

AN INVESTIGATION OF THE BLOOD SUPPLY TO THE LONG
BONES IN THE JIG WITH PARTICULAR REFERENCE TO THE
ARTERIAL SUPPLY OF THE EPIPHYSIS AND THE EPIPHYSEAL
PLATE.



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An Investigation of the Blood Supply to the Long
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Supply of the Epiphysis and the Epiphyseal Plate.

by

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During the Second World War there were approximately 1,000,000 pigs being kept in England and Wales, but by 1964 this number had increased to 5,800,000 (Noble, 1964). This increase in numbers has been accompanied by a reduction in the number of pig rearing establishments; the cottager's sty has been replaced by the automated pig-house.

Although they benefit both the industry and consumer, mass production methods of pig-keeping bring their own special problems. Due to steadily rising costs, profit margins are small or non-existent unless animals put on weight efficiently and quickly. Hence morbidity is almost as important as mortality, because diseased animals have a higher food conversion ratio and take longer to reach slaughter weight. The consequent increase in the cost of feeding, labour and housing is economically unacceptable.

One important disabling condition which has been accentuated by intensive methods of pig rearing, is leg weakness. It is common and present in various forms which cause discomfort, pain and lameness.

Post mortem examination of pigs affected by leg weakness shows pathological changes on the articular surfaces of bones and lesions associated with the epiphyseal plate. (Hupka, 1959; Walker, 1964; Fell, 1966.) The articular surfaces are pitted and ulcerated, while in the epiphyseal plate, necrosis of cartilage precedes changes in the metaphyseal tissue adjacent to the epiphysis. One possible/

possible cause of these defects is obviously a deficient circulation to the extremities of the long bones.

Little study has been made of the anatomy of arteries supplying the extremities of the long bones in domestic animals. Fleming (1891), Ellenberger and Baum (1943) and Grossman (1961) deal with the main nutrient artery within the shaft of the bone and with the periosteal blood vessels, and say little about or ignore the metaphyseal-epiphyseal blood supply. Their accounts define the principal nutrient arteries and those terminal branches which anastomose with a periosteal network. They consider that any arteries which enter the bone at the extremities play only a supporting role.

Trueta and Cavadias (1964) show that after occlusion of the main nutrient artery in the radius of the rabbit, the bone is nourished by periosteal vessels in the young animal, and by the metaphyseal-epiphyseal vessels in the adult. In the young animal, they show that the metaphyseal vessels cannot maintain the bone marrow and inner layers of the cortex of the shaft; however, after fusion of the growth cartilage and union of the metaphyseal and epiphyseal arteries, the whole bone can be maintained by the metaphyseal-epiphyseal vessels.

The blood supply to a long bone may be conveniently divided into the extrinsic vessels carrying the blood to the bone, and the intrinsic vessels which distribute the blood within its substance.

Anatomical/

Anatomical information on the extrinsic and intrinsic blood supplies to the extremities of the long bones in the pig is negligible. Fleming (1891), Ellenberger and Baum (1891) (1943), Bradley (1920) and Grossman (1961) rarely mention the vessels in domestic animals, and none even refer to those in the pig. This deficiency, and the possible importance of these vessels in relation to leg weakness, prompted the present anatomical study of the normal vascular patterns in the extremities of the long bones of the pig.

As an example of an extrinsic blood supply, the distribution of the genicular arteries in the pig was chosen for investigation, particular attention being paid to the epiphyseal blood supplies of the bones forming the stifle joint. The most detailed descriptions of the genicular arteries are given by Keen (1949), Davies and Davies (1962) and Walls (1964) in the human, and by Greene (1955) in the rat. The terminology used in this thesis is based on these descriptions.

The intrinsic vessels in the epiphyseal and articular cartilages are demonstrated by a variety of techniques, and for this part of the study the distal ends of the radius, ulna, femur and tibia, and the proximal ends of the humerus and tibia of the pig are used.

MATERIAL AND METHODS

Material was obtained from Large White and Large White-Landrace cross pigs of ages from birth to about seven months old. The correct age and breed were easily ascertained in the younger animals which were received alive and then destroyed by intravenous thiopentone sodium injection. The limbs from the older pigs were acquired fresh from the abattoir; information about these specimens was limited.

The techniques carried out were:-

- (a) the dissection of six hind limbs intra-arterially injected with latex;
- (b) the preparation of angiograms from eight hind limbs intra-arterially injected with radio-opaque emulsion;
- (c) the clearing, by means of the Spalteholtz method, of thick sections of the extremities of bones from eight fore limbs and twelve hind limbs intra-arterially injected with radio-opaque emulsion; and
- (d) the preparation of histological sections from the extremities of various limb bones from pigs of different ages.

2.1 Injection Techniques

Specimens were injected using the apparatus shown (Fig. 1); in all cases the injection pressure used was 200 mm. Hg. In young pigs the caudal vena cava was severed and perfusion was carried/

carried out through the abdominal aorta. Isolated hind limbs from the larger pigs were perfused through the external iliac artery.

As soon as possible after death, warm isotonic saline was run through the vascular bed of each cadaver or isolated limb, which was then perfused with one of the various injection media. 0.9% saline used to perfuse several of the limbs had 0.05% sodium nitrite added as a vasodilator; this seemed to improve penetration although its efficiency was not checked by controlled experiments. After injection the limbs were fixed and stored in 10% formalin solution.

Six hind limbs were injected with rubber latex (T. Gerrard and Co., Ltd., London). These were excellent for dissecting and examining the courses of the larger arteries. They were of no use for the examination of vessels less than approximately 0.5 mm. in diameter since the latex hardened prematurely and blocked the smaller branches. Latex injected specimens were used in the study of the topography of the extrinsic vessels.

Eight hind limbs were injected with radio-opaque emulsion before making antero-posterior and latero-medial angiograms. Various preparations of barium sulphate were tried as the injection fluid, and it was found that 50% to 75% aqueous solutions of "Micropaque" (Damancy and Co., Ltd.,) or "Fotogel" (Evans Medical Supplies, Ltd.,) gave the most successful penetration. These angiograms were used to confirm the results of the dissection of the latex injected limbs. The limiting factor was the poor definition on the X-ray plate caused by the thickness of the specimens/

specimens being used, and the relatively small difference in radio-opacity between the injection media and the bone. It was found, however, that if the extremities of the bones being examined were fixed, dissected out, cut into thick sections, decalcified, and cleared by the Spalteholtz method, they were excellent for optical examination of the vessels within the substance of the bone. This technique of injecting and clearing bones was therefore used upon an additional number of fore and hind limbs.

2.2 Decalcification Methods

After fixation in 10% formalin for at least 48 hours, the extremities of the radio-opaque injected bones were dissected out and cut by hand-saw into sections 5 - 10 mm. thick which were then decalcified. In every case a chemical test was applied to a sample of the decalcifying fluid used, in order to assess the degree of decalcification of the material.

The test was to add dropwise concentrated ammonium hydroxide solution (S.G. 0.880) to 5 ml. of the decalcifying fluid until it was neutralised. 0.5 ml. of a saturated solution of ammonium oxalate was then added. The absence of turbidity after five minutes was taken as an indication of the absence of calcium ions in the fluid and hence complete decalcification of the bone.

Several different methods of decalcification were employed; they were as follows:-

2.21/

2.21 Decalcification by formol-nitric acid solution

Formalin (40% formaldehyde)	5 ml.
Nitric Acid (S.G. 1.41)	10 ml.
Distilled Water	85 ml.

This fluid decalcified the bone sections in four to seven days, but the oxidising effect of the nitric acid caused a very dark brown discoloration of the tissue by nitrous acid compounds which rendered the bone sections opaque and of little use for the examination of the courses of the finer vessels in cleared specimens. Stabilisation of the formol-nitric acid solution with 0.1% urea produced no noticeable improvement in results, although by changing the fluid daily more satisfactory specimens were obtained.

2.22 Decalcification by formol-formic acid solution

Formalin (40% formaldehyde)	9 ml.
Formic Acid (S.G. 1.2)	10 ml.
Sodium Chloride	0.8 gm.
Tap Water	81 ml.

The bone sections were decalcified by three or four changes of this solution for ten to twenty days; there were no artefacts to interfere with the examination of the injected arteries and results were excellent.

2.23 Decalcification by formol-formic acid solution in the presence of an ion exchange resin.

Formalin/

Formalin (40% formaldehyde)	10 ml.
Formic Acid (S.G. 1.2)	15 ml.
Distilled Water	75 ml.

The ion exchange resin used was "Zeo-Karb 225" (Hydrogen form) cation exchange resin manufactured by the Permutit Company Limited.

The resin was layered over the bottom of a glass dish and covered by the decalcifying fluid. The sections of bone were placed on a disc of filter paper over the resin as shown in the diagram (Fig. 2). The proportion of resin used was about one-tenth the volume of the fluid and twice the volume of the material to be decalcified.

The results obtained by this method were as good as those obtained using no ion exchange resin, and complete decalcification was achieved in only 48 to 72 hours using one batch of fluid and resin.

The only disadvantage in using the ion exchange resin was that the chemical test could not be done directly on the decalcifying fluid; but if the specimens were placed in a small volume of fresh fluid for two to three hours without the ion exchange resin being present, and the chemical test performed upon this fluid, a positive result could be obtained.

The large amount of fat present in the pig bones slowed down the decalcification processes. In several specimens the fat was removed by immersing the sections in three to four changes of acetone/

acetone for about 48 hours before decalcifying. This increased the rate of the actual decalcification process, but the whole procedure took longer since the sections had to be hydrated in descending grades of ethanol before they could be decalcified. It was therefore decided to discontinue this process.

When the bone sections had been decalcified they were washed in running tap water overnight to remove residual decalcifying fluid, then dehydrated by passing them through ascending grades of ethanol and cleared in benzene before finally being stored in methyl salicylate.

The process adopted was:-

70% Ethanol		48 hours
96% "		48 hours
Absolute Ethanol I		24 hours
" "	II	24 hours
" "	III	24 hours
Benzene I		24 hours
"	II	24 hours

Methyl salicylate as storage fluid.

2.3 Histological Techniques

The histological work done for this study was directed towards the distribution of blood vessels within the epiphyses, and in particular, upon finding any connection between epiphyseal and metaphyseal blood vessels within the epiphyseal plate. Perfect fixation/

fixation was not required, since only the courses of blood vessels were being examined and not the detailed histology and cytology of the tissue. Although tissues were procured and fixed in as fresh a state as possible, it was found that acceptable results were obtained from material which had been dead for two or three days.

Blocks were cut from the portions of bone being examined with a hack-saw and then fixed in 10% formalin/60% ethanol solution in a vacuum embedding bath for six to eighteen hours. This method of fixation was chosen for its rapidity. Some blocks were fixed in a 10% buffered formalin solution for forty-eight to seventy-two hours.

After fixation the blocks of tissue were washed for several hours in tap water, then decalcified in a formol-formic acid solution in the presence of an ion exchange resin. The decalcifying fluid used contained 10% formic acid, but otherwise the technique employed was similar to that previously described for the decalcification of the thick injected bone sections.

After decalcification the blocks of tissue were washed in tap water overnight, then dehydrated, cleared and embedded by the following procedure in an Elliot automatic tissue processor:-

70% Ethanol/

70% Ethanol	24 hours
96% "	2 hours
Absolute Ethanol I	2 hours
" " II	2 hours
Chloroform I	4 hours
" II	4 hours
Paraplast Wax I	2 hours
" " II	2 hours
" " III	2 hours

Impregnation of the blocks by the waxes was done in a vacuum embedder and "Paraplast" (Biological Research Inc., Bridgeton, Mo., U.S.A.) was found to give superior results to normal paraffin waxes in the preparation of the bone sections. It was found that cooling the "Paraplast" wax blocks with ice made section cutting easier, although the manufacturers state that this is not necessary.

Sections were cut six to ten micrometers thick and stained by the following methods:- Culling (1963)

1. Mayer's haematoxylin and eosin
2. Masson's trichrome stain
3. Toluidine blue
4. Periodic acid Schiff (de Tomasi)
5. Alcian blue

Some sections were not stained, but were mounted in glycerine and examined by the phase contrast microscope.

Serial/

Serial sections of bone were made in order to make a more detailed study of the vascular channels within the developing epiphysis and epiphyseal plate. The preparation of serial sections of bone presented two main difficulties:-

(a) The blocks contained both decalcified bone and cartilage; this caused folding and distortion of the sections since tissues of different degrees of hardness were passing over the knife blade at the same time.

(b) It was difficult to produce good ribbons of sections especially at predetermined section thicknesses because of folding and fragmentation.

In order to overcome these problems, bones from new-born pigs were used where possible, since the epiphyses were mainly cartilaginous and less trouble was experienced with folding of sections due to differing tissue densities. Also the cartilage from new-born piglets is softer than that of older animals.

Blocks of tissue from older pigs were softened by Lendrum's technique (Culling 1963) between the wash in water and dehydration. The treatment was to immerse the block in 4% aqueous solution of phenol for two to three days.

The difficulty in obtaining good ribbons for mounting on large slides was so great that the procedure finally adopted was to float out and mount each section individually.

Fragmentation of some of the sections was probably due to the presence/

presence of a large amount of blood and bone marrow in the block. This fragmentation was reduced considerably by the application of ice to the surface of the block between every two to three sections.

Great difficulty was also experienced in cutting sections at predetermined thicknesses. Sections cut too thinly fragmented; those cut too thick had small folds running through them. An optimum section thickness had therefore to be found for each block during trimming; this was generally in the region of 7 μ m.

Apart from the above, the method used in the preparation of serial sections was the same as that described previously for the other histological work; that is, fixation in 10% buffered formalin, decalcification in formol-formic acid in the presence of an ion exchange resin, and embedding in vacuo in "Paraplast".

Some of the serial sections were stained with Masson's trichrome, but later sections stained by alcian blue with chloratine fast red as counterstain (Lison, 1954) were found to be far superior in that the relationships between the blood vessels and adjacent tissues were better demonstrated.

Black and white or coloured photographs were made throughout the course of this investigation to serve as a permanent record of the results.

3.0/

3.0

OBSERVATIONS3.1 Extrinsic Vessels

Specimens injected with rubber latex show the arterial distribution to the bones and periosteum, but, due to the poor penetration of the latex, few injected vessels can be seen within the substance of bones examined.

The general picture presented by these preparations is of a large artery entering the shaft of the bone, the medullary or nutrient artery, with a secondary or accessory nutrient artery in some cases. The extremities are supplied by a large number of smaller arteries (Fig. 3).

Examination of the blood supply to the stifle region in the pig reveals that there are eight vessels which are distributed so that they justify the name of genicular arteries, the Superior Middle (SMG), Inferior Middle (IMG), Lateral Superior (LSG), Lateral Inferior (LIG), Medial Superior (MSG), Medial Inferior (MIG), Descending (DG), and Superficial Medial (SupMG). These vessels are constant, and in addition, ascending branches of the Posterior Femoral artery (PFA) and recurrent branches of the Anterior Tibial artery (RAT) play an important part in supplying the stifle region.

The course and distribution of the vessels which supply the stifle region will now be described. Their general pattern has been summarised diagrammatically in figures 4, 5 and 6 which are intended to clarify the following text. Photographs of actual specimens/

specimens illustrating many of the points are given as figures and the labelling code given below applies to all illustrations.

ALM - Anterior Lateral Meniscal
 AMM - Anterior Medial Meniscal
 AT - Anterior Tibial
 DG - Descending Genicular
 F - Femoral
 IMG - Inferior Middle Genicular
 LC - Lateral Circumflex Femoral
 LIG - Lateral Inferior Genicular
 LS - Lateral Sural
 LSG - Lateral Superior Genicular
 LVC - Lateral Vertical Channel
 MIG - Medial Inferior Genicular
 MS - Medial Sural
 MSG - Medial Superior Genicular
 MVC - Medial Vertical Channel
 P - Popliteal
 PFA - Ascending branches of the Posterior Femoral
 PFD - Descending branches of the Posterior Femoral
 PLM - Posterior Lateral Meniscal
 PMM - Posterior Medial Meniscal
 PT - Posterior Tibial
 RAT - Recurrent branches of the Anterior Tibial
 RT - Recurrent Tibial
 RTar/

RTar - Recurrent Tarsal

S - Saphenous

SMG - Superior Middle Genicular

SupMG - Superficial Medial Genicular

3.11 Superior Middle Genicular Artery (SMG)

This vessel arises from the Popliteal artery (P) at the level of the condyles of the femur and passes forwards piercing the posterior aspect of the fibrous portion of the joint capsule and runs between the anterior and posterior cruciate ligaments to enter the intercondyloid fossa of the femur (Figs. 4 and 5). It then continues over the non-articular fossa on the tibial spine to enter the fat pad deep to the patellar ligament, where it anastomoses with branches of the Superficial Medial Genicular artery (SupMG), recurrent branches of the Anterior Tibial artery (RAT) and branches of the Medial Inferior Genicular artery (MIG) (Figs. 6 and 8). Finally, it enters the proximal extremity of the tibia through a large foramen situated in the non-articular area behind the tibial tuberosity.

The branches given off along the course of the Superior Middle Genicular artery are:-

(a) Within the proximal portion of the intercondyloid fossa, a large branch which enters a foramen in the distal epiphysis of the femur.

(b) Throughout the course of the Superior Middle Genicular artery, small branches are distributed to the adjacent fascia, joint capsule, ligaments, and to small foramina in the intercondyloid fossa/

fossa of the femur and the non-articular fossa on the tibial spine. Some of these branches form anastomoses with branches of the Inferior Middle Genicular artery (IMG).

(c) On entering the fat pad deep to the patellar ligament, two large branches, one medial and one lateral, run proximally under the patellar ligament towards the patella. They supply the fat pad, the patellar ligament and the patella, and also form several important anastomoses with other arteries which supply the stifle joint. Keen (1949) named these vessels the Medial (MVC) and Lateral Vertical channels (LVC).

In some specimens (2/6) the Superior Middle Genicular artery enters the distal epiphysis of the femur directly, and does not run over the fossa on the tibial spine to reach the fat pad deep to the patellar ligament. In these cases the Medial Inferior Genicular artery forms the Medial and Lateral Vertical channels, and then by anastomosing with recurrent branches of the Anterior Tibial artery and branches of the Superficial Medial Genicular artery, it forms the large vessel which enters the foramen at the junction of the proximal epiphysis of the tibia with the epiphysis of the tibial tuberosity (Fig. 9).

3.12 Inferior Middle Genicular Artery (IMG)

The origin of this artery is from the Popliteal, distal to the origin of the Superior Middle Genicular artery (Figs. 4 and 5). It pierces the posterior aspect of the fibrous portion of the joint capsule/

capsule and runs lateral to, or through, the substance of the posterior cruciate ligament to enter a foramen in the posterior portion of the central fossa of the tibial spine under the posterior ligament of the medial meniscus (Fig. 5).

It supplies small branches to the joint capsule and adjacent ligaments, and some twigs which enter a number of small foramina in the fossa of the tibial spine. Anastomoses are formed by these branches with small branches from the Superior Middle Genicular artery and with arterial twigs arising directly from the Popliteal artery.

3.13 Lateral Superior Genicular Artery (LSG)

In the pig the Lateral Superior Genicular, Lateral Inferior Genicular (LIG) and Lateral Sural (LS) arteries generally arise from a common trunk which has its origin from the Popliteal artery at the level of the lateral epicondyle of the femur and runs laterally and distally over the epicondyle (Fig. 4). In one of the specimens the Lateral Sural artery arises directly from the Popliteal.

The Lateral Superior Genicular artery is the first branch of this common trunk. Its course is over the lateral epicondyle of the femur where it divides into a proximal branch and distal branches (Figs. 6 and 12).

The proximal branch runs in the lateral femoropatellar ligament to supply the patella, where it joins the Lateral Vertical channel (Fig. 6). This branch gives off arterial twigs which run into the vastus/

vastus lateralis muscle, where they anastomose with ascending branches of the Posterior Femoral artery; other twigs supply the periosteum and fascia over the postero-lateral aspect of the distal end of the shaft of the femur and terminate by entering the metaphysis of the femur in this region.

The distal branches of the Lateral Superior Genicular artery run downwards over the lateral epicondyle, supplying the periosteum and bone in this region. They then continue, supplying the fat pad deep to the patellar ligament, the joint capsule, and anastomose with the Lateral Vertical channel and with twigs from the Anterior Lateral Meniscal artery within the tendon of origin of the extensor digitorum longus and peroneus tertius muscles. They also form anastomoses with twigs from the Lateral Inferior Genicular artery within the substance of the lateral collateral femoro-tibial ligament.

In one of the specimens the proximal and distal branches of the Lateral Superior Genicular artery arise as two separate vessels from the common trunk of the Lateral Inferior Genicular and Lateral Sural arteries.

3.14 Lateral Inferior Genicular Artery (LIG)

This artery originates from the common trunk with the Lateral Sural artery. Its course is anterior to the plantaris and gastrocnemius muscles under the origin of the popliteus muscle, and laterally and distally over the lateral epicondyle of the femur, where/

where it gives off arterial twigs which supply the adjacent fascia and joint capsule (Figs. 4, 5, 7, 10 and 12). These twigs anastomose with branches of the Lateral Superior Genicular artery within the lateral collateral femoro-tibial ligament.

The main trunk of the Lateral Inferior Genicular artery then forms an anastomosis on the lateral aspect of the stifle joint with a recurrent branch of the Anterior Tibial artery (Figs. 6, 10 and 12). Where these vessels join, two Lateral Meniscal arteries arise.

The Anterior Lateral Meniscal artery (ALM) runs forwards over the lateral meniscus supplying arterial twigs to it and also to the tendon sheath and tendon of origin of the peroneus tertius and extensor digitorum longus muscles. These twigs, which in some cases arise directly from the Lateral Inferior Genicular, anastomose with distal branches of the Lateral Superior Genicular artery within the tendon. The Anterior Lateral Meniscal artery continues over the lateral meniscus and ends by anastomosing with a branch of the Medial Inferior Genicular artery or a meniscal branch from the Lateral Vertical channel (Fig. 5).

The Posterior Lateral Meniscal artery (PLM) is small and runs back on the lateral meniscus, where it sometimes (3/6) forms an anastomosis with a lateral meniscal vessel which springs directly from the Popliteal artery at the level of the menisci.

3.15 Medial Superior Genicular Artery (MSG)

This artery is the smallest of the normal genicular vessels and/

and usually arises directly from the Popliteal artery at the level of the medial epicondyle of the femur (Figs. 4, 14 and 15). In some pigs (2/6) the Medial Superior Genicular artery originates from a short common trunk with the Medial Sural artery (MS) and the Medial Inferior Genicular artery.

The Medial Superior Genicular artery runs over the medial epicondyle of the femur proximal to the limit of the joint capsule. Its branches supply the joint capsule, the periosteum and the bone of the medial epicondyle, and form anastomoses with branches of the Descending Genicular (DG) and Anterior Medial Meniscal arteries (AMM), and with the Medial Vertical channel.

3.16 Medial Inferior Genicular Artery (MIG)

The Medial Sural artery and the Medial Inferior Genicular artery come from a common trunk which is a branch of the Popliteal just before the latter passes into the interosseous space (Figs. 4, 7 and 13). In one case the Medial Inferior Genicular artery is a direct branch of the Popliteal and in two other specimens it forms a common trunk with the Medial Superior Genicular artery.

The course of the Medial Inferior Genicular artery is medial and anterior, close to the outer edge of the medial meniscus, where it gives off a Posterior Medial Meniscal artery (PMM), then an Anterior Medial Meniscal artery (AMM), and several small twigs to the proximal epiphysis of the tibia. In some cases (2/6) the Superficial Medial Genicular artery originates from the Medial Inferior/

Inferior Genicular artery in this region. The Medial Inferior Genicular artery then continues into the fat pad under the patellar ligament where it forms two main branches.

(a) The proximal branch sends out twigs which anastomose with the Anterior Lateral Meniscal artery and with the Medial and Lateral Vertical channels.

(b) The distal branch is the larger, and runs towards the tibial tuberosity where it forms anastomoses with branches of the Superficial Medial Genicular artery and recurrent branches of the Anterior Tibial artery, then joins the Superior Middle Genicular artery as that vessel enters the foramen on the epiphysis of the tibia (Figs. 6 and 8).

When the Superior Middle Genicular artery does not form the Vertical channels, these are supplied by the large distal branch of the Medial Inferior Genicular artery (Fig. 9). In such cases the Medial Inferior Genicular artery has no direct anastomosis with the Superior Middle Genicular artery.

The Anterior Medial Meniscal artery runs over the medial meniscus supplying it and the medial femorotibial ligament, and during its course forms anastomoses with twigs from the Superficial Medial Genicular and the Medial Superior Genicular arteries. The Posterior Medial Meniscal artery runs from the Medial Inferior Genicular artery posteriorly on the medial meniscus where it anastomoses with a medial meniscal arterial twig which arises directly/

directly from the Popliteal artery (Fig. 5).

All these vessels running circumferentially on the menisci are the perimeniscal vessels of Policard (Barnett, Davies and MacConaill, 1961).

3.17 Descending Genicular Artery (DG)

Often known as the Arteria Genu Suprema, this vessel arises from the Femoral artery mid-way between the origins of the Saphenous artery and the ascending branches of the Posterior Femoral artery, at a level just proximal to the medial supracondyloid crest of the femur (Fig. 4). It runs anteriorly round the distal end of the shaft of the femur under the vastus medialis muscle, where it supplies a branch to that muscle and a branch to the periosteum and fascia over the medial aspect of the distal end of the femur.

The former branch, to the vastus medialis muscle, gives off a number of fine vessels which run on to the medial aspect of the patella, supplying the periosteum and anastomosing with the Medial Vertical channel which terminates in this region by entering the substance of the patella (Figs. 6, 8, 14 and 15).

The latter branch, that at the distal end of the femur, breaks up into a number of smaller branches, some of which anastomose with twigs from the Medial Superior Genicular, while the rest enter foramina on the distal epiphysis of the femur close to the medial ridge of the trochlea (Figs. 14 and 15).

From/

From the vastus medialis muscle the main trunk of the Descending Genicular artery continues round the shaft of the femur under the rectus femoris and vastus lateralis muscles to which it supplies branches; it then curves distally under cover of the vastus lateralis muscle, runs over the base of the patella, to which it supplies two or three small twigs, and enters the lateral aspect of the free surface of the patella. Just before entering the patella, the Descending Genicular artery anastomoses with an ascending branch of the Posterior Femoral artery which runs in the substance of the vastus lateralis muscle (Figs. 6 and 14). The Descending Genicular artery also sends small branches over the lateral aspect of the patella which anastomose with the Lateral Vertical channel.

On the anterior surface of the shaft of the femur, under the rectus femoris muscle, the Descending Genicular sends a branch proximally up the shaft to supply the adjacent periosteum and the rectus femoris muscle, before ending by anastomosing with a branch of the Lateral Circumflex Femoral artery (LC).

3.18 Superficial Medial Genicular Artery (SupMG)

The Superficial Medial Genicular artery is the largest superficial branch of the Saphenous artery (Figs. 6, 8, 9 and 13). Its origin, however, varies in that it can arise from the Medial Inferior Genicular artery (2/6), or even directly from the Popliteal (1/6).

Its/

Its course is over the medial aspect of the proximal end of the tibia towards the tibial crest which it supplies along with the distal end of the patellar ligament. The terminal branches form anastomoses with recurrent branches of the Anterior Tibial artery, branches of the Medial Inferior Genicular artery, and branches of the Superior Middle Genicular artery where this vessel enters the proximal epiphysis of the tibia.

3.19 Ascending Branches of the Posterior Femoral Artery (PFA) and Recurrent Branches of the Anterior Tibial Artery (RAT)

The Descending Genicular artery has an equivalent on the lateral aspect of the femur which arises from an ascending branch of the Posterior Femoral artery. It is distributed to the adjacent periosteum and fascia over the distal end of the femur, and forms branches which enter the distal epiphysis of the femur close to the lateral ridge of the trochlea (Fig. 11).

The lateral aspect of the proximal epiphysis of the tibia receives blood from recurrent branches of the Anterior Tibial artery; these branches anastomose with the Lateral Inferior Genicular, the Medial Inferior Genicular and terminal branches of the Superficial Medial Genicular (Figs. 6 and 11).

3.2 Intrinsic Vessels

3.21 Macroscopic Arrangement

Angiograms of specimens injected with radio-opaque emulsion were made and examined. These specimens were then dissected out and/

and sections of the epiphyses five to fifteen millimeters thick were cut, decalcified and cleared.

The angiograms demonstrate about the same number of arteries as the dissections of the latex injected limbs, i.e. the angiograms show the main nutrient artery, accessory nutrient arteries and arteries entering the extremities of the bones (Figs. 21 and 22). A few vessels within the epiphyseal cartilages are also visible on these angiograms, but the smaller blood vessels can be better demonstrated only in the cleared specimens (Figs. 23 to 30).

In the rubber latex injected specimens the terminal branches of the extrinsic arteries supplying the distal epiphysis of the femur, the patella, and the proximal epiphysis of the tibia are easily seen as they pass through numerous foramina to enter the substance of the bones. By tracing these terminal branches back to their parent vessels definite regions can be assigned to the named arteries supplying the stifle region (Figs. 16, 17, 18, 19 and 20). These areas are remarkably constant, and the named arteries supplying them exhibit a far greater variation in their origins than in their distributions over the surfaces of the bones which they supply.

The arteries entering the extremities of the bones are classified by their relationship to the epiphyseal plate, those on the metaphyseal side being named the metaphyseal arteries and those on the epiphyseal side the epiphyseal arteries. There are a large number/

number of epiphyseal arteries, but very few metaphyseal vessels; in the proximal end of the tibia there are over one hundred epiphyseal arteries with only eight to twelve metaphyseal vessels entering the bone below the epiphyseal plate.

The maximum possible diameter of the metaphyseal and epiphyseal vessels was determined by measuring the diameter of the foramina through which they passed; this was accomplished by comparing the foramina with monofilament nylon and copper wire of various gauges. The diameters of the foramina varied from 0.1 mm. to 1.0 mm., although most lay between 0.2 mm. and 0.7 mm. In almost every case these arteries were accompanied by veins when they passed through the foramina.

The periosteal blood vessels take up very little of the injection mass due to their small calibre, but examination of dried bones shows that the foramina through which the periosteal vessels enter the cortex are significantly smaller than those which carry the metaphyseal and epiphyseal arteries into the substance of the bone; the largest of these periosteal foramina is only about 0.05 mm. (50 μ m.) in diameter.

Measurements of the size of the nutrient foramen in the shaft show a range of 1.2 mm. to 1.6 mm. for the femur, tibia, humerus and radius in the pig.

Thick sections of the extremities of long bones which have been intra-arterially injected with radio-opaque emulsion, decalcified/

decalcified, then cleared by the Spalteholtz method show the epiphyseal arteries running into the substance of the bone towards the ossifying centre of the epiphysis where they form numerous branches which anastomose with each other (Figs. 23, 24 and 25). Most of these vessels are concentrated towards the periphery of the spongy bone of the epiphyseal centre and towards the articular surfaces and epiphyseal cartilages. Long arterioles originate from this arterial lattice and these in turn, by further branching and anastomosing, form secondary arteriolar networks from which the tissues are supplied via arterioles, arterial precapillaries and capillaries.

It is from the arteriolar networks that the epiphyseal side of the cartilage plate is supplied by blood. Small arterioles, arterial precapillaries and capillaries arise from arterioles near the plate, form anastomoses between each other, then plunge into the cartilaginous substance of the epiphyseal plate for almost one third of the thickness of the plate before curving back as venous vessels to join the venous drainage of the epiphysis (Figs. 25, 26, 27 and 28). In injected specimens from new born piglets there appear to be no capillaries between the arteriolar supply and the venous drainage. In some preparations it can be seen that the arterial precapillaries or small arterioles appear to expand when they reach their greatest depth within the epiphyseal cartilage (Figs. 26 and 27).

The/

The nature of the venous drainage associated with the arterial supply to the epiphyseal plate was not examined using injection techniques, but measurements on histological preparations indicate that the vessels involved are mainly venous precapillaries (prevenules) rather than venules.

The osseous tissues within the epiphysis are supplied from the arteriolar network via precapillaries and capillaries (Figs. 23 and 28).

The metaphyseal arteries form anastomoses on the surface of the bone with periosteal vessels. These anastomoses are through small arteries and arterioles. The metaphyseal arteries then enter their respective foramina and, like the epiphyseal arteries, are accompanied by satellite veins.

On passing through the cortex of the bone, the metaphyseal vessels form arterial anastomoses with longitudinal branches of the medullary artery. Most of these anastomoses are on the endosteum or within the spongy bone adjacent to it. The large arterial loops thus formed give rise to numerous smaller arteries which run longitudinally up, between the spicules of young cancellous bone and the columns of calcified cartilage, towards the epiphyseal plate (Fig. 29). Large arterial loops are also formed by anastomoses between branches of the medullary artery. These are found towards the longitudinal axis of the bone and curve towards the metaphysis. Small arteries also arise from these loops and run longitudinally to supply the metaphyseal region (Fig. 30).

These/

These longitudinal arteries of the metaphysis appear to be end arteries, forming no anastomoses at arterial or arteriolar levels. They finally arborise forming a capillary bed which supplies the metaphyseal region. Arising from this bed are the capillaries which enter the lacunae left by the dead cartilage cells in the metaphyseal side of the epiphyseal disc (Fig. 31).

Around the periphery of the epiphyseal plate, within the perichondrium and adjacent periosteum, there are anastomoses formed between arteriolar branches of the epiphyseal, metaphyseal and periosteal arteries (Figs. 23 and 29).

3.22 Microscopic Structure and Arrangement

The histological work done was concentrated upon the study of the contents of the larger vascular channels in the epiphyseal plate, whose presence has been demonstrated in the injected specimens.

The various techniques and staining methods used upon the tissues all cause considerable shrinkage and distortion of the venous elements between the very rigid cartilaginous walls of the canals. This may be due to physical shrinkage, but is probably also caused by the loss of arterial blood pressure before fixation. In some cases the blood vessel walls are obviously constricted, in others there was a tearing or loss of the contents of these vascular channels.

The canals within the epiphyseal cartilage are 40 - 350 μm . in diameter, with most measuring about 100 μm . They contain arterial/

arterial and venous vessels embedded in delicate connective tissue (Figs. 32, 33, 34 and 35). The cartilage of the walls of the canals contains a higher proportion of a Periodic acid Schiff positive material than the surrounding cartilage. It is also faintly eosinophilic and has a fibrous structure. Hence it was concluded that the walls of the canals contained a higher concentration of polysaccharide and more collagen fibres than the surrounding matrix.

The arteries measure only about 10 - 20 μm . in diameter in the fixed and stained preparations. The venous vessels measure from 8 - 120 μm . in diameter. These measurements indicate a considerable shrinkage of the tissues within the canals, and such measurements are useless in interpreting results. It is better to base the findings entirely upon the structure of the walls of the blood vessels rather than upon inaccurate and artificial measurements.

Sections cut obliquely through vascular channels also tend to give a false impression; this is particularly so with the thick Spalteholtz cleared sections where a blood vessel may appear to pass through the cartilaginous plate from epiphysis to metaphysis (Figs. 27 and 28).

The measurement of the overall diameter of a cartilage canal is fairly accurate so vessels under 100 μm . in diameter can be accommodated in them. These include arterioles, arterial precapillaries/

precapillaries, capillaries, venous precapillaries and sinusoids. The histological features distinguishing these vessels from each other may be summarised as follows (Ham and Leeson, 1965; Arey, 1957):-

Arterioles have a thin tunica intima with a very fine internal elastic membrane. The tunica media is relatively thicker than that of any other blood vessel, having one to five layers of smooth muscle cells.

Arterial precapillaries consist of an endothelial tube encircled by a scattered layer of smooth muscle cells.

Venous precapillaries (or prevenules) have an endothelial lining surrounded by connective tissue and in the larger a few smooth muscle cells. Precapillaries never show complete coats in section, the presence of such indicates an arteriole or venule.

Sinusoids have an irregular endothelium and contain fixed macrophages. Only a network of reticular fibres intervenes between the endothelium and the surrounding parenchyma.

Capillaries consist only of a layer of endothelial cells resting upon a network of reticular fibres and embedded in the surrounding tissues.

Another/

Another type of vessel which must be considered is the so-called muscular capillary which forms arteriovenous bridges now commonly termed preferred channels (Ham and Leeson, 1965). These muscular capillaries bear a superficial resemblance to capillaries, but have scattered along their walls smooth muscle cells that represent a continuation of the musculature of arterioles. It is most likely that these vessels are structurally identical with arterial precapillaries and for the purpose of this study will be considered as such.

Using the above criteria the histological picture of the contents of the vascular channels within the epiphyseal cartilages may now be more properly interpreted and evaluated without having to rely upon measurements.

Within the channel, usually occupying a fairly central position, there is a small arteriole having the characteristic thick muscular wall and measuring in the stained sections 10 to 20 μ m. in diameter (Figs. 32, 33 and 35). The small diameter is certainly due to shrinkage, since the muscular wall of the vessel is complete although thin, having only one or two layers of smooth muscle cells.

Towards the periphery of the channel there are two to five venous precapillaries (prevenules). These are very thin walled and not unlike sinusoids, but a few smooth muscle cells can be seen and there is no evidence of phagocytic cells within the endothelial/

endothelial walls (Figs. 33 and 35). In the histological sections the diameter of these pre-venules ranges from about 8 - 120 μ m.

The arterioles and pre-venules are embedded in loose connective tissue and undifferentiated mesenchymal cells within the cartilage canal. Occasionally, within this connective tissue, arterial precapillaries and true capillaries can be identified (Figs. 33 and 34) in new born piglets, but these appear to be associated with the nutrition of the tissue in the immediate vicinity of the canal, since they do not penetrate the walls of the canal and arborise in the surrounding cartilage.

Examination of serial sections confirms much of the above information. The course of a cartilage canal is easily followed, and it can be seen that the canal does not pass from the epiphysis to metaphysis. Further, it becomes obvious that many of the canals do not form loops. From near the end of a canal five to seven columns of the metaphyseal cartilage cells are directed towards the metaphysis (Fig. 36).

The collagen forming the wall of the canal does not curve abruptly around the end of the canal, but tapers off in a conical fashion towards the metaphysis producing septa between the columns of cartilage cells (Figs. 36, 37 and 38). These septa, being fibrous in structure, eosinophilic and giving a fairly strong positive result with Periodic acid Schiff reagent, are probably composed mainly of collagen. Near the end of a cartilage canal, close/

close to the metaphysis, the arteriole within the canal terminates by doubling back as a number of pre venules (Fig. 37).

The manner in which the arteriole arborises is not easy to determine, but the serial sections show that, after the arteriole cannot be distinguished as such within the canal, the lumen of the canal is filled with a mass of mesenchymal cells which appear to be of a fairly undifferentiated type. This cellular mass is perforated by numerous tiny blood vessels, which are mainly capillaries, but the presence of a few smooth muscle cells show that some arterial precapillaries are present. A number of small pre venules are also evident within this cellular mass. It seems, therefore, that the termination of a cartilage canal contains a vascular bud which consists of a network of capillaries together with mesenchymal cells (Fig. 39). The dilated structures seen in the injected specimens (Figs. 25, 26, 27 and 28) are probably artefacts caused by the filling of the capillary tufts by the injection media, and also leakage of the media through capillary walls ruptured by the pressure of the injection.

In addition to the cartilage canals within the future epiphyseal plate in the developing pig, canals of an identical structure were observed throughout the cartilage which in the future would form the articular surfaces and articular cartilages over the extremities of the long bone (Fig. 28). These canals ended in capillary tufts under the articular surfaces.

Smaller/

Smaller vascular channels in the epiphyseal plate and in the articular cartilage lie directly under the epiphyseal bone layer, often within it. These channels contain only capillary loops which appear to be associated with the bone and the zone of adjacent cartilage rather than the deeper cartilaginous layers.

4.0

DISCUSSION

The genicular arteries in the pig have not before been the subject of a special investigation. Indeed, there do not appear to be any original papers giving details of the genicular arteries in any of our domestic animals. Although textbooks of human anatomy contain full descriptions, these seem to be derived from unquoted early sources. It is therefore not surprising that books of reference on veterinary anatomy give very little information on the genicular arteries, and that none of them contain descriptions of these vessels in the pig.

Percivall (1832) and M'Fadyean (1884) say nothing of the vessels supplying the stifle joint in the horse. Fleming (1891) mentions only "articular branches" from the Popliteal artery, and Bradley (1920) dismisses the blood supply to the region of the stifle joint in the horse with the following sentence, "The collateral branches of the popliteal artery are small, and distributed to the joint and adjacent muscles." Ellenberger and Baum (1943) describe the "arteria articularis genu suprema", the "arteria articularis genu medialis et lateralis", and the "arteria articularis genu anteriores et posteriores", but give only a very brief description of these vessels.

Miller (1948) describes in the dog the "arteria genu suprema", the "superficial medial genicular artery", the "deep medial genicular artery" and the "lateral genicular artery". Grahame (1959) mentions only the "arteria genu suprema" in the dog.

Grossman/

Grossman (1961) describes the "arteria genu suprema", the "arteria genu media", and the "arteria genu lateralis distalis" in the horse and mentions the formation of an arterial plexus on the stifle. May (1964) mentions only the "genu suprema" in the sheep, and Habel (1951) does not describe any of the genicular vessels in the ox.

This ignorance of the details of blood supply to the joints of domestic animals is somewhat surprising, since the knowledge of a rich vascular supply to joints and associated epiphyses is well established. In 1743 William Hunter described the blood supply of the articular cartilages and introduced the term "Circulus Articuli Vasculosus". Harris (1933) chose the knee joint of a new-born human to illustrate this arterial circle and expanded Hunter's term to "circulus vasculosus articuli et epiphyseos" since the epiphyses as well as the joint are supplied with blood from it. The surgical importance of the human genicular vessels is indicated by Smillie (1962) who states that the Lateral Inferior Genicular artery is vulnerable during menisectomy, as severe haemorrhage occurs into the joint when this vessel is not ligatured; and that the fate of the anterior cruciate ligament after rupture depends upon the Middle Genicular artery.

The genicular arteries in the human knee have been described by Sharpey, Thomson and Cleland (1866); Heitzman (1887); Cleland and Mackay (1896); Sorbotta and McMurrich (1907); Adachi (1928); Keen/

Keen (1949); Davies and Davies (1962); Walls (1964); and Lockhart, Hamilton and Fyfe (1965). Greene (1955) gives a fair amount of detail about the genicular arteries in the rat.

The fullest recent accounts are given by Greene (1955); Davies and Davies (1962); Walls (1964) and Lockhart, Hamilton and Fyfe (1965) who classify the genicular arteries as medial and lateral superior and inferior arteries, a middle genicular artery and the genu suprema or descending genicular artery. It is upon these descriptions that the terminology used in this thesis is based. Since there are only six genicular arteries in the human, and eight vessels are seen in the pig, the relation between the nomenclatures is somewhat confused. In Table I an attempt is made to equate the present terminology with that of earlier authors. The additional vessels found in the pig are the Superficial Medial Genicular artery, as described by Miller (1948) in the dog, and the sub-division of the Middle Genicular artery into superior and inferior vessels. Poirier (1904) mentions that the Middle Genicular artery is often double in the human, and Sharpey, Thomson and Cleland (1866) state that, "there are sometimes several small middle articular branches".

The adjectives superior and inferior have been used by most workers; these have been retained, although it would perhaps be better to substitute for them the terms proximal and distal.

The dissections show that numerous large anastomoses are formed by the genicular arteries, and, as in the human, a definite pattern of/

of arteries is formed. This pattern may be briefly described as follows:-

The genicular arteries form vertical channels at the sides of the patella, which are joined by arterial arches above and below the patella. These arches are joined by three other arteries; from above, a branch of the Lateral Circumflex Femoral artery and an ascending branch of the Posterior Femoral artery; from below, an anterior recurrent branch of the Anterior Tibial artery.

Anterior recurrent branches of the Anterior Tibial artery seem to replace the circumflex fibular artery which is found in the human and has been described by Walls (1964) as originating from either the Anterior or Posterior Tibial arteries.

Attention is drawn to the importance of the anastomoses formed by the genicular arteries in the human by Lockhart, Hamilton and Fyfe (1965) who state that the anastomoses around the knee "can provide a collateral circulation if either the femoral artery in the subsartorial canal or the popliteal artery is blocked, but is usually inadequate if both vessels become occluded".

With regard to the epiphyseal blood supplies, the anastomoses formed by the genicular and associated arteries occur in three different places:-

(1)/

- (1) before reaching the periosteum or bone
- (2) on the periosteum
- (3) within the substance of the bones

The first two types of anastomoses are numerous, and the rich arterial plexus which is formed sends many branches through foramina in the epiphyseal and metaphyseal cortices to supply the substance of the bone, each arterial branch entering a foramen along with a satellite vein. The extent of this blood supply to the epiphysis and metaphysis may be judged from the numbers and sizes of the foramina observed.

Arey (1957) gives the following measurements of the diameter of the blood vessels in the human:-

Arteries	over 300 μm .
Arterioles	40 - 300 μm .
Arterial precapillaries	12 - 40 μm .
Average capillary	8 μm .
Venous precapillaries	12 - 200 μm .
Venules	200 - 1000 μm .
Veins	over 1000 μm .

Using this classification and naming the vessels as they enter the substance of the bone through their respective foramina, and, if one-half to one-third of each foramen contains an arterial vessel and the rest is occupied by venous tissue, then it may be said/

said that the nutrient and accessory nutrient vessels to the medulla of the bone are true arteries, the metaphyseal and epiphyseal vessels are true arteries although a few are small enough to be classified as large arterioles, and the periosteal blood vessels are mainly capillaries, precapillaries and a few small arterioles.

All the measurements were made on bones taken from pigs slaughtered at bacon weight, 80 - 100 Kgm. Examination of bones from younger animals show that the epiphyseal-metaphyseal foramina are actually larger, and thus indicate a relatively much richer blood supply to the less mature, rapidly growing bones.

Bones from adult boars and sows, after fusion of the epiphyseal plate, show a reduction in numbers of metaphyseal and epiphyseal foramina indicating a reduced metaphyseal-epiphyseal blood supply to mature bone.

This last observation could imply that the medullary-periosteal blood supply to a long bone is mainly associated with maintenance of the tissue, while the metaphyseal-epiphyseal blood supply is responsible for carrying most of the nutrients to the regions of growth in young developing bones. Many writers (Bradley, 1920; Ellenberger and Baum, 1943; and Grossman, 1961) deal with the main nutrient artery to the shaft of the bone and with the periosteal blood vessels, but say little about or ignore completely the epiphyseal-metaphyseal blood supply.

The/

The picture usually presented by veterinary anatomical textbooks is of a principal nutrient artery, the terminal branches of which form free anastomoses with a periosteal network, while any arteries which enter the bone at its extremities are considered as playing only a supporting role.

Grossman (1961), giving a general description of the blood supply to a long bone, says, "It is customary to recognise two sets of arteries - the periosteal and the medullary".

The description of the blood supply to bone as given by Fleming (1891) is more complete:-

"The arteries of bones belong to three orders - a distinction based on volume and the extent of their distribution.

The arteries of the first order penetrate to the interior of the medullary canal of long bones, by a particular orifice - the nutrient foramen. They soon divide into two branches, which break up into a network that lines the walls of the canal and enters the tissue of the medulla. This network communicates with arteries of the second order, which go to the spongy tissue of the extremities of the long bones, penetrating them by numerous nutritive foramina that surround the epiphyses. Lastly, the arteries of the third order are branches of the periostic/

periostic network that enters the superficial Haversian canals. These canals may be considered, strictly speaking, as a third category of nutrient conduits. In the flat and short bones there are no arteries of the first order."

In 1870, J. Wilson Johnston in "Strangeway's Veterinary Anatomy" wrote, "Bones are supplied by blood in three ways - by periosteum, small arteries entering the ends, and nutrient or medullary arteries".

This sentence, perhaps accidentally, does give the key to the order of functional importance of the various routes by which blood may enter a long bone, although Brookes and Harrison (1956) and Brookes (1957) suggest that the main blood supply to the cortical bone of the shaft comes from the venous drainage of the medulla through the cortex and that periosteal arterialisation is pathological.

Trueta and Cavadias (1964) showed that, in the radius of the rabbit, the medullary artery is the main vessel to the shaft, supplying the whole of the marrow and inner two-thirds of the cortex, but not the outer part of the cortex nor the extremities. They also showed that under experimental conditions the periosteal vessels supply only the outer part of the cortex if other supplies are cut off, and that the metaphyseal vessels alone cannot maintain the marrow and deep half of the cortex, but after union with the epiphyseal/

epiphyseal vessels on fusion of the growth cartilage in the adult rabbit, they carry enough blood to supply all the marrow and bone.

The epiphyseal-metaphyseal blood supply must therefore be of importance in the adult as well as in the young animal; its function in the adult being to supply those parts of the bone to which the forces are applied, viz., articular surfaces, attachments of ligaments, and the origins and insertions of many tendons.

In the light of the above the epiphyseal-metaphyseal blood supply now takes on a two-fold importance in the young animal - that of supplying nutrients to a region of maximum growth of a long bone, and of maintaining those parts of a long bone subjected to the greatest mechanical stresses.

This arterial network can, however, be divided into definite regions on the epiphyseal periosteum, each region being supplied by a specific artery, a collateral blood supply only entering that region from its periphery, or from within the substance of the epiphysis. Each of these regions on the bones examined for this work is fairly large, from which it could be inferred that, in the event of an obstruction to the single main arterial supply to a periosteal region, the collateral blood supply from its periphery is probably less important than the collateral supply from the substance of the epiphysis itself. In such a case a considerable strain could be put on the blood supply to the epiphysis as a whole, certainly in the area affected and especially to the outer portion
of/

of epiphyseal cortex and its periosteum. The nutrition of the bone will then depend upon the anastomoses formed by the epiphyseal-metaphyseal arteries within the substance of the bones which they supply.

The epiphyseal blood enters the epiphysis through a large number of different arteries, arterial and arteriolar anastomoses being formed before and after these arteries enter the substance of the bone. The blood supply to the metaphysis comes mainly from the medullary artery which forms only a few arterial and arteriolar anastomoses with a relatively small number of metaphyseal arteries.

The differences in the epiphyseal and metaphyseal blood supplies are greatest at the epiphyseal plate. In the immature pig the epiphyseal surface of the plate is covered by a mesh of arteries and arterioles which sends capillary loops and arterioles forming capillary tufts through the adjacent third of the thickness of the cartilaginous substance of the plate. The tufts appear as dilated extremities of the arterioles. Morgan (1959) described similar capillary loops in the epiphyseal plate of the rabbit.

The blood supply to the metaphyseal surface of the cartilaginous plate comes from the longitudinal metaphyseal arteries, anastomoses are only formed between them at capillary level and they are very long in relation to their diameter. This latter fact could predispose these vessels to embolism formation and/

and since they are end arteries, this would cause infarction, especially towards the axis of the bone where there is no chance of a collateral blood supply coming from periosteal vessels.

Harris (1933) describes such end arteries in the human and compares the blood supplies to the metaphyseal and epiphyseal sides of the cartilaginous plate. He states, "The small arteries in the bony epiphysis are not end-arteries, and it is this free anastomosis which leads to the important differences in the type of lesion found in the bony epiphysis as distinct from the diaphysis. The lesion in the shaft or diaphysis tends to be of the character of localised infarction; in the epiphysis it tends to be widespread destruction".

The capillaries which eventually supply the metaphyseal side of the cartilaginous plate do not penetrate the plate, but enter the spaces left by hypertrophied cartilage cells, and appear to be in direct continuity with the lumina of the venous sinusoids of the bone marrow.

Most of the work done on the structure and development of the blood supply to bone has been concentrated upon the shaft or diaphysis; little attention has been paid to epiphyseal blood supply.

The vascularisation of the epiphyses of long bones commences either late in foetal life or, in many bones, after birth. In the human embryo, ossification centres and vascularisation of the diaphyses/

diaphyses are evident at about the eighth week of intra-uterine life, whereas most epiphyseal centres of ossification and vascularisation are not established until after birth.

Clarkson (1896), Parsons (1904) and Trueta (1966) have shown that the process of epiphyseal vascularisation and ossification is similar to that of the diaphysis; however, there has been some doubt and disagreement as to the source of the vascular precursors for the epiphysis. Parsons (1904) and Jordan (1952) describe the development of the epiphyseal blood vessels as being merely an extension of the diaphyseal blood supply, vessels growing from the zone of advancing ossification into the centre of the cartilaginous cap of the epiphysis. Hamilton, Boyd and Mossman (1962), Ham and Leeson (1965) and Trueta (1966) describe a process similar to, but separate from the process of diaphyseal vascularisation, i.e. a periosteal bud consisting of capillaries and mesenchymal cells which grow inwards from the periosteum towards the epiphyseal centre of ossification.

Haines (1933) describes communicating canals which grow from the epiphysis to diaphysis and not vice versa, but records only one, apparently fairly constant, communicating canal at the distal end of the femur in the pup, but says that there are none in the proximal epiphysis of the tibia. Of all the material examined for this study only one section shows anastomosis between metaphyseal and epiphyseal blood supplies in the pig. This anastomosis is in the/
the/

the axial portion of the distal end of the femur of a pig slaughtered for bacon at about 200 days old. At this age the distal femoral epiphysis still requires a long time before fusion. Payton (1931, 1933) states that fusion of the distal end of the femur in the pig occurs between 647 days and 729 days old.

Harris (1933), noting the earlier work of Lexer, Kuliga and Türck (1904) who depicted anastomoses between the arterial fields of the diaphysis and the epiphysis traversing the epiphyseal cartilage, and after a series of experiments on growing animals, concluded that the vascular territories of the epiphysis and diaphysis are distinct and no vessels penetrate the growth cartilage.

The serial sections made from bones of new-born piglets support the view that the cartilaginous epiphyseal primordium is invaded by a large number of vascular buds from the epiphyseal periosteum. No diaphyseal tissues could be demonstrated growing in through the metaphysis to form the early bony epiphysis as described by Parsons and Jordan. Trueta (1966) showed that the vascular endothelium can directly and without intermediates, be responsible for the production of osteoblasts which in due course become osteocytes.

The development of an epiphyseal centre of ossification and its vascularisation may therefore be summarised as follows:-

(1)/

- (1) Chondrolysis and calcification of the cartilage.
- (2) The growth of vascular buds from the periosteum.
- (3) The formation of primitive cancellous bone.

The results of the work done for this study show that, after the epiphyseal centre of ossification has formed and during the subsequent growth in length of the bone, cartilaginous channels containing blood vessels can be readily demonstrated within the epiphyseal plate, and within the cartilage forming the future articular surfaces. The blood vessels which plunge through the epiphyseal plate towards the metaphysis do not anastomose with the metaphyseal blood supply. This fact may be of some clinical importance. Cope (1920) wrote:-

"It is well known that the cartilaginous epiphysis presents a barrier to the progress of infection and malignant disease, but so far I have been able to find very little evidence that the bony fusion-line acts in a similar manner."

Haines (1933) describing the formation of communicating canals states that, "If the end of a simple canal should lie in the growing cartilage it may persist while the cartilage around it is calcified and then eroded, thus coming into secondary continuity with the young marrow and forming a communicating canal." Communicating canals, are, however, rarely observed so the development and subsequent growth of the common non-communicating cartilage/

cartilage canals are probably different from that visualised by Haines.

Hurrell (1934) suggested that the growth of the vascular canals within the epiphyseal plate was under a chemotaxic influence set up by starvation of cells and metabolic waste products in the cartilage. This, he suggested, caused the bending of the canals into the zone of rapidly growing cartilage cells. The histological picture obtained in this study of the cartilage canals and their relationship with the columns of cartilage cells which diverge fan-like from near the termination of the canal towards the metaphysis suggests an alternative mode of development.

The blood vessels within an established cartilage canal cannot without active growth in length of their walls and chondrolysis of the epiphyseal cartilage become nearer to the metaphysis since the vascular network from which they originate lies upon the calcified cartilage on the epiphyseal side of the epiphyseal plate. As the bone grows in length, the ends of the cartilage canals will thus remain in the same position relative to the epiphyseal side of the epiphyseal plate, the developing columns of cartilage cells appearing to drift past the canal towards the metaphysis. The cartilage canal, by either providing more nutrients to the cartilage cells adjacent to it and thus increasing their rate of division, or by having a chondrogenic layer of cells in its wall, may act as a focus for increased chondrogenesis. If there is this greater/



greater cell production in relation to the cartilage canal this will disturb the even flow of cartilage cells from the epiphyseal side of the cartilaginous plate, since room must be available for the cells formed near the canals themselves. This suggestion would account for the columns of cartilage cells appearing to bend towards the cartilage canals. Trueta (1966) quoting the earlier work of Trueta and Amato (1960), Trueta and Trias (1961) and Trueta and Buhr (1963) showed in the rat that the only source of nourishment of the growth cartilage is the blood of the epiphyseal vessels. The metaphyseal vessels play little or no part in the nourishment of the cartilage cells, but that, on the contrary, the presence of these vessels is necessary to enable the hypertrophy and removal of the cells of the cartilage columns in the normal process of growth. A reduction in the epiphyseal blood supply causes damage and disintegration first to the germinative and proliferative cells, and finally to the whole cell columns. A reduction in the metaphyseal blood supply inhibits calcification, and the cartilage cells continue in their hypertrophic phase, not disintegrating; with this cell preservation, the cartilage thickens and resembles the changes seen in rickets.

It does not seem likely, however, that the only function of these epiphyseal blood vessels is to nourish the cartilage. The present observations indicate that one function of vessels within the cartilage canals is to supply the chondrogenic mesenchymal cells/

cells within the canal, particularly those associated with the capillary tuft at the end of the canal adjacent to the metaphysis and articular surfaces.

It would seem that these vascular elements within the epiphyseal plate and articular cartilages are, in fact, numerous vascular buds growing into the plate in the developing pig in the same way as an isolated vascular bud grows into the cartilaginous shaft of a long bone to form the diaphyseal ossification centre. The vessels within the cartilage of a developing epiphysis are probably as closely associated with the ossification and future blood supply to the developing epiphyseal bone as they are with the nutrition of the cartilage in the growing animal.

It must not, however, be assumed that the cartilage is independent of these blood vessels, since the observations show a relationship between the canals and the proliferative columns of cartilage cells.

The smaller capillary loops, particularly those in older animals, seem to be directly responsible for supplying nutrients to the epiphyseal plate. Morgan (1959) showed in the rabbit's tibia that the epiphyseal surface of the plate is supplied by blood from the subchondral loops of the epiphyseal arteries, the loops appearing at the summit of 8 - 12 columns of proliferating cartilage cells.

During their development the vascular canals appear to advance
by/

by chondrolysis brought about by the differentiation of some of the cells within the vascular bud into chondroclasts, or the direct action of the capillary endothelium. A period then follows during which the growth of the vascular canals appears to be minimal. They grow with the epiphyseal plate, maintaining a fairly constant relationship with its epiphyseal surface. This is the period during which the cartilage columns grow outwards from the walls of the canal. The different histochemical reactions of the wall of the canal may be due to a selective removal of some elements of the matrix, or they may be caused by the synthesis of collagen in the walls of the canal. In older animals, parts of these collagenous walls become calcified, then after replacement by bone the original lumen of the canal becomes an areolar space within the bony epiphysis.

Extensions of the collagenous walls of the canals extend right through the zone of proliferating cartilage towards the metaphysis. These septa often cause folding and artefacts of a histological section which may give the impression that blood vessels penetrate from metaphysis to epiphysis.

Hurrell (1934) noting the descriptions of earlier workers of diaphyseal-epiphyseal channels and blood vessels, described one or two of these vessels, but decided that their formation is accidental, they only remain patent for a short time, and they are functionless. He also describes "the connective tissue septa in the zone of cell rows" and points out that they are related to the vascular channels.

The/

The pattern of blood supply to the epiphyseal plate may be summarised as follows:-

1. The epiphyseal side of the plate is supplied by many arteries forming numerous anastomoses at arterial, arteriolar and capillary levels, many arterioles and capillary loops plunging into the substance of the cartilage.
2. The metaphyseal side of the disc is supplied by end arteries forming anastomoses only at the capillary level. None of these capillaries actually enter the substance of the cartilage. The peripheral portion of this side of the epiphyseal disc does, however, have a collateral blood supply coming through arteriolar anastomoses formed between metaphyseal arteries with epiphyseal and periosteal vessels.

In effect, this means that, in the event of a general or localised circulatory inefficiency, it would be expected that the axial portion of the metaphyseal side would be that part of the epiphyseal disc to be placed under the greatest stress due to its poor collateral blood supply, and hence it would be that part most likely to be damaged. The lesion in the distal end of the ulna of the pig described by workers from the Rowett Research Institute (Jones, Fell and Walker, 1964) may be an example of this since it starts/

starts as a necrosis of the cartilage on the metaphyseal side of the epiphyseal plate. This type of lesion in the human was described by Harris (1933) who says:-

"The vessels of the diaphysis are virtually 'end arteries', with but poor anastomoses between their respective territories. Thus the phenomena of disease in the juxta-epiphyseal zone or metaphysis are essentially phenomena of infarction. It is this infarction of a wedge of bone, the base of which abuts on to the epiphyseal cartilage which determines the radiographic appearance of a lesion in the metaphysis of the long bones. It is this infarction which militates against the diagnosis of the nature of a bony lesion in terms of radiographic appearance alone."

5.0

SUMMARY

1. The blood vessels supplying the stifle joint of immature pigs have been studied by dissection, injection methods, radioangiography and histology. A less detailed study has been made of the ends of other long limb bones.

2. The stifle joint is supplied by eight genicular arteries with contributions from adjacent vessels such as the posterior femoral and anterior tibial. The extensive anastomoses occurring between all these vessels and their detailed topography is described.

3. The epiphyseal side of the cartilaginous epiphyseal plate is supplied from a network of epiphyseal arteries which form numerous anastomoses at arterial, arteriolar, and capillary levels.

Capillary loops and arterioles terminating in capillary tufts penetrate the growth cartilage from the epiphyseal side.

4. The metaphyseal region is supplied by arteries which are derived from the main nutrient artery, periosteal vessels and those few metaphyseal arteries which penetrate the metaphysis. In the peripheral portion of the metaphyseal surface of the epiphyseal cartilage, these sources of blood anastomose with each other; but the axial region of the metaphyseal surface is supplied only by branches of the main nutrient artery.

5. The long narrow metaphyseal vessels are end arteries and form vascular loops which occupy the spaces left by the degenerating hypertrophied cartilage cells and do not actually penetrate the epiphyseal cartilage.

6. During the growth period, there is no evidence of extensive communication between the epiphyseal and metaphyseal vessels through the epiphyseal cartilage.

7. The significance of these vascular patterns in relation to pathological changes in the epiphyseal cartilage is discussed.

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TABLE I

The Terminologies used in the Descriptions of the
Genicular Arteries by Various Authors.

FIG	WALLS 1964 - HUMAN	GREENE 1955 - RAT	MILLER 1948 - DOG	ELLENBERGER & BAUM 1891 - DOG	SHARPEY, THOMSON & CLELAND 1866 - HUMAN
DESCENDING GENICULAR	DESCENDING GENICULAR or GENU SUPREMA	ARTERIA GENU SUPREMA	ARTERIA GENU SUPREMA	ARTERIA ARTICULARIS GENU SUPREMA	ANASTOMOTICA MAGNA
LATERAL SUPERIOR GENICULAR	LATERAL SUPERIOR GENICULAR	LATERAL SUPERIOR GENICULAR	LATERAL GENICULAR	ARTERIA ARTICULARIS GENU LATERALIS	UPPER EXTERNAL ARTICULAR
LATERAL INFERIOR GENICULAR	LATERAL INFERIOR GENICULAR	LATERAL INFERIOR GENICULAR			LOWER EXTERNAL ARTICULAR
MEDIAL SUPERIOR GENICULAR	MEDIAL SUPERIOR GENICULAR	MEDIAL SUPERIOR GENICULAR	DEEP MEDIAL GENICULAR	ARTERIA ARTICULARIS GENU MEDIALIS	UPPER INTERNAL ARTICULAR
MEDIAL INFERIOR GENICULAR	MEDIAL INFERIOR GENICULAR	MEDIAL INFERIOR GENICULAR			LOWER INTERNAL ARTICULAR
SUPERIOR MIDDLE GENICULAR	MIDDLE GENICULAR	MIDDLE GENICULAR	-	ARTERIAE ARTICULARES GENU POSTERIORES	MIDDLE or AZYGOS ARTICULAR
INFERIOR MIDDLE GENICULAR			-		
SUPERFICIAL MEDIAL GENICULAR	-	-	SUPERFICIAL MEDIAL GENICULAR	ARTERIAE ARTICULARES GENU ANTERIORES ?	-

FIGURE 1

Apparatus used for Intra-arterial Injection of
Specimens.

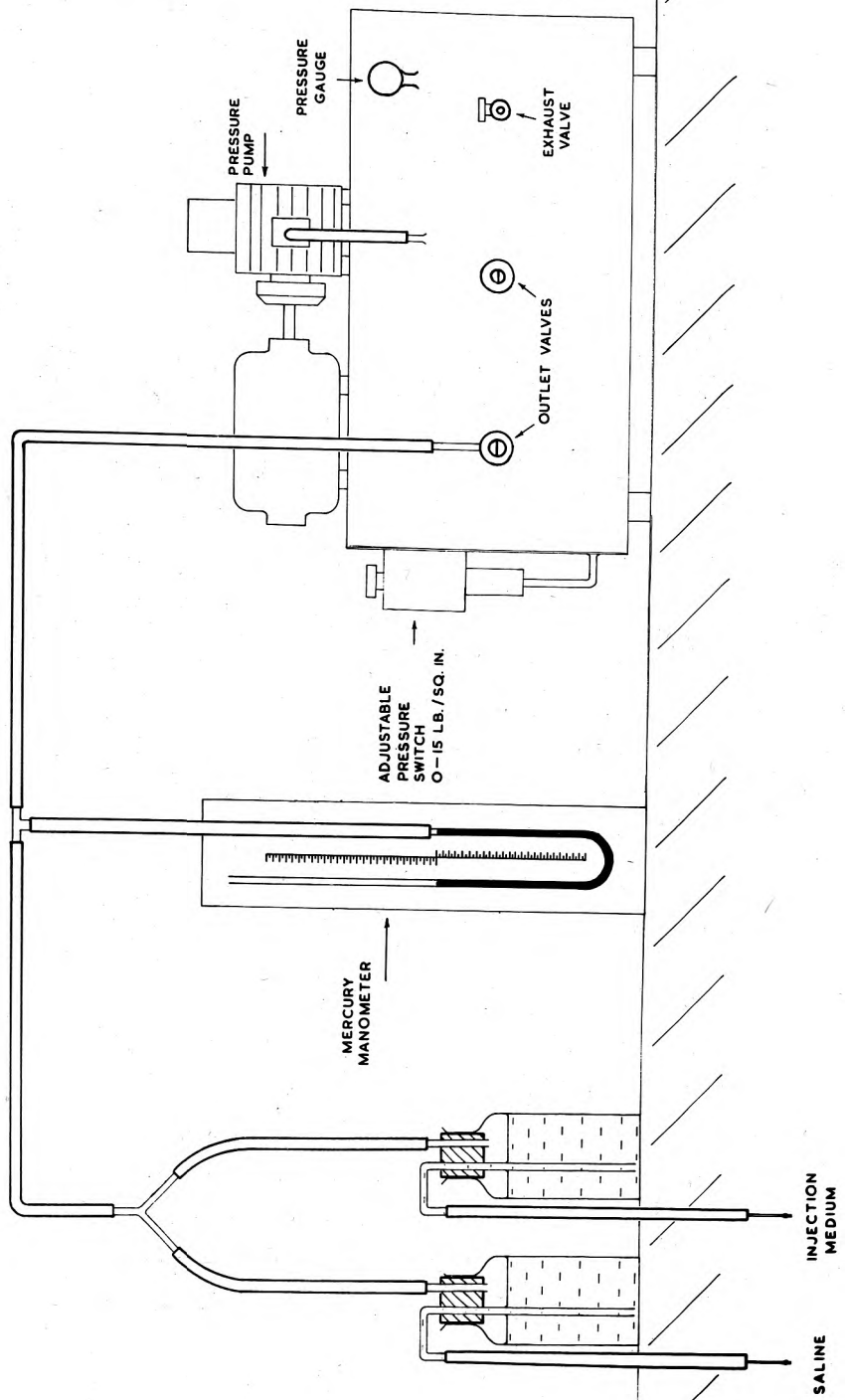
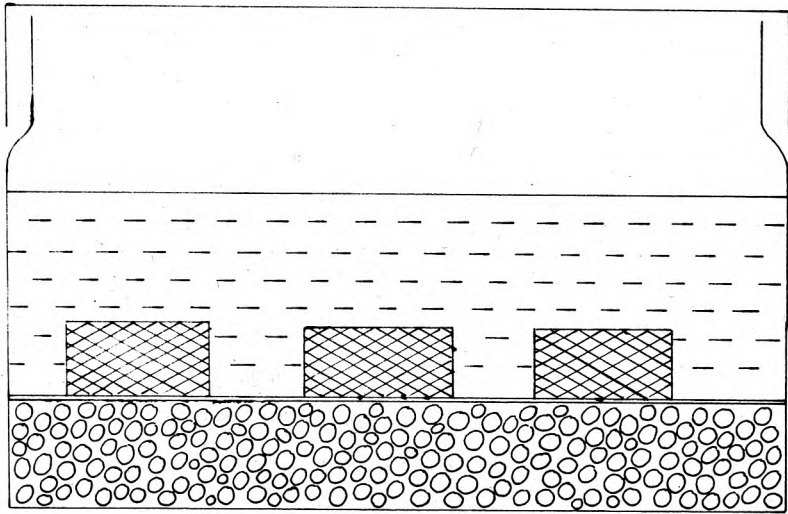


FIGURE 2

**Arrangement used for the Decalcification of Bone by
Formol-formic acid Solution in the Presence of an
Ion Exchange Resin.**



DECALCIFYING FLUID

BLOCKS OF TISSUE
FILTER PAPER DISC

ION EXCHANGE RESIN

FIGURE 3

The Distribution of Arteries to a Long Bone.

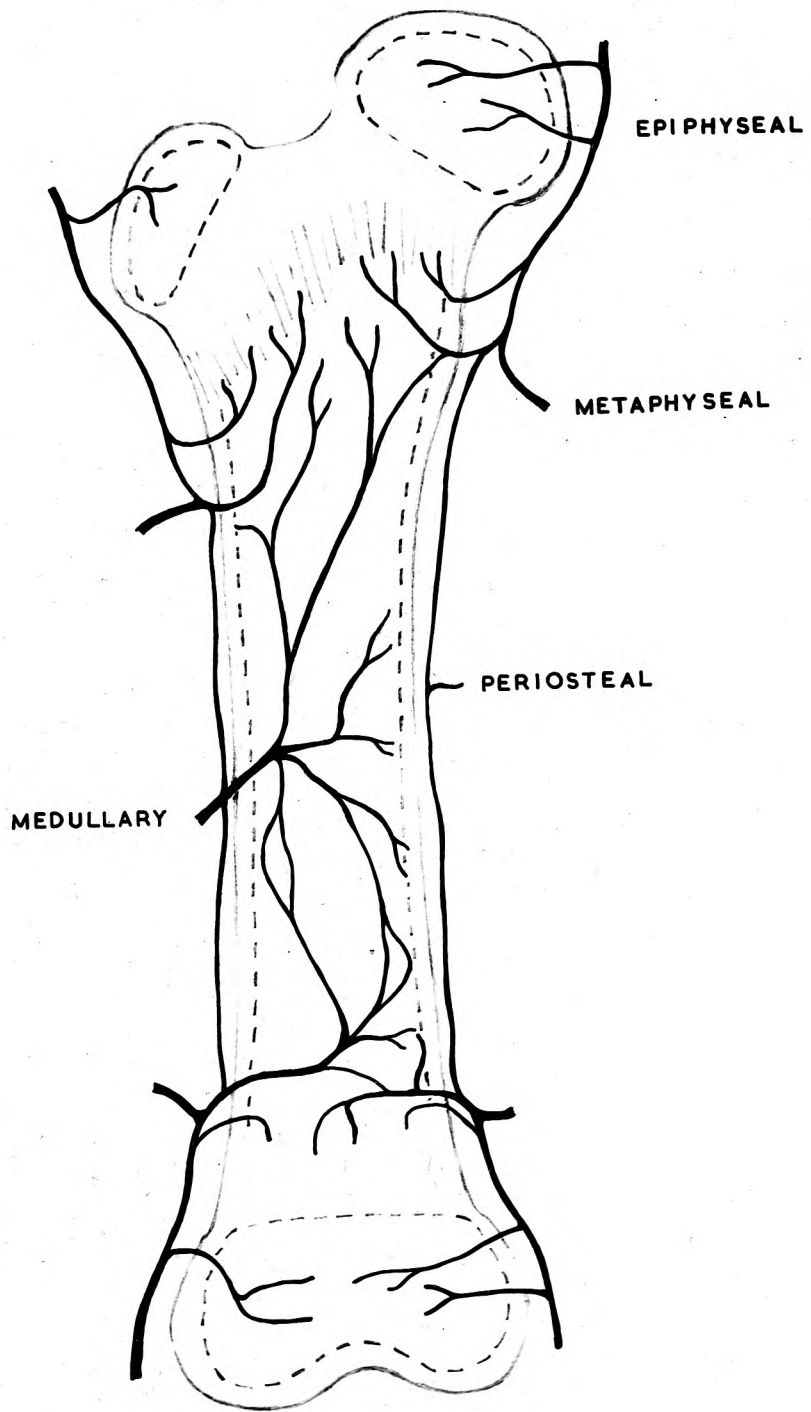


FIGURE 4

**The Posterior Aspect of the Stifle Joint Showing
the Origins of the Genicular Arteries. (Left Limb).**

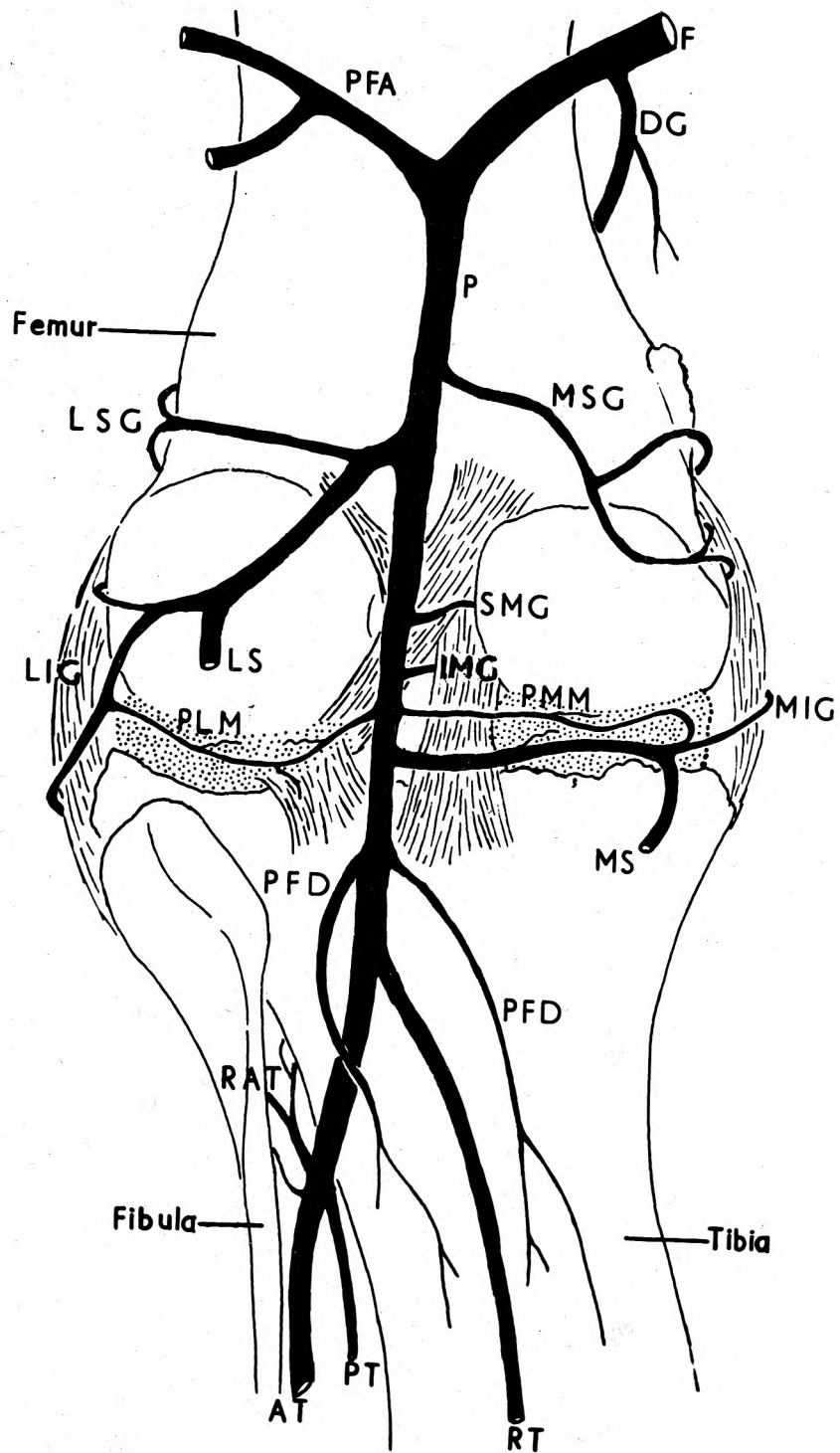


FIGURE 5

The Proximal End of the Tibia Showing the Blood Supply
to the Menisci. (Right Limb).

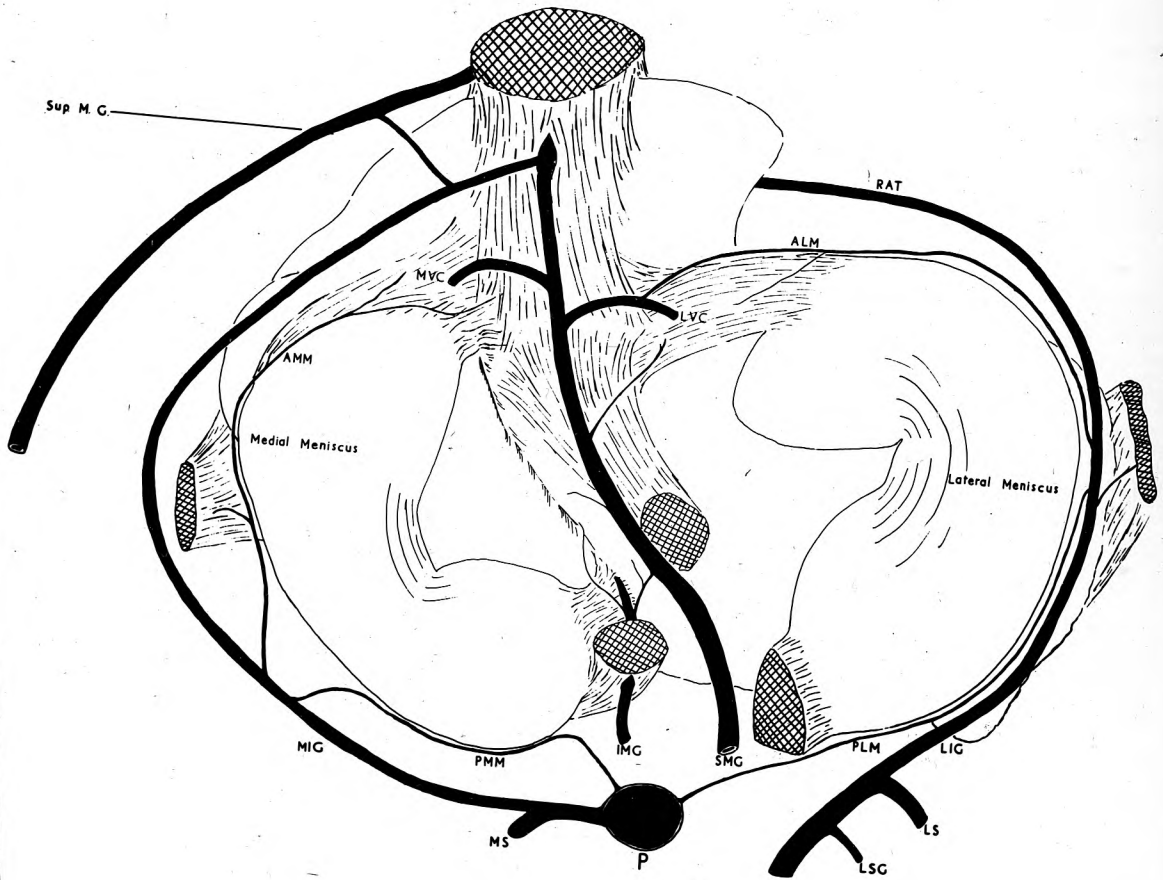


FIGURE 6

**The Distribution of Arteries over the Anterior
Aspect of the Stifle Joint. (Right Limb).**

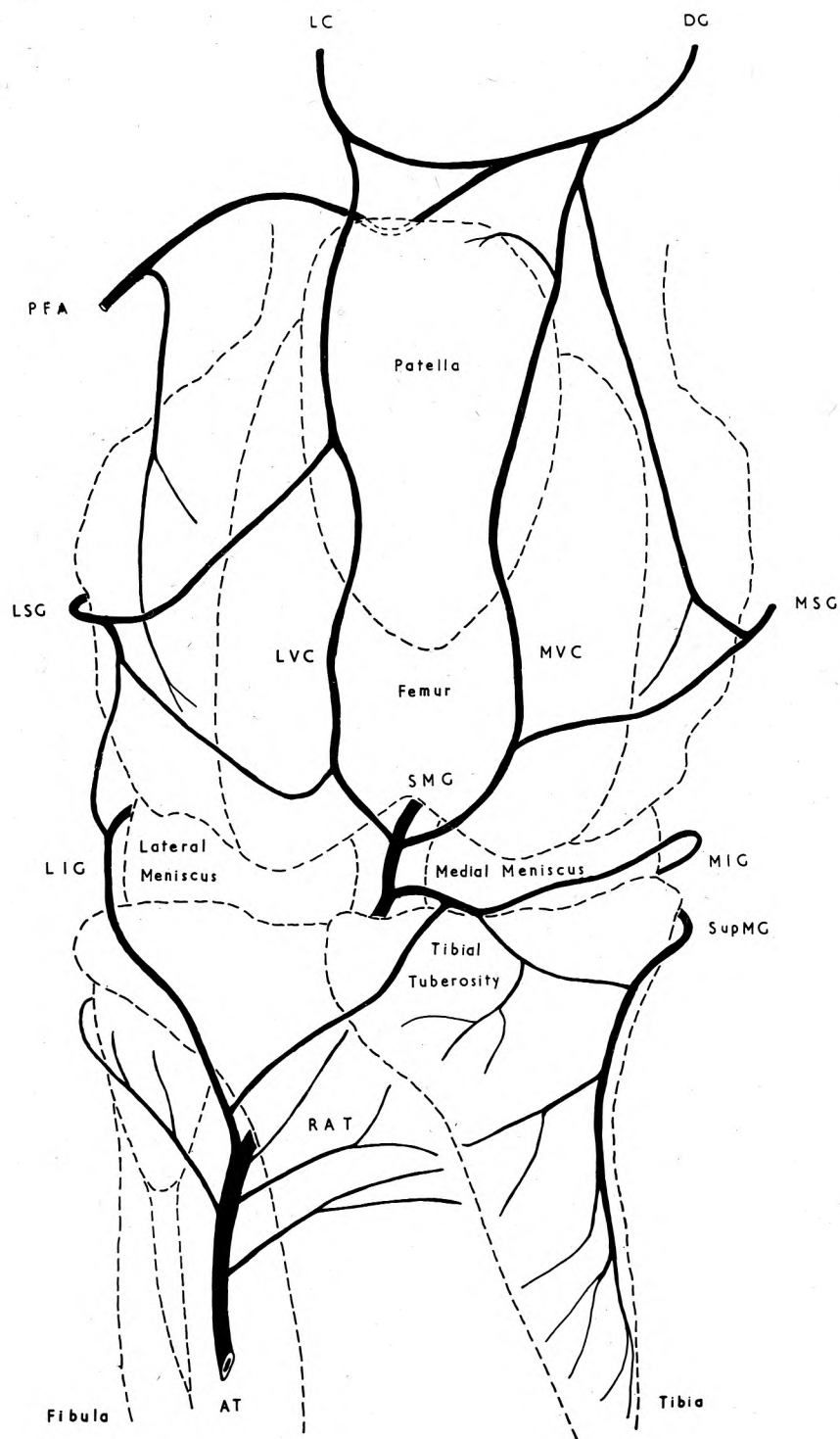
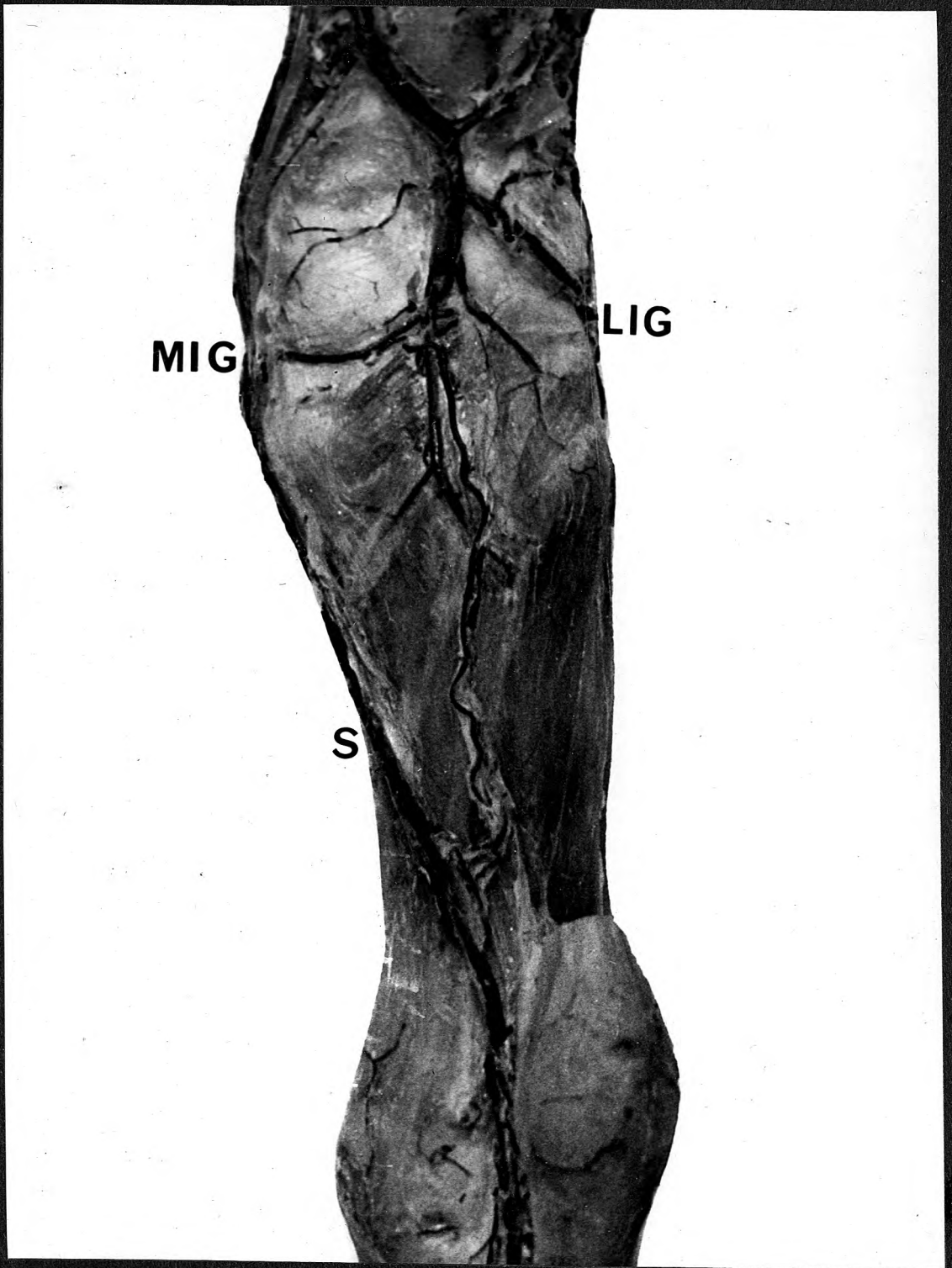


FIGURE 7

The Posterior Aspect of a Right Hind Limb.
(Arteries Latex Injected).

This photograph shows the vessels illustrated - Figure 4.



MIG

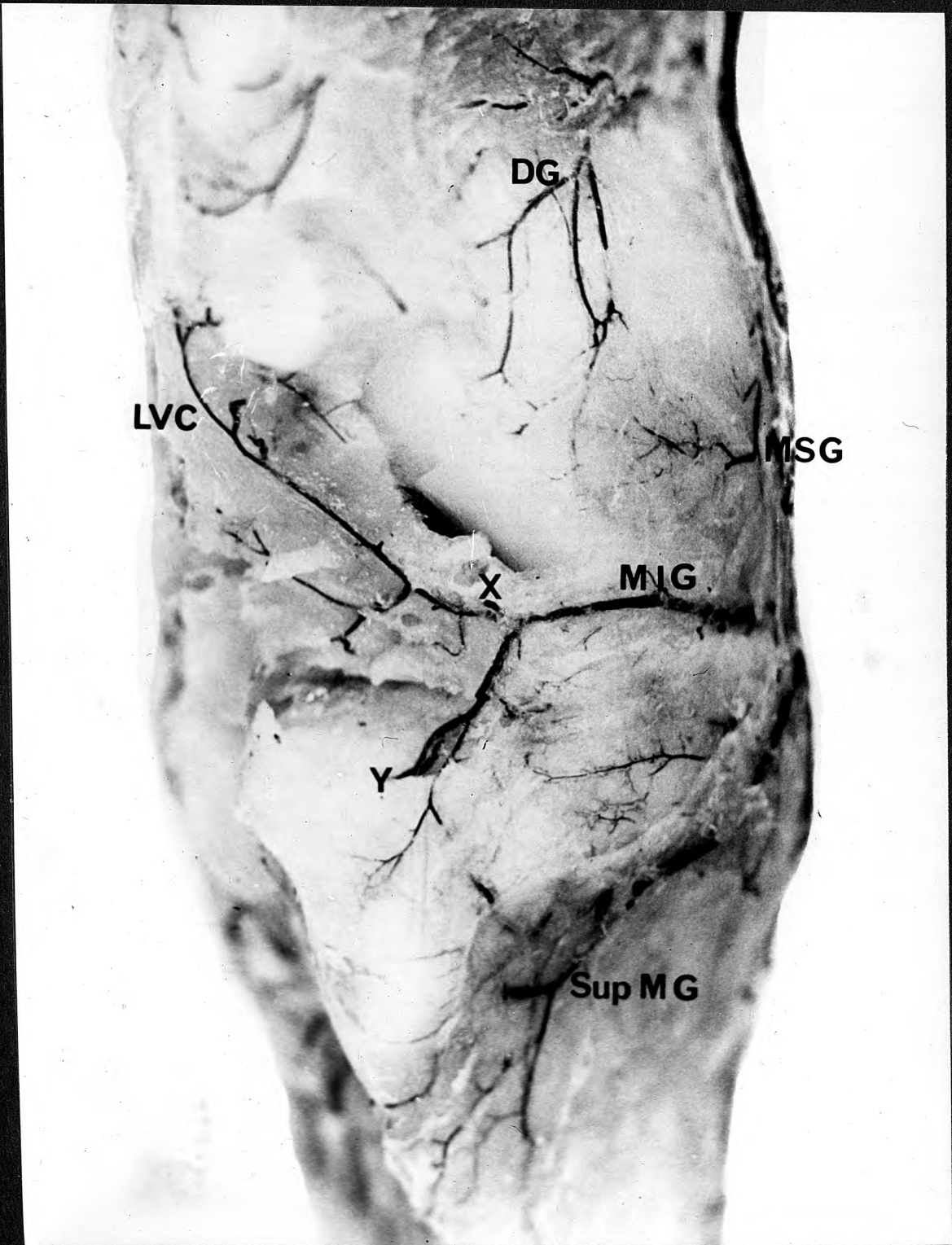
S

LIG

FIGURE 8

**The Anterior Aspect of the Right Stifle Region.
(Arteries Latex Injected).**

- X - Superior Middle Genicular artery (SMG) emerging from between the femoral condyles.
- Y - Artery entering foramen at the junction of the proximal epiphysis of the tibia with the epiphysis of the tibial tuberosity.



DG

LVC

MSG

X

MIG

Y

Sup MG

FIGURE 9

The Anterior Aspect of the Left Stifle Joint.
(Arteries Latex Injected).

- Y - Artery entering foramen at the junction of the proximal epiphysis of the tibia with the epiphysis of the tibial tuberosity.
- Z - Cut end of the Medial Vertical channel (MVC).

In this specimen the Medial Inferior Genicular artery (MIG), not the Superior Middle Genicular artery (SMG), forms the Medial and Lateral Vertical channels (MVC and LVC).

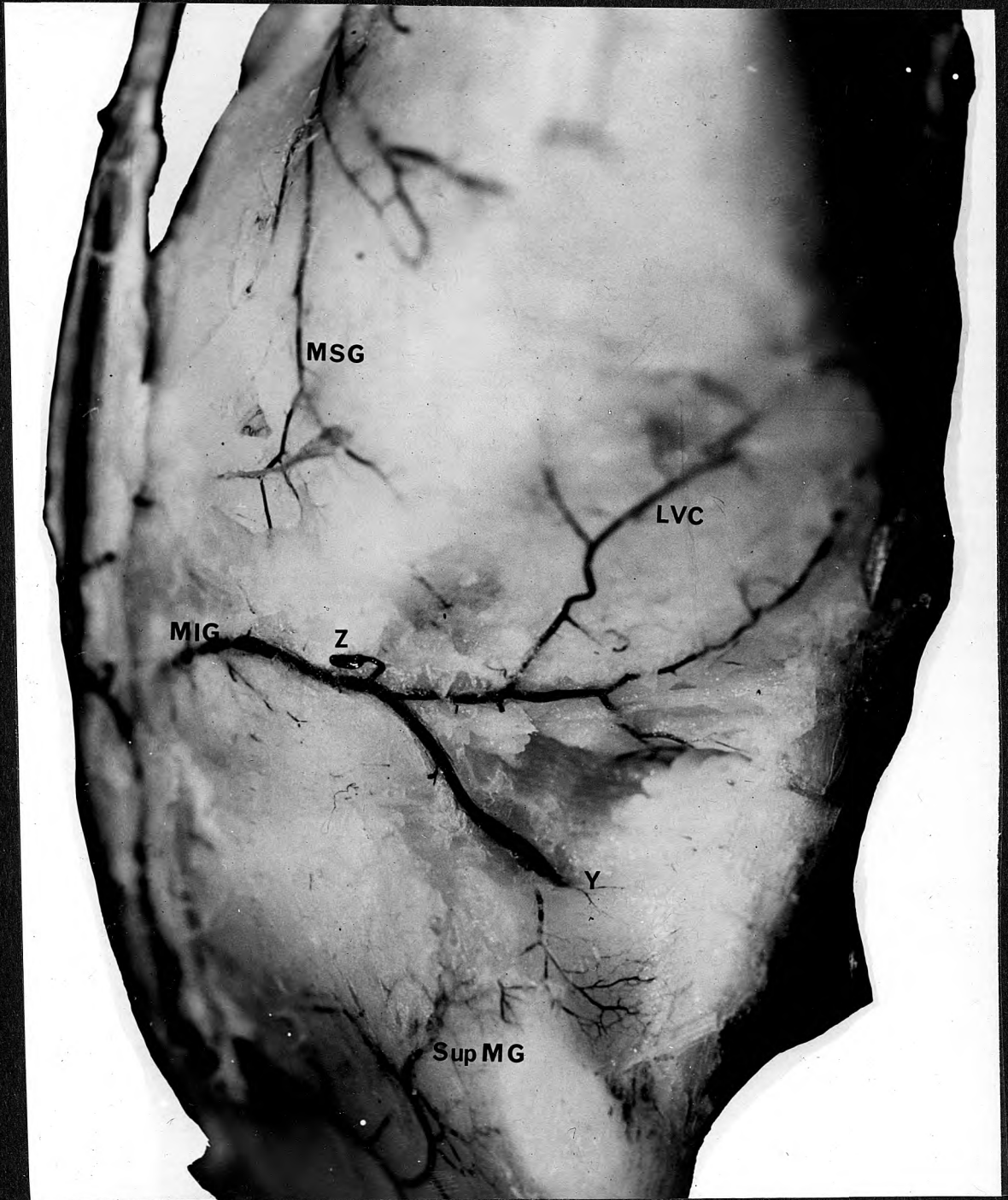


FIGURE 10

The Lateral Aspect of the Right Stifle Region
(Arteries Latex Injected).

The anastomosis between the Lateral Inferior Genicular artery
(LIG) and a recurrent branch of the Anterior Tibial artery (RAT)
is shown.

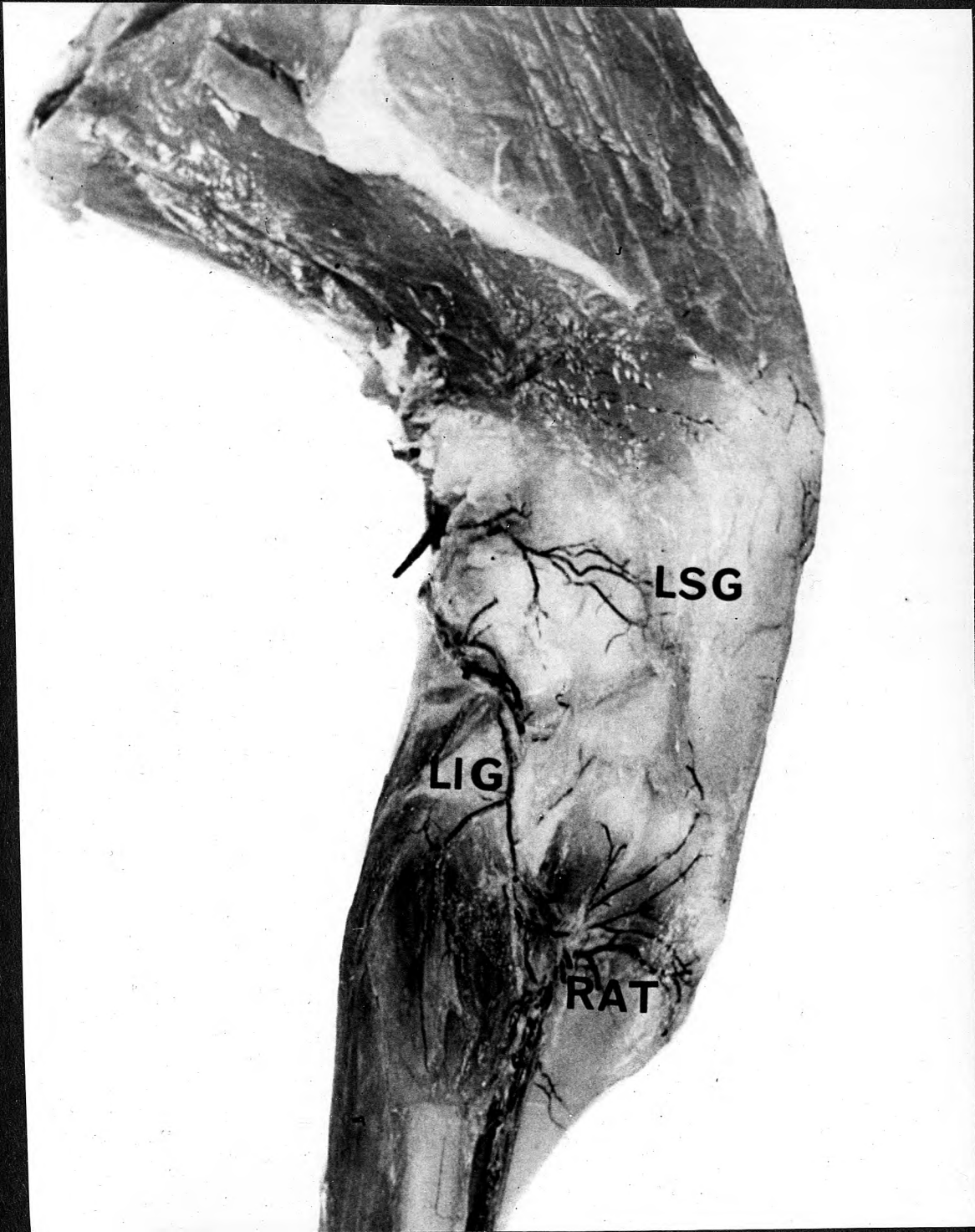


FIGURE 11

**The Lateral Aspect of the Left Hind Limb.
(Arteries Latex Injected).**

An ascending branch of the Posterior Femoral artery (PFA) is distributed over the distal epiphysis of the femur close to the lateral ridge of the trochlea.

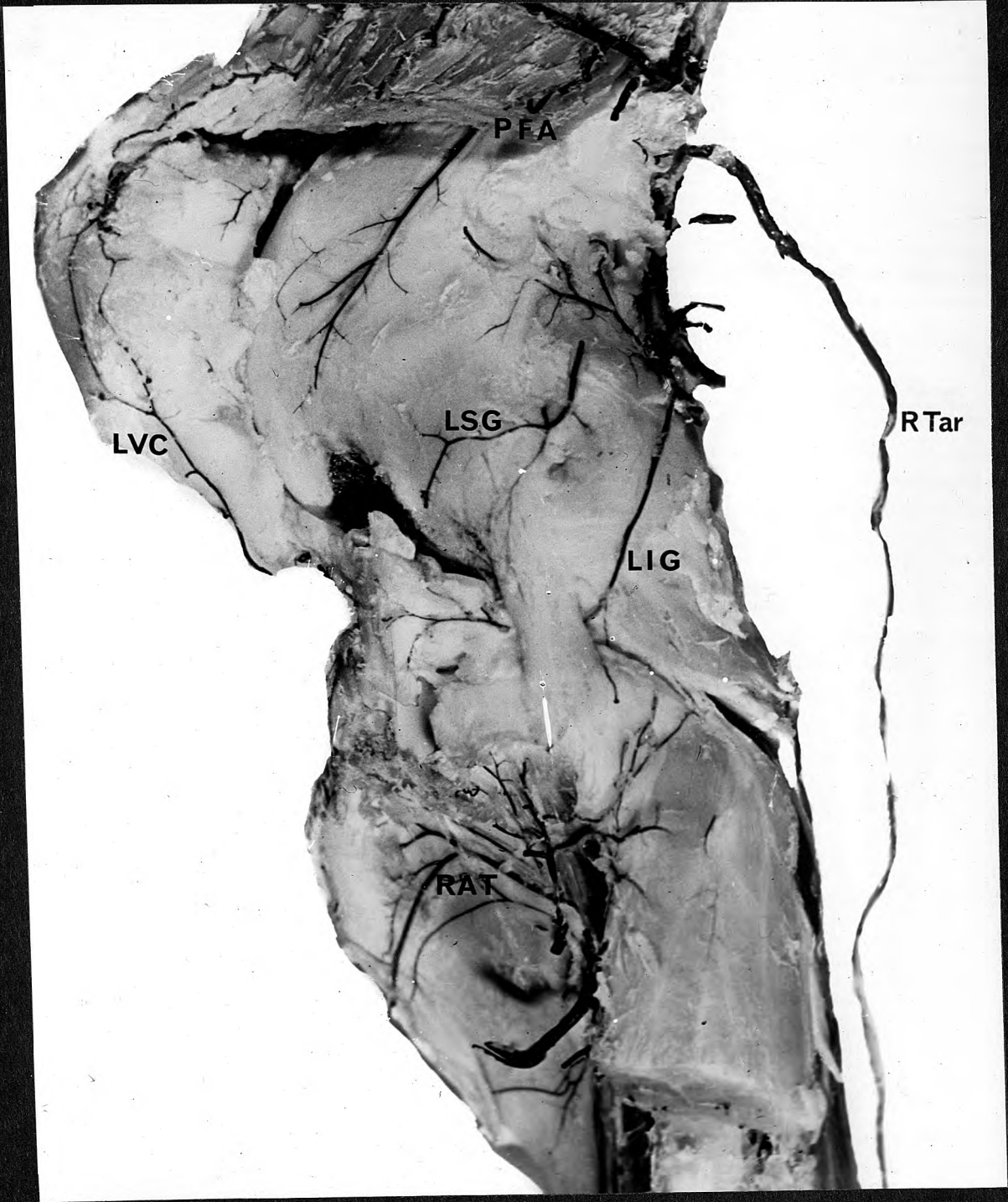


FIGURE 12

The Lateral Aspect of the Left Stifle Region.
(Arteries Latex Injected).

The anastomosis between the Lateral Inferior Genicular artery (LIG) and a recurrent branch of the Anterior Tibial artery (RAT) is shown.

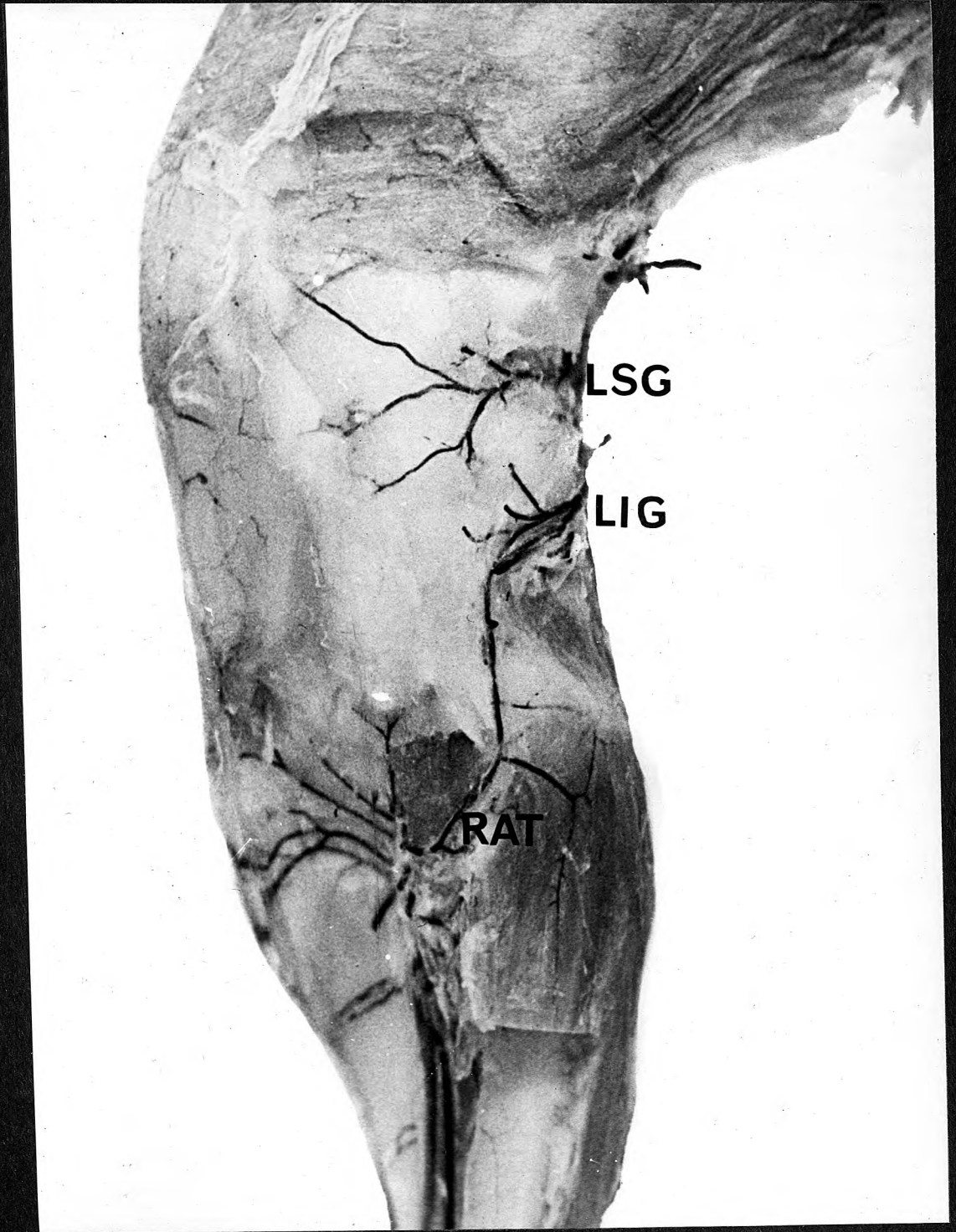
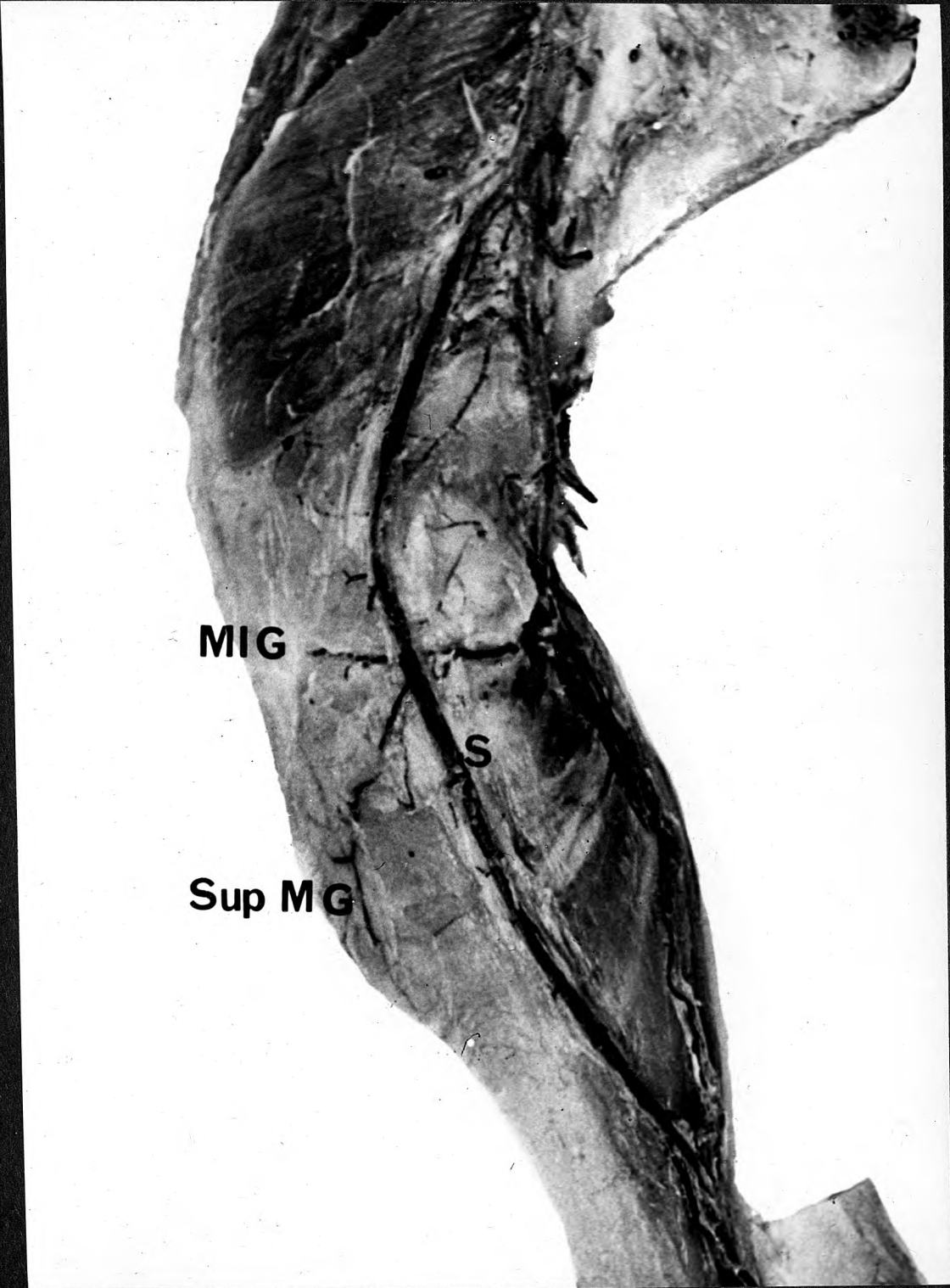


FIGURE 13

The Medial Aspect of the Right Stifle Region.
(Arteries Latex Injected).



MIG

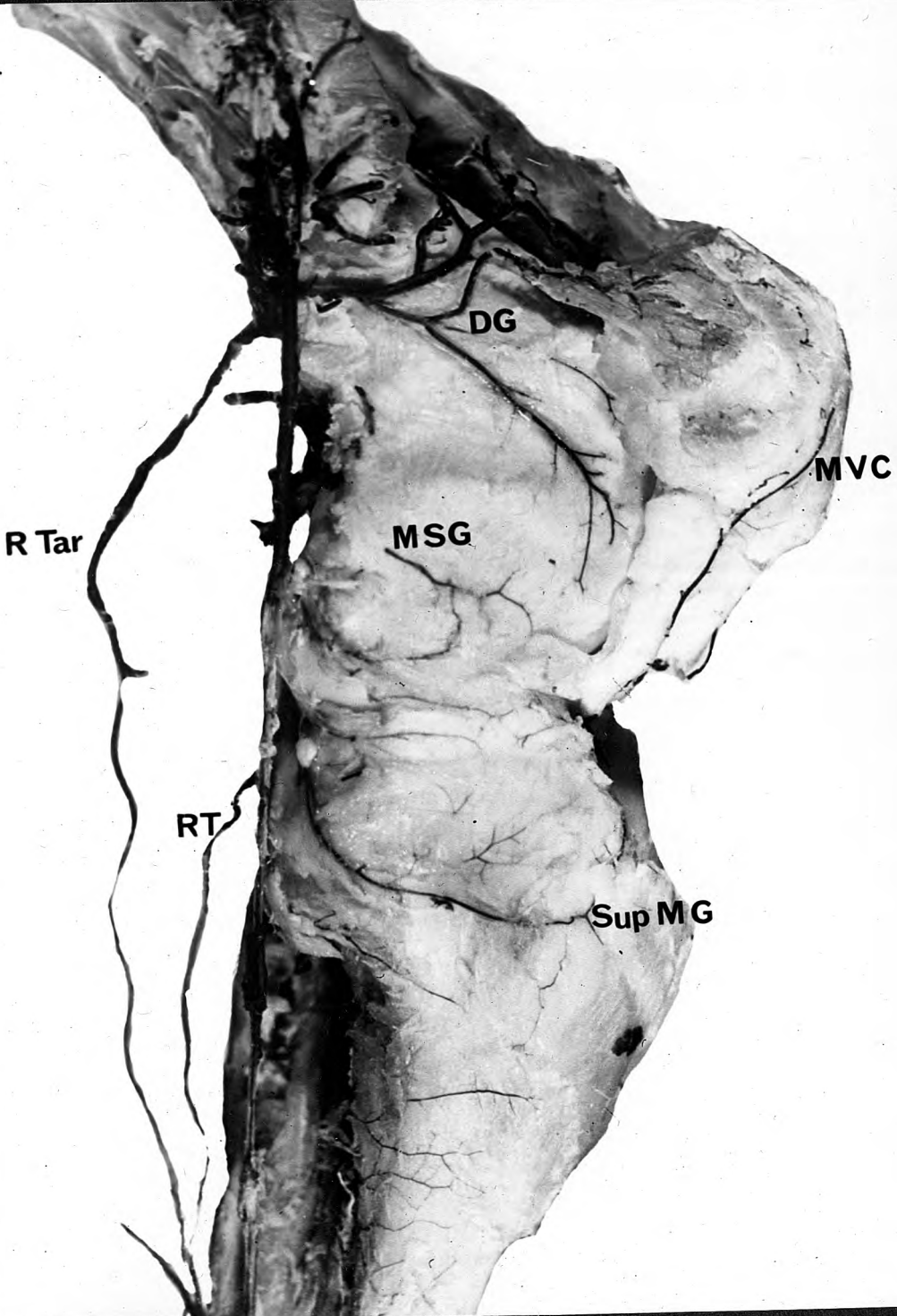
S

Sup MG

FIGURE 14

The Medial Aspect of the Left Hind Limb
(Arteries Latex Injected).

Large branches of the Descending Genicular Artery (DG) run proximal to the patella towards the lateral aspect where they anastomose with branches of the Lateral Circumflex Femoral artery (LC) and ascending branches of the Posterior Femoral artery (PFA).



R Tar

DG

MVC

MSG

RT

Sup MG

FIGURE 15

**The Medial Aspect of the Left Stifle Region.
(Arteries Latex Injected).**

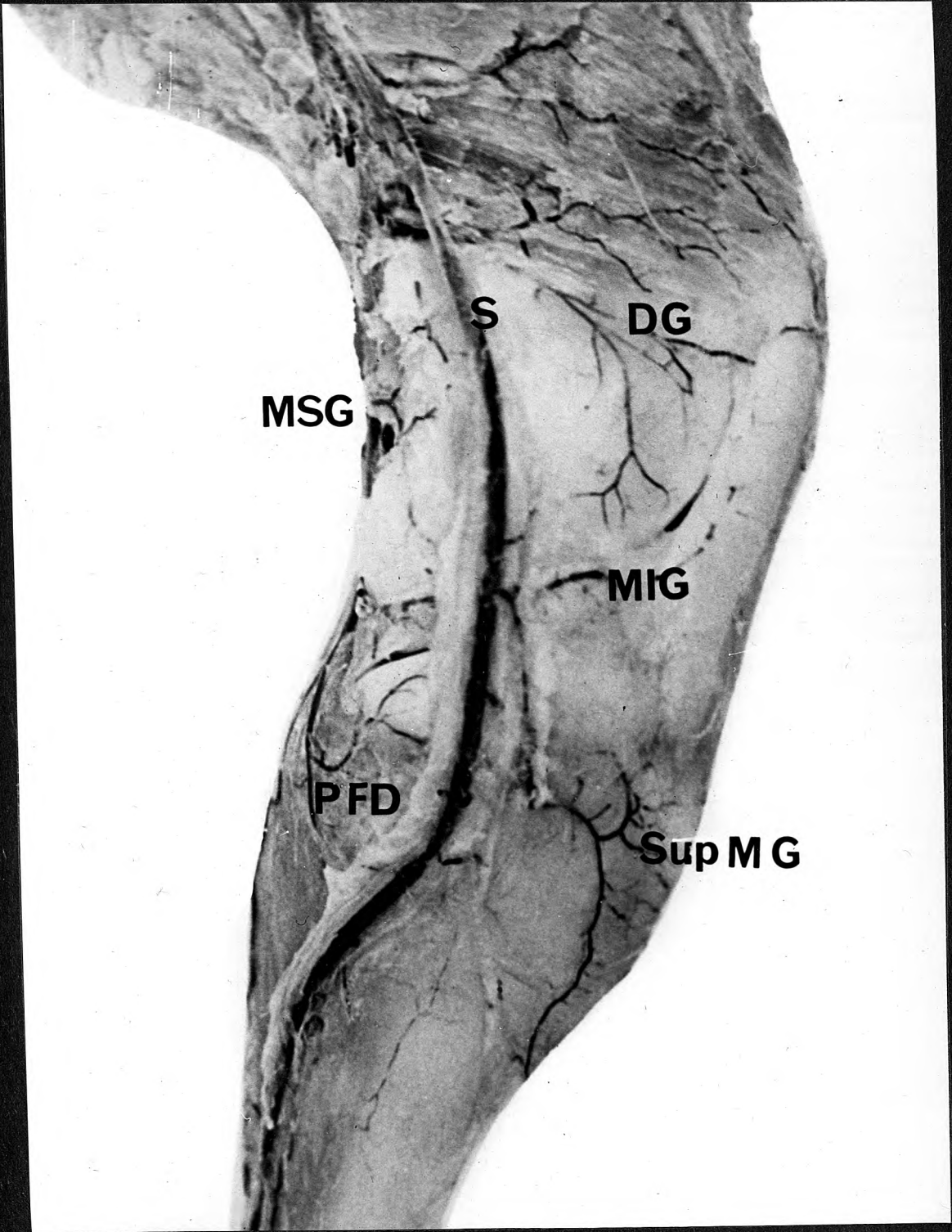


FIGURE 16

The Regional Distribution of Arteries to the Periosteum of the Lateral Aspect of the Distal Epiphysis of the Femur and Patella. (Left Limb).

FIGURE 17

The Regional Distribution of Arteries to the Periosteum of the Medial Aspect of the Distal Epiphysis of the Femur and Patella. (Left Limb).

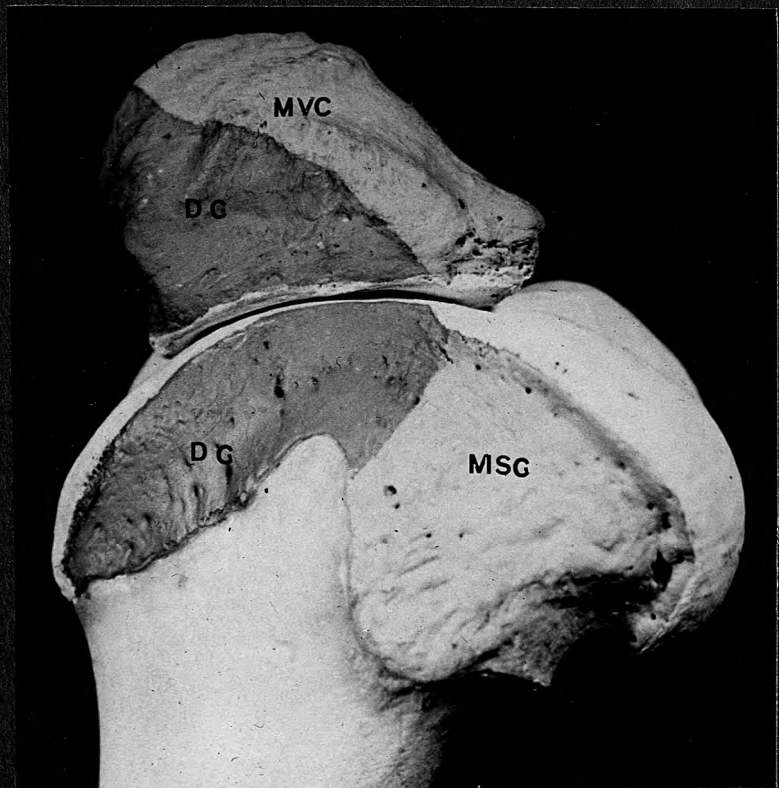
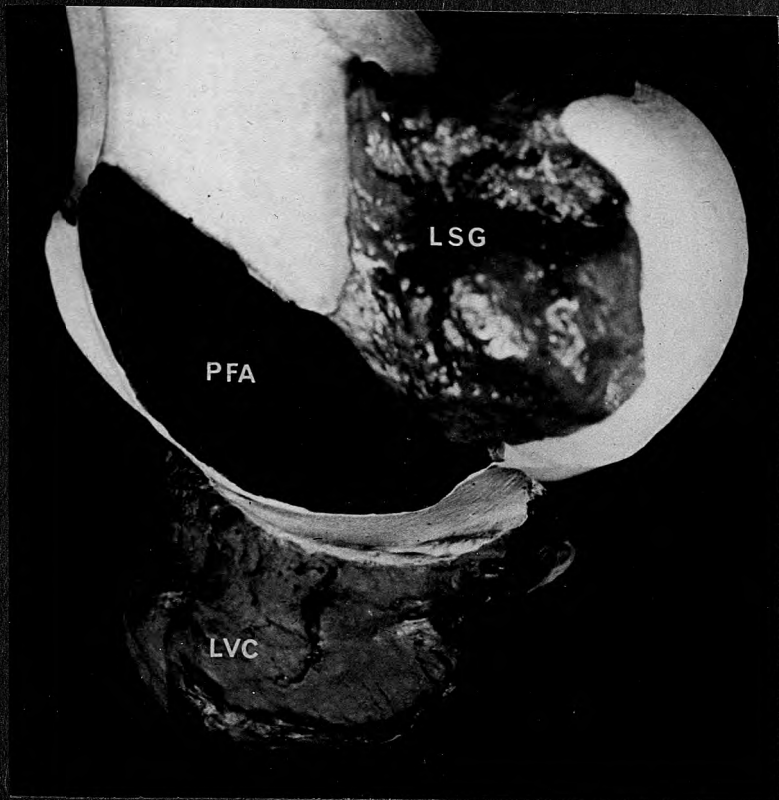


FIGURE 18

The Regional Distribution of Arteries to the Periosteum of the Anterior Aspect of the Proximal Epiphysis of the Tibia. (Left Limb).

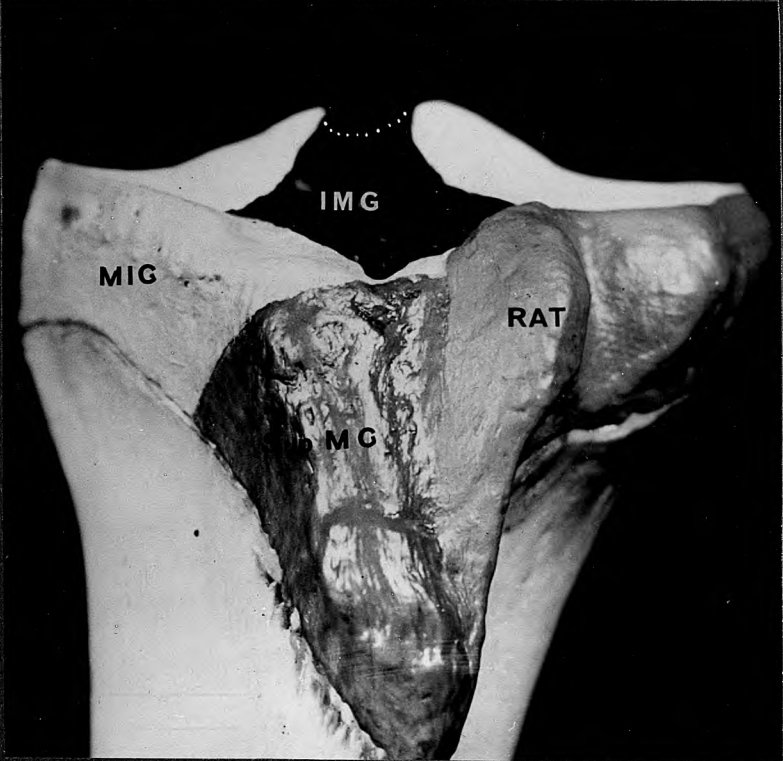


FIGURE 19

The Regional Distribution of Arteries to the Periosteum of the Posterior Aspect of the Distal Epiphysis of the Femur. (Left Limb).

FIGURE 20

The Regional Distribution of Arteries to the Periosteum of the Posterior Aspect of the Proximal Epiphysis of the Tibia. (Left Limb).

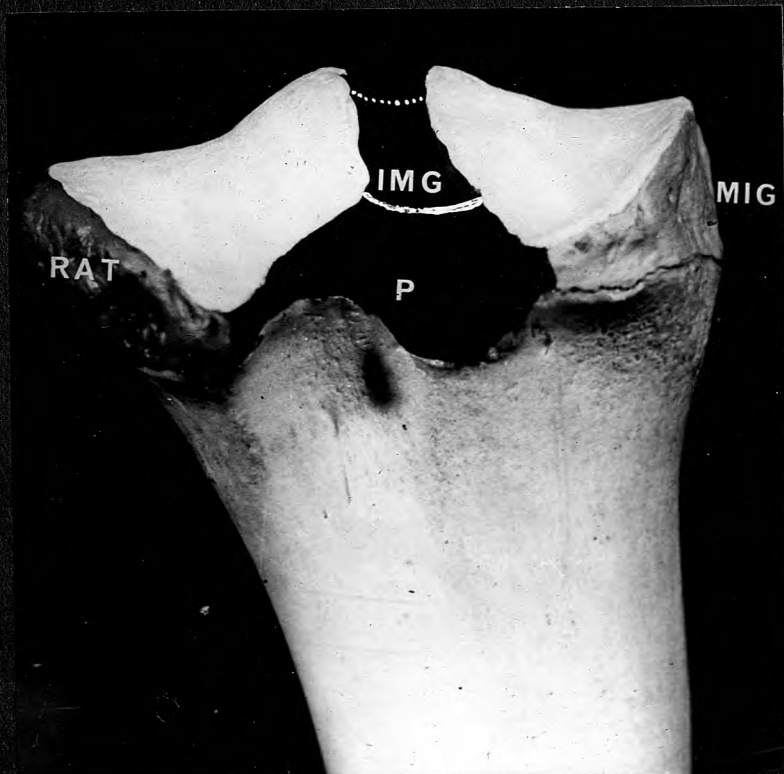
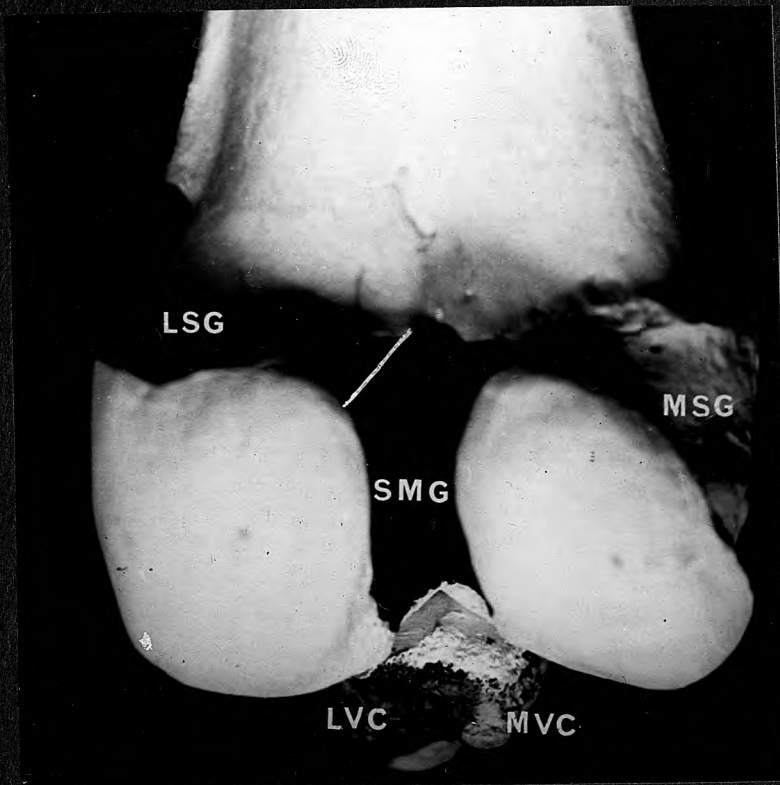


FIGURE 21

**Latero-medial Angiogram of Right Hind Limb of a
Four Week Old Pig**

**Injected with 75% "Micropaque" at 200 mm Hg; 65 KV;
100 m/a; 0.12 sec.**



FIGURE 22

Latero-medial Angiogram of Left Hind Limb of a
Three Month Old Pig

Injected with 100% "Micropaque" at 200 mm Hg; 65 KV;
100 m/a; 0.2 sec.



FIGURE 23

Section of Proximal End of Tibia Showing the Arteries
within the Epiphysis

(x 3.5). Radio-opaque injected and cleared.



FIGURE 24

Section of Proximal End of Tibia

In the upper portion of the photograph the rich arterial supply may be seen in the epiphysis, while the lower part, which is the metaphysis, is supplied by only fine, elongated end arteries from the shaft of the bone.

(x 6). Radio-opaque injected and cleared.

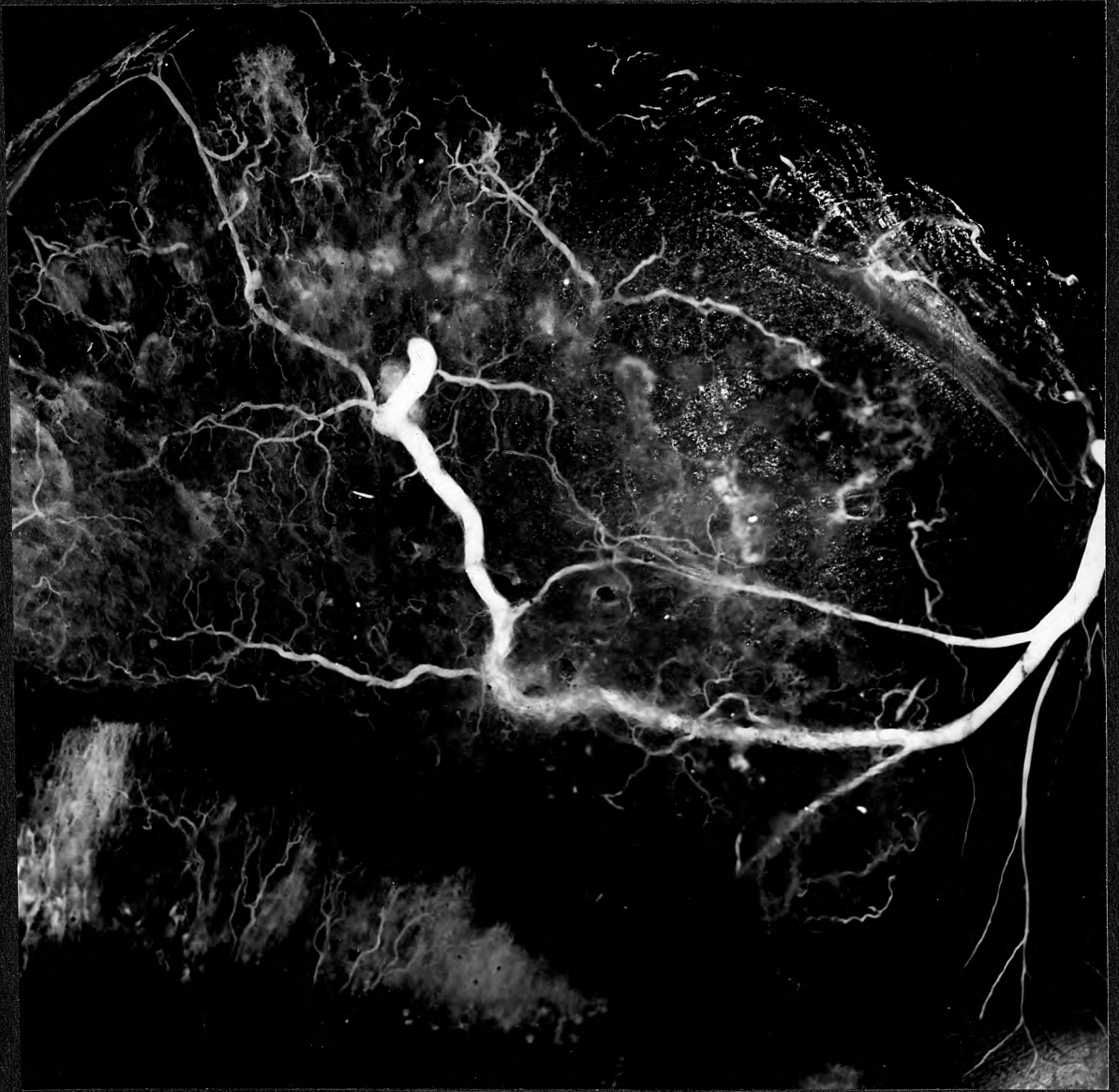


FIGURE 25

Section of Proximal End of Tibia

A large epiphyseal artery runs across the upper portion of the photograph; from it arterioles arise, which, after forming numerous anastomoses with each other, send capillary loops into the cartilaginous plate. On the metaphyseal side of the plate the injection mass has passed through the capillaries and filled the venous sinusoids of the bone marrow.

(x 10). Radio-opaque injected and cleared.

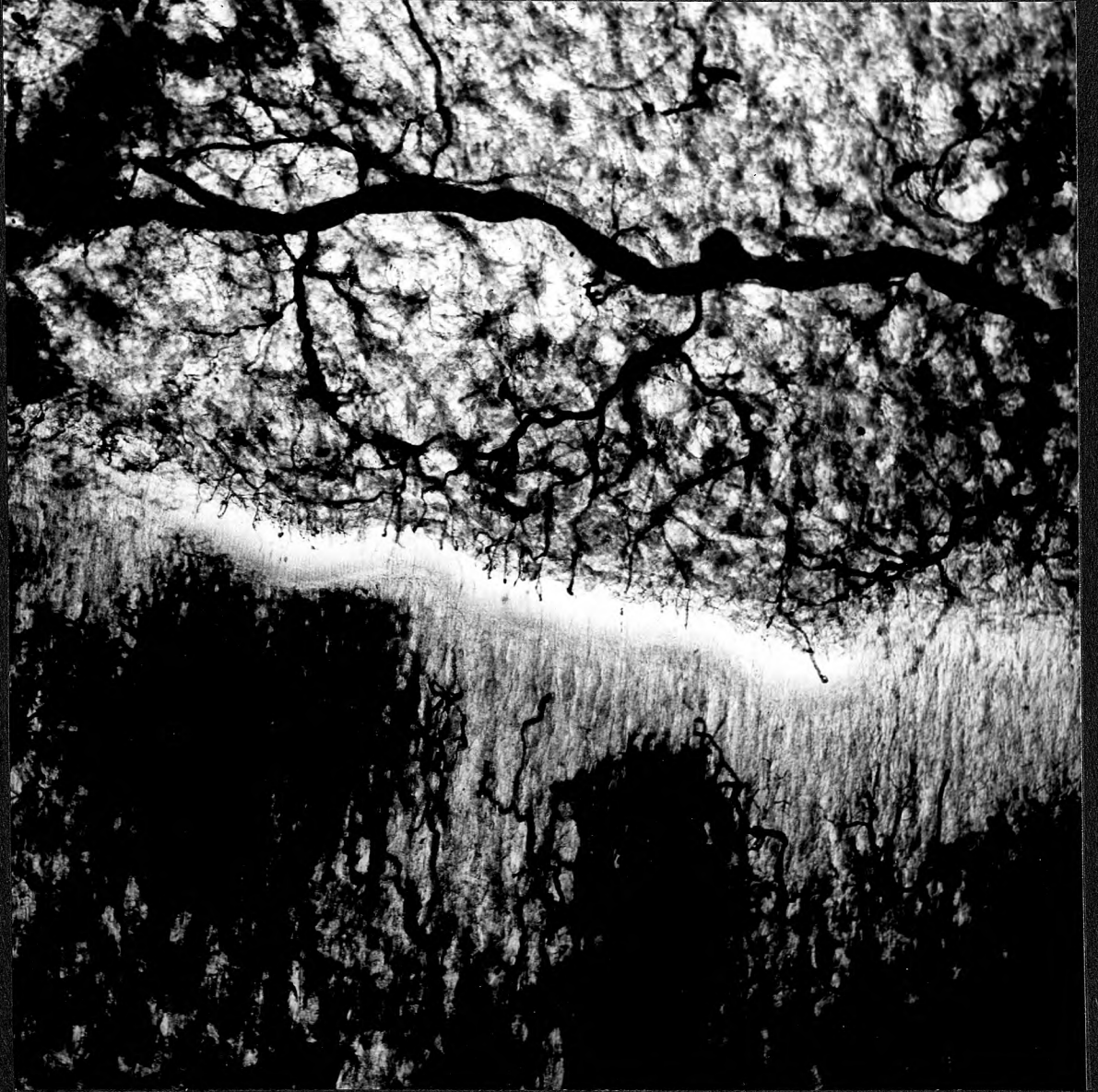


FIGURE 26

Section of Proximal End of Tibia

Arterioles and capillary loops may be seen entering the cartilaginous substance of the plate. Note the dilated ends to some of the arterioles and the numerous anastomoses formed upon the epiphyseal surface of the cartilaginous plate.

(x 25). Radio-opaque injected and cleared.



FIGURE 27

Section of Proximal End of Radius

The expanded ends of the injected arterioles are easily seen within the epiphyseal plate

(x 50). Radio-opaque injected and cleared.



FIGURE 28

Section of Proximal End of Tibia

A capillary bed is visible within the bony centre of the epiphysis. The lower part of the photograph shows the vascular channels within the epiphyseal plate and the upper shows the vascular channels which penetrate the region of the articular cartilages.

(x 25). Radio-opaque injected and cleared.



FIGURE 29

Section of Proximal End of Tibia

The loop formed by the anastomosis of a metaphyseal with a medullary artery gives rise to numerous longitudinal metaphyseal arteries, which are end arteries.

(x 6). Radio-opaque injected and cleared.



FIGURE 30

Section of Metaphysis from Proximal End of Tibia

Arterial loops from the medulla of the bone send longitudinal metaphyseal arteries to supply the metaphyseal region.

(x 10). Radio-opaque injected and cleared.



FIGURE 31

Section of Metaphysis

Capillaries entering lacunae left by hypertrophied cartilage cells. The blood seems to empty directly into the venous sinusoids of the bone marrow.

(x 1,120). Masson's Trichrome.

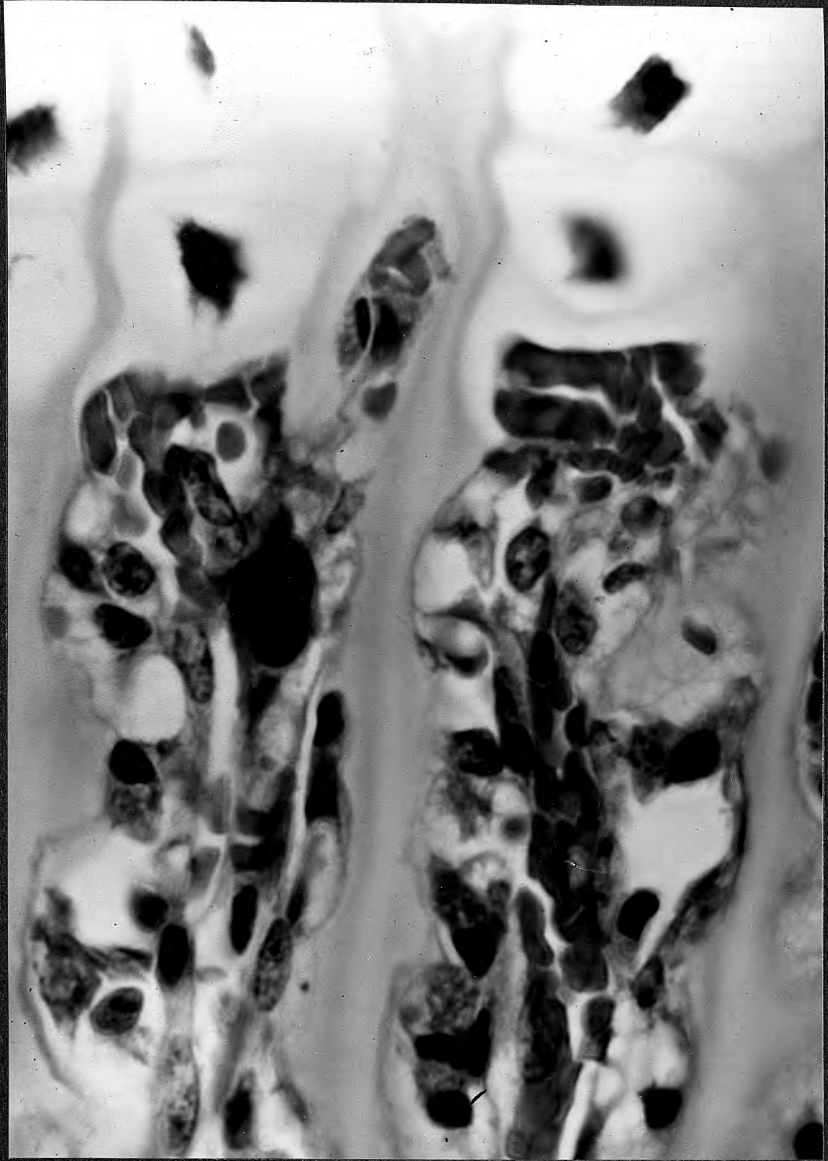


FIGURE 32

Epiphyseal Cartilage Showing Vascular Canals

(x 400). Lison's Alcian Blue.

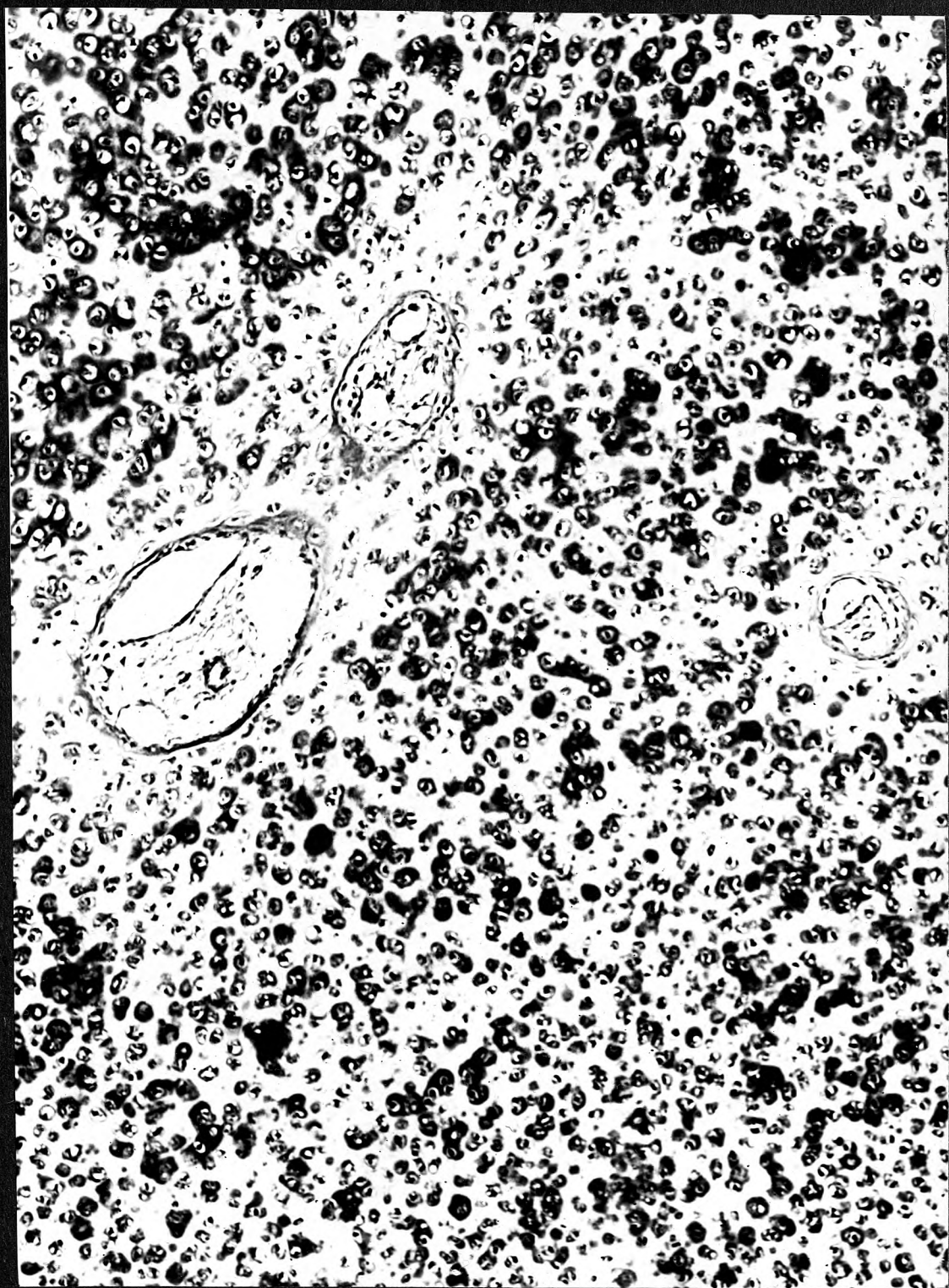


FIGURE 33

Transverse Section of Cartilage Canal

An arteriole, pre-venules and a capillary can be seen within the connective tissue of the canal.

(x 1,300). Lison's Alcian Blue.



FIGURE 34

Transverse Section of Large Cartilage Canal

This canal contains a large arteriole, probably one of the vessels running into and supplying the epiphysis. Arterial precapillaries, pre venules and capillaries may be seen within this canal.

(x 500). Lison's Alcian Blue.



FIGURE 35

Longitudinal Section of Cartilage Canal

The arteriole is central and the pre-venules are peripheral. The walls of the venous vessels contain a few smooth muscle cells but no phagocytic cells, so they are pre-venules rather than sinusoids.

(x 1,880). Lison's Alcian Blue.



FIGURE 36

The Termination of a Cartilage Canal

The left half of the photograph shows a canal cut in longitudinal section, the collagen extends from the walls of the canal as a connective tissue septum which runs towards the metaphysis. The right half of the photograph shows a connective tissue septum which has been sectioned obliquely.

(x 330). Lison's Alcian Blue.



FIGURE 37

The Termination of a Cartilage Canal

The collagen of the walls forms connective tissue septa within the zone of proliferating cartilage. The columns of cartilage cells curve characteristically towards the canal.

The arteriole (shaded) within the canal terminates by forming a capillary tuft which drains into a number of pre-venules.

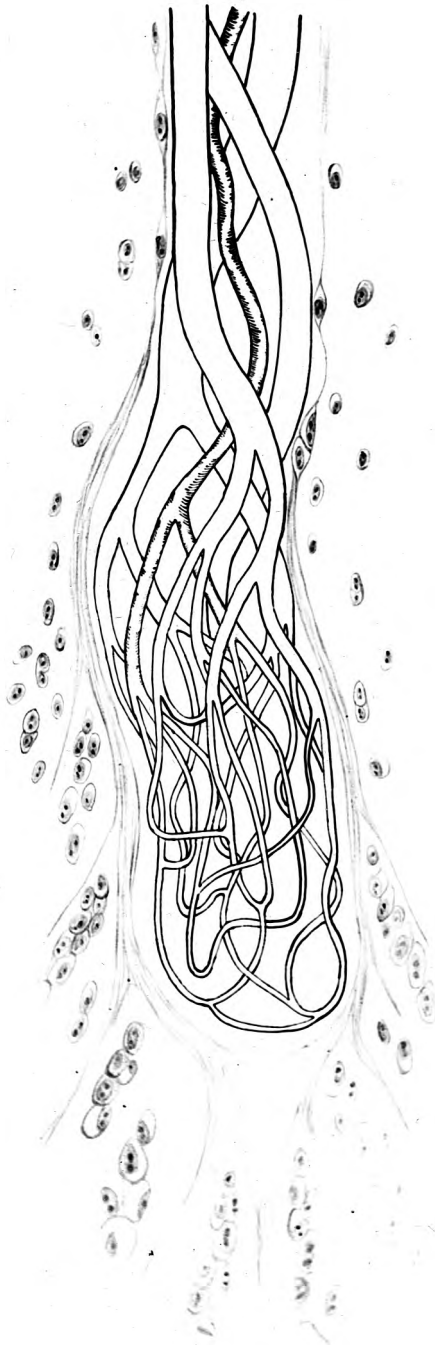


FIGURE 38

Epiphyseal Cartilage

Eosinophilic septa within the cartilage.

(x 180). Haematoxylin and Eosin.

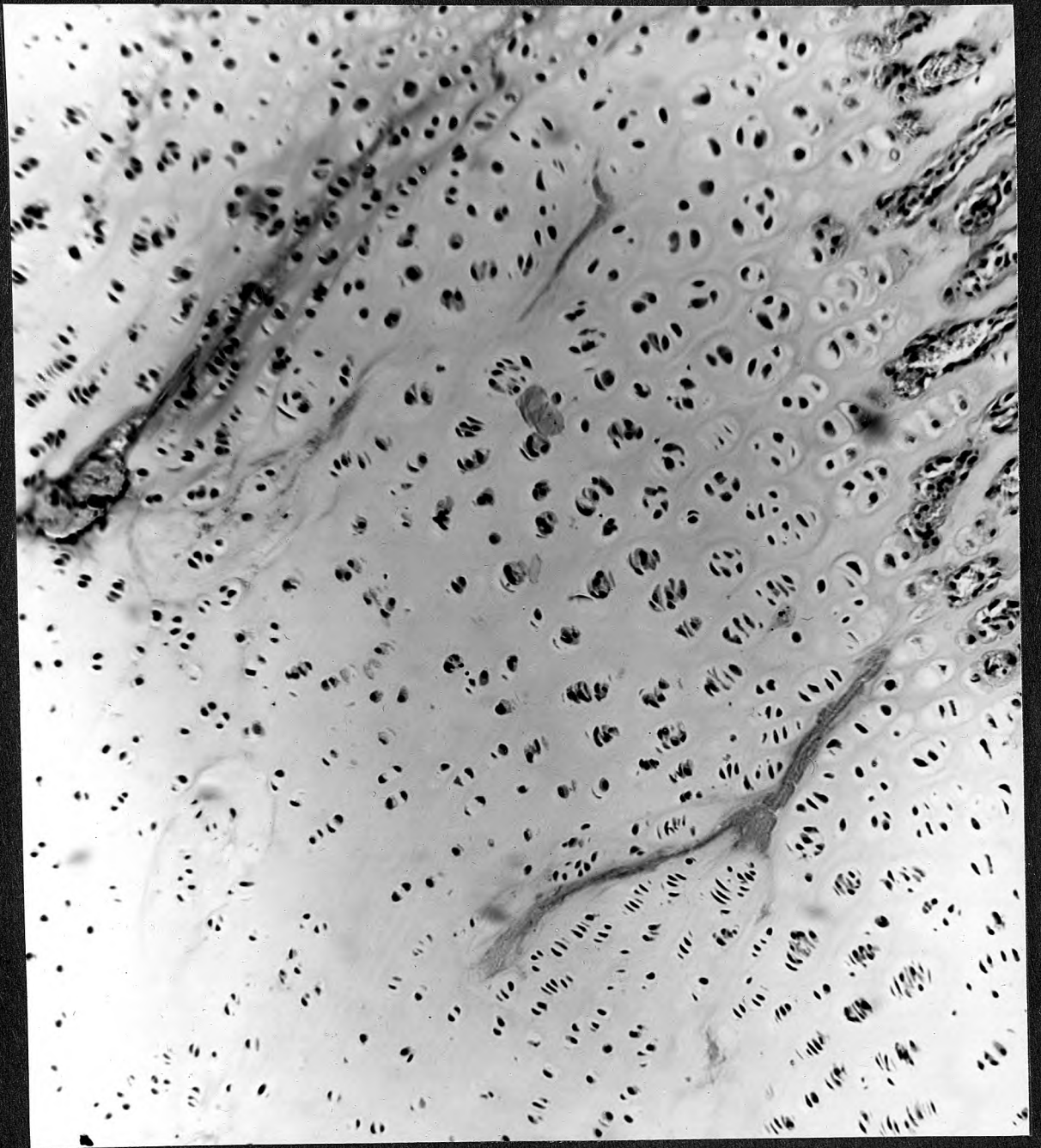


FIGURE 39

Transverse Section of Vascular Bud

This shows the tuft of blood vessels, mainly capillaries and pre-venules, surrounded by mesenchymal cells, which is found at the termination of a vascular channel.

(x 1,300). Lison's Alcian Blue.

