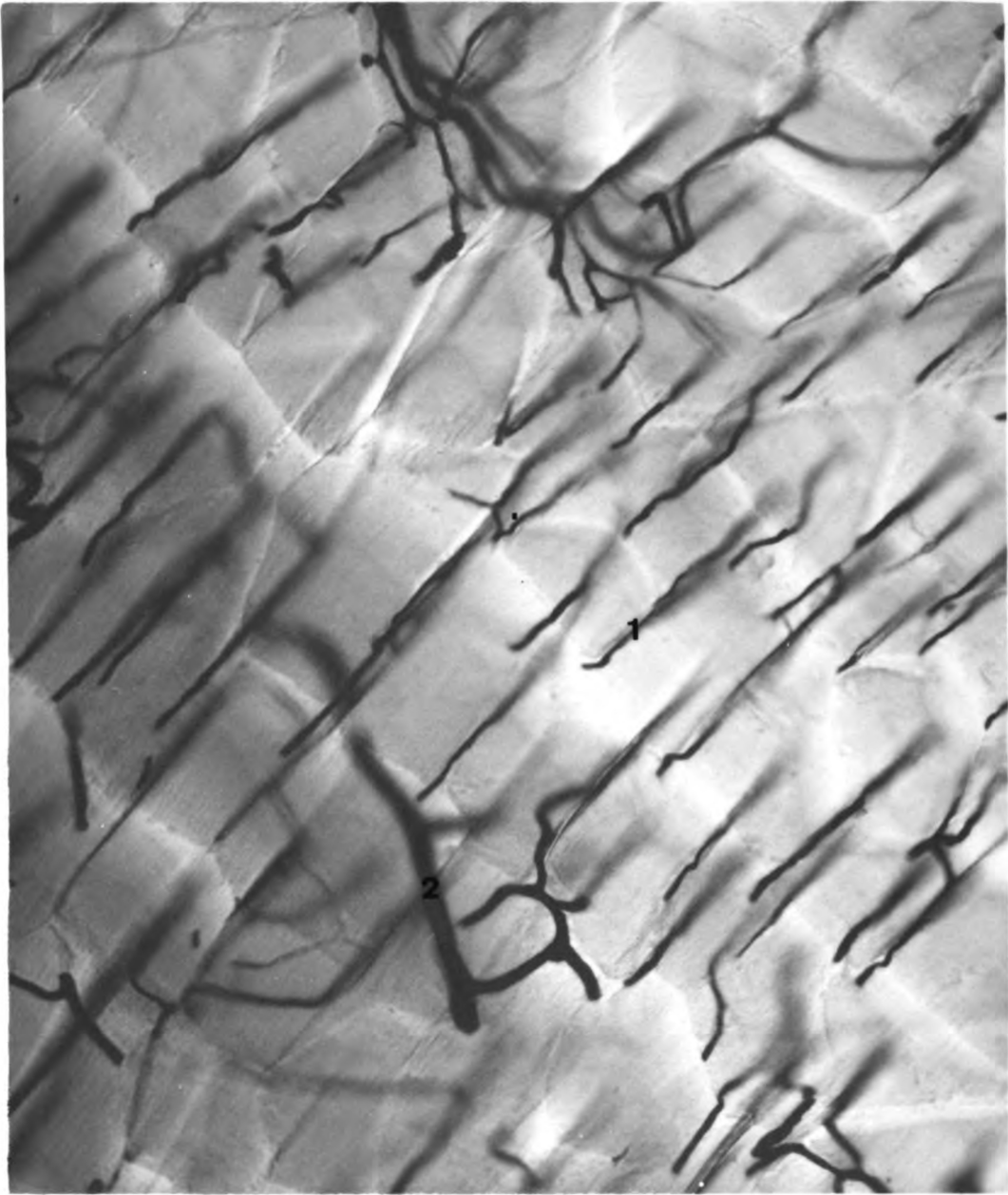


Plate 9. Longitudinal view of muscle fibers showing parallelism of the capillaries. 1, capillary; 2, metarteriole. PAS stain; x 306.

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~~10-2-1966~~