Origin and development of Bruch’s membrane in monkey fetuses: an electron microscopic study

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The appearance of the components of Bruch’s membrane in staged monkey fetuses was investigated with the electron microscope. The formation of this structure is closely associated with the morphological and cytological changes of the adjoining tissues. The sequence of events in the primate is as follows: (1) establishment of basal lamina of the pigment epithelium on the twenty-seventh day of gestation (optic cup stage); (2) appearance of some aggregated collagen fibers with nodosities on the thirty-eighth day of gestation; (3) layering of some collagen fibers with striations on the forty-sixth day of gestation; (4) appearance of the first immature elastic fibers on the forty-ninth day of gestation; and (5) incomplete formation of basal lamina of the choriocapillary endothelium on the fifty-fourth day of gestation. The observations are discussed in relation to published work which has a bearing on Bruch’s membrane formation.

Key words: basal infoldings, basal lamina, Bruch’s membrane, choriocapillaris, collagen fibers, fibroblasts, elastic fibers, pigment epithelium.

There are many reports concerning the fine structure of Bruch’s membrane in the adult human and animals. Its development was also investigated electron microscopically. These studies dwelt mainly on the developing choriocapillaris, pigment epithelium, and photoreceptor outer segment, which were well discussed in detail, but they paid only subsidiary attention to the precise sequence of events in Bruch’s membrane formation. The origin of each of its components was not examined in great detail.

Admittedly, choriocapillaris, Bruch’s membrane, pigment epithelium, and photoreceptor are interrelated during the gestational stages, and the development of Bruch’s membrane cannot be discussed without examination of neighboring tissues.

In this study, the developmental changes of Bruch’s membrane in various ages of monkey fetuses were examined and particular attention was paid to the appearance and the probable origin of each of its constituents.

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Materials and methods

Macaca mulatta fetuses of precisely known gestational stages were studied. The gestational period of this species is 160 to 162 days. Monkey embryos and fetuses of 27, 28, 29, 32, 38, 43, 46, 49, 54, 60, 67, 76, 80, 90, 100, 110, 126, 131, 143, and 160 days in utero were examined.

The posterior region of the eyeballs was immersed in cold 3.5 per cent glutaraldehyde in 0.5 M phosphate buffer at pH 7.4 immediately after removal of the embryo or fetus by Caesarean section. The tissues were postfixed for one hour in 1 per cent OsO₄ in 0.5 M phosphate buffer, dehydrated in a graded alcohol series, cleared in propylene oxide, and embedded in Epon. Thin sections were cut with a Porter-Blum Ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Siemens Elmiskop I. The 1 μ thick Epon-embedded sections for light microscopy were stained with Azur II.

Results

Gestation (G)-27 days (M66). The primary optic vesicle did not yet form the optic cup. Some sinusoids were observed near the neuro-epithelial layer in the surrounding mesenchymal tissue. The sinusoids were formed by a few endothelial cells which were united with combinations of short tight junctions and gap junctions. Fenestrations were already present in their walls (Fig. 1). The cytoplasm of the sinusoids contained ribosomes, mitochondria, Golgi apparatus, vesicles, and few profiles of granular endoplasmic reticulum. They were situated among widely distributed early fibroblasts having prominent oval nuclei with one or two nucleoli, polyribosomes, and mitochondria. The cytoplasmic projections of both types of mesenchymal cells were only slightly developed. Among these cells there were a few short scattered fibrils.

G-28 (M62) and 29 (M64) days. The optic vesicle has been converted into an optic cup, the outer and inner layers of which were separated at each other only at the periphery. The lens vesicle has appeared.

The outer layer of the optic cup was composed of multilayered pigment epithelial cells which had large oval nuclei with one or two nucleoli, mitochondria, ribosomes, premelanosomes, a few melanosomes, and some profiles of granular endoplasmic reticulum. At 28 days (M62) cells of the inner layer of the pigment epithelium were already joined near their apical terminations by the future Verhoff’s or “R” membrane, consisting of short segments of gap and occludens type junctions, followed by zonulae or maculae adherentes, then tiny gap junctions. This general pattern, set down at this early embryonic stage, was observed in the succeeding specimen examined.

The outermost layer of pigment epithelial cells was covered with continuous basal lamina, whereas the endothelium and the fibroblasts had none as yet. There were many processes of mesenchymal cells and some short fine fibrils within the narrow space (future Bruch’s membrane) between the pigment epithelium and fibroblasts or endothelium. Some of the fibrils were attached to the basal lamina of the pigment epithelium and to the fibroblasts, but they were observed particularly around the fibroblasts. A few scattered aggregated collagen fibrils of 291 A diameter and no measurable periodicity were also noted in this space.

G-32 days (M55). The outer and inner layers of the optic cup were still separated, but the lumen was progressively reduced in size. In the pigment epithelium a few cell processes were noted at the apex (Fig. 2). Melanosomes had increased in number and the basal lamina became well defined. Basally the plasmalemma had a smooth contour without infoldings. A few fine fibrils and many filipodia were still observed among the poorly differentiated fibroblasts.

G-38 (M65), 43 (M47), and 46 (M41) days. The outer and inner layers of the optic cup were closely apposed, the outer
Fig. 1. *Low-power electron micrograph of a sinusoid in the mesenchymal tissue behind the optic stalk in the region of the future choriocapillaris. The lens placode has not yet invaginated at this stage (27 days gestation). ×3,800. Inset: Enlargement of endothelial wall of the same sinusoid showing fenestrations (arrows). ×30,400. *Abbreviations: bl, basal lamina; Br, Bruch’s membrane; Cap, capillary; col, collagen fibrils; cp, cored pit; E, erythrocyte; el, elastic fibrils; ELM, external limiting membrane; En, endothelial cell (capillary); er, endoplasmic reticulum; f, fibrils; fib, fibroblast; g, glycogen granules; Gc, Golgi complex; jc, junction; L, lumen; m, mitochondria; me, melanosome; Mes, mesenchymal cells; OS, outer segments; PE, pigment epithelial layer. Magnification bar is 1 μm unless otherwise indicated.

(pigment epithelium) being multilayered and consisting of cuboidal cells with large round nuclei containing one or two nucleoli. At 43 and 46 days of gestation many glycogen granules occurred, especially in the basal portion of the cells. Fine filaments were homogeneously distributed, but smooth-surfaced endoplasmic reticulum was scarce. Apical processes were few and short. At the forty-sixth day of gestation regions of the apical surfaces of the pigment epithelial cells were occasionally
Fig. 2. Low-power electron micrograph of the pigment epithelium and developing choriocapillaris of the monkey embryo at 32 days of gestation. A continuous basal lamina (bl) lines the pigment epithelium. The tight junctions (Verhoef's membrane) near its apical surface (arrows) are present. Endothelial channels of the choriocapillaries (En) are incomplete. ×2,200.

united to the apposing apical portions of the neuroepithelium by macula occludens-like and gap junctions. Such conjugations were not observed later in gestation. Pigment epithelial cells were interconnected apically by zonulae or maculae occludentes followed by incomplete zonulae or maculae adherentes, basal to which were again tight, or gap junctions. Punctate desmosomes united the lateral cell surfaces basally to these.

Some fibroblasts had invaded the site of the future Bruch's membrane, their processes approaching very closely the basal lamina (Fig. 3). The choriocapillaris had an irregular arrangement but there were a few well-formed vessels. Their endothelium was similar in fine structure to the
Fig. 3. Site of the presumptive Bruch's membrane at 43 days of gestation contains fibroblast cell processes, fine filaments and their aggregations. Near the surface of the endothelial cell fine fibrils are clumped in a manner suggestive of those forming collagenous and elastic tissues. Glycogen (g) is present. Tubular presumed preelastin fibrils are encircled. x32,000.

fibroblast, containing large, round nuclei with one or two nucleoli, many ribosomes, vesicles, fine filaments, and granular endoplasmic reticulum. Endothelial cells were united by macula occludens-like structures, however, often there was no complete fusion to the five layers constituting the adult junction. There were tiny gaps alternating with fused portions. Some of the cells elongated their cytoplasm to form a flattened lumen. Basal lamina was not observed around the capillary endothelium, but there were occasionally a few short, fine fibrillar tufts attached to it and the fibroblasts. In the 43-day stage accumulations of fine fibrils, about 186 Å in diameter and with hollow cores, were present near the plasmalemma of a few endothelial cells and fibroblasts. Their occurrence was rare, but they suggested the physical configuration of early elastic microfibrils (Fig. 3). Fenestrations covered with a single membrane, and pores at the inner side of the endothelium were also present. It is of interest to note here that in a much earlier stage (28 days gestation), at the site of the future choriocapillaris, where there were only a few blood channels with lumina surrounding the optic cup, such vessels had already several fenestrations. In the 46-day stage (Fig. 4), an incomplete fibrillar coat appeared over some endo-
Fig. 4. Low-power electron micrograph of a portion of pigment epithelium and presumptive Bruch's membrane in the 46-day fetus. Bruch's space has scattered collagen fibers, some in parallel arrangement, irregular tufts of basal lamina-like filaments adjacent to the plasma membrane of the endothelial cells, and fine fibrils (f) in irregular patches, further away from the endothelial cell membrane. ×10,500. Inset: Light micrograph of the region from which the electron micrograph was taken. The pigment epithelium is multilayered. The future "external limiting membrane" is partly defined. ×800.

...thelial cells and the collagen fibers, occurring randomly scattered in the previous stages, showed some indication of parallel layering near the fibrocyte or endothelial cell. The average diameter of such collagen fibers with nodosities was 361 Å.

C-49 (M46) and 54 (M50) days. The outer layer of the optic cup was formed of monolayered pigment epithelium. A few mature pigment granules were present in the cytoplasm.

In the prospective Bruch's membrane short collagen fibers ranging in average diameter from 312 Å to 361 Å were randomly scattered near the pigment epithelium. Fine fibrils, some intermixed with collagen fibers, had increased in number around the fibroblasts and endothelium, however, they were not widely dispersed. There were still some tiny fibroblast processes present and small amounts of elastin-like units, though widely spaced from each other, were noted at the 49-day stage (Fig. 5). The fibroblasts had very active Golgi complexes, enormously dilated cisternae of rough surfaced endoplasmic reticulum filled with a granular substance, and clumps of glycogen scattered throughout the cytoplasm. Patches of basal lamina-like material appeared near the endothelial plasmalemma in the 54-day gestational stage (Fig. 6).

C-60 days (M27). A few short apical cell processes of the pigment epithelium were present in the narrow space between neuro-epithelial layer and pigment epithelium, which occasionally had basally located Golgi complexes. Collagen fibers had increased in number on the outer (choroidal) side of the basal lamina and occupied a wider space than that of the previous stage. Fibroblasts or their processes did not approach the pigment epithelium, thus maintaining the narrow future Bruch's...
Fig. 5. Electron micrograph of early Bruch's membrane showing two patches of elastin-like material among the collagen fibrils. Much glycogen (g) is present in this 49-day gestation stage. x37,200.

area. Granular endoplasmic reticulum with dilated cisternae had increased in amount both in fibroblasts and endothelium. Almost continuous basal lamina encircled the capillaries, but in some places the same structure was often observed around the fibroblasts. Collagen fibers were present mainly between fibroblasts and pigment epithelium and to a lesser extent between fibroblasts and choriocapillaris. Fenestrations were frequent at the pigment epithelial side.

Elastic fiber patches among the collagen fibers still appeared as small amorphous spots. The average diameter of the tubular fibrils surrounding such patches, was 119 Å.

G-67 days (M42). The photoreceptor inner segment had differentiated slightly from the neuro-epithelium, containing a basal body at the distal portion. The apical cell processes of the pigment epithelium had increased in number and length. The future Verhoeff's membrane became more
Fig. 6. Electron micrograph of portions of developing inner segments, pigment epithelium, Bruch's membrane and choriocapillaries in the 54-day gestation fetus. Basal-lateral Golgi complexes (Gc) are present in the pigment epithelium. The future Bruch's membrane shows occasional strips of aggregated fibrils having the morphologic appearance and staining properties of elastin fibers (el) near the endothelium of the capillaries. Collagen fibers (col) with periodicity, as well as patches of basal lamina-like material (bl) near the endothelial plasma-lemma occur. x6,000.

 accentuated and the fine fibrils radiating into the cytoplasm from this junction were also more prominent.

Between the pigment epithelium and choriocapillaris there were many collagen and fine fibrils attaching to the endothelium and fibroblasts. The amount of elastic fiber units had also somewhat increased. They had an irregular outline and were still located near the choriocapillaris. Some presumed elastic fibers were grouped around the invaded fibroblasts and between the fibroblasts and the choriocapillaris.

G-76 (M59) and 80 (M43) days. Slightly differentiated photoreceptor inner segments were in contact with many apical cell processes of the pigment epithelium, which had an increase in mature pigment granules, smooth surfaced endoplasmic reticulum, and mitochondria. Golgi apparatus was laterally and occasionally basally located and well developed, but the basal infoldings had not yet appeared.

Fibroblasts and their processes were still invading, mainly between the elastic tissue
Fig. 7. Electron micrograph of a portion of pigment epithelium and Bruch's membrane in the 100-day gestation fetus. Elastin elements (el) and collagen fibers with faint striation are shown in Bruch's membrane. \( \times 19,000 \). Inset: Light micrograph of the same region from which the electron micrograph was prepared, showing incipient outer segments reaching into the pigment epithelium (arrows). \( \times 800 \).

...and the choriocapillaris. Where there were no fibroblasts or their processes, elastin patches were aligned parallel to the endothelium and at a little distance from it. Fenestrations of the endothelium became gradually more numerous at the inner wall.

G-90 (M26) and 100 (M61) days. Neither the basal lamina of the pigment epithelium nor the shape of the basal infoldings were changed from the previous stages, but
smooth-surfaced endoplasmic reticulum and mature pigment granules were augmented. Fibroblasts with well-developed profiles of granular endoplasmic reticulum, surrounded by fine fibrils and debris, were partially lined by basal lamina-like material. Their invasion into the collagenous layer of Bruch's membrane was decreasing on the one hundredth day and there were some connections between the presumed elastic microfibrils and fibroblasts. Collagen fibers in Bruch's membrane at 100 days of gestation had an average diameter of 684 Å and definite periodicity (Fig. 7).

G-110 (M68) and 126 (M53) days. Golgi apparatus and smooth endoplasmic reticulum of the pigment epithelium were still developing but rough-surfaced endoplasmic reticulum was gradually diminishing. Pigment granules were mostly of the mature type, interspersed with a few melanosomes and premelanosomes.

Basal infoldings were well formed by the one hundred twenty-sixth day. Basal lamina followed the line of the tips of approximating infoldings but did not line the cell membranes in their extent.

Fibroblasts were not observed between the pigment epithelium and the choriocapillaris. Elastic fibers had increased to form an elastic "layer" having a linear arrangement with many disruptions. The pigment epithelium possessed almost the same structure as it does in the adult.

G-131 (M56), 143 (M54), and 160 (M31) days. Outer segments were developing to form structures like those on the one hundred sixtieth day of gestation just prior to birth. Numerous apical cell processes of the pigment epithelium surrounded the distal portion of the outer segments. Pigment granules were mostly of the mature type. Profiles of smooth-surfaced endoplasmic reticulum occupied most of the pigment epithelial cytoplasm, while rough-surfaced endoplasmic reticulum had further decreased in amount. The lamellated inclusion bodies, already occasionally present on the one hundred thirty-first day of gestation, occurred more frequently on the one hundred sixtieth day (Fig. 8 inset).

The elastic fiber layer was in quasi-parallel array to the base of the pigment epithelium and located a little nearer to the choriocapillaris. In general, the basal lamina of the pigment epithelium and choriocapillaris, the collagenous layer, and basal infoldings of the pigment epithelium did not change much during these stages, differentiating to almost adult structure near the end of gestation (Fig. 8).

Comments

Conventionally, Bruch's membrane may be subdivided into the following parts: (1) basement membrane of the retinal pigment epithelium, (2) inner collagenous zone, (3) elastic layer, (4) outer collagenous zone, and (5) basement membrane of the choriocapillaris. The first layer is of retinal derivation, the last layer is absent in the intercapillary zone, and, according to some views, actually pertains to the choriocapillary endothelium.

There are some morphological differences which show continuous changes in the adult Bruch's membrane between the posterior pole and the ora serrata. Nakaiizumi reported that in the human it was thicker in the peripheral one-third of the choroid by virtue of an increase in collagen in the inner and outer collagenous zones. Takei also observed the increased thickness of the collagenous layers, especially the inner one, the decrease of both the interruptions in the elastic layer and the basal infoldings of pigment epithelium near the ora serrata, when compared with those of the posterior pole. These changes correspond to the regional diminution of retinal function. In the present observations, therefore, we limited the examination to the posterior zone where retinal function would be best reflected.

Basal lamina of the pigment epithelium.

The basal lamina of the pigment epithelium had already appeared even in the earliest stages of rat fetuses, namely the thirteenth and sixteenth days of gestation, and was also noted in the present work,
Fig. 8. Electron micrograph of a portion of a pigment epithelial cell and Bruch’s membrane of a monkey fetus just prior to birth (160 days gestation) Bruch’s membrane has a well defined, interrupted elastic layer (el) between the irregularly distributed collagenous aggregates. There is a continuous basal lamina over the fenestrated endothelial cell (En). ×48,000. Inset: Low-power electron micrograph of apical portion of the same area showing ingested outer segment material. ×4,500.
namely on the twenty-seventh gestational day of the monkey embryo.

It was postulated that basement membranes at the base of epithelial cells in general are an epithelial secretion, and are not a condensation of ground substance. Cohen reported that the basement membranes completely enclose the optic and lenticular primordia from their inception, that they are well-defined on neuroectodermal structures before appearing on nearby blood vessels, and that the ectodermal basement membrane exists prior to lens formation at the eighth gestational day in the mouse. We confirmed his findings in the monkey embryos ranging from the twenty-seventh to the thirtysixth gestational days. The basal lamina in our specimen was clearly defined at the outer layer of the optic cup (future pigment epithelial layer) prior to the lens placode formation, when the site of the future Bruch’s membrane was occupied by sinusoids and immature fibroblasts and their processes. These observations support morphologically the possibility of epithelial secretion of basal lamina.

Collagenous layer. In the extracellular space between pigment epithelium and sinusoids from the twenty-seventh to the forty-third gestational days, some fibroblasts and filipodia had invaded and were surrounded by a few fine filaments and fibrils which increased in number and size and by the forty-sixth day formed collagen-type fibers. These fibroblasts, with cytoplasmic organelles indicating active protein synthesis, were usually at a narrow distance from the pigment epithelium, and the fine fibrils occurred mainly at the pigment epithelial side of the fibroblasts.

Involvement of fibroblasts in the production of collagen fiber precursors and mucopolysaccharides which form the interfibrillar ground substance was established by many biochemical, histochemical, and radioautographic studies. It is reasonable to assume that the collagen and fine fibrils seen between pigment epithelium and choriocapillaris in our series are the production of neighboring fibroblasts, but morphologic evidence alone cannot prove whether the basal lamina around the choriocapillary endothelium is formed by the endothelium or by fibroblasts. Between the sixtieth and eightieth days of gestation (midterm) we noted in many pigment epithelial cells a change of the Golgi apparatus to the basal position, very near to the basal lamina. Trelstad interpreted such a shift in the chick corneal epithelial cells as indicative of their participation in the production of connective tissue or ground substance precursors. Examination of our material does not allow any conclusions to be drawn as to the extent of pigment epithelial involvement in the production of these components of Bruch’s membrane.

During the second half of gestation, the collagenous layer gradually increased in fiber density and the basal lamina of the choriocapillary endothelium became well-defined in later stages, whereas the basal lamina of the pigment epithelium maintained its structure almost unchanged in the whole series of monkey fetuses.

Basal lamina of the choriocapillary endothelium. The sinusoids of the early choriocapillaris were irregularly distributed with only a few channels having flattened lumina with occasional fenestrations at the inner side and with no basal lamina around their cytoplasm on the thirty-eighth day of gestation. There were even fewer pores in corresponding vessels on the twenty-eighth gestational day. By the fifty-fourth day of development basal lamina-like structure lined faintly most of the choriocapillary endothelium.

Takei and Smelser observed in the developing tunica vasculosa lentis of rabbit and monkey fetuses that there was no basal lamina at the capsular side of the tunica vasculosa lentis on the fifty-fourth gestational day of the monkey, where there were only a few fibroblasts. The basal lamina gradually increased surrounding the whole capillary wall in later stages. It is believed that the basal lamina of the

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choriocapillary endothelium is formed by a secretion of the endothelium, but the possibility of the fibroblast partaking in the formation of the basal lamina cannot be excluded, since the fibroblasts has a fibrogenic or cementogenic ability, and there often occurred a basal lamina-like structure around its cell membrane in the earlier stages of our series.

Fibroblast and endothelium of choriocapillaris are both of mesenchymal origin, having morphological features characteristic of fibrogenic cells, namely a large prominent nucleus with one or more equally prominent nucleoli, the presence of an extensive Golgi apparatus, and net of rough-surfaced endoplasmic reticulum, vesicles and vacuoles of varying sizes and cytofilaments of 50 to 80 A in diameter.

Elastic layer. Presumptive elastic microfibrils appeared on the forty-ninth gestational day, at first very scantily, near the choriocapillaris. They congregated into irregular, small, amorphous spots surrounded by short fine fibrils, and clumps were increasing in amount up to approximately the one hundred tenth day. They became arranged in an interrupted line roughly parallel to the choriocapillaris and divided the collagenous mass into outer and inner portions. The elastic fibers were located near the fibroblasts which invaded mainly between elastic fiber and choriocapillaris at later stages, whereas the elastin groups occurred dispersed around the fibroblasts in the early stages. Fine fibrils were seen both around the endothelium and the fibroblasts attached to the already existing elastic fibers. The amorphous zone of elastic fibers has a collagenous property, while elastic microfibrils are noncollagenous in nature.

Ross and Greenlee found early elastic fiber formation to consist of parallel aggregates of fibrils which sometimes appeared to be beaded and were approximately 100 A in diameter. These aggregates often developed in close proximity to cells that appeared to be typical fibroblasts.

Our observations indicate both fibroblast and endothelium to be closely related to the formation of the early elastic layer in Bruch's membrane.

As noted by Braekevelt and Hollenberg, marked increase of basal infoldings of pigment epithelium is the most characteristic sign of the onset of differentiation resulting in a greatly increased surface area available for transport at a time when the nutrient requirements of the outer retina are rapidly increasing.

The collagenous and elastic layers are almost completely formed prior to the differentiation of photoreceptors (Fig. 7). Gradual increase in the number of fenestrations of the choriocapillaris, of smooth surfaced endoplasmic reticulum, and of apical cell processes in pigment epithelium, are mostly accomplished before photoreceptor formation. They continue in development after the appearance of photoreceptors, but the changes are not so marked as those that occurred previously.

Leeson stated that anatomically and developmentally, choriocapillaris, lamina elastica, pigment epithelium, and outer segment are closely related and from the nutritional aspect, these layers should perhaps be considered as a unit.

Light micrographs were prepared by Miss Mary E. Rayborn of the Department of Ophthalmology Research, Columbia University.

REFERENCES


