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THE DEVELOPMENT OF THE NEURAL CREST IN THE CEPHALIC REGION OF *XIPHOPHORUS HELLRI*

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ABSTRACT

The site, time of segregation and migratory pathways of the neural crest cells are studied in the head region of seven developmental stages (0.7; 1.0; 1.5; 2.0; 3.0; 4.2 and 6.0 mm total length) of the teleost fish *Xiphophorus helleri*. Moreover, the changes in the distribution of the extracellular matrix components during the migration of the neural crest cells and their differentiation are also considered.

The formation, segregation and migration of the neural crest cell. take place after complete formation of the neural keel in an antero-posterior direction. The migration of the neural crest cells at the head region occurred in three migratory pathways. The first pathway is ventral along the brain and notochord. The second pathway is lateral in the sub-ectodermal space. The third pathway, contralateral pathway, is across the dorsal midline of the fore-brain and mid-brain.

Non-sulfated glycosaminoglycans are the main extracellular matrix (ECM) components, that may facilitate the neural crest cell migration and invasion to form the corneal endothelium and some cartilages of the chondrocranium. Sulfated glycosaminoglycans may provoke the arrest of the crest cells to form the iris melanophores.

The cranial neural crest cells are aggregated at certain levels to form the presumptive proximal ganglionic primordia of 5th (trigeminal), 7th (facial) 9th, (glossopharyngeal) and 10th (vagus) cranial nerves. Neutral polysaccharides are seen to be the predominant matrix components that probably provoke the crest cell aggregation to form these structures and stromal fibroblasts.

INTRODUCTION

The neural crest first appears as a band of cells or a meshwork of synchronously pulsating cells. The formation and migration of the

neural crest cells are fundamental events during embryogenesis and controlled by roles of each component of the surrounding microenvironment

Newsome (1976), Fauquet *et al.* (1981), Le Douarin (1982) and Ziller *et al.* (1983) reported that the neural crest cells are pluripotential at the time of their migration. They may give rise to different phenotypes, including craniofacial skeletal and connective tissue, peripheral and sensory ganglia, adrenal medulla, calcitonin-producing cells, and glial and schwann cells as well as various types of pigmented cells, except those of the pigmented layer of the retina.

Johnston *et al.* (1979), Duband & Thiery (1982), Jacobson & Meier (1984) and Jaskoll *et al.* (1991) described the migratory behaviour of the cranial neural crest cells of Amphibia and Aves. They reported that the neural crest cells migrate mainly in lateral pathway found between the surface of the ectoderm and underlying mesenchyme, although small numbers of the neural crest cells migrate also ventrally between the brain and endomesenchyme.

Bolender *et al.* (1980); Hay (1980); Newgreen *et al.* (1982) and Tosney (1982) reported that glycosaminoglycans (GAGs) have been localized in the neural crest cell migration pathways of mouse and chick embryos at the cranial level. The embryonic glycosaminoglycans (GAGs) are primarily of the non-sulfated type (e.g. hyaluronic acid, the most common one) and sulfated type (e.g. chondroitin sulfate) and other types may occur.

MATERIAL AND METHODS

A. Source of material used in the present work :

The samples of the ovoviviparous cyprinodont fish, *Xiphophorus helleri* (swordtail) were obtained from local shops specific in the breeding of "Decoration fish" at Tanta City, El-Gharbia Governorate and identified according to Sterba and Habil (1973). Males and Females of this species were allowed to breed in dechlorinated water aquarium (90 x 30 x 50 cm) at 24-26°C areated by a suitable air pump. They were fed on an artificial diet (e.g. animal-based components). Early ontogenetic stages (0.7; 1.0; 1.5; 2.0; 3.0; 4.2 and 6.0 mm total length) were obtained from the abdominal cavity of the mothers after rapid dissection.

B. Preparation of serial sections :

The stages of swordtail fish embryos were fixed in different fixatives including carnoy, Bouin's solution and buffered formalin.

To the carnoy, 0.5% Cetyl Pyridinium Chloride (CPC) and 0.25% Polyvinyl Pyroblidone (PVP) were added to maximally preserve glycosaminoglycans (Concklin, 1963 and Derby, 1978).

Embryos were dehydrated, cleared and embedded in paraffin wax. Serial transverse sections (5-7m) of the embryos were prepared. Some of the sections were stained with haematoxylin and eosin for general histological examination. Other sections were stained with the following histochemical stain;

a. 1% Alcian blue (A.B) (pH 2.6) for detection of sulfated and non-sulfated glycosaminoglycans (Scott & Dorling, 1965).
b. 1% Alcian blue (A.B) (pH 1.0) for detection of sulfated glycosaminoglycans only (Culling, 1975).
c. 1% Alcian blue (A.B) (pH 2.6) followed by periodic acid Schiff for the detection of different carbohydrate classes (Culling, 1975).
d. Van Gieson's stain (Unna's variant) for detection of collagen (Culling, 1975).

A good series of transverse sections of each of the studied stages was chosen and photographed by using research microscope.

RESULTS

Stage I (0.7 mm total length) :

This is the earliest stage obtained from the abdominal cavity of the female during the breeding season. The larva has just escaped from the egg capsule and retained a large amount of yolk.

In the cephalic region of the swordtail fish embryo, Xiphophorus helleri, ridges of cells are visible on the dorso-lateral sides of the neural keel, which is initially formed and consists of a solid cord of cells (Fig. 1). These ridges of cells may represent the neural crest cells (Sadaghiani & Vielkind, 1989). The presumptive neural crest cells can be detected arising from the dorso-lateral sides of the neural keel and are segregated in a narrow cell-free space between the neural keel and optic bud (Fig. 1).

Stage II (1.0 mm total length) :

In this stage, the optic vesicle has evaginated from the brain at the levels of the fore-brain and mid-brain (Fig. 2). The ectoderm which is tightly opposed to the primary brain and optic vesicle, creates a dorsal furrow that will lead to a narrow space for the segregation of the neural crest cells (Fig. 2). The latter appear in this stage as masses of pleiomorphic and not oriented cells; while the

neural tube cells are columnar and oriented. On the other hand, the early migration of the neural crest is observed dorso-lateral to the brain and ventrally in a narrow space between the brain and optic vesicles (Fig. 2). The migration of the neural crest cells occurs below the level of the notochord (Fig. 2).

The optic region, shows non-sulfated glycosaminoglycans (GAGs) in the membranes surrounding the midbrain, optic vesicle and notochord (Fig. 3). Moreover, little glycosaminoglycans and collagen are observed also in the narrow space between the brain and optic vesicle (Fig. 3). However, the sulfated glycosaminoglycans show the same distribution in the membranes of the structures described above, although with less stain intensity (Fig. 4). However, it is nearly absents in the brain-optic vesicle space (Fig. 4). On the other hand, the neutral polysaccharides are absent in the basement membranes of the brain, optic vesicle and the interspace between these two membranes (Fig. 3). These results indicate that in the optic region, non-sulfated glycosaminoglycans are the main extracellular matrix components of the early migratory spaces of the neural crest cells and their lining membranes during the early appearance and segregation of the neural crest cells. On the other hand, the sulfated glycosaminoglycans have a slight representation to this process at the head level, while the neutral polysaccharides have no contribution at all.

Stage III (1.5 mm total length) :

The olfactory placode shows its first appearance in this stage. It is separated from the ectoderm as a compact mass of cells (Fig. 5). On the other hand, the optic cup has formed as a result of the invagination of the outer wall of the optic vesicle (Fig. 6).

Several masses and individual neural crest cells migrate in the space between the fore-brain and olfactory placode (Fig. 5). Moreover, they are closely associated with the membranes of the brain and the above mentioned sensory organs (Figs. 5 & 6).

The most important feature of the present stage is the elevation of the ectoderm over the dorsal sides of the brain, olfactory placodes and optic cups (Figs 5 & 7). Several individual crest cells are found dorso-lateral to the olfactory placode, optic cup, pre-otic, post-otic and brain (Figs. 5, 6 and 7). The first sign of the lateral (i.e. sub-ectodermal) migration of the neural crest cells in the cranial region is represented by the presence of the neural crest cells lateral to the olfactory placode (Fig. 5), and of the contralateral migration at

the dorsal midline of the fore-brain (Fig. 5) and the mid-brain (Fig. 6).

The ventrally migrating neural crest cells in the otic region are closely associated with the endoderm that will develop into the pharyngeal pouches (Fig. 7).

As in the previous stage, the non-sulfated glycosaminoglycans are the main matrix components that contribute to the increase of the migratory spaces of the neural crest cells.

Stage IV (2.0 mm total length) :

In this stage, the larva is well developed than previously and is still retaining a large amount of yolk. The mouth, gills and fins have not yet developed, but the tail is considerably elongated. The eyes are non-pigmented and can be seen easily on either sides. The olfactory placode is well developed (Fig. 8). The optic cup has detached from the brain to form the retina, and the lens placode became rounded and detached from the overlying ectoderm to form the lens (Fig. 9). Stream of individual loose and irregular neural crest cells, the mesectoderm cells, are migrating intensively in the brain-olfactory placode space (Fig. 8), and brain-eye space (Fig. 9). On the other hand, the close association of the otic vesicle and the hind-brain seems to prevent the migration of neural crest cells in between them in this stage (Fig. 10). The neural crest cells are called mesectoderm (Le Douarin, 1982) by this stage.

The first aggregation of the cranial neural crest cells, or mesectoderm, at this stage appear as a compact structure of densely packed neural crest cells, lying immediately ventro-lateral to the otic vesicle (Fig. 10). This position is occupied by the ganglion of the 7^{th} cranial nerve (facial nerve) in other vertebrates (Le Douarin, 1982), suggesting that the neural crest cells, or mesectoderm, may contribute to the formation of the proximal ganglionic primordium of the 7^{th} cranial nerve (Fig. 10).

Non-sulphated glycosaminoglycansm, intensely stained with Alcian-blue, are detected in the membranes of the brain, ectoderm and the periphery of the ganglion of the 7^{th} cranial nerve as well as on the surface of the endoderm that will develop into the pharyngeal pouches in the otic region (Fig. 11). On the other hand, the neutral polysaccharides are abundant in the matrix of the ganglion of the 7^{th} cranial nerve (Fig. 11).

However, the sulfated glycsoaminoglycans are nearly absent in the matrix of the proximal ganglionic primordium of the 7th cranial nerve (facial nerve) (Fig. 12).

It may be concluded from these results that the non-sulfated glycosaminoglycans probably play the major role in the increase of the ventral and lateral migration of the neural crest cells and their localization at the cephalic region, but a little role in their aggregation to form the ganglion of the 7th cranial nerve, comparable to the prominent role of the neutral polysaccharides. Also, the sulfated glycosaminoglycans have no contribution to these processes.

Stage V (3.0 mm total length) :

In this stage, the retina and lens began to differentiate, and the pigmented cells of retina have developed. The ventral migration of the neural crest cells, or mesectoderm, in the brain-eye space is drastically reduced than previously (Fig. 13). Some neural crest cells, or mesectoderm are, destined to form the corneal endothelium, and have started to migrate into the presumptive area of the primary corneal stroma (Fig. 13). It is to be noted that some neural crest cells, or mesectoderm, migrate and invade the undifferentiated retinal pigmented epithelium (Fig. 13). Thus, the neural crest cells, or mesectoderm, may contribute to the formation of the pigment layer of the retina.

It is of interest to note that numerous neural crest cells, or mesectoderm, are aggregated in this stage as compact, masses of cells at certain sites in the post-otic region (Fig. 14). These sites are occupied by the proximal ganglionic primordia of the 9th (glossopharyngeal), and 10th (vagus) cranial nerves among other vertebrates (Noden, 1975 & 1978; Narayanan & Narayanan, 1980), suggesting that the neural crest cells mesectoderm, may contribute to the formation of the proximal ganglia of these cranial nerves.

The optic region shows a network of fine filamentous strands surrounding the neural crest cells and the mesenchymal cells in the brain-eye interspace which are rich in neutral polysaccharides (Fig. 15) and sulfated glycosaminoglycans (Fig. 16).

It is concluded from these results that, at the optic region, the extracellular matrix contains predominantly sulfated glycosaminoglycans and neutral polysaccharides but no non-sulfated glycosaminoglycans, in consequence with the reduction of the neural crest cell, migration in the head region.

Collagen material is observed in the presumptive corneal region and the membrane of brain, but little in the filamentous strands

in the brain-eye space (Fig. 17). These results indicate that the collagen material, present in the corneal region, has an important role in the early migration of the neural crest cells into this region.

Stage VI (4.2 mm total length):

The present stage is characterized by the formation of the primary meninges (Fig. 18), inner and outer, which surround the brain. Some neural crest cells are seen interspersed in the inner and outer meninges as well as the ectoderm, suggesting a possible contribution of the neural crest cells to the formation of the meninges and melanocytes, respectively.

In this stage the neural crest cells are condensed on the surface of the pigmented epithelium of the retina (Fig. 19), where they contribute to the formation of the sclera. Other neural crest cells are aggregated as a compact, strongly packed mass of cells at the same region between the brain and the retina to form the ganglion of 5th cranial nerve.

It is observed that non-glycosaminoglycans and collagen materials present in the ectoderm, sub-ectodermal space and to a lesser extent, in both inner and outer meninges (Fig. 20, 21). Also, some neutral polysaccharides are observed in the region of the inner and outer meninges (Fig. 21). Thus, these results indicate that the ectoderm and meninges contain predominantly non-sulfated glycosaminoglycans and collagen materials and little neutral polysaccharides, suggesting a major role of these extracellular matrix (ECM) components in the neural crest cell invasion into the ectoderm and meninges, and their later differentiation into the ectodermal melanocytes and some parts of the meninges.

Stage VII (6.0 mm total length):

This larva is well developed and retains a small amount of yolk. The crest cells of this stage show several distributions as compared with the previous stages.

The neural crest cells have migrated to the corneal stroma, which is swelled in this stage, just beneath the corneal epithelium, where the cells probably form the corneal endothelium (Fig. 22). Moreover, other neural crest cells are accumulated approximately above the corneal endothelium. This position is occupied by the stromal fibroblasts in other vertebrates (Le Lievre & Le Douarin, 1975; Le Lievre, 1978 and Johnston *et al.*, 1979), suggesting that the stromal fibroblasts are derived from the neural crest cells in the swordtail fish. On the other hand, some neural crest cells are

interspersed in the iris mesenchyme (Fig. 22) and are observed invading some of the chondrocranial elements suggesting that they contribute to the formation of the iris melanophores and some cartilages of the chondrocranium.

The sections of the head region revealed that the non-sulfated glycosaminoglycans are intensively present in the iris mesenchyme and corneal epithelium, particularly in its basal lamina. Also, it is found in the matrix of some cartilages as trabecula communis, palatoquadrate, symplectic, basihyal and on their margins, as well as in association with the mesenchymal cells surrounding them (Fig. 24). Moreover, mixed amounts of non-sulfated glycosaminoglycans and neutral polysaccharides are present in association with the corneal endothelium (Fig. 23), but the neutral polysaccharides only are observed in the developing stromal fibroblasts.

The sulfated glycosaminoglycans are absent in the developing corneal stroma, where the neural crest cells migrate to form the corneal endothelium. However, some are distributed in the iris mesenchyme and the corneal epithelium (Fig. 25).

These results indicate that the non-sulfated glycosaminoglycans and neutral polysaccharides are the prominent extracellular matrix components during the formation of corneal endothelium. However, the neutral polysaccharides only may play the major role during the formation of the stromal fibroblasts, while the non-sulfated glycosaminoglycans seem to play an important role in the formation of the iris melanophores as they are intense in the iris mesenchyme. Also, both types of glycosaminoglycans are the main extracellular matrix components that contribute to the differentiation of the neural crest cells into certain skeletal elements. However, neutral polysaccharides do not seem to play such a role.

DISCUSSION

The study of the development of the neural crest cells of the cephalic region in the swordtail fish, *Xiphophorus helleri*, has shown that it more or less follows the general teleostean and other vertebrate pattern. However, it shows some features of special interest which have not been described before.

In the species studied, *Xiphophorus helleri*, the neural crest cells first appear after the formation of the neural keel and start their segregation as ridges of cells dorso-lateral to the neural keel, the brain and neural tube. This is also true for the lamprey (Langille & Hall,

1987), birds (Anderson & Meier, 1981; Le Douarin, 1982: Vincent & Thiery, 1984 and Bronner-Fraser, 1986) and mammals (Tan & Morriss-Kay, 1985 and Agamy, 1988). However, in Amphibia (Spieth & Keller, 1984 and Sadaghiani & Thiebaud, 1987) and some mammals (Tan & Morriss-Kay, 1985), the appearance of the neural crest cells and neural tube is in continuation with each other, where the crest cells first appear as three swellings, on each side, of the neural primordium or as a solid cord of cells dorsal to the neural tube.

In *Xiphophorus helleri* as in most vertebrates, e.g. amphibians (Jacobson & Meier, 1984 and Sadaghiani & Thiebaud, 1987), and mammals (Vermeij-Keer & Poelman, 1980; Nichols, 1981 and Tan & Morriss-Kay, 1985), the olfactory region is free from the crest cells at the early stages of development, where the crest cells migrate to the olfactory region (1.5 mm total length), later than those of the optic region (0.7mm total length) and the otic region (1.0 mm total length).

The neural crest cells of the swordtail fish *Xiphophorus helleri*, at the head level, migrate mainly through three well defined pathways; ventrally, laterally and contralaterally. The first pathway occurs along the brain and notochord at the head region (1.0 mm total length). The second pathway appears in the sub-ectodermal space in the head region (1.5 mm total length) but the third pathway takes place across the dorsal midline of the fore-brain, mid-brain (1.5 mm total length). In this respect, the swordtail fish, *Xiphophorus helleri* is similar to the neut (Jacobson & Meier, 1984), chick embryo (Le Douarin, 1982) and mouse (Johnston & Bunge, 1981 and Erickson & Weston, 1983). In some birds the cranial neural crest cells migrate laterally and contralaterally (Noden, 1975 and Duband & Thiery, 1982).

The increase of the extracellular matrix components, and non-sulfated glycosaminoglycans, is related to the segregation and migration of the crest cells, respectively in the head region. In this respect, *Xiphophorus helleri* is similar to the chick embryos (Solursh, 1976 and Derby, 1978), quail embryos (Pintar, 1978) and rat embryos (Morriss & Solursh, 1978 and Morriss-Kay *et al.*, 1986). Such increase is in agreement with the expansion of the cell-free spaces (ventral, lateral and contralateral pathways) created by the progressive development of the cephalic organ primordia. This is certainly supported by works on avian embryos (Pintar, 1978 and Bolender *et al.*, 1980) and rat (Erickson *et al.*, 1989), where the pathway selected by the crest cells of a particular region appears to be

predetermined by the availability of these spaces. In other studies on birds (Bilzour & Hay, 1988 and Perris *et al.*, 1991), the presence of little amount of non-sulfated glycosaminoglycans in early crest cell migratory spaces (pathways) may open the spaces for the migration of the crest cell.

In the swordtail fish *Xiphophorus helleri*, as in most vertebrates (Le Lievre, 1978; Johnston et al., 1979; Bagnara *et al.*, 1979 and Sadaghiani & Thiebaud, 1987), the neural crest cells probably form the presumptive connective tissues adjacent to the eye including the endothelial and stromal fibroblasts of the cornea. Also, they contribute to the formation of the sclera, pigmented layer of the retina and the iris melanophores. On the other hand, the increase of non-sulfated glycosaminoglycans and absence of collagen and sulfated glycosaminoglycans in the corneal stroma (i.e. below corneal epithelium) may facilitate the migration of the crest cells to the developing cornea to form the stromal fibroblasts and the corneal endothelium. The presence of some sulfated glycosaminoglycans in the matrix of iris probably cause the arrest of the invading crest cells and formation of the iris melanophores. These findings confirm and support those of Tucker (1986) in anuran amphibians, Toole & Trelstad (1971), Birk *et al.* (1986), Funderburgh *et al.* (1986) and Duband et al. (1986) in chick embryos, and Agamy (1988 & 1991) in mouse embryos.

In the swordtail fish, *Xiphophorus helleri*, as in some other vertebrates (Harvey *et al.*, 1933) and (Le Douarin, 1982), the crest cells contribute to the formation of the inner and outer meninges. Moreover, the matrix of the inner and outer meninges contains mainly non-sulfated glycosaminoglycans and collagen which probably facilitate the invasion of the crest cells into these structures (Toole & Trelstad, 1971 and Newgreen *et al.*, 1982). However, the presence of some sulfated glycosaminoglycans may reduce the number of invading crest cells into these structures (Tucker, 1986) and (Brauer *et al.*, 1985).

In the present study, the neural crest cells of *Xiphophorus helleri* contribute to the formation of certain sensory ganglia of the cranial nerves; several neural crest cells are aggregated as compact structures of densely-packed cells which form the proximal ganglionic primordia of the 5th (trigeminal nerve), 7th (facial nerve), 9th (glossopharyngeal) and 10th (vagus) cranial nerves as is the case in the chick (Hamburger, 1961; Noden, 1975 & 1978; Narayanan & Narayanan, 1980 and Le Douarin, 1982).

In the swordtail fish *Xiphophorus helleri,* the extracellular matrix components of neutral polysaccharides, show great contribution to the aggregation of the crest cells that form the proximal ganglia of the cranial nerves and the crest-derived stromal fibroblasts as well as the corneal endothelium. However, glycosaminoglycans have no contribution to the matrix of the cranial nerve ganglia. Similar results were obtained in anuran amphibians (Tucker, 1986), birds (Duband *et al.,* 1986 and Perris *et al.,* 1991) and mammals (Erickson & Weston, 1983 and Sternberg & Kimber, 1986).

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LIST OF FIGURES

Fig. (1) Photomicrograph of a transverse section through the optic region of stage 0.7 mm length of Xiphophorus helleri showing the arising of the neural crest cells (N.C.C) from the dorso-lateral sides of the neural keel (N.K) and their segregation in the narrow neural keel-optic bud (OP. B) space.
Hx & E
x 1000

Fig. (2) Photomicrograph of a transverse section through the optic region of stage 1.0 mm length of Xiphophorus helleri showing the segregation of the neural crest cells (N.C.C) from the dorso-lateral sides of the fore-brain (F.B), and their early migration in the space between the fore-brain, optic vesicle (OP. V) and below the level of the notochord (N).
Hx & E
x 1000

Fig. (3) Photomicrograph of a transverse section through the optic region of stage 1.0 mm length of Xiphophorus helleri showing the presence of non-sulfated glycosaminoglycans (▶▶) in the membranes surrounding mid-brain (M.B), optic vesicle (OP. V) and notochord (N). Little amount is seen in the brain-optic vesicle space.
A.B (pH 2.6)/P.A.S.
x 1000

Fig. (4) Photomicrograph of a transverse section through the optic region of stage 1.0 mm length of Xiphophorus helleri showing less stain intensity of sulfated glycosamnoglycans (▶) in the membranes of the mid-brain (M.B), optic vesicle (OP. V). They are absent in the brain-optic vesicle space.
A.B. (pH 1.0)
x 1000

Fig. (5) Photomicrograph of a transverse section through the olfactory region of stage 1.5 mm length of Xiphophorus helleri showing several masses and individual neural crest cells (N.C.C) migrating in the space between the fore-brain (F.B) and olfactory placode (OL.P) and dorso-lateral to the olfactory placode. Some cells are present at the dorsal midline of the fore-brain (F.B).

Hx & E
x 1000

Fig. (6) Photomicrograph of a transverse section through the optic region of stage 1.5 mm length of Xiphophorus helleri showing some neural crest cells migrating in the space between the mid-brain (M.B) and optic cup (OP. C). The ectoderm (EC) is elevated over the dorsal side of the mid-brain and optic cup. Leaving a space for the contralateral subectodermal migrations of flat neural crest cells (N.C.C).
Hx & E
x 1000

Fig. (7) Photomicrograph of a transverse section through the pre-otic region of stage 1.5 mm length of Xiphophorus helleri showing some neural crest cells (N.C.C) migrating rostrally to the otic vesicle (i.e. in the pre-otic (PR.OT) region). They migrate on either sides of the hind-brain (H.B).
Hx & E
x 1000

Fig. (8) Photomicrograph of a transverse section through the optic region of stage 1.5 mm length of Xiphophorus helleri showing intense non-sulfated glycosaminoglycans (▶▶) in the space between the brain (BR) and optic cup (OP. C) as well as in the membranes of the brain, optic cup. It is also present in the sub-ectodermal space (S.S).
A.B. (pH 2.6)/P.A.S.
x 1000

Fig. (9) Photomicrograph of a transverse section through the pre-otic region of stage 1.5 mm length of Xiphophorus helleri showing intense non-sulfated glycosaminoglycans (▶▶) on the surface of the placodal thickening (P.T) and in the membranes of the brain (BR), as well as between the brain and placodal thickening.
A.B. (pH 2.6)
x 1000

Fig. (10) Photomicrograph of a transverse section through the otic region of stage 2.0 mm length of Xiphophorus helleri showing the close association of the otic vesicle (OT.V) and the hind-brain (H.B). Numerous neural crest cells, mesectoderm cells, (MES) are aggregated as densely

packed cells to form the proximal ganglionic primordium of the 7th cranial nerve (G.VII).
Hx & E
x 1000

Fig. (11) Photomicrograph of a transverse section through the pre-otic region of stage 2.0 mm length of Xiphophorus helleri showing intense non-sulfated glycosaminoglycans (▶▶) in the membranes of the brain (BR), endoderm (EN) and the periphery of the ganglion of the 7th cranial nerve. Intense neutral polysaccharides (>⇒) are detected in the matrix of this cranial ganglion.
A.B. (pH 2.6)/P.A.S
x 1000

Fig. (12) Photomicrograph of a transverse section through the pre-otic region of stage 2.0 mm length of Xiphophorus helleri showing the absence of sulfated glycosaminoglycans in the matrix of the 7th cranial nerve (G.VII), and on the surface of the endoderm that forms the pharyngeal pouches (EN).
A.B. (pH 1.0)
x 1000

Fig. (13) Photomicrograph of a transverse section through the optic region of stage 3.0 mm length of Xiphophorus helleri showing the migration of the neural crest cells, mesectoderm, (MES) in the brain-eye space and sub-ectodermal space, and primary corneal stroma (C.S), as well as their invasion into the undifferentiated retinal pigmented epithelium (U.R.P.E).
Hx & E
x 400

Fig. (14) Photomicrograph of a transverse section through the post-otic region of stage 3.0 mm length of Xiphophorus helleri showing the aggregation of the neural crest cells, mesectoderm (MES) as compact, strongly-packed masses of cells to form the presumptive proximal ganglia of the 9th (G. IX) and 10th (G.X) cranial nerves.
Hx & E
x 1000

Fig. (15) Photomicrograph of a transverse section through the optic region of 3.0 mm length of Xiphophorus helleri showing intense neutral polysaccharides (>⇒) in the network of

fine filamentous strands (F.F.S) in the brain-eye space where the migration of the neural crest cells, mesectoderm, is arrested.
A.B. (pH 2.6)/P.A.S.
x 1000

Fig. (16) Photomicrograph of a transverse section through the optic region of 3.0 mm length of Xiphophorus helleri showing intense sulfated glycosaminoglycans (▶) in the network of fine filamentous strands (F.F.S) in the brain-eye space where the migration of the crest cell, mesectoderm, is arrested.
A.B. (pH 1.0)
x 1000

Fig. (17) Photomicrograph of a transverse section through the optic region of 3.0 mm length of Xiphophorus helleri showing little collagen material (—▶) in the network of fine filamentous strands (F.F.S) of the brain-eye space where the migration of the neural crest cells, mesectoderm, is arrested. Collagen material present in the presumptive corneal stroma (C.S).
Van Gieson's stain
x 400

Fig. (18) Photomicrograph of a transverse section through the optic region of stage 4.2 mm length of Xiphophorus helleri showing the brain which is surrounded by the inner (I.M) and outer (O.M) meninges. Some neural crest cells, mesectoderm, (MES) are interspersed in the inner and outer meninges as well as in the ectoderm (EC).
Hx & E
x 400

Fig. (19) Photomicrograph of a transverse section through the optic region of stage 4.2 mm length of Xiphophorus helleri showing the neural crest cells, mesectoderm, (MES) condensed on the surface of the pigmented epithelium of the retina (R.P.E) to form the presumptive sclera (SC). Other crest cells are condensed together to form the proximal ganglion of the 5th cranial nerve (G.V).
Hx & E
x 1000

Fig. (20) Photomicrograph of a transverse section through the head region of 4.2 mm length of Xiphophorus helleri showing non-sulfated glycosaminoglycans (▶▶) in the ectoderm (EC), sub-ectodermal space (S. S), and inner (I. M) and outer meninges (O.M). Little neutral polysaccharides (⇒) are seen in the region of inner and outer meninges.
A.B (pH 2.6)/P.A.S
x 1000

Fig. (21) Photomicrograph of a transverse section through the head region of 4.2 mm length of Xiphophorus helleri showing the collagen material (→) in the ectoderm (EC) and in both the inner (I.M) and outer meninges (O.M).
Van Gieson's
x 1000

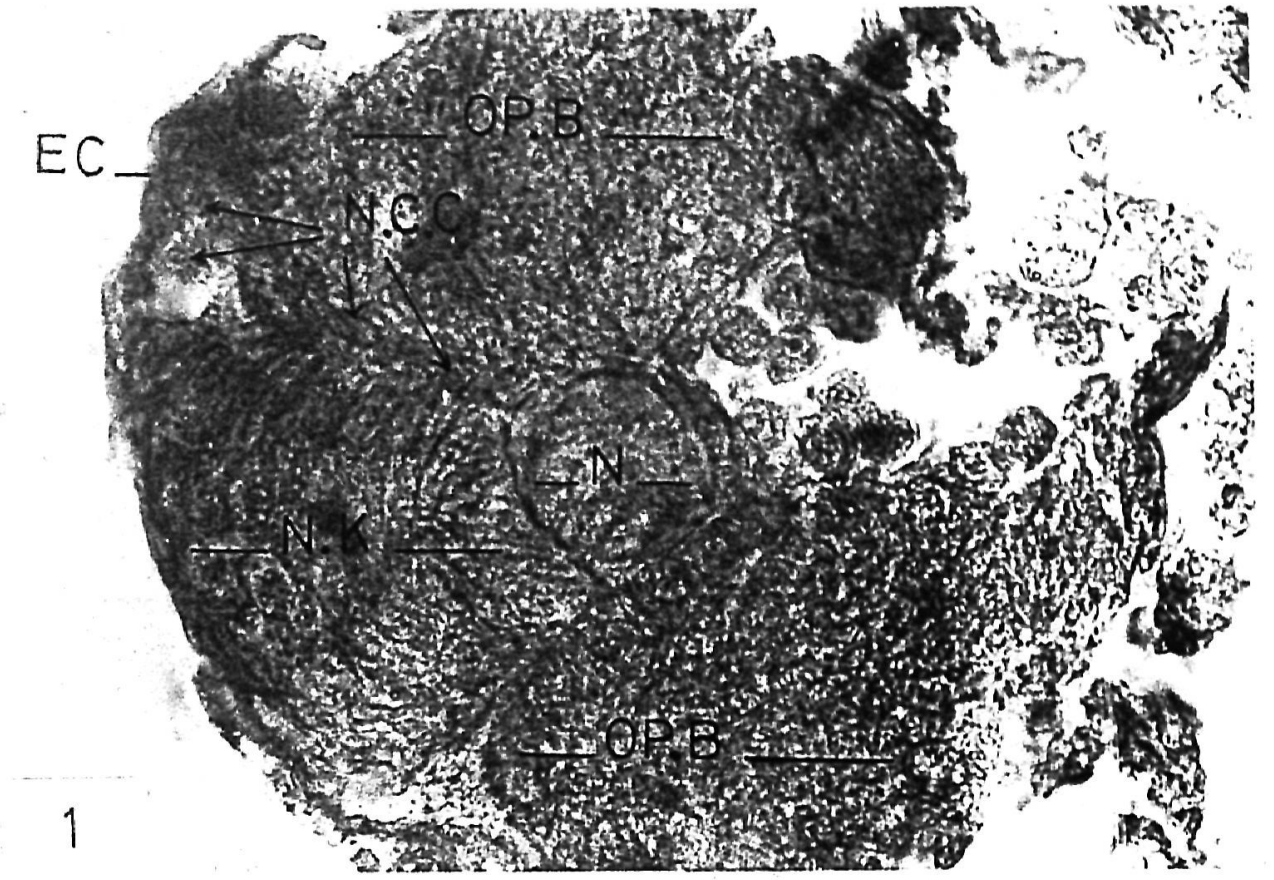
Fig. (22) Photomicrograph of a transverse section through the optic region of stage 6.0 mm length of Xiphophorus helleri showing the neural crest cells, mesectoderm, (MES) migrating beneath the corneal epithelium (C.EPI) to form the presumptive corneal endothelium (C.EN). Other crest cells are accumulated to form the presumptive stromal fibroblasts (S.F), while some crest cells are interspersed in the iris mesenchyme (IR.MS) to form the presumptive iris melanophores.
Hx & E
x 400

Fig. (23) Photomicrograph of a transverse section through the optic region of stage 6.0 mm length of Xiphophorus helleri showing the non-sulfated glycosaminoglycans (▶▶) in the iris mesenchyme (IR.MS), corneal epithelium (C. EPI) and its basal lamina (B.L). Neutral polysaccharides (⇒) present in the stromal fibroblasts (S.F). The two mentioned extracellular matrix components are found in the corneal endothelium (C.E.N).
A.B. (pH 2.6)/P.A.S
x 1000

Fig. (24) Photomicrograph of a transverse section through the optic region of stage 6.0 mm length of Xiphophorus helleri showing the non-sulfated glycosaminoglycans (▶▶) in the matrix and on the margins of the trabecula communis

(T.C), palatoquadrate (PQ), and symplectic (SY) as well as basihyal (BH). It is also present in the mesenchymal cells that surround these cartilages.
A.B (pH 2.6)/ P.A.S
x 1000

Fig. (25) Photomicrograph of a transverse section through the optic region of stage 6.00 mm length of Xiphophorus helleri showing some sulfated glycosaminoglycans (▶) in the corneal epithelium (C.EPI) and iris mesenchyme (IR.MS). The corneal stroma (C.S), is free of it.
A.B (pH 1.0)
x 1000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N
N.K
OP.B
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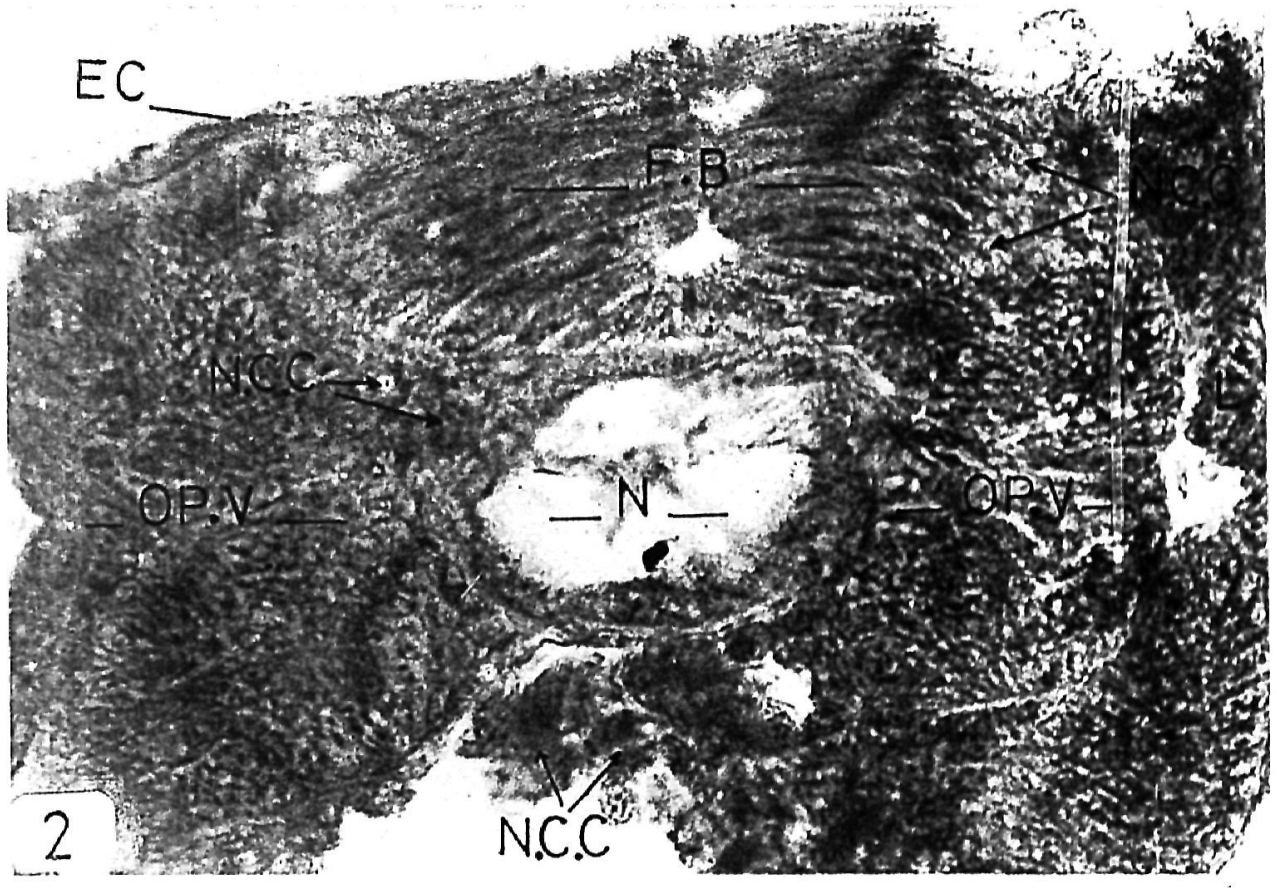
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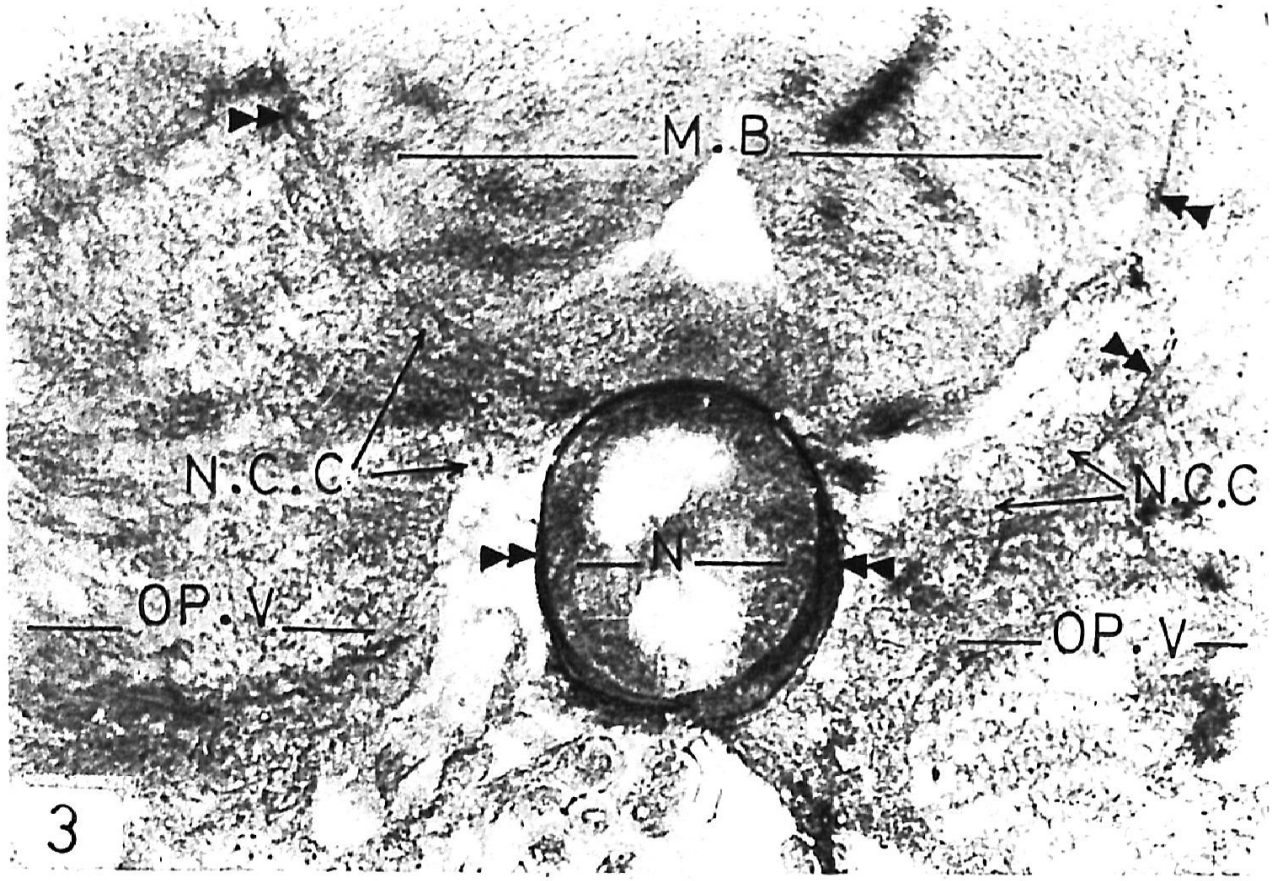
OP.V
N.C.C
2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OP.V
OP.V
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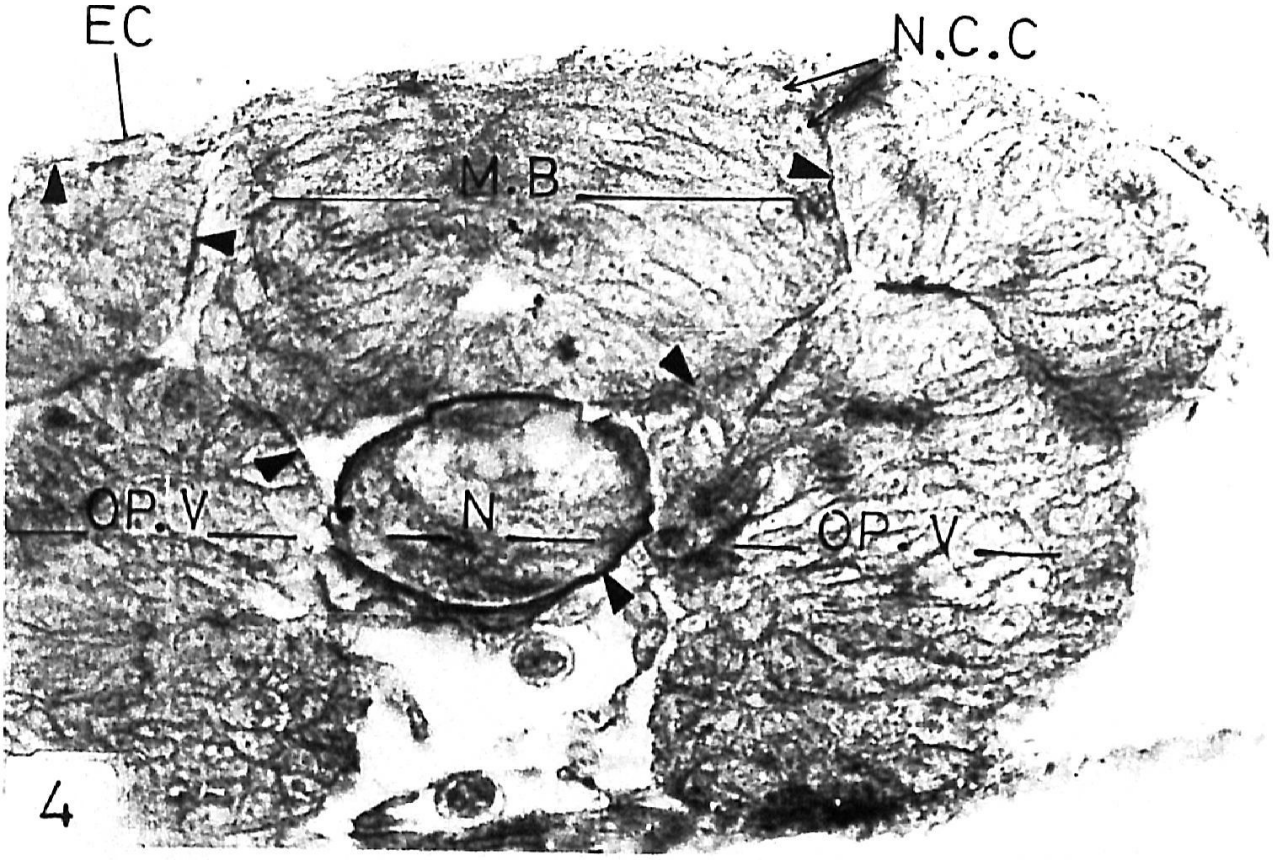
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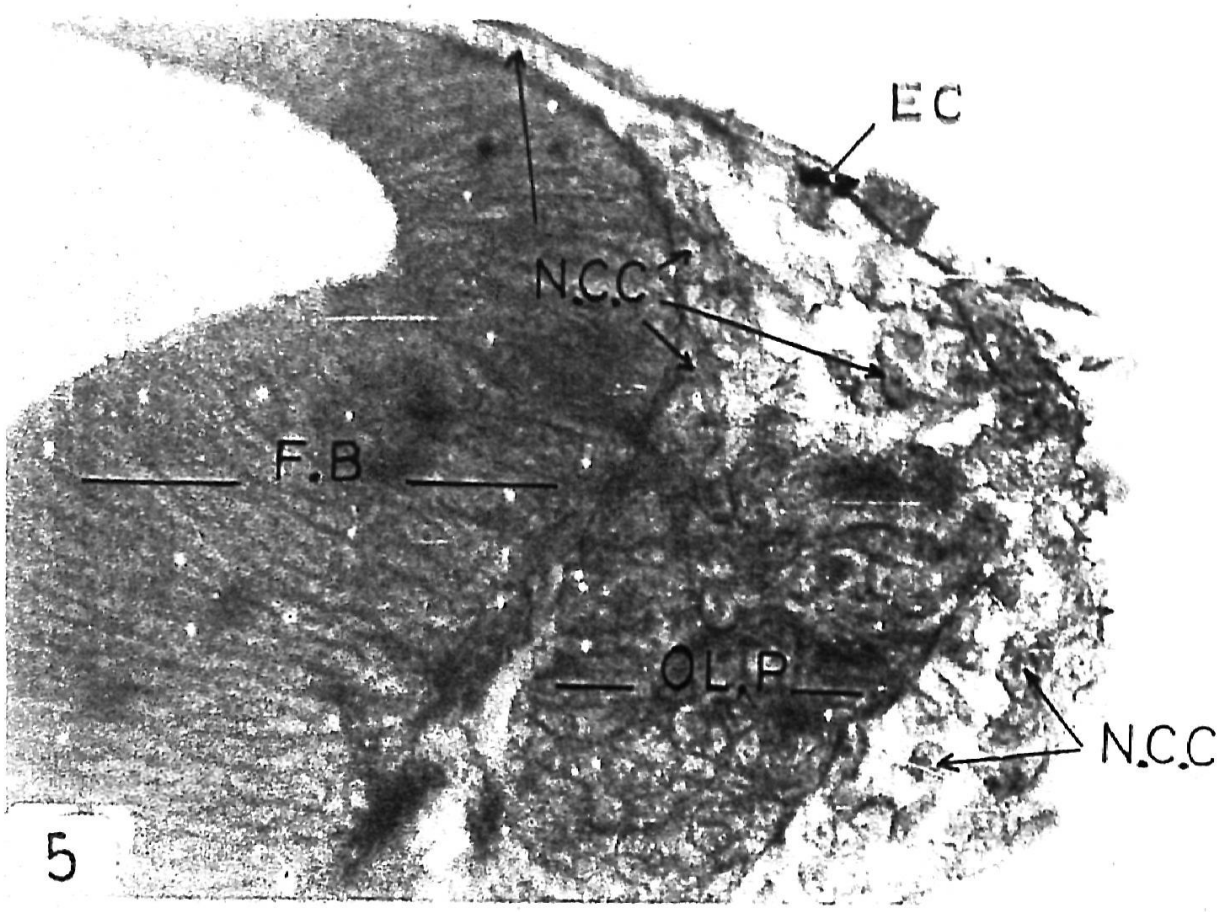

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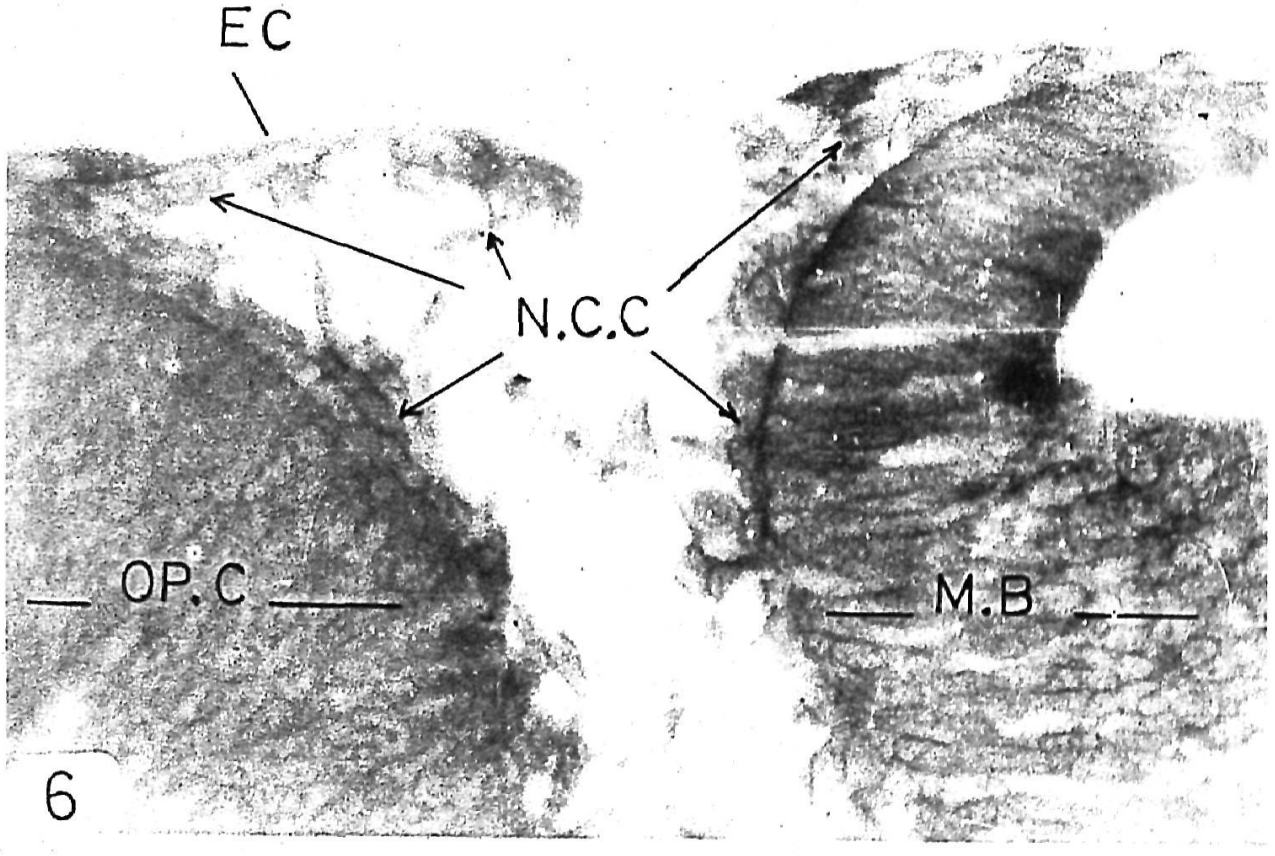

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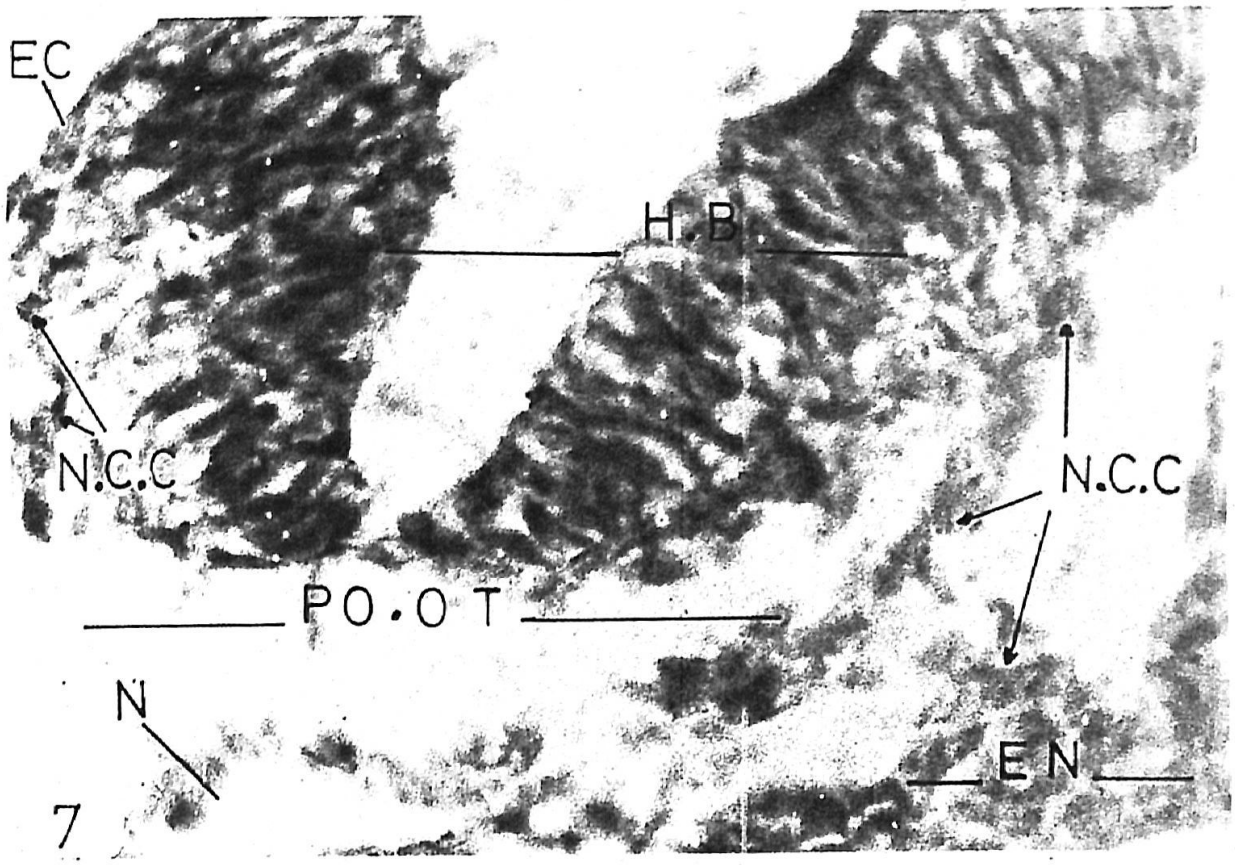

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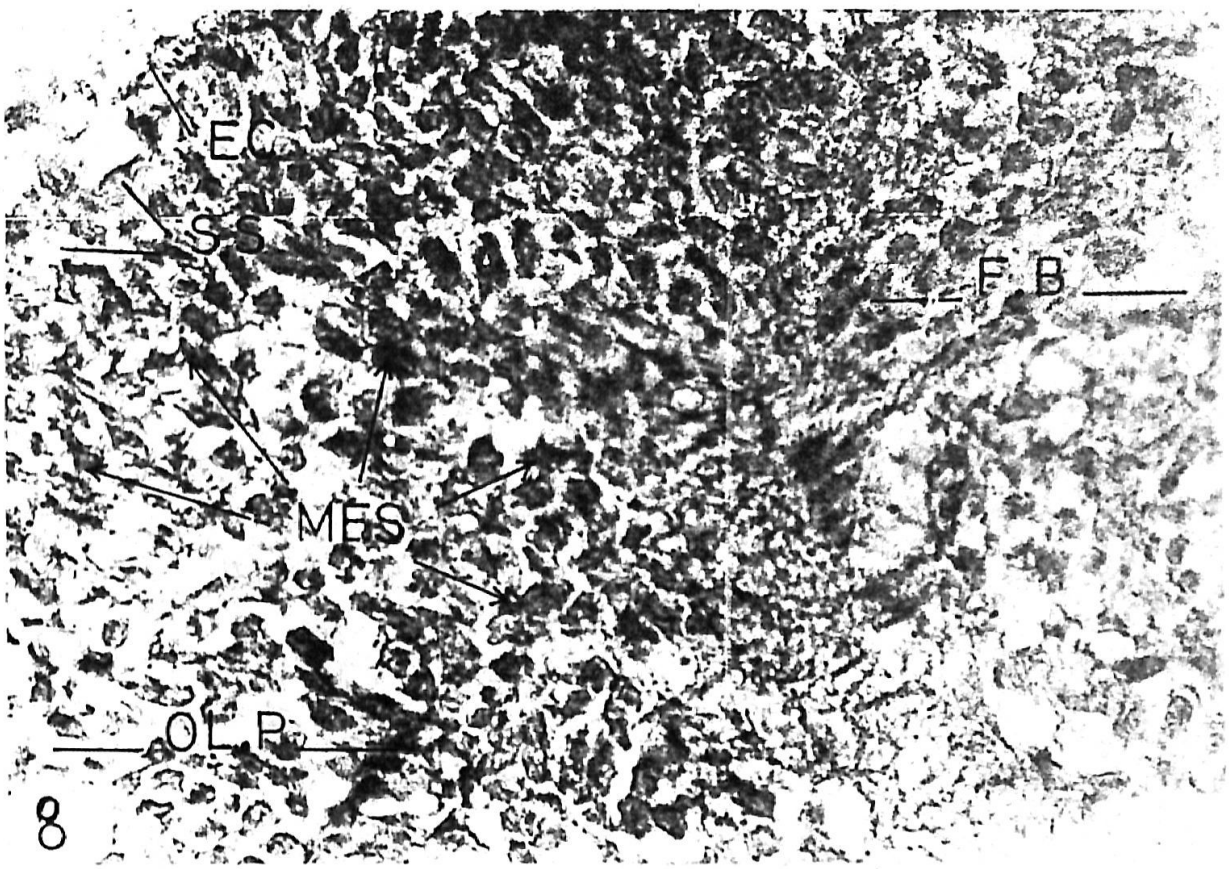

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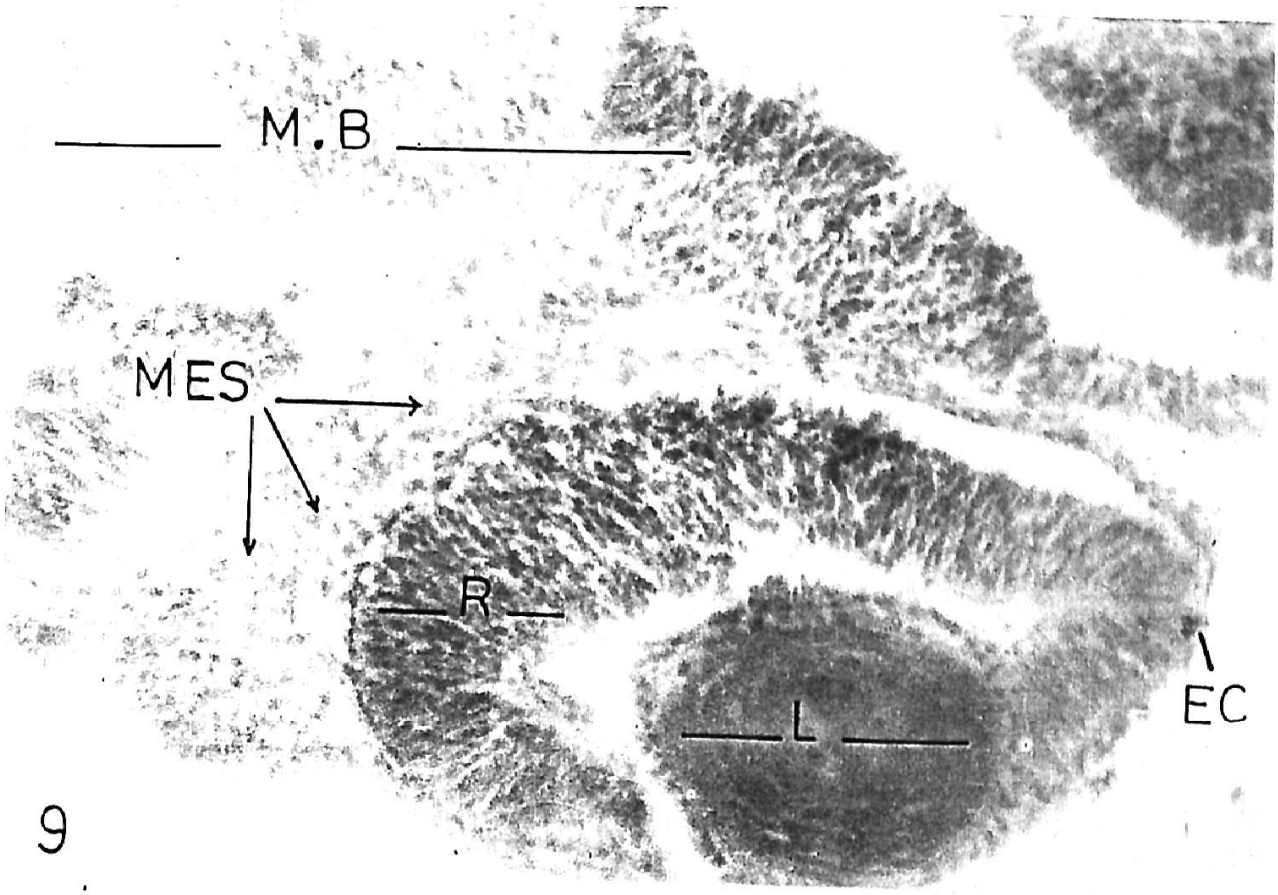

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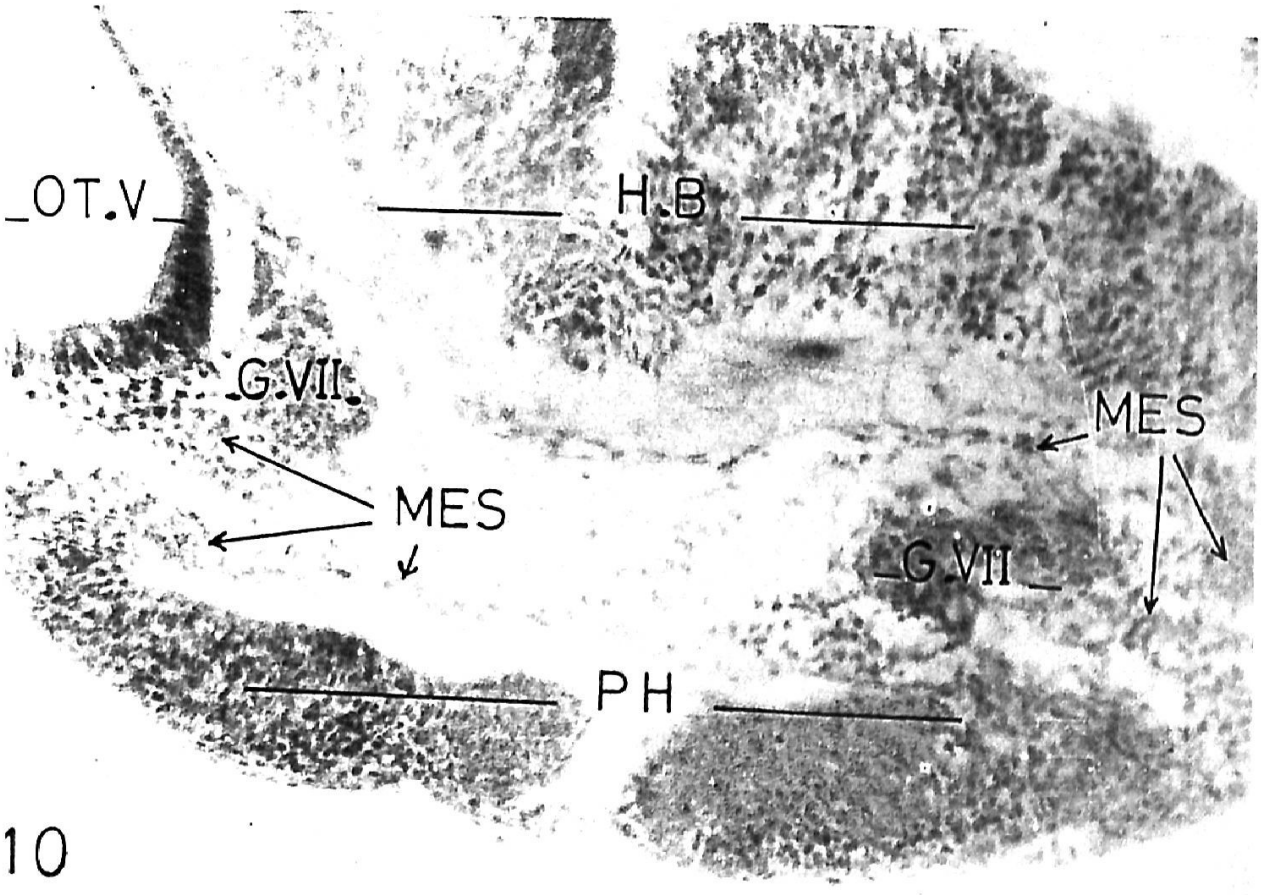

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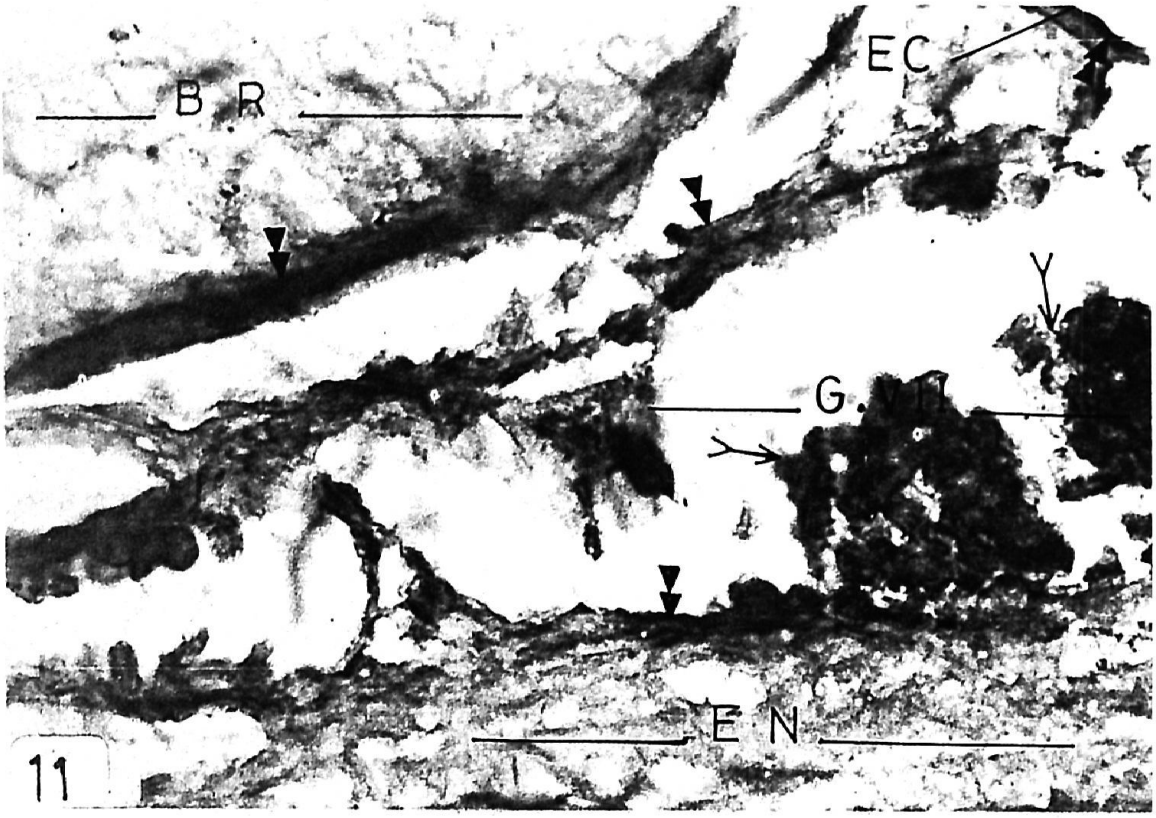

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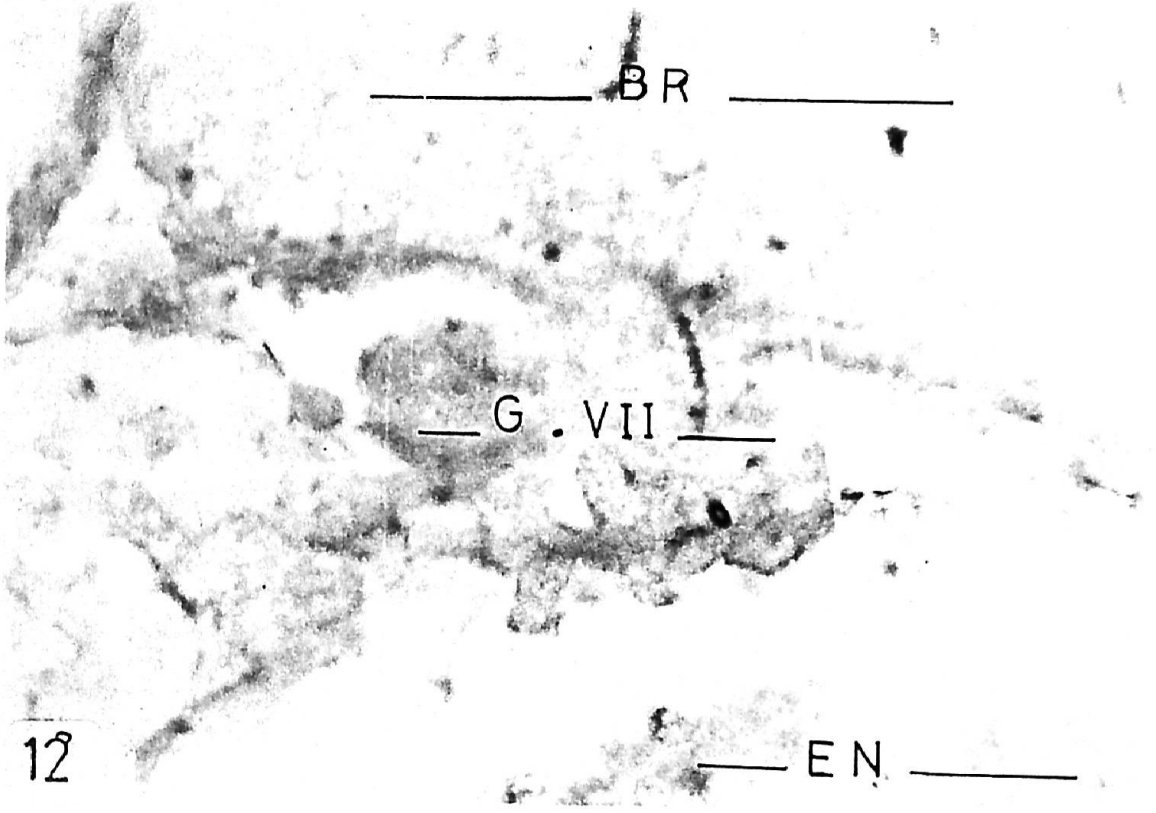

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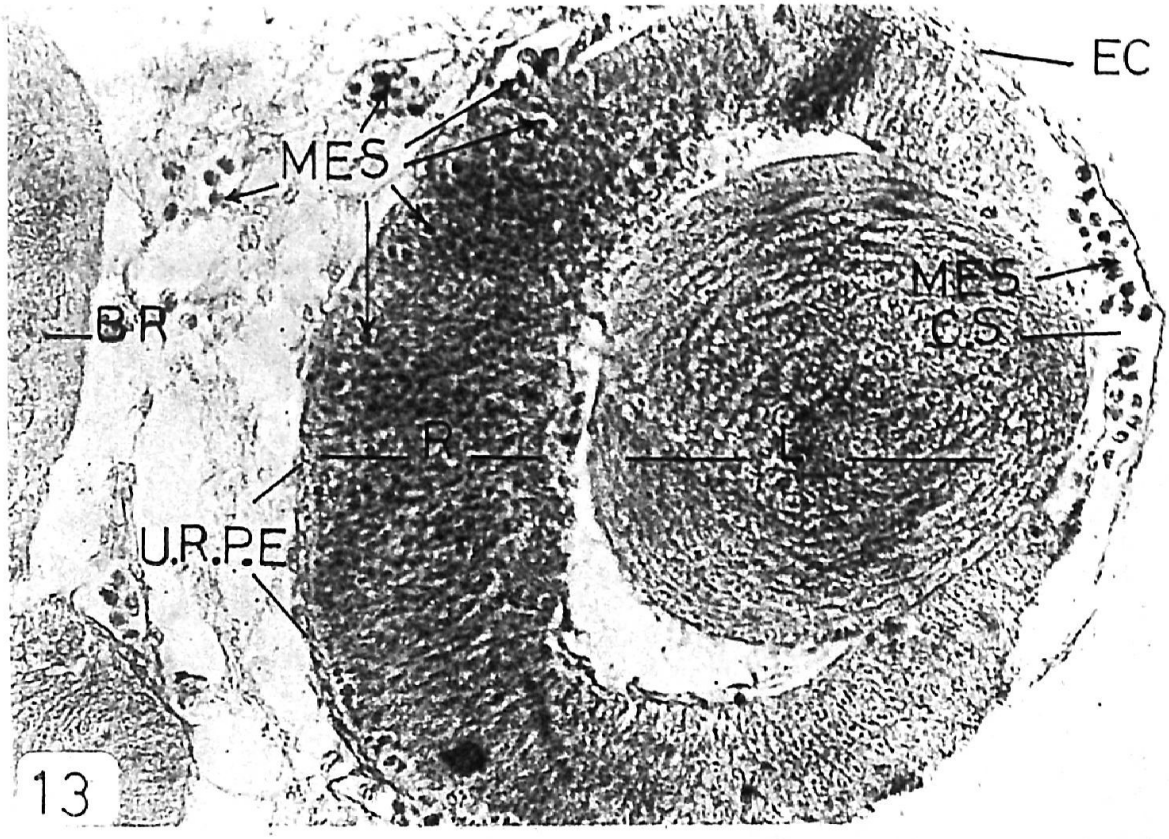

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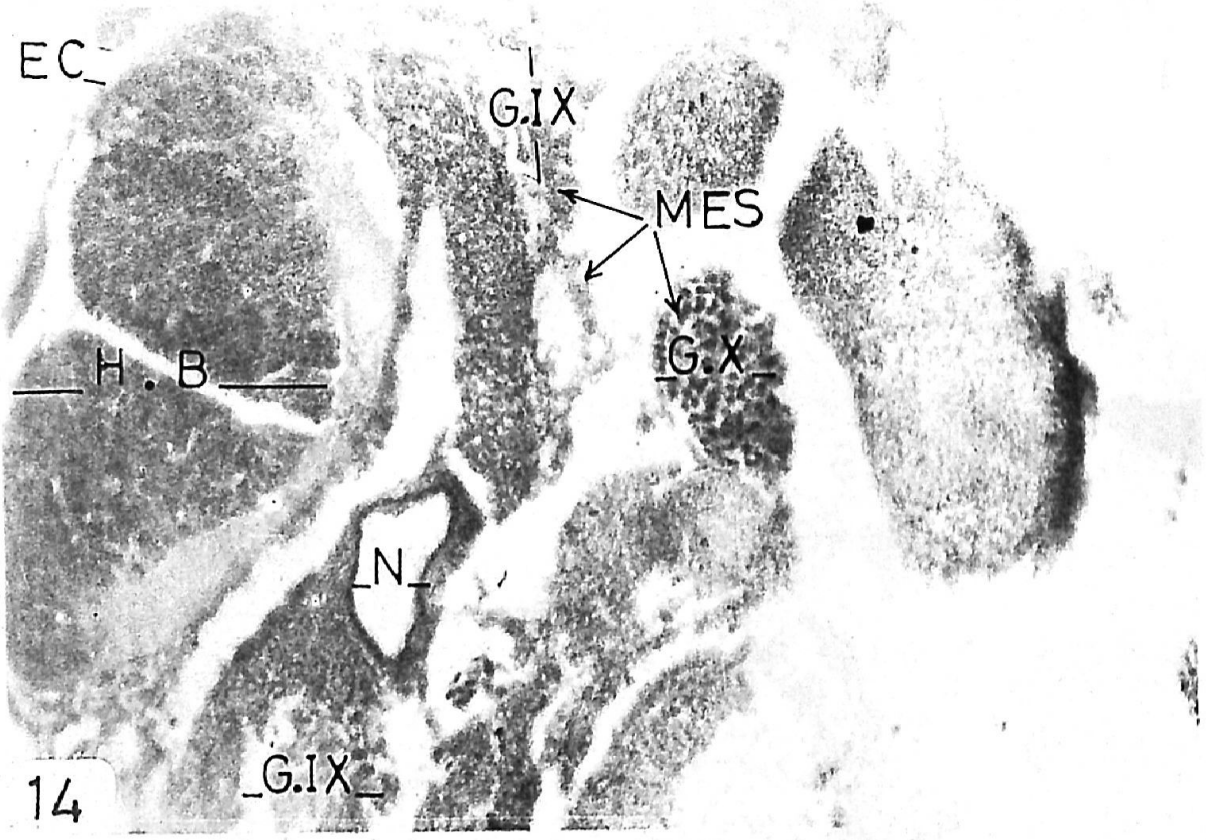

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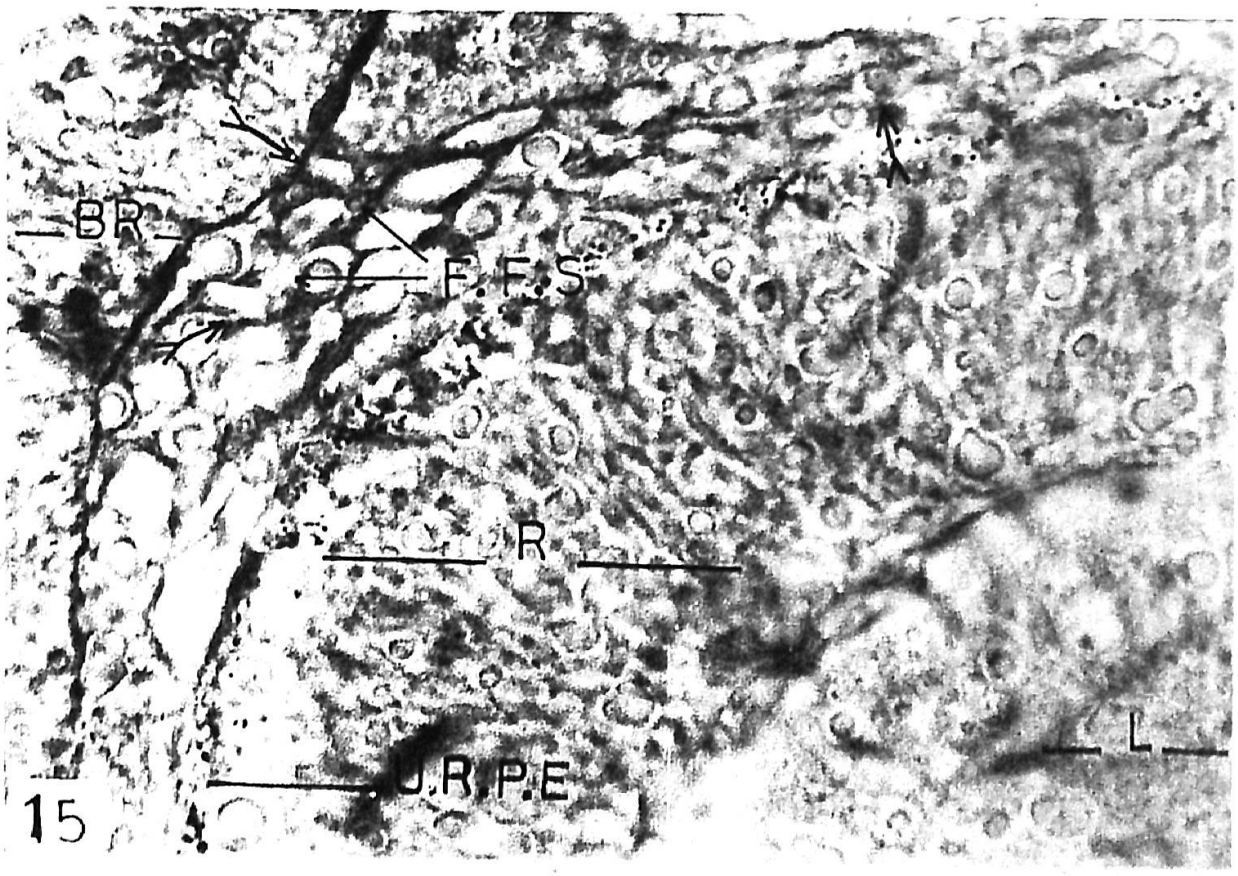

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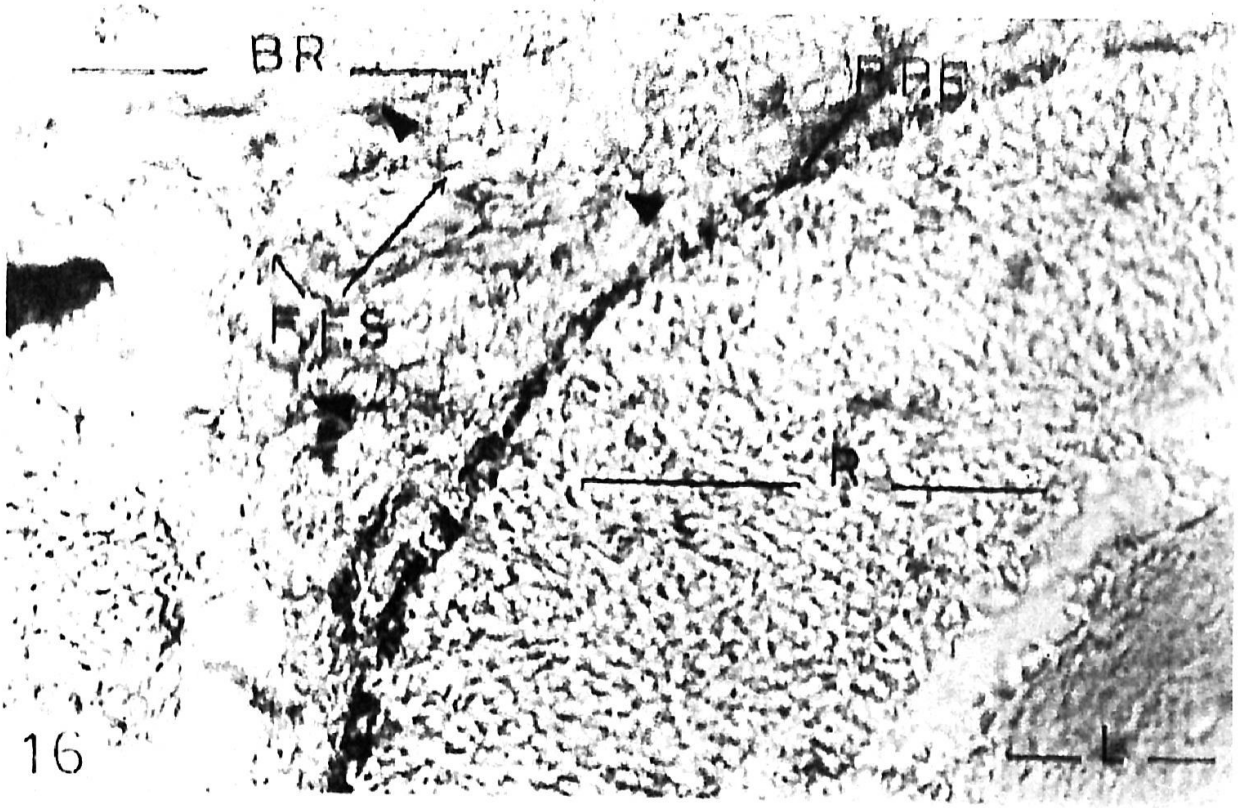

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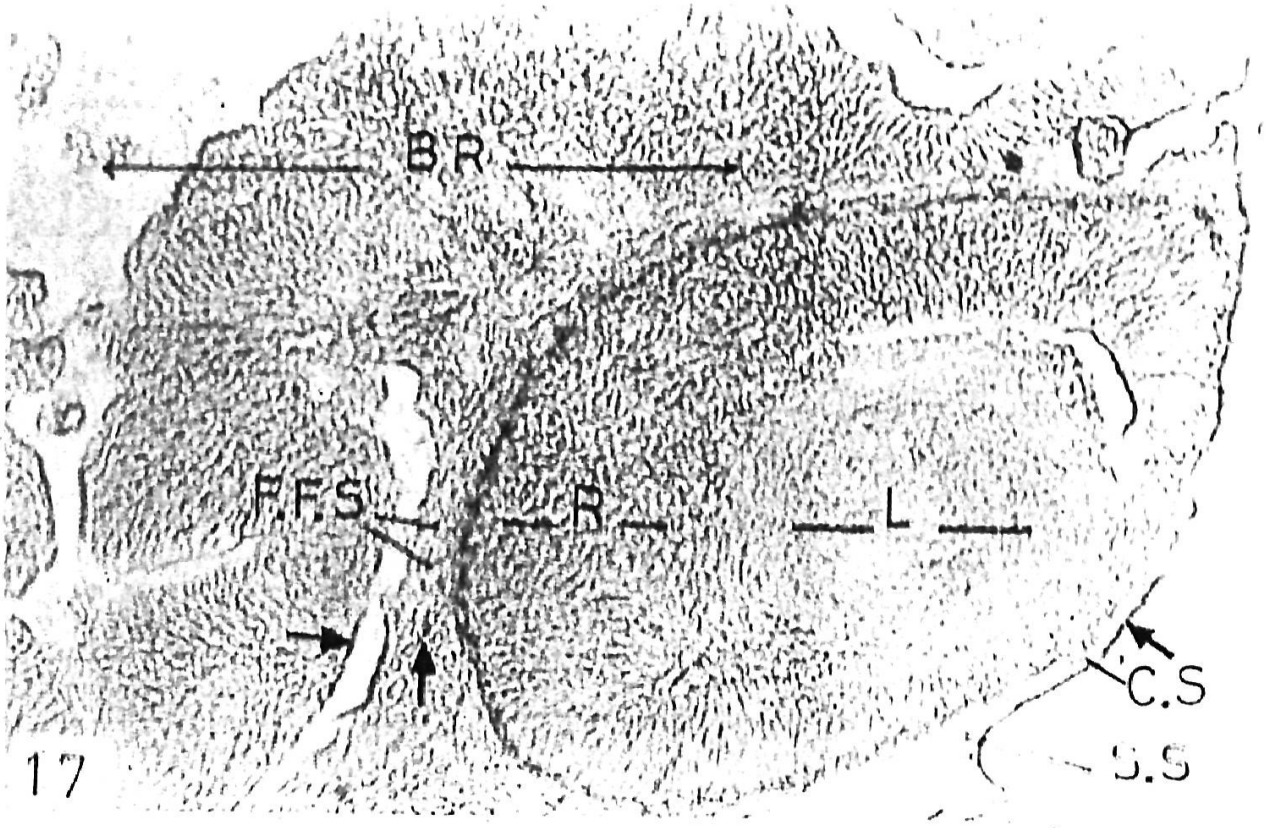

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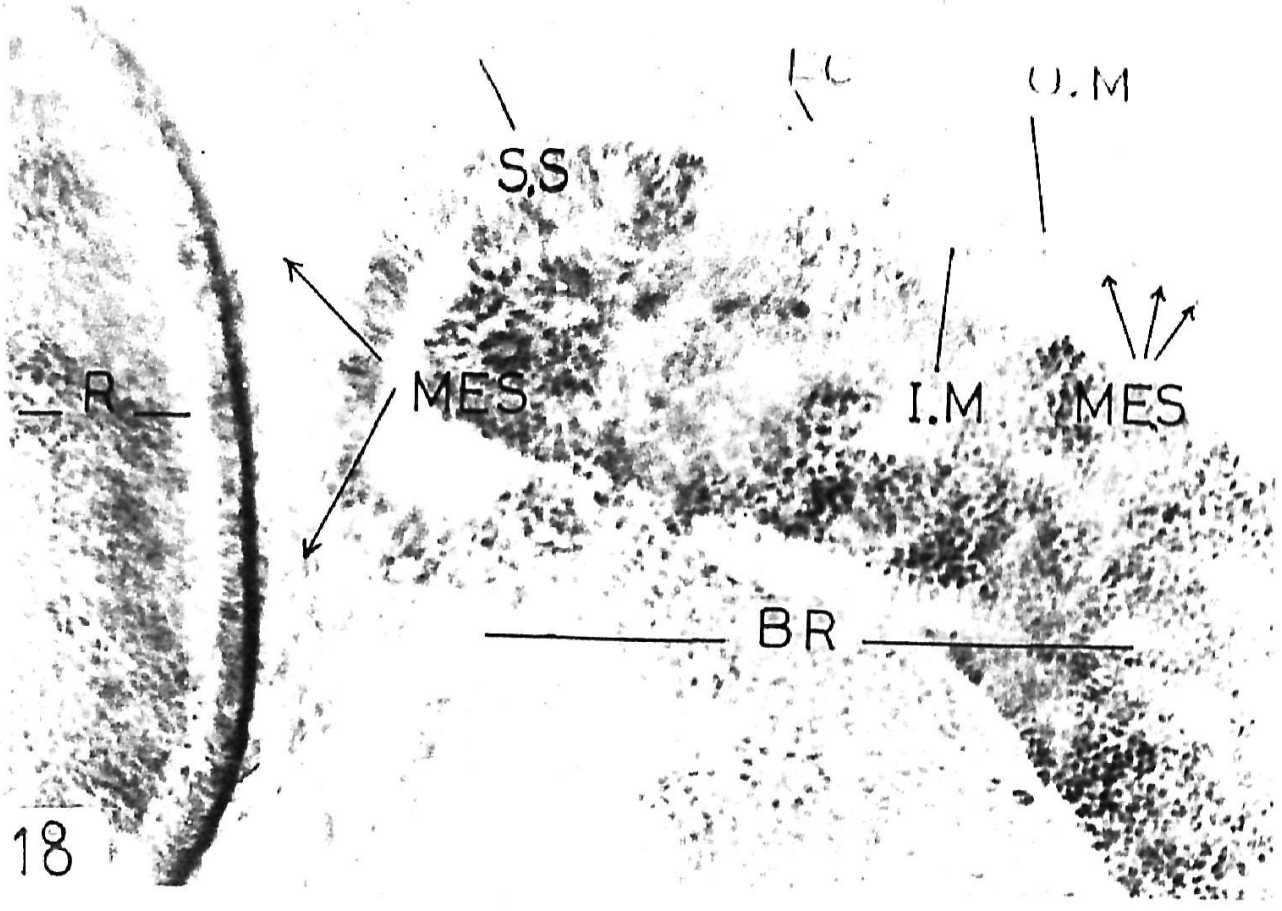

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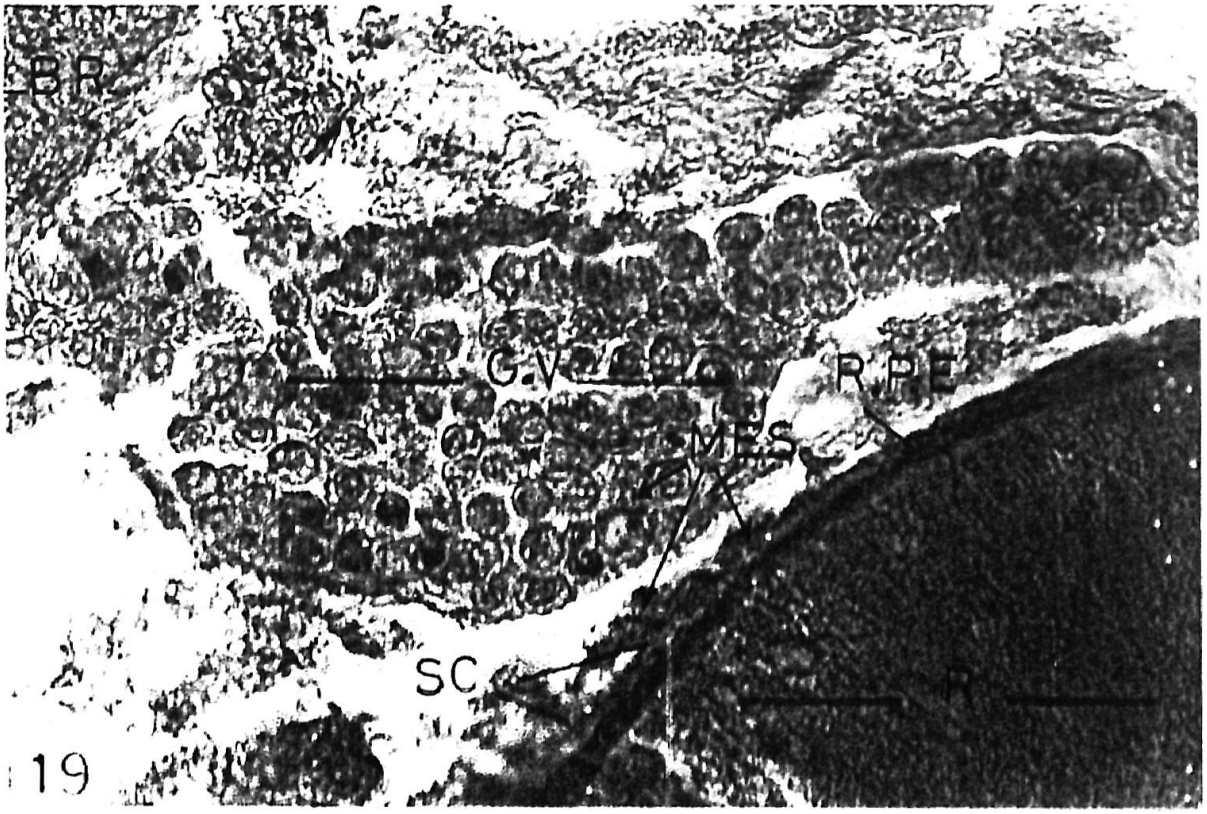

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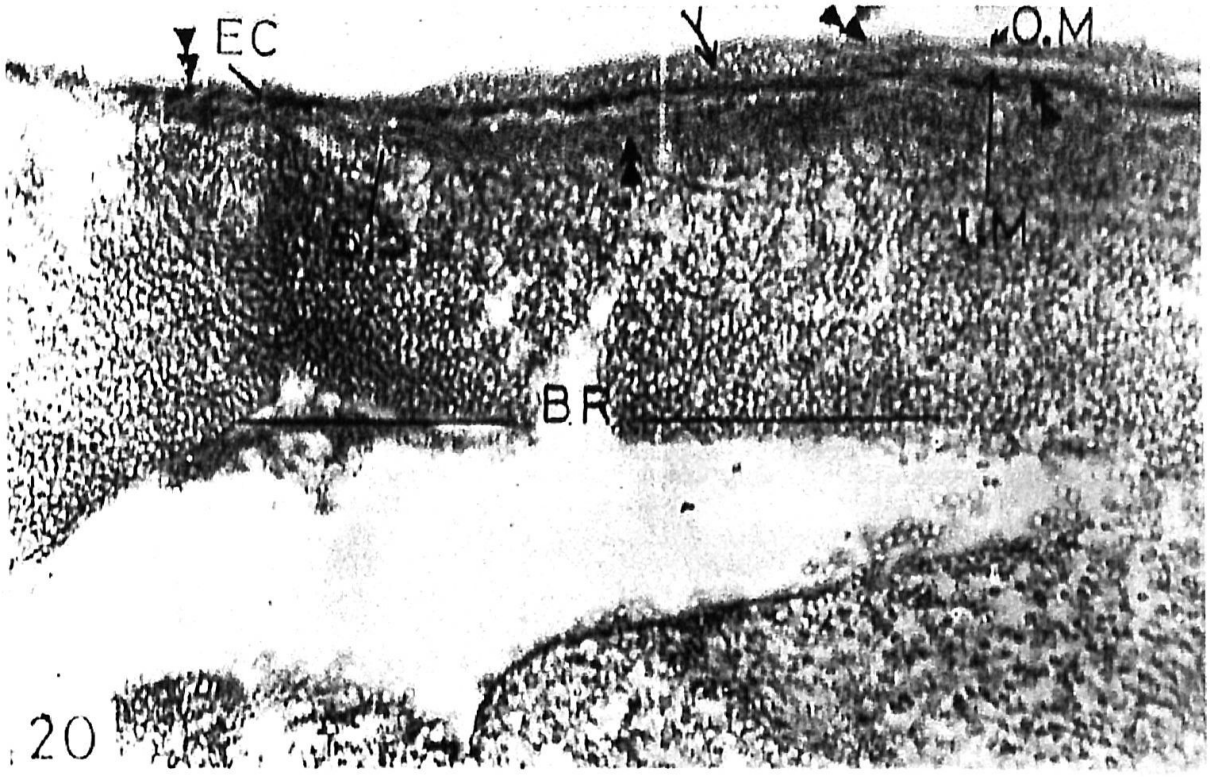

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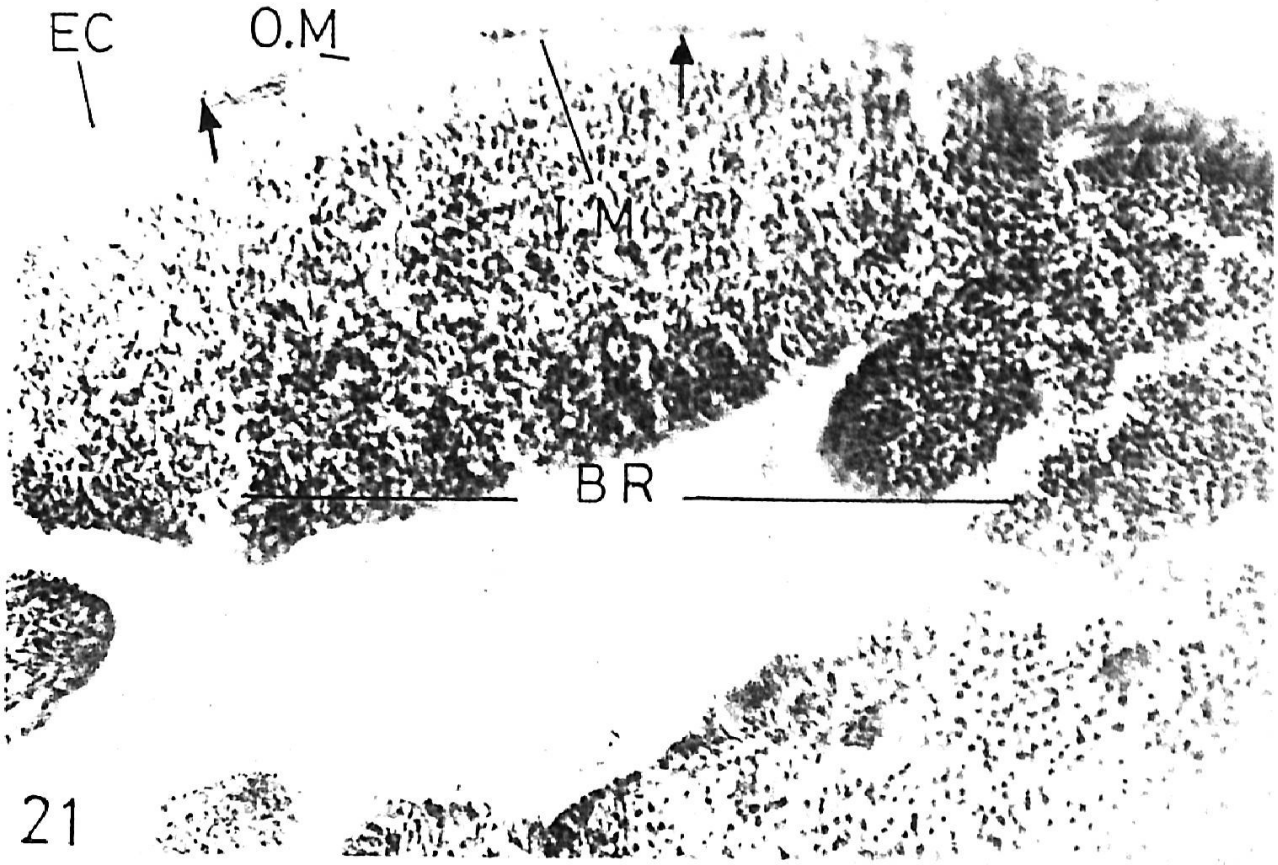

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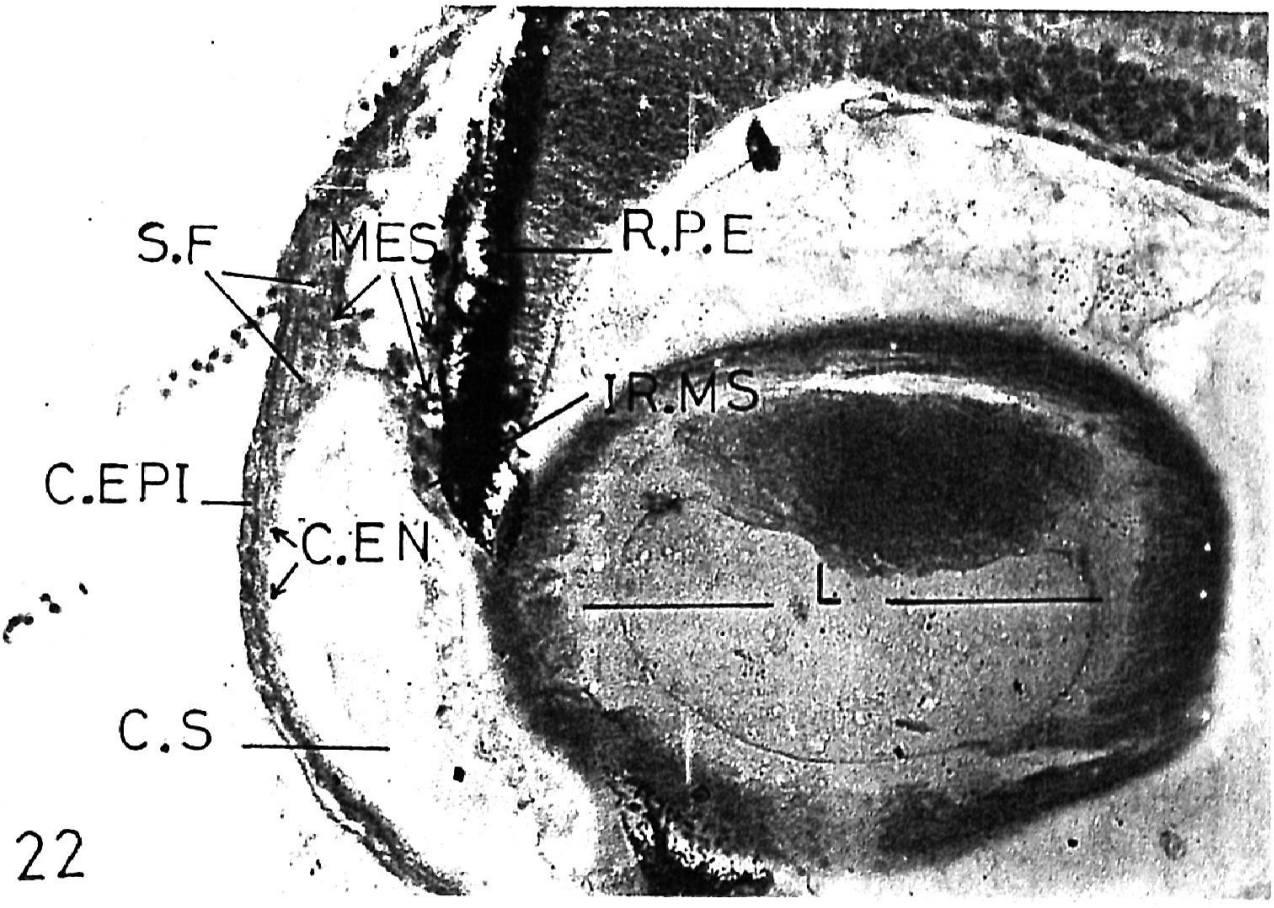

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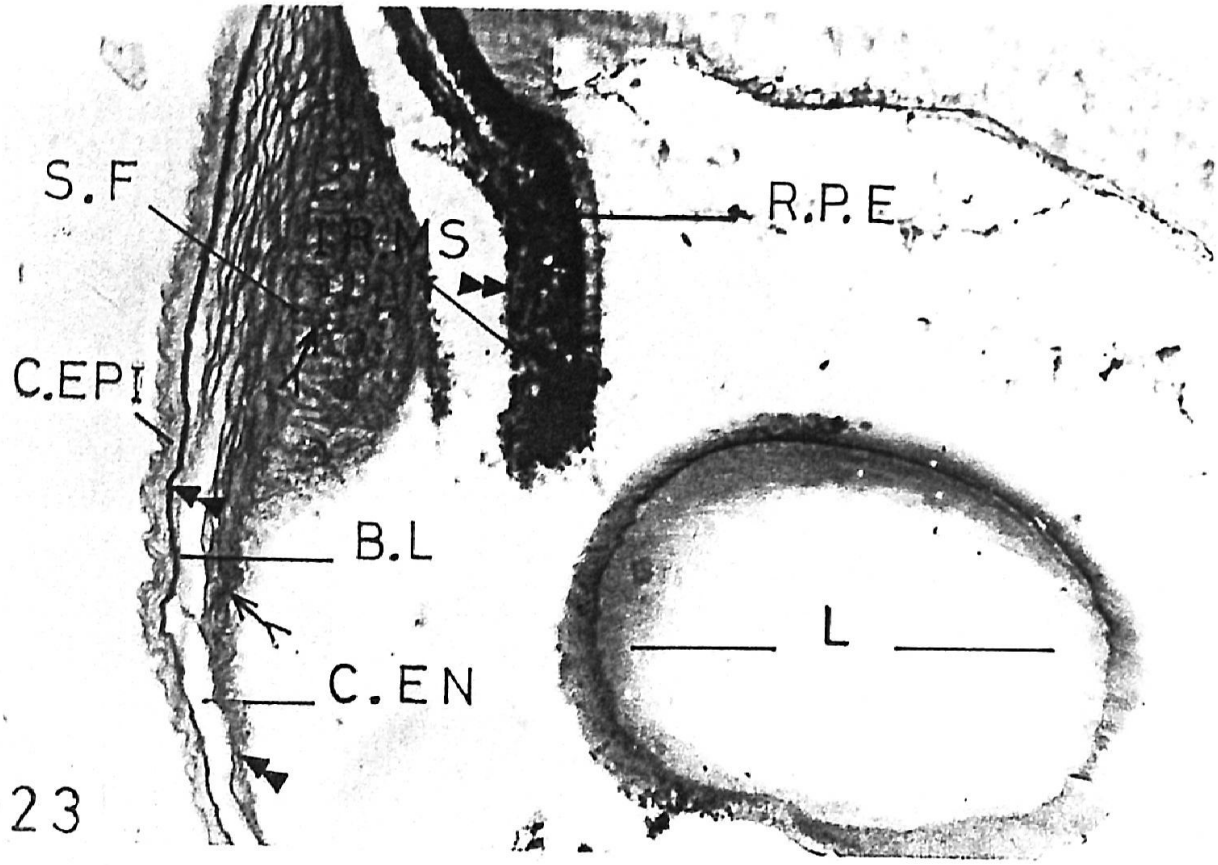

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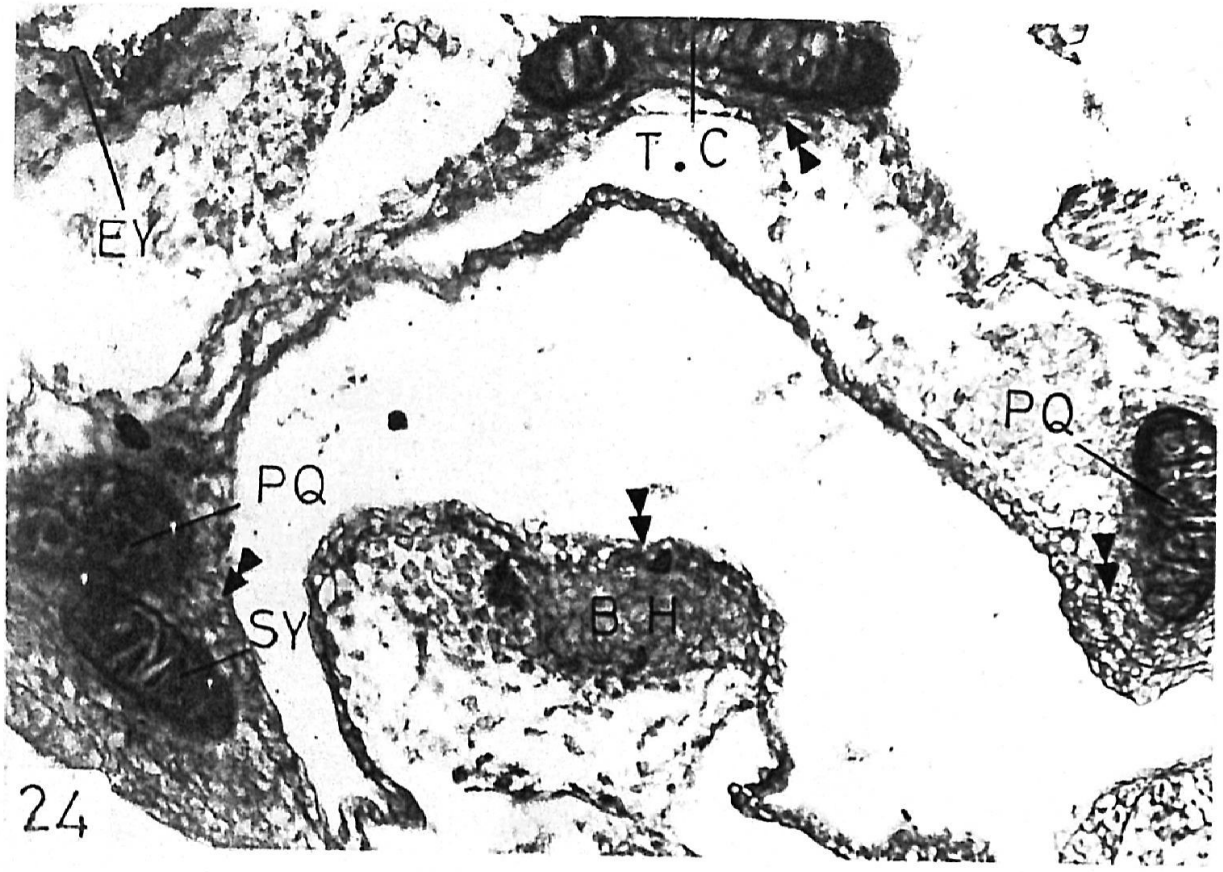

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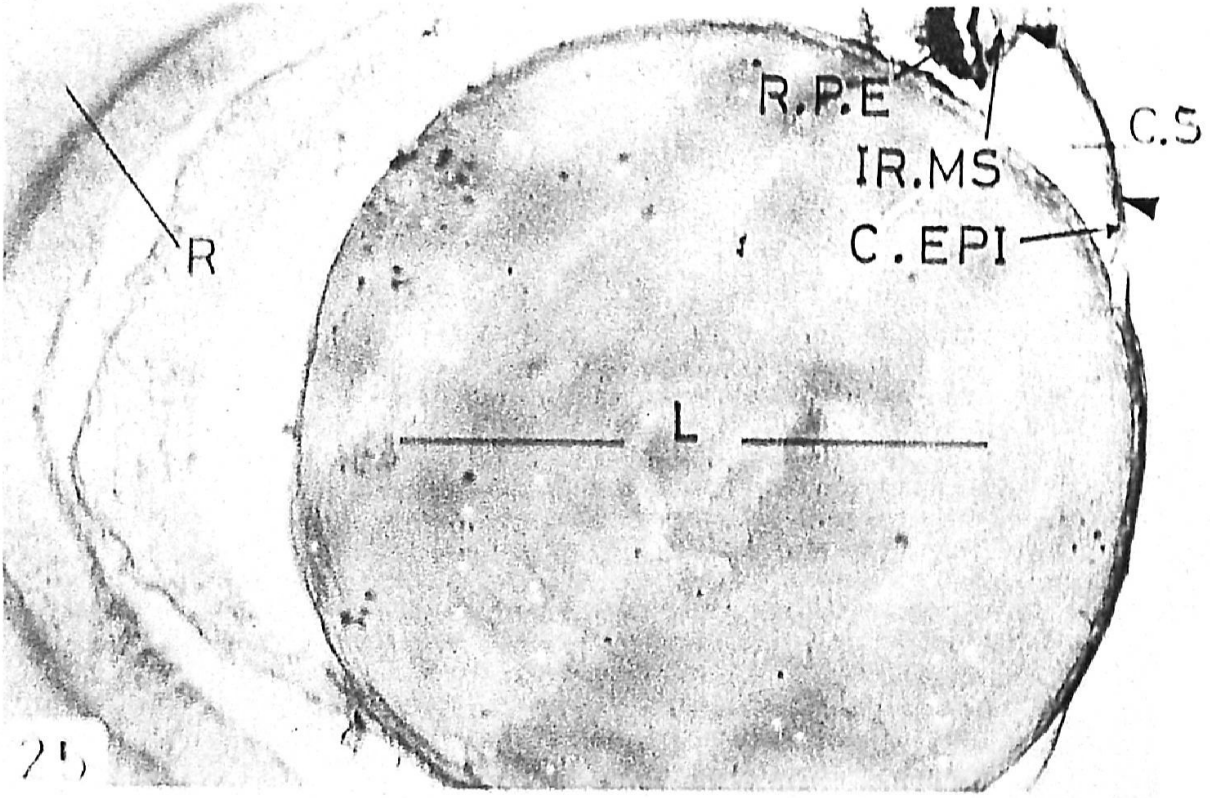
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