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Development of the femur of the cat

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Preparation of Tissue for Microscopical Examination.

For the preparation of material for the microscopical study of the development of bone, the following methods and processes were used:-

Paraffine Method.

I Fixation and Decalcification of Tissue. Three methods were used in this stage. The first was the decalcification and fixation of the tissue at the same time by means of chromic acid. The second was the use of chromic with the addition of nitric acid to hasten decalcification. The third method was the fixation of the tissue with Muller's fluid and decalcification by means of V. von Ebner's hydrochloric acid and sodium chloride method. The Muller's fluid was changed as soon as it became turbid and was renewed from day to day to maintain perfect transparency. The tissue remained in the fluids from two to four weeks and that in chromic acid was transferred to distilled water for a short time

II Hardening of Tissue. After the fixation and the decalcification, the tissue was placed in seventy per cent. alcohol and kept in the dark, the alcohol being changed whenever it became deeply tinged until it remained colorless.

III. Staining of tissue. Following the hardening, the tissue was transferred for staining to borax carmine or Delafield's hæmatoxylin from twenty-four to forty-eight hours. The principal advantage in using the carmine stain was its great penetrating properties, that it did not overstain and that the stain was permanent. Hæmatoxylin, on the other hand, was found feeble in its power of penetration and there was a possibility of overstaining.

IV. Differentiation of Stain. When borax carmine was used, the tissue was transferred to acid alcohol for twenty-four hours. The object of the acid solution was to effect differentiation and fixation of color. When hæmatoxylin was used, the tissue was transferred to distilled water for twenty-four hours.

V. Dehydration and Saturation with Chloroform. The tissue was subsequently changed to seventy per cent. alcohol-twenty four hours-then to ninety per cent-twenty four hours-from ninety to ninety-five twenty-four hours; and was then dehydrated in absolute alcohol twenty four hours. The latter step was very important, because it was first necessary to free the tissue of all traces of water in order to insure the free entrance of chloroform with which paraffin is perfectly miscible. The tissue was left in pure chloroform until the alcohol was replaced by chloroform which was indicated by the tissue sinking toward the bottom of the bottle.

from the chloroform, the tissue was introduced into a saturated solution of paraffine in chloroform, from two to three hours.

VI. Embedding. From the solution of paraffine in chloroform, the tissue was removed to pure melted paraffine which had a melting point of about fifty degrees Centigrade, until it had ban completely filled with the embedding mass and all the chloroform had ban driven off. The latter point was determined by bubbles failing to follow the introduction of a heated iron rod into the melted paraffine. When all the chloroform had ben driven off, the tissue was removed to another solution of pure melted paraffine of proper consistence for cutting for thirty minutes and cooled rapidly.

VII. Mounting. After embedding, the blocks were trimmed suitably for cutting, sectioned in microtome and sections were fixed to slide by collodion over oil. The paraffine was removed by benzole, the balsam applied and cover glass lowered in position.

Celloidin Method

I. Fixation and Decalcification of Tissue. The processes used were the same as in the paraffine method

II. Dehydration and Infiltration with Ether. After fixation and decalcification, the tissue was run through the ascending series of alcohols, thoroughly dehydrated by absolute alcohol and placed in a mixture of equal

parts of alcohol and ether.

III Embedding. From the solution of alcohol and ether, the tissue was transferred to celloidin of the consistence of a thick syrup for several days. The tissue was removed to small box moulds, covered with a solution of celloidin and hardened by means of seventy per cent. alcohol.

IV Cutting. The cutting was done by means of the "Slidunk" microtome, while both knife and tissue were flooded with seventy per cent. alcohol, the sections being immediately transferred to seventy per cent. alcohol.

V Staining. Dehydration and Mounting of Tissue. From the seventy per cent. alcohol, the sections were placed in hæmatoxylin - ten to fifteen minutes - then in eosin - two to three minutes - and washed in distilled water - five minutes. The sections were dehydrated by the ascending series of alcohols, placed in chloroform for clearing and mounted in balsam.

Development of the Femur.

The development of bone includes a series of phenomena whose sequence is determined by invariable laws. The composition and properties of foetal bone are entirely different from those of adult bone. The foundation of the skeleton is mapped out in an early embryonic stage, but we are able to recognize the parts representing the future bones only by their position and form. At an early period, the entire framework is almost uniform in structure. It consists of mesoblastic cells with a small amount of intercellular substance. Very soon however many bones are modeled in solid cartilage, surrounded by a membrane, the perichondrium, and resemble more or less the future bone.

In a long bone, the process of ossification commences in the centre and proceeds towards the extremities which for some time remain cartilaginous. The extremity does not, however, become joined to the shaft of the bone until growth has ceased, but remains separated by a layer of cartilaginous tissue termed epiphysial cartilage.

The first step toward the ossification of a cartilage bone is an enlargement of the

embryonal cartilage cells at the so called centres of ossification and the formation, at the same time, of a relatively larger amount of hyaline cartilage matrix. This is followed by a rearrangement of the enlarged cells into vertical rows, the cells becoming smaller the more distant they are removed from the centres of ossification. Between these tapering lines of cartilage cells, a calcareous deposit is formed. This deposit may also extend between the cells of the columns and thus the cells, either singly or in groups, are enclosed in calcified matrix. Such single cells and aggregations of cells are the primary areolæ of Sharpey. (Plate I Fig. 7.)

While this change is going on within the cartilage, an osseous deposit takes place on the outside of the cartilage immidiately beneath the perichondrium. This latter contains many osteoblasts, collected chiefly on the inner side next to the cartilage. It is through their agency that the bony layer is formed on the surface of the cartilage. The osteoblasts are the descendants of the mesoblastic cells of the embryo. Successive deposits of earthy material are laid down upon the surface of the cartilage and some of the osteoblasts are included between these, forming the bone cells of the lacunae.

The next step consists in the prolongation

of the vascular osteogenetic tissue of the periosteum into the middle of the cartilage, one or more apertures being excavated by absorption in the new formed bony layer. This process is known as the vascularization of the cartilage. Whenever these ingrowths come in contact with the calcified walls of the primary areolae, the latter are absorbed and form larger spaces called the secondary areolae of Sharpey or the medullary spaces of Müller. The cartilage cells abruptly disappear and their places are filled with embryonic marrow, consisting of osteoblasts and vessels derived from the osteogenetic layer of the periosteum. The fate of the cartilage cells has not been determined. Some histologists hold that after undergoing division they become osteoblasts; others that they are absorbed and have no part in the formation of bone. (Plate I, Fig. 7.)

Thus through the action of the osteogenetic layer of the periosteum, we have enlarged spaces, the secondary areolae, the walls of which are still formed by the calcified cartilage matrix. In many places the vertical partitions of calcified cartilage matrix, lying between the columns of cells remain and project into the marrow cavities as irregular trabeculae.

As the calcification of the cartilage matrix

extends toward the ends of the shaft; advancing always in the same manner, we find the osteoblastic tissue closely following, supplanting the cartilage cells of the primary areolae and absorbing parts of their walls, so as to throw two or more together to form secondary areolae. In this way, a great part of the primary bone is at once removed. (Plate I, Fig 7.)

The secondary areolae are further enlarged by a partial absorption of their walls, but at the same time the latter begin to thicken elsewhere by the deposition of layers of true bone. This deposition increases in amount gradually toward the middle of the shaft. Some of the osteoblasts arrange themselves on the surface of the new bone and through their agency, the walls of the space becomes gradually covered with osseous substance. Upon this new layers may be deposited. (Plate II.)

In the adult bone, some of these cavities with their walls remain to form the cancellous tissue of the extremities, but in the axis of the shaft of the bone nearly all of this structure is finally removed by absorption to give place to the medullary canal. This absorption and that of the primary areolae, in fact of all bone, is caused by the agency of certain large cells, which from their functions have been termed by Kölliker osteoclasts. (Plate II)

the marrow of the young bone is red in color. It is derived from the osteogenic layer of the periosteum and is made up of a delicate connective tissue, arterioles and numerous capillaries which form a network. Within the meshes of this supporting tissue are numerous marrow cells, fat cells and many osteoclasts.

While absorption is taking place in the centre of the bone and the medullary canal is being formed, the Haversian spaces adjoining the medullary canal are more or less enlarged as a preparation for the formation of the Haversian systems. Immediately after, new bone is deposited on the walls of the enlarged spaces and by the addition of successive layers, the spaces are reduced to small tubes containing a blood vessel, osteoblasts and embryonic connective tissue. These tubes are the Haversian canals. At the same time the bone gradually increases in girth by the deposition of new layers of periosteal bone. Small spicular extend outward from the last bony layer into the ground substance of the osteogenic layer of the periosteum. When finally the ends of the spicules unite and enclose a vessel and osteoblasts, the latter arrange themselves upon the walls of this space and depositions of osseous material is the result. These circumferential lamellae differ from

the first formed bone in being more compact and more regularly lamellar. The blood vessels which pass from the periosteum into the bone, pierce these circumferential lamellae. At first the blood vessels are not surrounded by concentric lamellae, but after a time absorption takes place, succeded by a redeposition of concentric lamellae, and true Haversian systems are formed. From the very beginning of deposition of osseous layers upon the walls of the Haversian spaces, some of the osteoblasts are included between the lamella. At first they are only partially surrounded by osseous substance and lie in little recesses or bays; after a time however the edges of the entrance to these recesses unite. Thus the osteoblast is completely surrounded by osseous material and remains permanently fixed, forming the bone corpuscles of the lacunae. The mode in which the canaliculi are formed is not known.

As the cartilage goes on steadily increasing in dimension by interstitial growth, the bone has a tendency to become larger toward the extremities. The bone has the least width at the centre, where the ossification began and where there is the largest deposit of periosteal bone. As the ossification of the cartilage approaches the end, the periosteal bone accompanies it

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and serves to prevent the lateral expansion of the cartilage. When however the ossifycation reaches the extremities of the shaft, the enlarged cartilaginous end has extended itself over the subperiostial layer so that this, with the osteoblastic tissue which accompanies it, seems to lie in a groove in the cartilaginous head of bone. This groove is filled with vascular tissue of the periosteum, osteoblasts, and osteogenic fibres, which have a longitudinal direction. The tissue of this groove is gradually converted into cartilage and then into bone.

During this period of growth, the articular ends remain cartilaginous; at length however independant centres of ossification appear and the end is completely ossified, with the exception of a narrow layer of cartilage which seperates the epiphysis from the shaft. This layer of cartilage, as was mentioned at the beginning, remains unossified until the bone ceases to grow. Meanwhile, the bone increases in length by the ossification of this cartilage, which continues to grow at the same time.

As we study the developing bone, we perceive that the law of growth is

carried out very systematically. The change in shape, which the bone undergoes in the process of growth, is by the deposition of new bone by the osteoblasts in one place and a simultaneous absorption by the osteoclasts in another. As more and more bone is removed from the interior by the process of absorption, new bone is continually being deposited on the exterior. This process of correction - of removal in one place and building up in another - continues until the bone has attained at length the shape and size which it is destined to retain during life.

Plate I.

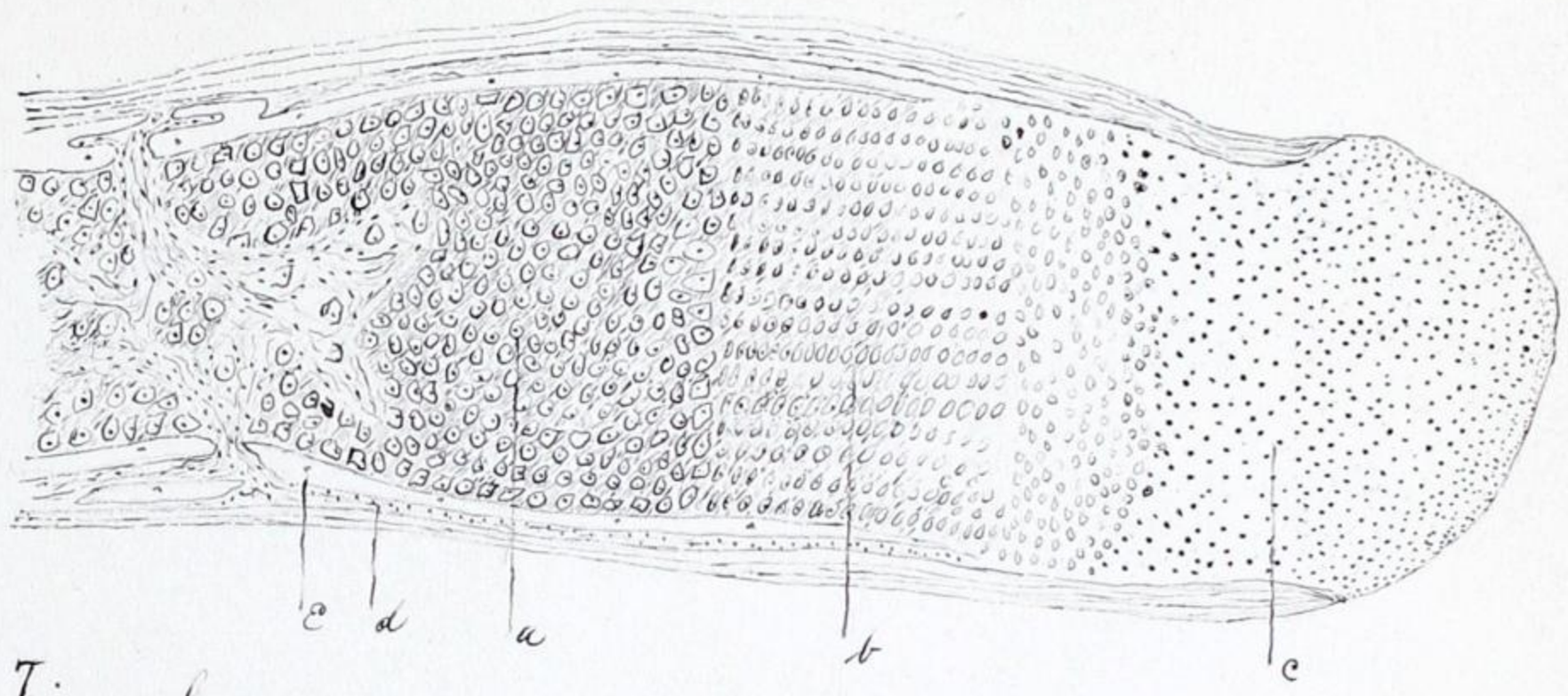


Fig. 1. Section of metatarsal bone of a foetal kitten. Enlarged cartilage cells with calcified matrix, a; b, cartilage cells arranged in rows; c, cartilage cells irregularly arranged; d, layer of osteoblasts; e, periosteal bone.

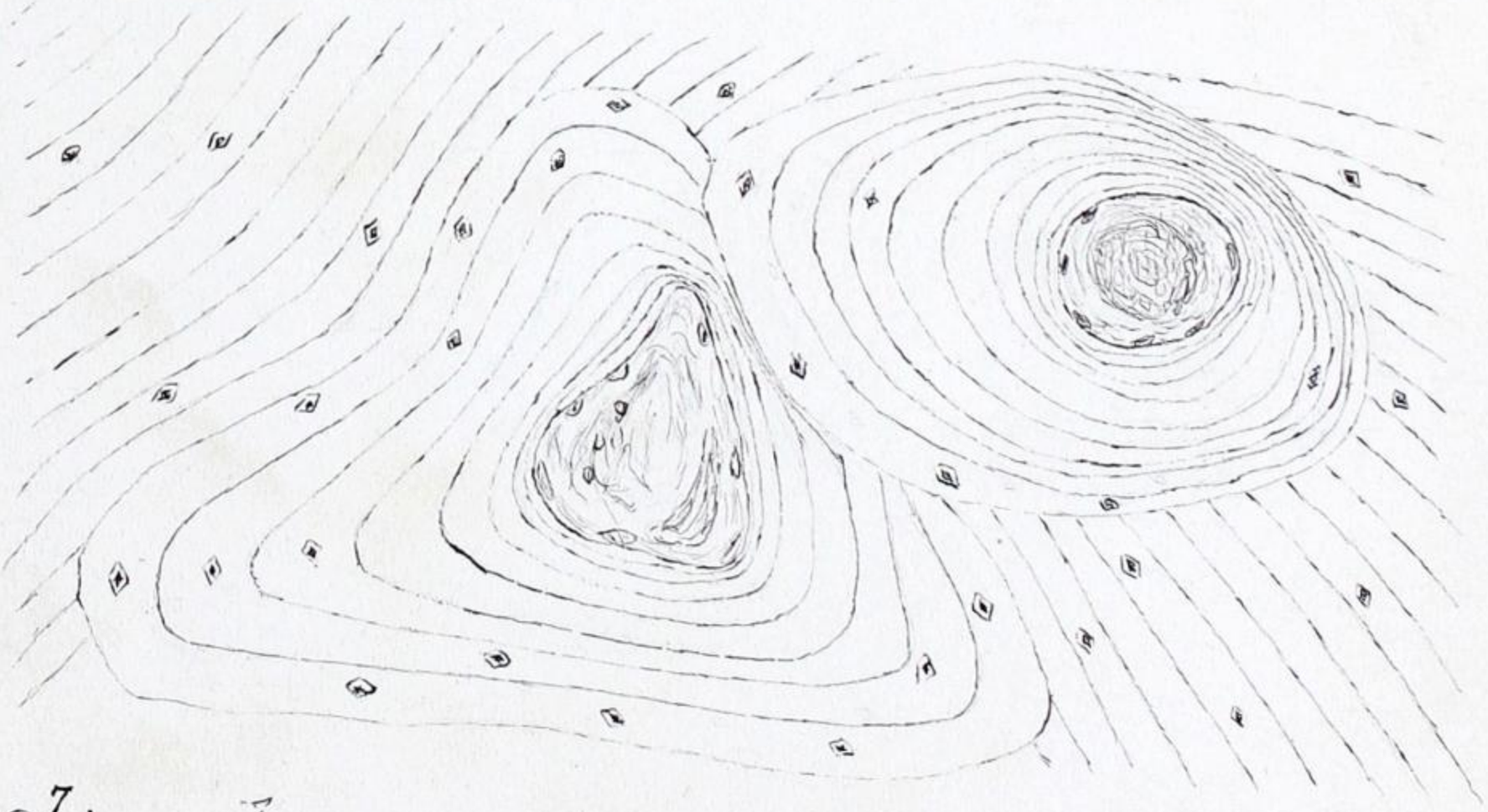
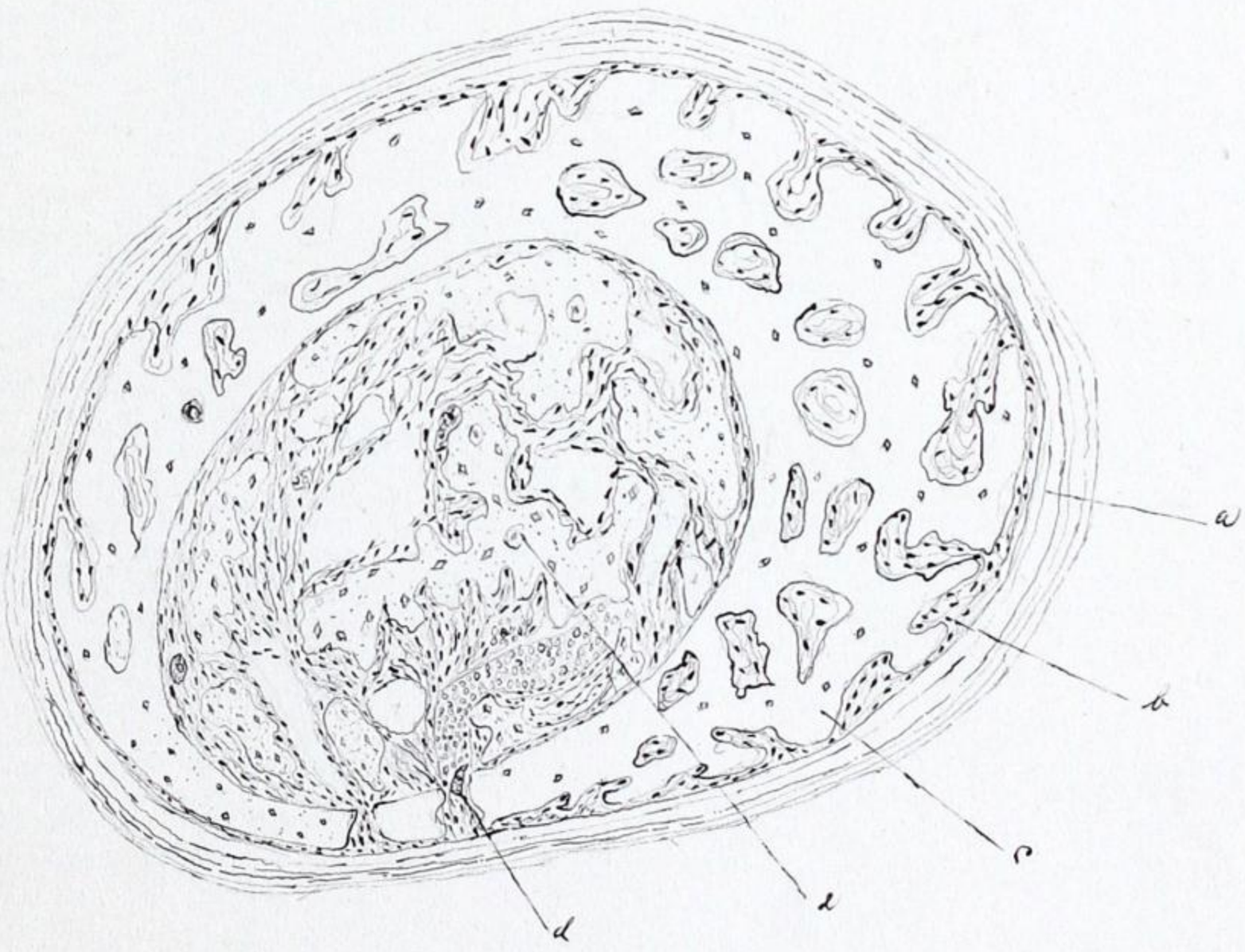


Fig. 2. Transverse section of the femur of a cat, showing haversian systems. Magnified 530 diameters.

Plate II.



Transverse section of the femur of a foetal kitten, magnified 705 diameters. Periosteum, a; b, layer of osteoblasts by which bone is formed; c, periosteal bone; d, osteoclast which has absorbed the periosteal bone, and made passage for osteoblasts and blood vessels of periosteum; e, enlarged cartilage cell.