قسم التشريح
كلية الطب - جامعة أسيوط
رئيس القسم : أ٠د/ فتحي زكي ٠

النمو الجنيني لمفصل الركبة عند الكتكوت

رفعت شحاته

أجريت هذه الدراسة على خمسين جنينا (Gallus gallus) ٠ وقد قسم هذا العدد الى عشرا مجموعات ، كل منها يتكون من خمسة أجنة ، أخذت عند أعمارا ٥ ، ٦ ، ٧ ، ٨ ، ١٠ ، ١٢ ، ١٤ ، ١٦ ، ١٨ ، ٢٠ يوما ٠

بعد اجراء العمليات الهستولوجية المعتادة أخذت قطاعات بسمك ١٠ ميكرون وصبغت بالهيماتوكسلين والايوسين ثم فحصت بالمجهرا الضوئي ٠

تضمنت النتائج وصف مراحل نشوء وتكوين المفصل خلال النمو الجنيني وتمت مناقشتها مع نتائج الأبحاث الأخرى ٠

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EMBRYONIC DEVELOPMENT OF THE CHICK STIFLE JOINT

(With 28 Figs.)

By
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SUMMARY

A total number of 50 chick embryo was used in the present work to describe the embryonic development of the stifle (knee) joint. The embryos were divided into 10 groups, each consists of 5 embryos taken at the ages of 5, 6, 7, 8, 10, 12, 14, 16, 18 and 20 days of incubation.

Fixation, decalcification and histological processing were done. Serial longitudinal sections were cut at 10 um. thick.

Embryonic stages were described and discussed.

INTRODUCTION

Extensive histological and histochemical studies on the embryonic development of the human knee joint were present (HAINES, 1947; GRAY and GARDNER, 1950; ANDERSEN, 1961 a; GARDNER and O'RAHILLY, 1968; SLEDGE, 1975).

Concerning the normal embryonic development of the knee joint in experimental animals, few reports are present. The present investigation aims to obtain a detailed information on the normal embryonic development of the stifle (knee) joint in chickens that are widely used as experimental animals in medical researches.

MATERIAL and METHODS

Fertilized eggs of chicken (Gallus gallus) were obtained from a local hatchery. They were incubated in an electric incubator, at 37±1°C, and daily rotated. Humidity was roughly adjusted by putting a petri dish containing water in the incubator. The start of the incubation was designated day one.

A total number of 50 chick embryos was used in the present study. They were divided into 10 groups, each consisting 5 embryos, taken at 5, 6, 7, 8, 10, 12, 14, 16, 18 and 20 days of incubation.

The early embryonic stages (5-10 days of incubation) were taken as a whole, while the late ones (12-20 days of incubation) were decapitated, skinned, and their pelvic limbs were used.

The specimens were fixed in 10% formalin saline for 24 hours. The late embryonic stages were decalcified in EDTA, PH 7, 2.

Histological processing and embedding in paraffin were carried on as usual (DRURY and WALLINGTON, 1980). Serial longitudinal sections were cut at 10 um and stained with haematoxylin and eosin.

RESULTS

INTERZONE

In 5 days old chick embryo, the two skeletal primordia, the femur and the tibia are connected together by a dense mesenchymal tissue known as the interzone (Fig. 1). The interzone is continous peripherally with the general mesenchyme.

The interzone is homogenous and avascular. Its mesenchymal cells are closely packed together and oriented at different planes (Fig. 2).

In 6 days old chick embryo, the interzone loses its homogenous character and becomes differentiated into three layers, two chondrogenous layers separated by an intermediate one (Fig. 3).

Each chondrogenous layer is dense and continous peripherally with the perichondrium. Its cells are aligned parallel to the subjacent skeletal primordium (Fig. 4).

The intermediate layer is loose centrally, but dense peripherally where it becomes continuous with the surrounding general mesenchyme. Its cells are oriented at different planes (Fig. 4).

MORPHOGENESIS

Most of the constituents of the stifle (knee) joint appear at the 7th day of incubation.

The joint capsule appears as a condensation of mesenchymal tissue which surrounds the joint area and separates if it from the general mesenchyme. Caudally, it is represented by a dense mesenchymal tissue band that is continuous proximally and distally with the perichondria of the femur and tibia, respectively (Fig. 5). Cranially, it is represented by the primordium of the common extensor tendon which appears as a dense mesenchymal tissue band passing distally to the cenemial crest (Fig. 5).

The cenemial crest appears as a cranial outgrowth from the proximal end of the tibia (Fig. 5).

Examination of serial sagittal sections, at this stage, reveals that the articular surfaces of the femur and tibia are narrow and slightly convex (Fig. 5).

The primordia of the intra-articular ligaments and the mensici appear in the intermediate layer of the interzone. Each meniscus is an ill-defined dense mesenchymal tissue mass (Fig. 25).

The peripheral part of the intermediate layer of the interzone becomes loose and vascular. It merges peripherally with a part of the general mesenchyme that is enclosed within the primordium of the joint capsule. The latter tissue is also loose and well vascularised. Both form the synovial mesenchyme (Figs. 5 & 21).

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At this stage of development, the joint cavity makes its first appearance as two clefts, femoro-patellar and femoro tibial (Fig. 5). The femoro-patellar cleft appears in the synovial mesenchyme located between the distal end of the femur and the primordium of the common extensor tendon (Fig. 5). The femoro-tibial cleft appears between the femur and tibia (Fig. 5). The first cleft is more longer and wider than the second.

In 8 days old chick embryo, the following can be observed.

The femoro-patellar and femoro-tibial clefts fuse together forming the joint cavity (Fig. 6 & 7).

The intra-articular ligaments appear as dense cellular bands (Fig. 7). The meniscus becomes a well-defined wedge shaped mass whose base is directed externally (Fig. 6).

The extent and convexity of the articular surfaces of both femur and tibia increase (Figs. 6 & 7).

The synovial mesenchyme, in both crainal and caudal portions of the stifle (knee) joint becomes distinct (Figs. 6 & 7).

The patella appears as an ill-defined chondrifying mass in the proximal part of the cranial portion of the synovial tissue (Fig. 7).

In 10 days old chick embryo, the patella becomes a well-defined quadrangular mass of still chondrifying tissue (Fig. 8).

In 10 and 12 days old chick embryos the area proximal to the patella and located between the extensor muscles and the distal end of the femur is occupied by a vascular loose mesenchymal tissue (Figs. 8 & 9).

In 14 and 16 days old chick embryos, the joint cavity becomes more extensive than that of the previous stage and had extended proximal to the patella forming the proximopatellar pouch (Figs. 10 & 11).

HISTOGENESIS

The Chondrgenous Layer

In 8 days old chick embryo, each of the articular surfaces of both femur and tibia is covered centrally with the thick chondrogenous layer of the interzone that is continous peripherally with the intracapsular perichondrium (Fig. 12). This layer consists of cells which are small, spindle shaped and closely packed together (Fig. 12). They are aligned parallel to the subjacent cartilage.

In 14 days old chick embryo, the layer covering the articular surface is still thick and consists of the same cells, previously described, embedded in connective tissue fibres aligned parallel to the surface (Fig. 17).

In 20 days old chick embryo, the articular surface is chondrified. The superficial cells are small spindle shaped and vary from 2-6 in number. Connective tissue fibres are absent (Fig. 13).

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Synovial Tissue

In 8 days old chick embryo, the synovial mesenchyme is vascular (Fig. 14). The superficial layer that lines the joint cavity consists of synovial cells which are variable; they are small and spindle shaped at some sites but they are large and ovoid at other sites (Fig. 14). The surface of synovial mesenchyme is ragged (Fig. 14).

In 10 days old chick embryo, the surface of synovial mesenchyme remains ragged at some sites but becomes smooth at other sites (Fig. 15). It is thrown into vascular synovial folds, the cranial synovial fold being the largest one (Fig. 8).

During further development, there is a gradual increase in the vascularity of the synovial mesenchyme. In 12 days old chick embryo, blood capillaries extend to the superficial surface of the synovial mesenchyme being frequentely separated from the joint cavity by a single layer of flattened cells (Fig. 16).

In 14 days old chick embryo, the synovial tissue becomes differentiated into two layers, intima and subintima (Fig. 17). The intima appears as a thick layer composed of small spindle shaped cells embedded in collagen fibres. The subintima becomes looser than that of the previous stage and shows thin, irregularly disposed connective tissue strands (Fig. 17).

In 16 days old chick embryo, the subintima becomes a loose areolar tissue that is generously rich in fat cells (Fig. 18).

In 18 and 20 days old chick embryos, the intima and subintima become well developed (Fig. 19 & 20). The subintima appears as an adipose connective tissue composed of fat cells organised into groups which are separated and supported by incomplete connective tissue septa (Fig. 19 & 20). This picture of the synovial tissue is present all over the joint. The surface of the synovial tissue is smooth.

Joint Capsule, Intra-articular Ligaments and Menisci

The caudal part of the joint capsule, the common extensor tendon and the intra-articular ligaments run the same course of histogenesis. The common extensor tendon is described as a representative of them.

In 7 days old chick embryo, the primordium of the common extensor tendon appears as a dense mesenchymal tissue band whose cells are aligned parallel to the longitudinal axis of the tendon (Fig. 21).

In 8 days old chick embryo, the mesenchymal cells are converted into fibroblasts with thin collagen fibres in between (Fig. 22).

In 14 days old chick embryo, the common extensor tendon reveals that its collagen fibres become thick (Fig. 23).

In 20 days old chick embryo, its collagen fibres become thicker than those of the previous stage and disposed in bundles (Fig. 24).

Each meniscus appears in 7 days old chick embryo as a dens mesenchymal tissue mass whose cells are irregularly disposed (Fig. 25).

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In 8 days old chick embryo, the mesenchymal cells differentiate into fibroblasts which deposite thin connective tissue fibres (Fig. 26).

In 14 days old chick embryo, the meniscus reveals that its connective tissue forms bundles which are disposed in different directions (Fig. 27).

In 20 days old chick embryo, the connective tissue fibres become thicker than those of the previous stage (Fig. 28).

DISCUSSION

Interzone

The present results reveal that the homogenous interzone appears in 5 days old chick embryo. These results are confirmed by FELL and CANTI (1934) in chick embryo. GARDNER and O'RAHILLY (1968) in human embryo and MOHAMED and GABR (1986) in rabbit embryo, found that the homogenous interzone appears in stages 17 or 18 of human embryo and in 16 days old rabbit embyro, respectively. Mechanical stress brought about by the femur and tibia may be essential for the development of the interzone (FELL and GANTI, 1934; HOLDER, 1977).

The present findings reveal that the interzone, in 6 days old chick embryo, loses its homogenous character and becomes differentiated into three layers; two chondrogenous layers separated by an intermediate one. The same findings are obtained by FELL and CANTI (1934). Simillar findings are also obtained by HAINES (1947), DRACHMAN (1969) and SLEDGE (1975).

The Chondrogenous Layer

The present work reveals that each of the articular surfaces of the femur and tibia, in 8 days old chick embryo, is covered with the chondrogenous layer, centrally and with the intracapsular perichondrium, peripherally. The cells of this covering layer are small spindle shaped, closely packed together and aligned parallel to the articular surface. In 14 days old chick embryo, these cells lay down collagen fibres arranged parallel to the articular surface. In 20 days old chick embryo, the collagen fibres are disappeared. The covering layer becomes thinner than those ofthe previous stages, and is 2-6 cells thick.

The disappearance of the collagen fibres and the reduction in the thickness of the covering layer lead to a suggestion that these cells especially the deep ones are converted into chondroblasts which deposite cartilage matrix, into which the collagen fibres are embedded. So the covering layer begins a gradual incorporation into the subjacent cartilage.

HAINES (1947), GRAY and GARDNER (1950), ANDERSEN (1961 a) in human embryo and MOHAMED and GABR (1986) in rabbit mentioned that the covering layer is converted directly into cartilage. While SLEDGE (1975) stated that the covering layer lay down collagen fibres and this structure remains up to the time of birth.

ANDERSEN (1961 a) and SLEDGE (1975) in man, suggested that the articular cartilage may be derived from the chondrogenous layer of the interzone and the intracapsular perichondrium.

Joint Cavity

The present observations show that joint cavity makes its first appearance as two clefts; a peripheral (femoropatellar) and a central (femoro-tibial). The former is more extensive than the latter. This suggests that joint cavity commences peripherally. This suggestion agrees with WILLIS (1962), HAMILTON and MOSSMAN (1972) and MOHAMED and GABR (1986), but contradicts ANDERSEN (1961 a, 1962 a&b, 1963), who stated the joint cavity makes its first appearance centrally.

Concerning whether the initial appearance of the joint cavity is due to accumulation of fluid or cellular degeneration, different opinions are present. The present work suggests that the initial appearance of the joint cavity is due to a accumulation of fluid as it is preceded by vascular invasion of the synovial mesenchyme. This vascular invasion increases tissue oxygenation which causes release of lysosomal enzymes and consequentely accumulation of fluid (ANDERSEN, 1964; HENRICKSON and COHEN, 1965).

While HAINES (1947), LEVER and FORD (1958) and WILLIS (1962) claimed that it is due to cellular degeneration, COPEMAN (1970) mentioned that both accumulation of fluid and cellular degeneration contribute to the initial apperance of the joint cavity.

Regardles the precise nature of the process of joint cavitation, it is well established that it depends upon mechanical factors especially joint motion (DRACHMAN and SOKOLOFF, 1966; PERSON, 1983).

Synovial Tissue

The present work shows that the synovial mesenchyme makes its first appearance as a well vascularized loose mesenchyme in 7 days old embryo. It does so in 20 days old rabbit embryo (MOHAMED and GABR, 1986) and in 34 mm. stage of human embryo (HAINES, 1947; ANDERSEN, 1961 a).

In respect of the source of origin of the synovial mesenchyme different opinions are present. The present work clarifies that the synovial mesenchyme has a double source of origin; the peripheral part of the intermediate layer of the interzone and the part of the general mesenchyme that becomes well vascularised and entrapped within the joint capsule. MOHAMED and GABR (1986) however demonstrated that the whole of the intermediate layer of the interzone; in 20 days old rabbit embryo, becomes vascularised and shares, with the part of the general mesenchyme that is cut off by the joint capsule, in the formation of the synovial mesenchyme. While, other authors stated that the synovial mesenchyme possesses a single source of origin, either from the peripheral part of the intermediate layer of the interzone (ANDERSEN, 1961 a, 1962 a, 1963) or from the thin strip of general mesenchyme that is entrapped within the joint capsule (COPEMAN, 1970).

The present study demonstrates that the synovial tissue, all over the joint cavity, is finally differentiated, in 18 and 20 days old chick embryos, into adipose type. This is according to KEY (1932) who distinguished three types of synovial tissue; fibrous, areolar and adipose depending upon the structure of the subintima. On the other hand, the synovial tissue is areolar in type in the knee joint of rabbit embryo (MOHAMED and GABR, 1986).

CHICK STIFLE JOINT

Joint Capsule, Intra-articular

Ligements and Menisci

The present work shows that the common extensor tendon and the intraarticular ligaments appear in 7 days old chick embryo as dnse mesenchymal tissue bands. Both differentiate into fibrous tissue in 8 days old chick embryo. The same stages are described by HAINES (1947) and ANDERSEN (1961 a) in human embryos and MOHAMED and GABR (1986) in rabbit.

The menisci appear in 7 days old chick embryo as dense mesenchymal tissue mass. Then it is differentiate into fibrous tissue in 8-20 days old chick embryos, and is differentiated into fibro-cartilage in human (HAINES, 1947; ANDERSEN, 1961 a) and rabbit (MOHAMED and GABR, 1986).

CONCLUSION

From the present study, it can be concluded that:

1- The stifle (knee) joint makes its appearance by the formation of the interzone which is at first, homogenous and avascular. Then after it loses its homogenous character and becomes differetiated into 3 layers; two chondrogenous layers separated by a loose intermediate one.

2- The chondrogenous layer of the interzone may take part in the formation of the future articular cartilage.

3- The intermediate layer of the interzone gives rise to the intra-articular ligaments and the menisci.

4- The synovial mesenchyme has a double source of origin; the peripheral part of the intermediate layer of the interzone and a part of mesenchyme entrapped within the joint capsule.

5- The synovial cavity begins peripherally.

6- The initial appearance of the joint cavity may be attributed to accumulation of fluid.

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ABBREVIATIONS

F. :Distal end of femur.
AHI : Avascular homogenous interzone.
CH : Chondrogenous layer of the interzone
CC : Cenemial crest.
FT : Femoro-tibial cleft.
JC : Caudal part of the joint capsule.
SM : Synovial mesenchyme.
P : Patella.
SF : Synovial fold.
IS : Intima of synovial tissue.
FC : Fat cells.
ICP : Intra capsular perichondrium.
T. : Proximal end of tibia.
TLI : Thre layered interzone.
I : Intermediate layer of the interzone
FP : Femoro-patellar cleft.
CET : Common extensor tendon.
IL : Intra-articular ligaments.
M : Meniscus.
PP : Proximo-patellar pouch.
CSF : Cranial synovial fold.
SS : Subintima of synovial tissue.
CS : Connective tissue septa.

CHICK STIFLE JOINT

EXPLANATION OF FIGURES

Fig. (1): A longitudinal section of the pelvic limb, in 5 days old chick embryo, showing that the interzone is avascular and homogenous Hematoxylin & Esoin X 31 1/4.

Fig. (2): High-power of a part of the interzone, seen in figure (1), showing that its cells are closely packed together and oriented at different planes. Hematoxylin & Eosin X 320.

Fig. (3): A longitudinal section of the pelvic limb, in 6 days old chick embryo, showing that the interzone is differentiated into 3 layers; two chondrogenous layers separated by an intermediate one Hematoxylin & Eosin X 31 1/4.

Fig. (4): High-power of a part of the interzone, seen in figure (3), showing that the intermediate layer is loose centrally but dense peripherally. Hematoxylin & Eosin X 320.

Fig. (5): A longitudinal sectin of the stifle (knee) joint, 7 days old chick embryo. Hematoxylin & Eosin X 31 1/4.

Fig. (6): A longitudinal section of the stifle joint, in 8 days old chick embryo. Hematoxylin & Eosin X 31 1/4.

Fig. (7): A longitudinal section of the stifle joint, in 8 days old chick embryo. Hematoxylin & Eosin X 31 1/4.

Fig. (8): A longitudinal section of the stifle joint, in 10 days old chick embryo. Hematoxylin & Eosin X 31 1/4.

Fig. (9): A longitudinal section of the stifle joint, in 12 days old chick embryo. Hematoxylin & Eosin X 31 1/4.

Fig. (10): A longitudinal section of the stifle joint, in 14 days old chick embryo. Hematoxylin & Eosin X 31 1/4.

Fig. (11): A longitudinal section of the stifle joint, in 16 days old chick embryo. Hematoxylin & Eosin X 31 1/4.

Fig. (12): A part of the longitudinal section of the stifle joint in 8 days old chick embryo, showing the chondrogenous layer covering the articular surface of the femur. Hematoxylin & Eosin X 320.

Fig. (13): A part of the longitudinal section of the stifle joint, in 20 days old chick embryo, showing that the chondrogenous layer is chondrified. Its superficial cells are small, spindle shaped and disposed in rows 2-6 cells thick. Hematoxylin & Eosin X 320.

Fig. (14): The synovial mesenchyme of the stifle joint, in 8 days old chick embryo, showing its synovial cells which may be small and sphindle shaped or large and ovoid. Hematoxylin & Eosin X 320.

Fig. (15): The synovial mesenchyme of the stifle joint, in 10 days old chick embryo. Hematoxylin & Eosin X 320.

Fig. (16): The synovial mesenchyme of the stifle joint, in 12 days old chick embryo, revealing that it is generously vascularised. (see the arrows). Hematoxylin & Eosin X 320.

Fig. (17): A part of a longitudinal section of the stifle joint, in 14 days old chick embryo, showing the chondrogenous layer, the intima and the subintima of the synovial tissue. It also reveals the presence of collagen fibres in the intimal and also in the subintima in the form of thin connective tissue strands disposed irregularly. Hematoxylin & Eosin X 320.

Fig. (18): The synovial tissue of the stifle joint, in 16 days old chick embryo, showing its intima & subintmia. The subintima is generously rich in fat cells. Hematoxylin & Eosin X 320.

Fig. (19): The synovial tissue of the stifle joint, in 18 days old chick embryo. It shows that the intima is a thick layer of collagen fibres aligned paraltel to the surface with small spindle shaped cells disposed in between. The subintima is an adipose connective tissue. Hematoxylin & Eosin X 320.

Fig. (20): The synovial tissue of the stifle joint, in 20 days old chick embryo. Notice that the fat cells in the subintima are larger when compared with those in the previous figure. Hematoxylin & Eosin X 320.

Fig. (21): A longitndinal section of the common extensor tendon, in 7 days old chikc embryo. It shows that it is a dense mesenchymal tissue band. Hematoxylin & Eosin X 320.

Fig. (22): A longitudinal section of the common extensor, in 8 days old chick embryo, showing that its cells are fibroblasts and disposed parallel to its longitudinal axis with thin connective tissue fibres in between. Hematosylin & Eosin X 320.

Fig. (23): A longitudinal section of the common extensor tendon, in 14 days old chick embryo, showing that its collagen fibres are thick. Hematoxylin & Eosin X 320.

Fig. (24): A longitudinal section of the common extensor tendon, in 20 days old chick embryo, showing that its collagen fibres are thick and disposed in bundles. Hematoxylin & Eosin X 320.

Fig. (25): The meniscus, in 7 days old chick embryo. It is a dense mesenchymal tissue mass whose cells are irregularly disposed. Hematoxylin & Eosin X 320.

Fig. (26): The meniscus, in 8 days old chick embryo. It is composed of fibroblasts with thin connective tissue fibres between them. Hematoxylin & Eosin X 320.

Fig. (27): The meniscus, in 14 days old chick embryo, showing that its connective tissue bundles are arranged in different directions. Hematoxylin & Eosin X 320.

Fig. (28): The meniscus, in 20 days old chick embryo, showing that its connective tissue fibres are thicker than those of the previous stage (compare with figure 27). Hematoxylin & Eosin X 320.

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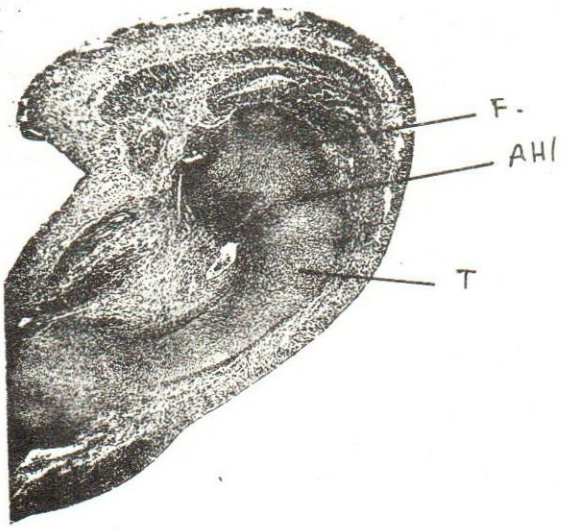

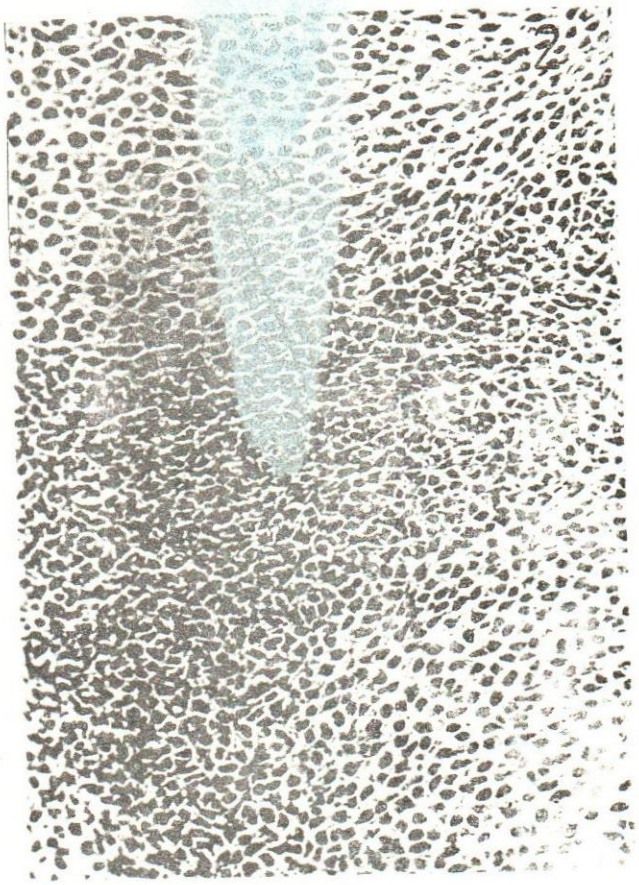

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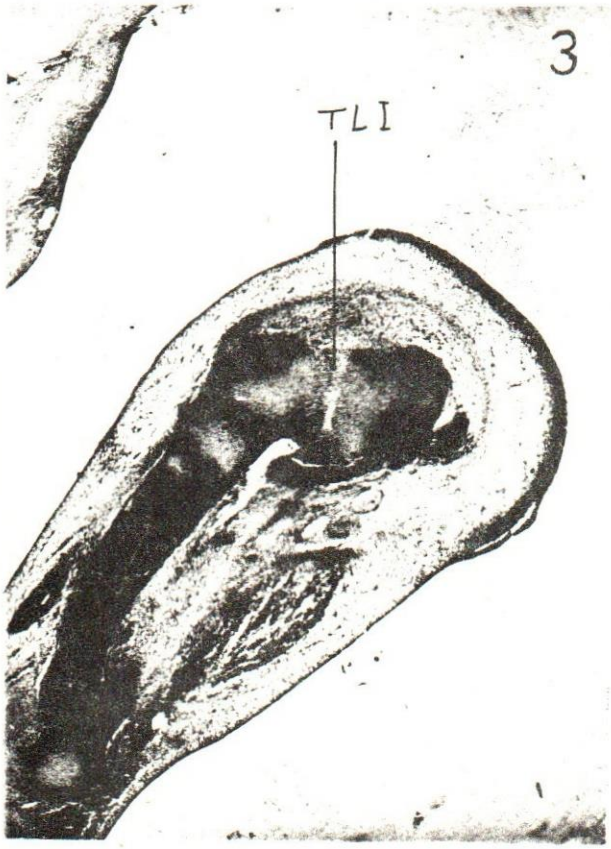

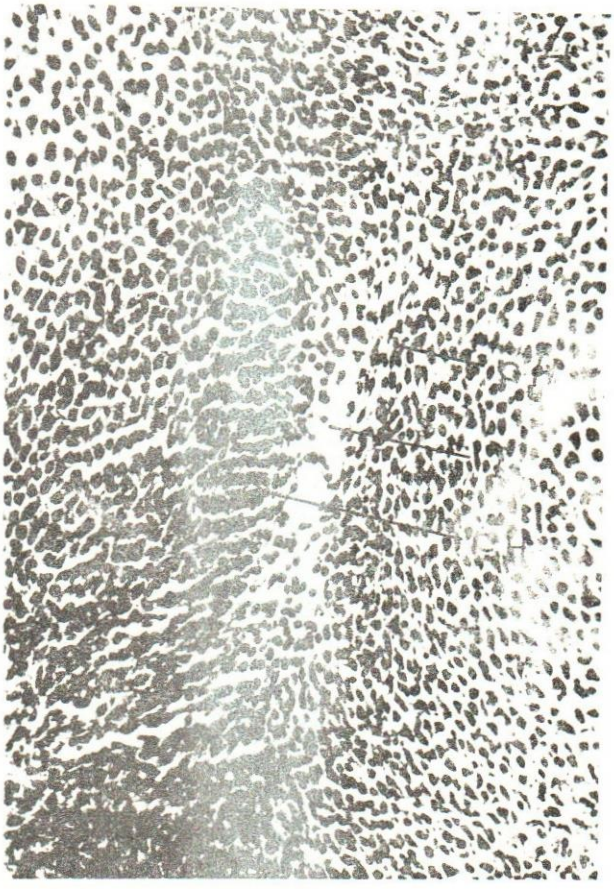

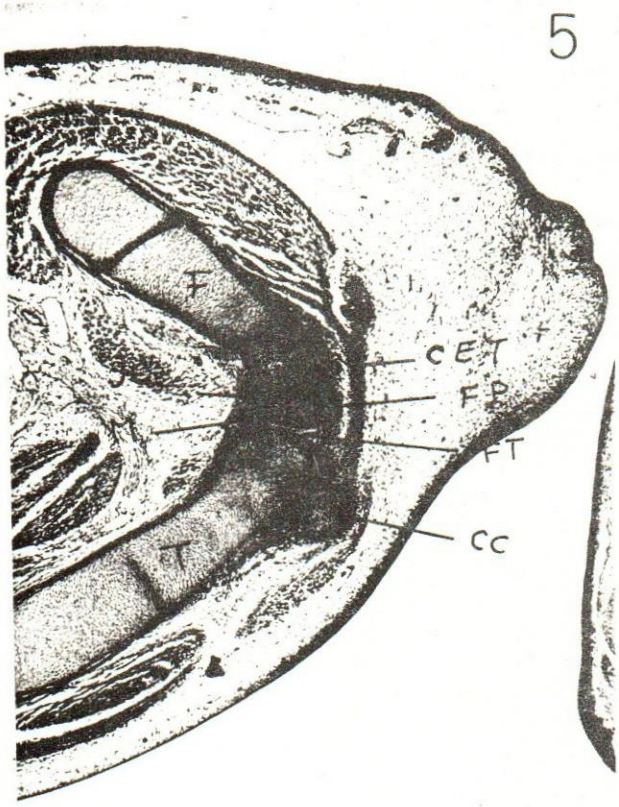
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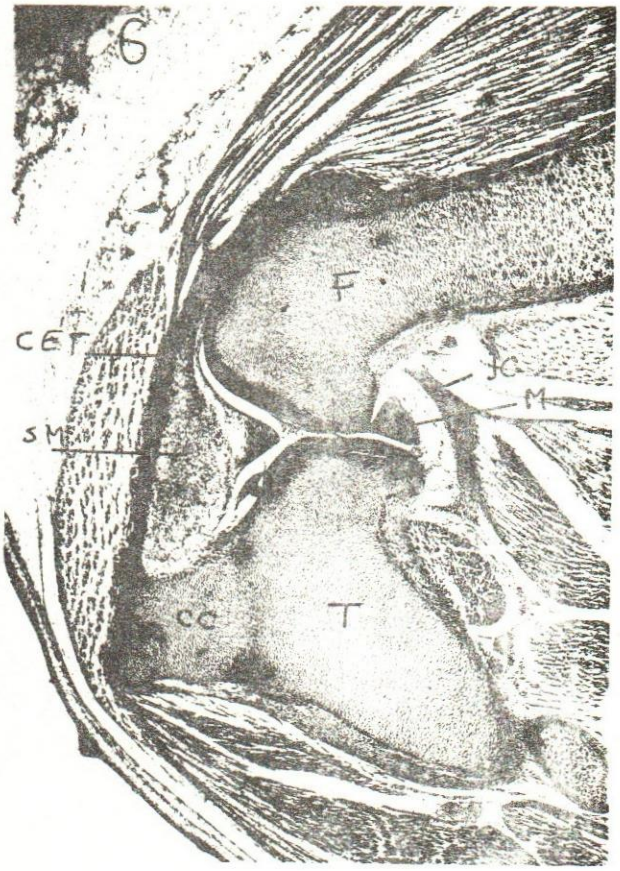
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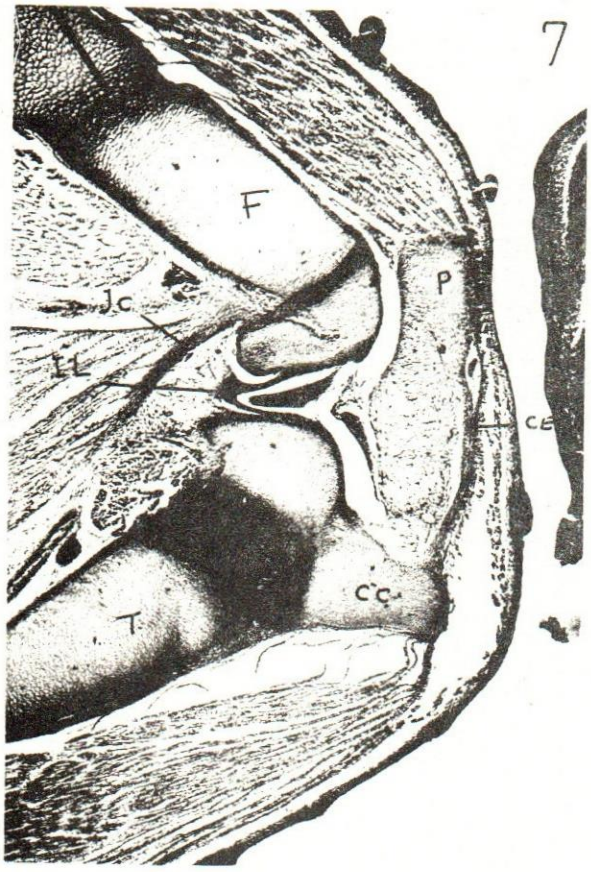
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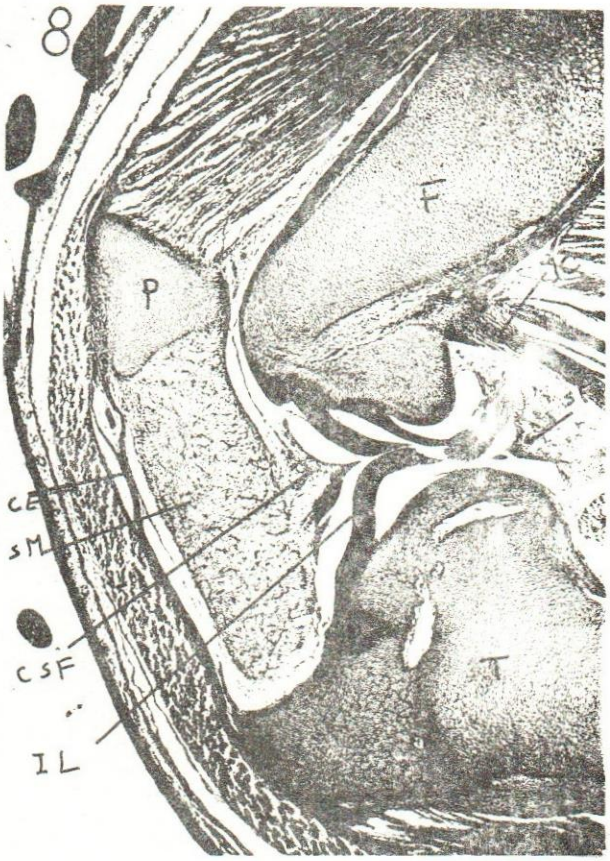
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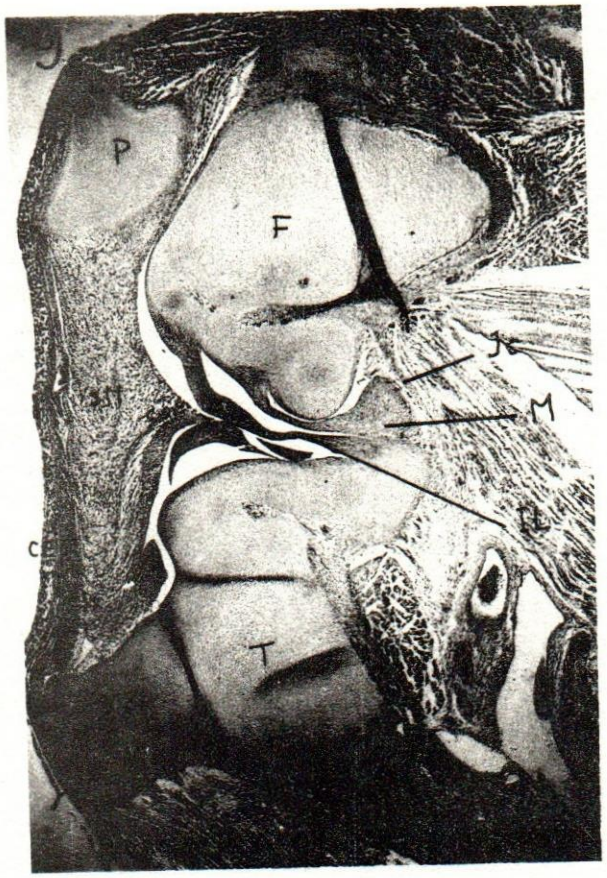

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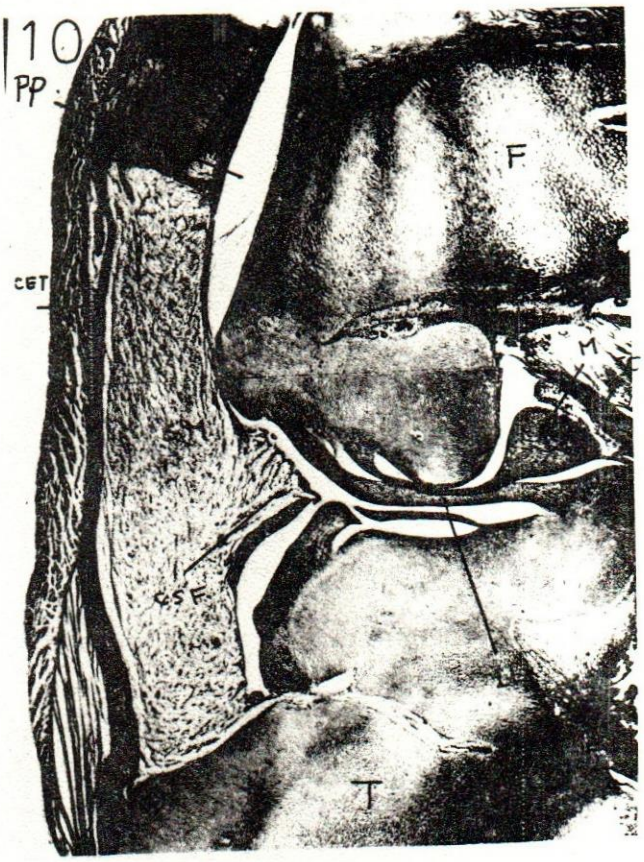

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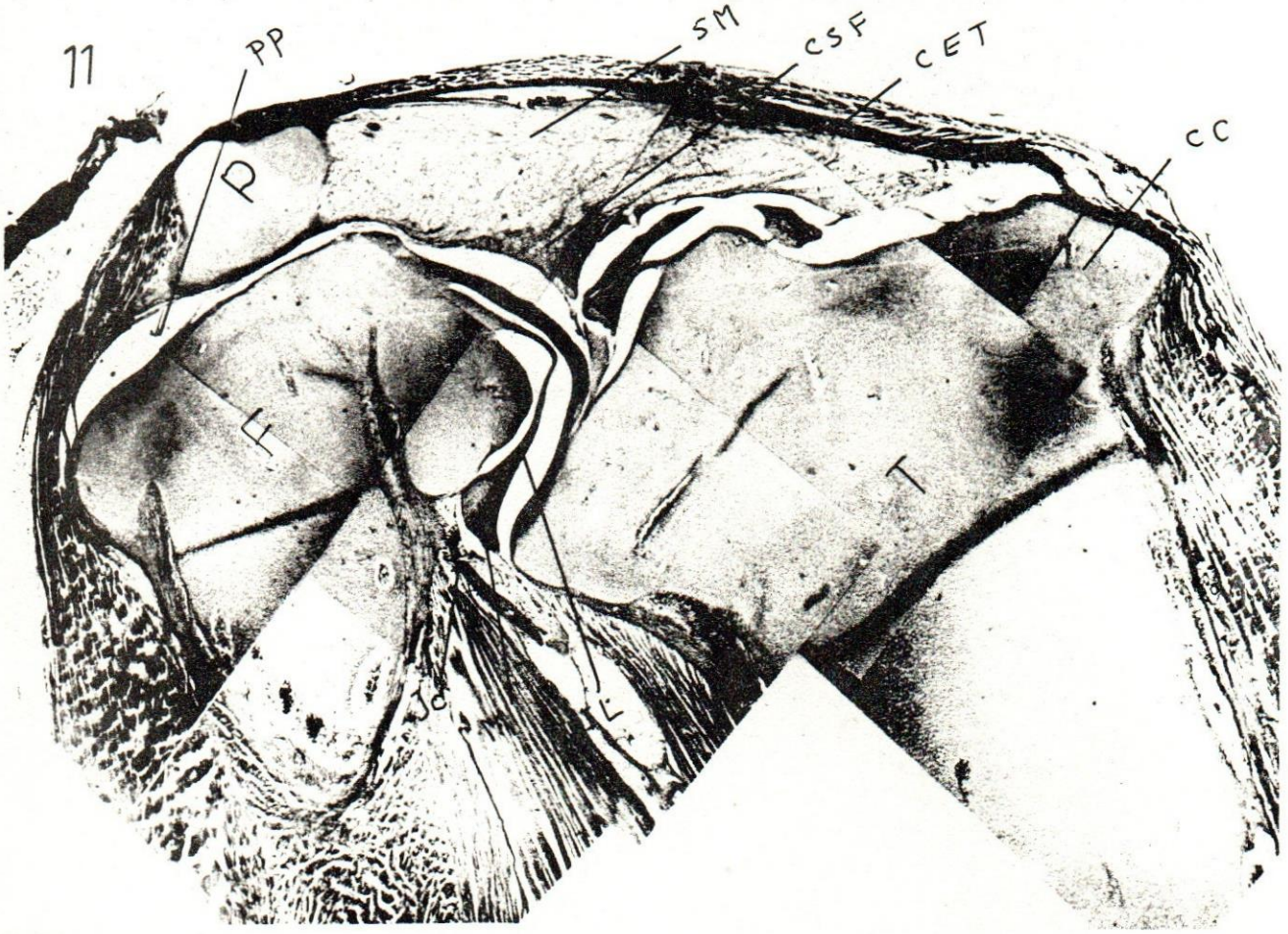

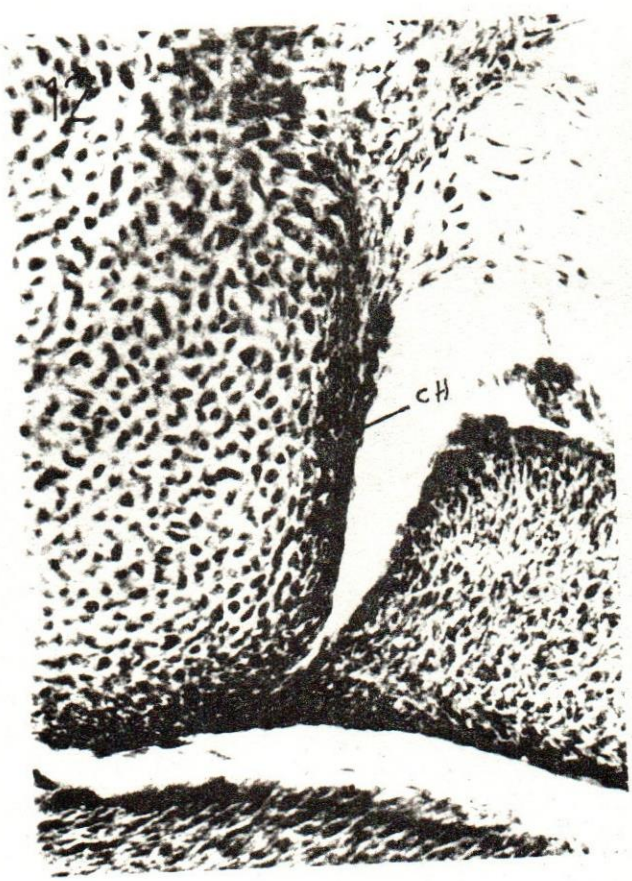
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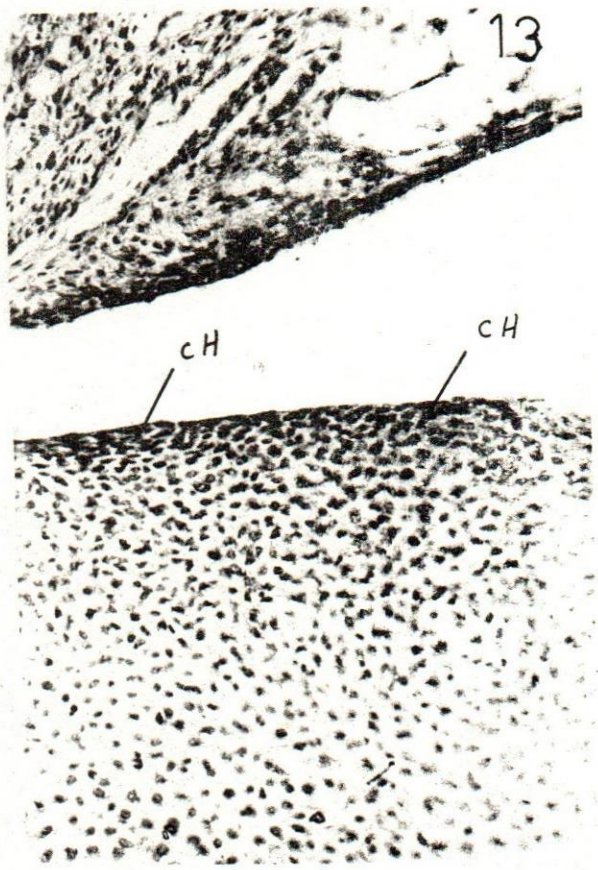
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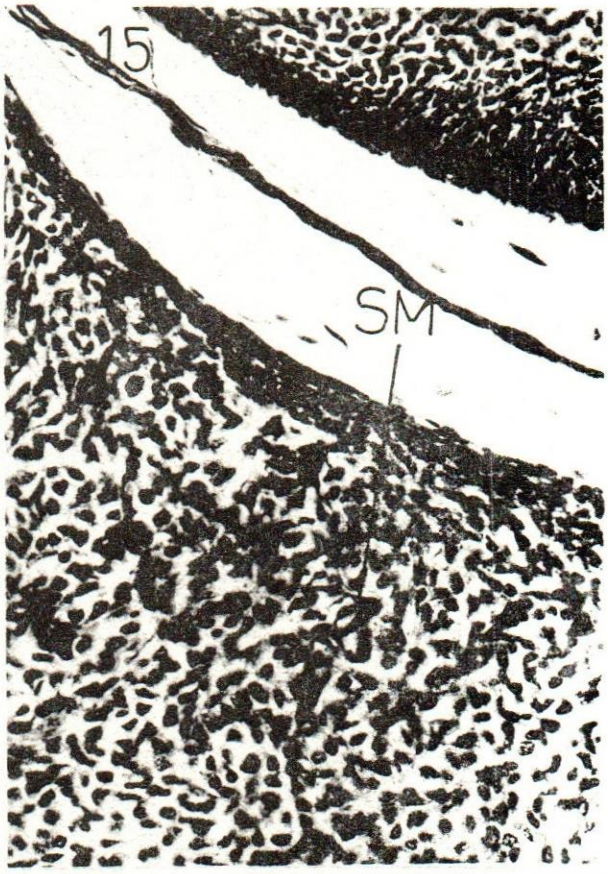
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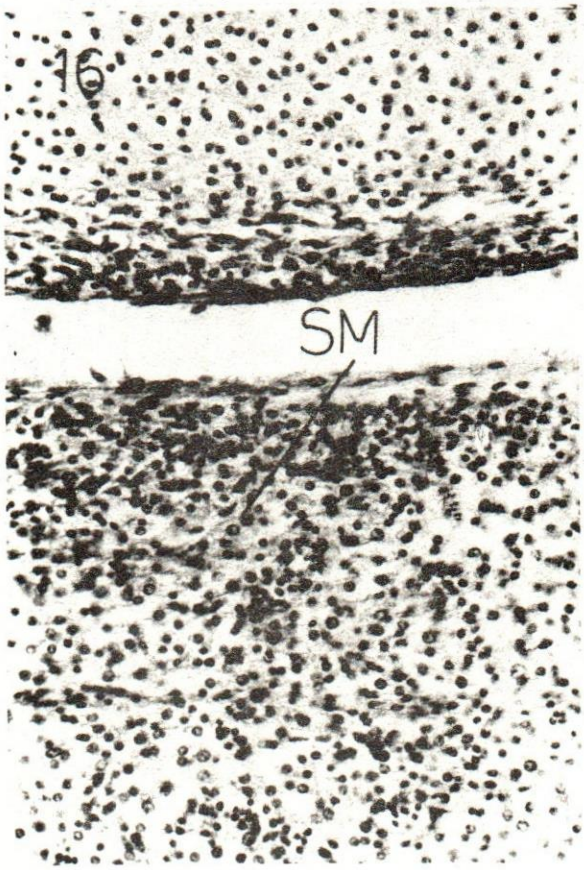
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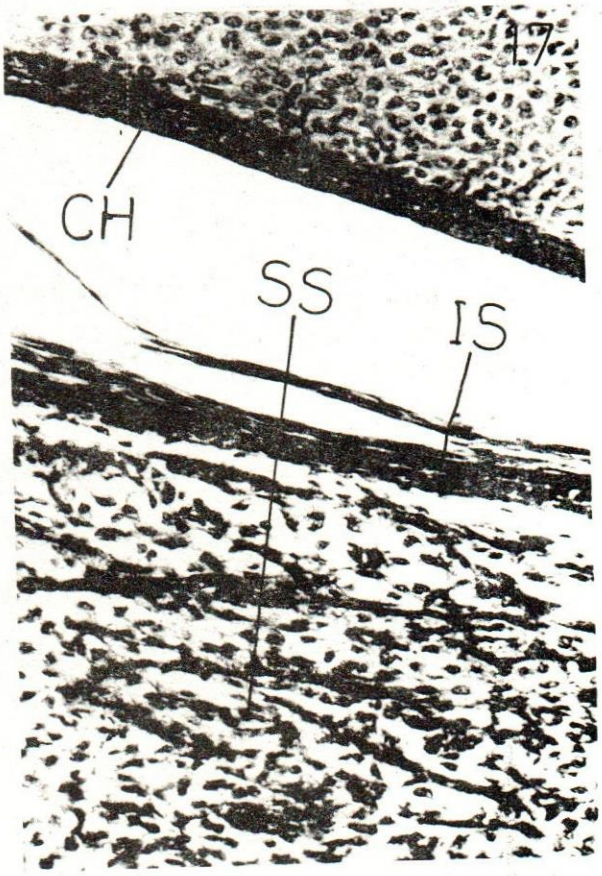
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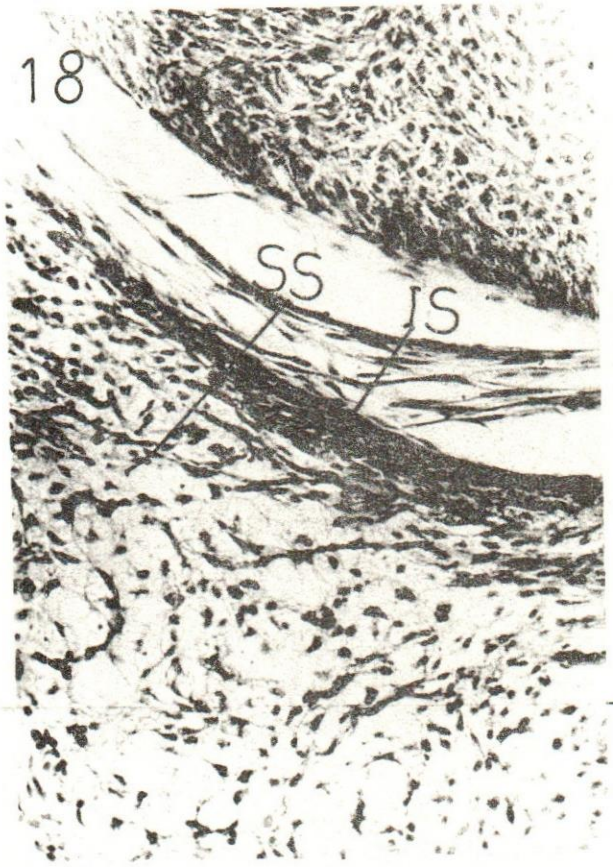
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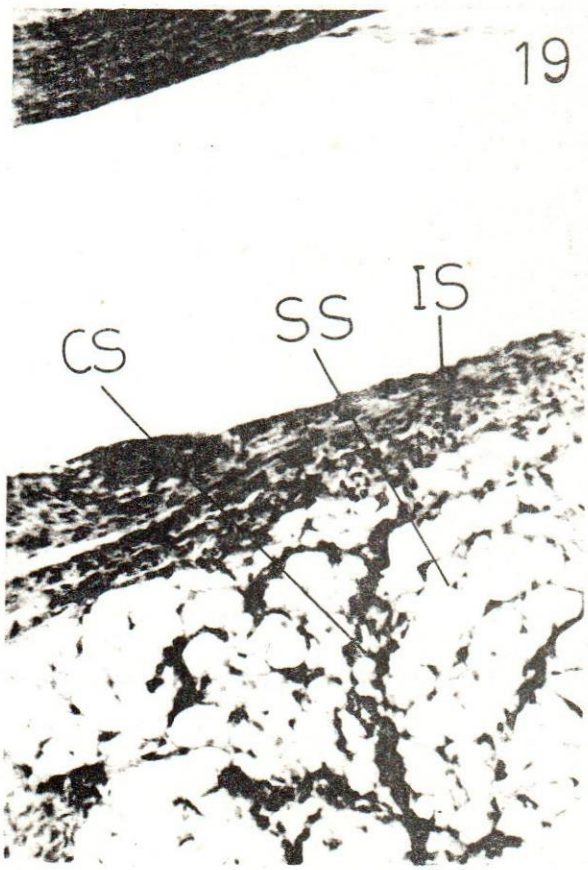
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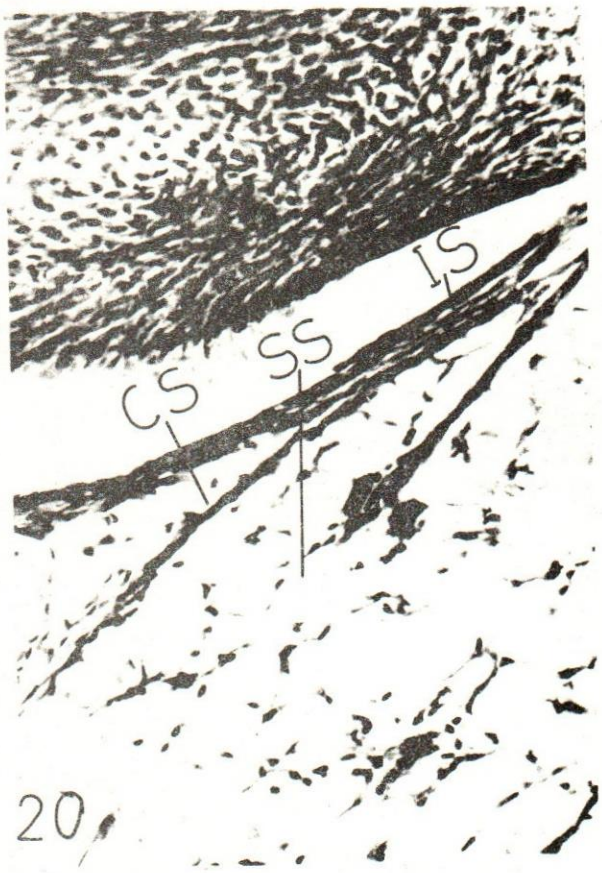
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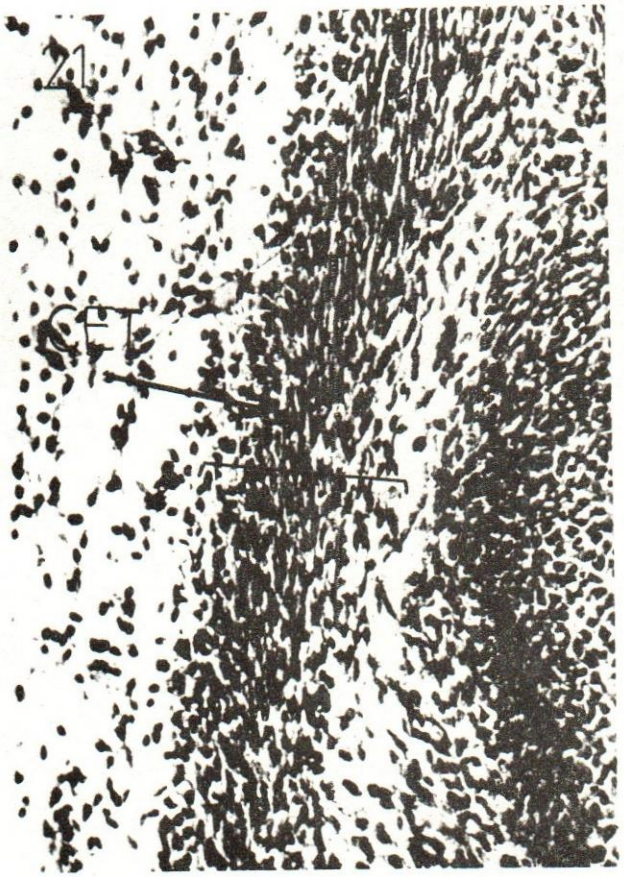
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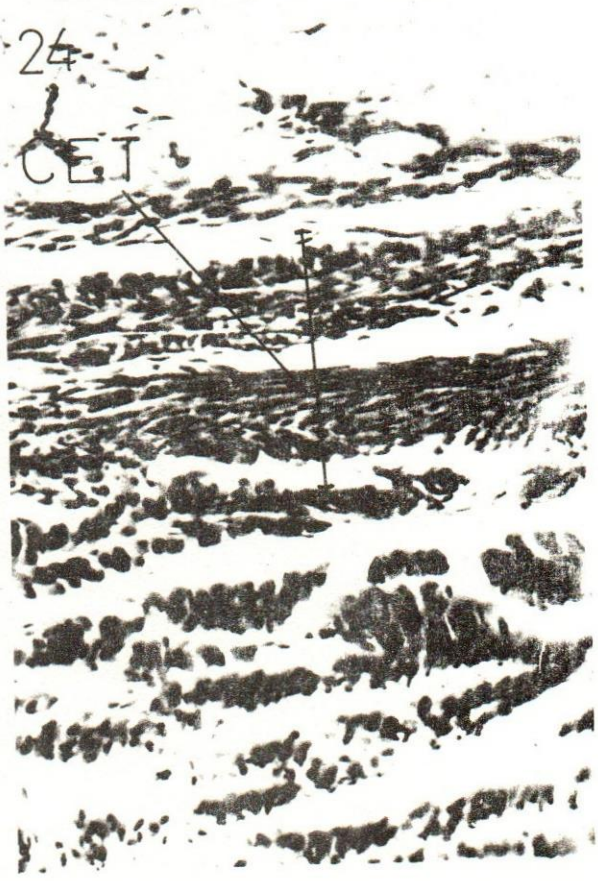
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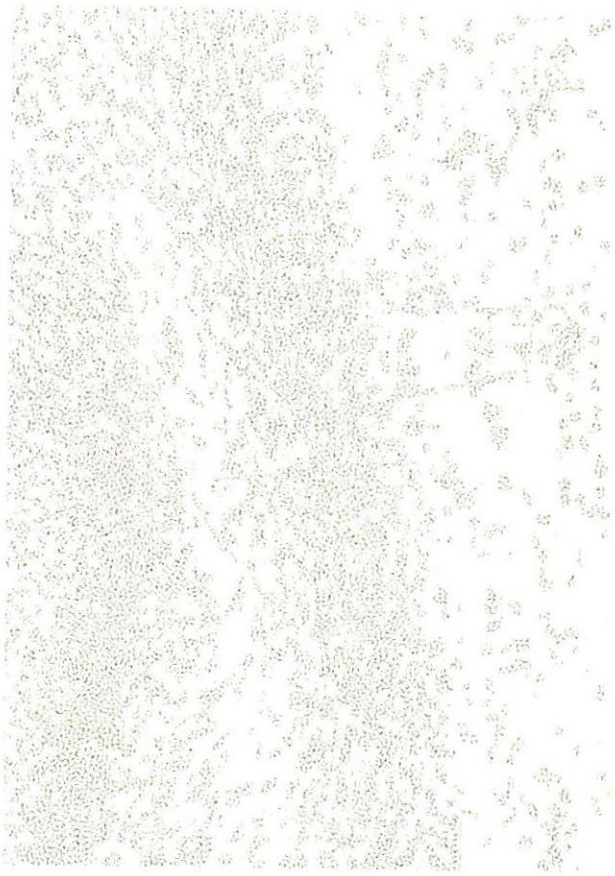

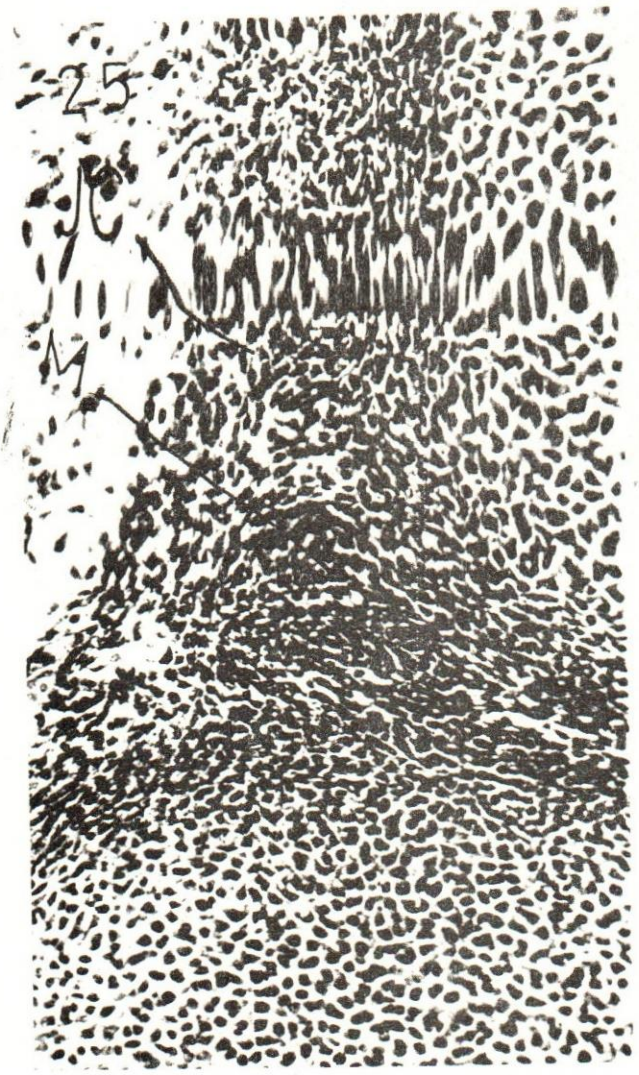
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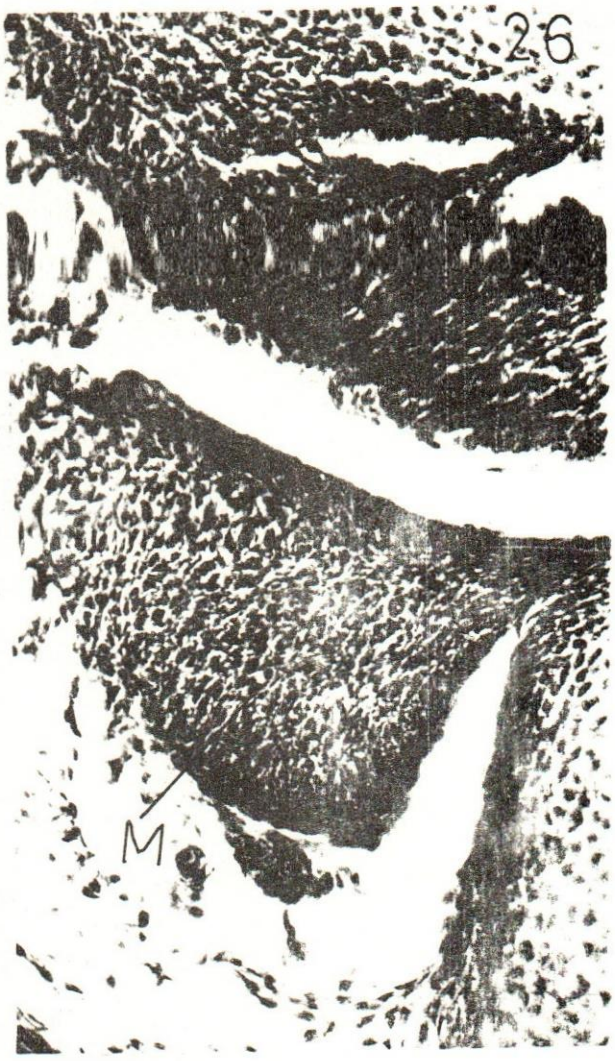
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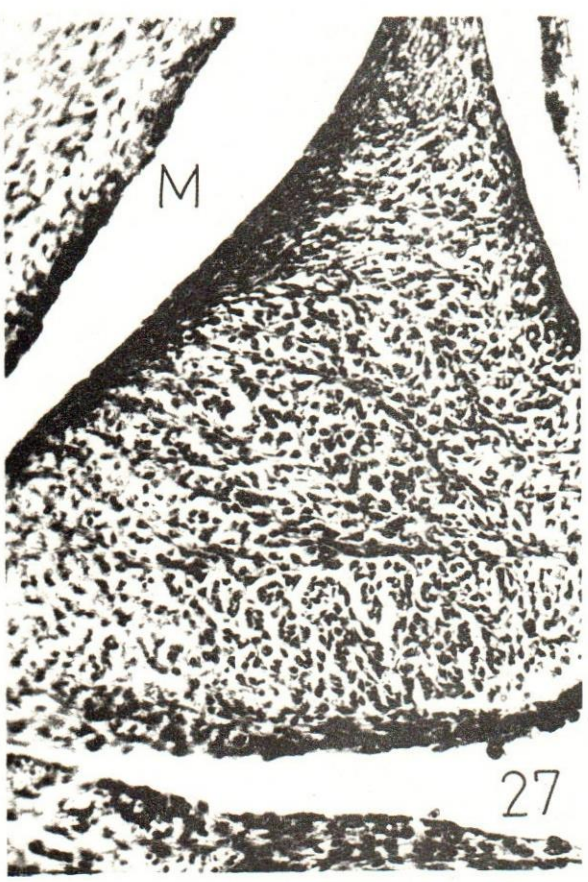
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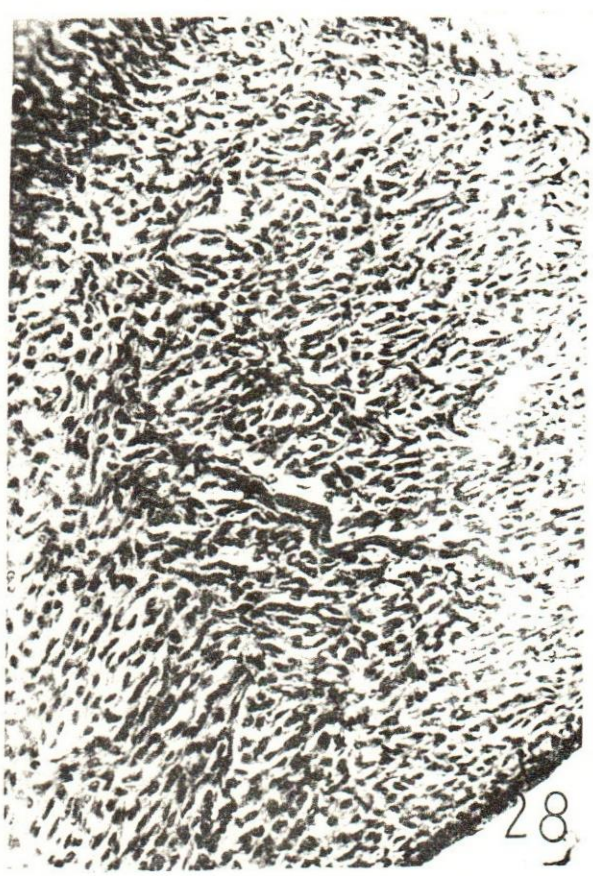
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