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# DISTRIBUTION OF EXTRACELLULAR MATRIX COMPONENTS DURING EARLY DEVELOPMENT OF THE EYE AND EAR OF THE MOUSE

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## ABSTRACT

*The extracellular matrix contains many components including glycosaminoglycans, collagen, fibronectin, etc.., which significantly affect morphogenesis of different tissues. The distribution of glycosaminoglycans, collagen and fibronectin was studied during the early development of mouse eye ear using histochemical, electron microscopy, immunoflourescence, and immunoelectron microscopy methods.*

*Glycosaminoglycans and fibronectin were found in the basal lamina of the optic vesicle. These components, in addittion to collagen forms, were also found in the space between the vesicle and lens placode. These components increased gradually during the formation of the optic resicle and lens placode. During the formation of the retina and lens, there was an increase in hyaluronic acid, fibronectin and collgen, while sulphated glycosaminoglycans decreased.*

*The basal lamina of the otic placode as well as the surrounding matrix contained glycosaminoglycans, fibronectin and collagen which increased gradually. After otic vesicle formation, the increase of sulphated glycosaminoglycans was obvious.*

*The possible role of these extracellular matrix components in morphogenesis was discussed.*

## INTRODUCTION

The extracellular matrix (ECM) has received an increasing amount of

attention as a potential regulator of cell proliferation and differentiation and phenotypic stabilization in a variety of tissues (for review, see Gospadarowicz *et al.*, 1978; Wartiovaara *et al.*, 1979; Hay, 1981; Armstrong, 1982; Bissel *et al.*, 1982; Ekblom *et al.*, 1986). Among the major defined components of the ECM are glycosaminoglycans (GAGS), proteglycans, collagens, and glycoproteins, incuding fibr onectin, laminin, cytotactin, entactin, laminin, tenascin, and nidogen (Crossin *et al.*, 1986; Mayne and Burgeson, 1987; schuppan and Hahn, 1978).

GAGS consist of linear polymers of disaccharide units, while proteoglycans are composed of several GAG side chains linked to a protein core Toole, 1981; Hook *et al.*, 1984; 1984; Fransson, 1985). GAGS are classified into nonsulphated GAGS (represented by hyaluronic acid) and sulphated GAGS (including chondroitin sulphate, keratan sulphate, heparan sulphate, and dermatan sulphate). GAGS and proteoglycans have been demonstrated to be involved in a number of embryonic processes, including skeletone development (Toole, 1972), chick eye morphogenesis (Toole and Trelstad, 1971; Meier and Hay, 1973), mouse lens morphogenesis (Webster *et al.*, 1983), and cushion cell migration (Markwald *et al.*, 1984).

Fibronectin is a glycoprotein which forms a significant component of the ECM and plays an important role in many morphogenetic processes including migration of cardiac cushion cells (Icardo and Manasek, 1983, 1984), primordial germ cells (Heasman *et al.*, 1981; Wylie and Heasman, 1982), and neural crest cells (Duband *et al.*, 1986; Agamy, 1988) as well as differentiation, in vitro, of neurons (Loring *et al.*, 1982), myoblasts (Garnder and Fambrough 1983) and chondrocytes (Weston *et al.*, 1979). Involvelment of matrix glycoproteins in cells and tissue adhesion processes at specific developmental times has been demonstrated in

embryonic chick eye (Hendrix and zwaan, 1974, 1975; kurkinen *et al.*, 1979) and in embryonic mouse tooth germ (Thesleff *et al.*, 1979; Lesot *et al.*, 1981).

Collagen is considered as a major matrix component in connective tissue. There are about eleven distinct types of collagen (Aumailley *et al.*, 1985; Morriss *et al.*, 1986; Schuppan and Hahn, 1987). The specific collagen type present depends upon the structure involved and the age of the animal (Fessler and Fessler, 1978; Stryer, 1981). Collagen types vary in predictable temporal sequence at specific spatialities during mouse odontoblast differentiation (Lesto *et al.*, 1981), and in embryonic chick eye development (Hay *et al.*, 1969; Von der Mark *et al.*, 1977; Linsenmayer *et al.*, 1986; Fitch et al., 1988). Type VI collagen is a major component of the adult human cornea (Zimmeran *et al.*, 1986).

Morphogenesis of the vertebrate eye begins when bilateral optic vesicles from the embryonic forebrain come into close proximity with cephalic ectoderm (Rugh, 1986; Pei and Rhodin, 1970; McAvoy, 1980). No direct contact was observed between the cell membranes of the optic vesicle and cephalic ectoderm in chick (weiss and jackson, 1961; Hendrix and Zwaan, 1975 ) or mice (Cohen, 1961). The optic vesicle / presumptice lens interface area in chink has been demonstrated to be rich in glycoproteins (Hendrix and Zwaan, 1974, 1975; Kurkinen *et al.*, 1979), collagen fibres (Silver and wakely, 1974), and GAGs (Hendrix and Zwaan, 1975). Using histochemical techniges webster *et al.* (1983) have demonstrated GAGs in the same area of mice.

The Ear of vertebrates develops as an ectodermal invagination, the otic placode, which then forms the otic vesicle that differentiate into the inner ear (Balinsky, 1965; Kent, 1987).

Since ECM has been reported to play important roles in morphogenesis, the present work was aimed to examine the ECM components (GAGs, fibronectin and collagen) during the early development of eye and ear of mouse embryo, with particular emphasis placed on temporal and spatial distribution patterns of these molecules.

## MATERIAL AND METHODS

### Embryos

Embryos aged 9 to 12 days (23 - 52 somites) of gestation were obtained by timed matinges, the day vaginal plugs were discovered being counted as day 0 of gestation (Bronson *et al.*, 1966). The mice used were non-agouti black (aa++) derived from the stocks maintained at the MRC, Radibiology Unit, Harwell, England. Embryos were dissected out of the uteri into phosphate buffered saline (PBS) (pH 7.4). The ECM components, GAGs, fibronectin, and collagen were detected using histochemical, electron microscope, immunofluorescence and immunoferritin methods.

### Histochemistry

Embryos were fixed in 10% formalin containing 0.5% cetyl pyridinum chloride and 0.25% polyvinyl pyrollidone (SIGNA) to preserve GAGs (Scott and Dorling, 1965; Derby, 1978). The embryos were dehydrated in ethanol, embedded in paraffin, and serially sectioned at 7 um. Sections were mounted in sets of 3 sections on glass slides, and alternate slides were stained with : 1) haematoxylin and eosin for general histology, 2) alcian blue (pH 2.6) for detection of whole GAGs, or 3) alcian blue (pH 1.0) for detection of sulphated GAGs (Scott and Dorling, 1965; Culling, 1975.

**Transmission electrom microscopy (TEM)**

Embryos were fixed in Karnovsky's fluid containing 0.2% rhuthenium red (SIGMA), and postfixed in 1% osmic acid containing 0.05% ruthenium red to preserve GAGS (Luft, 1971; Hay, 1978). The head regions were embedded in Spurr resin and ultrathin sections were stained with uranyl acetate and lead citrate (Venable and Coggeshal, 1965) and examined in a Phillips transmission electron microscope.

**Scanning electron microscopy (SEM)**

Embryos were fixed in Karnovsky's fluid containing 0.5% cetyl pyridinum chloride and 0.25% polyvinyl pyrollidone. The embryos were dehydrated and freeze fractured in liquid nitrogen (Humphreys *et al.*, 1974). The specimens were then critical point dried, coated with gold and viewed in a Joel scanning electron microscopse.

**Immunofluorescence**

Embryos were fixed in 10% formalin in PBS, embedded in O.C.T medium (Lab - Tek), and sectioned at 10 um. using a Reichert cryostat at - 20°C. Frozen section were mounted on glass slides and incubated with fibronectin-antibody in PBS containing bovine serum albumin (SIGMA) for 1 hour (Duband *et al.*, 1986). Control sections were incubated with normal rabbit serum or with PBS. All sections were washed in PBS, incubated with fluorescein isthiocyanate (FITC) - conjugated goat anti-rabbit IgG (SIGMA), mounted in 90 % glycerol in PBS, and examined in Leitz microscope.

### Immunoferritin method

Embryos were fixed in 1% formaldehyde and 0.5% glutaraldehyde and were cut into small segments (less than 3 mm). Specimens were incubated with fibronection - antibody, washed in PBS, incubated with ferritin - conjugated goat antirabbit IgG, and postfixed in 1 % osmic acid (Mayer *et al.*, 1981). Specimens were treated with normal rabbit serum or PBS in place of the fibronectin - antibody. Specimens were dehydrated and embedded in Spurr resin and ultrathin sections were stained with uranyl acetate and lead cit-rate and viewed in a Phillips transmission electron microscope.

### Designation of stages

For convenience in interpreting results, three stages of early eye and ear development were devised according to developmental changes in the eye and ear. Embryos were identified based on a combination of the number of somites and distinguishing morphological charachteristics.

## RESULTS

### 9 day embryos (23 - 26 somites)

A bilateral optic vesicle was evaginated from the brain at the level of the prosencephalon (Fig 1, 2). The outer wall of the optic vesicle became flattened toward the ecoderm which represents the presumptive lens actoderm

### Distribution of GAGs and collagen

In sections stained with alcian blue at 2.6 (stains all GAGs) ( Fig. 18), it

appears that GAGs were abundant in the basal laminae of both the optic vesicle and ectoderm. A slight alcian blue stain was found between the basal laminae. In adjacent sections stained with alcian blue at pH 1.0 (stains only suphated GAGs) (Fig. IC), the stain was less intense and had a similar distribution to that of the whole GAGs.

TEM examination (Fig. 4A) showed that the ECM materials located between the optic vesicle and ectoderm consists of :1) 5nm filaments, representing hyaluronic acid, which were few in number, 2) 30 - 50 nm granules, representing sulphated GAGs, which were concentrated in the basal lamina of the optic vesicle and were found in few numbers in the space between the optic vescile and ectoderm, and 3) 20 - 60 nm fibrils associated with GAG granules and may represent procollagen forms and collagen fibrils.

Specimens examined by SEM (Fig. 4B) showed that the space between the optic vesicle and ectoderm was occupied by collagen fibrils (30 - 60nm in diametr). Few GAG granules were scattered among the collagen fibrils, and this confirms the results obtained form alcian blue stained sections and TEM sections.

**Distribution of fibronection**

Frozen sections treated with fibronectin antibody (Figs 2A,B) showed fluorescien staining in the basal lamina of the optic vesicle and the presumptive lens ectoderm and in the space between them. Control sections treated with normal serum showed no fibronectin staining (Figes 2C, D), confirming the specifity of the fibronectin - antibody.

In ultrathin sections of immunferritin-treated embryos, it appears that both

the basal lamina of the optic vesicle and the space between the optic vesicle and ectoderm were rich in fibronectin (Fig. 4c).

As regards to the region of the ear, frozen sections showed fibronectin staining in the basal lamina of the otic placode and the surrounding matrix (Figs 3A, B). Similar distribution of GAGs and fibronectin was observed by using the other techniques of the present study.

**10 day embryos (32-35 somites)**

The outer wall of the optic vesicle has invaginated inwards and an optic cup was formed (Fig. 5a). The actoderm facing the optic cup has thickened and invaginated to from the lens placode. The basal laminae of the these two structures became in close proximity to each other. The otic placode was closed forming the otic vesicle (Fig. 6).

**Distribtion of GAGs and collagen**

In sections stained with alcian blue (pH 2.6) (Fig.5B), the basal laminae of the optic vesicle and lens placode were intesely stained. The space between. these two laminae was also positive for alcian blue staining. In adjacent sections stained with alcian blue (pH 1.0) (Fig. 5C), the sulphated GAGs had approximately the same pattern of stain distribution as the whole GAGs, with a reduced intensity of the stain.

TEM ultratin sections (Fig. 8A) showed that the basal lamin of the optic cup is associated with hyaluronic acid and sulphated GAGs. There was an increase in the amount of both GAG types in the space between the cup and lens placode. Collagen

fibrils (40-70 in diameter) were observed in that space and were decorated with hyaluronic acid filaments and sulphatedGAG granules.

In cryofractured specimens examined by SEM (Fig. 8B), the spcae between the optic cup and lens placode was found to have a network of 50 - 120nm collagen fibrils. These fibrils were slightly beaded (indicating the presence of hyaluronic acid) and were associated with sulphated GAG granules. There was an increase of hyaluronic acid and sulphated GAGs compared with the previous stage.

The basal lamina of the otic vesicle was intensely stained with alcian blue (pH 2.6) and the stain was relatively more intense in the part of the basal lamina facing the ectodern (Fig. 6A). The space surrounding the otic vesicle was also positive for alcian blue staining. Alcian blue staining at pH 1.0 of adjacent sections (Fig. 6B) showed a similar distribution of sulphated GAGs but the stain was less intense. Results obtained from TEM and SEM examination confirmed the pattern of GAG distribution showed by the alcian blue staining. Ultrastructure distribution of GAGs was similar to that explained in figures 8 A, B.

**Distribution of fibronectin**

In frozen sections, the basal laminae of the otic vesicle and the ectoderm were intensely stained for fibronectin (Fig. 7). Also, the space surrounding the otic vesicle was rich with fibronectin. The fibroectin distribution in the optic cup and lens placode was similer to that of GAGs.

TEM sections showed that the basal lamina of the optic cup and the space between the optic cup and lens placode were rich with fibronectin (Fig. 8C). Fibronectin was also found to be associated with collagen fibrils of different

diameters. The distribution of fibronection in the otic region was similar to that in the optic region.

**12 day embryos (50-52 somites)**

The optic cup has completely detached from the brain to form the retina (Fig. 9A). Also, the lens placode has completely encircled and detached from overlying ectoderm to from the lens. The otic vesicle was embedded deeper into the space between the ectoderm and extended to form the inner ear parts (Fig. 11A).

**Distribution of GAGs and collagen**

In sections stained with alcian blue at pH 2.6 (Fig. 9B), the basal laminae of the retina and lens were richly stained. Also, the space between the retina and lens was richly stained with alcian blue. In adjacent sections stained with alcian blue at pH 1.0 (Fig. 9C), the distribution of the sulphated GAGs was again similar to that of the whole GAGs, but with notably less stain intensity.

In ultrathin sections of embryos treated with rhuthenium red (Fig. 12A), the space between the retina and lens was rich with a large number of 3-5nm hyaluronic acid filaments. However, the 30-50nm sulphated GAG granules were less in quantity than in the previous stage both in the retinal basal lamina and the space between the retina and lens.

SEM examination showed that the space between the retina and lens contained 50-120nm collagen fibrils (Fig. 12B). These fibrilswere mostly beaded, indicating the presence of high content of hyaluronic acid. 30-50nm sulphated GAG granules were also associated with the collagen fibrils.

Paraffin sections, in the otic region stained with alcian blue at pH 2.6 (Fig. 11A) showed that the basal lamina of the ear as well as the matrix surrounding the ear were rich with alcian blue staining. In adjacent sections stained with alcian blue pH 1.0 (Fig. 11B), the sulphated GAGS have a similar distribution to the whole GAGs. These suphated GAGs were densely stained with alcian blue at pH 1.0 (Fig. 11B), indicating the high content of sulphated GAGs in the matrix surrounding the developing ear. There was disturbance in alcian blue staining in some parts of the basal lamina.

### Distribution of fibronectin

In frozen sections, the basal laminae of both the retina and lens were intensely stained for fibronectin. Also, the space between the retina and lens had an abundant amount of fibronectin (Fig. 10).

TEM sections (Fig. 12C) confirmed the presence of high amount of fibronection in both the retinal basal lamina and the space beteween the lamina and lens. Fibronectin was also associated with collagen fibrils.

## DISCUSSION

### Identification of ECM molecules

The staining of the whole GAGs with alcian blue at pH 2.6 and of the sulphated GAGs with alcian blue at pH 1.0 was confirmed by other investigators (Scott and Dorling, 1965; Turely et al., 1985; Agamy, 1988).

In truthenium red-treated embryos, the GAG-rich ECM contained 3-5 nm filaments which were identified as hyaluronic acid, and 20-50nm granules which

were identified as sulphated GAGs according to Hay (1978), Tucker (1986) and Agamy (1988).

In case of SEM, the beaded filaments were identified as collagen fibres associated with hyaluronic acid, and the 30-50 nm granules were identified as sulphated GAGs according to Turcker (1986) and Agamy (1988).

The specifity of the fibronectin-antibody used in the present study was confirmed by using control specimens.

**Role of ECM in eye development**

The inductive influence of the optic vesicle on lens formation has experimentally demonstrated in amphibians (Lewis, 1904, 1907), chickens (Mckeehan, 1958), and mice (Muthukaruppan, 1965; Van der Starre, 1978). A well defined interspace between the optic vesicle and cepalic ectoderm has been observed with TEM in chick (Weiss and Jackson, 1961; Hendrix and Zwaan, 1972) and mice (Cohen, 1961) with no reports of direct contact between the cell membranes. However, Johnston *et al.* (1979) reported that there is a focal fusion of these basement membranes in chick lens morphogensis and Mckeehan (1951) claimed that this fusion is the apparent reason for lens placode invagination. Characteristic features of optic vesicle-induced ectoderm were typically found in those cells in close association with the optic vesicle (Zwaan and Hendrix, 1973), suggesting causality due to proximity. In the present study, no focal contact was observed between the basal laminae of the optic vesicle and placode, suggesting that the influence of the optic vesicle on lens formation may occur through the ECM found between these two structures.

The results of the present study demonstrated that the basal lamina of the optic vesicle contains GAGs and fibronectin prior to its close apposition with the cephalic ectoderm. Chondroitin sulphate was detected in the mouse optic vesicle by enzymatic testing (Webster *et al.*, 1983). Also, a large increase in cetyl pyridinium chloride-precipiable materials appeared in the margin of the chicken optic vesicle during lens placode invagination (Hilfer and Yang, 1980; Yang and Hilfer, 1982).

The early interface matrix between the optic vesicle and lens placode, as observed in the present study, contained hyaluronic acid, sulphated GAGs and fibronectin associated to the basal laminae and to collagn fibrils. Using different techniques, this interface matrix of chick was reported to contain glycoproteins (Hendrix and Zwaaan, 1974, 1975; Kurkinen *et al*; 1979). GAGs (Yang and Hilfer, 1982) and Collagen (Silver and Wakely, 1974), GAGs were demonstrated in the same matrix of mice (Webster *et al.*, 1983). In anophthalmic mice, abnormal contact between the optic vesicle and lens placode was attributed to a deficiency in sulpated GAGs on the basal lamina of the optic vesicle (webster *et al.*, 1984). Webster *et al.* (1983, 1984) proposed that the initial triggering mechanism of lens mrophogenesis consists of a cross-linking and polymerization of optic vesicle-associated GAGs to ectodermal-associated glycoproteins resulting in a firm attachment between those structures. However, these authors did not specify which GAGs or which glyroproteines could be involved.

Indeed, the presence of fibronection in the optic vesicle/lens placode interface may well provide the attachment power required. Fibronectin alone, or in association with other ECM molecules, promotes cell adhesion (Couchman *et al.*, 1982; Erickson and Turley, 1983; Duband *et al.*, 1986; Barondes, 1985; Brown and

Juliano. 1987). Fibronectin is found in association with embryonic basement memberanes, but much less fibronectin is found in adult basement membranes (Wartiovaara and Vaheri, 1980; Hynes and Yamada, 1982; Furcht, 1982), suggesting the role of fibronecti in develeopment.

Collagen first appeared between the eye rudimnts as small fibrils which increased gradually in diameter and quantity. Some investigators classify collagens into basement membrane collagens (collagen type IV) and interstitial collagens (other types) (Timple *et al.*, 1981; Timpl and Dziadek, 1986) according to their location. Others classify collagens into fibril-forming, network-forming, and microfibril-forming collagens according to their ultrastructure (Mayne and Brugeson 1987). The collagen fibrils which first appeared in the present study may be procollagen forms or certain collagen types which than are transformed into oter types as development proceeds.

Thus, ECM molecules or factors transferred from the optic vesicle or interactions of both controls the inductive influence of the optic vesicle to early lens morphogenesis. The firm attachment by ECM molecules provides optimal conditions for that inductive effect.

Later stage of eye development is characterized by incresae of hyaluronic acid and fibronectin and decrease of sulphated GAGs in the matrix between the retina and lens. The obvious increase of hyaluronic acid could be morphogenetically important in terms of the enormous expanding capacity of hyaluronic acid when binds to water. The movement of embryonic cardiac cushion cells (Markwold *et al* ., 1978), sclerotomal cells (Toole, 1972), palate cells (Greene and pratt, 1976) and neural crest cells (Agamy, 1988) take place in hyaluronic acid-rich matrices.

Also, fibroblast invasion into the corneal stroma coincides with the swelling of the stroma due to the presence of hyaluronic acid (Hay and Revel, 1969; Toole and Trelstad, 1971). Thus, the increase of hyaluronic acid between the retina and lens may well cause expansion of the space between these structures leading to the formation of the viterous body. Dense collogen fibres may give the mechanical support for that process. The decrease of sulphated GAGs, observed in the present study, was correlated with the appearance of more collagen fibres. A similar pattern was observed in salivary glands and mouse eye using hitochemical studies (Bernfield and Banerjee, 1972 Webster *et al.*, 1982). Yang and Hilfer (1982) demonstrated that disruption of GAG synthesis at the time of early lens morphogeneisis resulted in marked morphological alterations to the eye rudiments. In addition, the appearance of GAGs is delayed in aphakia mice (Webster *et al.*, 1986).

Thus, the temporal and spatial distribution of GAGs, fibronectin and collagen is important for normal eye development.

**Role of ECM in ear development**

Each ear starts with just one rudiment, the otic placode, a situation which is different from that of eye development which involves a more complicated process, including interactions between the optic vesicle and lens placode. The basal lamina of the otic placode and the matrix surrouding it contained GAGs, fibronectin and collagen fibrils. The mechanism of invaginaation of the otic plaode is still obsure. There was a gradual increase in these ECM molecules in the basal lamina of the otic vesicle as well as in the sorrounding matirx. Disruptions occrred in the GAG found on the basal lamina, followed by branching of the otic vesicle. It is known that ECM, including basal laminae, play a crucial role in morphogensis as many tissues

undergo folding within the epithelial sheets. Changes in ECM composition appear to coincide directly with developmental changes in a number of these interacting systems. For example, epithelial branching during salivary gland development is promoted by degrading the basal lamina in selective regions and allowing new branches to develop by destabilizing the epithelium (Bernfield and Banerjee, 1982; Smith and Bernfield, 1982). Similar observations were reported for mammary gland development (Gordon and Bernfield, 1980). The importance of GAGs and collagens in branching processes was implicated in inhibitor studies (Spooner and Faubion, 1980; Thompson and Spooner, 1983).

In conclusion, it appears that spatial distribution of ECM molecules coinicdes with the morphogenesis of the eye and ear of the mouse. Analysis of the mechanisms of this effect could provide insight into the means by which extracellular molecules regulate intracelluar events.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AB | Alcian blue |
| E | Ear |
| ECM | Extracellular matrix |
| FB | Fore brain |
| GAG | Glycosaminoglycans |
| HB | Hind brain |
| HE | Haematoxylin and eosin |
| L | Lens |
| LP | Lens placode |
| OPC | Optic Cup |
| OPV | Optic vesicle |
| OtP | Otic placode |
| Otv | Otic vesicle |
| R | Retina |

## EXPLANATION OF FIGURES

Fig. (1) : Paraffin transverse serial sections through the optic region of 9 day embryo. x150

(A) shows the optic vesicle (Opv) evaginted laterally from the fore-brain (FB). HE stain.

(B) The basal laminae of the optic vesicle (arrows) and ectoderm (arrowheads) are stained with alcian blue. AB stain (pH 2.6).

(C) The basal laminae of the optic vesicle (arrows) and ectoderm (arrowheads) have less stain intensity than that of Figure 1B. AB stain (pH 1.0).

Fig . (2) : Frozen transverse serial sections through the optic region of 9 day embryo. x 175.

(A) Phase contrast photograph showing the optic vesicle (DpV) evaginated from the fore-brain (FB).

(B) Fluorescence photograph showing fibronectin staining in the basal lamina of the optic vescil (arrows) and in the surrounding matrix.

(C) Phase contrast photograph of a control section.

(D) Fluorescance photograph of the control section treated with normal serum showing no fibronectin to insure the specifity of the fibronectin-antibody.

Fig. (3) : Frozen transverse section through the otic region of 9 day embryo showing the otic placode (OtP) invaginating inward from the ectoderm (E) at the side of the hind - brain (HB). x 175.
(A) Phase contrast photograph.
(B) Fluorescence photograph showing fibronectin staining in the basal lamina of the otic vesicle (arrows) and in the surrounding matrix.

Fig. (4A) : TEM photograph through the optic region of 9 day embryo treated with ruthenium red showing 30-50 nms sulphated GAG granules in the basal lamina of the optic vesicle (arrowheads) and in the space between the optic vesicle and ectoderm (arrows). Hyaluronic acid filaments (curved arrows) and collagen fibrils (open arrows), are found in that space. x 15000.

Fig. (4B) : SEM Pohotograph of 9 day embryo cryofractured at the optic region showing collagen fibrils (arrows) decorated with suephated GAG granules (arrowheads) in the space between the opic vesicle (OpV) and ectoderm. x 12500.

Fig. (4C) : TEM photograph through the optic region of 9 day embryo treated with the immunferritin method showing fibronectin staining in the basal lamina of the optic vesicle (arrowheads) and in the extracellular space (arrows). x 15000.

Fig. (5) : Paraffin transverse serial sections through the optic region of 10 day embryo. x 150.
(A) Shows the optic cup (OpC) in close association with the lens placode (LP). HE stain
(B) The basal lamina of the optic cup ( arrows) and lens placode ( arrowheads) are stained with alcian blue. AB stain (pH 2.6).

(C) The distribution of alcian blue stain is similar to that in Figure 5B, but with less stain intesity. AB stain (pH 1.0)

Fig. (6) : Paraffin transverse serial sections through the otic region of 10 day embryo showing the encircled otic vesicle (OtV) between the hind brain (HB) and ectoderm. x150
(A) Alcian blue staining is located in the basal lamina of the otic vesicle (arrows) and in the surrounding matrix. AB stain (pH 2.6)
(B) The alcian blue staining of both the basal lamina of the optic vesicle (arrows) and the surrounding matrix is less intense than that of Figure 6B. AB stain (pH 1.0).

Fig. (7) : Frozen transverse section through the otic region of 10 day embryo showing fibronectin - staining in the basal lamina (arrows) of the otic vesicle (OtV) and in the space surrounding the otic vesicle. x175.

Fig. (8A) : TEM photorgraph through the optic region of 10 day embryo treated with ruthenium red showing sulphated GAG granules in the basal lamina (arrowheads) of the the optic cup (OpC) and in the space (arrows) between the optic cup and lens placode. Hyaluronic acid filaments (curved arrows) and collagen fibrils (open arrows) are found in that space. x 15000.

Fig. (8B) : SEM Photograph of 10 day embryo cryofractured at the optic region showing beaded collagen fibrils (open arrows) and sulphated GAG granules (arrows) in the space between the optic cup (OpC) and lens placode. x 12500.

Fig. (BC) : TEM Photograph through the optic region of 10 day embryo treated with immunoferritin showing fibronectin labelling in the basal lamina (arrowheads) of the optic cup and lens placode. Collagen fibrils (open arrows) have fibronection. x 15000.

Fig. (9) : Paraffin transvers serial sections through the optic region of 12 day embryo. x 150.

(A) shows the formation of the ectoderm. He stain.

(B) There is intense alcian blue stain in the basal laminae of the retina (arrowheads) and lens (arrows) and in the space between them. AB stain (pH 2.6)

(C) The distribution of alcian blue stain is similar to that found in Figure 9B, but the stain intensity is much less. AB stain (pH 1.0).

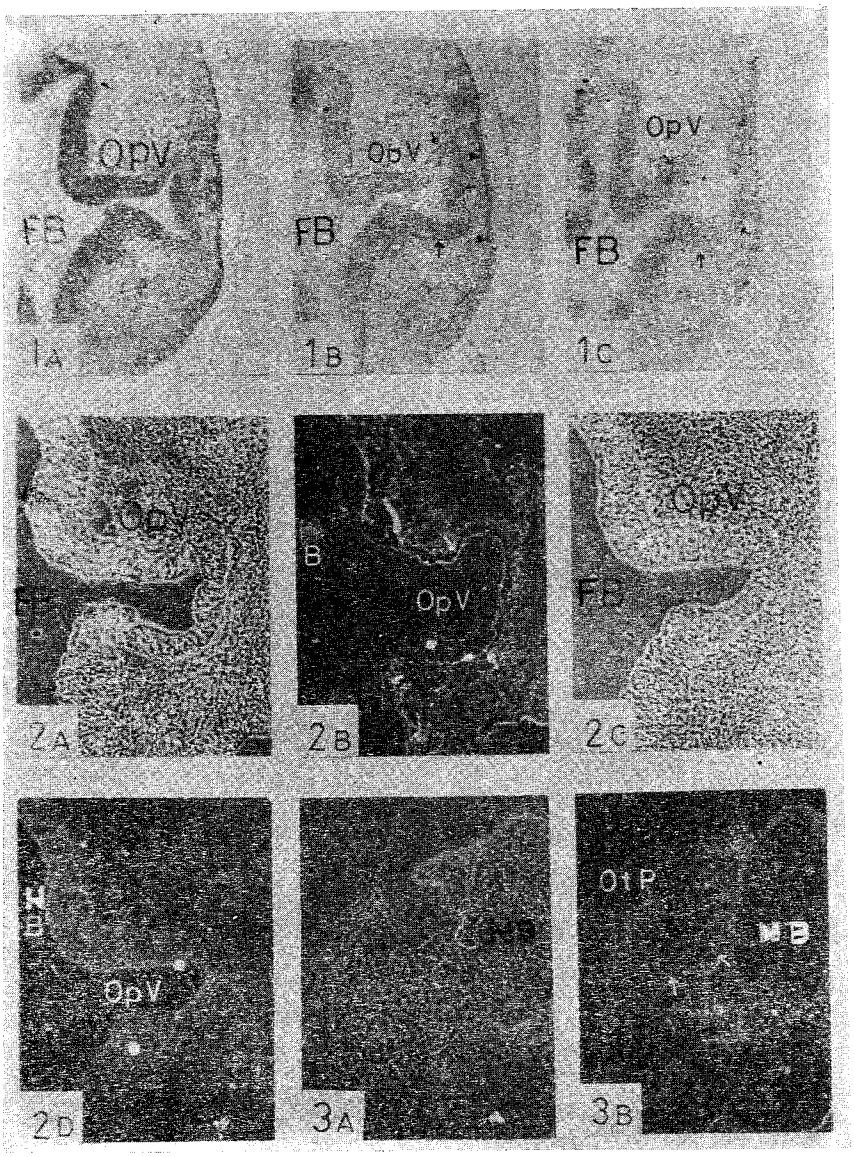
Fig. (10) : Frozen transverse section through the optic region of 12 day embryo showing intense fibronectin - staining in the basal lamina of the retina (arrowheads) and lens (arrows)and in the space between the retina and lens. x 150.

Fig. (11) : Paraffin transverse serial sections through the otic region of 12 day embryo. x 150
(A) The ear (E) has enlarged and the basal lamina of the ear (arrows) as well as the surrounding matrix are rich with alcian blue staining. Disruprion are observed in the GAGs of the basal lamina (stars) AB stain (pH 2.6)
(B) The alcian blue staining has a similar distribution to that in Figur 11A, and is onle slightly less in intensity. AB stain (pH 1.0)

Fig. (12A) : TEM photograph through the optic region of 12 day embryo treated with ruthenium red showing the higher content of hyaluronic acid filaments (curved arrows) and the lower content of the sulphated GAG granules (arrows and arrowheads) compared with the previous stage. x 15000.

Fig. (12B) SEM photograph of 12 day embryo cryofractured at the optic region showing frequently beaded collagen (arrows) in the matrix betwen the retina (R) and lens. x 12500.

Fig. (12C) : TEM photograph through the optic region of 12 day embryo treated with immunofrritin showing dense fibronectin labelling in the basal lamina (arrowheads) of the retina (R) and in the space (arrows) between the retina and lens. x 15000.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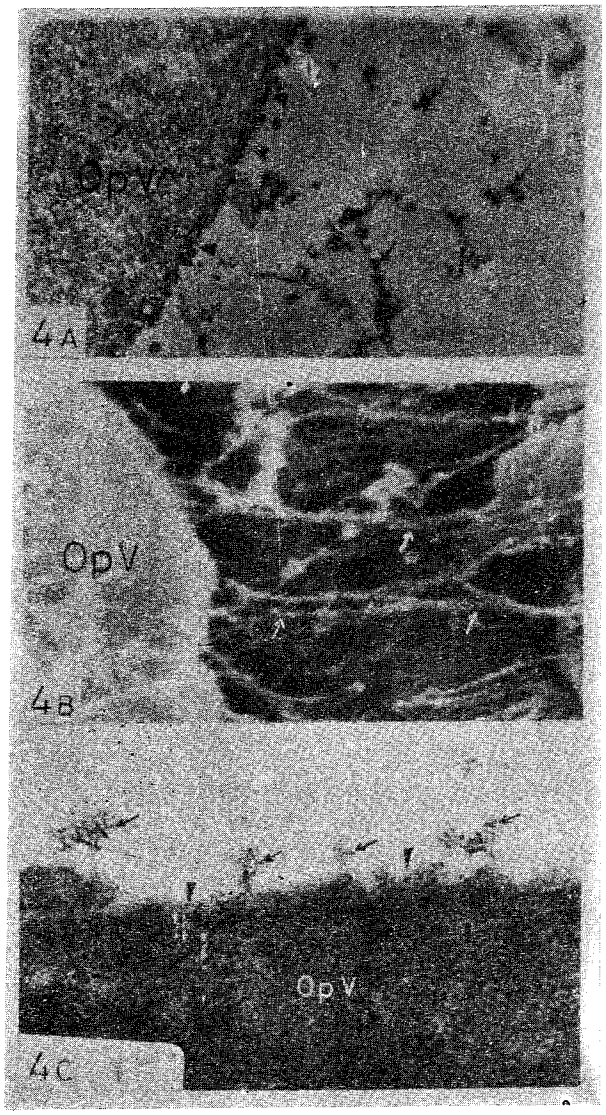
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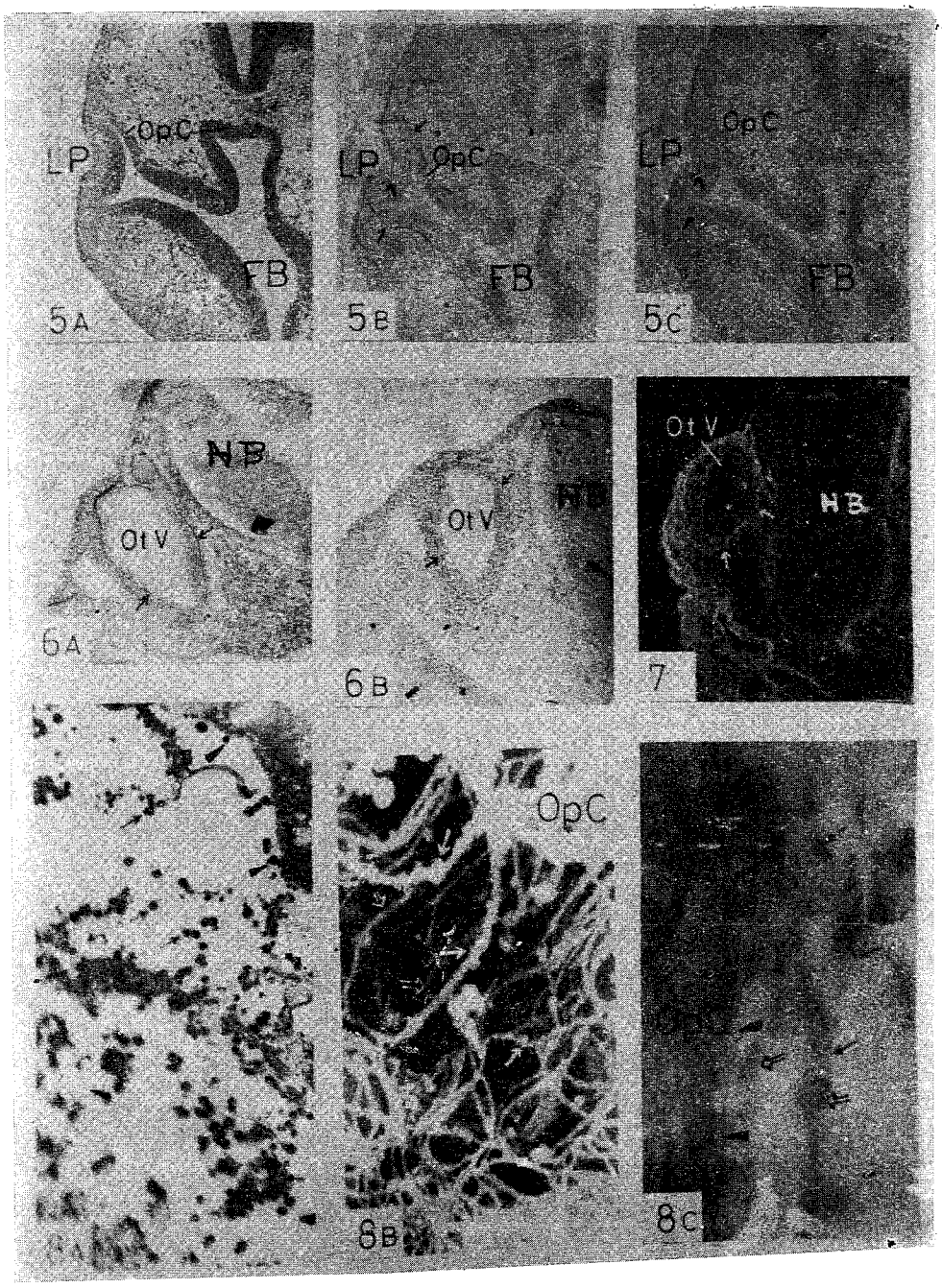
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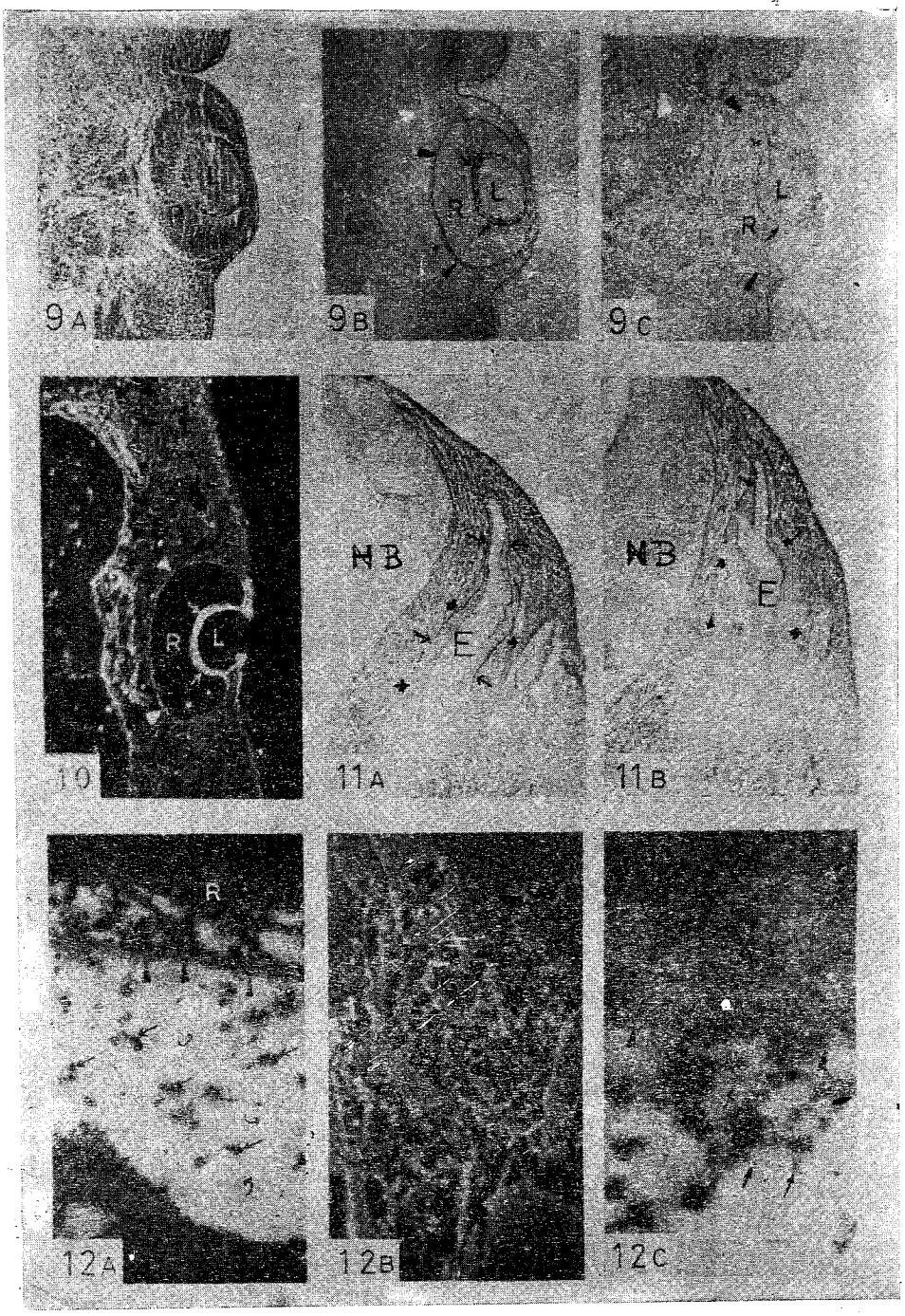
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# توزيع مكونات المادة بين الخلوية اثناء التكوين الجنينى المبكر للعين والاذن فى الفأر

عصام الدين عجمى
قسم علم الحيوان - كلية العلوم - جامعة المنوفية

تحتوى المادة بين الخلوية على العديد من المكونات والتى تشمل الجليكوزأمينوجليكانات والكولاجين والفيبرونكتين وغيرها.. وتؤثر هذه المكونات على تكوين وتشكيل ونمو الأنسجة المختلفة. ولقد تم دراسة توزيع هذه المكونات الثلاث أثناء مراحل النمو الجنينى المبكر للعين والأذن فى الفأر.

إحتوى الغشاء القاعدى للحوصلة البصرية على جليكوزأمينوجليكانات وفيبرونكتين، كما وجدت هاتان المادتان بالإضافة إلى بعض خيوط الكولاجين فى الفراغ الواقع بين الحصلة البصرية والإكتوديرم المكون للعدسة. حدث زيادة فى كميات هذه المواد فى الغشاء القاعدى للفنجان البصرى وبداءة العدسة. أثناء تكوين الشبكية والعدسة حدث زيادة كبيرة فى كمية حامض الهيالورونيك ونقص فى كمية الجليكوز أمينوجليكانات الكبريتية كما حدث زيادة فى كمية الفيبرونكتين وذلك فى الغشاء القاعدى الموجود بين الشبكية والعدسة. كما حدث زيادة فى عدد وقطر خيوط الكولاجين..

أحتوى الغشاء القاعدى للبداءة السمعية على جليكوزأمينوجليكانات رفيبرونكتين، وإزدادت تدريجياً كمية هذه المواد بالإضافة إلى الكولاجين حول الحوصلة السمعية . عند تمدد الحوصلة السمعية لتكوين الأذن الداخلية حدث زيادة فى كمية الجليكوزأمينوجليكانات الكبرتية بالمقارنة بالمواد الأخرى .

تم مناقشة أهمية هذه المواد فى تكوين العين والأذن للفأر.