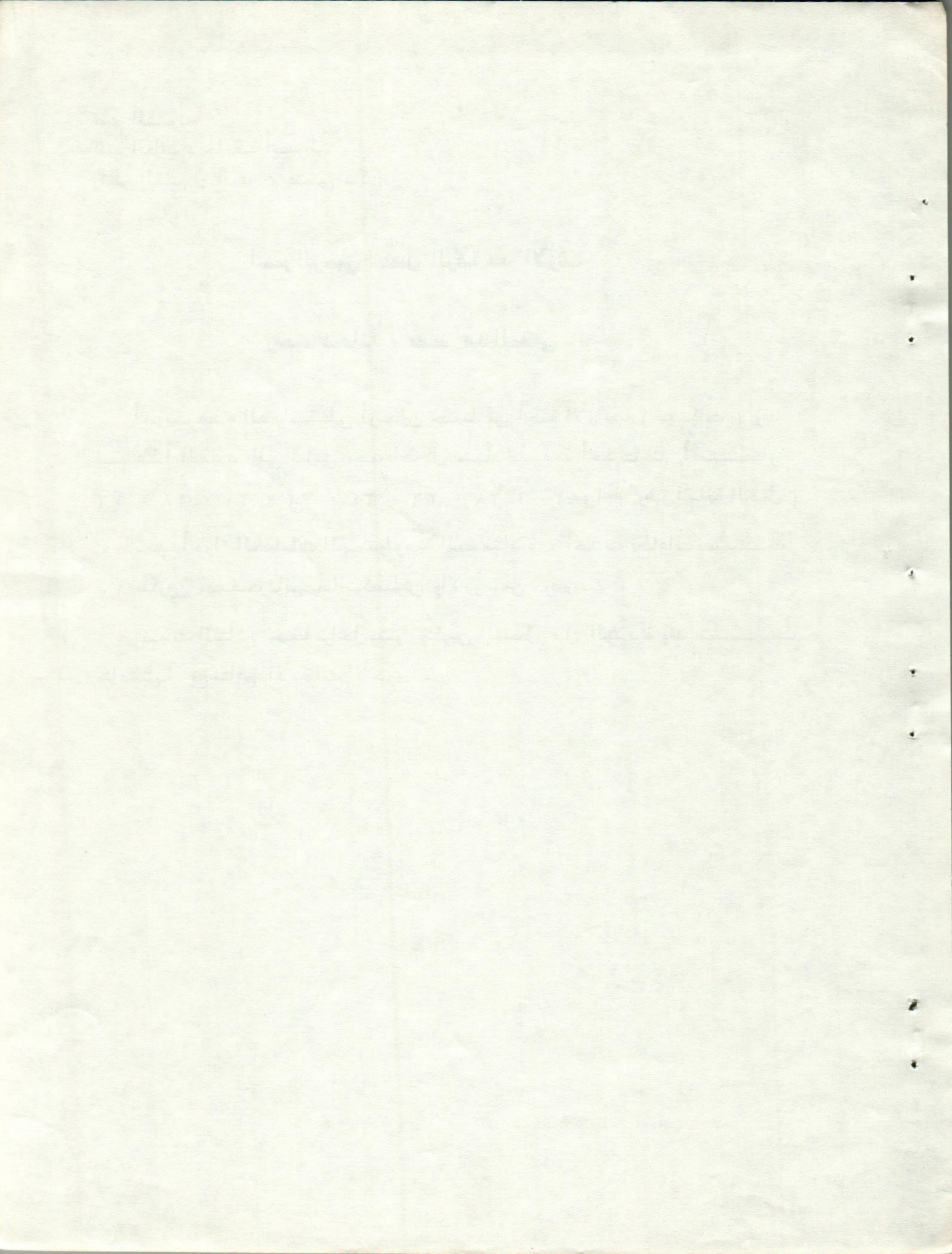


قسم التشريح  
كلية الطب - جامعة أسيوط  
رئيس القسم : د. د / فتحى ذكى

## النمو الرحمى لمفصل الركبة عند الأرنب

رفعت شحاتة ، محمد عبد المبنى

أجريت هذه الدراسة على أربعين جنينا من أجنة الأرنب ( بوسكات ) وقد قسم هذا العدد الى ثمانى مجموعات كل منها خمسة ، أخذت عند أعمار ١٦ ، ١٨ ، ٢٠ ، ٢٢ ، ٢٤ ، ٢٦ ، ٢٨ ، ٣٠ يوما ( وهو نهاية الحمل ) بعد إجراء العمليات الهستولوجية المعتادة ، أخذت قطاعات بسمك ١٠ ميكرون وصبغت بالهيما توكسيلين والايوسين ودرست .  
تضمنت النتائج وصف مراحل نشوء وتكوين المفصل قبل الولادة وقد تمسست مناقشتها مع نتائج الأبحاث الأخرى .



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**PRE-NATAL DEVELOPMENT OF THE KNEE JOINT OF RABBIT**  
(With 28 Figs)

By  
**R.S. MOHAMED and M.A. GABR**  
(Received at 22/4/1985)

**SUMMARY**

A total number of 40 Boscat rabbit embryos was used in the present work to study the prenatal development of its knee joint. The embryos were divided into 8 groups, each consists of 5 embryos, and taken at the ages of 16, 18, 20, 22, 24, 26, 28 and 30 days (new born).

After fixation, decalcification and histological processing, serially longitudinal sections of the knee joint were cut at 10um thick.

Stages of development were described and discussed.

**INTRODUCTION**

Many authors have described the prenatal development of human knee joint (WALMSLEY, 1940; WHILLIS, 1940; HAINES, 1947; GRAY and GARDNEER, 1950; O'RAHILLY, 1951; ANDERSEN, 1961 a).

Revising the above literature two questions were raised. Firstly, where the joint cavity begins, some authors found that the cavity begins peripherally (WILLIS, 1962; COPEMEN, 1970 and HAMILTON and MOSSMAN, 1972) while others mentioned that it begins its formation centrally (ANDERSEN, 1961, 1961 a, 1962 b, 1963). Secondly whether cell degeneration (HAINES, 1947; LEVER and FORD, 1958; WILLIS, 1962), accumulation of fluid (HAMILTON and MOSSMAN, 1972; HAM and CORMACK, 1979) or both (COPEMAN, 1970) is the factor preceding the appearance of synovial cavity.

The present study was undertaken to provide information about the prenatal development of the knee joint of rabbit that is widely used, as an experimental animal, in medical researches and also try to answer the previously mentioned two questions.

**MATERIAL and METHODS**

The present study was undertaken on Boscat rabbit (*Lepus caniculus*). The adult animals were maintained under normal conditions and were adequately fed with a sufficient diet (SANDFORD, 1977). The onset of pregnancy was considered to be the day at which mating occurred (McDONALD, 1977).

A total number of 40 rabbit embryos was used. They are divided into 8 groups, each consists of 5 embryos, taken at the ages of 16, 18, 20, 22, 24, 26, 28 and 30 (new born) days.

**MOHAMED and GABR**

The mothers were lightly anaesthetised with diethyl ether, their abdomens were opened and the embryos were extracted. The embryos were skinned and their knee joints were prepared and immersed at once in Bouin's fluid for 6-24 hours according to the age. Decalcification was done by neutral ethylene diamine tetra acetic acid (E.D.T.A.) Histological processing and embedding in paraffin was done as usual (DRURY and WALLINGTONS, 1976). Serial longitudinal sections were cut at a thickness of 10µm. and stained with haematoxyline and eosin.

**RESULTS**

In 16 days old rabbit embryo, a dense mesenchymal tissue mass connecting the ends of the chondrifying skeletal primordia appears (Fig. 1). It is known as the interzone.

The mesenchymal cells are packed together, oriented at different planes and have sparse cytoplasm and round or ovoid nuclei (Fig. 2). The interzone is homogenous and avascular.

In 18 days old rabbit embryo, the interzone loses its homogenous character and becomes differentiated into three layers; two chondrogenous layers separated by a loose intermediate one (Fig. 3).

Each chondrogenous layer shows transition to the underlying chondrifying tissue. It is continuous, at its periphery, with the perichondrium of its corresponding skeletal primordium.

The loose intermediate layer becomes vascular and continuous at its periphery with the surrounding general mesenchyme.

In 20 days old rabbit embryo, the three layers of the interzone become sharply distinct.

Each chondrogenous layer is still distinct from the underlying cartilage (Fig. 6) and continuous peripherally with the intracapsular perichondrium that is, in turn, continuous with the extracapsular perichondrium (Figs. 4,5). Both the chondrogenous layer and the intracapsular perichondrium form a complete investment for the articular end of each of the chondrifying skeletal primordia (Figs. 4,5).

The vascular and loose intermediate layer is continuous at its periphery with a vascular and loose mesenchymal tissue that forms the synovial tissue primordium (Figs. 4,5). The latter is bounded ventrally by the common extensor tendon primordium and dorsally by the flexor muscles primordia (Figs. 4,5).

The menisci (Fig. 7) and the cruciate ligaments appear as condensations of mesenchymal tissue in the loose intermediate layer of the interzone. The menisci are two wedge shaped masses while the cruciate ligaments appear as two bands crossing each other like the letter x (Figs. 4,5).

The common extensor tendon primordium appears as a band of dense mesenchymal tissue (Fig. 9) extending from the primordia of the extensor muscles to the tibia where it is continuous with its perichondrium. It is separated from the distal end of the femur by a loose mesenchymal tissue that is continuous distally with the synovial tissue primordium (Fig. 8).

The patella primordium appears in the proximal part of the common extensor tendon as a dense cellular mass whose cells are round in shape.

## PRE-NATAL DEVELOPMENT OF THE KNEE JOINT

The synovial cavity appears as a longitudinal cleft separating the patella primordium from the articular surface of the femur, patello-femoral part of the joint cavity proper, (Fig. 5). This cleft is not clear as it contains cells, some of them are degenerating.

In 22 days old rabbit embryo, the articular ends of both femur and tibia are still chondrifying (Fig. 10&11).

The patella becomes prominent but is also still chondrifying (Fig. 10).

The articular surfaces taken their characteristic shapes being greatly convex ventro-dorsally in the femur, slightly convex ventro-dorsally in the tibia and flat in the patella (Fig. 10).

Each articular surface is covered with a thin fibrillar layer within which flat cells are seen (Fig. 12). This fibrillar layer is separated from the articular surfaces in some areas especially the areas of apposition.

The cruciate ligaments are similar to those of the previous stage. The central part of each meniscus shows that its cells are separated by a little amount of a matrix while its peripheral part remains mesenchymal in structure (Fig. 13).

The patello-femoral part of the joint cavity extends proximally forming the craniopatellar synovial pouch that separates the common extensor tendon from the distal end of the femur (Fig. 10). At the same time the femoro-tibial part of the joint appears separating the menisci from the distal end of the femur, proximally, and from the proximal end of the tibia, distally (Fig. 10). Both parts of the joint cavity, patello-femoral and femoro-tibial, are still separated from each other by fusions present between the femoral condyles and the synovial tissue present ventral to it (Fig. 10). Joint cavities are not clear as they contain cellular strands and cellular debris.

The synovial tissue is vascular loose mesenchymal tissue and its surface is ragged.

The common extensor tendon is similar to that of the previous stage. The dorsal part of the joint capsule is ill defined.

In 24 days old rabbit embryo, the whole of the patella and also the articular ends of both femur and tibia become completely chondrified (Figs. 14&15). Cartilage canals appear in the articular ends of both femur and tibia (Figs. 14&15).

The chondrogenous layer of the interzone and the whole of the articular surface of the patella become indistinct and incorporated into the underlying hyaline cartilage (Fig. 16).

The fibrillar layer is still present in some areas, but separated from the articular surfaces, and is completely absent in other areas.

The cruciate ligaments and also the common extensor tendon are similar to those described in the previous stage.

The central part of each meniscus shows that the matrix is increased in amount. Some of its cells become differentiated into chondrocytes (Fig. 17). The peripheral part is still mesenchymal in structure.

The joint cavity becomes more extensive than that of the previous stage. The patello-femoral part of the joint becomes continuous with its femoro-tibial part (Fig. 14). The cavity extends distally separating the cruciate ligaments from the synovial tissue present ventral to them, the caudo-patellar synovial fold (Fig. 15). The joint cavity is still not clear as it contains cellular strands and cellular debris.

## MOHAMED and GABR

At this stage of development, the synovial tissue covers all parts of the joint cavity except the articular surfaces of patella, femur, tibia and the menisci and the proximal two thirds of the dorsal aspects of the cruciate ligaments (Fig. 15). The synovial tissue varies in thickness, vascularity and structure. In the cranio-patellar part of the joint cavity, it is thin, poorly vascular and consists of flat cells closely situated and resting directly on the underlying common extensor tendon. In other parts, it is thick, vascular and thrown into small folds. In these parts the synovial tissue has a ragged surface and consists of two layers, a synovial intima composed of widely separated synovial cells which are large and ovoid and a subintima composed of loose areolar tissue (Figs. 18&19).

At the site of reflection of the synovial tissue from the periphery of the capsule to the articular cartilage, the synovial cells are suddenly replaced by chondrocytes while the underlying loose areolar tissue, subintima, becomes rich in fibroblasts and thin collagen fibres and then merge into the hyaline cartilage.

In 26 days old rabbit embryo, the knee joint (Fig. 21) is similar to that of the previous stage except that.

The differentiation of the common extensor tendon, and cruciate ligaments into fibrous tissue becomes distinct and vascularised.

The dorsal part of the capsule appears as a thin strand of fibrous tissue.

The joint cavity becomes more extensive than that of the previous stage.

In 28 days old rabbit embryo, the joint (Fig. 22) is similar to that of the previous stage except that:

The peripheral part of each meniscus becomes differentiated into fibrous tissue (Fig. 23).

In new born rabbit, the knee joint (Fig. 24) is similar to that of the previous stage except that:

A primary centre of ossification appears in the distal end of the femur and also in the proximal end of the tibia (Fig. 24).

Cartilage canals appear in the patella (Fig. 24).

Each of the articular surfaces is smooth and its surface cells are flattened in contrast to the deep round chondrocytes (Fig. 25).

The fibrillar layer is completely disappeared.

The synovial subintima becomes more vascular and more looser than that of the previous stage fibroblasts and thin collagen fibres are seen (Figs. 26&27).

The common extensor tendon and the cruciate ligaments reveal that the collagen fibres become more thick than those of the previous stage. (Fig. 28).

## PRE-NATAL DEVELOPMENT OF THE KNEE JOINT

### DISCUSSION

#### Homogenous Interzone

The present work reveals that the interzone appears in 16 days old rabbit embryo. It corresponds to the articular disc of WALMSLEY (1940) and WHILLIS (1940) and to the interzone of HAINES (1947) who find it in 11-13 mm. stages of human embryos. It also corresponds to the mesenchymal disc of FELL and CANTI (1934) who find it in 5 days old chick embryo.

#### Three Layered Interzone

The present results show that the interzone loses its homogenous character and becomes differentiated into three layers, in 18 days old rabbit embryo. The same results are obtained by HESSER (1926); LANGER (1929); HAINES (1947); GRAY and GARDNER (1950); O'RAHILLY (1951) and ANDERSEN (1961 a), in 25-26 mm human embryos and also obtained by FELL and CANTI (1934) in 6 days old chick embryos. On the other hand, WHILLIS (1940) denied the differentiation of the interzone into its three layers.

#### Articular Surfaces

In 20 days old rabbit embryo, the present work finds that each of the articular surfaces of the femur and tibia is covered with the chondrogenous layer of the interzone centrally and a structurally similar layer derived from the intracapsular perichondrium peripherally. The articular surface of the patella is similar in structure to the chondrogenous layer of the interzone but not derived from it. In 22 days old rabbit embryo, these layers become completely chondrified and incorporated into the underlying hyaline cartilage. The same findings are obtained by WALMSLEY (1940) HAINES (1947); GRAY and GARDNER (1950); O'RAHILLY (1951) and ANDERSEN (1961 a). This leads to an opinion that the articular cartilage may be developed from the chondrogenous layer of the interzone and the intracapsular perichondrium (ANDERSEN, 1961; COPEMAN, 1970).

#### Joint Cavity and Synovial Tissue

As regards where the joint cavity first appears, either peripherally or centrally, contradicting opinions are present. The present study clarifies that the joint cavity commences peripherally as a cleft separating the patella from the distal end of the femur, the patello-femoral part and then centrally, its femoro-tibial part. These results agree with WILLIS (1962) and HAMILTON and MOSSMAN (1972) who stated that, it begins peripherally but contradict ANDERSON and BRO-RASMUSSEM (1961); ANDERSEN (1961 a); ANDERSEN (1962 a&b) and ANDERSEN (1963), who mentioned that, it begins centrally.

Concerning whether the appearance of the joint cavity is due to accumulation of fluid or cellular degeneration different opinions are present.

The present study attributes it to accumulation of fluid, however degenerating cells are detected in joint cavity. This means that both factors contribute to the appearance of the joint cavity. This agrees with Copeman (1970). Other investigation, on adult synovial joint, found that the synovial membrane cannot be the main source of synovial fluid as some mucinous substance is supplied to the synovial fluid by wear and tear of the articular cartilage (WARWICK and WILLIAMS, 1973). ANDERSEN (1963) and HAMILTON and MOSSMAN (1973) on the other

## MOHAMED and GABR

hand attribute it only to accumulation of fluid. While HAINES (1947); LEVER and FORD (1958) and WILLIS (1962) attributed it to cellular degeneration.

Concerning the underlying cause of the appearance of the joint cavity different opinion are mentioned.

FELL and CANTI (1934) found that when one of skeletal elements is reduced beyond a certain size, the joint fails to develop.

COPEMAN (1970) stated that pressure on the uterus results in anomalies of the joint.

HALL (1975) and PERSON (1983) found that skeletal muscle contractions of the embryo is essential for the development of synovial joints.

### Synovial Tissue

The present study found that the synovial tissue primordium appears in 20 days old rabbit embryo as the vascular loose intermediate layer of the interzone. Ventrally it is separated from the general mesenchyme by the flexor muscles. These findings agree with those obtained by HAINES (1947); GARDNER and GRAY (1950) and GARDNER and O'RAHILLY (1968).

According to HAINES (1947) in his developmental study and WARWICK and WILLIAMS (1973) and HAM and CORMACK (1979) in their studies on the adult synovial joints, the thickness, vascularity and structure of the synovial tissue depend upon the nature of the substratum upon which it lies. The present results reveal, in 24 old rabbit embryo, that it is thin, poorly vascular and consists of flat cells where it overlies the common extensor tendon. It is thick richly vascular and consists of two layers; intima and subintima. The intima consists of synovial cells which are large, ovoid or round and are separated from each other by narrow gaps which contain extensions from the underlying subintima. The subintima consists of loose mesenchymal tissue in 24 days and then becomes loose areolar tissue in newborn. At this stage of development, the synovial tissue is still ragged and thrown into few folds and villi.

### Fibrous Capsule, Cruciate Ligaments and Menisci

The common extensor tendon and the cruciate ligaments make their first appearance in 20 days old rabbit embryo as bands of dense mesenchymal tissue. The former separates the synovial tissue primordium from the general mesenchymal tissue while the latter arises as a derivative of the loose intermediate layer of the interzone. Then both differentiate into fibrous tissue in 26 days old rabbit embryo. The same stages are described by HAINES (1947) and ANDERSEN (1961 a).

The menisci appear in 20 days old rabbit embryo as two wedge shaped masses of dense mesenchymal tissue. The same were observed by HAINES (1947) and ANDERSEN (1961 a). Its central part becomes chondrified, in 24 days old rabbit embryo. While its peripheral part remains mesenchymal up to the stage of 28 days of prenatal life where the mesenchymal cells become differentiated into fibroblasts separated by thin collagen fibres interposed in a matrix.



## PRE-NATAL DEVELOPMENT OF THE KNEE JOINT

### Conclusion

From the present study, it is concluded that:

- 1- The knee joint makes its first appearance by the formation of the interzone that is, at first, homogenous and avascular. Then it loses its homogenous character and becomes differentiated into 3 layers; two chondrogenous layers separated by a loose vascular intermediate one.
- 2- The chondrogenous layers of the interzone become incorporated with the underlying hyaline cartilage of the articular end of the skeletal primordium and take part in the formation of the future articular cartilage.
- 3- The intermediate layer of the interzone gives rise to the synovial tissue and the intracapsular structures; the cruciate ligaments and the menisci.
- 4- The synovial cavity begins peripherally and then centrally.
- 5- The appearance of the joint cavity is attributed to accumulation of fluid that is secreted by the synovial tissue and is contributed to by cellular degeneration.

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## EXPLANATION OF FIGURES

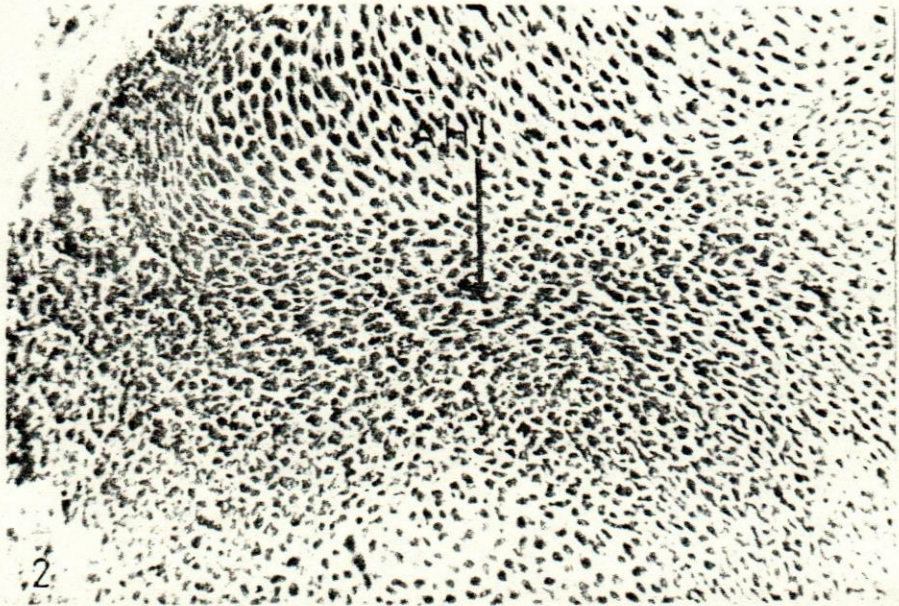
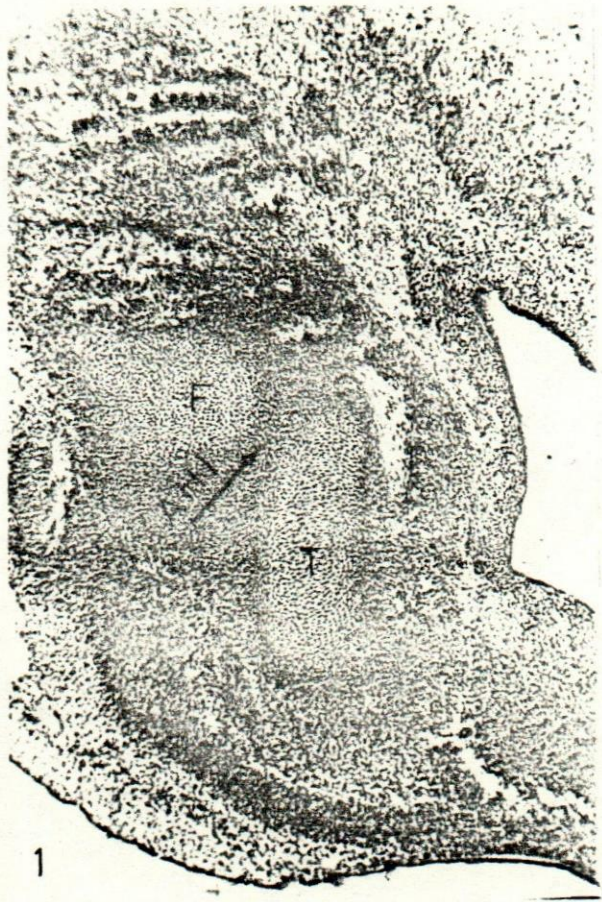
- Fig. (1):** Longitudinal section of the hind limb of 16 days old rabbit embryo showing avascular homogenous interzone (AHI) chondrifying femur primordium (F) and chondrifying tibia primordium (T). Hematoxylin & Eosin x 50.
- Fig. (2):** Interzone of 16 days old rabbit embryo. Its mesenchymal cells are packed together and oriented at different planes. Hematoxylin & Eosin x 200.
- Fig. (3):** Longitudinal section of the hind limb of 18 days old rabbit embryo showing that the interzone is differentiated into 3 layers; 1,2 chondrogenous layers (CH) 3- loose vascular intermediate layer (LI). Hematoxylin & Eosin x 50.
- Fig. (4):** A longitudinal section in the knee joint of 20 days old rabbit embryo showing; the distal end of femur (F), the proximal end of tibia (T), the chondrogenous layers of the interzone (CH), the loose intermediate layer (LI), the intracapsular perichondrium (IP), the extracapsular perichondrium (Ex. P), meniscus (M) and the common extensor tendon (C E T). Hx. & E. x 40.
- Fig. (5):** A longitudinal section in the knee joint of 20 days old rabbit embryo showing the cruciate ligament (C L), the patellofemoral part of the joint cavity (PF), the patella (P) and common extensor tendon (C E T). Hx. & E. x 40.
- Fig. (6):** A longitudinal section in the knee joint of 20 days old embryo showing the articular ends of femur and tibia. Each is covered with the chondrogenous layer of the interzone (CH) and separated by the loose intermediate layer (LI). Hx. & E. x 400.
- Fig. (7):** A longitudinal section in a meniscus of 20 days embryo. Hx. & E. x 400.
- Fig. (8):** The synovial tissue primordium (S.T.) in 20 days old rabbit embryo. Hx. & E. x 100.

## PRE-NATAL DEVELOPMENT OF THE KNEE JOINT

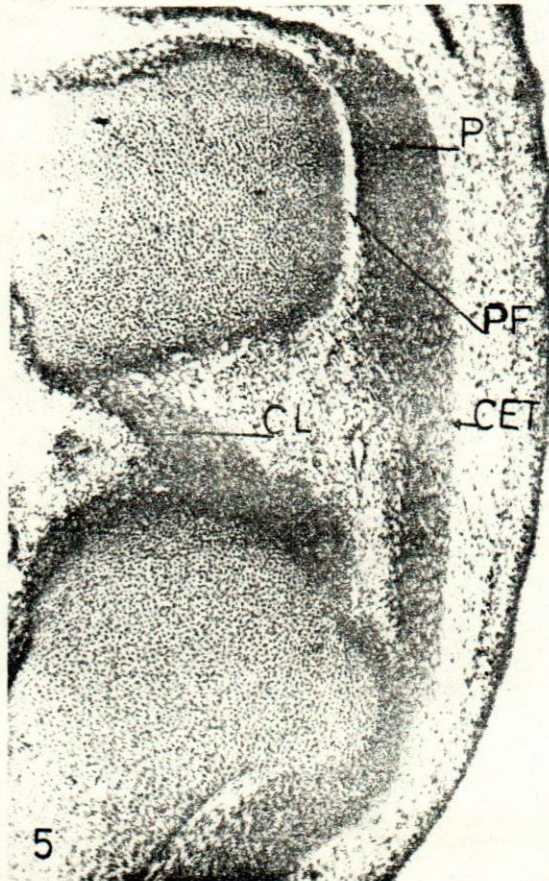
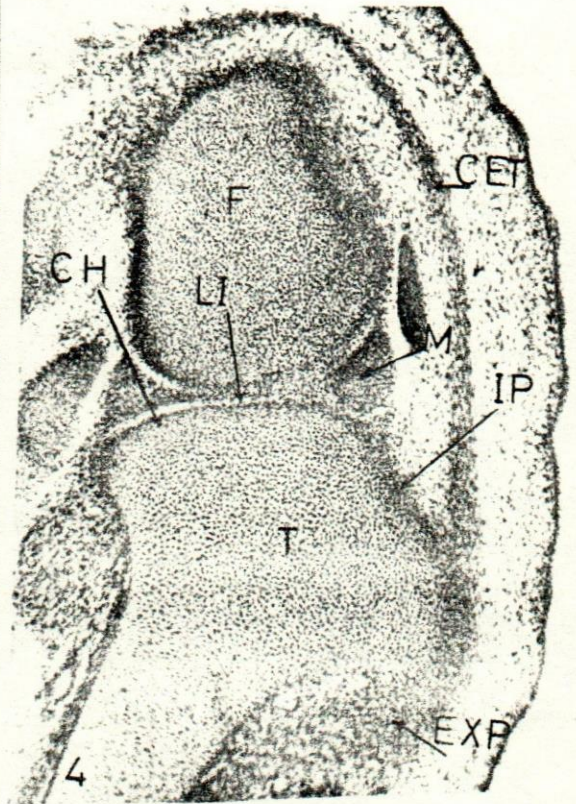
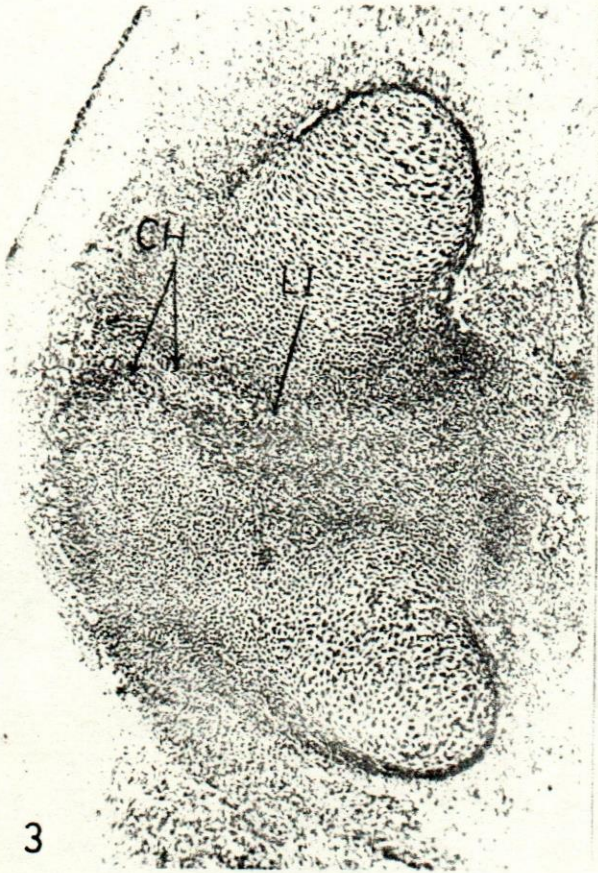
- Fig. (9):** A longitudinal section in the cruciate ligament, in 20 days old rabbit embryo. Hx. & E. x 400.
- Fig. (10):** A longitudinal section in the knee joint of 22 days old rabbit embryo showing; the articular ends of femur (F) and tibia (T), the patella (P), menisci (M), patello-femoral part of the joint (P.F.) and its cranio-patellar pouch (CP) the femoro-tibial part of the joint cavity (FT), synovial tissue (S.T.) and the common extensor tendon (C E T). Hx. & E. x 25.
- Fig. (11):** A longitudinal section of the knee joint of 22 days old embryo showing the cruciate ligaments (CL) and the synovial tissue (S.T.). Hx. & E. x 20.
- Fig. (12):** A longitudinal section in the knee joint of 22 days old embryo showing that the chondrogenous layer (CH) is still distinct from the underlying hyaline cartilage (H.C.). Fibrillar layer (F.L.) is also appearant. Hx. & E. x 400.
- Fig. (13):** A longitudinal section in the meniscus of 22 days old embryo showing that the cells of its central part are separated by a little amount of matrix. Hx. & E. x 400.
- Fig. (14):** A longitudinal section in the knee joint of 24 days old embryo showing that the chondrogenous layers (CH.I) become incorporated into the underlying hyaline cartilage (HC) of the articular ends of femur (F) and tibia (T), cartilage canals (C.C.) are appearant, all parts of the joint cavity form one continous compartment (P.F., C.P., T.F.) and synovial folds (S.F.) appear. Hx. & E. x 20.
- Fig. (15):** A longitudinal section in the knee joint of the same age showing cruciate ligaments (C.L.), caudo-patellar synovial fold (C.P.S.F.) and synovial villi and common extensor tendon (C.E.T.) Hx. & E. x 20.
- Fig. (16):** A longitudinal section in the knee joint of 24 days old rabbit embryo showing the articular surfaces of both femur (F) and tibia (T). The chondrogenous layer (CH.I) becomes incorporated into the underlying hyaline cartilage. Remenants of the fibrillar layer (F.L.) is still present. Hx. & E. x 400.
- Fig. (17):** A longitudinal section in the central part of a meniscus of 24 days old embryo showing that the intracellular matrix is increased. (Compare with fig. 12). Hx. & E. x 400.
- Fig. (18):** A longitudinal section in the caudo-patellar fold of synovial membrane (C.P.F.) of 24 days old rabbit embryo showing that it is differentiated into synovial intima (S.I.) and subintima (Sub. I). Hx. & E. x 100.
- Fig. (19):** A part of the same previous region caudo patellar fold is magnified 5 time. It shows synovial cells (arrows) widley separated resting on a loose mesenchymal tissue, the subintima (Sub. I). Hx. & E. x 400.
- Fig. (20):** A longitudinal section in a cruciate ligament of 24 days old rabbit embryo. It shows that it consists of fibroblasts with thin collagen fibres interposed in between them. Hx. & E. x 400.
- Fig. (21):** A longitudinal section in the knee joint of 24 days old rabbit embryo showing articular ends of femur (F) and tibia (T), the patella (P), cartilage canals (C.C.), Cruciate ligament (C.L.) synovial tissue (S.T.) and common extensor tendon (C.E.T.). Hx. & E. x 12.5.

## MOHAMED and GABR

- Fig. (22):** A longitudinal section in the knee joint of 28 days old embryo showing articular ends of femur (F) and tibia (T), cartilage canals (C.C.); patella (P) joint cavity (J.C.), menisci (M), synovial tissue (S.T.) and the common extensor tendon (C.E.T.). Hx. & E. x 20.
- Fig. (23):** A longitudinal section in the peripheral part of a meniscus of 28 days old embryo showing that it consists of fibroblasts with thin collagen fibres interposed in between. Hx. & E. x 400.
- Fig. (24):** A longitudinal section in new born rabbit showing that a primary centre (P.C.) of ossification in each of articular ends of femur (F) and tibia (T) is present, cartilage canals (C.C.) appear in the patella (P) and the synovial tissue especially its folds (caudo-patellar fold, C.P.F.) become richly vascularised. It also shows a cruciate ligament (C.L.). Hx. & E. x 25.
- Fig. (25):** A longitudinal section in a part of the articular end of the femur (F) showing that the surface layer of cartilage cells consists of flat cells (the arrow) in contrast to the deep round cells. Hx. & E. x 400.
- Fig. (26):** A longitudinal section in the caudo-patellar fold of synovial membrane in new born rabbit showing that blood vessels are numerous and of large diameter (compare with fig. 18). Hx. & E. x 100.
- Fig. (27):** A part of the same previous region, the caudopatellar synovial fold, magnified 4 times showing the intima (S.I.) and subintima (Sub I). The latter becomes loose areolar tissue. Hx. & E. x 400.
- Fig. (28):** A longitudinal section in a cruciate ligament of a new born rabbit showing that its collagen fibres become thicker than the previous stages (compare with fig. 20). Hx. & E. x 400.

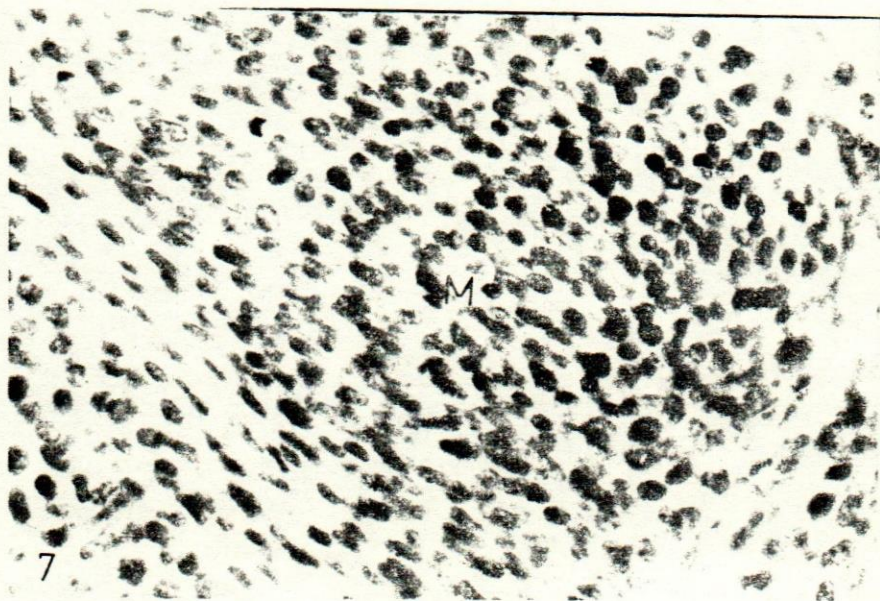
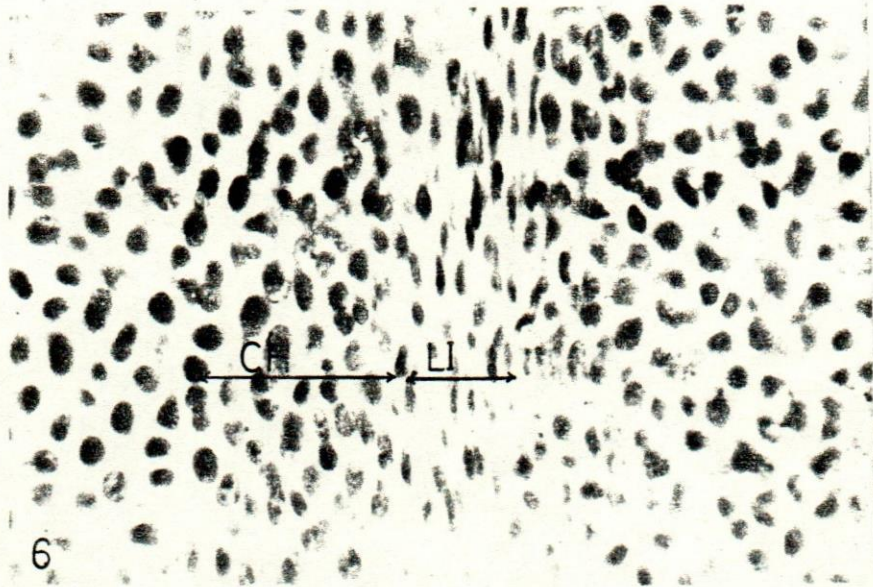




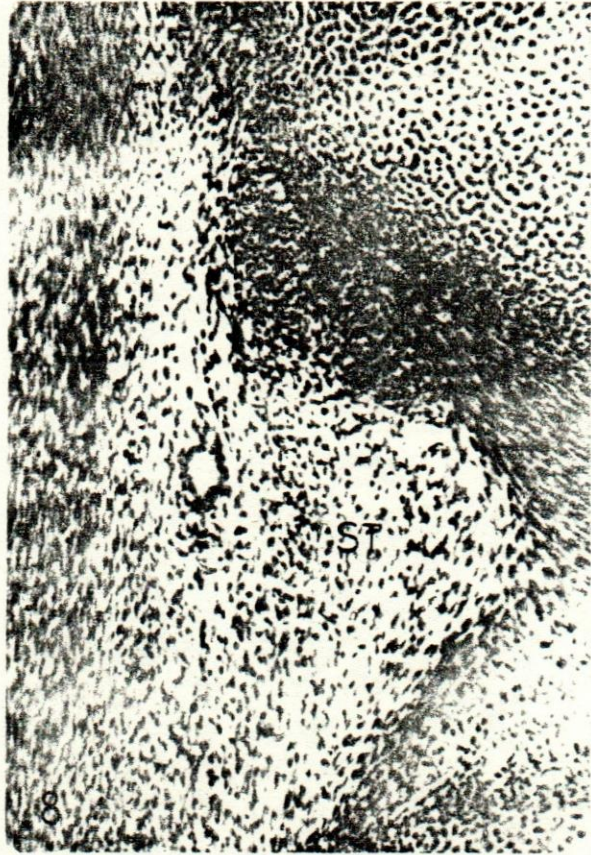




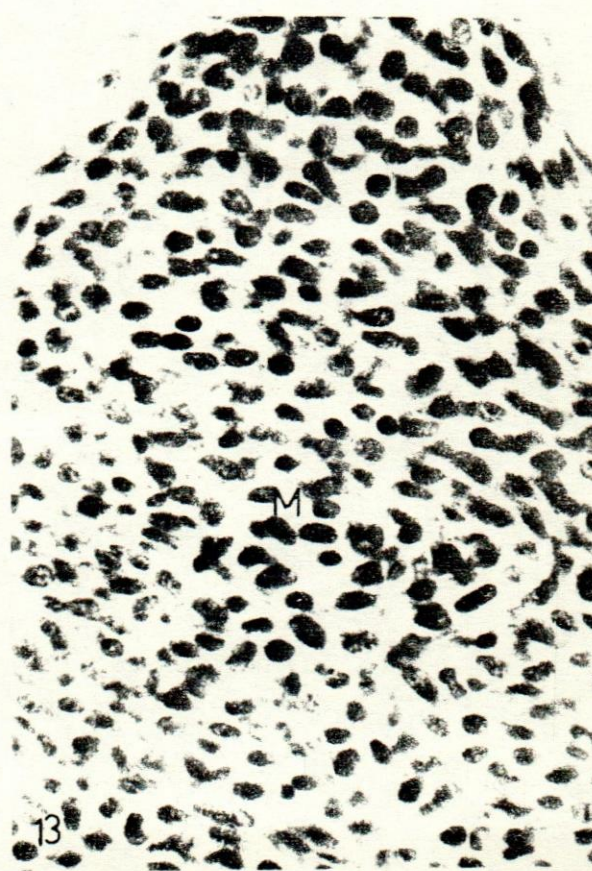
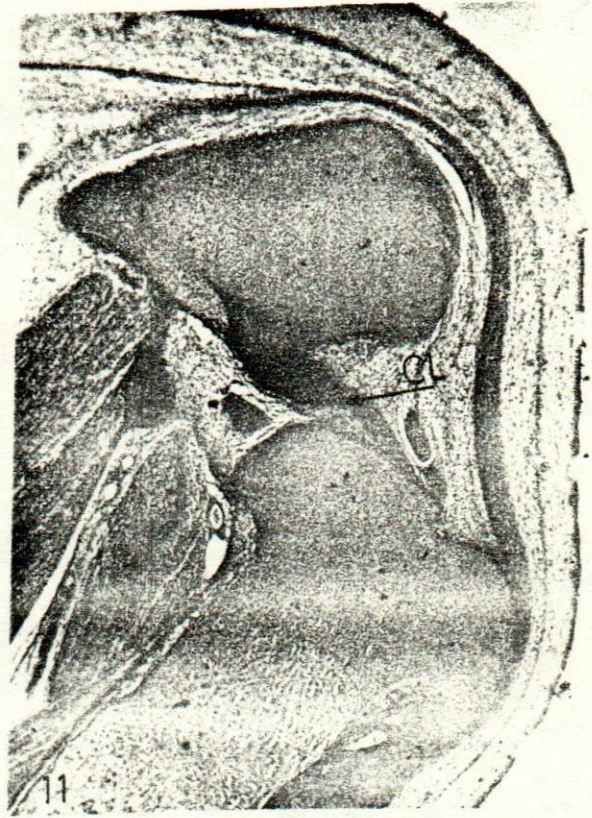
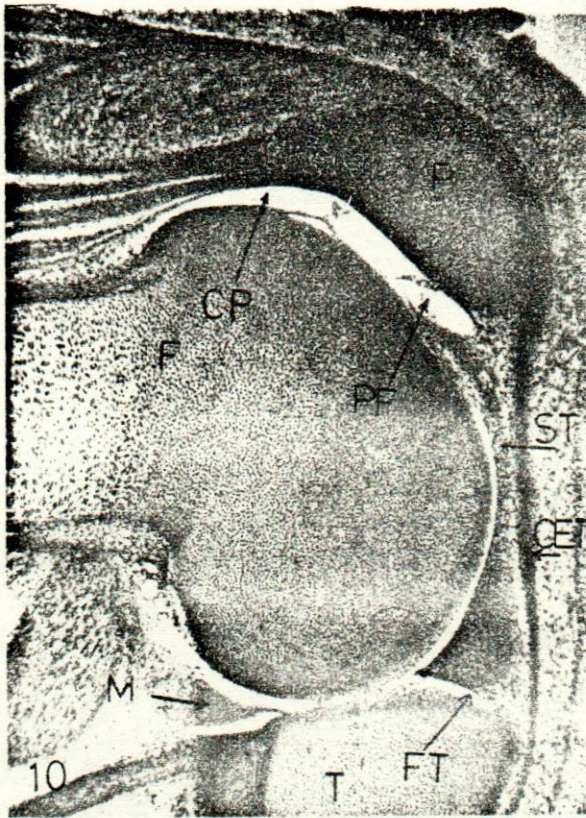




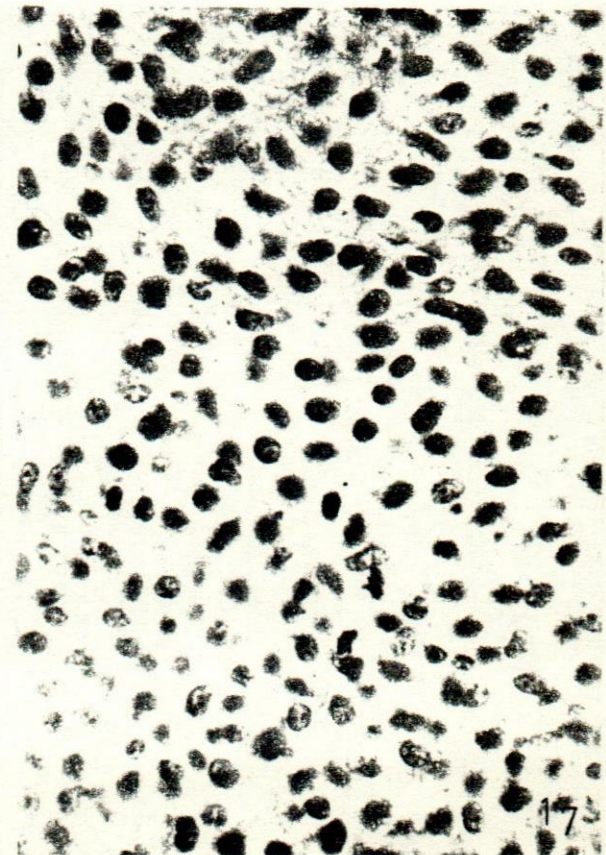
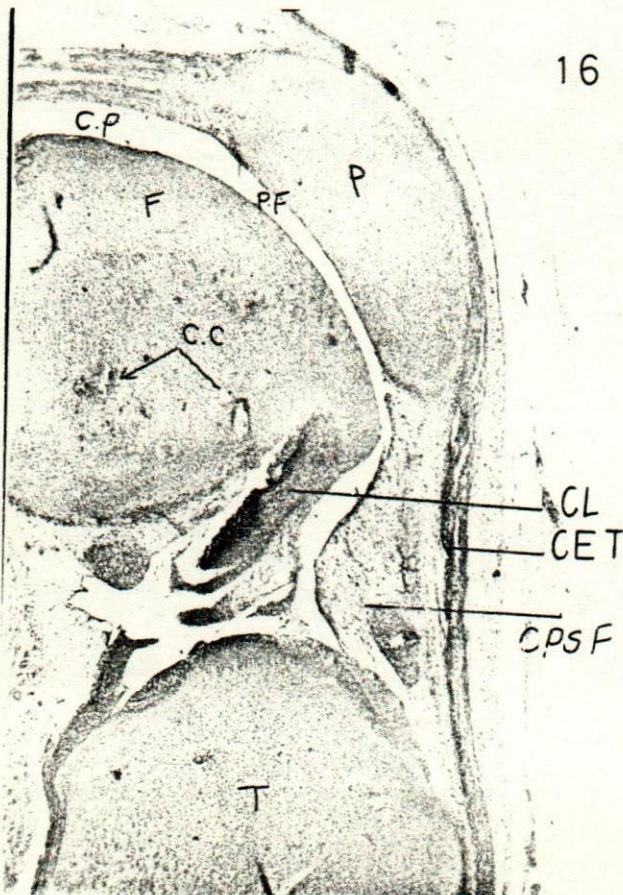
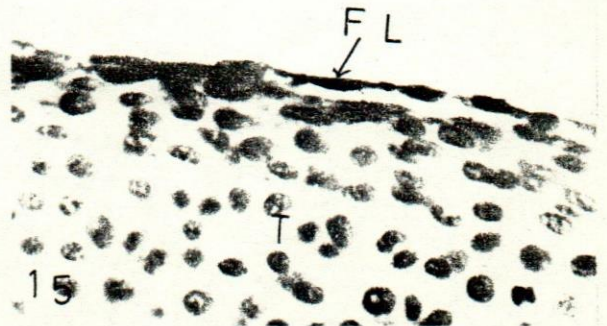
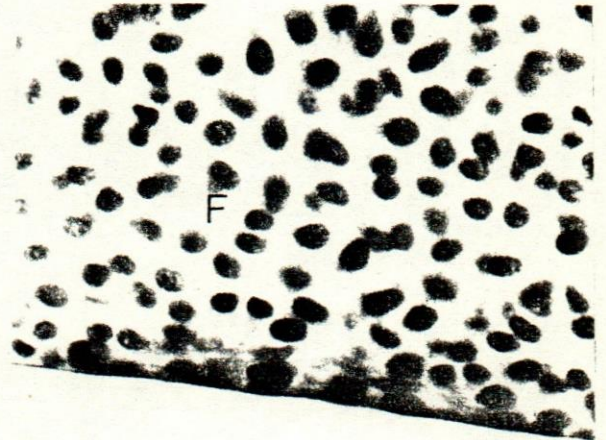
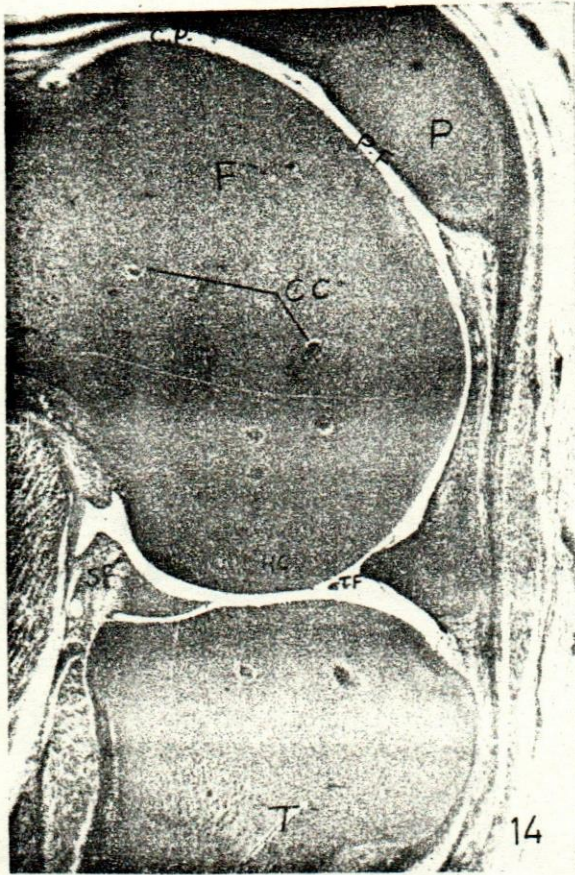


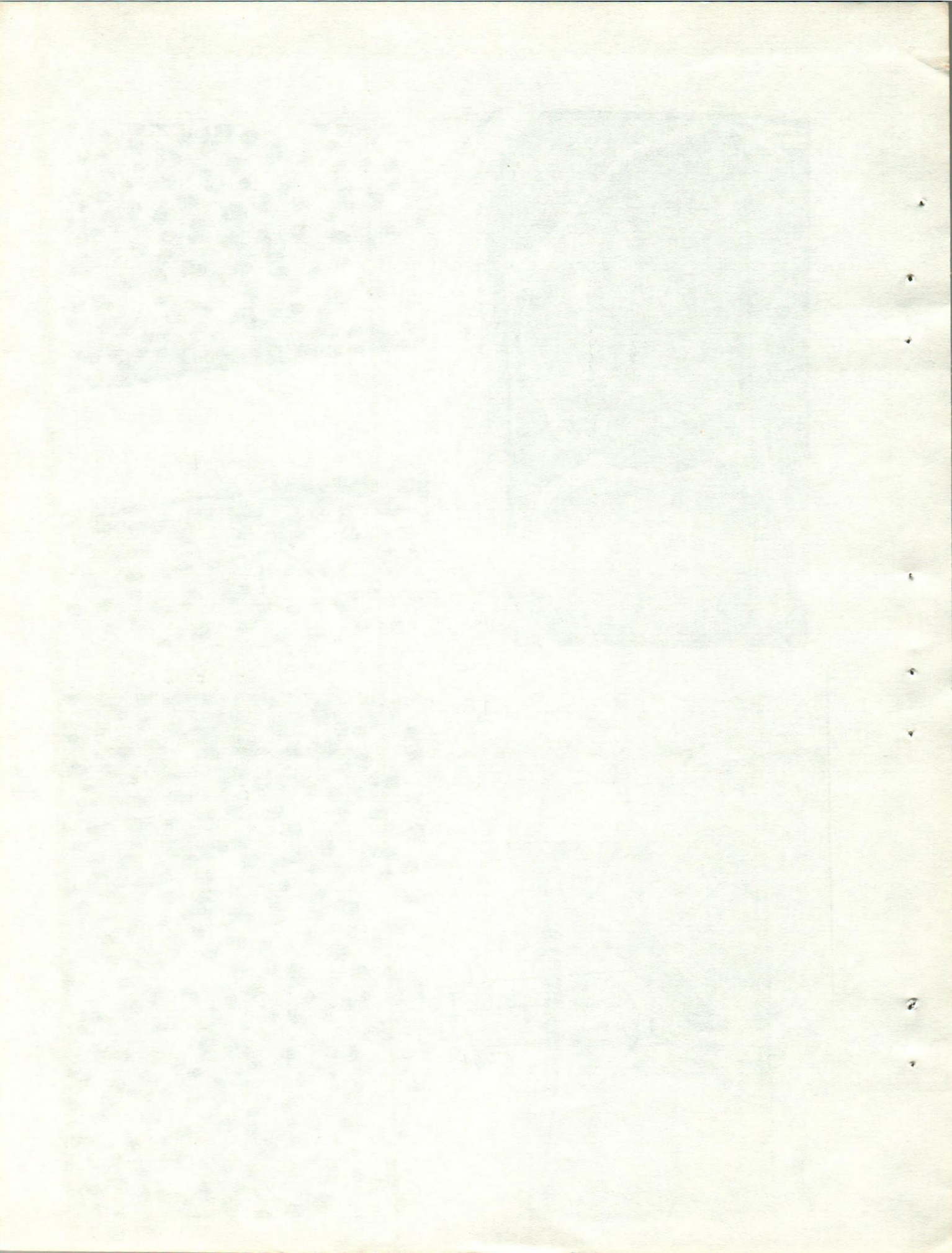














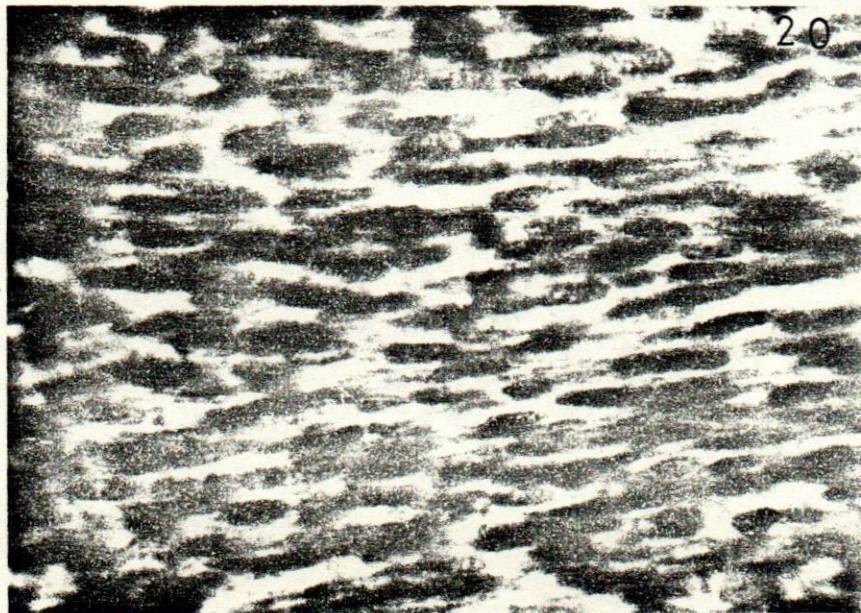
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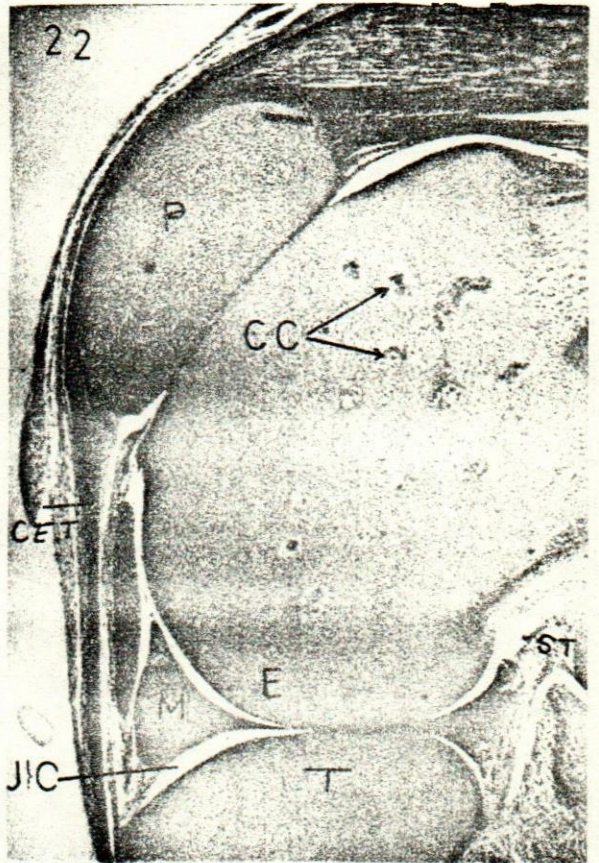
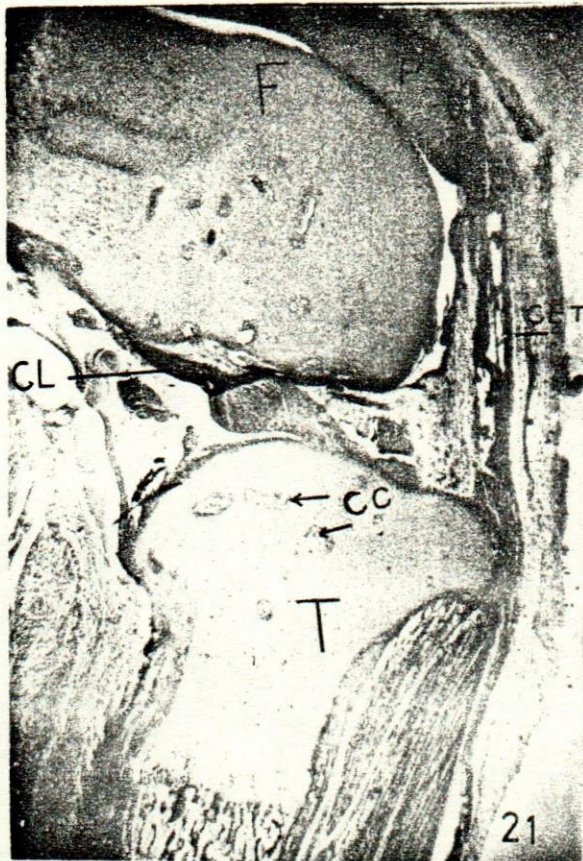
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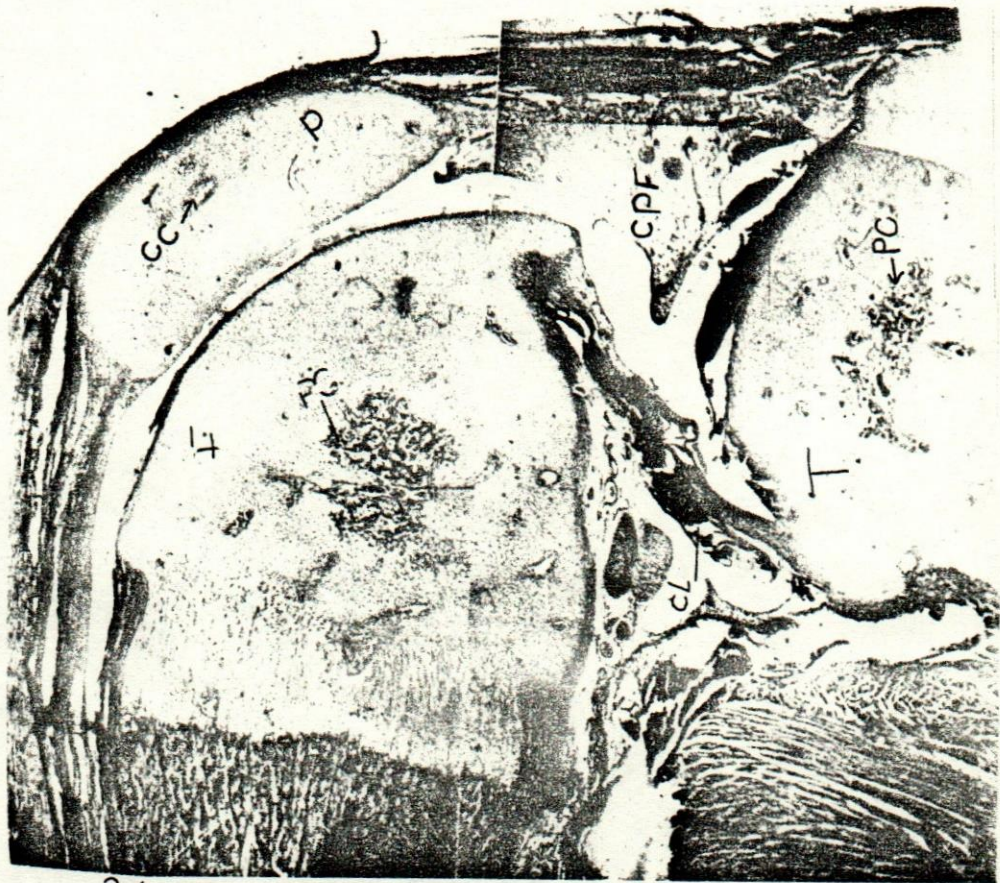
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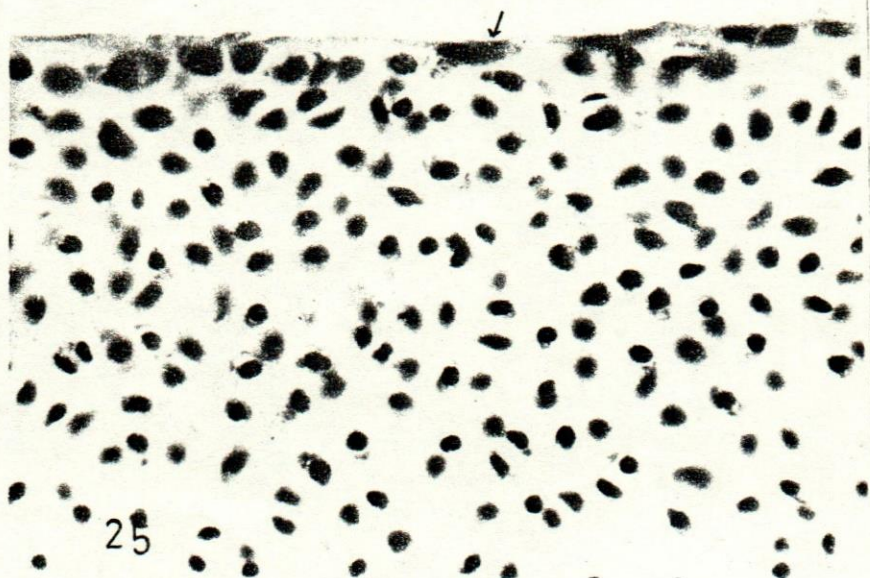








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Faint, illegible text or markings, possibly bleed-through from the reverse side of the page, located in the lower half of the document.

