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HISTOPATHOLOGICAL STUDY OF ENDOCHONDRAL OSSIFICATION OF FEMUR IN INFANTS

Histological Observation on 193 Autopsy Cases

BY

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ABSTRACT

The purpose of the present study was to clarify the fundamental histological changes in endochondral ossification and their morphogenesis in 190 infants with various kinds of diseases ranging in ages from birth to 14 months and in 3 aborted fetuses. Histopathological examination was performed on the epiphyseal plate and its precursor, that is, the diaphyseal growing part of the cartilaginous epiphysis at the lower end of the femur. Before the development of the secondary ossification center, the diaphyseal growing part showed a less distinct zonal differentiation in structure. With the progressive development of the secondary ossification center, the epiphyseal plate became well differentiated and showed a typical zonal differentiation. The cluster pattern of the proliferating cartilage cells was characteristic in the fetal life. There was a close relationship between the cluster pattern and the insufficient development of the secondary ossification center. The present study revealed that the cluster pattern, disturbances in the proliferation of the cartilage cells and calcification of the cartilage were most fundamental and influential in morphogenesis of the histological changes in the endochondral ossification in the younger infants.

INTRODUCTION

Endochondral ossification takes place in the epiphyseal plate as well as in the adjacent metaphysis and is a most essential process for the longitudinal growth of a long bone. This process consists of a coordinated sequence of many different processes: proliferation, maturation, calcification of the cartilage followed by the invasion by the connective tissue and capillaries from the adjacent metaphysis and the formation of the primary as well as secondary spongiosae in the metaphysis. In an infant, these areas are the site of tremendous cellular activity. Any interference with any one of these different processes, while the others continue, is rapdily reflected by an alteration in the normal histological appearance of these areas (Weinmann and Sicher[1]; Bourne[2]; Ham[3]; Collins[4]).

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The infant undergoes a most dramatic alteration in his environment at birth. This environmental change, probably affects the endochondral ossification and results in the morphologic alteration of the process. However, there are a few systematic studies on the histological change in the endochondral ossification during the period from birth to the end of the first year of life (Follis et al.[5]; Park[6]).

The present author made a histopathological study on the epiphyseal plate and metaphysis at the lower end of the femur in infants who died before birth and until one year and 2 months after birth to clarify the fundamental morphologic changes in endochondral ossification and their morphogenesis.

Material and Method

The femurs used in the present study were taken from the 190 autopsy cases of infants with various kinds of diseases ranging in ages from birth to 14 months of life (Tables 1 and 2). In addition, 3 aborted human fetuses were used. These femurs were sawed longitudinally into two pieces in the sagittal direction and fixed in 10% formalin. The lower part of the femur was used for the study. Decalcification was performed according to Plank and Rychlo's method[7] for two to ten days. The decalcified specimens were rinsed under running water for three days and then dehydrated and embedded in paraffin. The 6-micron sections were prepared and stained with hematoxylin-eosin. The femurs were histologically examined on the paraffin sections.

Table 1. Age distribution

Age	Number of cases
0 —24 hours	47 (maceration 3, abortion 3)
2 days — 7 days	82
8 days — 1 month	30
2 months — 6 months	25
7 months —14 months	9
Total	193

Observation

I Differentiation of epiphyseal cartilage plate

In the premature infants who were born during the period from 6 to 9 months of gestation, the major part of the epiphysis at the lower end of the femur was composed of the hyaline cartilage without the secondary

Table 2. Principal diseases in infants

Disease	Age				
	0–24 hr.	2–7 d.	8 d.–1 mon.	2–6 mon.	7–14 mon.
Prematurity	20	45	14	—	—
Placental dysfunction syndrome	4	2	—	—	—
Pregnancy toxicosis severe	2	4	—	—	—
Pulmonary lesion	49	60	14	11	3
Cardiovascular disease	9	23	6	2	2
Intracranial bleeding	2	15	2	—	—
Sepsis	1	6	6	10	1
Kernicterus	—	8	7	1	—
Erythroblastosis fetalis	6	3	1	—	—
Disease of the digestive tract	—	5	7	4	—
Disease of the liver and biliary system	—	—	1	2	4
Miscellaneous	2	5	1	5	2

Note: As several infants had more than 1 principal disease the total number of diseases in this table is more than 193.

ossification center. The diaphyseal part of the hyaline cartilage showed more or less a regular arrangement of the rows of cartilage cells parallel to the long axis of the femur and presented a less distinct zonal differentiation, that is, zone of the proliferating cartilage, zone of the maturing cartilage, zone of the calcified cartilage followed by the regular invasion by the connective tissue and capillaries from the adjacent metaphysis (Fig. 1). This diaphyseal part of the cartilaginous epiphysis histologically corresponds to "the growing zone of a long bone (Ham[3])" and can be considered as the precursor of the epiphyseal plate or the disk of a long bone in the younger adult. It is called the diaphyseal growing part in the present study.

This precursor of the epiphyseal plate showed some differences in structure from the well differentiated epiphyseal plate. One of the differences was the absence of the zone of the resting cartilage and another one was the cluster pattern of the cartilage cells in the zone of the proliferating cartilage (Fig. 2).

During the postnatal growing period the secondary ossification center gradually developed in the cartilaginous epiphysis and simultaneously the epiphyseal plate or disk also developed. With the progressive development of the secondary ossification center, the epiphyseal plate became more and more differentiated and came to be composed of the zone of the resting cartilage, zone of the proliferating cartilage, zone of the maturing cartilage

and zone of the calcified cartilage, followed by the invasion by the connective tissue and capillaries from the metaphysis (Fig. 3).

However, the cartilage cells tended to be arranged in clusters in the zone of the proliferating cartilage, occasionally in the zone of the maturing cartilage, until 14 months after birth.

The borderline between the zone of the resting cartilage of the epiphyseal plate and the adjacent cartilage of the epiphysis was generally not so distinct as in the well differentiated epiphyseal plate in the younger adult.

II Pathologic alteration in endochondral ossification in infants

Of the 193 infants 125 showed more or less remarkable pathologic changes in the endochondral ossification.

A. Histopathological changes in endochondral ossification in infants who died within 24 hours after birth

(1) Histological change of zone of proliferating cartilage

The most fundamental pathologic change in the endochondral ossification was the disturbance in the proliferation of the cartilage cells. This disturbance usually brought about a decrease in the size, number and longitudinal diameter of the cell cluster in the zone of the proliferating cartilage and most frequently exerted an influence on the structure of the zone of the maturing cartilage, too (Figs. 4 and 5).

In the premature infants, the cartilage cells tended to be arranged in the form of clusters and the intercellular cartilage matrix among the clusters constituted a coarse network in the zone of the maturing cartilage. The thick partitions among the clusters became calcified in the lower part of the zone and were continuous with the primary spongiosae in the adjacent metaphysis. The primary spongiosae showed an occasional interconnection.

In the mature infants the cartilage cells tended to be arranged in short columns and the longitudinal cartilage matrix between the columns showed an increased thickening in the zone of the maturing cartilage. The invasion of the lowermost part of the calcified cartilage by the connective tissue and capillaries took place in a relatively regular way. Some of the primary spongiosae increased in thickness, but the primary and secondary spongiosae were extended parallelly in the longitudinal direction.

In two macerated fetuses the lower part of the zone of the hypertrophied cartilage cells showed a zonal collapse (Fig. 6). These epiphyseal plates underwent a disturbance in the proliferation of the cartilage cells, too.

(2) Histological change of zone of calcified cartilage

Under normal conditions the most mature cartilage cells in the lower part of the zone of the maturing cartilage undergo degeneration, die and disintegrate, resulting in the disappearance of the cartilage cells from the lacunae. Simultaneously, the intercellular cartilage matrix of the lowermost layer of the zone gradually becomes calcified. The calcified cartilage is regularly resorbed by the osteoclasts and invaded by the connective tissue and capillaries from the adjacent metaphysis.

In the disturbed calcification of the maturing cartilage the metaphyseal surface of the zone of the maturing cartilage showed an irregular outline (Fig. 7). In addition, the matured cartilage cells in the lowermost layer of the zone did not show signs of a regressive change and the transverse intercellular cartilage matrix on the metaphyseal surface occasionally remained undissolved. The hypertrophied cartilage cells became increased in number, resulting in an irregular increase in the depth of the zone of the maturing cartilage.

The disturbance in the calcification occurred less frequently, together with the disturbance in the proliferation of the cartilage cells. Under these circumstances, the cartilage matrix partitions among the clusters of the matured cartilage cells became prominent. The primary and secondary spongiosae also showed an irregular increase in thickness.

(3) Histological change of metaphysis

The primary and secondary spongiosae did not show an increase in thickness because of the decreased deposition of the new bone on the surface of the spongiosae.

B. Histopathological changes in endochondral ossification in infants ranging in ages from 2 to 7 days

(1) Histological change of zone of proliferating cartilage

In the infants, the histological change in the zone of the proliferating cartilage principally did not differ from that of the same zone in the infants who died within 24 hours after birth. However, the disturbance in the zone of the proliferating cartilage occasionally exerted a more remarkable influence on the structure of the subsequent processes in the endochondral ossification (Figs. 8 and 9).

(2) Histological change of zone of maturing cartilage

During the disturbance of maturation, many of the maturing cartilage cells, except for a few in the lower part of the zone, showed more or less a deficient hypertrophy and caused an insufficient enlargement of the clusters and columns of the cartilage cells (Figs. 10 and 11).

At the same time, the thickening of the intercellular cartilage matrix occurred and resulted in the increased irregular thickening of the partitions, especially among the clusters or columns. These thick partitions were continuous with the thick primary spongiosae in the metaphysis.

(3) Histological change of zone of calcified cartilage

In the decalcified section, the blue stain of the cartilage with hematoxylin generally corresponds to the calcification. In the normally growing cartilage, calcification of the lower part (including 2 or 3 hypertrophied cartilage cells) of the zone of the maturing cartilage is coordinately followed by resorption and invasion by the connective tissue as well as the capillaries from the metaphysis.

In the case of slightly disturbed resorption the transverse intercellular partitions on the metaphyseal surface of the zone of the calcified cartilage are somewhat increased in thickness and in the stainability by hematoxylin. At that time the most matured cartilage cells in the zone of the maturing cartilage remained alive within the lacunae (Fig. 12).

In the case of markedly disturbed resorption of the calcified cartilage the blue stain of the intercellular cartilage matrix occasionally spread to the beginning part of the zone of the maturing cartilage and presented the pattern of "the growth retardation lattice (Park)[6]" (Fig. 13). Simultaneously, the disturbance in the proliferation of the cartilage cells occurred, too.

(4) Histological change in metaphysis

The trabeculae of the calcified cartilage remnants normally become increased in thickness by means of a new bone formation on their surface. The thickness and arrangement of these trabeculae, that is the primary spongiosae, were intimately dependent upon the thickness and arrangement of the partitions among the clusters in the zones of the maturing and calcified cartilages. When the thicker partitions constituted a coarse network in these zones, the primary spongiosae were arranged in network. On the other hand, when the longitudinal partitions between the clusters or the columns increased in thickness, the thick primary spongiosae were formed in the adjacent metaphysis.

The important change was more or less the remarkable disturbance of the bone deposition on the surface of the trabeculae of the calcified cartilage remnants. Under these conditions the primary spongiosae were composed of the calcified cartilage remnants only, even at the site far away from the metaphyseal surface of the epiphyseal plate. For this reason, when the trabeculae of the calcified cartilage remnants were very thin, most of them were resorbed, resulting in a remarkable decrease in the number of

the secondary spongiosae. In the case of a slight disturbance of the remodelling of the metaphyseal spongiosae, decrease in the number of the secondary spongiosae was not so distinct as in the case of normal endochondral ossification.

C. *Histopathological changes in endochondral ossification in infants ranging in ages from 8 days to 14 months*

The principal changes were poor differentiation of the epiphyseal plate, disturbed proliferation of the cartilage cells, defective calcification of the maturing cartilage and pathologic change in the metaphysis.

(1) Poor differentiation of epiphyseal plate

Poor differentiation of the epiphyseal plate presented histologically an ill defined zone of the resting cartilage and an occasional cluster arrangement of the cartilage cells in the zones of proliferation and maturation (Fig. 14). The poor differentiation of the plate was related to the retarded development of the secondary ossification center in the cartilaginous epiphysis. Under these conditions the primary and secondary spongiosae in the metaphysis were arranged in a coarse network pattern.

(2) Disturbed proliferation of cartilage cells

Disturbed proliferation of the cartilage cells resulted in the decrease in the number, size and longitudinal diameter of the cell clusters in the zones of the proliferating as well as the maturing cartilage (Fig. 14). Consequently, these zones decreased remarkably in depth. In the zone of the maturing cartilage the cartilage cells, except for the few hypertrophic cells in the lower part of the zone, showed simultaneously a disturbance in maturation. The clusters or columns did not sufficiently enlarge and between them many thick longitudinal partitions were present. These thicker partitions became the thick trabeculae of the calcified cartilage remnants in the metaphysis after the resorption of the other parts of the calcified cartilage. The thick trabeculae occasionally showed an interconnection with one another just below the metaphyseal surface of the epiphyseal plate. These trabeculae increased in thickness by continuous deposition of a new bone on their surface and became thick primary spongiosae with cartilaginous cores. The primary spongiosae frequently did not extend the length of the trabeculae. Under these conditions the development of the secondary spongiosae was generally poor, resulting in a decrease in the number. Markedly retarded proliferation of the cartilage cells occasionally resulted in the development of "the growth retardation lattice" in the zone of the maturing cartilage (Figs. 13, 14 and 15).

(3) Defective calcification of maturing cartilage

In the 2 infants respectively with severe giant cell hepatitis and internal hydrocephalus, the epiphyseal plate showed histological findings of typical rickets (Figs. 16, 17 and 18), that is, irregularly thickened epiphyseal plate, remarkable increase in the height of the clusters or columns of the maturing cartilage cells, irregular resorption of the cartilage and defective invasion of the connective tissue and capillaries from the adjacent metaphysis, and active new bone formation in the metaphysis.

In the other infants, considerable increase in the depth of the zone of the maturing cartilage was found (Fig. 19). Many of the cartilage cells in the zone were less hypertrophied. In the lower part of the zone of the maturing cartilage the majority of the hypertrophic cartilage cells remained alive within the lacunae and the transverse intercellular partitions on the metaphyseal surface of the epiphyseal plate frequently did not undergo resorption. The metaphyseal surface showed more or less an irregular outline. There was no remarkable disturbance in the proliferation of the cartilage cells. In the metaphysis the development of the primary as well as the secondary spongiosae was generally poor. These histological findings in the decalcified section suggest most probably a delayed resorption resulting from disturbed calcification in the lower part of the zone of the maturing cartilage.

(4) Pathologic change in metaphysis

The disturbed proliferation of the cartilage cells occasionally caused a decrease in the longitudinal growth of the epiphyseal plate and brought about the shortening of the primary spongiosae associated with an increased bone deposition on their surfaces. These changes resulted in the formation of the "transverse stratum (Park)[6]" and decrease in the number of the secondary spongiosae in the metaphysis (Fig. 20). Some enormously thick calcified cartilage remnants formed the so-called giant trabeculae by bone deposition on their surface. The shortening of the primary spongiosae was frequently found in the disturbed calcification of the maturing cartilage.

In the infants with prematurity, kernicterus, interstitial pneumonia, etc., the new bone deposition on the surface of the primary as well as the secondary spongiosae was markedly disturbed.

Discussion

1. Relation between differentiation of epiphyseal plate and development of secondary ossification center in cartilaginous epiphysis

In the younger infants endochondral ossification took place in the

diaphyseal growing part of the cartilaginous epiphysis. However, the histological structure of the endochondral ossification differed considerably from that of the typical epiphyseal plate in the older infants. The major part of this difference in the structure may be attributed to the insufficient development of the secondary ossification center in the cartilaginous epiphysis.

(1) Zonal differentiation of epiphyseal plate

In the premature infants ranging in gestational ages from 6 to 9 months, the distal epiphysis of the femur was composed of the hyaline cartilage alone, and the secondary ossification center did not yet develop there. In these infants, the longitudinal growth of the femur occurred in the diaphyseal growing part of the hyaline cartilage of the epiphysis. The diaphyseal growing part showed relatively a good zonal differentiation: zones of proliferation, maturation, calcification and metaphysis as in the well differentiated epiphyseal plate in the older infants. The zonal differentiation was less prominent in the aborted fetuses of the gestational age of 6 months, but later became more conspicuous in the infants who were delivered near the end of the full term.

However, the diaphyseal growing part of the cartilaginous epiphysis was ill defined before the secondary ossification center was developed sufficiently. At that time there was no occurence of the zone of the resting cartilage. With the progressive development of the secondary ossification center, the epiphyseal plate became well developed in structure and showed the typical zonal differentiation consisting of the zone of the resting cartilage, zone of the proliferating cartilage, zone of the maturing cartilage and the zone of the calcified cartilage and metaphysis.

(2) Cluster pattern of proliferating cartilage cells

The cluster pattern of the proliferating cartilage cells was another characteristic in the structure of the diaphyseal growing part of the cartilaginous epiphysis in the premature infants. This cluster pattern of the cartilage cells may be attributed to the trend to form a cartilage cell nest during the interstitial growth of the hyaline cartilage before the full development of the secondary ossification center in the epiphysis. The progressive development of the secondary ossification center in the epiphysis resulted in the typical zonal differentiation of the epiphyseal plate consisting of the zone of the resting cartilage, zone of the proliferating cartilage, zone of the maturing cartilage and the zone of the calcified cartilage. After the zone of the resting cartilage formed, the proliferating cartilage cells became arranged in the form of a column. The retardation in the development of the secondary ossification center in the epiphysis led to a delayed differentiation of the 4 zones described above, resulting in the persistence of

the cluster pattern of the proliferating cartilage cells.

Consequently, these findings indicate that there is a close relationship between the typical zonal differentiation of the epiphysal plate and the development of the secondary ossification center in the epiphysis. On the other hand, the arrangement of the proliferating cartilage cells in the epiphyseal plate depended greatly on the development of the secondary ossification center.

2. *Effect of disturbance of proliferation of cartilage cells on structure of epiphyseal plate and metaphysis*

In the younger infants including the premature ones, disturbance in endochondral ossification was reflected by various histological manifestations in the epiphyseal plate and its precursor, that is, the diaphyseal growing part of the cartilaginous epiphysis at the distal end of the femur.

Under these circumstances, the disturbance in the proliferation of the cartilage cells and in the calcification of the cartilage was considered to be most influential on the histological structure of the epiphyseal plate and the adjacent metaphysis. The disturbance in the proliferation of the cartilage cells led to the formation of the smaller clusters consisting of a small number of cartilage cells.

Among these smaller clusters, there was a wide intercellular and interclustery cartilage matrix. These wide intercellular and interclustery cartilage matrices, in turn, constituted the thicker intercellular paritions and the network of the broad interclustery partitions in the zones of the maturing and calcified cartilages.

The zone of the calcified cartilage consisting of the thicker intercellular cartilage matrix, especially of the thicker transverse intercellular partitions, formed a mechanical barrier and resisted much more the osteoclastic resorption than that consisting of the thin transverse intercellular ones. Retardation in the resorption of the provisional zone of calcification was occasionally associated with progressive extensive calcification in the zone of the maturing cartilage on the one hand and bone deposition on the surface of the calcified cartilaginous matrix just dissolved in the metaphysis on the other hand. These circumstances resulted in the development of the "calcified lattice" or the "growth retardation lattice (Park)[6]" in the epiphyseal plate or its precursor.

Park[6] insists that the growth retardation lattice is mainly attributed to the resistance to resorption because of the slowing of the growth of the cartilage plate of the epiphysis and heavy calcification of the thicker horizontal partitions of the cartilaginous matrix frame separating the cartilage cells from each other. The present study revealed that the disturbance in the proliferation of the cartilage cells is responsible for the increase in the

thickness of the transverse intercellular partitions in the zones of the maturing and calcified cartilages, too. Furthermore, the increase in the thickness of the interclustery partitions resulting from the disturbed proliferation of the cartilage cells might be greatly related to the formation of the transverse stratum as well as of the giant trabeculae (Park)[6] and to the formation of the network-like arrangement of the primary as well as the secondary spongiosae in the metaphysis.

3. Zonal collapse of matured cartilage cells

In the 2 macerated fetuses, zonal collapse involving several layers of the relatively well matured cartilage cells was found in the zone of the maturing cartilage. Aoike et al.[8] reported a similar change in the epiphyseal plate of the femur in the rats receiving repeated intraperitoneal injection of Thrombolysin. However, detailed episodes in the pregnant mothers were not available in these fetuses. Therefore, the actual mechanism of the development of the zonal collapse of the matured cartilage cells remains unknown.

4. Disturbance in calcification of epiphyseal plate

The disturbance in the calcification was always followed by disturbed resorption and invasion of the lowermost part of the zone of the maturing cartilage by the osteoclasts and connective tissue, as well as by the capillaries. Consequently, the zone of the maturing cartilage invariably became increased in depth and consists of a strikingly high column or cluster of enlarged cartilage cells. In the present study, a prominent thickening of the zone of the maturing cartilage associated with a less regular resorption of the lowermost part of the zone was less frequently observed. This change suggests a slight disturbance in calcification, although the Kossa staining had not been performed. Follis et al.[5] reported the prevalance of rickets consisting of slight to severe ones during each month of the first year and for the entire second year in 1303 children. According to their examination, prematurity has no effect on the prevalence of rickets in their study. Yamazaki[9] histologically examined the costochondral junction and the distal end of the radius as well as the ulna in the aborted fetuses and premature infants ranging in ages from 9 to 10 gestational months. He described that the prerachitic- or rachitic-like histologic change (irregular thickening of the zone of the hypertrophic cartilage cells and calcification confind to the lowermost part of the provsiional zone of calcification) frequently occurred in the premature infants. The present study indicated that there is no close relationship between the prerachitic change and prematurity.

The typical rachitic histologic change, that is, the irregular prominent

thickening of the zone of the maturing hypertrophic cartilage cells associated with a marked disturbance in calcification of the lowermost part of the zone, was found in an infant with severe giant cell hepatitis. This finding of the epiphyseal plate is considered to correspond to hepatic rickets (Gernstenberger)[10]. Watanabe[11] reported that the preexisting rickets is exaggerated by the severe hepatic lesion and the hepatic lesion is not the direct cause of the rachitic change. In the present study, quite a similar rachitic change was observed in the epiphyseal plate in the infant with internal hydrocephalus associated with subarachnoidal and cereral bleeding. This case also suggests a severe disturbance in calcification, although the laboratory data on the serum calcium and phosphorus were not available in this patient.

5. Pathogenesis of disturbance in endochondral ossification

It is generally accepted that endochondral ossification is influenced by various systemic factors: genetic factors (as in chondrodysplasia fetalis, osteogenesis imperfecta, marble bone disease, etc.), hormonal factors (as in the disorders of the secretion of the growth hormone, thyroxine, cortisone, estrogen, etc.), nutritional factors (vitamins A, C and D, and protein as well as the essential amino acids) (Bourne[2]; Collins[4]). Of these factors, the genetic and hormonal ones, and vitamins cause relatively characteristic histological changes in the epiphyseal plate and adjacent metaphysis. The cases examined in the present study had various kinds of diseases. However, there was no close relationship between the definite histological change in the endochondral ossification and a certain kind of disease. Park[6] histopathologically examined the costochondral junction and the epiphyseal plate of the humerus in children with various kinds of diseases. He divided these diseases into two groups, infective diseases and nutritional disturbance, and considered the latter as an important causative factor in the disturbance of the endochondral ossification in the children. The present study revealed that development of the secondary ossification center, zonal differentiation of the epiphyseal plate, and disturbance in proliferation and calcification are important fundamental factors in the morphogenesis of the various histological changes in the endochondral ossification in the infants. Furthermore, the metabolic disturbance in the various kinds of diseases may be responsible for the retardation of the development of the secondary ossification center and zonal differentiation of the epiphyseal plate, and disturbance in proliferation and calcification.

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References

1) Weinmann, J. P., and Sicher, H.: Bone and bones. 2nd ed. C. V. Mosby, St. Louis, 1955.
2) Bourne, G. H.: The biochemistry and physiology of bone. Academic Press, New York, 1956.
3) Ham, A. W., and Leeson, T. S.: Histology. 4th ed. Lippincott, Philadelphia and Montreal, 1961.
4) Collins, D.: Pathology of bone. Butterworths, London, 1966.
5) Follis, R. H. et al.: The prevalence of rickets at autopsy during the first two years of age. Bull. Johns Hopkins Hosp., 91: 480–497, 1954.
6) Park, E. A.: Bone growth in health and diseases. Arch. Disease Childhood, 29: 269–281, 1954.
7) Plank, J., und Rychlo, A.: Eine Schnellentkalkungsmethode. Zentr. Allgem. Pathol. Pathol. Anat., 89: 252–254, 1952–53.
8) Aoike, I. et al.: Relation between synovial joint and fibrolytic activity of synovial fluid. Bull. Tokyo Med. Dent. Univ., 13: 77–95, 1966.
9) Yamazaki, I.: Study on rickets in premature infants (Part 4). (in Japanese, English abstract). Acta Pediat. Japon., 64: 1104–1114, 1960.
10) Gerstenberger: Monatsschr. Kinderheilk., 56: 216, 1933. Cit. by Watanabe, T. *in* A supplement to the hepatic rickets. (in Japanese, English abstract). Acta Pediat. Japon., 59: 131–137, 1955.
11) Watanabe, T.: A supplement to the hepatic rickets. (in Japanese, English abstract). Acta Pediat. Japon., 59: 131–137, 1955.

Explanation of Figures

Plate I

Fig. 1. Less distinct zonal differentiation of diaphyseal growing part of cartilaginous epiphysis. Cluster pattern of proliferating cartilage cells in zone of proliferation and adjacent hyaline cartilage of epiphysis. Absence of zone of resting cartilage. Aborted fetus, 6 gestational months. K-3.

Fig. 2. Less distinct zonal differentiation of diaphyseal growing part. Cluster pattern of proliferating cartilage cells. Absence of zone of resting cartilage. Active bone formation on the surface of the primary and secondary spongiosae in the metaphysis. 13 days old, male. Congenital subendocardial fibroelastosis. SN-3362.

Fig. 3. Distinct zonal differentiation of epiphyseal plate: zones of resting cartilage, proliferation, maturation and calcification, and metaphysis. Columnar arrangement of proliferating cartilage cells in the epiphyseal plate. 7 months old, male. Purulent mediastinitis. SN-2099.

Fig. 4. Marked disturbance in proliferation of cartilage cells. Decreased number of small cell clusters and extensive cell-free cartilage matrix. Short clusters. Broad interclustery partitions forming a network in the zone of proliferation, maturation and calcification, and metaphysis. Stillborn, female. Placental dysfunction syndrome. SN-2317.

Plate II

Fig. 5. Disturbance in proliferation of cartilage cells. Prominent cluster pattern and network of interclustery partitions in the zone of maturation. Stillborn, female. Erythroblastosis fetalis. SN-3396.

Fig. 6. Zonal collapse of maturing cartilage cells. Marked disturbance in proliferation of cartilage cells. Extensive cell-free cartilage matrix and broad intercolumnar partitions. Thick primary and secondary spongiosae in metaphysis. Two giant trabeculae in right lower quadrant. Macerated fetus near full term, female. Placental dysfunction syndrome. SN-3764.

Fig. 7. Disturbance in calcification associated with disturbance in proliferation of cartilage cells. Irregular resorption of the lowermost part of zone of maturing cartilage. Irregularly thick intercolumnar partitions are continuous with irregularly thick primary as well as secondary spongiosae in the metaphysis. 30 minutes old, female. Albinismus universalis and pulmonary dystelectasis. SN-2520.

Plate III

Fig. 8. Marked disturbance in proliferation of cartilage cells. Decreased number of small clusters in the zone of proliferation and maturation. Broad interclustery partitions continuous with numerous thick primary spongiosae in the metaphysis. Slightly irregular resorption of the metaphyseal surface of the zone of maturing cartilage. 6 days old, male. Prematurity and subarachnoidal bleeding. SN-2132.

Fig. 9. Considerable disturbance in proliferation of cartilage cells. Irregular thick interclustery partitions forming network in the zone of proliferation and maturation. Network formation of the primary and secondary spongiosae. Less regular resorption of the zone of calcification. Giant trabeculae in metaphysis. 2 days old, female. Subgaleal bleeding. SN-2784.

Fig. 10. Remarkable disturbance in proliferation of cartilage cells. Decreased number of small clusters and irregularly broad interclustery partitions associated with progressive calcification. Short clusters. Considerably thick irregular primary spongiosae. 5 days old, female. Pulmonary dystelectasis and placental dysfunction syndrome. SN-2550.

Fig. 11. Disturbance in maturation of cartilage cells, associated with slight disturbance in proliferation. Thin primary spongiosae with minimum bone deposition in the metaphysis. 3 days old, male. Erythroblastosis fetalis. SN-1049.

Plate IV

Fig. 12. Disturbance in calcification associated with delayed resorption of the lowermost part of the maturing cartilage. Persistence of living cartilage cells in lacunae in the lowermost part of zone of maturing cartilage. 29 hours old, male. Hyaline membrane disease and aspiration of amniotic fluid. SN-2538.

Fig. 13. Calcified lattice in the zone of maturing and calcified cartilage. Disturbed proliferation of cartilage cells. Irregular resorption of calcified cartilage. Decreased number of primary spongiosae. 5 days old, male. Prematurity. SN-2208.

Fig. 14. Marked disturbance in proliferation of cartilage cells. Decreased number of small, short clusters and irregularly broad interclustery partitions connecting with irregular primary spongiosae in the metaphysis. 2 months old, male. Congenital megacolon and postoperative ileus. SN-1975.

Fig. 15. Marked disturbance in proliferation of cartilage cells. Irregularly thick intercellular and interclustery cartilage matrix. Formation of calcified lattice. Irregularly thick connecting primary spongiosae and decrease in number of secondary spongiosae. 7 months old, male. Hand-Schüller-Christian disease. SN-3490.

Plate V

Fig. 16. Typical rachitic change in diaphyseal growing part of cartilaginous epiphysis with poor development of secondary ossification center. Slight disturbance in proliferation of cartilage cells. Irregular outline of metaphyseal surface of the maturing cartilage. Abundant osteoid formation. No regular primary nor secondary spongiosae in metaphysis. 6 months old, male. Giant cell hepatitis and rachitis. SN-3510.

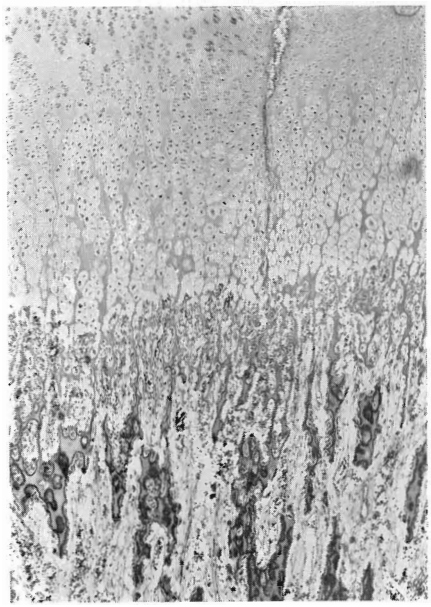
Fig. 17. Rachitic change of diaphyseal growing part of cartilaginous epiphysis. Moderate disturbance in proliferation of cartilage cells. Cluster pattern. Irregular outline of metaphyseal trabeculae with abundant osteoid deposition and scanty calcified cartilage core. 2 months old, male. Hydrocephalus internus with cerebral and subarachnoidal bleeding. SN-1465.

Fig. 18. Rachitic change of diaphyseal growing part of cartilaginous epiphysis. Increased thickening of zone of maturing cartilage. Slight disturbance in proliferation of cartilage cells. Irregular resorption line of the lowermost part of zone of maturing cartilage. Irregular metaphyseal trabeculae with abundant osteoid deposition. 2 months old, male. Hydrocephalus internus with cerebral and subarachnoidal bleeding. SN-1465.

Plate VI

Fig. 19. Slight rachitic change. Marked increase in depth of zone of maturing cartilage. Less regular resorption line. Persistence of living cartilage cells in lacunae in lowermost part of maturing cartilage. No remarkable change in metaphyseal trabeculae. 10 days old, male. Prematurity and kernicterus. SN-1875.

Fig. 20. Transverse stratums in lower part of metaphysis. Marked decrease in number of secondary spongiosae. 14 months old, male. After operations for omphalocele and atresia ani. SN-3436.



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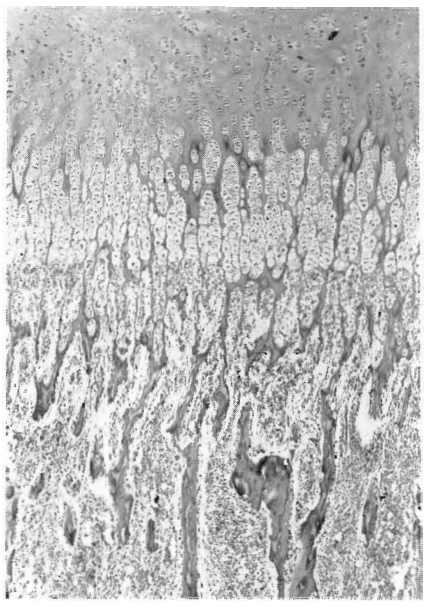

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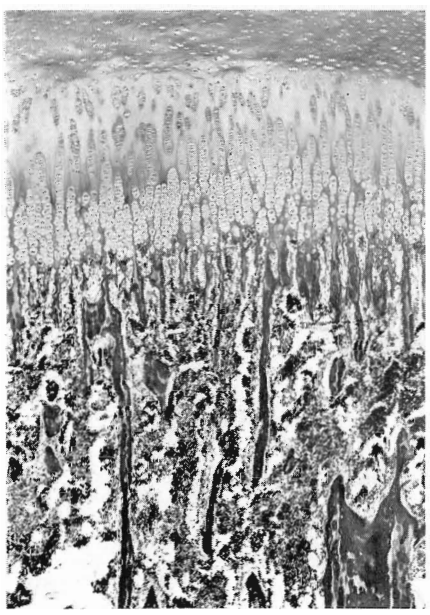

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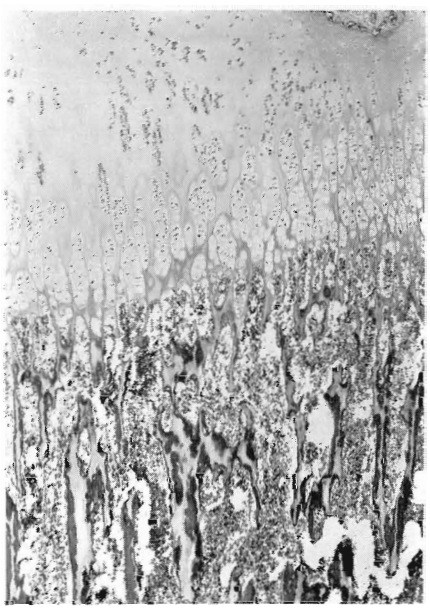

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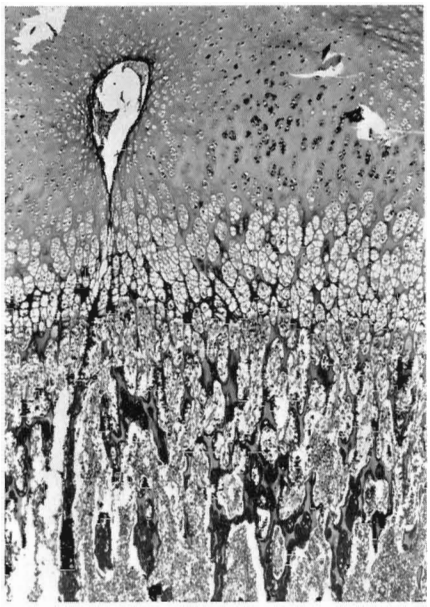

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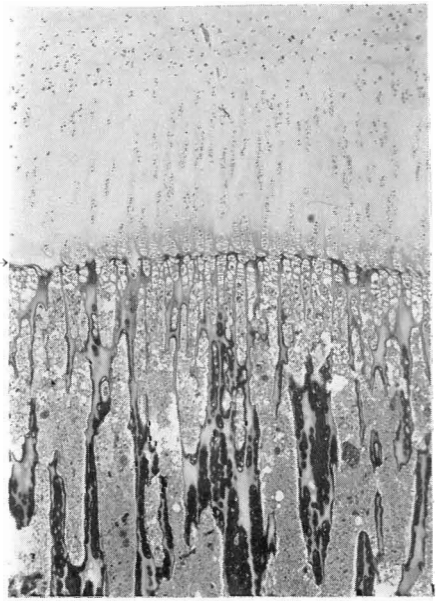

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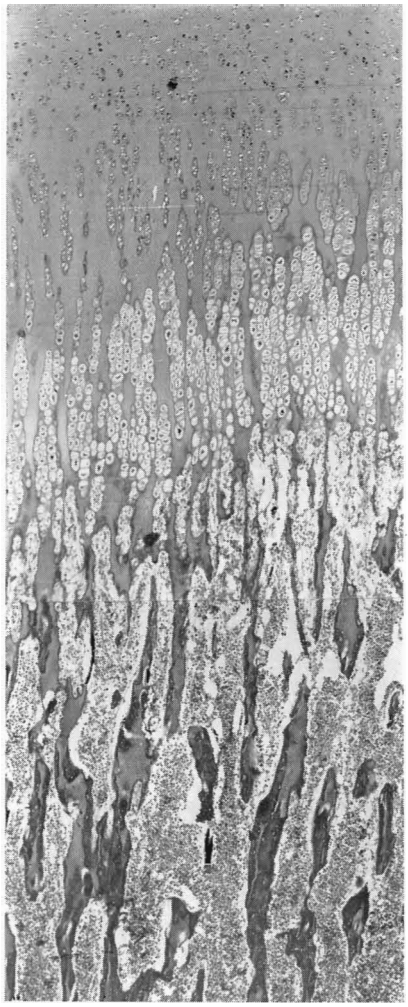

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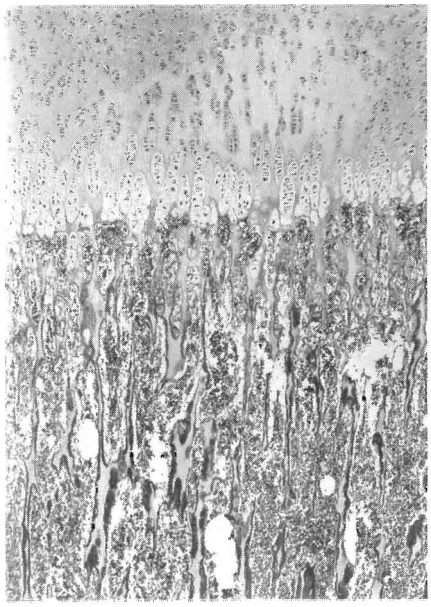

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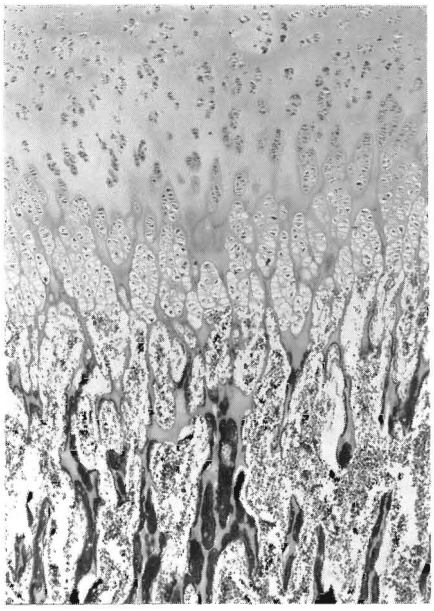

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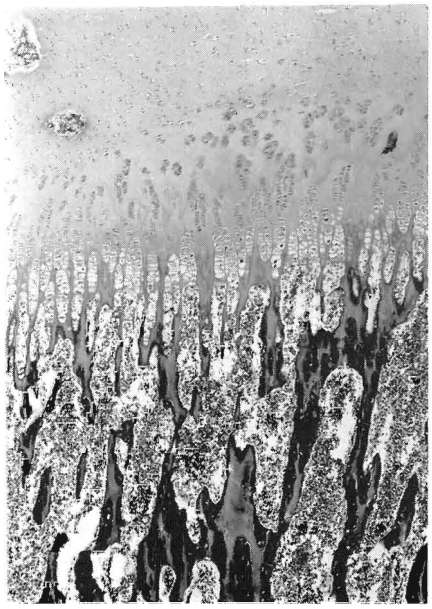
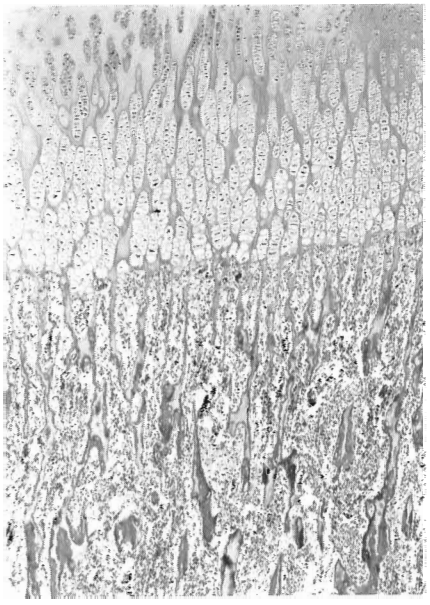


Fig. 10 (×30)

Fig. 11 (×30)



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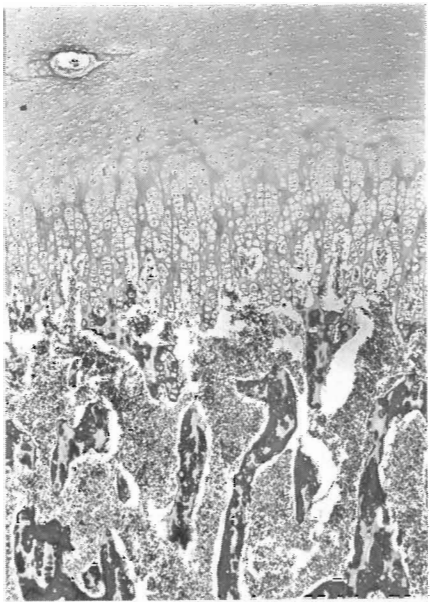

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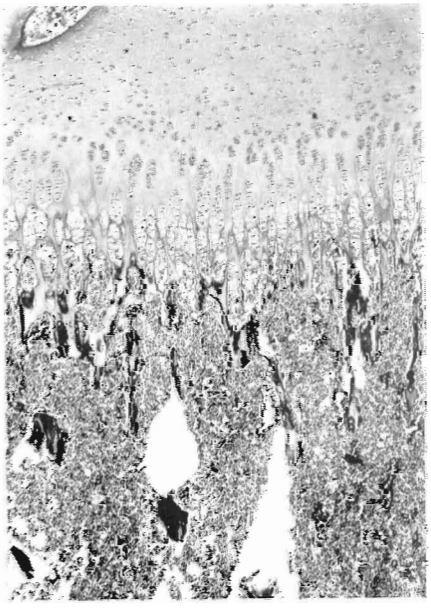

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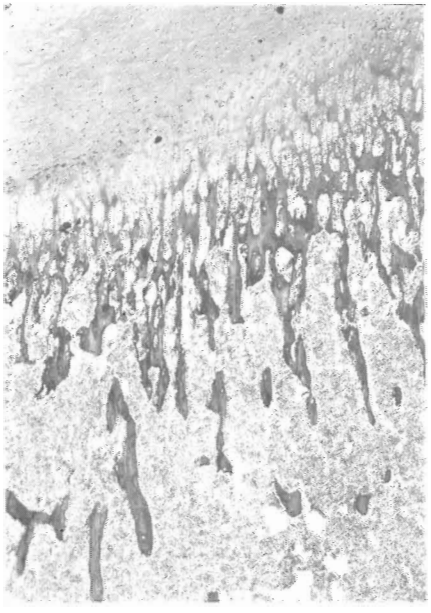

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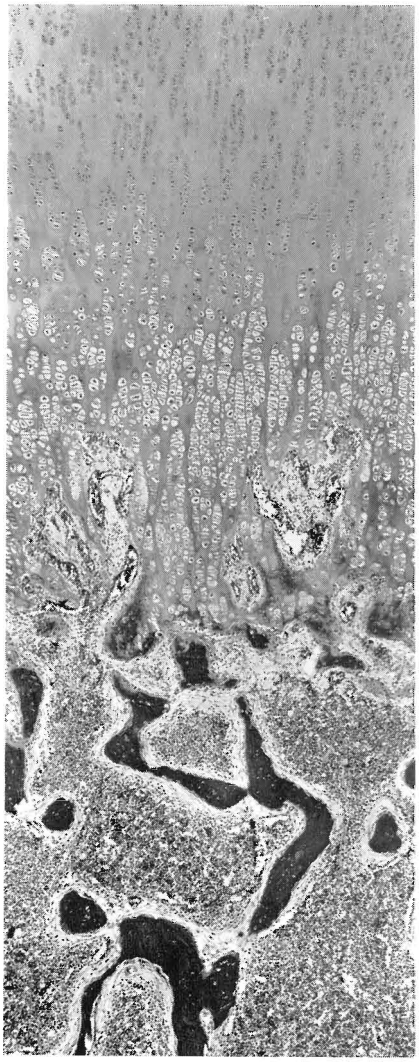

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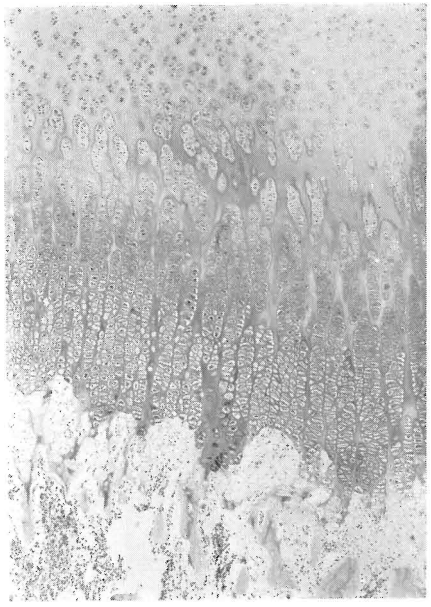

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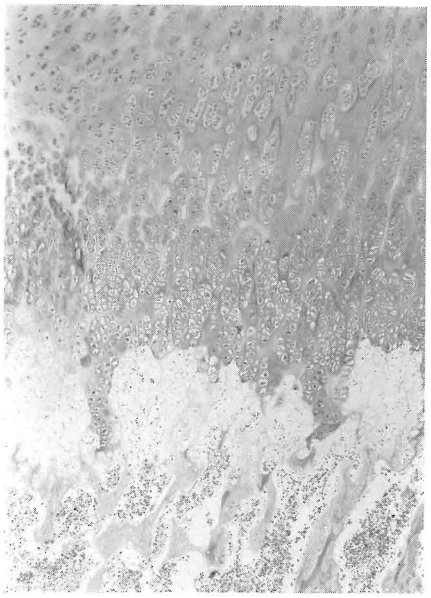

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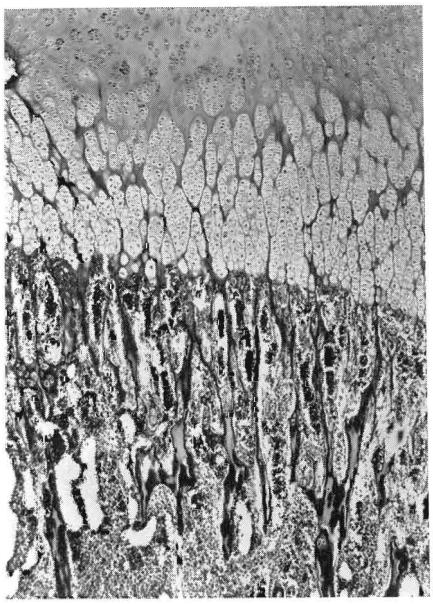

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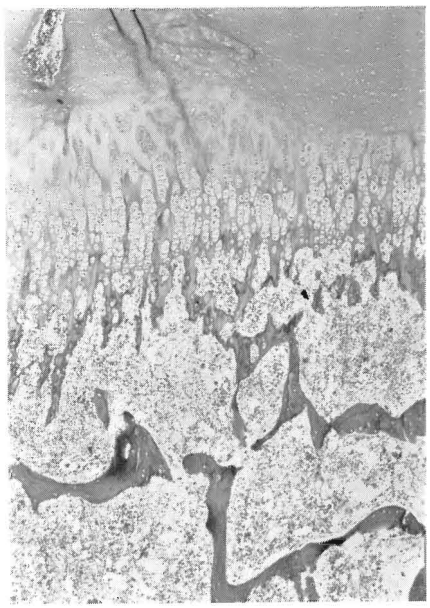

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