A MICROSCOPIC SURVEY OF THE FEMORAL ARTERY AND VEIN OF THE DOG

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A MICROSCOPIC SURVEY OF THE FEMORAL ARTERY AND VEIN OF THE DOG

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INTRODUCTION

Blood vessels, arteries in particular, are a hard working group of organs which have specific characteristics that set them apart from any other organ system. They have unique susceptibilities to disease; vary greatly in structure, and age at different rates. As was pointed out by Hare (1929), improper functioning blood vessels jeopardize the efficiency of the heart.

According to Lansing (1959), two out of three adult deaths are caused either directly or indirectly by cardiovascular disease. It is, therefore, reasonable to assume that a complete understanding of the normal structure of the arteries and veins is essential to our understanding of altered structure and function.

A great amount of work has been done on the histology of the vascular system of humans, but that of laboratory animals has been neglected. If it can be shown that the vessels of the dog are similar to those of the human, it would make cardiovascular research on this animal more meaningful in its translation to human disease.

Some vascular diseases, such as varicose veins and thromboses, are limited exclusively to humans. It is, therefore, a reasonable assumption that lower animals may have some morphological characteristic which helps prevent these problems. With this in mind, a

microscopic survey of the femoral artery and vein of the dog was conducted in an effort to demonstrate some of the morphological structures of these vessels.

LITERATURE REVIEW

FEMORAL ARTERY

Very little work has been done on the histology of the blood vessels of domestic animals, therefore, unless otherwise stated, the following account concerns human material.

The femoral artery is described by most authors as a medium sized muscular artery consisting of the usual three layers, tunica intima, tunica media, and tunica adventitia (Clark, 1958, Greep, 1966), although Trautmann and Febiger (1957) describe the femoral artery of domestic animals as an elastic artery. Three definite layers have been ascribed to the intima: endothelium, intermediate (subendothelial), and internal elastic membrane (Copenhaver, 1964). Henle, in 1841, was the first to describe the internal elastic membrane (Hass, 1939), and also the first to describe it as having a fenestrated appearance (Wright, 1963). As the size of the vessel increases in caliber, the number of fibers in the membrane also increases (Hass, 1939). It appears as a thick membrane with holes in it, but actually it consists of two or more layers of thick and thin fibers arranged in a network with overlapping openings, of which the larger fibers run parallel to the axis of the vessel (Huber, 1916, Gross, 1949, Hall, et al., 1955, Wright, 1963).

The media of the muscular artery is the predominant layer. Its main constituent is 25 - 40 layers of concentrically arranged smooth muscle (Copenhaver, 1964, Arey, 1968). It has been shown that the media is not arranged in circular rings of smooth muscle, as it at first might appear, but it actually consists of obliquely arranged fusiform muscle cells, which do not separate into distinct rings but rather is a continuous compact spiral structure (Strong, 1938, Clark, 1958). The thickness of the muscle coat seems to be somewhat proportional to the size of the vessel, but there may be considerable difference in arteries of the same size (Copenhaver, 1964).

Between the muscle layers are fibers of elastic, collagenous and reticular connective tissue, the amount varying with the caliber of the vessel. In the larger muscular arteries, the elastic tissue exists as fenestrated lamellae, while in some of the smaller muscular arteries, elastic fibers may be almost absent in the media. The collagenous fibers correspond to the reticular fibers seen surrounding individual muscle fibers (Bloom and Fawcett, 1968, Greep, 1966).

The adventitia consists of thick collagenous and elastic fibers which course predominately in a longitudinal direction and sometimes appear as groups of smaller fibers in transverse sections (Wright, 1963, Copenhaver, 1964, Greep, 1966). The portion next to the media abounds with prominent elastic fibers and is known as the elastica externa.

In this type of artery, the adventitia varies greatly in thickness. In some it is much thinner than the media, while in others it may be as thick or thicker than the media. The collagenous fibers of the adventitia extends into the surrounding connective tissue without a clear boundary (Maximow and Bloom, 1938).

All elastic tissue has essentially the same structure. It consists of dense ill-defined elastic fibers intermingled with fibers of collagen. These fibers have a tendency to split into fibrils which lie parallel to one another and in many cases cross (Gross, 1949). The content of elastic and collagen fibers of the femoral artery was found to be 34.3% ± 10 (Harkness et al., 1955). The elastic fiber content was assessed as 1-5% in the media and 10-30% in the adventitia and appeared constant at different ages for any one site (Wright, 1963).

With age, profound changes occur in the elastic tissue of blood vessels. Primary among these changes is fragmentation of the fibers in which the coarser plexuses break up into fibrils. Another change is an increase in thickness of the intima and atrophy of the media with the replacement of muscle and elastic tissue with collagen (Dock, 1950). The basic age change, however, seems to be calcification of the media which apparently occurs after the second decade of life (Blumenthal et al., 1950, Lansing, 1961). Correspondingly, there is an increasing resistance to stretch with age. This seems to have some bearing on atherosclerosis, because vessels not affected

by this disease, show a significant increase in elasticity in later age (Roy, 1880, Roach and Burton, 1959).

For a vascular disorder to occur, the structure of the vessel must in some way be impaired, such as calcification of the elastic tissue or the dissolution of the integrity of the intima or media (Duff, 1954). Differences in the prevalence of certain vascular diseases between man and dog have been noted. It was shown that arteriosclerosis of the intramural coronaries is more frequent in the dog, while atherosclerosis is much less prevalent (Detweiler, 1963). It would appear that there could be some morphological reason for this, although it has been shown that with increasing age the arterial wall of the canine takes on a truly sclerotic appearance. This is less common in animals of limited life span and is almost nonexistent in wild animals (Dahme, 1962). Carnivores and birds show the greatest degree of atherosclerosis and come close to the type seen in humans, however, they do have distinctive morphological differences. In contrast to human arteriosclerosis, cholesterol deposition seems to play a subordinate role in this disease in animals (Davies and Reinert, 1965).

Immediately after death, the structure of blood vessels is modified more greatly than perhaps any other organ in the body. The internal elastic membrane appears highly convoluted, and the lumen decreases markedly in size, sometimes varying as much as 25-100% from the living state (MacWilliam and McKie, 1908, Bunce, 1965).

The thickened wall contains loosely arranged contracted smooth muscle and tightly convoluted elastic fibers. Early speculation was that this phenomenon was caused by mechanical stimulation, cooling, and exposure to air (MacWilliam, 1902). Later, it was thought to be caused by the intrinsic elastic qualities of the vessel (Strong, 1938), but it is now held that this change is caused by a contraction of the smooth muscle component of the vessel and also by a decrease in length (Finerty and Cowdry, 1960, Galloway, 1936). In young persons, this decrease in length may be as much as 40% (Hesse, 1926).

It has been shown that by certain mechanical means the post-mortem vessel can be relaxed. MacWilliam (1902) accomplished this in several ways including freezing, sulphocyanide, ammonia vapors, heating at 38^{o}C. and kneading or stretching.

The waviness of the internal elastic membrane almost completely disappears when exposed to an intraluminal pressure equal to the systolic blood pressure however, in some cases, the folds do not entirely disappear and contrary to expectation, the vessel wall was identical at diastolic pressure to that of systolic pressure (MacWilliam and McKie, 1908, Galloway, 1936, Bunce, 1965). With the increase in pressure, the wall of the vessel becomes thin and compact: the relaxed smooth muscle cells are elongated and the elastic tissue becomes straightened.

Methods of experimentally distending vessels have been described as early as 1875 by Tait. The amount of change in thickness

appears to be related to the size and structure of the vessel with the small muscular arteries showing a greater proportionate increase in thickness when collapsed than did the larger elastic type arteries. The width of the distended media of the arteries varied from 36-70% less than the collapsed media. An intima was not found in the distended arteries. The endothelium was found pressed tightly against the internal elastic lamina. In the wall of the collapsed vessels, tissue of the media seemed to be forced through the fenestrations of the internal elastic membrane into the subendothelial layer and appeared to form an intima (Bunce, 1965). This same appearance was described in sections of vessels in a state of chemically induced vasoconstriction. The endothelial cells were quite irregular and bulged into the lumen, the smooth muscle cells next to the internal elastic lamina became deformed with the nuclei much shorter and the lamina itself was highly convoluted. The wall thickenss to lumen ratio in the convluted vessel was 1:5, while in the control (with intraluminal pressure) the ratio was 1:30, and no convolutions in the internal elastic membrane were evident (Wagner, 1962, Hayes, 1967).

Very sparse information on actual measurements of the thickness of blood vessels was found. Klingelhöffer and Meyer (1962) did a study on 30 humans in the third, fifth, and seventh decades of life. He found that the relationship between the inner surface and the vascular volume is more favorable for nutrition of the vascular wall of the arteries of the arm than it is for the femoral artery. The proportions

of the femoral artery, a thicker wall and larger radius, indicate a greater mechanical stress to the wall. He found the vessel wall to be 510 microns thick in the normal artery of those in their third decade and 750 microns when contracted. In the seventh decade, this had increased to 1,080 microns and 1,430 microns respectively. The tunica media of these same arteries during the third decade, was 472 microns at normal pressure and 699 microns when contracted, while in the seventh decade it measured 555 microns and 608 microns. It can be seen from these figures that the tunica media of the younger individual is the predominant layer and with ageing increases very little, yet the vessel wall more than doubles in thickness. These peculiarities of the femoral artery may be important in the vessel becoming sclerose later in life.

In a study of the arteries of the bat wing, the total cross sectional area of the vessel at different portions of the arterial bed showed a linear relationship from artery to capillary. These values varied greatly from those on fixed material (Wiedmann, 1962).

The vasa vasorum of arteries originates either from the parent artery or small neighboring vessels and is limited almost entirely to the adventitia (Arey, 1968, Bloom and Fawcett, 1968), however, in the femoral artery it is also seen in the outer third of the media.

FEMORAL VEIN

The femoral vein has been classified as both a medium sized (Copenhaver, 1964, Greep, 1966) and a large vein (Bloom and Fawcett,

1968, Arey, 1968).

The three coats of the venous walls are very difficult to distinguish due to a lack of limiting membranes. Variations in the structure of veins do not appear to be related to the size of the vessel but to the local mechanical conditions. As a result the microscopic structure of veins of the same caliber, or even different sections of the same vein are quite different, if they exist under different stress conditions. Muscular tissue is usually scarce, but when it does occur, it may be in all three layers. In the inner and outer layers, it may occur longitudinally or circularly. Since hydrostatic pressure is greatest in the limbs, muscular tissue is more abundant in the femoral and saphenous veins, and for the same reason elastic tissue is also highly developed (Pesonen, 1953). Franklin (1932, 1937) stated that the femoral vein possesses muscle and connective tissue in the wall, with longitudinal bundles in the adventitia, and spirally arranged collagenous fibers.

The veins of the extremities are usually well endowed with valves. Valves are formed from extensions of the intima, and in most cases are bicuspid, but occasionally a tricuspid valve is found (Powell and Lynn, 1951, Basmajian, 1952). The most common position for a valve is just distal to a tributary (Franklin, 1927, Arey, 1968). Valves at the entrance of a tributary are termed ostial valves, while those along the course of the vein are parietal valves (Rau, 1943).

It has been shown that at the site of a valve the vein assumes

an elliptical shape with the major axis of this ellipse paralleling the surface of the skin. The cusps are parallel to the elastic force (Edwards, 1936).

There seems to be an inverse proportion between the number of valves and the size of the vessel. They are very numerous in the deep veins and fewer in number in the femoral, popliteal and external iliac veins (Cockett, 1964).

An examination of the external saphenous vein by Bleicher and Weber (1932) showed an average number of 9.6 valves. This is quite a few more than the average of 3 found for the femoral (Basmajian, 1952). Basmajian found the following percentages: 0 valves - 1.3%, 1 valve - 7.9%, 2 valves - 23.8%, 3 valves - 33%, 4 valves - 23.8%, 5 valves - 3.9%, 6 valves - 6.6%. In another study, Powell and Lynn (1951) found one valve in the common femoral vein of 72% of his subjects and 1-4 valves in the superficial femoral of 100% of the subjects.

Using a comparative approach, Williams (1954) studied the valves in the limb veins of the dog, cat and rhesus macaque. He found the dog contained the most valves with an equal number in the fore and hind limbs. He felt that there should be more valves in the hind limbs of the monkey due to its erect posture but found an equal number here, also. This seems to support the views of Franklin (1937) and Kampmeier and Birch (1927) that the function of the valves is not to counter the affect of gravity. It seems likely that the lack of a compensatory mechanism in the lower limbs of erect animals would lead to an increase

in the disorders of their veins. There are two quite divergent views on the subject. It is felt by some that the absence or incompetence of valves in the lower limbs is an etiological factor of varicose veins (Edwards and Edwards, 1940, Egar and Wagner, 1949, Powell and Lynn, 1951). Basmajian (1952), on the other hand, felt that the presence or absence of valves proximal to the termination of the long saphenous vein is at most of theoretical importance. Van Cleave and Holman (1954) found a valve situated every 8.81 centimeters in normal veins and every 16.8 centimeters in varicose veins, thus indicating the number may affect the susceptibility of the vein to disease. In a later study by Mullarky (1964) two limbs with varicose veins were found to have normal valves above and below the sapheno-femoral junction as well as normal valves in the great saphenous vein. He found no evidence that the absence of valves in the femoral or iliac veins is a cause of varicose veins of the leg (Mullarky, 1964).

The media of veins is better supplied with vasa vasorum than that of arteries and these vessels may extend as far as the intima. This fact has been attributed to the poor quality of blood flowing through the veins (Franklin, 1937, Arey, 1968). Venous vasa originate at the junction of the outer and middle thirds of the media, and terminal arteriovenous anastomoses also occur at this junction in the femoral vein (Clark, 1965).

INNERVATION OF BLOOD VESSELS

Dogiel (1898) says that His (1892), Kolliker (1863), Cajal and

Sola (1891), Retzius (1892) and Bietti (1895) observed nerve networks in blood vessel walls, especially the arteries. Müller and Gläser (1913) found a nerve plexus in the adventitia, and in 1914, Glaser found nerve fibers in the adventitia, media and intima. Woolard (1926) thought the intra-muscular plexus to be a true net and described a pericellular rather than an intracellular ending about the smooth muscle cells of the media. Lewis (1927) summed up the knowledge of the time of vasoconstrictor nerves, in saying that all the fibers branch upon reaching the vessel forming intricate plexuses in the adventitia with a few fine fibers running into the media.

Ganglion cells were found in the adventitia of the aorta, renal artery, internal carotid and some arteries of organs, but they were not found in the vessels of the extremities (Muller and Glaser, 1913, Krogh, 1922, Truex, 1936).

By using degeneration to isolate each of the nerve components, Hinsey (1929) observed afferent nerves in the adventitia of small arteries and veins, and he also observed branches of these fibers in the perivascular connective tissue and adipose tissue. Small skein-like structures and coarser nerve fibers, which formed brush like arrangements, were also found in the surrounding tissue (Truex, 1936). Truex also found that the end organs in the adventitia are coarser than those of the perivascular region. He observed no specialized nerve endings in the media but did find slender fibers ending freely in the connective tissue between the muscle cells.

Later, it was observed that non-medullated fibers extend into the adventitia, between the adventitia and media, and also a few finely beaded fibers in the media. The medullated fibers end in the fat of the perivascular tissue (Miller, 1948, Polley, 1955).

Cheng and Kimura (1958) found thick undulated nerve fibers in the media of the dog's common carotid and abdominal aorta. Franklin (1937) found nerve fibers in close continuity with the sarcoplasm of muscle cells in the venous wall. He also observed Pacinian corpuscles in the vena cava, portal vein and certain of the cerebral veins. Terminal loops and encapsulated end organs were also observed in the adventitia of veins (Pereira, 1946). Thick undulating nerve fibers were found in the intima of the vena cava (Cheng and Kimura, 1958).

MATERIALS AND METHODS

The post-mortem study of the femoral artery and vein included 19 mongrel dogs, 10 males and 9 females, varying in weight from 6.8 to 22 kilograms. All 19 were used for study of the artery, and 18 of these were used for study of the vein. The animals were obtained from the Department of Physiology and the Department of Surgery of the College of Veterinary Medicine. They were euthanatized by intravenous or endocardial injection of either magnesium sulfate or sodium pentabarbitol. The age of the animals could not be determined in most cases, so only the weight and sex were recorded.

Prior to the actual research, a short pilot study was conducted on several animals to determine the best surgical technique, the best fixative and the most beneficial stains. The results will be enumerated in the following account.

Immediately after death, the femoral artery and vein on one side of the animal were removed. An incision was made on the medial surface of the hind limb, and the vessels were dissected free of the muscle and fascia. At this point, a U-shaped piece of aluminum (Figure 1) was placed in the incision with the prongs extending between the artery and vein. The vessels were then clamped to the holder with hemostats, one being placed proximal to the inguinalligament, and

the other distal to the point where the popliteal artery emerges. The use of the aluminum holder was necessary to eliminate longitudinal contraction of the vessels when they were cut free, since contraction markedly changed the thickness of the vessel wall. The choice of aluminum was made, because it is flexible enough to be fitted to the vessel length yet sturdy enough that its length will not be affected by the elasticity of the vessels. The vessels were then dissected free of the limb by cutting them on the outer edge of the aluminum holder.

After removal from the body, the vessels, while still attached to the holder, were placed in Bouin's fixative where they remained from 8 to 24 hours. Bouin's fixative was chosen because it was a mild yet fast acting fixative and acted with a minimum of distortion to the tissues.

After adequate fixation, a piece of both the artery and vein, 6-10 mm. in length, was removed from the upper third (near the deep femoral artery), the middle third, and the lower third (near the popliteal artery) of the vessels. These pieces were washed in several changes of 50% alcohol and stored overnight in 70% alcohol. After adequate washing, the tissue was cleared and dehydrated by the following method: two hours in tetrahydrafuran and water mixed in a 1:1 ratio, one and a half hours in each of two changes of pure tetrahydrafuran, and two hours in a 1:1 mixture of tetrahydrafuran and paraffin. The tissues were then infiltrated for 1-2 hours in a vacuum dessicator

and blocked in Paraplast*. Sectioning of these blocks was done at 6 microns, and the sections were affixed to slides. One slide from each representative group (upper, middle and lower third) was stained with hematoxylin and eosin and one from each group was stained with Weigert's resorcin-fuchsin elastic stain. This proved to be a most useful stain in examining the elastic tissue and in differentiating the layers of the vessels.

After the tissues were stained, the thickness of the tunica media and the tunica adventitia were measured by using a Bausch and Lomb microscope equipped with an occular micrometer. The criteria for the boundaries of the layers measured are as follows: The media was measured from the internal elastic membrane to the junction of the smooth muscle of the media and the elastic fibers of the adventitia. This point is quite clearly demonstrated with the resorcin-fuchsin stain. The measurement of the adventitia was from the muscle-elastic tissue junction to the point where the elastic fibers disappeared in the perivascular connective tissue. Since the adventitia blends so well with the surrounding fascia, this point was used as an arbitrary boundary. Four measurements of each of these layers was made, and an average figure attained. Since it is very difficult to accurately separate the coats of the vein wall (Pesonen, 1953), they were all included in one measurement, which was done in the same manner as those of the arteries, from the internal elastic membrane to the

* Sherwood Medical Industries, St. Louis, Missouri.

disappearance of the elastic tissue in the fascia.

When all the measurements had been taken, the figures were plotted on graphs, both linear and logarithmic, which compared the thicknesses to the weight of the animal. The method of the least squares was then used to determine the best straight line for the figures.

PERFUSION

According to Bunce (1965), blood vessels are altered more by post-mortem changes than any other organ in the body. They collapse, their walls thicken and the area of the lumen is reduced.

After the study was completed on the structure of the post-mortem vessels of these 19 animals, 5 animals, 1 male and 4 females, were studied to determine any structural changes which might occur with an intraluminal pressure of 100 mm. mercury. To do this, a cannula was inserted in the common iliac artery for introduction of the fixative and another in the common iliac vein for drainage of blood and excess fixative. Physiological saline was then injected into the system to clean out the excess blood and 10% formalin was injected into the vessels at 100-105 mm. mercury. The vessels remained in the animal, uninterrupted, for four to five hours after injection and were then dissected free in the same manner as described in the previous section. They were then placed in 10% formalin for at least 48 hours and processed in the same manner as the Bouin's fixed tissue with the alcohol washings being eliminated. These vessels were

measured in exactly the same manner as the non-perfused vessels, and they too were plotted on graphs and compared to the others.

VALVES IN VEINS

In order to determine the number of valves in the femoral vein, this vessel was dissected out of 15 limbs by fastening them to the aluminum holders and fixing them in 10% formalin for at least 48 hours. At the end of this time, the veins were placed under a dissecting microscope and split lengthwise. This process adequately exposed the valves for enumeration.

COLLATERAL STUDIES

In conjunction with the aforementioned work, a number of closely related studies were also conducted. Several representative slides were stained with Bielchowsky's stain to demonstrate the reticular network of the vessel wall. For the demonstration of nerve fibers and nerve endings, the Davenport rapid silver method was employed (Conn and Darrow, 1946). Observations were also made on the number of layers of elastic tissue in the tunica media and the tunica adventitia and on the vasa vasorum.

OPTICAL AND PHOTOGRAPHIC EQUIPMENT

Observations for this study were made on a Bausch and Lomb binocular microscope and on an American Optical binocular microscope.

The photomicrographs were made with a Carl Zeiss Photomicroscope* which was equipped with an automatic exposure setting device. The photographic film used was Adox KB14**. The film was enlarged in printing to the magnifications noted on the figures.

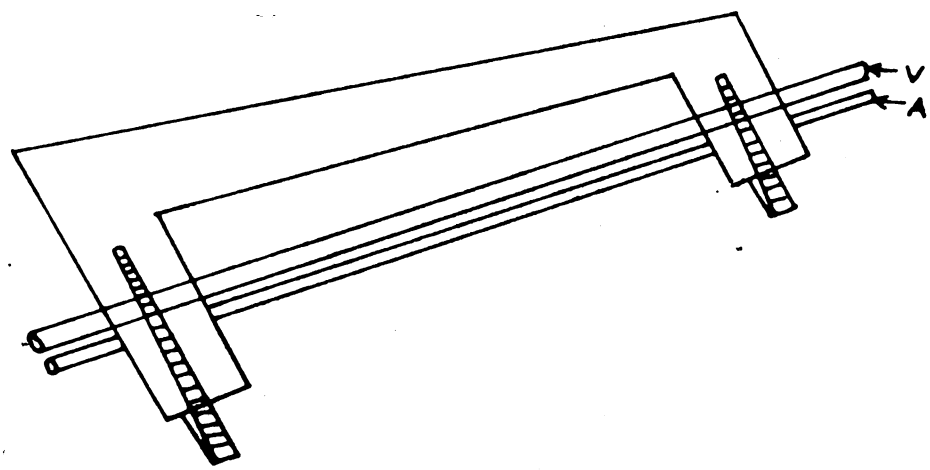
* Carl Zeiss, Oberkochen, Wuerttemberg, Germany.

** Adox Fotowerke, Frankfurt, Germany.

FIGURE 1

Aluminum holder used for maintaining normal length of femoral artery (A) and vein (V).

FIGURE 1



OBSERVATIONS AND CONCLUSIONS

FEMORAL ARTERY

INTIMA

The intima of the femoral artery of the dog varies in appearance with changes in the intraluminal pressure. The intima of the vessel with no intraluminal pressure consists of an endothelium, two or three layers of connective tissue and is limited by a very prominent internal elastic membrane. The intima of the perfused vessel, on the other hand, consists of an endothelium and an internal elastic membrane, with only occasional connective tissue fibers between them. The nuclei in the endothelium of the relaxed vessel are quite prominent and rounded, while those of the perfused vessels are in close apposition to the internal elastic membrane and are elongated. The internal elastic membrane of the relaxed vessel is very convoluted, with the folds extending in both circular and longitudinal directions (Figure 2, 3). This configuration is caused by post-mortem contraction of smooth muscle in the vessel in both longitudinal and circumferential directions (Finerty and Cowdry, 1960, Galloway, 1936). These folds become less prominent in the perfused vessel, and in some cases completely disappear (Figure 4).

At first glance, the internal elastic membrane appears to be one

thick elastic fiber, but upon closer examination, it can be seen that it is actually composed of several layers of finer fibers. This may be evidenced at certain points along the membrane where the layers are separating (Figure 5). Located in the internal elastic membrane are numerous fenestrations which are created when openings in adjacent layers of elastic tissue coincide (Figure 6, 7). Only limited amounts of connective tissue appear between the endothelium and internal elastic membrane of the perfused vessel. An explanation for this might be that as the vessel relaxes, some of the tissue from the media, connective tissue and smooth muscle, migrates through the fenestrations into the intima, however, when the vessel is distended by an intraluminal pressure, this material seems to be forced back into the media (Figure 8, 9). These observations indicate that the subendothelial layer described in many textbooks may be a product of tissue preparation rather than a naturally occurring morphological feature.

MEDIA

The media of the canine femoral artery is very well delineated with the internal boundary being the internal elastic membrane and the external boundary the junction between smooth muscle of the media and large elastic fibers of the adventitia (Figure 3). As in the intima, the structure of the media also varies slightly between relaxed and perfused vessels. The main constituent of the media is 18-40 layers of smooth muscle. In the relaxed vessel, the nuclei of the muscle

fibers are of the cork-screw shape, indicating contraction, while in the perfused artery, the nuclei are quite elongated (Figure 8, 9). Between the muscle fibers is a network of elastic, collagenous and reticular connective tissue. Viewed in cross section, the elastic tissue seems to be arranged in three to five distinct layers of fine fibers (Figure 4), but when the vessel is cut longitudinally, it can be seen that the elastic tissue is actually an interwoven net, running in all directions (Figure 10). Since the media contains this network of elastic fibers, the femoral artery of the dog appears to be of the transitional type rather than the muscular classification it is usually given.

There is more collagenous connective tissue in the media than elastic, but like the elastic tissue, this does not occur in great amounts either. It is found coursing randomly between the muscle fibers. Also found in the media, is a network of reticular fibers around the muscle fibers. These fibers are not oriented in any one direction and appear to enclose the muscle fibers (Figure 11).

ADVENTITIA

The adventitia of the artery consists of collagenous connective tissue with a large number of thick elastic fibers coursing predominently in a longitudinal direction (Figure 3, 10). These fibers are much thicker than those found in the media, and resemble the internal elastic membrane in appearance. Although they are very sparse, reticular

fibers are also found in the adventitia. These fibers course randomly between the elastic and collagenous fibers (Figure 11). The collagenous fibers of the adventitia, which are quite extensive, lie between the large elastic fibers and extend into the connective tissue of the perivascular region. The collagenous fibers of the two layers blend very well and make the external boundary of the adventitia quite hard to distinguish (Figure 3).

VASA VASORUM

The vasa vasorum of the artery extends in a predominately longitudinal direction and is found almost exclusively in the adventitia (Figure 4, 12). In only one case a longitudinal nutrient vessel was observed in the media. Cases were observed, however, where nutrient arterioles originated from the parent vessel and ran obliquely through the media and ended in the outer adventitia (Figure 14, 15, 16, 17). The internal elastic membrane of the nutrient vessel is an extension of the external elastic membrane of the parent vessel and is incorporated in the nutrient arteriole over its entire length. Near the junction of the connective tissue of the adventitia and perivascular tissue, the vessel connects with a capillary network. It is necessary to inspect longitudinal sections to observe this type of arrangement, and it was seen in two of the specimens examined.

NERVES

Several nerve fibers were observed coursing through both the adventitia and the media of the femoral artery. At intervals along

these fibers several small fibers branched from the main fiber. A few fibers appeared to extend as far as the intima but the majority ended in the media. The majority of these terminals were simply free endings but a few net-like endings were observed.

MEASUREMENTS

Measurement of the walls of the femoral arteries showed a decrease in thickness distal to the inguinal ligament. In the media there was a 13% decrease in the average thickness between the upper third and the middle third of the artery and a 19% drop between the upper third and the lower third. The adventitia decreased 14% in average thickness between the upper and middle thirds of the artery and 32% between the upper and lower thirds (Appendix 1). When plotted against body weight, the thickness of the layers at the three levels along the artery increased logarithmically as the weight increased (Figure 25-30).

The perfused vessels showed a definite decrease in wall thickness. This decrease differed, however, between the media and the adventitia. The thickness of the media decreased 42% in the upper one third, 37% in the middle one third, and 49% in the lower third when compared to the thickness of comparably sized animals (Appendix 1, 3). When plotted against the body weight, the thickness of the media of the perfused vessels increased logarithmically as the weight increased and the calculated straight line ran approximately parallel to that of the

relaxed vessel but was displaced 600 to 1000 microns lower on the graph (Figure 25, 26, 27).

A different arrangement was found in the adventitia of the artery. The adventitia of the animals observed showed a decrease in average thickness, but as the animal size increased, the decrease in thickness was much less pronounced. The average thickness for the group of animals observed showed a decrease of 37% in the upper third of the perfused vessel from that of the relaxed vessel, 36% in the middle third, and 34% in the adventitia of the lower third (Appendix 1, 3). The calculated straight line for the adventitia of the perfused animals is not parallel to that of the relaxed vessels, but intersects the latter at the 12-18 kg. point on the abscissa (Figure 28, 29, 30).

FEMORAL VEIN

INTIMA AND MEDIA

The femoral vein of the dog closely resembles the generally accepted description of the medium sized vein (Figure 18, 19). It is very difficult to distinguish between the three layers of the wall, especially between the intima and media. There is no internal elastic membrane, so the collagenous connective tissue of the intima and media blend together. Interspersed between the collagenous fibers are 3-8 layers of smooth muscle and a few longitudinally directed elastic fibers (Figure 20, 21). Few reticular fibers were observed.

ADVENTITIA

The adventitia, which constitutes the main portion of the vein wall, is composed of collagenous connective tissue with a network of large elastic fibers within it. These elastic fibers resemble quite closely those seen in the artery and are disposed in much the same manner (Figure 18, 20). The external boundary of the adventitia of the vein, like that of the artery is quite indistinct and for the same reason. The collagen fibers of the adventitia blend with those of the perivascular region preventing a clear line of demaracation (Figure 17).

VASA VASORUM

The vasa vasorum of the vein was located entirely in the adventitia and was more extensive than that seen in the artery (Figure 13, 20). It was also evident that there were more small vessels supplying the perivascular connective tissue of the vein than there were to the same region of the artery (Figure 19).

Increasing the intraluminal pressure of the vein did not have nearly as much effect on its appearance as it did on the artery. One difference, however, was seen in the collagenous tissue of the adventitia. In the relaxed vessel, this tissue was fairly compact in nature, but in the perfused vein it appeared to be much looser. In order to accommodate the increase in diameter, the fibers seemed to pull away from each other as the vessel was distended (Figure 13 vs 20).

NERVES

The main network of nerves of the vein wall was observed in the adventitia but a few fibers extended into the media. These networks appeared quite similar to those found in the artery but not as extensive.

MEASUREMENTS

It was felt that the thickness of the venous wall would decrease as the measurements proceeded distally from the inguinal ligament, for as one proceeds in this direction, the venous pressure increases, being greatest at the most distal point. This, however, was not the case. Measurement of the venous wall showed that, like the artery, it decreased in thickness as measurements proceeded distally from the inguinal ligament. The average thickness decreased 8% from the upper third to the middle third and 20% from the upper third to the lower third (Appendix 2). As in the arteries, the veins increased in thickness logarithmically as the body weight increased (Figure 31, 32 33).

Perfusion caused a change in the thickness of the femoral vein wall comparable to the change noted for the adventitia of the femoral artery (Appendix 4). The average wall thickness was less for these animals than for the comparably sized group with relaxed veins, but the straight lines calculated for the two groups were not parallel but, as in the arterial adventitia, intersected. In this case the point of intersection was at the 9-11 kg. point on the abscissa.

VALVES

The most common sites for valves in the femoral vein were the entrances of tributaries, the most prominent being the entrance of the deep femoral. The valves were all of the bicuspid type (Figure 22, 23) and in addition to the valves, an occasional small reservoir, or invagination, was found in the wall. Of the 15 femoral veins examined for valves, all had at least one, and the following distribution occurred: one (6.7%) had one valve, four (26.7%) had two valves, eight (53.3%) had three valves, and two (13.3%) had four valves (Appendix 5 and Figure 34). The average for this group was 2.8, which is quite similar to the average of 3 found in the femoral vein of humans (Basmajian, 1952).

SUMMARY

A study was made of the microscopic structure of the femoral artery and vein of the dog in an attempt to elucidate the similarities and differences between their morphology and that of similar vessels in man. It was hoped that by doing this study, vascular research on the dog may be more meaningful in its interpretation to human disease.

The vessels were examined in both a normal post-mortem state and after perfusion with formalin at a pressure of 100 mm mercury. One morphological difference observed in the femoral artery of the dog was 4-6 layers of elastic tissue in the media. This feature indicates that the artery should be classified as a musculo-elastic type rather than the muscular class it is usually given. This type of classification has been suggested by Dankmeijer and Haefsmit (1967) to distinguish the transitional arteries from those which are strictly muscular or elastic.

Measurements were made on the thickness of the arterial and venous walls at various points. It was found that as the body weight of the animals increased, the thickness of the vessel walls increased logarithmically.

After perfusion of the vessels, there were marked changes in the architecture of the vessels such as near elimination of the convolutions of the internal elastic membrane of the artery, a decrease in

sub-endothelial connective tissue, elongation of the nuclei of the endothelium and the muscle of both the artery and vein, and an overall decrease in thickness of the vessel walls. This decrease varied in magnitude, however, between the arterial media, arterial adventitia and venous wall.

The vasa vasorum of both the artery and vein were limited primarily to the adventitia with a few nutrient vessels extending into the media.

Small nerve fibers and small nerve endings were observed in both the adventitia and media of the arteries and veins. After examination of 15 veins, an average of 2.8 valves were found with the most common position being distal to the entrance of a tributary. This number is in close agreement with that found for man.

Other than the few layers of elastic tissue found in the media of the femoral artery, no distinct morphological differences were observed between these two vessels in the dog and the same two in man.

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FIGURE 2

Longitudinal section of the femoral artery. Note the numerous circular folds of the internal elastic membrane (IEM).

X 33 Weigert's resorcin-fuchsin.

FIGURE 2

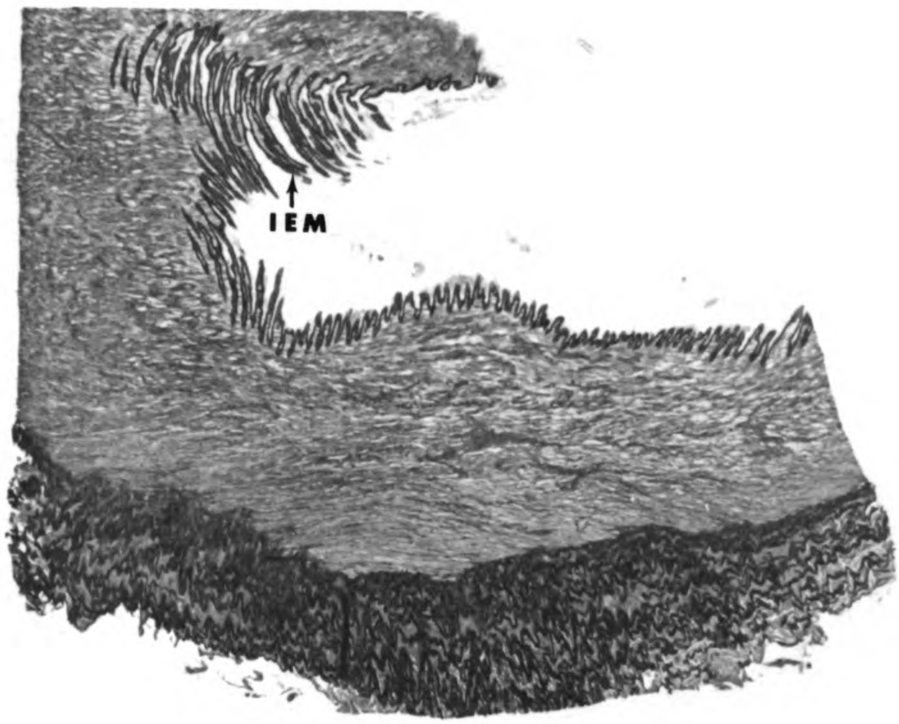


FIGURE 3

Cross section of femoral artery demonstrating the boundaries of the media with the adventitia (MB) and the adventitia with the perivascular connective tissue (AB). Note, also, the distribution of the dark staining elastic tissue and the highly convoluted internal elastic membrane (IEM).

X 170 Weigert's resorcin-fuchsin.

FIGURE 4

Cross section of femoral artery perfused at 100 mm. Hg demonstrating the network of elastic tissue (ET) in the media and adventitia and an internal elastic membrane (IEM) which is much less convoluted than the similar structure of the relaxed vessel (Figure 3). Note the nutrient vessel (NV) in the adventitia.

X 210 Weigert's resorcin-fuchsin.

FIGURE 3

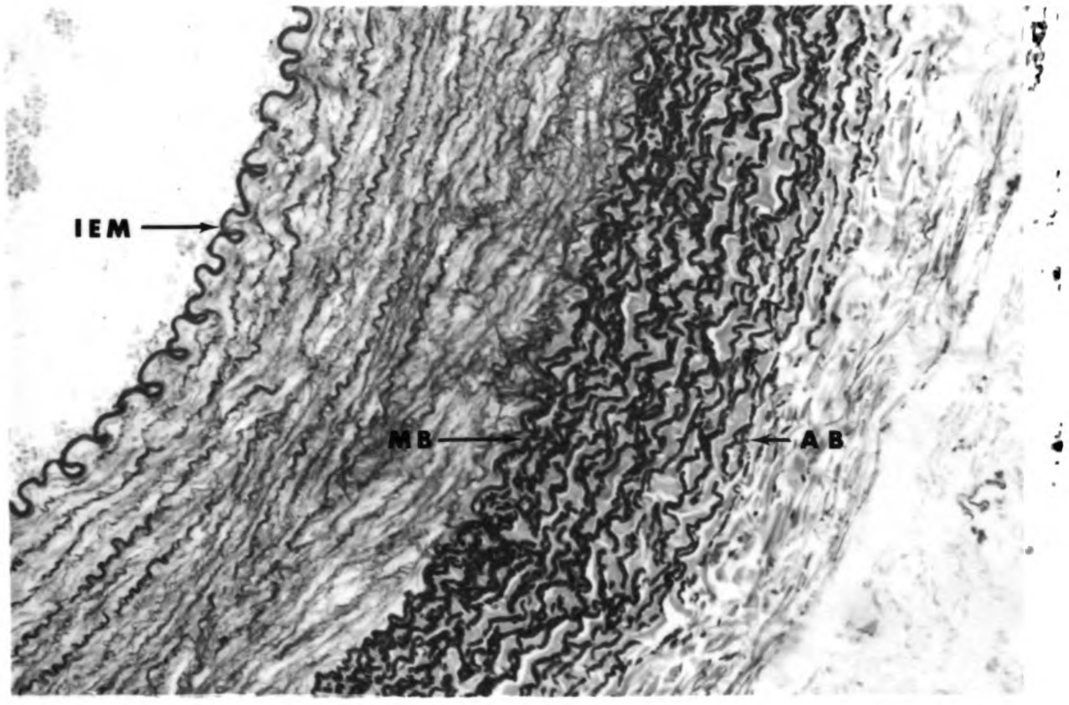


FIGURE 4

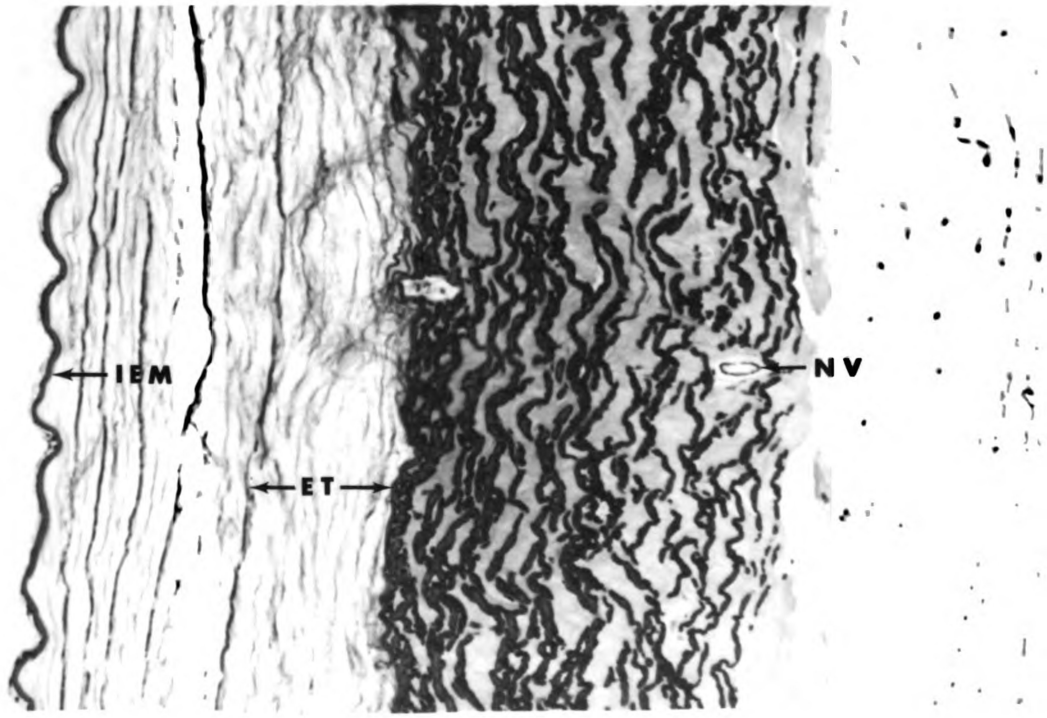


FIGURE 5

Separation of the internal elastic membrane (IEM) of the femoral artery.

ET elastic tissue X 850 Weigert's resorcin-fuchsin.

FIGURE 5

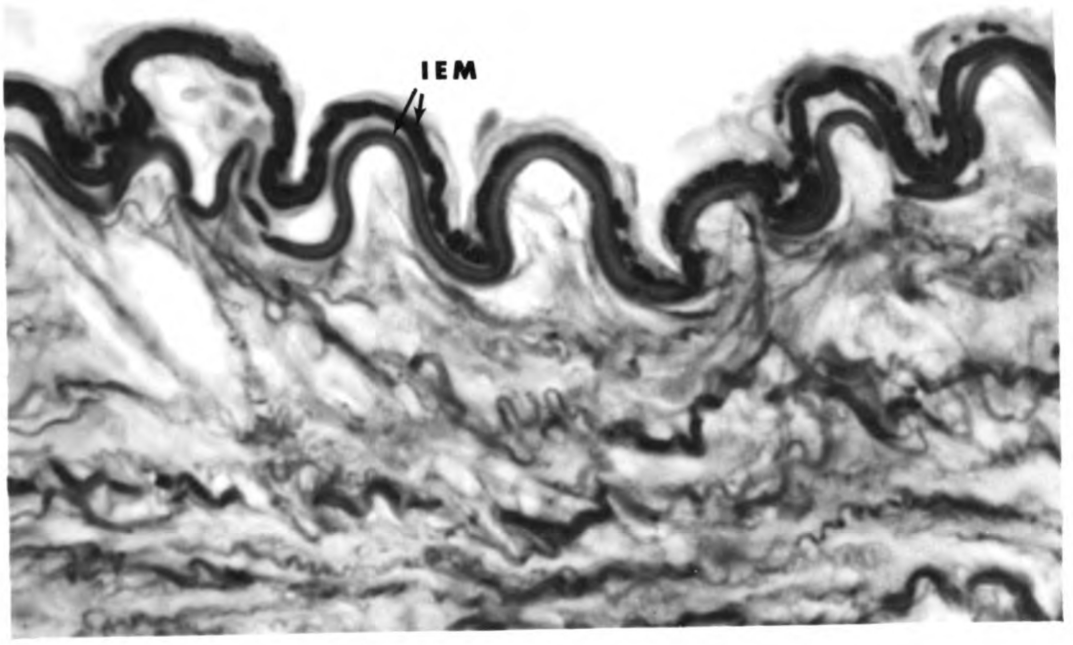


FIGURE 6

Internal elastic membrane of femoral artery. Note the fenestration.

X 850 Weigert's resorcin-fuchsin.

FIGURE 7

Same section as Figure 6. Due to adjustment of fine focus the fenestration has closed (F).

X 850 Weigert's resorcin-fuchsin.

FIGURE 6

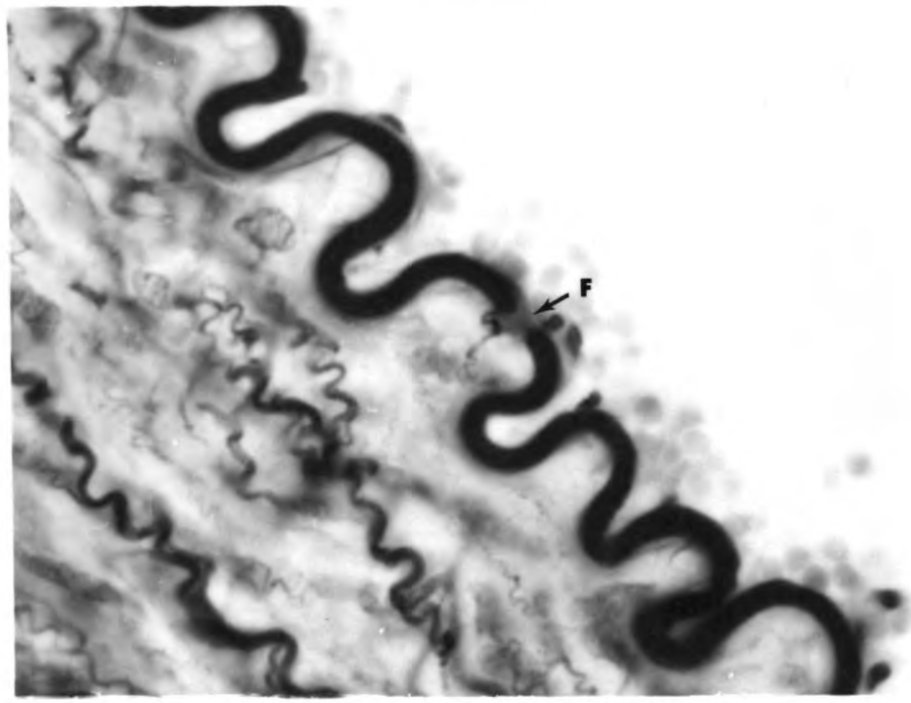


FIGURE 7

FIGURE 8

Intima and media of relaxed femoral artery. Note the large amount of connective tissue and the round nuclei of the intima (I), and the corkscrew shaped nuclei (N) of the smooth muscle in the media. Note, also, the highly convoluted internal elastic membrane (IEM).

X 530 Hematoxylin and Eosin.

FIGURE 9

Intima and media of artery perfused at 100 mm. Hg. Note very thin intima (I), few convolutions of the internal elastic membrane (IEM) and the elongated nuclei of the smooth muscle in the media (N).

X 530 Hematoxylin and Eosin.

FIGURE 8

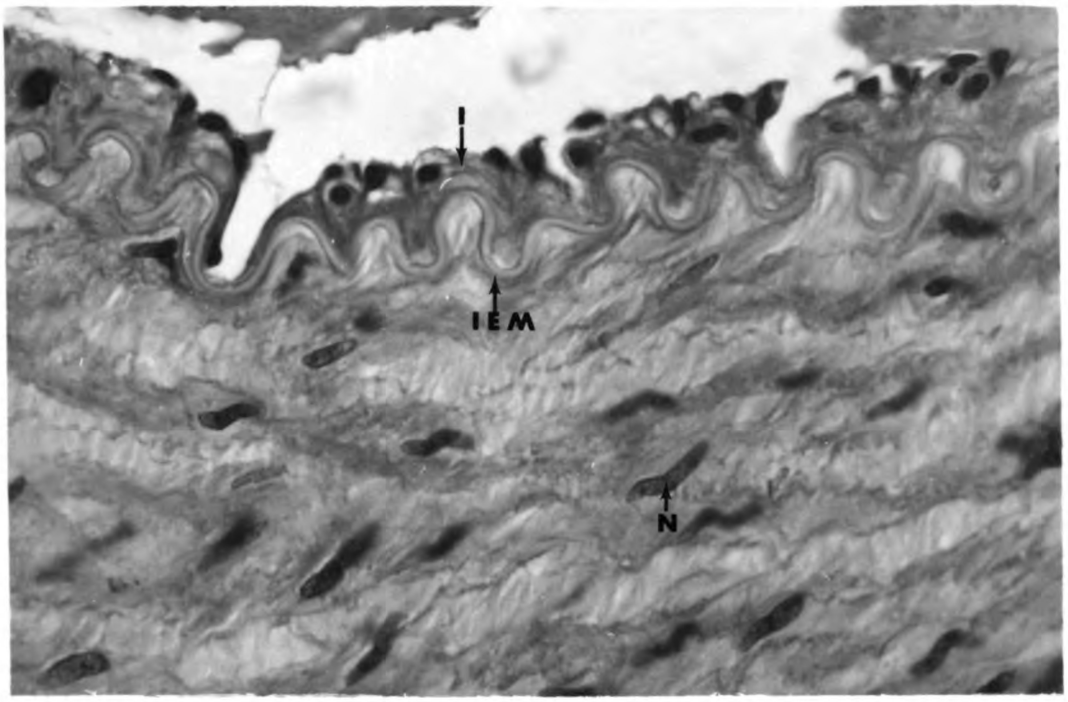


FIGURE 9

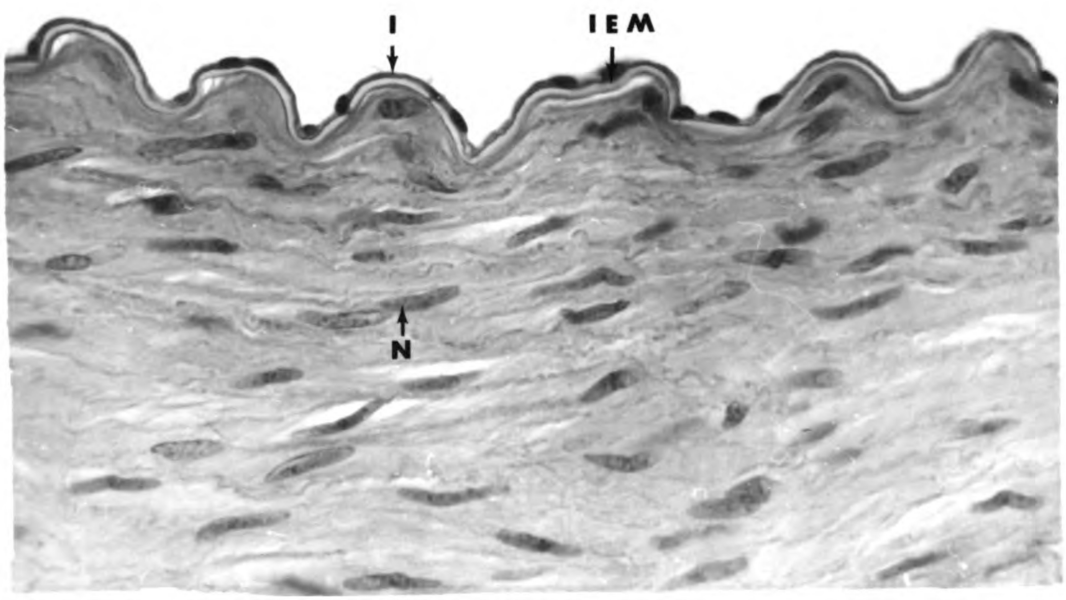


FIGURE 10

Longitudinal section of a perfused femoral artery. Note the predominatly longitudinal direction of the dark elastic fibers (EF) and the flat internal elastic membrane (IEM).

X 210 Weigert's resorcin-fuchsin.

FIGURE 11

Longitudinal section of relaxed femoral artery. Note the abundance of the dark staining reticular fibers in the media (M) compared to the few fibers in the adventitia (A).

X 210 Bielschowsky's reticular stain.

FIGURE 10

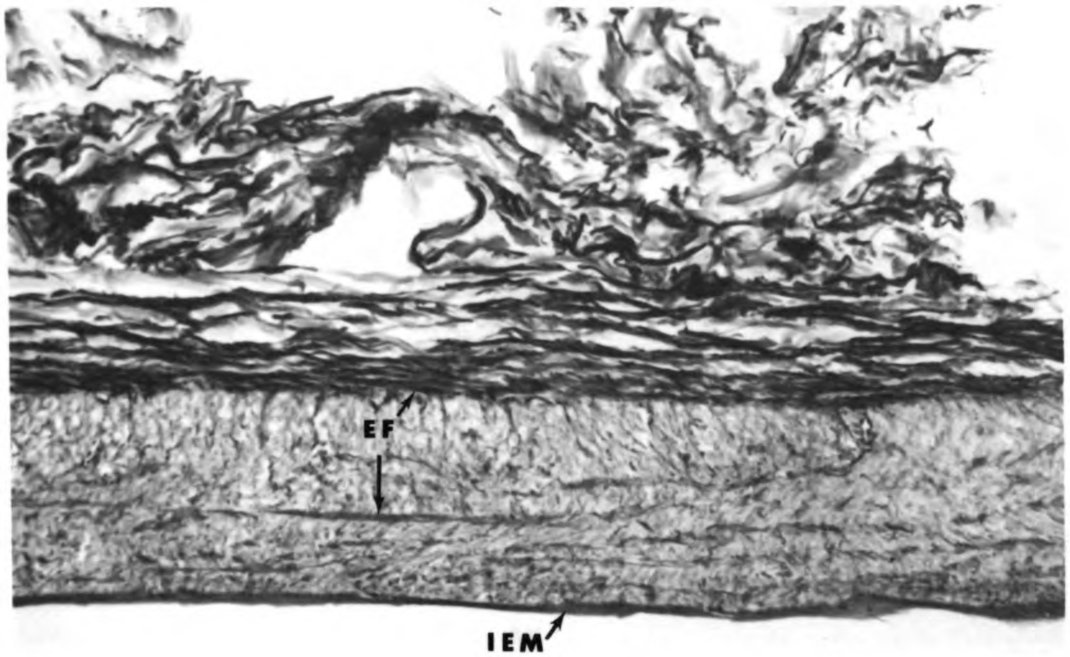


FIGURE 11

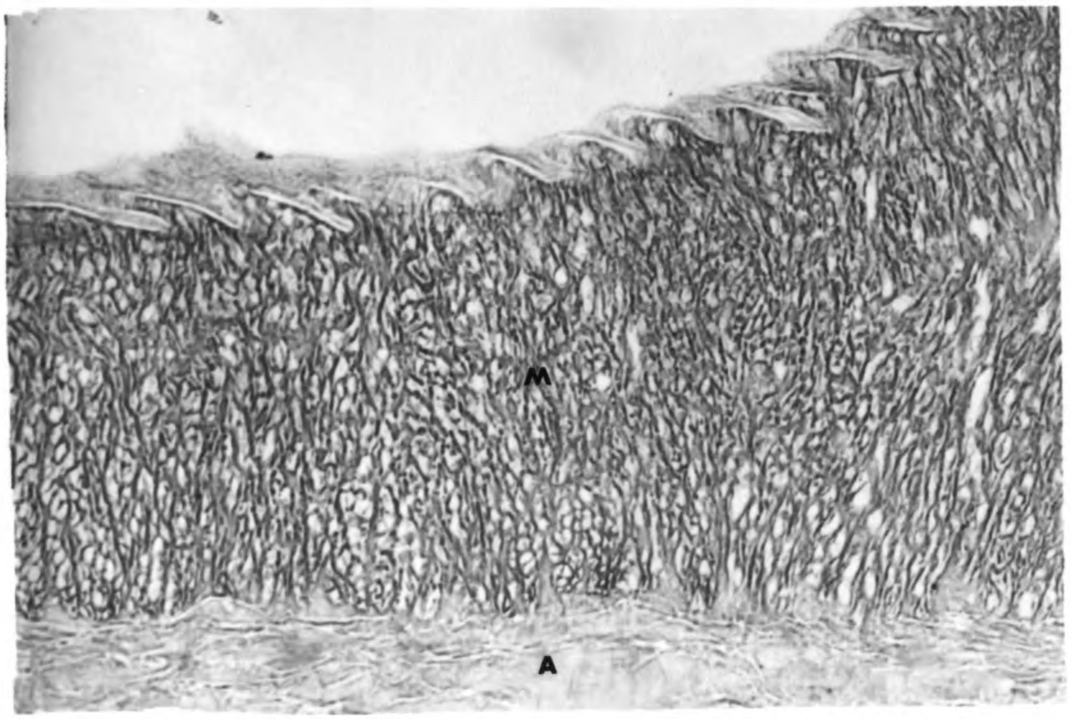


FIGURE 12

Cross section of femoral artery. Note nutrient vessel (NV) in the adventitia and convoluted internal elastic membrane (IEM).

X 210 Weigert's resorcin-fuchsin.

FIGURE 13

Cross section of femoral vein. Note the nutrient vessels (NV) in the adventitia and the distribution of the dark elastic fibers (EF).

X 330 Weigert's resorcin-fuchsin.

FIGURE 12

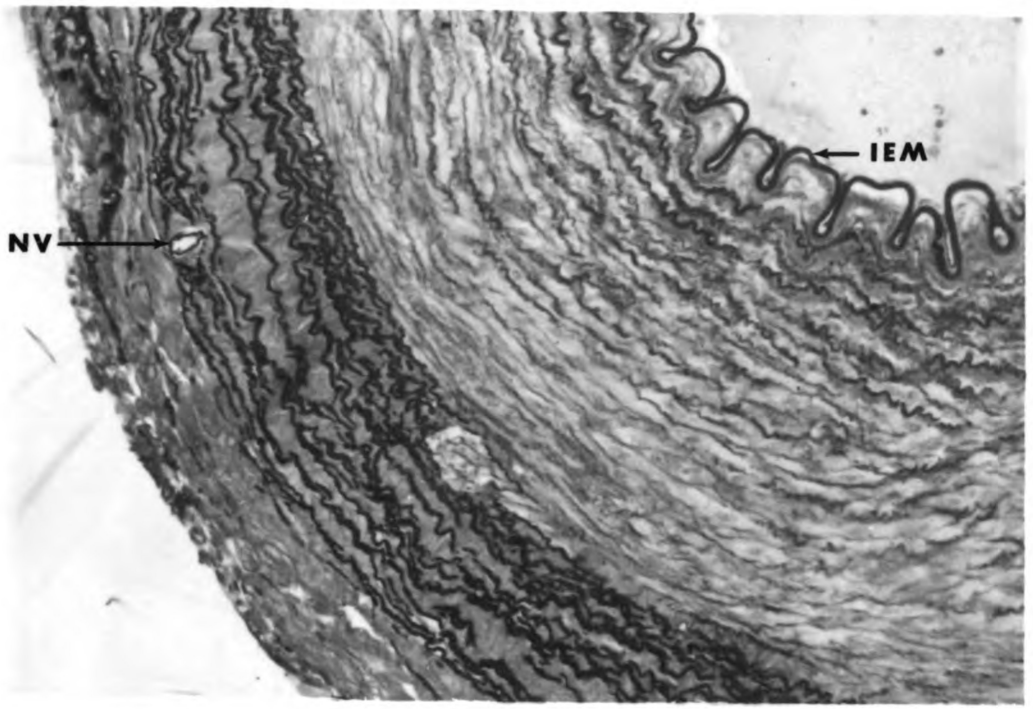


FIGURE 13

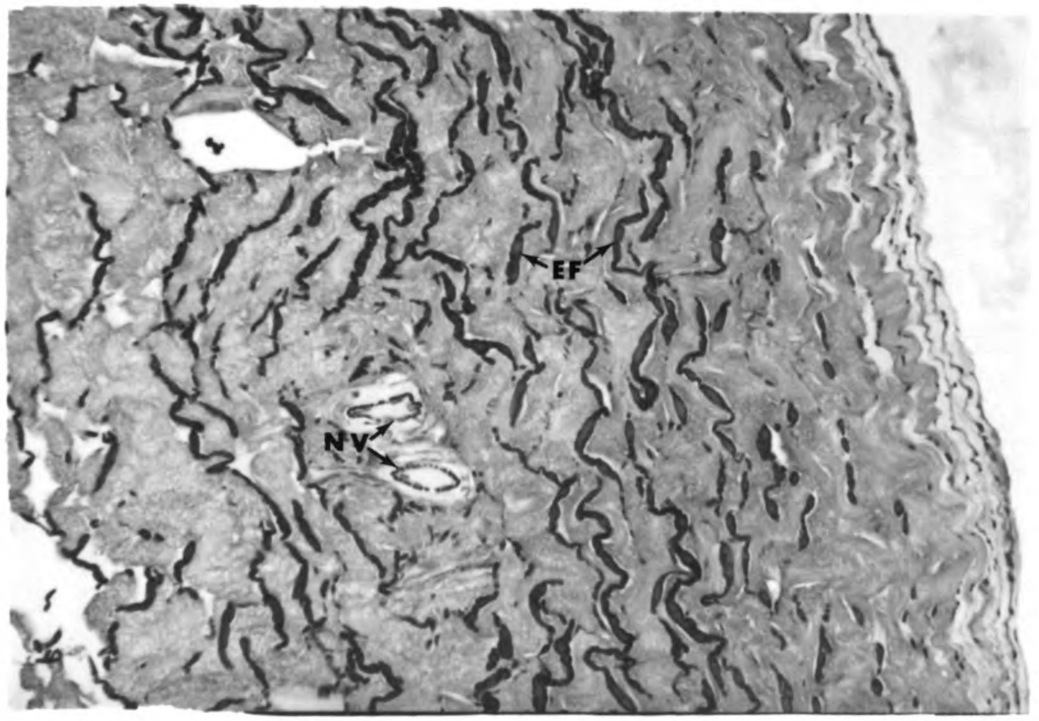


FIGURE 14, 15

Longitudinal section of femoral artery. Note the origin of a nutrient arteriole from the intima of the parent vessel (NA).

X 33 Weigert's resorcin-fuchsin.

FIGURE 14

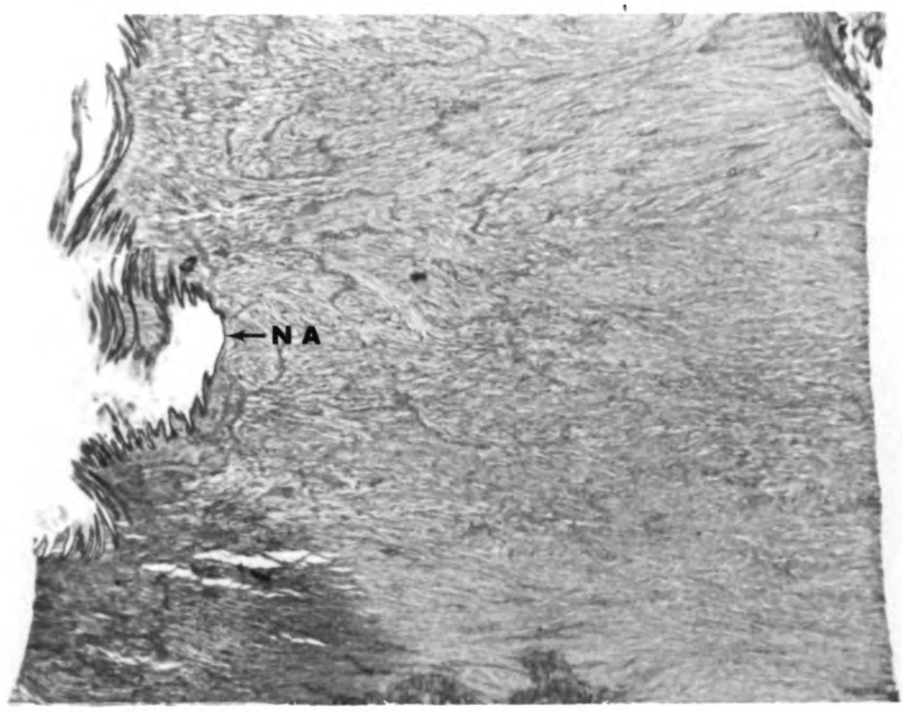


FIGURE 15

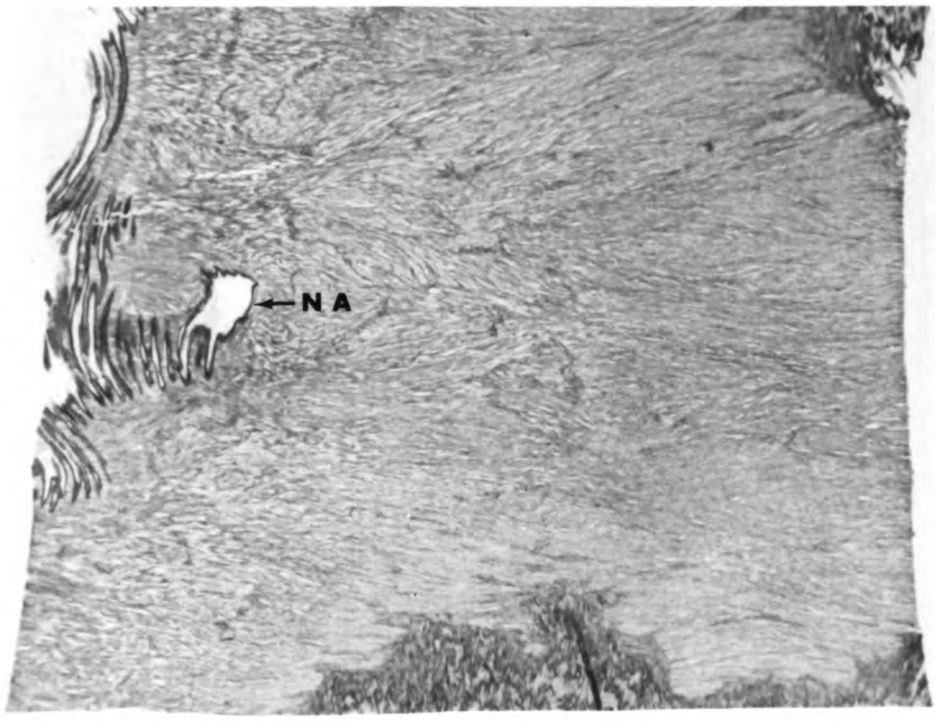


FIGURE 16, 17

Continuation of longitudinal series of Figure 14, 15 demonstrating a nutrient arteriole (NA) as it courses obliquely through the media (M) and approaches the adventitia (A).

Figure 16 X 43 Weigert's resorcin-fuchsin.

Figure 17 X 53 Weigert's resorcin-fuchsin.

FIGURE 16

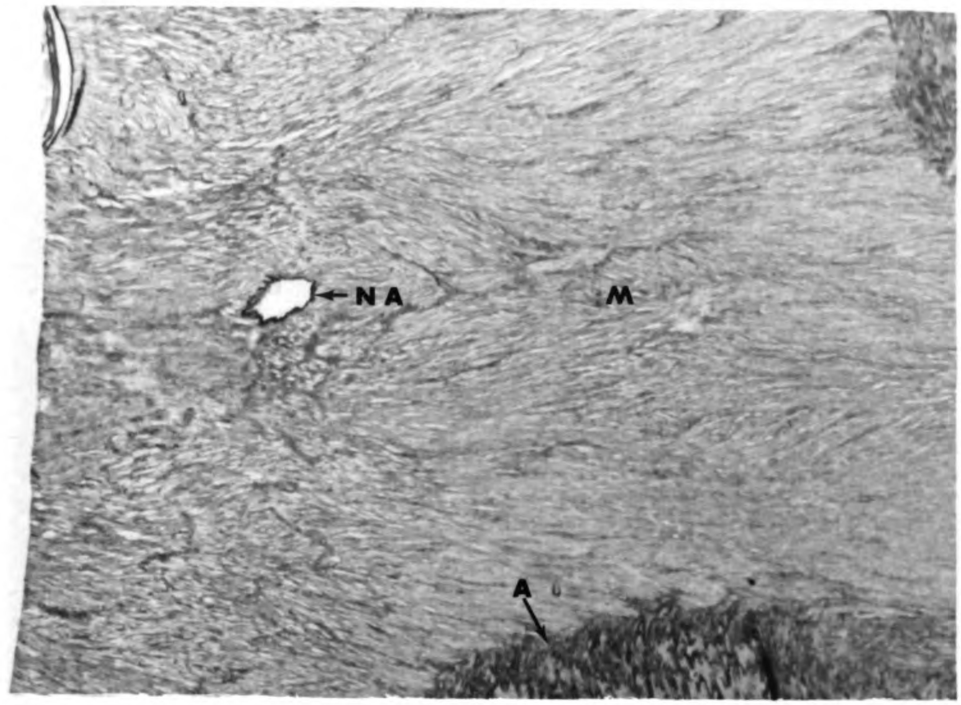


FIGURE 17

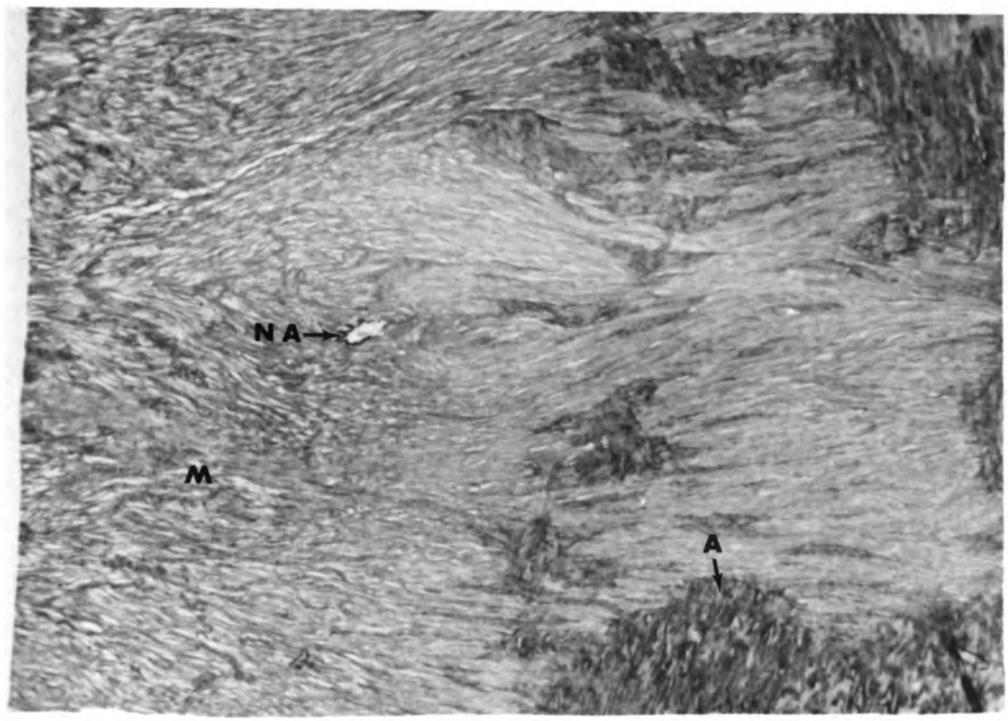


FIGURE 18

Cross section of femoral vein. Note the extent of media (M) and adventitia (A), and the distribution of the dark elastic fibers (EF).

X 170 Weigert's resorcin-fuchsin.

FIGURE 19

Cross section of the femoral vein. Note the abundance of perivascular vessels (PV).

X 33 Hematoxylin and Eosin.

FIGURE 18

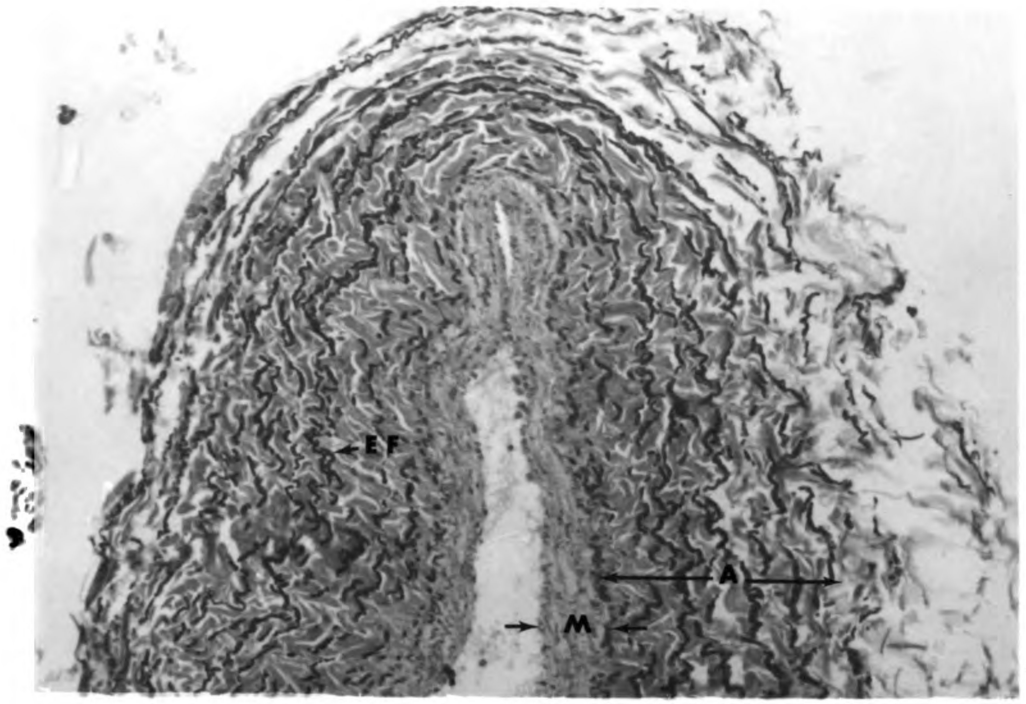


FIGURE 19

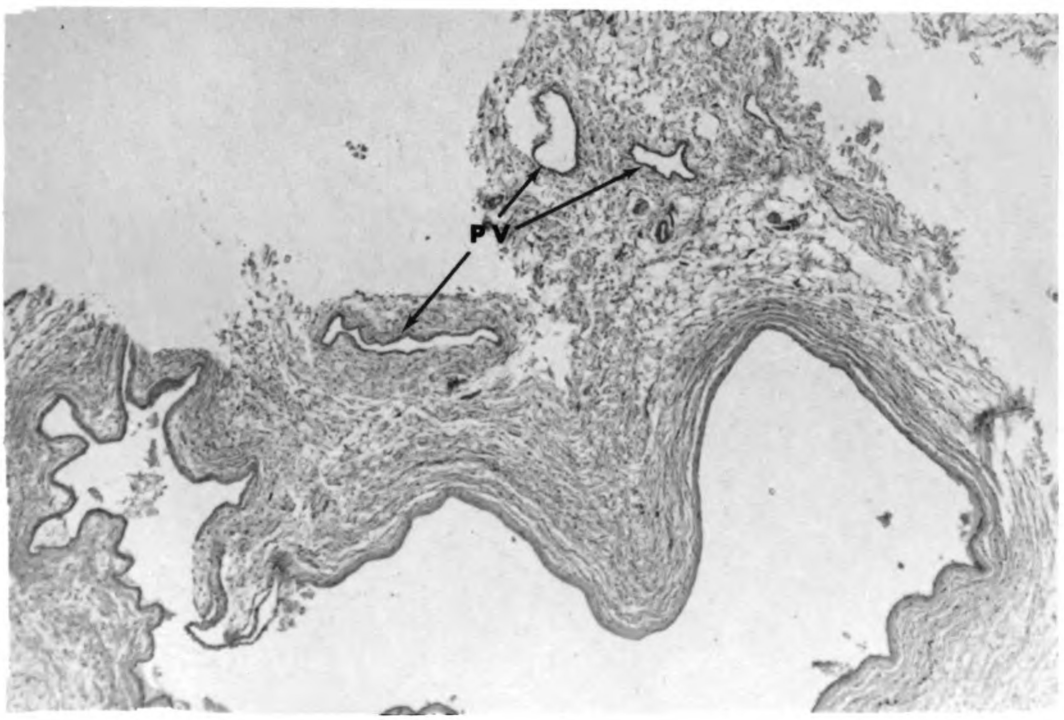


FIGURE 20

Cross section of femoral vein. Note the extent of media (M) and adventitia (A), and the nutrient vessel (NV) in the adventitia. (EF) - elastic fibers.

X 170 Weigert's resorcin-fuchsin.

FIGURE 21

Cross section of perfused femoral vein. Note the smooth muscle of the media (SM) and elongated nuclei of the endothelium (N). (M) - media. (A) - adventitia.

X 530 Hematoxylin and Eosin.

FIGURE 20

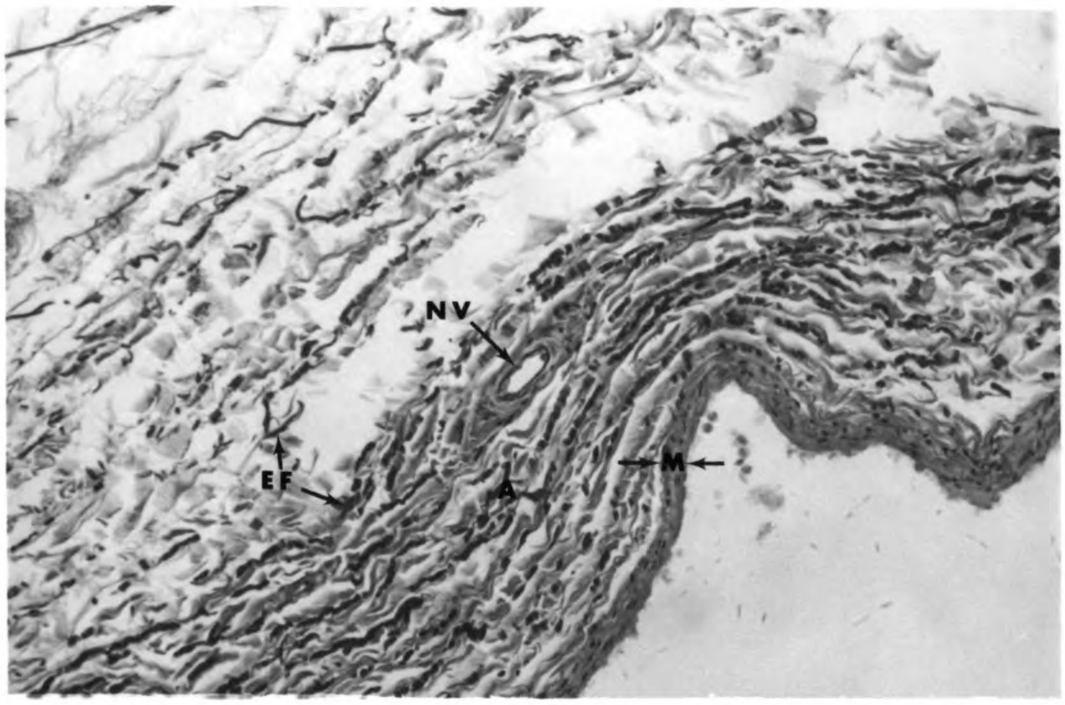


FIGURE 21

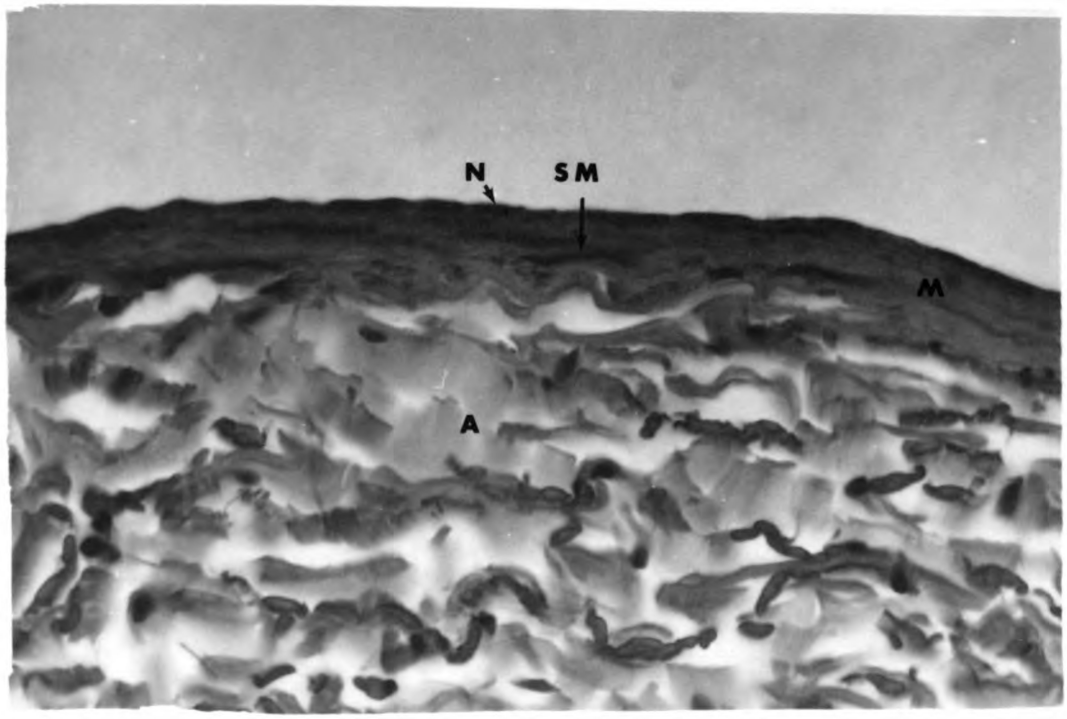


FIGURE 22

Valve in femoral vein (V). See Figure 24 for level of section.

X 33 Weigert's resorcin-fuchsin.

FIGURE 23

Valve in femoral vein (V). Note origin from intima (O).

X 170 Hematoxylin and Eosin.

FIGURE 22

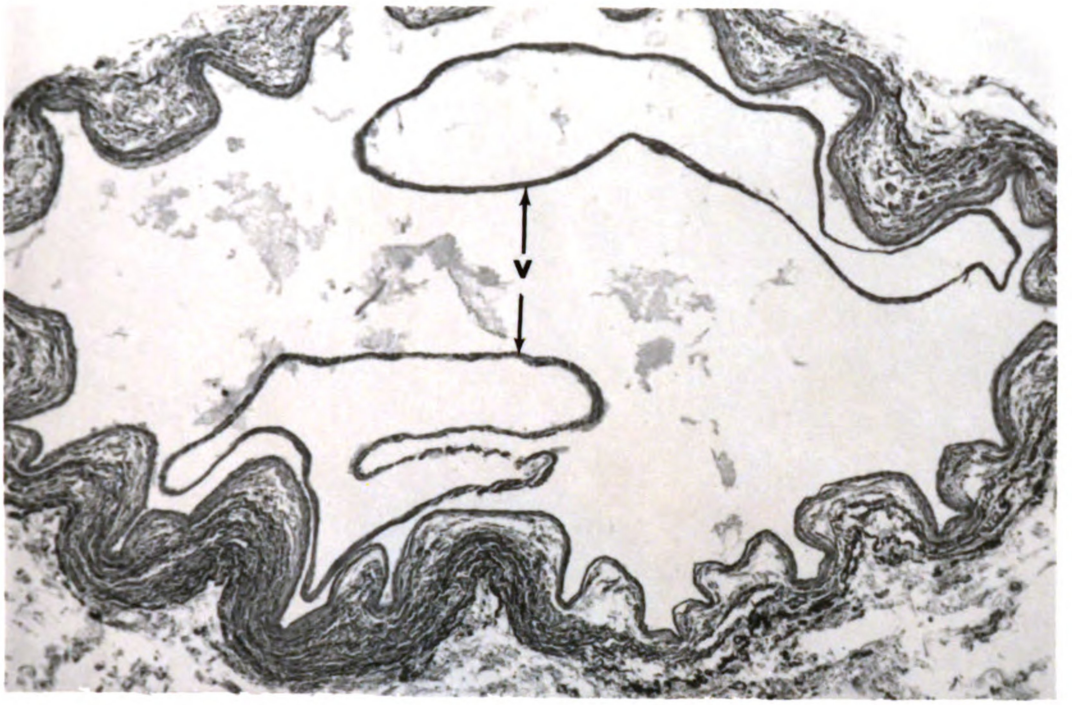


FIGURE 23

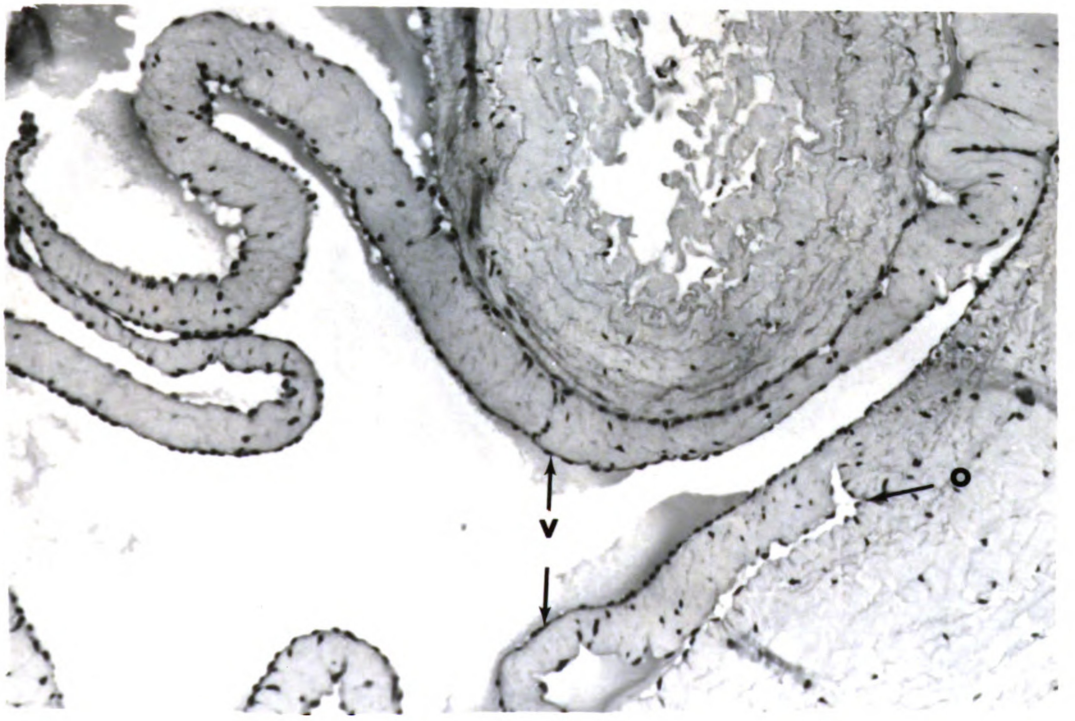


FIGURE 24

Level of section of Figure 22.

FIGURE 24

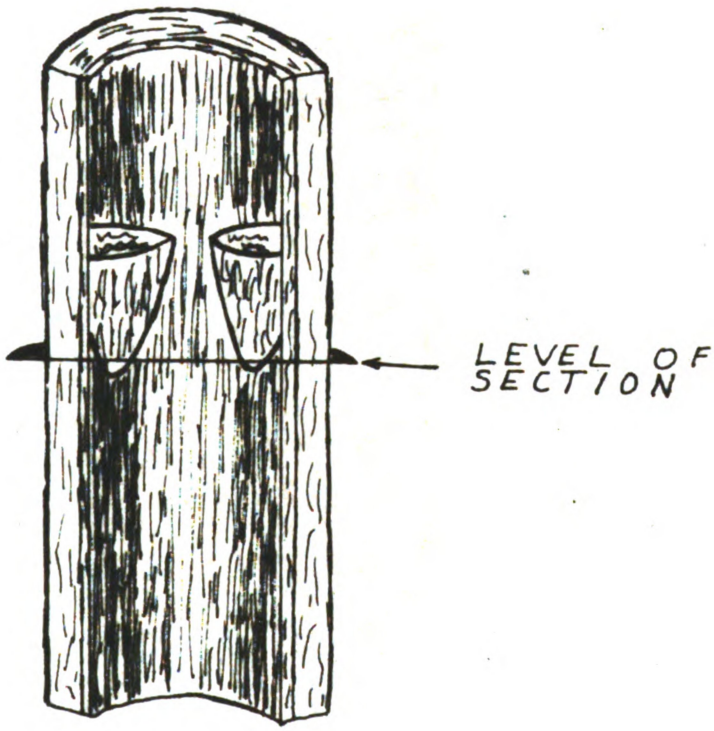


FIGURE 25

THICKNESS OF TUNICA MEDIA OF FEMORAL ARTERY

UPPER THIRD

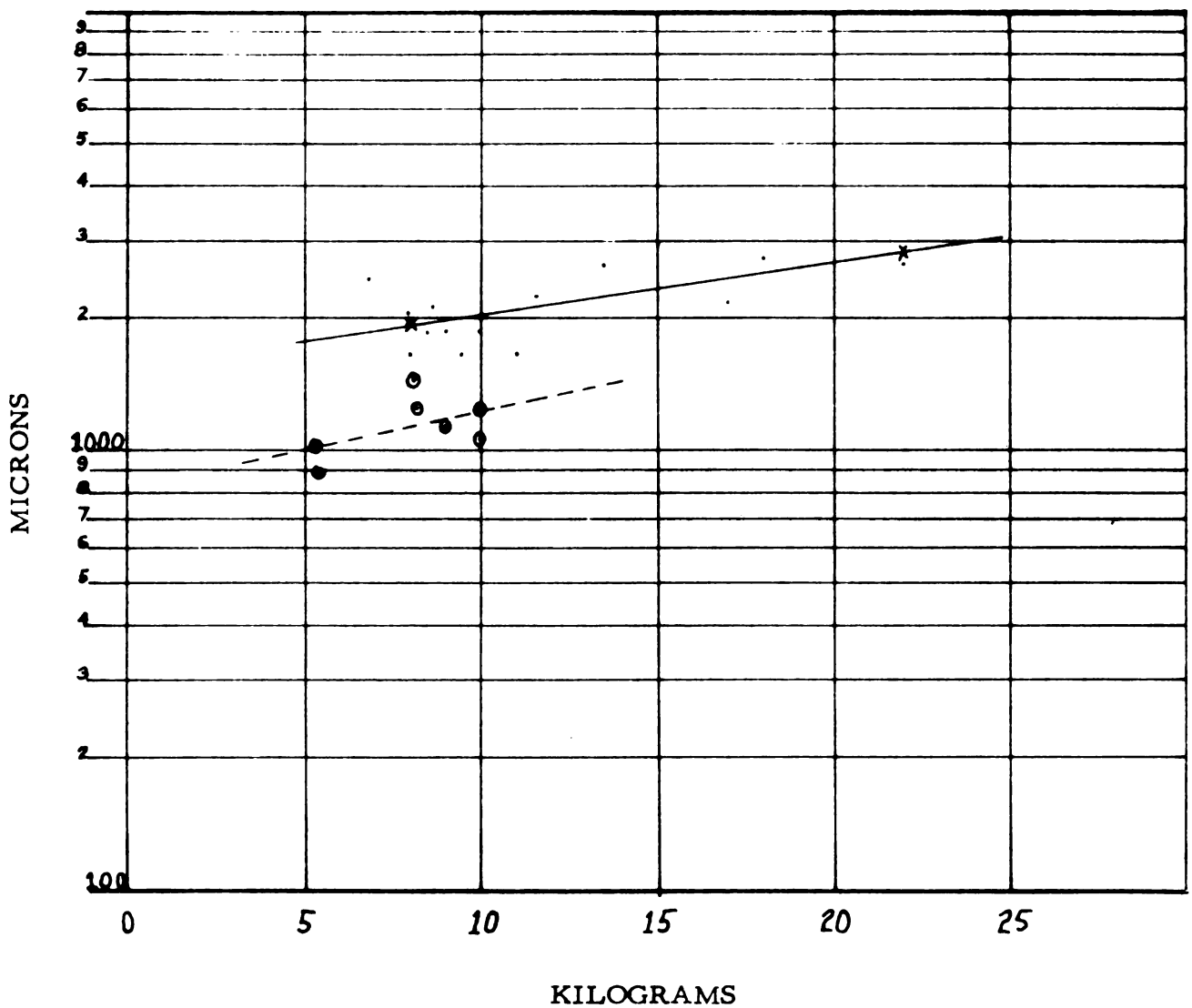
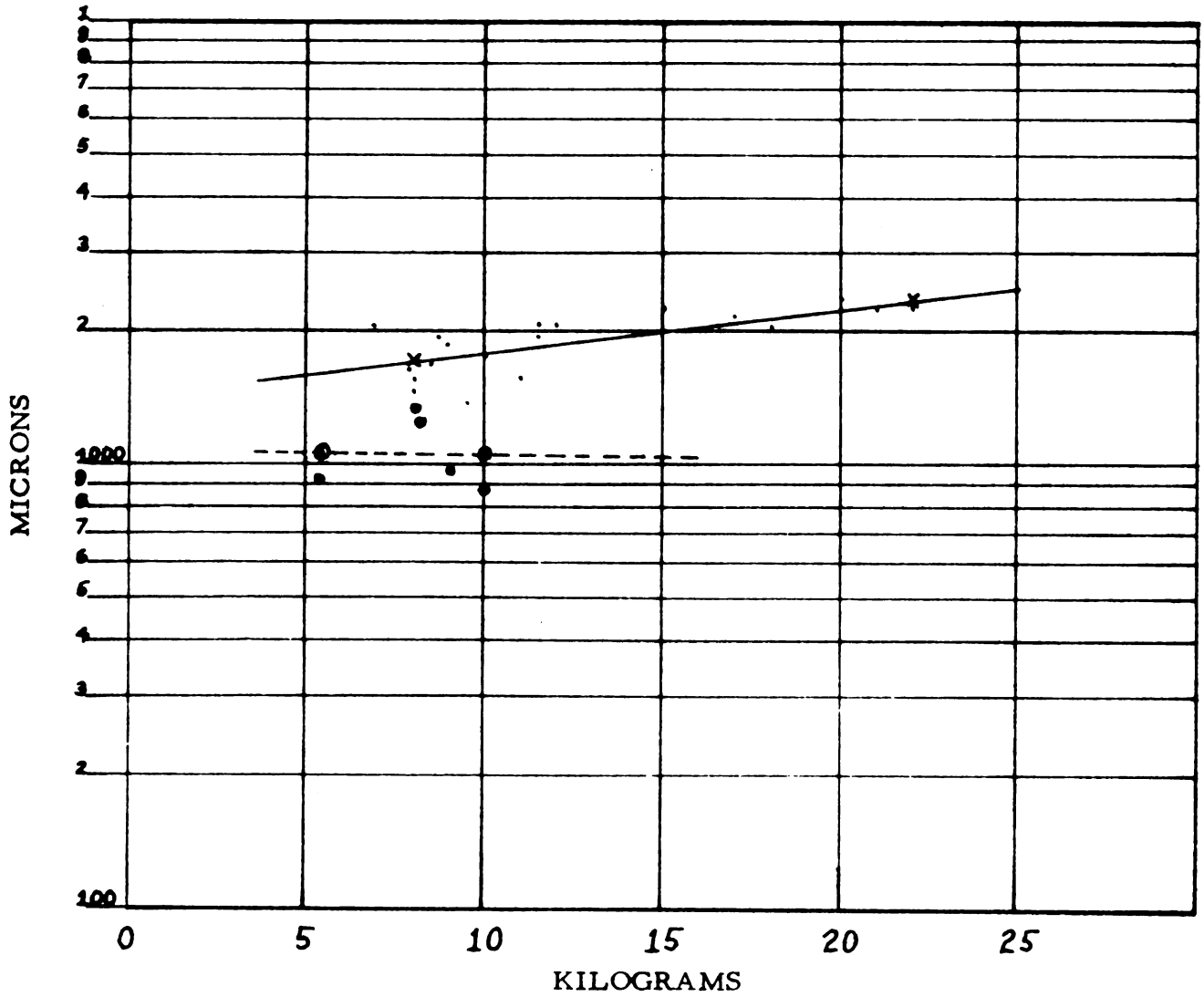


FIGURE 26

THICKNESS OF TUNICA MEDIA OF FEMORAL ARTERY

MIDDLE THIRD



·——RELAXED

●----PERFUSED

FIGURE 27

THICKNESS OF TUNICA MEDIA OF FEMORAL ARTERY

LOWER THIRD

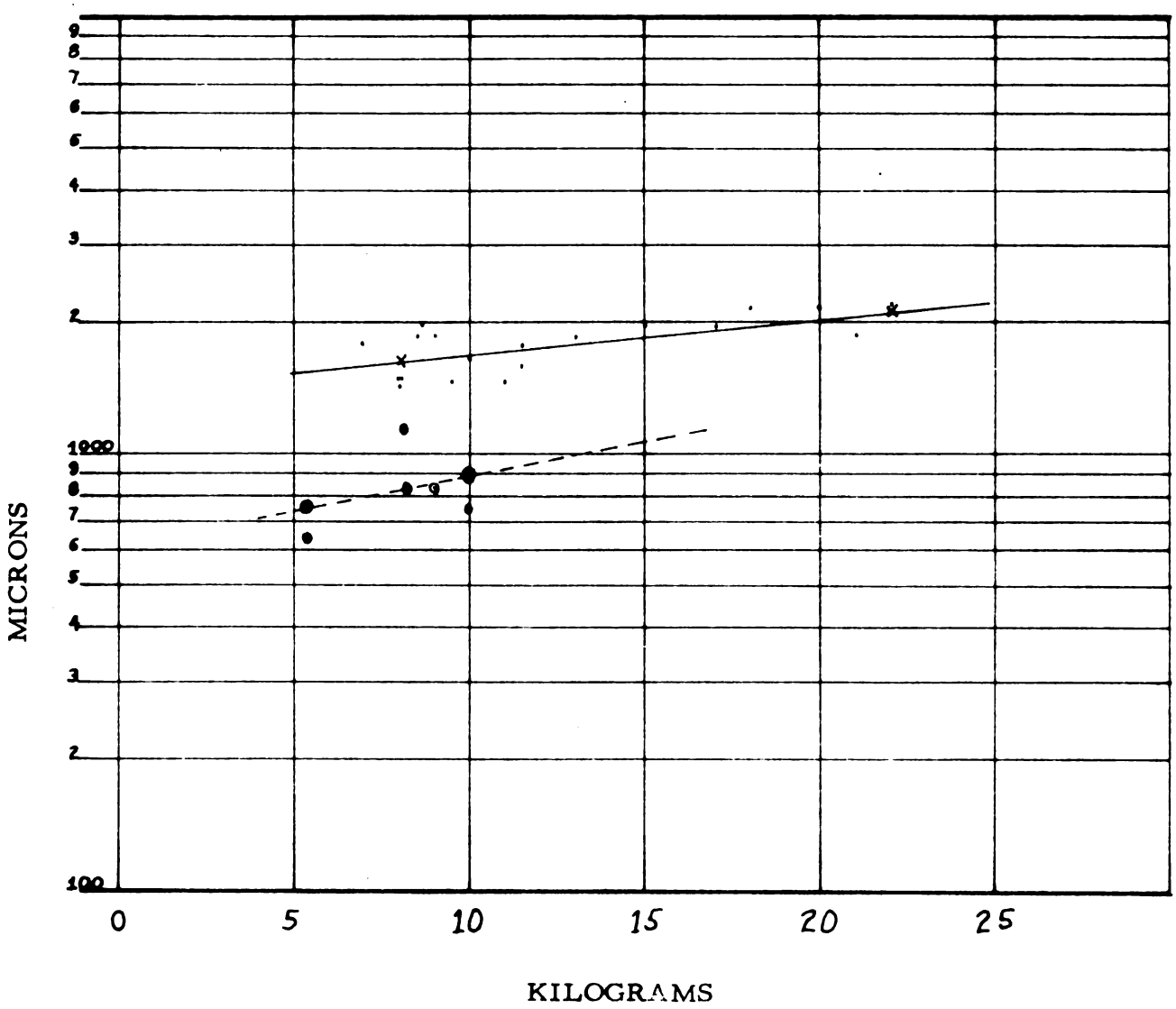


FIGURE 28

THICKNESS OF ADVENTITIA OF FEMORAL ARTERY

UPPER THIRD

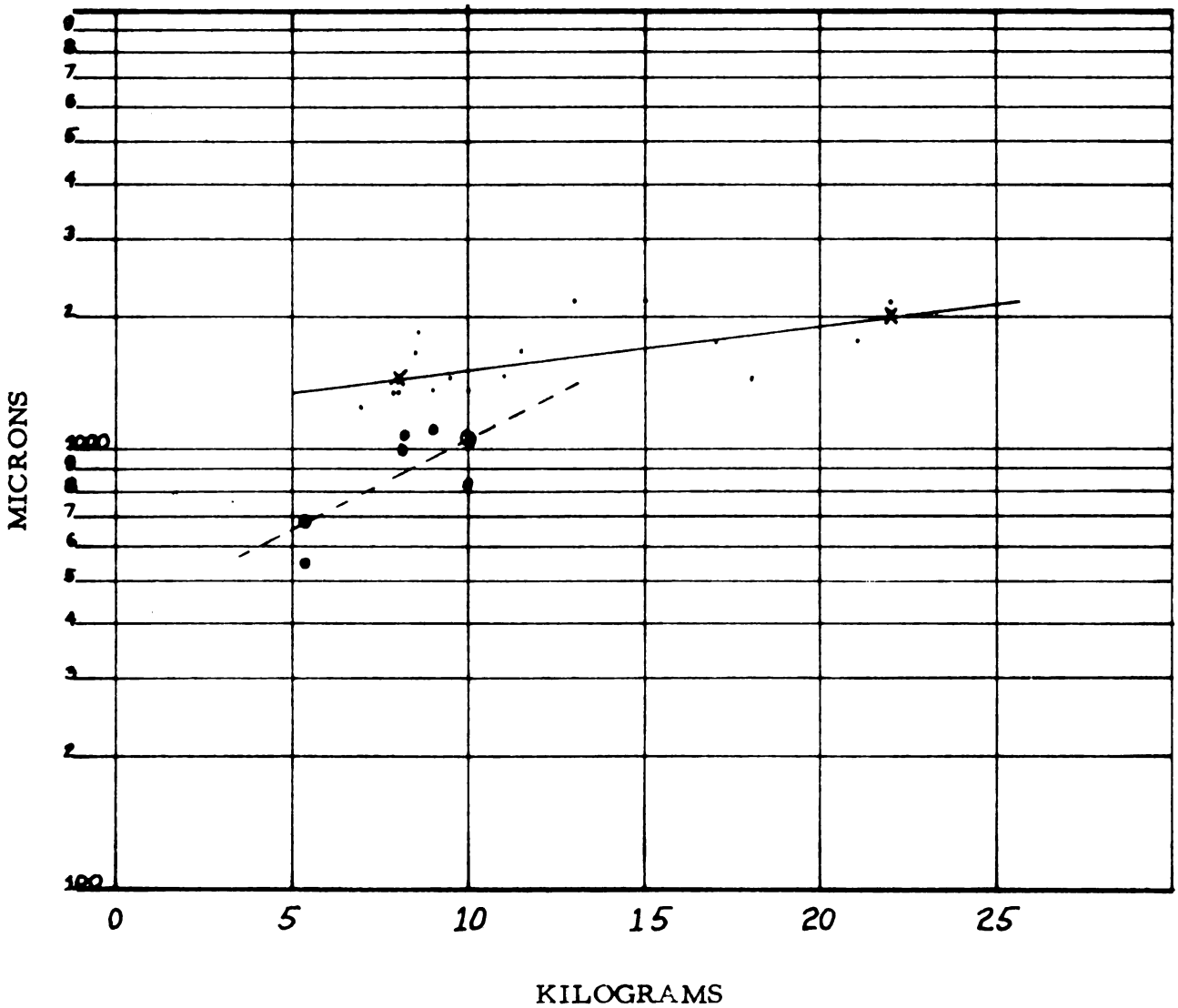


FIGURE 29

THICKNESS OF ADVENTITIA OF FEMORAL ARTERY

MIDDLE THIRD

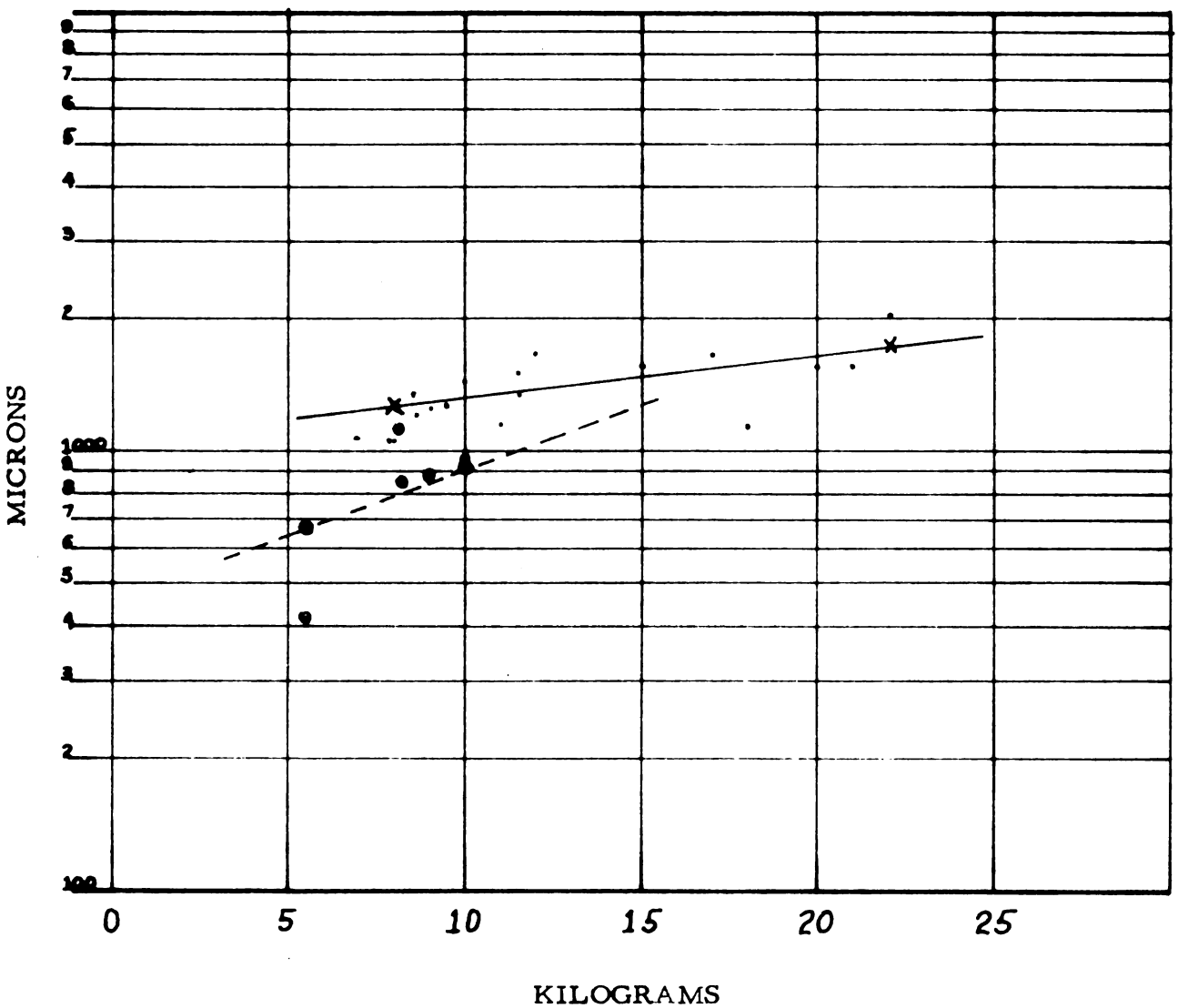


FIGURE 30

THICKNESS OF ADVENTITIA OF FEMORAL ARTERY

LOWER THIRD

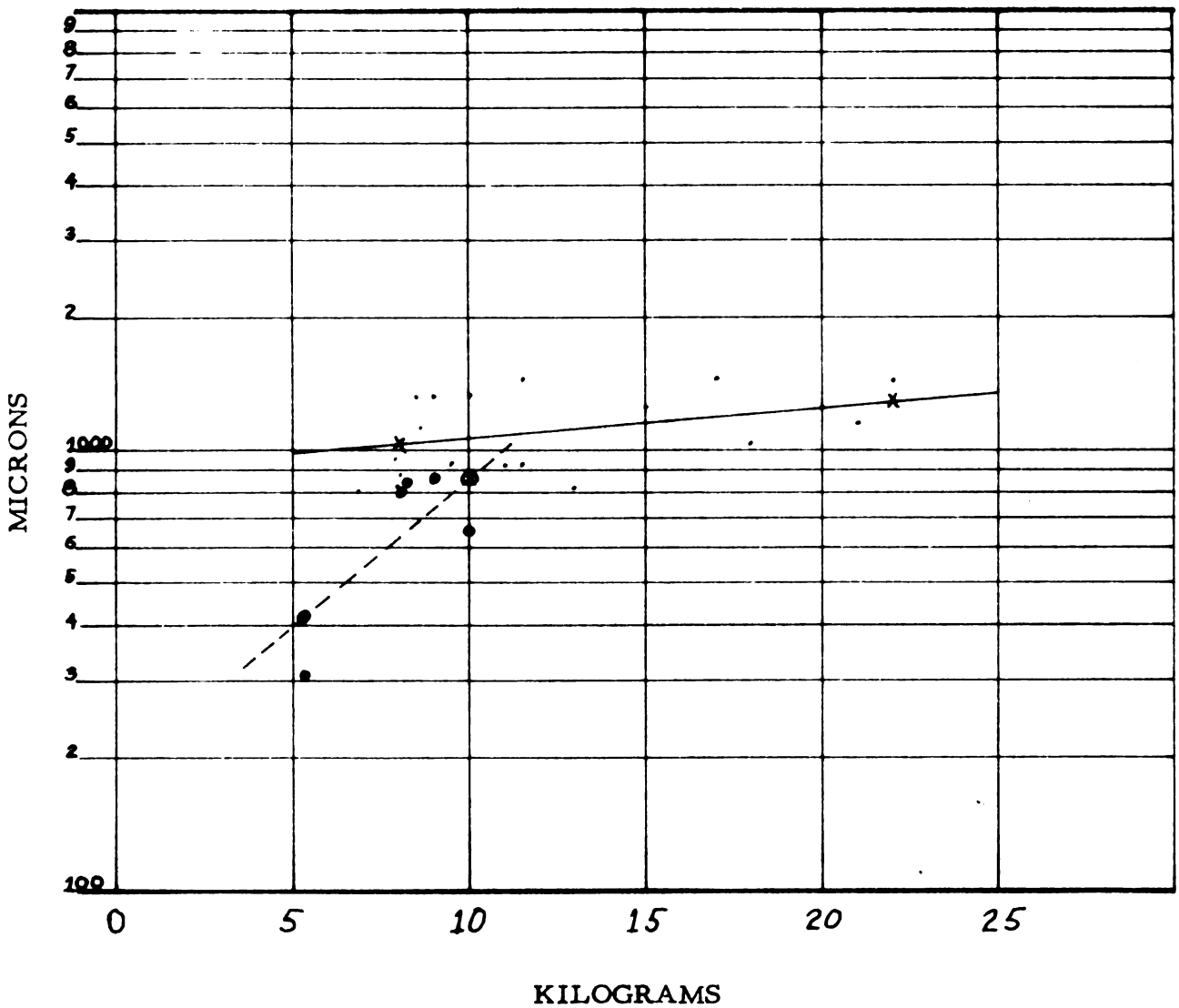


FIGURE 31

THICKNESS OF THE FEMORAL VEIN

UPPER THIRD

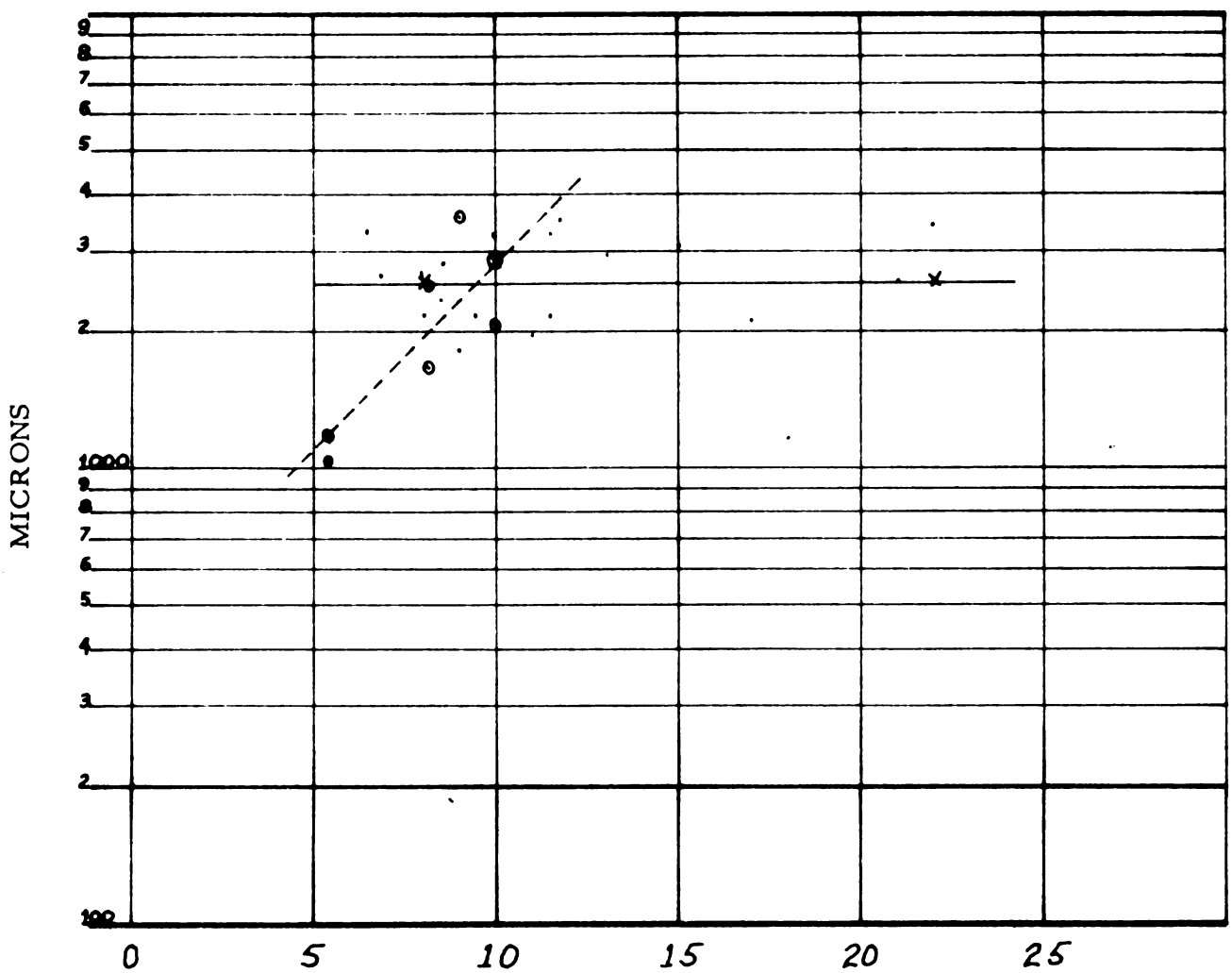


FIGURE 32

THICKNESS OF THE FEMORAL VEIN

MIDDLE THIRD

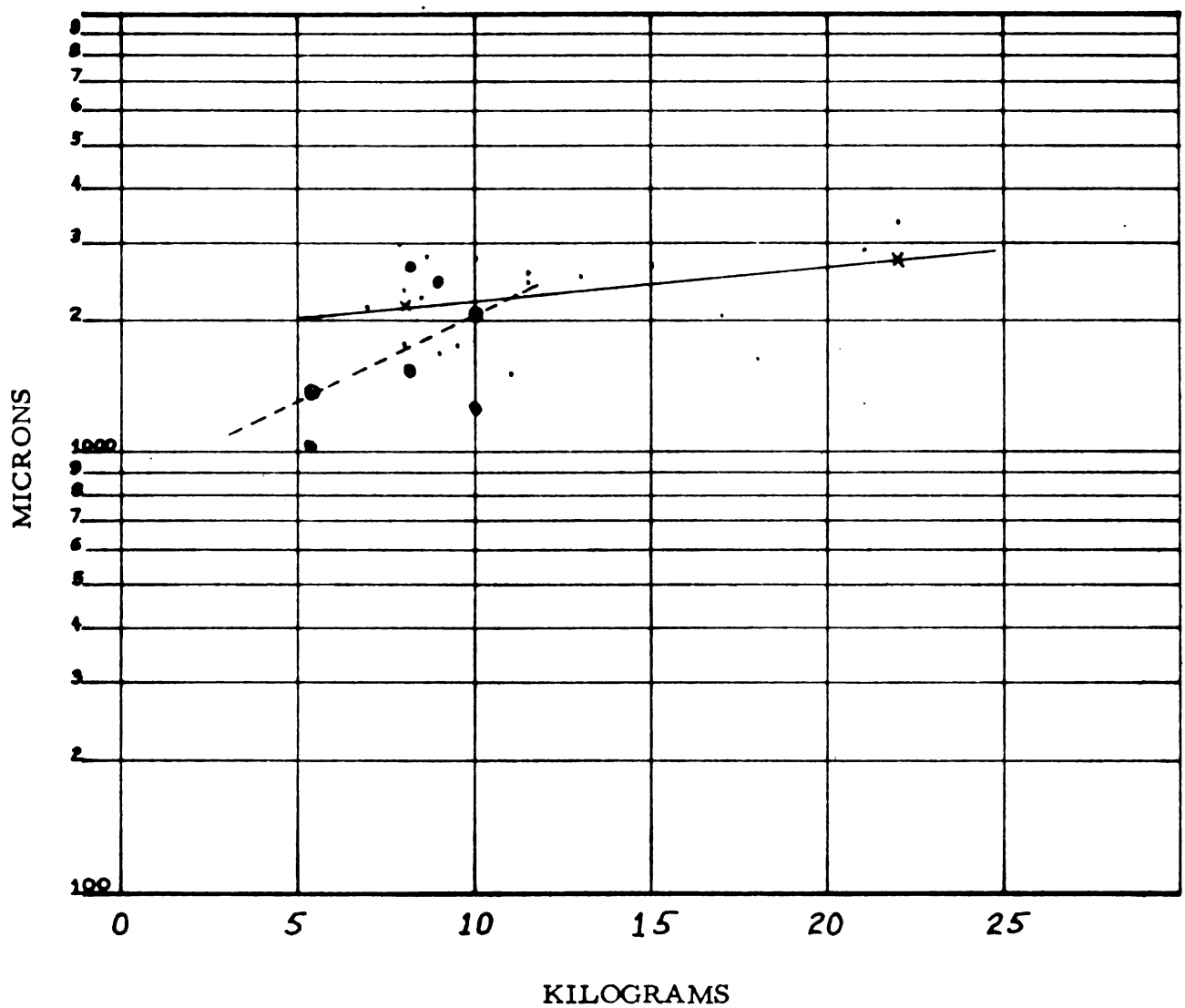


FIGURE 33

THICKNESS OF THE FEMORAL VEIN

LOWER THIRD

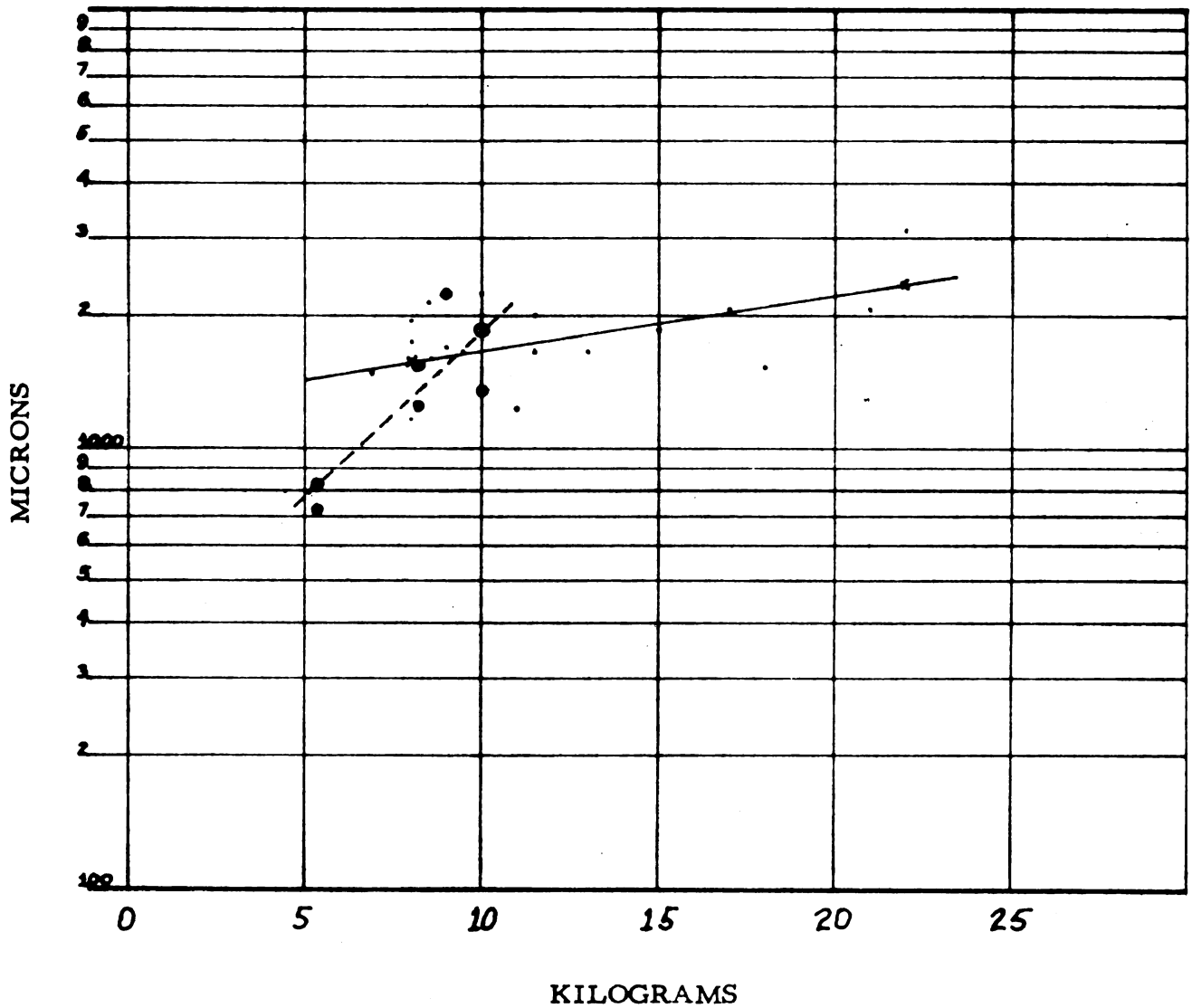
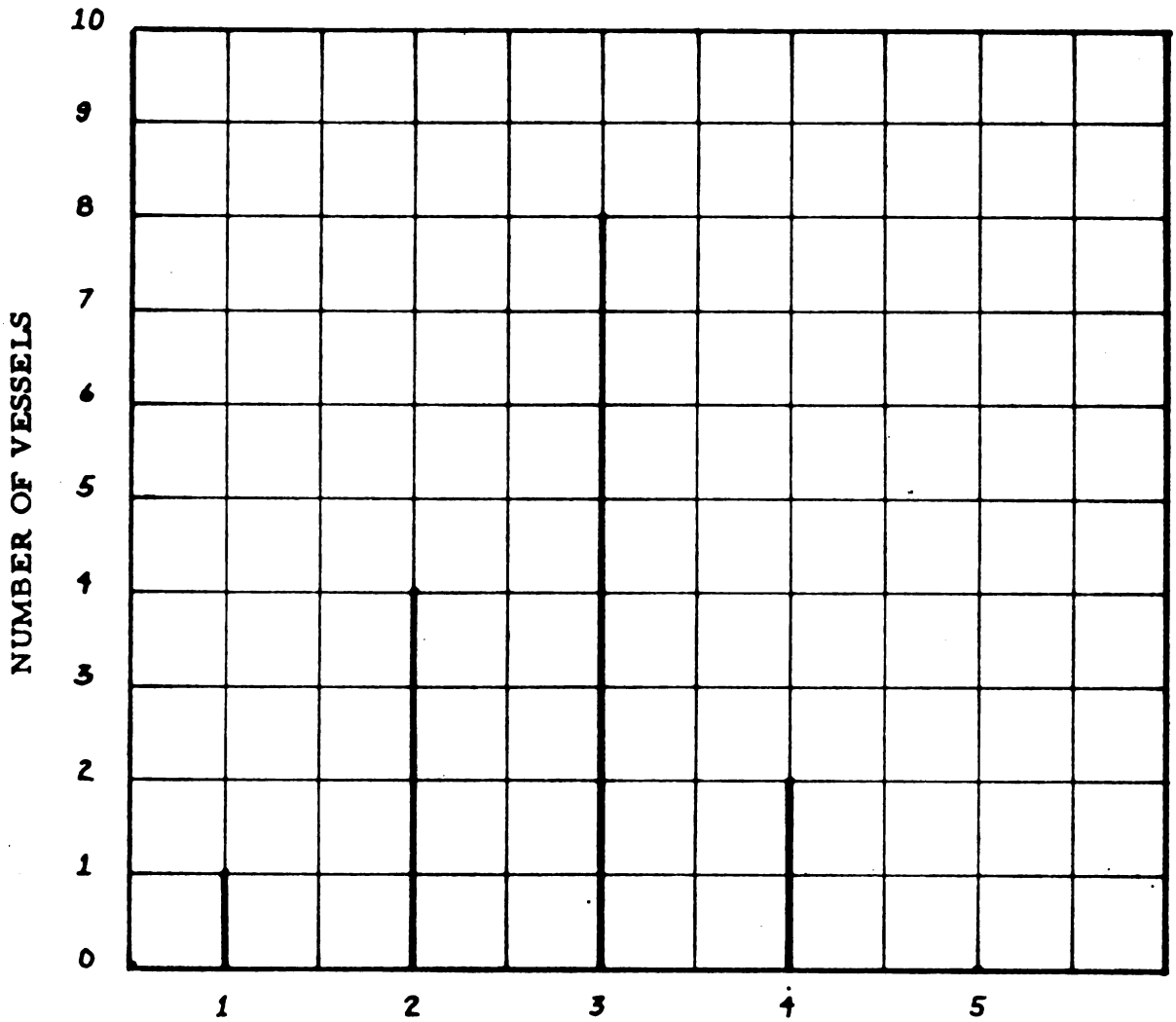


FIGURE 34

DISTRIBUTION OF VALVES IN THE FEMORAL VEIN



APPENDICES

APPENDIX 1

MEASUREMENTS OF ARTERIAL WALLS

Animal	Weight (kg.)	Sex	Media (μ)	Adventitia (μ)
2 *upper	13	Female	2665	2155
*middle	13	Female	2050	1640
*lower	13	Female	1847	820
2-1*upper	18	Female	2768	1435
*middle	18	Female	2050	1129
*lower	18	Female	2155	1026
3 upper	6.8	Male	2461	1231
middle	6.8	Male	2050	1078
lower	6.8	Male	1796	820
4 upper	11	Female	1640	1435
middle	11	Female	1539	1129
lower	11	Female	1435	923
5 upper	9	Female	1949	1385
middle	9	Female	1949	1231
lower	9	Female	1847	1332
6 upper	8.5	Female	1847	1640
middle	8.5	Female	1640	1332
lower	8.5	Female	1847	1332
7 upper	22	Male	2665	2155
middle	22	Male	2258	2050
lower	22	Male	2155	1435
8 upper	10	Female	1847	1332
middle	10	Female	1745	1435
lower	10	Female	1640	1332
9 middle	11.5	Male	1949	1332
lower	11.5	Male	1590	820
10 upper	9.5	Male	1640	1435
middle	9.5	Male	1332	1180
lower	9.5	Male	1435	924
11 *upper	11.5	Male	2258	1692
*middle	11.5	Male	2050	1500
*lower	11.5	Male	1798	1435
1 lower	20	Male	2155	1231
1 middle	20	Male	2360	1540
12 upper	15	Female	2990	2165
middle	15	Female	2265	1545
lower	15	Female	1959	1236
13 upper	21	Male	2680	1750
middle	21	Male	2265	1545
lower	21	Male	1853	1133

Animal		Weight (kg.)	Sex	Media (μ)	Adventitia (μ)
14	upper	17	Male	2165	1750
	middle	17	Male	2165	1649
	lower	17	Male	1959	1441
15	upper	8	Male	1649	1340
	middle	8	Male	1545	1030
	lower	8	Male	1400	876
16	upper	8	Male	1959	1441
	middle	8	Male	1441	1236
	lower	8	Male	1441	825
17	upper	7.9	Female	2060	1340
	middle	7.9	Female	1649	1030
	lower	7.9	Female	1441	978
18	upper	8.6	Female	2125	1855
	middle	8.6	Female	1910	1700
	lower	8.6	Female	1959	1133

*Fixed with formalin.

AVERAGE WEIGHT 12.44 kg.

AVERAGE THICKNESS (μ)	Media	Adventitia
Upper	2198	1619.88
Middle	1906	1385
Lower	1774	1108.15

APPENDIX 2

MEASUREMENTS OF VEIN WALLS

Animal	Weight (kg.)	Sex	Thickness (μ)
2 *upper	13	Female	2968.5
*middle	13	Female	2505
*lower	13	Female	1641.5
2-1*upper	18	Female	1156
*middle	18	Female	1621.5
*lower	18	Female	1500
3 upper	6.8	Male	2660
middle	6.8	Male	2127
lower	6.8	Male	1487.5
4 upper	11	Female	1959
middle	11	Female	1500
lower	11	Female	1212
5 upper	9	Female	1800
middle	9	Female	1700
lower	9	Female	1700
6 upper	8.5	Female	2364.5
middle	8.5	Female	2264.5
lower	8.5	Female	2055
7 upper	22	Male	3400
middle	22	Male	3390
lower	22	Male	3080
8 upper	10	Female	3300
middle	10	Female	2770
lower	10	Female	2258
9 upper	11.5	Male	2155
middle	11.5	Male	2461
lower	11.5	Male	1640
10 upper	9.5	Male	2155
middle	9.5	Male	1745
lower	9.5	Male	1640
11 *upper	11.5	Male	3280
*middle	11.5	Male	2538
*lower	11.5	Male	2000
12 upper	15	Female	3090
middle	15	Female	2680
lower	15	Female	1855
13 upper	21	Male	2575
middle	21	Male	2895
lower	21	Male	2060

Animal		Weight (kg.)	Sex	Thickness (u)
14	upper	17	Male	2100
	middle	17	Male	2060
	lower	17	Male	2060
15	upper	8	Male	2163
	middle	8	Male	1750
	lower	8	Male	1750
16	upper	8	Male	3295
	middle	8	Male	2370
	lower	8	Male	1959
17	upper	7.9	Female	2600
	middle	7.9	Female	3000
	lower	7.9	Female	1130
18	upper	8.6	Female	2800
	middle	8.6	Female	2835
	lower	8.6	Female	1599

*Fixed with formalin.

AVERAGE WEIGHT 12.02 kg.

AVERAGE THICKNESS (u)

Upper	2506.85
Middle	2403.36
Lower	1920.86

APPENDIX 3

ANIMALS USED AND MEASUREMENTS

OF THE WALLS OF PERFUSED ARTERIES

Animal		Weight (kg.)	Sex	Media (μ)	Adventitia (μ)
19	upper	9	Female	1133	1100
	middle	9	Female	979	875
	lower	9	Female	824	875
20	upper	8.2	Female	1236	1000
	middle	8.2	Female	1340	1100
	lower	8.2	Female	824	800
21	upper	10	Male	1030	824
	middle	10	Male	875	926
	lower	10	Male	762	669
22	upper	8.2	Female	1441	1082
	middle	8.2	Female	1236	824
	lower	8.2	Female	1133	824
23	upper	5.4	Female	875	567
	middle	5.4	Female	927	412
	lower	5.4	Female	646	309

AVERAGE WEIGHT 8.16 kg.

AVERAGE THICKNESS (μ)	Media	Adventitia
Upper	1143	914.6
Middle	1071.4	827.4
Lower	837	695.4

APPENDIX 4

ANIMALS USED AND MEASUREMENTS OF THE WALLS OF PERFUSED VEINS

Animal		Weight (kg.)	Sex	Thickness (μ)
19	upper	9	Female	3605
19	middle	9	Female	2470
19	lower	9	Female	2215
20	upper	8.2	Female	2575
	middle	8.2	Female	2680
	lower	8.2	Female	1545
21	upper	10	Male	2060
	middle	10	Male	1278
	lower	10	Male	1390
22	upper	8.2	Female	1649
	middle	8.2	Female	1546
	lower	8.2	Female	1236
23	upper	5.4	Female	1030
	middle	5.4	Female	1030
	lower	5.4	Female	721

AVERAGE WEIGHT 8.16 kg.

AVERAGE THICKNESS (μ)

Upper	2183.8
Middle	1800.8
Lower	1421.4

APPENDIX 5

ANIMALS USED AND NUMBER OF VALVES IN THE VEINS

Animal	Weight (kg.)	Sex	Number of Valves
1	11.5	Male	4
2	9.5	Male	3
3	8.5	Male	4
4	15	Female	3
5	22	Female	2
6	22	Female	3
7	22	Female	3
8	22	Female	2
9	18	Male	2
10	18	Male	2
11	15	Female	3
12	15	Female	1
13	5.5	Female	3
14	8.2	Male	3
15	8.2	Male	3