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Vascularity of the proximal humeral chondroepiphysis and its role in epiphyseal ossification

Robert F. Hempton
Yale University

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Robert F. Hampton

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AND
ITS ROLE IN EPIPHYSEAL OSSIFICATION

by

Robert F. Hempton
B.S., University of California, Berkeley, 1970

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" . . . thou knowest not how the bones do grow in the womb of her that is with child."

--Book of Ecclesiastes

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INTRODUCTION

This study will investigate the ossification of the epiphyses of long bones. Particular attention will be directed toward the role of the blood supply in this process. Most discussions of ossification of long bones are based on observations of the process within the diaphysis and metaphysis. Briefly, the ossification of long bones is believed to be the consequence of the nutritional death of the chondrocytes in the avascular cartilaginous model of the future bone. As a result, blood vessels (the irruption arteries) invade the tissue, bringing with them osteoblasts to form the primary center of ossification. It is assumed that the secondary center of ossification develops in the epiphysis in a similar manner. However, it will be shown that this is not a valid extrapolation.

In contrast to the avascular initial cartilage model, the epiphyses are well vascularized. They contain cartilage canals; that is, passages within the cartilage matrix that contain blood vessels and loose connective tissue. These cartilage canals allow nourishment of the chondrocytes. In the presence of this extensive blood supply, the epiphyses develop secondary ossification centers. They begin to ossify long after the diaphyses have begun to ossify, with most secondary centers appearing after birth, some as late as adolescence.

Thus, any theory that says ossification can <u>only</u> begin in an avascular zone either must be incorrect or should be modified to account for the additional

factors that appear to be operative in the epiphyses.

Therefore, the following theories and factors will be discussed and investigated:

1. Ossification
   - intramembranous
   - endochondral
     - diaphyseal - the primary ossification center
     - metaphyseal - the growth plate (physis)
     - epiphyseal - the secondary ossification center
2. Blood Supply
   - adult pattern
   - developing patterns
     - diaphyseal
     - metaphyseal
     - epiphyseal
3. Cartilage Canals
   - structure
   - types (terminology)
   - origin
   - metaphyseal-epiphyseal communications
4. Role of Cartilage Canals in Ossification of the epiphyses

OSSIFICATION

Ossification is the formation of bone. The major mechanisms are recognized: intramembranous, when the ossification occurs directly within a pre-existing connective tissue model, and endochondral, when there is a pre-existing cartilage model. In the second mechanism, the bulk of the cartilage is removed as bone is formed. However, the actual deposition of osseus material is essentially the same in both types of bone formation [1].

The first theory of long bone formation was presented by Henri Duhamel de Monceau in 1742 [36]. He said that the periosteum was solely responsible for the formation of new bone. Further early supporters of the periosteal theory were Syme, Flourens, and Ollier in 1840, 1842, and 1867, respectively [36]. Duhamel was opposed by Haller of Lausanne in 1763 [36]. Haller stated that the blood vessels played a direct role in the formation of bone. He was supported by John Hunter. Hunter's works were not published until after his death, although they were probably presented in his lectures as early as 1754 [38]. In 1798 his experiments were presented to the Royal Society by Sir Everard Home, and in 1835 his works were published under the editorship of Sir James Palmer[13]. A quote from the latter, regarding the healing of fractures, is: " . . . the arteries and absorbents by their action, model the part, and form it into a cellular substance, in which the arteries deposit calcareous earth. It is first formed into cartilage and then into bone." [13] Later, he noted that soft tissues required only 48 hours to form union, whereas bones took much longer, one of the reasons being that "they cannot be brought so closely into contact, therefore a larger quantity of new matter must become vascular." [13]

In 1845, Sir John Goodsir presented his theory of "centres of nutrition" or "centres of germination", by which he meant the key cells in the development

of the organism and its organs: "The masses of cells in the corpuscles (lacunae), I am inclined to consider as the nutritive centres, germinal centres, or germinal spots of the texture. . . . each of these soft germinal masses is the centre of attraction for the proper nutriment of bone, and is the active agent in withdrawing this from the vessels and appropriating it, . . . for the formation of new calcigerous cells . . ."[6] He denied the osteogenic function of the periosteum, attributing it to bone cells clinging to the membrane[6].

In 1867, Gegenbaur gave the name "osteoblasts" to Goodsirs "germinative centres".[36] Additional strong support for the importance of the osteoblast was William Macewen's book "The Growth of Bone; Observations on Osteogenesis", published in 1912.[23]

The fourth theory of bone formation was the humoral theory of Leriche and Policard in 1926.[36] This was similar to the theory of Haller and Hunter.

Today it is recognized that each of these aforementioned elements plays an integral role in osteogenesis, with no single theory being sufficient to explain all aspects of skeletal formation and maturation.

G. L. Streeter's "A Review of the Histogenesis of Cartilage and Bone" [25] is an excellent summary of the early stages of bone formation. He defined stages, called horizons, not by gestational age but by morphological development [34]. The term horizon was chosen because of its connotation of a "plane of reference" [34]. Gardner [5] has supplemented Streeter's data by correlating the horizon with gestational age. In general, the gestational age in days is equal to twice the horizon number plus one. The following description of the early developmental stages of the human arm is a summary of their findings. In an embryo of 21 to 29 pairs of somites (horizon XII), the arm bud first appears.

At horizon XV, there is an early collection of cells termed the "skeleto-muscle condensation", which consists of tightly packed, relatively undifferentiated cells with round to oval nuclei and cytoplasm that appears syncytial. By horizon XVI, skeletal anlagen can be distinguished from muscle, which contains nerve trucks. There are definite muscle groups and nerve branches in horizon XVII, and by horizon XVIII, there are definite individual muscles and the early cartilage is in the rough shape of the future bones.

The cartilage cells within the skeletal anlagen pass through five stages or phases, as Streeter called them. In general, as the cells mature and pass through the first four phases, they proliferate, increase in size, progressively vacuolate, and secrete matrix. In phase five, they appear to liquify and disintegrate. More specifically, the phase one cells are only slightly larger than the blastemal cells of the skeletomuscle condensation. The phase one cells are close together, actively dividing, and have just a small amount of intercellular substance. The cells in phase two are flattened,so that in a longitudinal section of the developing bone they appear to be long, slender cells with their long axis directed transversely to the long axis of the bone. This is probably related to the direction of cell division of these actively multiplying cells. By phase three the cells are about three times larger than the cells of the earlier stage, and they are now cuboidal with extensive vacuolization. The surrounding matrix is much increased in amount. The phase four cells are the largest and are nearly completely vacuolated. The matrix is conspicuous, resembling a honeycomb. By phase five, the last stage, the cells appear to be liquifying and disintegrating. There are some empty lacunae within the matrix.

Returning to the description of the overall morphogenesis of the arm, it is seen that by horizon XVIII, the first two phases of chondrocytes are

present. By horizon XIX, the first three phases are present. Horizon XX is most notable for the prominent appearance of an "epiphyseal knob" at the proximal end of the humerus, that is, a much larger epiphyseal diameter relative to the diaphyseal diameter than at later stages of development. By horizon XXI, the first four stages of chondrocytes are present and the shoulder joint has become distinct. Horizon XXII is defined by the appearance of the primary osseous shell with osteoblasts and an overlying fibrous layer. This bony shell is called the primary bone collar and is entirely external to the cartilage model. Horizon XXIII is defined by the presence of all five stages of chondrocytes, the oldest and most mature in the middle of the diaphysis, the least mature at the extremities, with an orderly progression inbetween.

The next step is the vascular invasion of the diaphysis in this central zone of hypertrophic and degenerating cartilage cells by an irruption artery, with the subsequent formation of bone and marrow within the diaphysis. In the perivascular connective tissue are pluripotential mesenchymal cells that are carried along with the vessels into the interior of the diaphysis. Some of these cells differentiate into the hematopoietic elements of the marrow, while others become osteoblasts.[1] Further, in the rat, it has recently been reported that some chondrocytes de-differentiate and subsequently transform themselves into osteoblasts.[12, 28] The vascular invasion of the diaphysis marks the end of the embryonic period and the beginning of the fetal stage.[35]

Growth in the length of the long bones proceeds at the growth plate by an analogous process. At any one time a similar sequence of cartilage cell stages is seen as one looks from the epiphyseal to the diaphyseal side of the growth plate.

As the marrow cavity of the diaphysis and metaphysis approaches the epiphysis, an intermediary zone, the physis or growth plate, becomes structurally defined. Cells are continually produced on the epiphyseal side; and, as they are displaced towards the diaphysis by the production of newer cells, they secrete matrix, hypertrophy, and finally are replaced by the advancing blood vessels of the marrow. Osteoid and new bone are laid down on the partially degenerated matrix. With time these chondro-osseous trabeculae are reworked and the cartilage/fetal bone complex is completely removed and replaced by mature bone. However, if the humoral concentrations of calcium and phosphate are diminished, or if there are various metabolic defects, ossification of the matrix can be adversely affected (e.g., rickets or osteopetrosis).[1]

A discussion of ossification of the epiphyses will be preceded by a discussion of blood supplies to the various parts of bones.

BLOOD SUPPLY

The blood supply of mature long bones is classically described as consisting of three components: diaphyseal, metaphyseal-epiphyseal system, and periosteal.[27]

The diaphyseal circulation is composed of one or more nutrient arteries that enter through the cortex and divide, sending branches up and down the marrow cavity and supplying both the marrow and the cortex (expecially the endosteum).[25,41]

The metaphyseal - epiphyseal system is composed of a number of arteries that enter the ends of the bone. The metaphyseal arteries tend to enter in a longitudinal direction through the obliquely angled cortical surface, while the epiphyseal vessels tend to enter more radially from the pericapsular arterial system.[41] There is a good degree of anastomosis between these two

systems, as well as with the diaphyseal supply.[41]

The third component is the periosteal plexus of arteries and veins that communicate with numerous capillaries and venules in the cortex.[25] The role of the periosteal blood supply is unclear at the present time.[41] Flow through the cortex usually is considered to be centrifugal.[16, 29] However, there may normally be a sizeable centripetal component, and in time of damage to the other arterial supplies, the periosteal supply may increase in importance[25,29].

The above presentation of the blood supply pertains to the fully developed long bone. However in considering the subject of ossification, the immature (and developing) blood supply must be considered. This is quite different and time-dependent.

Initially the cartilage anlage of the entire bone is avascular. The first vascular penetration, which is by the irruption arteries, is into the diaphysis and marks the beginning of the fetal stage. This occurs at about 28-30 mm. crown-rump (CR) length, which is at about 9 weeks after the start of the last menstrual period.[5,33] The irruption arteries are variable in number and position and are superceded by the nutrient artery.[5] The nutrient artery develops when the fetus is about 60 millimeters in crown-rump, which corresponds to a gestational age of about 12 weeks from the last menstrual period.[7] Branches of the nutrient artery then develop within the enlarging marrow cavity to supply the diaphyseal cortex and also the diaphyseal side of the central portion of the developing growth plate.[38]

Metaphyseal vessels penetrate the cortex only in the late fetal stages.[2] They supply the metaphyseal cortex and the metaphyseal side of the periphery of the growth plate.[38] Before the appearance of the metaphyseal vessels, the nutrient artery is the sole source of blood to the marrow and cortex.[2]

The vascularity of the epiphysis is unique in that the blood vessels lie within cartilage rather than bone and this cartilage persists rather than immediately ossifying as in the diaphysis and metaphysis.

The proximal epiphysis of the humerus is invaded by blood vessels when the fetus is about 45 millimeters in crown-rump length. [2] This is about 10 days after the diaphysis is invaded. By this time the shoulder joint is distinct, [35] and no vessels enter the epiphysis by way of the articular surface.[14] Most of the vessels enter from the region of the attachment of the joint capsule to the anatomical neck of the humerus. [14,41,44] Blood vessels commonly enter at or near the insertion of a ligament, e.g., the ligamentum teres, the cruciate ligaments or, in the humerus, the rotator cuff insertion.[11,14] Also vessels can penetrate the epiphysis from any other area that is covered with periosteum; in man these vessels enter the epiphysis at fairly regular intervals. [21] The question of an epiphyseal blood supply from vessels in the diaphysis and/or metaphysis is complex and unresolved and will be discussed later.

Once into the epiphysis, the blood vessels lie in the peculiar structures known as "cartilage canals", which will be described in the following section.

Cartilage Canals

Contrary to present day textbooks [1,9,20], which state either that cartilage is completely avascular or that it is functionally avascular (i.e., the vessels that are in the cartilage are merely passing through), blood vessels in cartilage have been known since William Hunter's first report of them in 1743 [14,21]. He made them visible by injecting the vascular system with colored wax. He also noted that he could see them only in

"very young subjects" and not in adults.[21] In 1815, Howship is reputed to have originated the term cartilage canals. [21] In 1827, E.H. Weber reported the presence of blood vessels in the costal cartilage of both newborn and adult humans. [14] After that a large German literature on cartilage canals developed, but the subject was ignored (except for Hunter's first reference) by the English literature until Parson's studies were published in 1905. [14] Since then the literature has grown significantly.

Exactly what is a cartilage canal? In the most general terms it is a channel in hyaline cartilage that contains blood vessels. [8, 14] This cartilage may be either permanent or temporary. An example of permanent cartilage with cartilage canals is the cartilaginous portions of the ribs, as mentioned above.[21] (Although William Hunter did not observe blood vessels in the ribs of adults, this may have been a technical difficulty since they have been well documented by later researchers).[21] The archetype of the temporary cartilage with cartilage canals is the epiphysis.

The detailed structure of the cartilage canals is a matter of some dispute. There is a consensus that the passage in the cartilage contains an arteriole, a venule, and several capillaries between them.[5] These capillaries may be predominantly at the end of the canal within the cartilage, in which case they form a structure called a glomerulus. [24] Glomeruli range from simple to complex depending on the number of branches at the end of the arteriole and the number of capillary loops.[44] The complexity of the glomerulus has been correlated with the presumed activity of the surrounding chondrocytes, being especially developed just above the growth plate.[44] In addition, capillaries anastomose the arteriole and the venule along the whole length of the canal.[24,44] This has been presumed to indicate that the

canal serves a nutritive function throughout its entire length.[44] (The glomerular and capillary structures described above have been studied mostly in dogs and a few other animals. Differences between species do exist.[8,21] The exact structure in humans is not yet known).

In addition to blood vessels, cartilage canals contain a perivascular web of loose connective tissue which is in continuity with the perichondrial connective tissue.[14] Also, unmyelinated nerves have been reported three times[5, 32,44] and the possibility of small lymphatics once.[44]

The question of the origin of the cartilage canals, that is, how they are formed, has been even more in dispute than the question of their structure. An early theory was that the blood vessels were passively included in the cartilage as the cartilage enlarged by appositional growth and that these vessels played no role in the metabolic economy of the cartilage or its ossification.[9] Haines[8] was the strongest proponent of this view.

However, it has now been shown conclusively that cartilage canals must actively grow into the epiphysis.[2, 14, 21] The fact that the canals end blindly within the cartilage[2, 10, 14] is evidence that they were not just surrounded by cartilage as the cartilage grew but rather that a functional unit with both inflow and outflow of blood had to grow into the cartilage.[2]

The mechanism of ingrowth is thought to be chondrolysis, but the agent of this action has not yet been identified. Numerous authors have proposed that the endothelial cell has the ability to erode cartilage.[11, 14, 24] According to this theory, a capillary glomerulus develops at the epiphyseal-perichondrial interface and actively lyses the cartilaginous matrix to form a channel into the epiphysis.[24] Other authors have suggested that the loose connective tissue cells, rather than the endothelial cells, are the cells

which actually lyse the cartilage. [2, 22] Finally, several authors have suggested that the chondrocytes do not die, but remain active and lyse themselves free of the cartilaginous matrix, allowing the ingress of the blood vessels and de-differentiating to more primitive cell types in the process.[2, 22] These de-differentiated cells can then re-differentiate into more specialized cells; these could include osteoblasts as well as chondrocytes again. [12, 22, 28]

Those authors who believe that the chondrocytes die as the cartilage canals invade the epiphysis generally believe that the osteoblasts arise from the loose connective tissue brought in with the vessels from the perichondrium, just as occurs in the diaphysis.

For clarity, the following terms will be introduced at this time. A penetrating canal is a cartilage canal that enters the epiphysis from its perichondral surface. A communicating canal is a cartilage canal that crosses the growth plate, thus allowing vascular communication between the epiphyseal and the diaphyseal and metaphyseal blood supplies. A perforating canal is a cartilage canal that exists from the metaphyseal aspect of the epiphyseal ossification center, passing through or "perforating" the lamella of bone at the border of the ossification center and supplying the epiphyseal side of the growth plate. By definition, perforating canals do not completely traverse the growth plate. The cartilage canal is the focus of a functional unit similar to the osteon of mature bone. The term chondron will be devised to describe this canal system, which included blood vessels, undifferentiated mesenchyme, and the differentiating pericanalicular cartilage.

The question whether cartilage canals cross the growth plate is still not entirely resolved. Many authors have stated that such a communication never exists. In 1968, J. Trueta in Oxford, stated this quite definitely.

He said that he had never observed a communicating canal in over 2000 growth plates from several species of mammals. [39] Haraldsson, in his study of the distal humeral epiphysis in the human, did not see blood vessels cross the growth plate until the growth plate was being replaced by bone at the time of epiphyseal fusion. [10] With the opposite observation, many other researchers have reported the existence of communicating canals. Hurrell said that they exist in the humeral head of the human at birth, but that they are functionless. [14] He supposed that they arise by incorporation of part of the vascularized epiphysis into the growth plate as the diaphysis grows in length. [14] Trueta, somewhat at variance with his later writings, reported in 1959, "From the last stages of intrauterine life up to the first six months, in some epiphyses, when the growth cartilage is established but not yet limited by bone on the epiphyseal side, vessels from the metaphysis penetrate the 'anlage', perforating the pre-existing growth plate." [37] He then added that the growth plate was a barrier to blood vessels after 8 to 18 months of age until the time of fusion of the epiphyses. [37] He did not mention to which epiphyses he was referring, although photographs of the femoral head were shown.

Thus, it seems that there is probably a time-dependent factor at work here - the communications forming and then disappearing. However, this time - dependency is not well defined and is, therefore, one of the areas of investigation of this study.

The blood supply to the growth plate from the epiphyseal and metaphyseal sides after the epiphyseal ossification center is well established is shown in figures 1 and 2, respectively.

The Role of Cartilage Canals in the Ossification of the Epiphyses

Just as there were several early theories regarding ossification of

primary centers, so there are conflicting reports and theories regarding secondary ossification centers within the epiphyses.

First, there are those who ignore the existence of cartilage canals altogether and state that epiphyseal ossification begins in an avascular matrix.

Second, there are those who admit the existence of cartilage canals but say that ossification begins in an avascular area of the cartilage or that the cartilage canals are not directly involved in the process of ossification. Some important supporters of this view are Haines [8], Hurrell [14], Ring [30], Levene [21], and Trueta [39].

Finally, there are those who say both that the cartilage canals exist in the epiphyses and that they are intimately involved in the endochondral ossification process. These authors include the following: Mashuga [24], Haraldsson [10], Gray and Gardner [7], and Wilsman and VanSickle [43].

It should be repeated that there are two types of cartilage in which cartilage canals appear: permanent and temporary. Therefore, the appearance of cartilage canals does not make ossification inevitable, and there must be other factors at work, too, e.g., hydrostatic pressure within the epiphysis, as suggested by Kummer [18].

The scope and findings of this study will now be presented.

MATERIALS AND METHODS

Developing proximal humeri were obtained from human fetuses ranging in age from 15 weeks from the onset of the last previous menses to full-term. Gestational age was estimated from crown-rump length by using the results of Streeter's work in 1920 [33]. Additional humeri from infants up to 24 months

of age were obtained. Other developing bones were studied as appropriate, e.g., phalanges, metacarpals, matatarsals, and proximal tibia.

For simplicity and to avoid confusion, pre-natal materials will be referred to by gestational age (from the last menses) in weeks, and post-natal material will be referred to by post-natal age in months.

All specimens were fixed in 10 per cent formalin, partially decalcified in 5 per cent formic acid, washed in water, dried in alcohol and xylene, and then embedded in paraffin. Serial sections 6 to 10 microns in thickness and in the frontal plane were cut and mounted on glass slides. The mounted specimens were stained with various stains including the following: hematoxylin and eosin (H and E), Mallory's phosphotungstic acid hematoxylin (PTAH), periodic acid - Schiff leucofuchsin (PAS), and Safranin O - fast green (SOF). The sections were then examined grossly (by eye and low power microscope) and histologically (with overall magnification ranging from 50 to 750X).

TABLE I: TABULATION OF AVAILABLE MATERIAL

	SPECIMEN	SEX	AGE (weeks)	STAINS USED
Pre-natal	1	M	15	H and E
	2	F	16 1/2	PTAH
	3	M	19	PAS
	4	M	21	H and E
	5	M	28	H and E, PTAH
	6	M	39	H and E, PTAH
			(months)	
Post-natal	7	M	2	H and E, PTAH
	8	F	2	H and E, PTAH
	9	F	7	H and E
	10	F	15	H and E, SOF
	11	F	24	H and E
	12*	M	1	H and E

* This specimen was obtained from a one month old male with cystic fibrosis who died of disseminated staphylococcal osteomyelitis.

The histories of the other specimens are not known.

RESULTS

On gross and low power microscopic (less than 10X) examination of the humeral heads, the following observations were made:

Specimen 1 (15 weeks): There is a large epiphyseal head relative to the diameter of the shaft. The capsule and joint space are well defined. The largest cartilage canals are in a band just distal to the plane of insertion of the joint capsule. Smaller canals are located in the epiphysis distal to this plane (i.e., in the area of the greater tuberosity). Canals are seen more proximally (i.e., within the head itself) (figure 3).

Specimen 2 (16 1/2 weeks): The head of this specimen is grossly avascular. The cartilage stains lightly compared with surrounding connective tissues. (figure 4).

Specimen 3 (19 weeks): The epiphysis is now much more vascular, with the exception of the greater portion of the head within the plane of the capsular attachment, which remains relatively avascular. The most complex, branched canal is in the plane of attachment of the capsule. There are two large canals just proximal to the growth plate and two canals appear to cross the growth plate. (figure 5).

Specimen 4 (21 weeks): The epiphysis is now in about the same proportion to the shaft as it is in a child or adult. Most of the epiphysis is now quite well vascularized. The only exception to this is a shell of cartilage beneath the articular surface that is still avascular. The canals, where they are present, are much more developed. They are larger and more branched; their boundaries are more defined. Canals lie close to the growth plate but do not clearly cross it. (figure 6).

Specimen 5 (28 weeks): There is little change from the previous specimen, except that now the borders of the cartilage canals are very distinct. Also, there appears to be one cartilage canal that sends a branch through the growth plate. (figure 7).

Specimen 6 (39 weeks): The proximal epiphysis is grossly vascular with the exception, as before, of the sub-articular cartilage. Canals enter the medial side of the head from the capsular attachment and the lateral border of the head from the perichondrium. There is a secondary center of ossification forming just medial to the center of the head. There are cartilage canals both around it and within it. The growth plate has assumed its final dome-like shape, bulging into the head of the humerus. Just above the growth plate is either a system of canals or a zone of weakness which has torn. A communicating canal is near the medial borders of the growth plate. (figures 8 and 9).

Specimen 7 (2 months): This specimen is of the same general shape as the previous one. The growth plate has the same shape and is in the same relative position. However, this specimen is not as well canalized and there is no ossification center in any of the sections examined. One and possibly two communicating canals are near the center of the growth plate, and a vascular communication between marrow space and epiphysis is at the lateral border of the growth plate. (figure 10).

Specimen 8 (2 months): Similar to previous specimen. No ossification center is seen. Possibly three communicating canals cross the growth plate. (figure 11).

Specimen 9 (7 months): The original (medial) secondary ossification center is now quite large. A lateral, smaller secondary ossification center

has appeared. Both ossification centers are penetrated by several cartilage canals. The superior borders of both secondary ossification centers are fairly uniformly convex; however, the inferior and more central-facing borders are flatter and more irregular. (figures 12 and 13).

Specimen 10 (15 months): Both secondary ossification centers have grown in size, especially the lateral. Their borders are as described for the previous specimen. The two centers are separated by a plate of cartilage, which a canal traverses allowing vascular communication between the two centers. (figure 14).

Specimen 11 (24 months): The two centers are nearly joined. The superior surface of the medial ossification center is rather smoothly curved to conform with the overlying articular surface. The superior surface of the lateral ossification center, lying beneath the rotator cuff insertion is a bit flatter. The inferior and central borders conform to the underlying growth plate. No communicating canals are seen.

On histological examination of the specimens, the following observations were made:

Specimen 1 (15 weeks): The chondrocytes of the humeral head are relatively undifferentiated. They are in early phase 3 (according to Streeter's definition). There is a moderate amount of lightly staining cartilaginous matrix. The larger vessels in the cartilage canals, an arteriole and venule, are surrounded by loose connective tissue, which contains many small capillaries and which gradually blends into the surrounding cartilage. (figure 15).

Specimen 2 (16 weeks): The cartilage cells are at roughly the same stage of differentiation. In most sections there are no canals, but on several there are some rather poorly-defined canals located near the medial

and lateral attachments of the joint capsule. In proceding from the head towards the shaft, the cartilage cells become more differentiated and finally hypertrophic just proximal to the advancing diaphyseal vessels.

Specimen 3 (19 weeks): The cartilage cells are generally more developed. Their lacunae are larger and the cartilaginous matrix is darker staining, indicative of increased mucopolysaccharide content. The canals have a more distinct boundary, with a more darkly staining shell of matrix around them. Numerous capillaries containing red blood cells are seen near the canal walls in the loose connective tissue around the arteriole and larger venule. The chondrocytes immediately adjacent to the cartilage canals are hypertrophic relative to the general population of chondrocytes farther away from the canals. The vascular endothelium is separated from the cartilage matrix by 1-2 cell thicknesses of loose connective tissue. Various degrees of gradation between connective tissue cells and chondrocytes can be seen, with these intermediate cell types located on the surface of the cartilage matrix. The growth plate is well defined, with all stages of growth plate chondroctyes present. Two cartilage canals in the epiphysis send communicating vessels across the plate. (figure 16-20).

Specimen 4 (21 weeks): The cartilage canals are now well defined; the differentiation between cartilaginous matrix and loose connective tissue substance is quite distinct. The thin-walled vessels lie nearly adjacent to the matrix, separated only by a layer of cells which are intermediate in type and half enclosed in matrix. The capillary endothelium may lie in intimate contact with the cartilage matrix in some places. A cartilage canal just above the growth plate gives off a very thin vessel, filled with red blood cells which crossed most of the growth plate before it is lost from the sections.

Specimen 5 (28 weeks): By this stage, all the chondrocytes are well-differentiated; matrix is plentiful, and the cartilage canals are also well-defined, with a slightly more cellular and hyperchromic matrix surrounding them. The well-developed growth plate is crossed by a blood vessel of large caliber, passing from the cartilage canal above to the marrow below. The columns of hypertrophic cells in the growth plate are locally disrupted by its passage. (figure 21).

Specimen 6 (39 weeks): The most notable feature of the specimen is that a secondary center of ossification has begun to form. It is in a very vascular region. The endothelium is in intimate contact with the free surfaces of cartilaginous matrix and there is increased calcification of the matrix near these surfaces. Vessels in this region are of varying caliber, but all have only a single layer of endothelium. Numerous small capillaries are in the loose connective tissue within the canals. Cells at the margins of the canals are surrounded by small deposits of bone. One section appears to show the initial steps of ossification without any vascular connections. However, this area corresponds to and is just above the ossifying area of an adjacent section which clearly does have a vascular supply. In addition, blood cells are seen within the spaces, implying a vascular supply. Hypertrophic cartilage cells are most prominent in the area surrounding the newly forming ossification centers, just surrounding the cartilage canals, especially in the areas of the future ossification centers, and, of course, in the growth plate just above the metaphyseal blood vessels. (figures 22 and 23).

Specimen 7 (2 months-male): This specimen does not yet have an ossification center, although the head is well-vascularized. A large communicating canal is in the center of the growth plate, and several others are near the edges. (figure 24).

Specimen 8 (2 months-female): Throughout the matrix, the lacunae are generally larger than in earlier specimens; they are even more hypertrophied around the cartilage canals, and the matrix is more hyperchromic there. The canals are larger with bigger vessels than in earlier specimens. Numerous capillaries are dispersed throughout the loose connective tissue. There is one region of matrix which is apparently "avascular", in which there is a hypertrophic group of cells. However, examination of the corresponding region on the next slide shows that a cartilage canal lies just out of the plane of section of the first slide. A narrow blood vessel leaves a cartilage canal near the growth plate and probably crosses the plate, although this is not completely seen. The distance between canals averages 1.0 millimeters. (figures 25-27).

Specimen 9 (7 months): This specimen contains two secondary centers of ossification, the medial being larger than the lateral. In one section, three cartilage canals are seen entering each ossifying center. One canal, entering the superomedial border of the medial ossification center contains a very prominent and straight arteriole in the center and a larger, more thin-walled and dilated vein to the side. The borders of the secondary ossification centers, on the microscopic level, are very irregular. Although there are some hypertrophic chondrocytes, there are no columns of cells. Calcification of the cartilaginous matrix seems to be retarded immediately adjacent to the chondrocytes and their lacunae. (figures 28 and 29).

Specimen 10 (15 months): This specimen shows extension of the changes noted in the previous one. The two secondary ossification centers are larger but still separate. In each section there are more cartilage canals entering each ossification center, but this is a function of the increased size of the

ossification centers, since the distance between canals is still the same. One canal connects the two centers. There is an area of increased calcification between the borders of the lateral ossification center and a nearby cartilage canal. In general the borders of the ossification centers are still irregular, although not as much as before, and there is an area where the hypertrophying chondrocytes are arranged in columns. Probably there were several communicating canals, but this is not certain because of damage to the sections. (figures 30 and 31).

Specimen 11 (24 months): There are no major microscopic changes from the previous specimen. The borders of the two ossification centers show slightly more organization. Calcification is still less near the lacunae than in other parts of the matrix near the borders of the ossification centers. No canals or vessels are seen crossing the growth plate.

Additional Observations

1. A humerus was obtained from a one-month old male with cystic fibrosis who died of disseminated staphylococcal osteomyelitis. On gross examination the head of the humerus was at roughly the same stage of development as the two-month old specimens previously described. There was no secondary center of ossification. In the medial part of the proximal metaphysis, there was an area of obvious suppuration. The growth plate above this area was completely destroyed by the suppurative process, and the epiphyseal cartilage above was being directly involved by extension of the suppuration. The cartilage canals in this area seemed to be the leading pathways for the spread of the infection and suppurative process. (figure 32).

Histologically, the marrow was hyperplastic with mostly polymorphonuclear

leukocytes. The leukocytes filled the lacunae in the growth plate which had previously contained the hypertrophic chondrocytes. The matrix was also eroded by varying amounts. On the medial side the matrix of the growth plate was completely destroyed, and the leukocytes extended into the epiphyseal cartilage.

On the lateral side, the growth plate was intact. However, there was minimal invasion of the metaphyseal aspect of the growth plate by the leukocytes of the hyperplastic marrow. Just above the growth plate on the epiphyseal side, there were numerous leukocytes within the cartilage canals. In the other cartilage canals of the epiphysis, except for those in the medial side of the epiphysis that were directly invaded by suppuration, there was no such increase in leukocytes within the vessels or perivascular tissue.

2. Sections of the first toe of the 28-week fetus were examined. The sections included all the structures from the diaphysis of the first metatarsal to the diaphysis of the distal phalanx. All of the cartilaginous epiphyses included in these sections contained cartilage canals with the exception of the distal end of the proximal phalanx. Thus, in this case, there is a one-to-one correspondence between the presence of cartilage canals and the later development of a secondary ossification center. The distal epiphysis of the proximal phalanx, the only epiphyseal cartilage in these sections without a canal system, characteristically does not develop a secondary ossification center.

DISCUSSION

The overall pattern of vascularization of the proximal humerus observed in this study is consistent with the earlier reports of Gardner [5] and Gray and Gardner [1]. They reported the earliest appearance of cartilage canals in the

proximal humerus at a crown-rump length of 37 millimeters, which corresponds to approximately 10 weeks of age (dating from the last menstrual period). The earliest specimen in this study was 15 weeks of age, and it had several small canals at its medial and lateral sides, near the insertion of the joint capsule. The canals entered the epiphysis from the perichondrial surface, especially near the insertion of the joint capsule. Within the epiphysis, the canals were uniformly distributed, except for the early stages when the cartilage of the anatomical head tended to have fewer cartilage canals than the rest of the epiphysis. However, with time, this too became well vascularized, except for a thin shell beneath the articular surface, which remained avascular. The vascularity of the epiphysis remained constant even though its size was increasing, because the canals were increasing both in number and in complexity. Additional canals entered from the perichondral surface, and the canals within the epiphysis developed branches. The fact that the cartilage canals of older specimens were observed to have branches, whereas the canals of younger specimens did not, i.e.(that there is a topological difference between the younger and the older canals) is additional evidence that the canals are the product of an active, formative process, rather than vessels that are simply passively included in the epiphysis as it enlarges by appositional growth. This argument for the active growth of cartilage canals was put forth by Levene [21], based on his observations of the human proximal tibial epiphysis. His finding is thus supported by observations of the proximal humeral epiphysis in this study.

Communicating canals, i.e., cartilage canals which crossed the growth plate, were seen in this study of the proximal humerus in specimens between the ages of 19 weeks gestation (from the last menstrual period) to 15 months after birth.

These time limits are not very precise because of the limited number of specimens that were available for examination. Also, the variability of these limits has not yet been determined. These limits will be refined as additional materials become available.

Numerous earlier studies have both observed and failed to observe vascular canals crossing the growth plate. The present study is complementary to the study by C. Levene. [21] He reported that, in the proximal tibia of man, communicating canals do cross the growth plate, first in the center, then more peripherally. He also reported species differences both in the overall patterns of canals within the epiphyses (of proximal tibiae) and also in the existence of communicating canals.

Trueta's study [37] in 1959, was also in agreement with the present study. In that study, he described how osteomyelitis (which begins in the metaphysis) can spread to the epiphyses in infants because of communicating canals across the growth plate. That finding was confirmed in this study. Specimen 12, from the one-month old infant with osteomyelitis, showed the destructive process crossing the growth plate. However, Trueta's later studies [38,39], which were performed in older specimens, stated categorically that communicating canals did not exist, and because they failed to mention his earlier work, they were misleading.

Brookes [2] recognized the existence of communicating canals, but stated also that they "no longer are in evidence" after the formation of the specialized subchondral circulation.

Some studies that failed to demonstrate cartilage canals may have failed for reasons of technique, since blood vessels may not always fill during injection studies. The results of the injection studies by Trueta [38, 39]

and Haraldsson [10] must be evaluated with this possibility in mind. Also, the circulation may have been studied at a time that was either before or after the period during which the communicating canals existed. Thirdly, in the case of studies on species other than man, the lack of communicating canals may have been a correct finding (but correct only for the species studied), or, again, the studies may have failed for one of the reasons described above.

The time period for the existence of communicating canals through the growth plate of the proximal humerus (19 weeks of gestation to 15 months post-natal) should only be considered valid for the proximal humerus. This is the time period which includes the initial formation and development of the two secondary centers of ossification within the epiphysis of the proximal humerus. Several authors, including Trueta [37] and Brookes [2], have called attention to the possibility that the existence of communicating canals may be dependent upon the stage of development of the adjacent secondary center of ossification. However, Trueta's report [37] was confusing because he did not mention to which epiphysis he was referring when he said that there ceased to be a vascular communication across the growth plate at about the age of 6 months. It seems as though he was generalizing, probably from his studies of the proximal femur. However, it is well known (and well summarized by Schmid [31]) that the different epiphyses develop and mature at quite different times. Therefore, the time period of existence of communicating canals through other growth plates still needs to be studied and correlated with the time of appearance of the adjacent secondary center of ossification.

Within the canals, thin walled arterioles, venules and capillaries were observed, surrounded by loose connective tissue. At the border between

the loose connective tissue and the cartilage matrix, there were cells which appeared to be intermediate in type between the connective tissue cells within the canals and those within the cartilage matrix. The cartilage matrix in these areas was hyperchromic compared with the matrix farther away from the canals. Lufti [22] in his study of the development of the proximal tibia in the chicken said that the hyperchromic matrix around the canals in that epiphysis was associated with the differentiation of mesenchymal cells to chondrocytes and the formation of new cartilaginous matrix. Thus, it appears that a similar process might be occuring in the human and that the cartilage canals might be a source of chondrocytes for the growing epiphysis.

It was also observed that the chondrocytes around the cartilage canals were often hypertrophied, whereas the chondrocytes elsewhere in the matrix were not hypertrophied. Thus, the chondrocytes around the cartilage canals resembled the chondrocytes in the growth plate just above the zone of calcification and therefore, on histological grounds, a relationship between the cartilage canals and epiphyseal ossification is suggested.

Nerve fibers and lymphatic vessels wer e not seen. However, no special stains for these structures were used and no special search was made for them.

An intimate association between the blood supply of the proximal epiphysis and the formation of the secondary ossification center in the medial side of the epiphysis was observed. (The lateral ossification center was never seen at such an early formative stage, because specimens of the appropriate age could not be obtained). The whole epiphysis was well-vascularized from early fetal life onward. The only area that was not so well vascularized was

the cartilage just below the articular surface (and it is an area that does not normally ossify). The cartilage canals within the proximal epiphysis were surrounded by hypertrophic chondrocytes and hyperchromic matrix reminiscent of the zones of the growth plate just above the zone of ossification. When the secondary ossification center first appeared, it contained obvious vascular spaces lined with endothelium and filled with erythrocytes. The endothelium seemed to be in intimate contact with the surface of the cartilage matrix, and the matrix had the increased density of staining characteristic of increased calcium content. There were some areas in which the endothelium was separated from the cartilage matrix by a narrow cleft, but these spaces appeared to be artefactual, resulting from the fixation and handling of the tissue.

This finding of the close association between the blood vessels and the ossification of the epiphysis is similar to the finding of Wilsman and VanSickle [43] in the canine proximal epiphysis. Previous studies of the ossification of epiphyses in human materials have not reported this finding. Most of those studies relied primarily upon injection techniques to observe the overall vascular pattern (Haines [8], Levene [21], Trueta [63], Waugh [42]), and, thus, although presumptive evidence about the role of blood vessels in ossification of the epiphyses was obtained (from which erroneous conclusions could be drawn, e.g. Haines [8], vide infra), histologic information showing the changes of ossification occuring in contact with the blood vessels in the cartilage canals was lacking. One earlier study, by Gray and Gardner [7], of the pattern of the cartilage canals in the proximal humerus of the human did include a small, low power photomicrograph of the proximal humerus with an early ossification center, but there was no discussion at all of any relationship of that ossification center to any blood supply.

The observation that the earliest ossification of the epiphysis occurs in close relation to the blood vessels refutes the theory that avascularity must necessarily precede ossification. Earlier observations that seemed to indicate that ossification occured between, i.e., apart from, rather than around, i.e., near, cartilage canals may have been in error because of the method used or because of inadequate observation. For example, Haines [8] in his 1933 article on cartilage canals, studied their location by injection. He noted that there were no anastomoses between the canals and that there were "avascular laminae" between groups of canals. He then examined older specimens and saw that the ossification centers had formed in the places where his "avascular laminae" had been. However, by the time of observation, the ossification centers were already penetrated by several canals, and there is no evidence that the avascular lamina existed up until or after the time of formation of the ossification center. Trueta is another investigator who has supported Haines' conclusion that ossification of the epiphysis occured in the least vascular, most nutritionally-deprived part of the epiphysis, the part between rather than adjacent to the cartilage canals.

Caffey [23] reported in 1961 from radiographic studies that ossification of the epiphyses begins as several small foci which later coalesce to form the main ossification center. Waugh [42], in 1958, in his injection study of the tarsal navicular, failed to see separate foci, but hypothesized nonetheless that they might occur around several nearby cartilage canals in the center of the navicular. VanSickle [40] showed this to be the case in the anconeal process of the dog [38].

In this study multiple centers of ossification were not seen. However, hypertrophic chondrocytes and hyperchromic matrix reminiscent of that found

near the calcifying zone of the growth plate were found around many cartilage canals, especially those in the more central areas of the epiphysis. It seems probable that with the limited number of specimens examined that the stage of multiple small foci was not present, although this stage may and undoubtedly does exist.

The head of the humerus has two secondary centers of ossification. The medial one lies under the articular surface; the lateral one lies below the greater tubercle. According to Caffey [3], based upon radiographic evidence, the epiphyseal ossification centers begin to develop within the following times:[2]

Medial:	birth - 2 months in girls
	birth - 3 months in boys
Lateral:	5-10 months in girls
	5-27 months in boys

If there is a relationship between the maturation of the secondary ossification center and the disappearance of the communicating canals across the growth plate, it might be expected that communicating canals would persist longer in the male. This hypothesis could not be tested in the present study because of the shortage of suitable specimens; however, it will be investigated as specimens become available. Another interesting question is whether the infantile type of osteomyelitis, described by Trueta [37], occurs at a far older age in males than in females. This would be expected if communicating canals persist longer in the male because of the later formation of the secondary ossification center.

The two secondary centers of ossification in the proximal humerus were still separated by a plate of cartilage in the oldest specimen (24 months of age) examined. They had not yet fused into a single center of ossification.

The standard textboods of radiology and the radiological diagnosis of bone diseases do not discuss the fusion of secondary centers of ossification[3]. Thus, further questions about the ossification of epiphysis are raised: When do the two secondary centers of ossification of the proximal humerus fuse together? What factors affect the time of fusion? And are there any peculiarities about this fusion, e.g., is it different from the fusion of an epiphyseal ossification center with the bony metaphysis when the growth plate is obliterated? Again, these questions will be investigated as additional specimens become available.

The finding of epiphyseal ossification in association with a rich blood supply in the human humeral head supports a new theory for epiphyseal ossification, that is, a vascular rather than an avascular theory. In the light of modern knowledge that bone is a metabolically active tissue and also that ossification is an even more metabolically demanding process than simply maintaining bone, there is even more reason to support a vascular theory. Because of the long delay between the appearance of the cartilage canals in early fetal life and the appearance of the secondary ossification centers, there must be other factors as well that play a role in determining the time and site of development of the ossification center. Kummer [18] supports the view that biomechanical factors, especially hydrostatic pressure within the epiphysis, are very important. The fact that there are two major forces applied to the proximal humerus, one pressure and the other traction, and that there are two secondary ossification centers within the proximal humeral epiphysis, one beneath the articular surface where the pressure is greatest and the other beneath the greater trochanter where the most traction is exerted, is very suggestive that such a process is functioning.

CONCLUSIONS

1. The cartilaginous head of the humerus is not avascular at the time that ossification occurs. Cartilage canals invade the epiphyseal cartilage by about ten weeks of gestation. The cartilage canals grow in complexity by branching and in total length, as the volume of the humeral head increases, so that the density of blood vessels per unit volume of cartilaginous matrix remains both uniform in distribution and constant in time. Ossification of the humeral head does not occur until approximately full-term, or shortly thereafter.

2. Ossification first appears in the human humeral head in a region of cartilaginous matrix that is in close contact with numerous thin-walled blood vessels (a cartilage glomerulus). This is the first histological demonstration of this relationship between blood vessels and ossifying epiphyseal cartilage in human material.

3. Multiple, separate microscopic foci of ossification were not seen. However, hypertrophic chondrocytes and hyperchromic cartilagenous matrix, which are typical of the pre-ossific zone of the growth plate, were seen around many cartilage canals. Thus, it seems likely that multiple microscopic foci of ossification probably precede the formation of a secondary center of ossification, which would enlarge by coalescence of these foci. As additional materials become available, the specimens will be examined to answer this question.

4. Two major centers of ossification develop in the humeral head. The medial one develops first, just before or within the first few months following birth, and lies under the articular surface. The lateral one appears

between the second and seventh months of post-natal life in females and lies beneath the greater tubercle (tuberosity). The two separate secondary ossification centers were still distinct in an infant of 24 months, although vascular communications existed. The vascular communications between the two secondary ossification centers probably played a role in the eventual fusion of the centers, as hypertrophic cells were observed along the course, together with minimal extension of the ossification process along the canal from each ossification center. These vascular communications also improved the blood supply to the medial ossification center, which may be an important factor in explaining the relative rarity of avascular necrosis of this region compared to the lower extremity analogue (the capital femoral epiphysis).

5. Communicating canals were definitely seen in many sections; frequently several were seen in the same section. The existence and frequency of communicating canals appears to be dependent upon the degree of maturation of the secondary ossification center. They were first seen in the proximal humerus at about 19 weeks of gestation and persisted until 15 months after birth. It was during this period that the secondary centers of ossification developed within the humeral head. It is hypothesized that there may be a similar relationship between communicating canals and the development of secondary centers of ossification in other epiphyses.

Specimen 12 is the first histological demonstration in human material of osteomyelitis destroying the growth plate of an infant and spreading into the epiphysis, with cartilage canals as the leading points of extension of the suppuration. This supports Trueta's hypothesis, formulated in 1959, but not deomnstrated by any specimens, that osteomyelitis of the infant spreads

to the epiphysis by passage through the blood vessels that cross the growth plate.

6. There are several interesting "experiments of nature" that direct attention to the relationship between cartilage canals and the ossification of epiphyses.

In normal phalanges, cartilage canals form only in the proximal epiphysis, and this is the only end of the phalanx to develop a secondary center of ossification. But in experimental hypothyroidism in the rat, cartilage canals form in both ends of the phalanx and both ends develop secondary ossification centers (pseudoepiphyses). Similarly, a characteristic of hypothyroidism in humans is the development (radiologically) of secondary epiphyseal ossification centers at both ends of the phalanges, matacarpals, and metatarsals.

In chondrodystrophia calcificans congenita, commonly known as "stippled epiphyses", the epiphyses develop multiple small centers of ossification. These calcified areas probably occur around cartilage canals, but for some unknown reason do not coalesce to form a single secondary ossification center.

7. A complex epiphyseal vessel network adjacent to the growth plate, similar to that described by Trueta, was not seen in the specimens studied. Histological sections did show a few vessels in the resting/germinal layers of the growth plate. In the older specimens (e.g. 24 month proximal humerus, 15 month proximal tibia), many more capillary-sized vessels were seen between the growth plate and the subchondral plate of the secondary ossification center. Thus, like the presence or absence of communicating canals, the complexity of the epiphyseal circulation, especially that portion juxtaposed to the growth plate, may be related to the structural maturation of the epiphysis.

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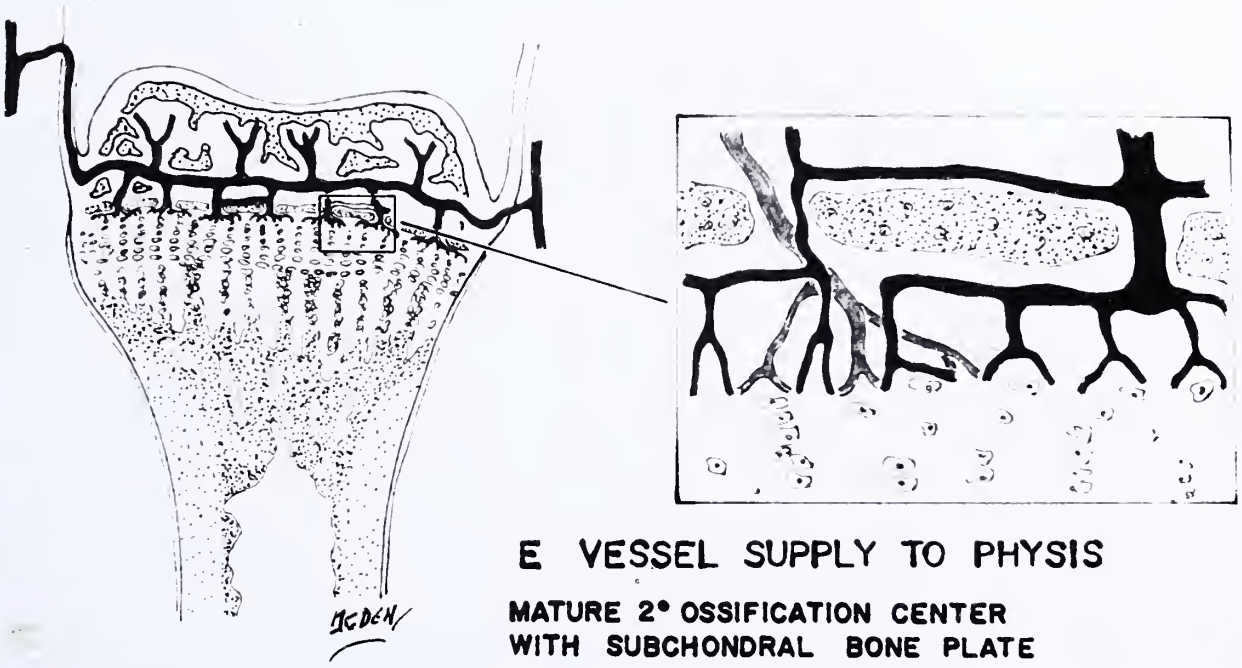
FIGURE 1:

Schematic diagram of the blood supply to the growth plate from its epiphyseal side (i.e. vessels) at the stage when the epiphyseal ossification center is well established. Note the perforating canals through the bony subchondral plate to supply the zones of resting and proliferating chondrocytes.

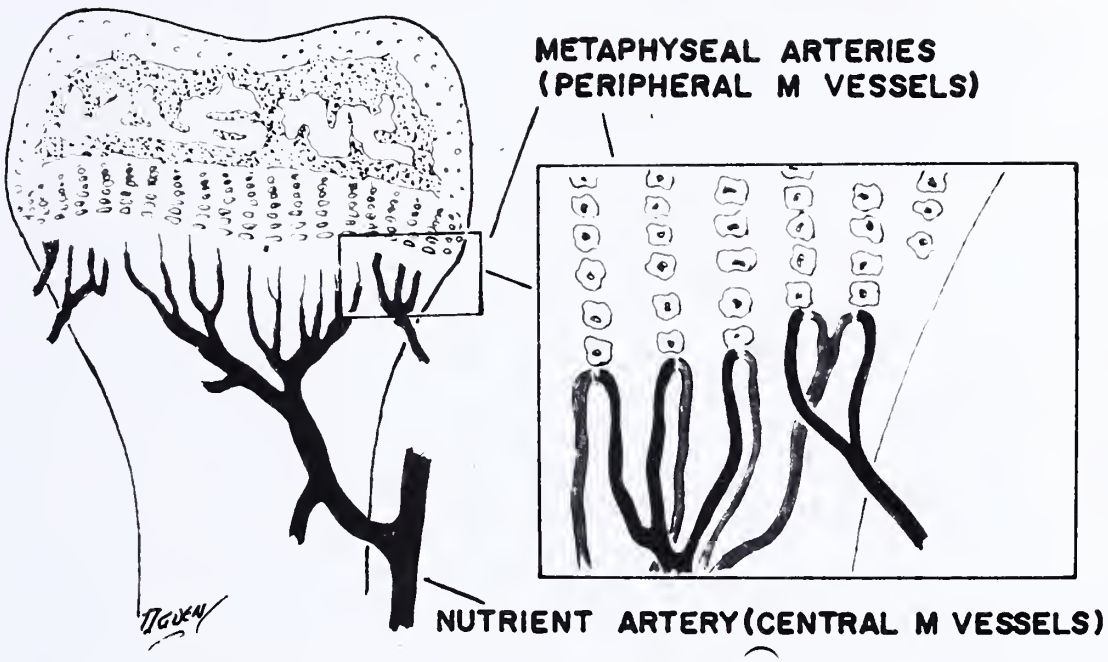


E VESSEL SUPPLY TO PHYSIS

MATURE 2° OSSIFICATION CENTER
WITH SUBCHONDRAL BONE PLATE

FIGURE 2:

Schematic diagram of the blood supply to the growth plate from its metaphyseal side. The developmental stage is the same as in figure 1.



M VESSEL SUPPLY TO PHYSIS

FIGURE 3:

15 week specimen. Photomacrograph of humeral head.

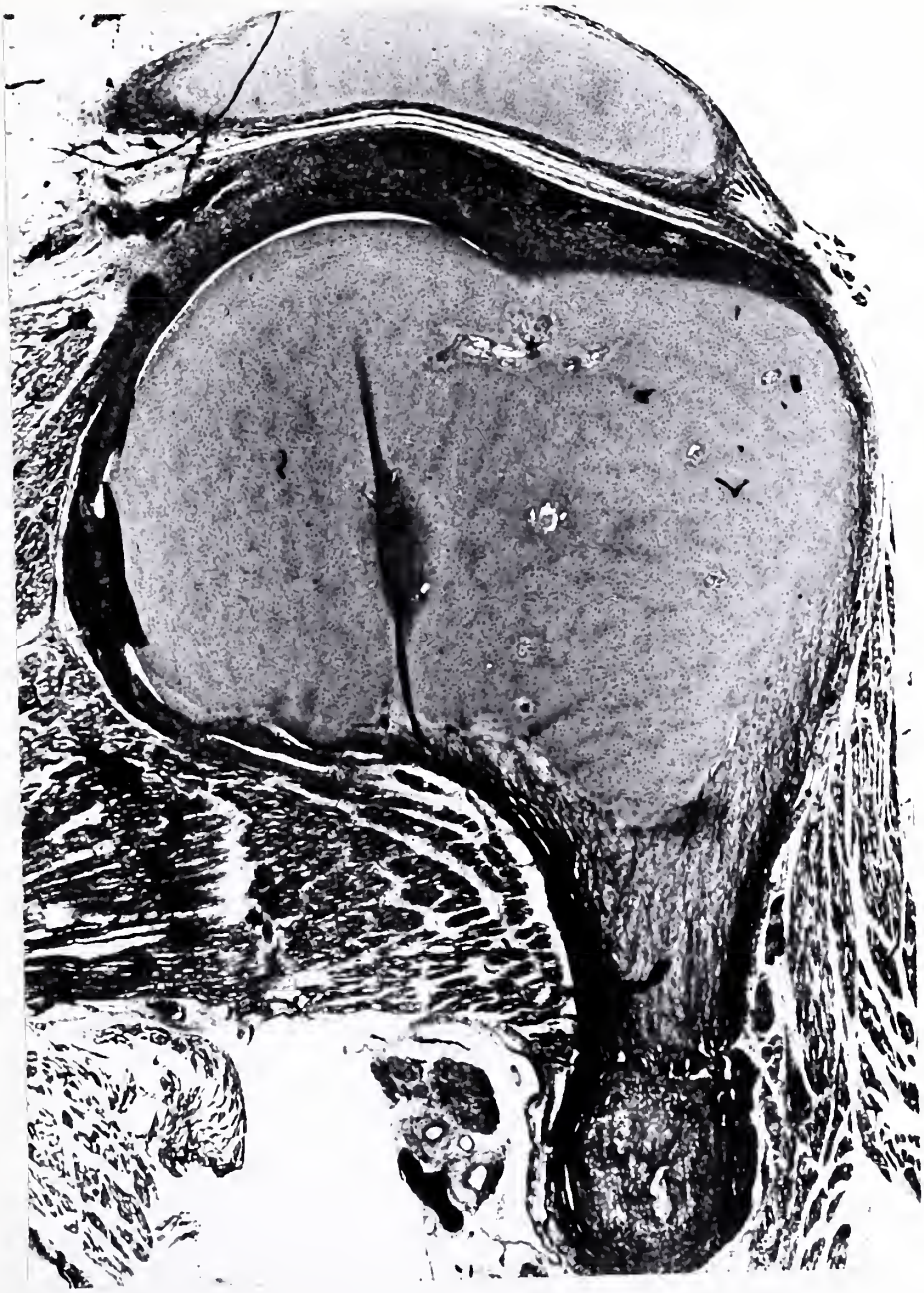


FIGURE 4:

16 1/2 week specimen. Photomacrograph of humeral head.

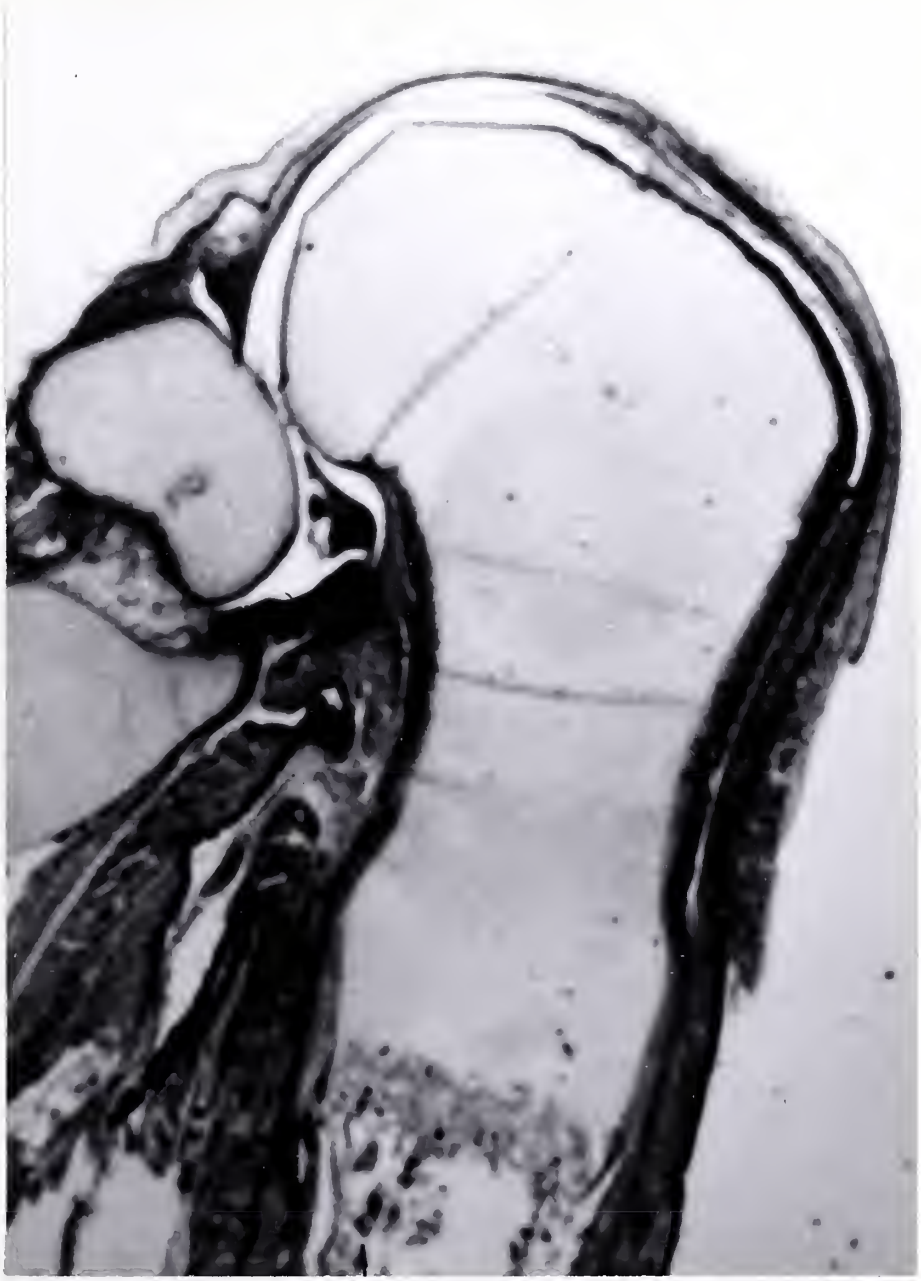


FIGURE 5:

19 week specimen. Photomacrograph of humeral head.

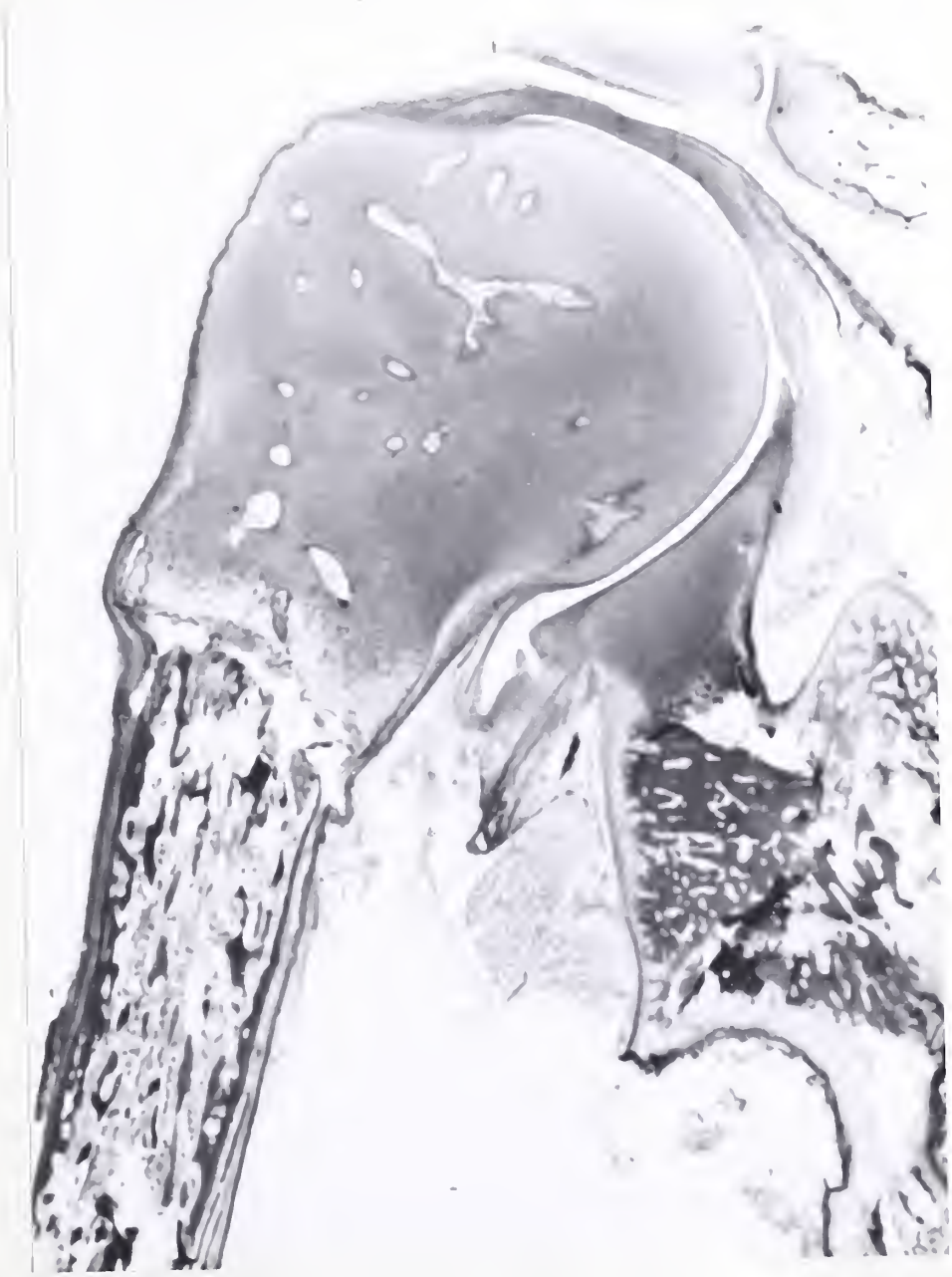


FIGURE 6:

21 week specimen. Photomacrograph of humeral head.

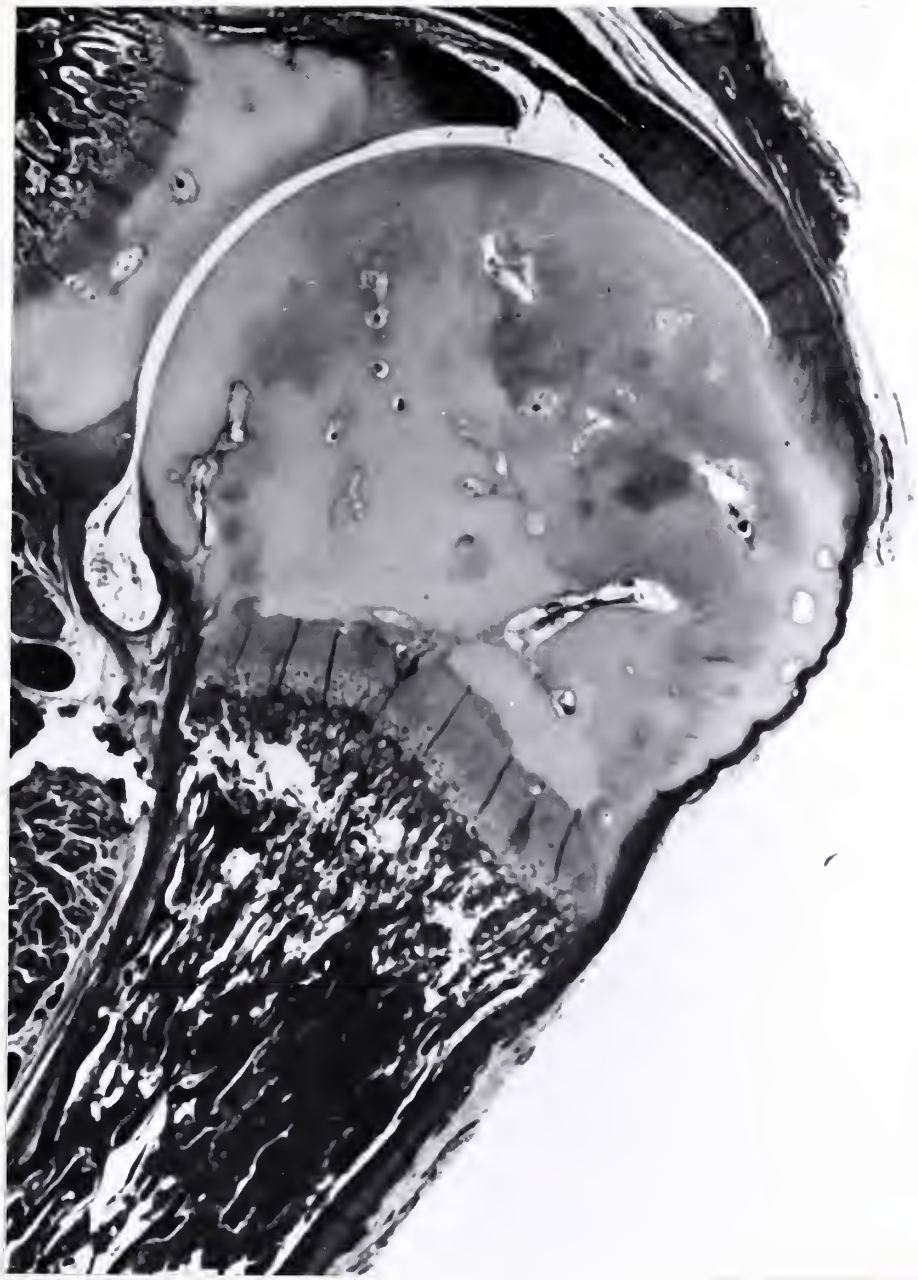


FIGURE 7:

28 week specimen. Photomacrograph of humeral head.

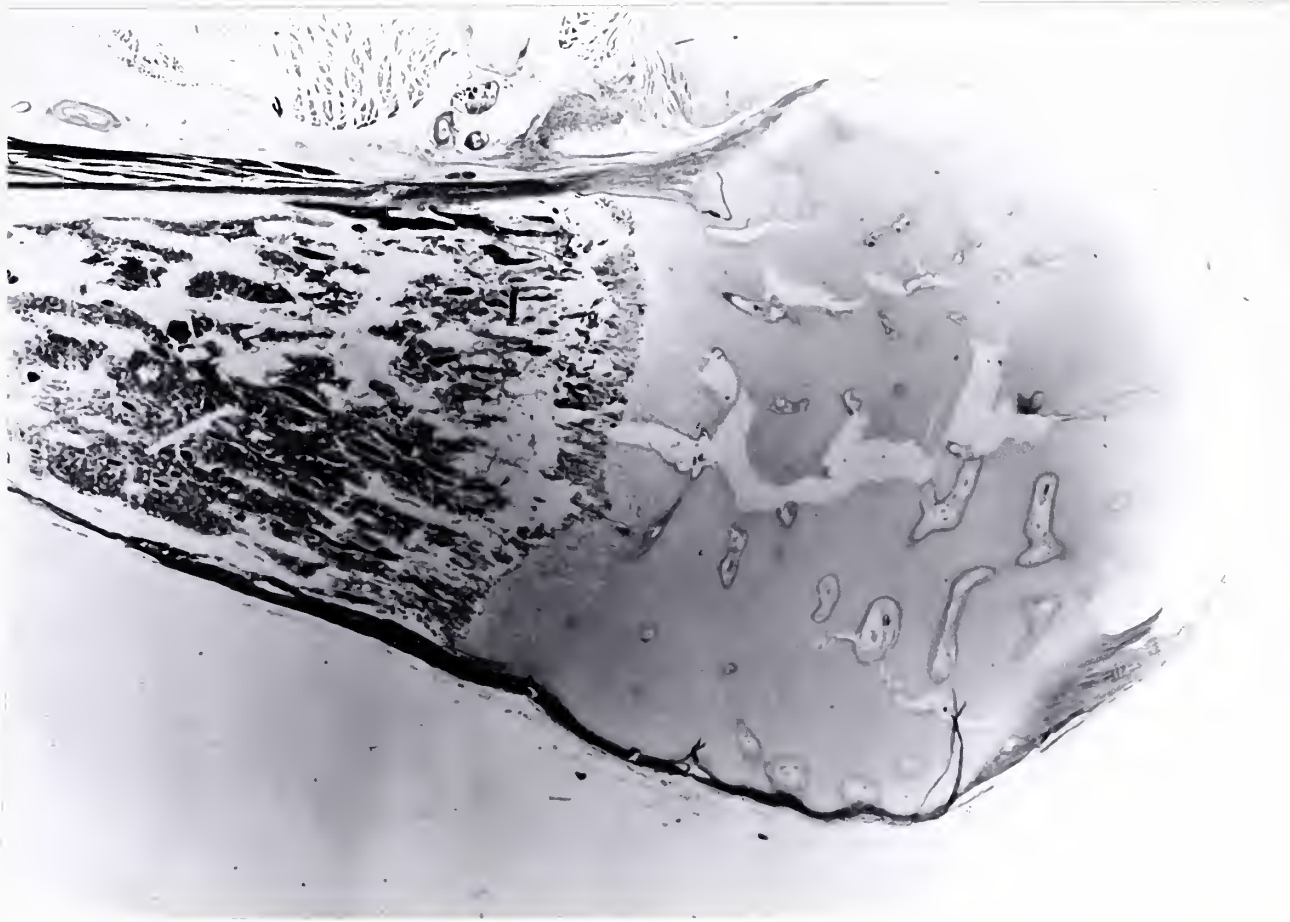


FIGURE 8:

39 week specimen. Photomacrograph of humeral head.

FIGURE 9:

39 week specimen. Closer view of the area of early ossification.

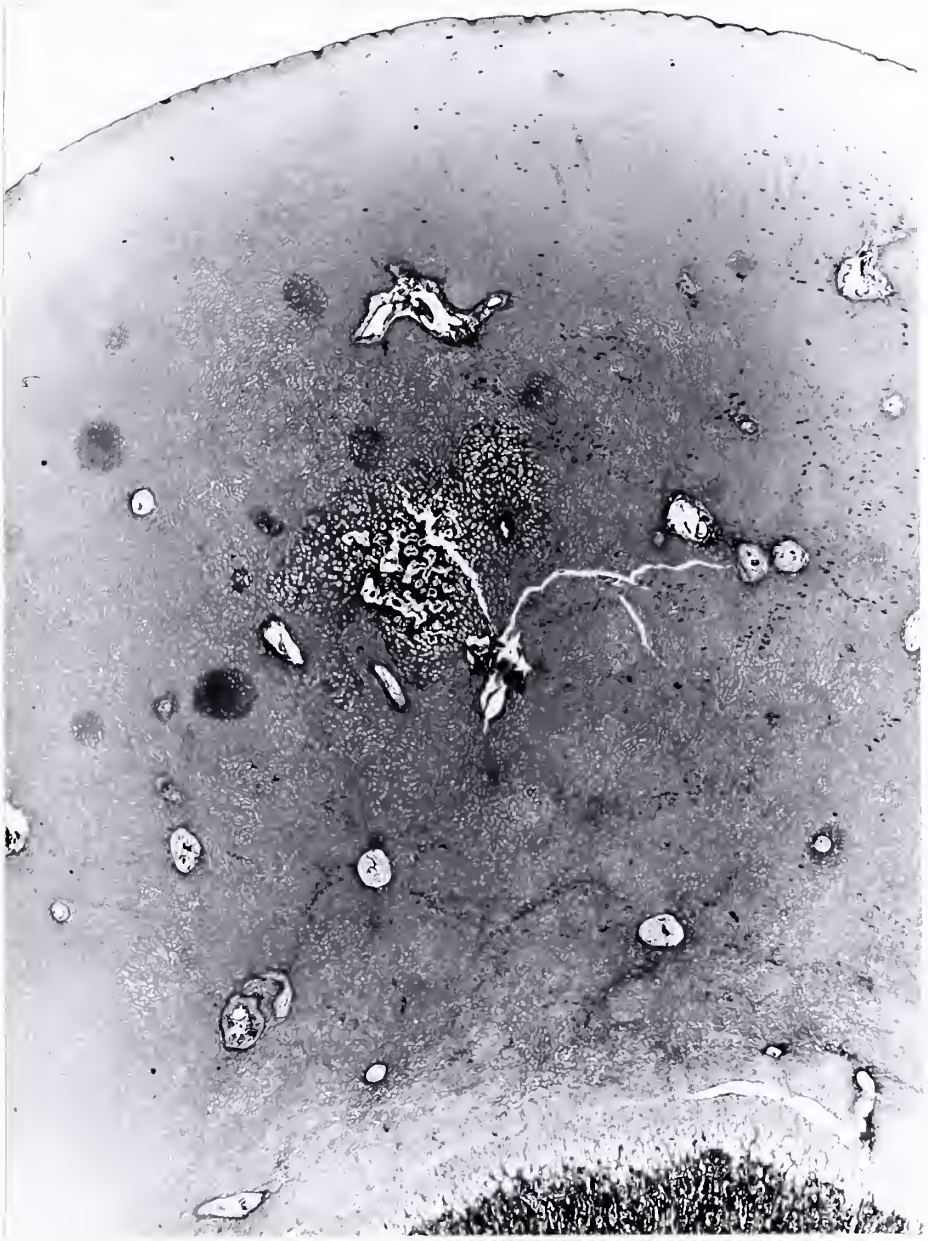


FIGURE 10:

2 month male specimen. Photomacrograph of the humeral head.

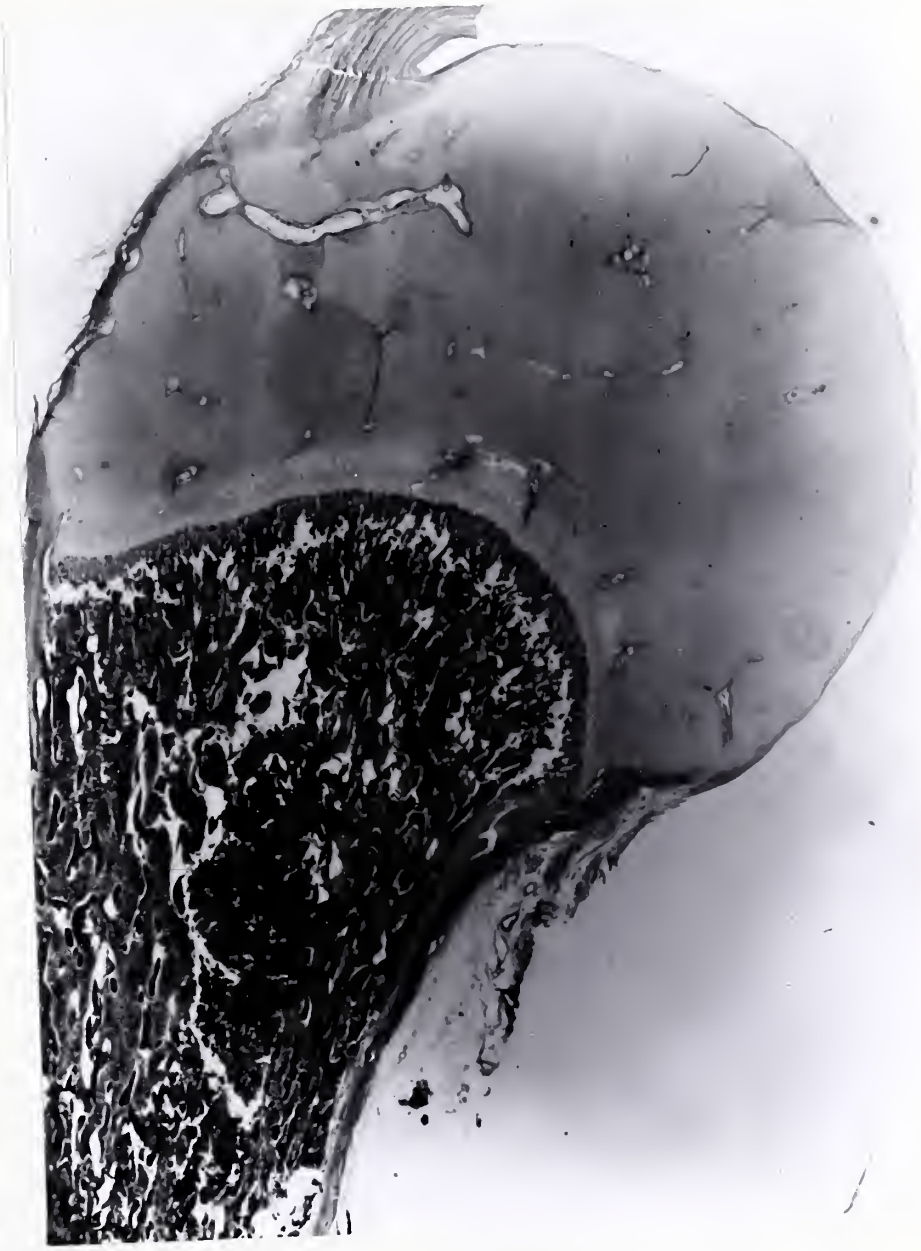


FIGURE 11:

2 month female specimen. Photomacrograph of the humeral head.

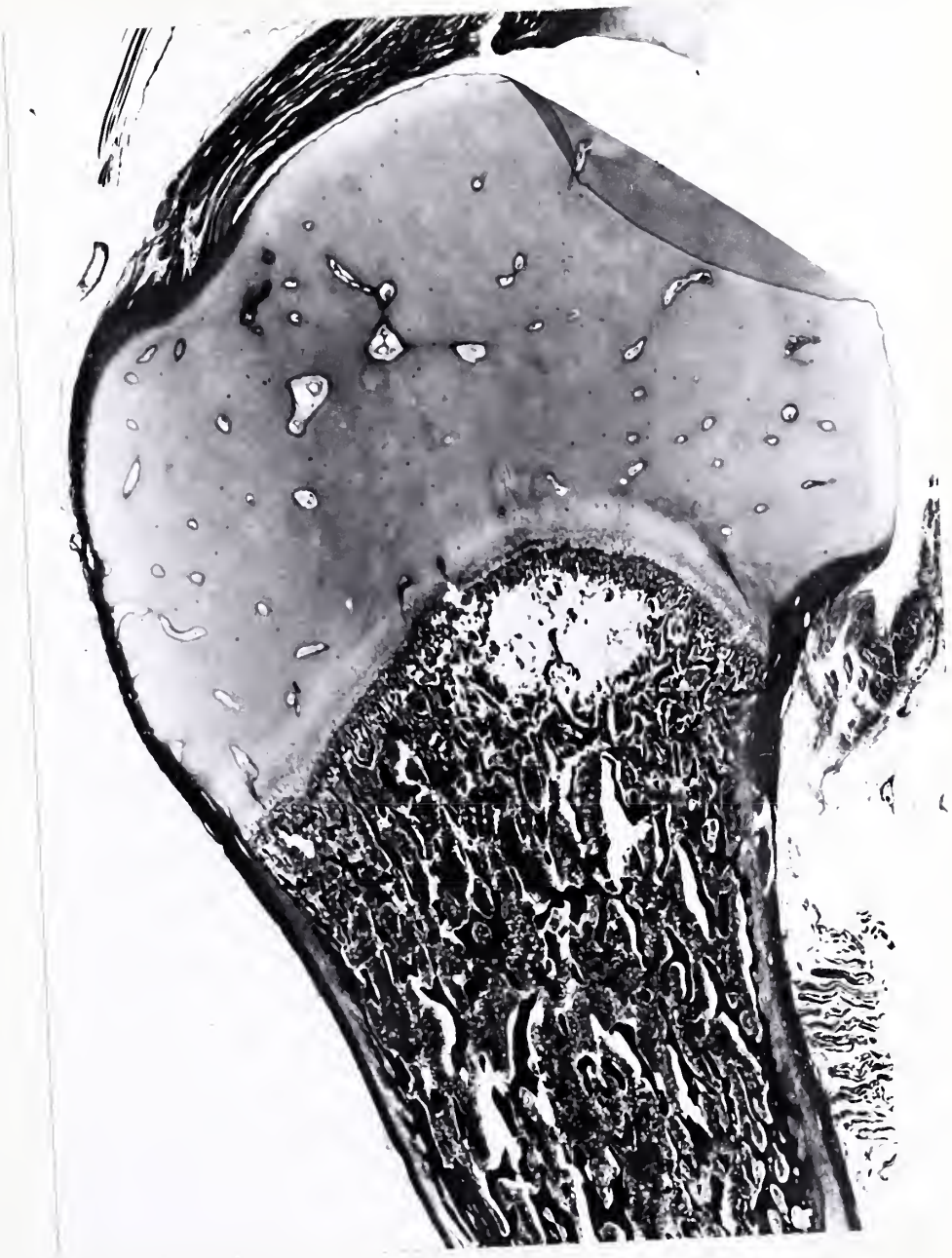


FIGURE 12:

7 month female specimen. Photomacrograph of the humeral head.

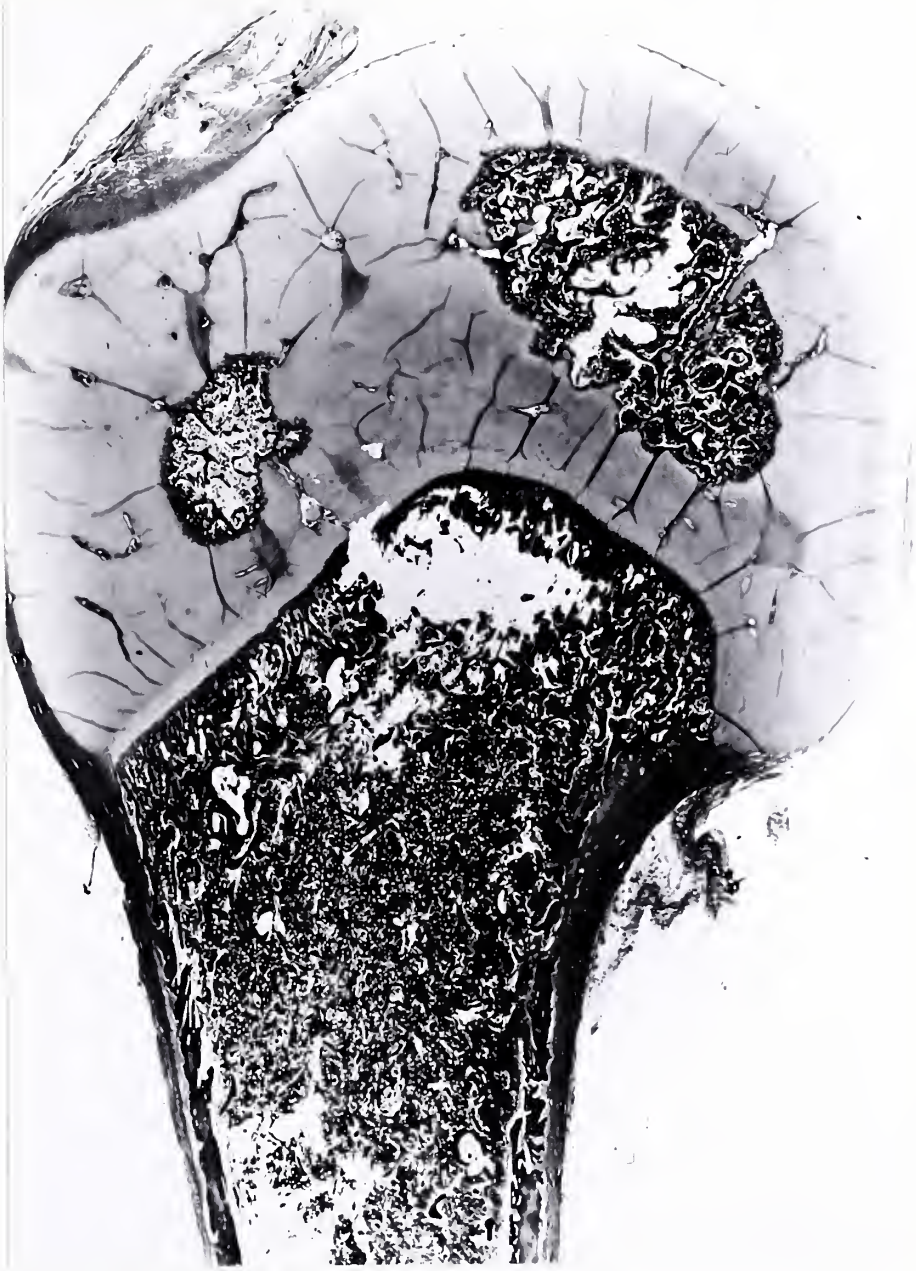


FIGURE 13:

7 month female specimen. Closer view of the lateral ossification center.

FIGURE 14:

15 month female specimen. Photomacrograph of the humeral head.

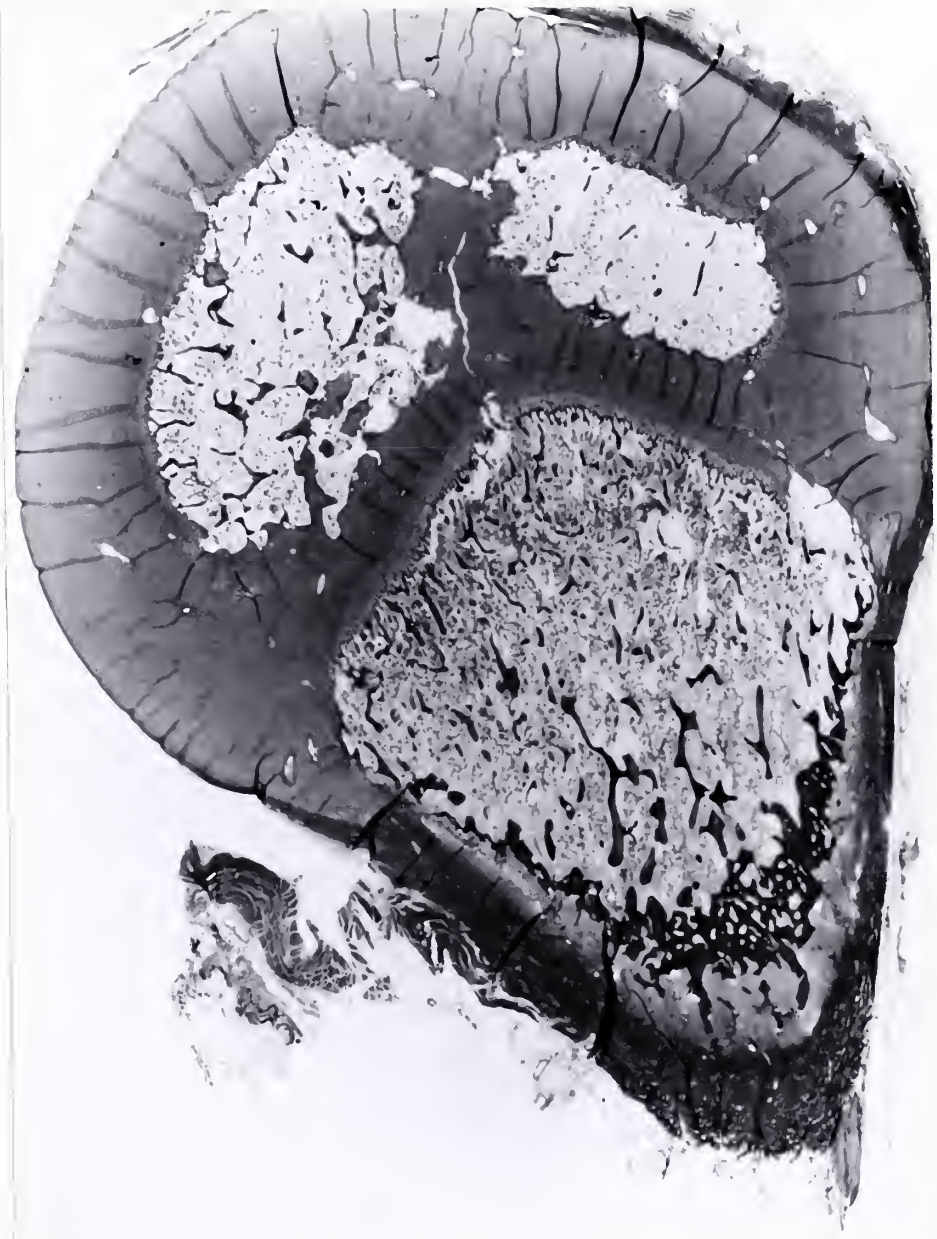
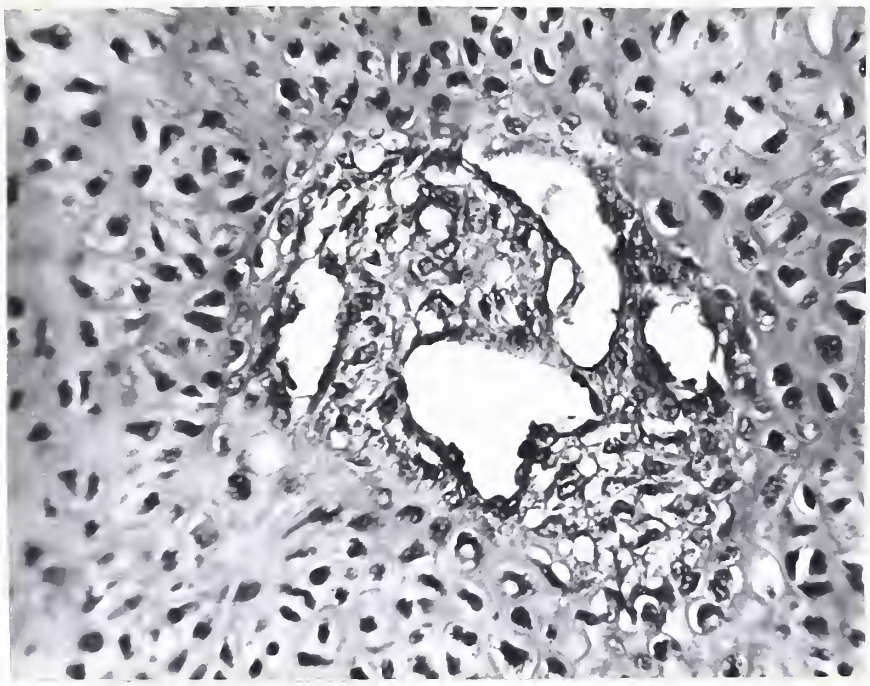


FIGURE 15:

15 week specimen. Medium power view of a typical cartilage canal.

FIGURE 16:

19 week specimen. View of growth plate showing two communicating canals.



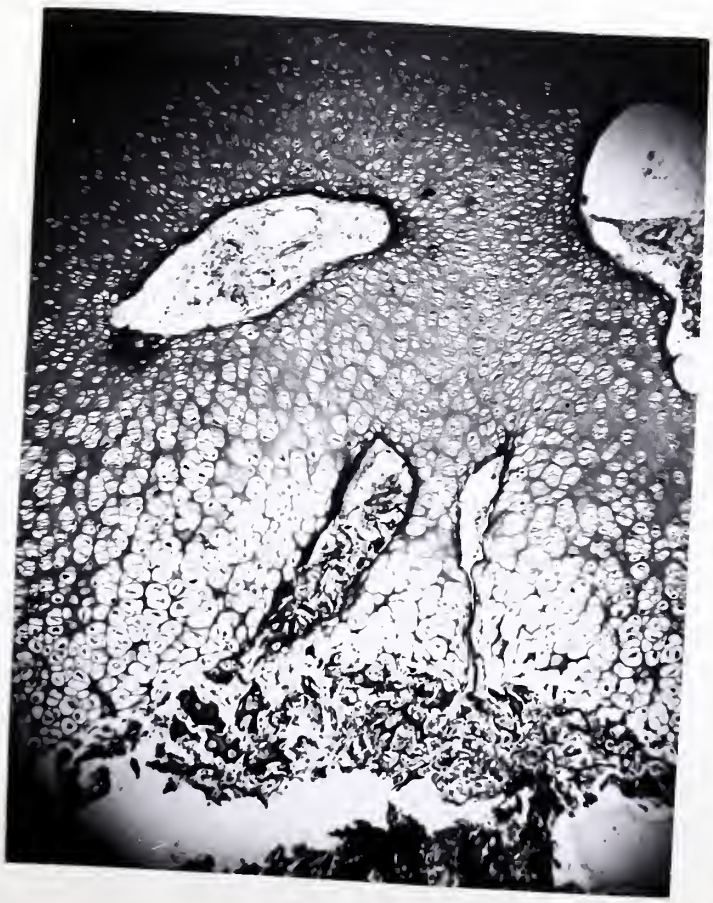
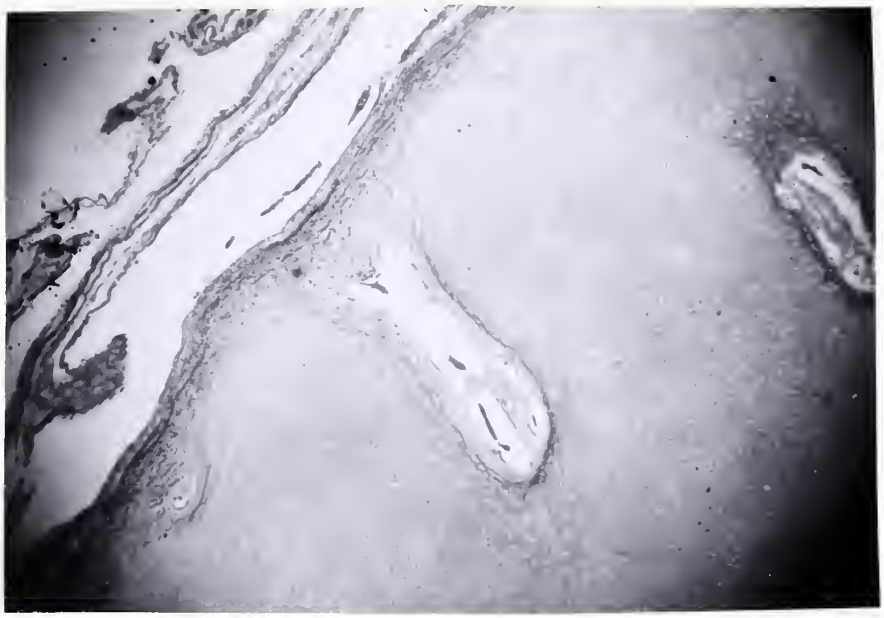


FIGURE 17:

19 week specimen. Canal entering from lateral border of head.

FIGURE 18:

19 week specimen. Branched canal within the head.



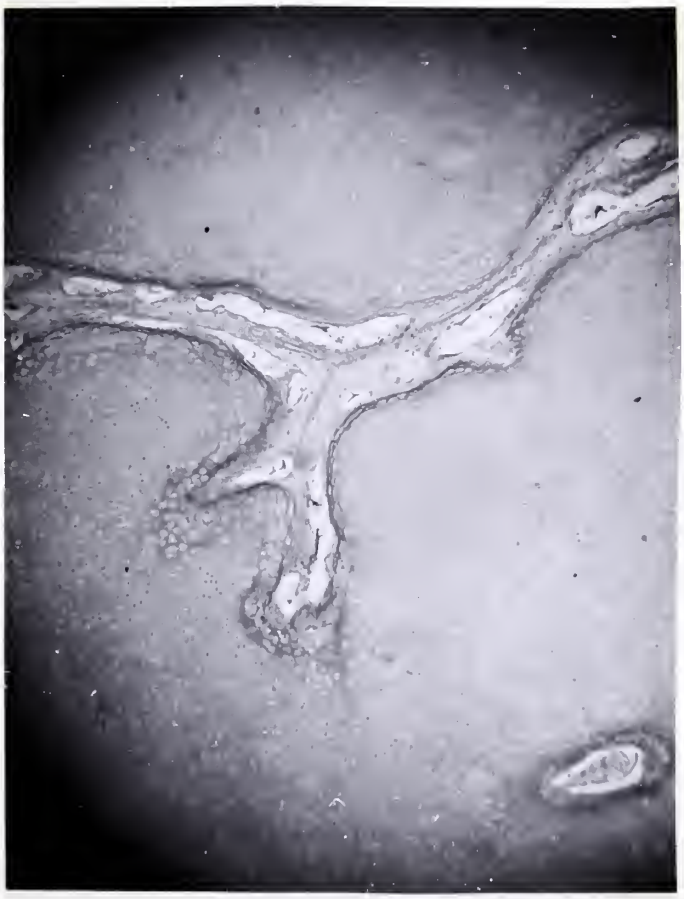
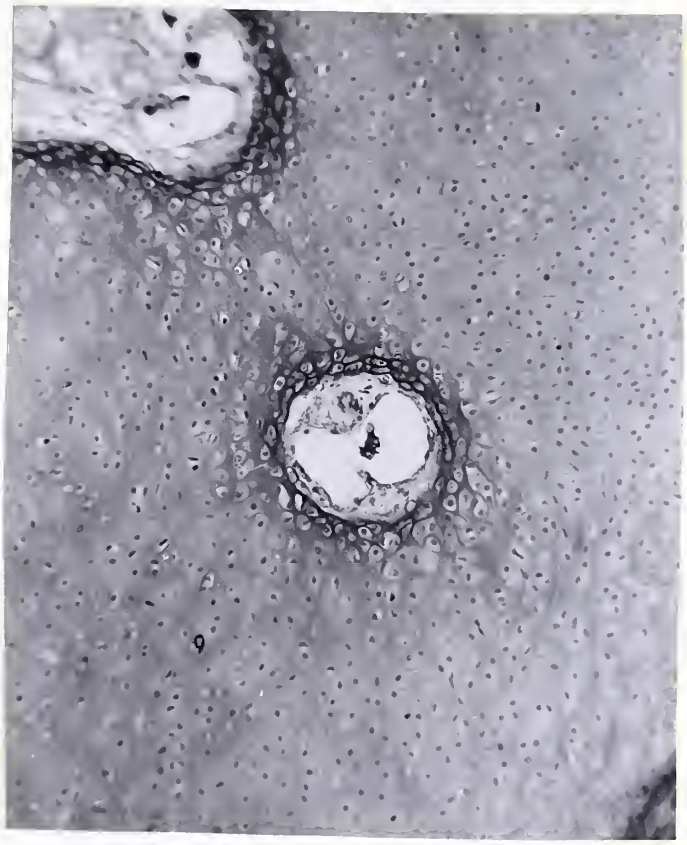


FIGURE 19:

19 week specimen. Slight cellular hypertrophy and hyperchromaticity of the matrix around a cartilage canal.

FIGURE 20:

19 week specimen. Higher power, showing intermediate cell types at the boundary of the canal and the matrix.



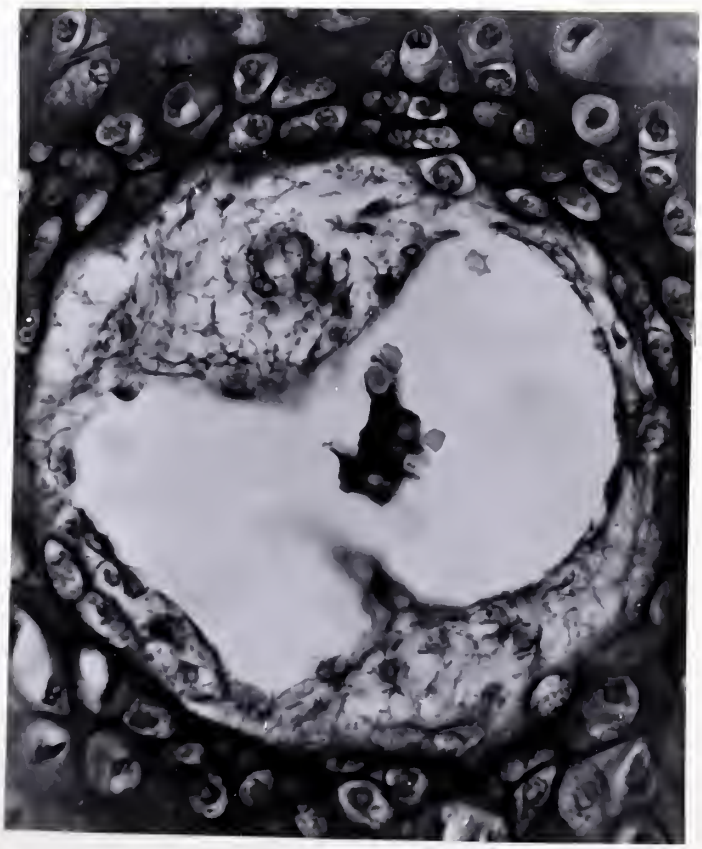


FIGURE 21:

28 week specimen. A wide communicating canal.

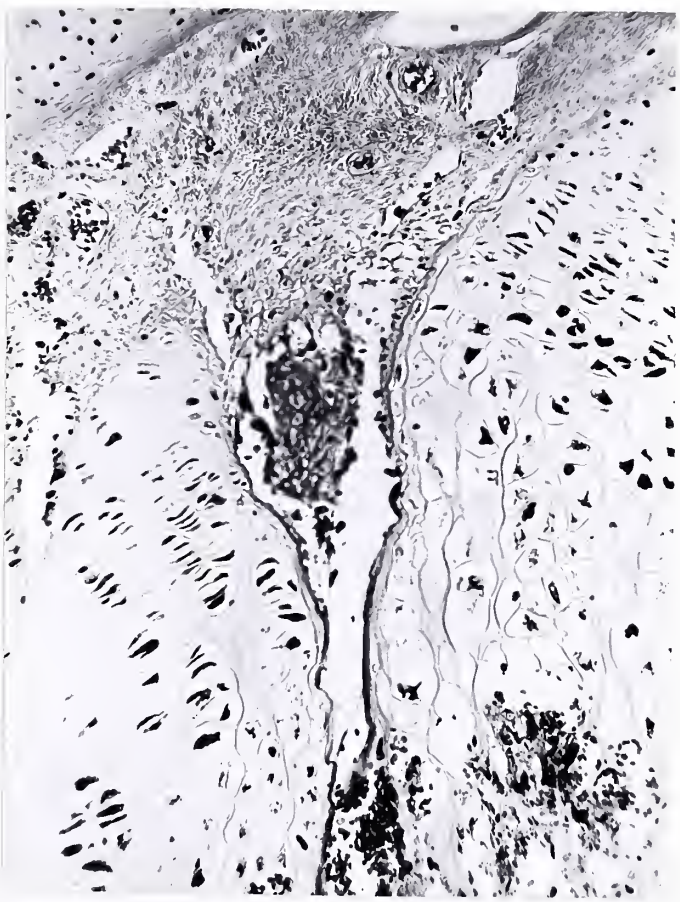
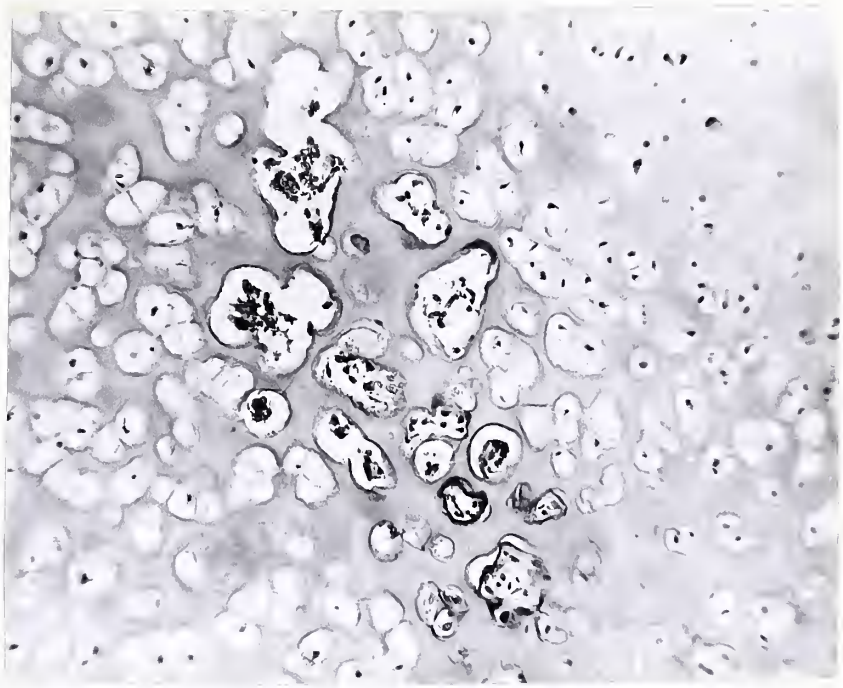


FIGURE 22:

39 week specimen. The early center of ossification. Erythrocytes are visible in the vascular space at the top of the field. Many capillaries are present in the center of the field.

FIGURE 23:

Higher power, showing the intimate relationship between the endothelium and ossification.



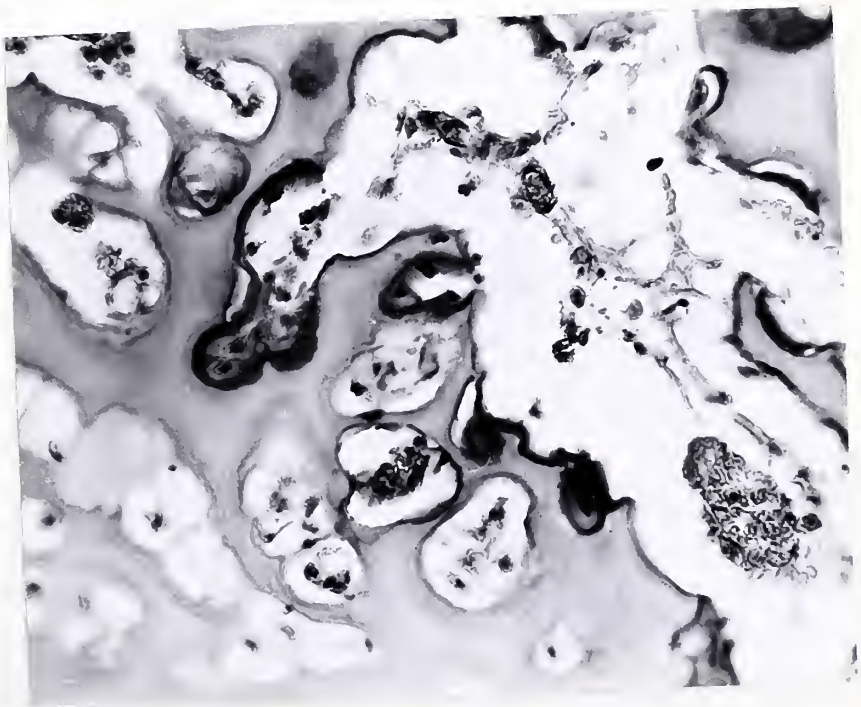
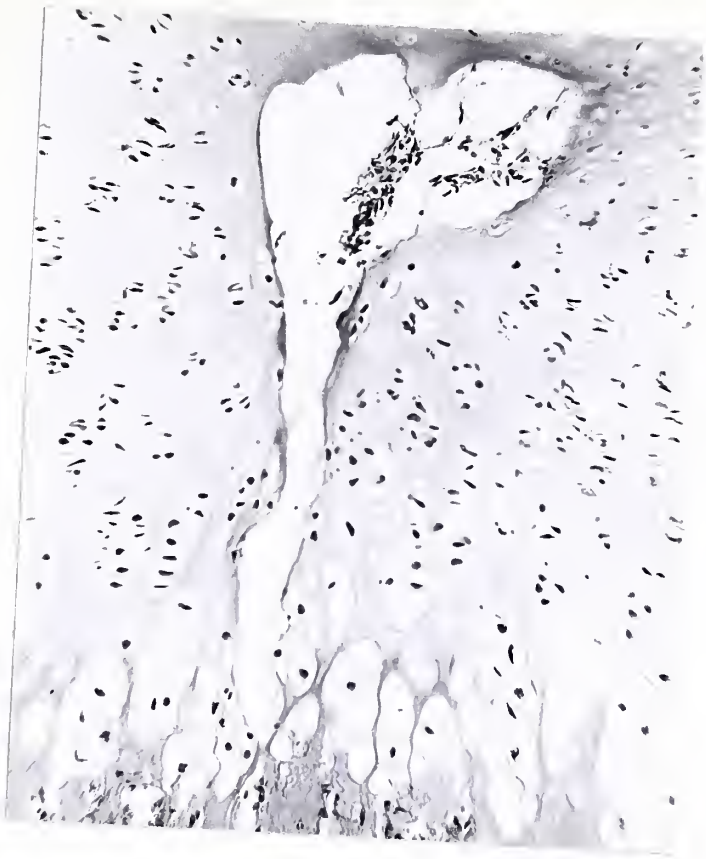


FIGURE 24:

2 month male specimen. A communicating canal. Note the hyperchromic matrix along the borders of the passage.

FIGURE 25:

2 month female specimen. Hypertrophied chondrocytes around the cartilage canal. Note increased intercellular distances and matrix compared to figures 19, 20.



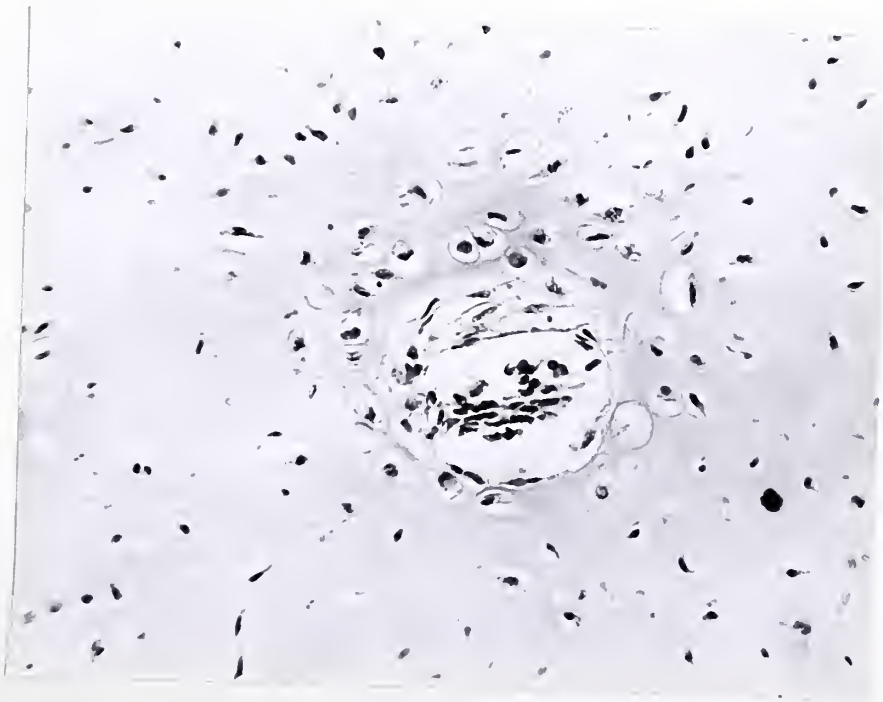
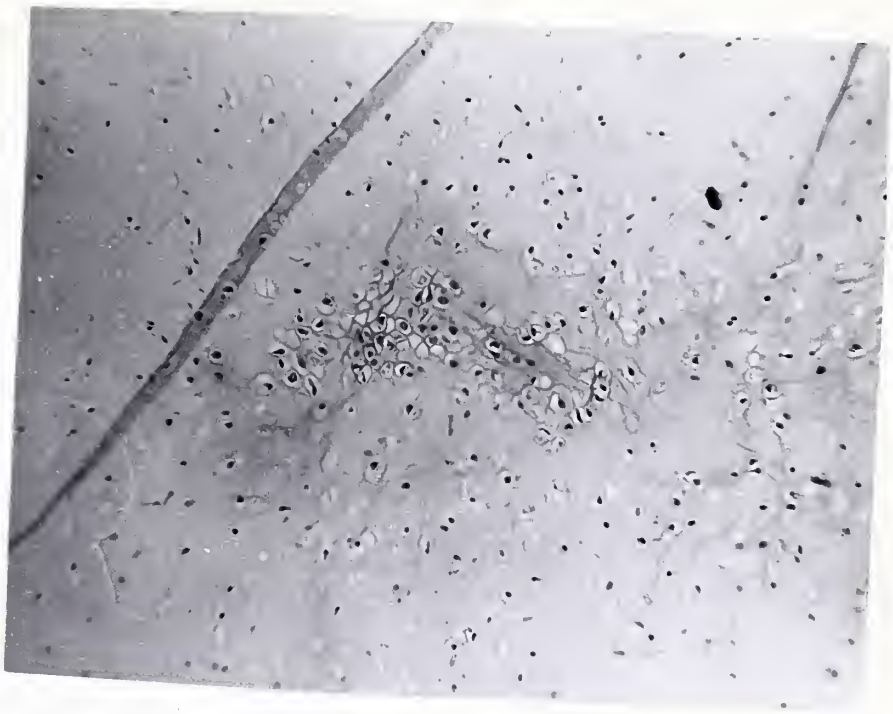


FIGURE 26:

2 month female specimen. Area of hypertrophied chondrocytes in an apparently avascular region. However, see next photograph.

FIGURE 27:

2 month female specimen. Close relationship of hypertrophied cells to the cartilage canal, which lies near them but out of the plane of the previous section.



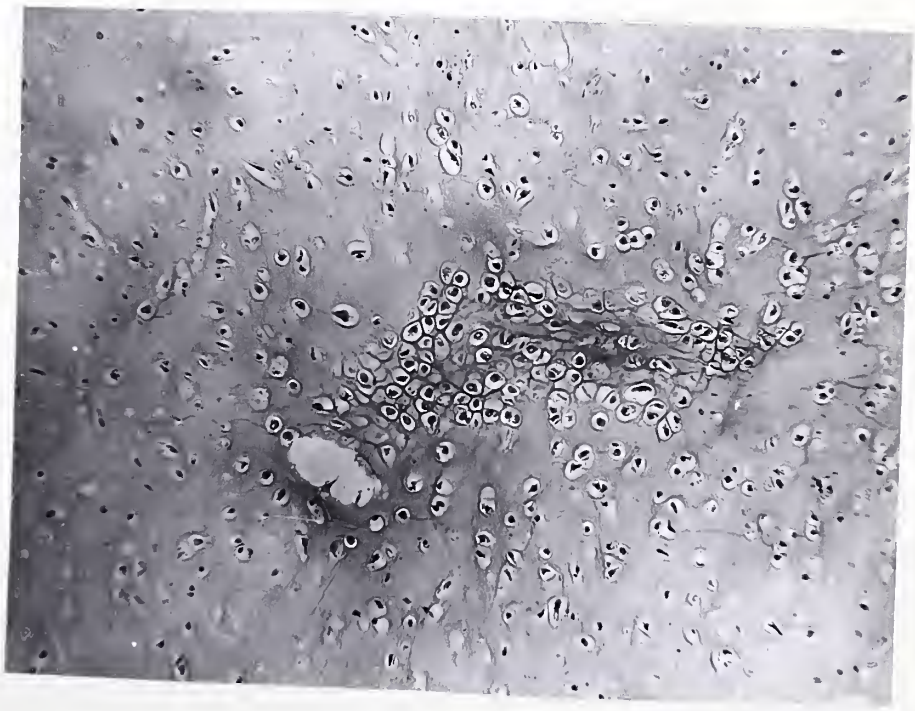


FIGURE 28:

7 month female specimen. The entrance of a canal into the medial ossification center. A narrow arteriole is visible in the center of the canal.

FIGURE 29:

7 month female specimen. Increased zone of calcification between the ossification center and a nearby canal.

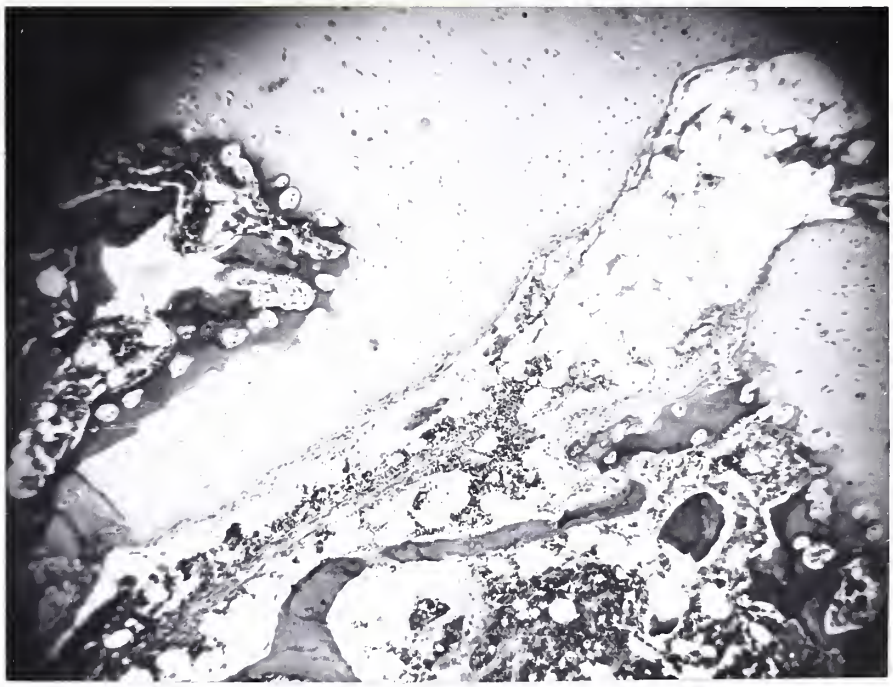
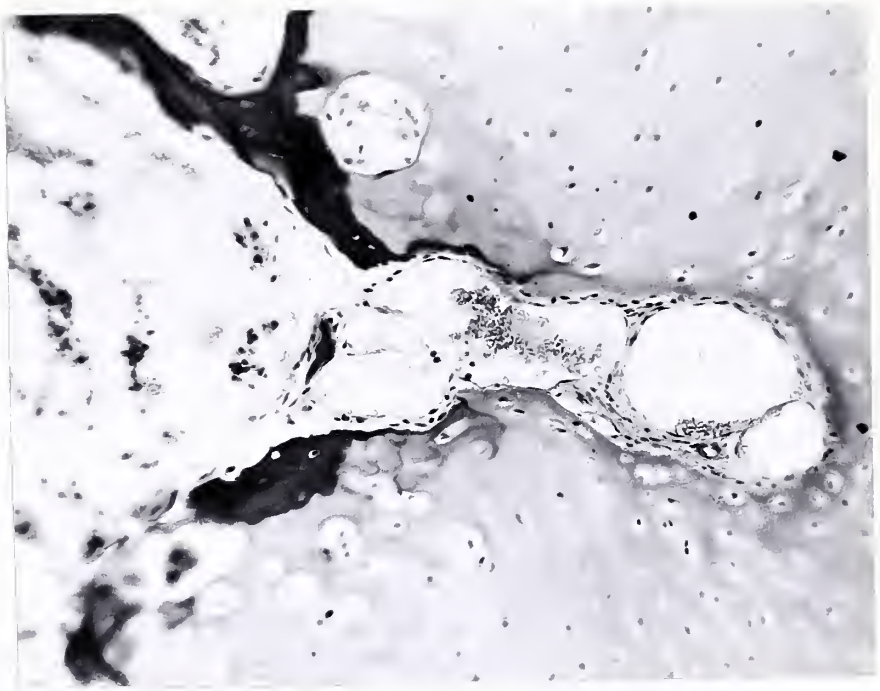


FIGURE 30:

15 month female specimen. Canal entering lateral ossification center.

FIGURE 31:

15 month female specimen. Increased calcification of the cartilaginous matrix between the ossification center and a nearby cartilage canal.



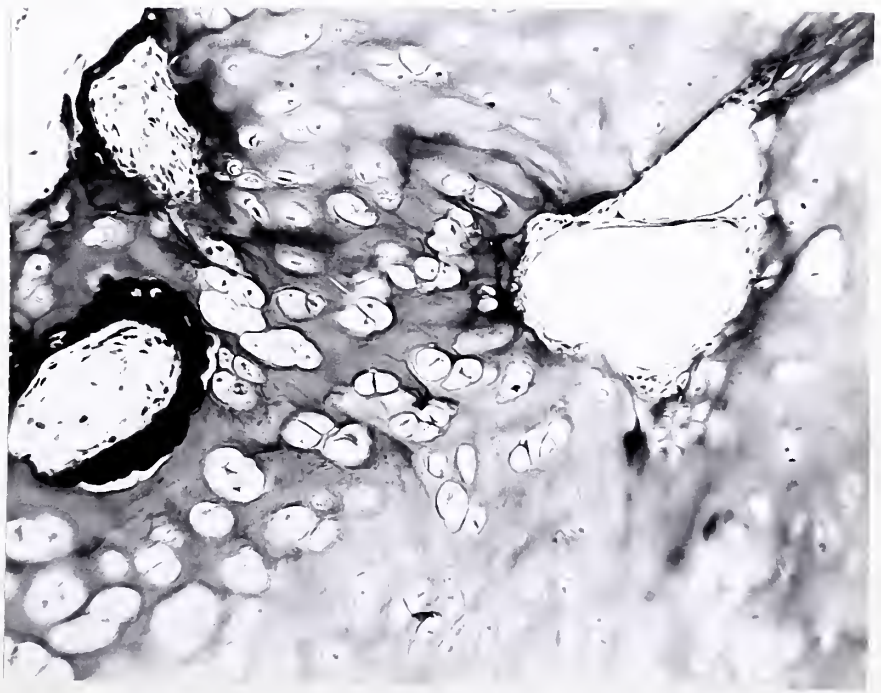


FIGURE 32:

1 month male specimen with disseminated osteomyelitis, showing an area of erosion of the growth plate near the medial border (left).

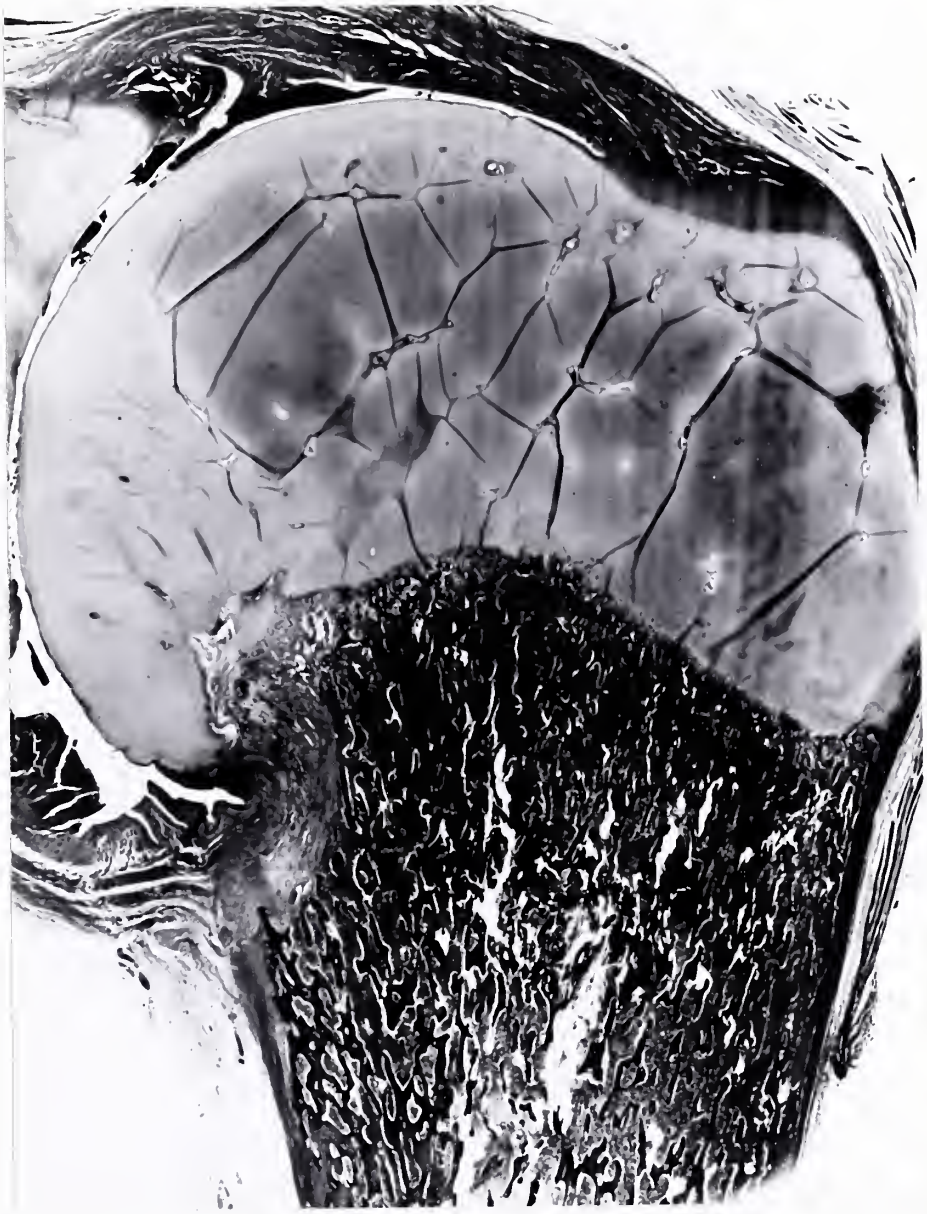
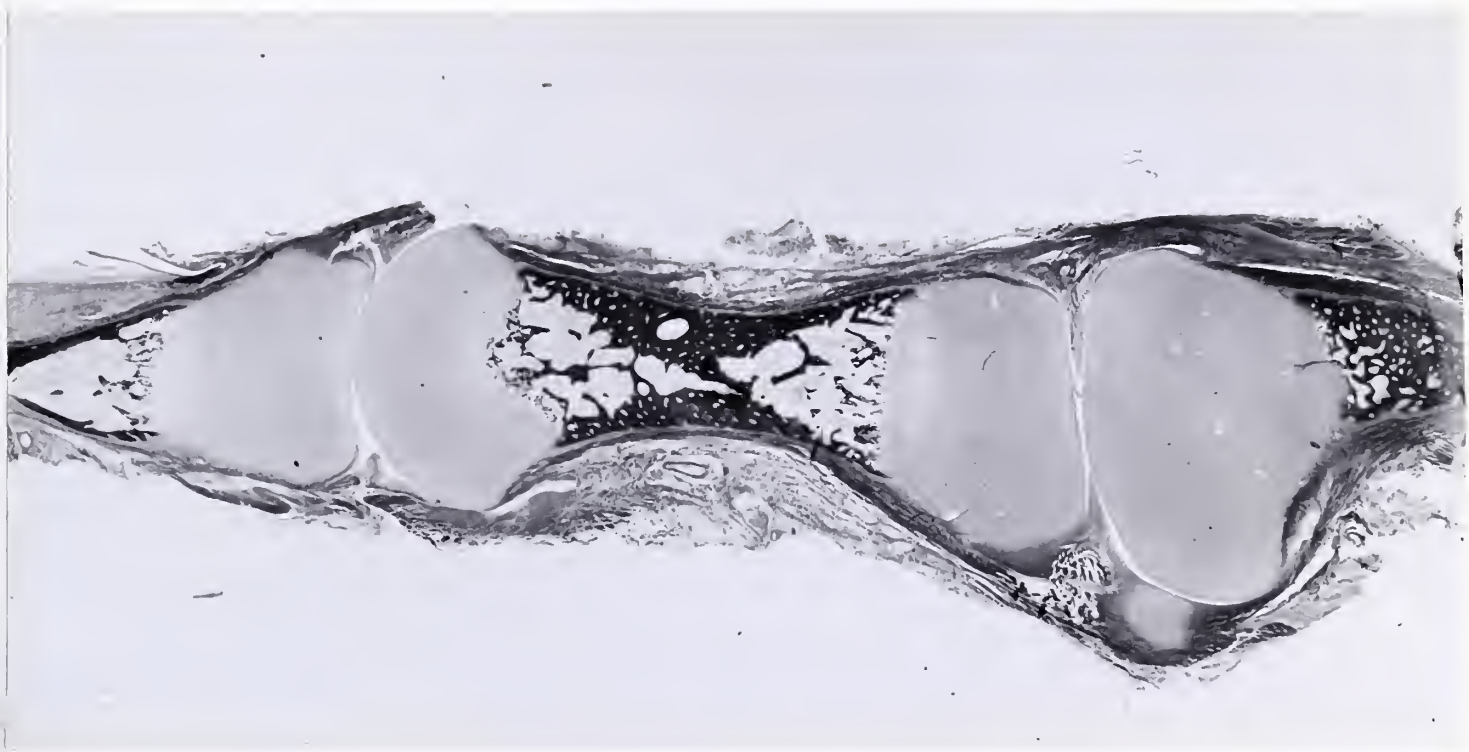


FIGURE 33:

39 week specimen. A sagittal section of the first toe, including distal metatarsal and both phalanges. Note that the distal epiphysis of the proximal phalanx, which does not develop a secondary ossification center, has no cartilage canals, whereas the proximal epiphysis, which does develop an ossification center, has a canal system.



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