Human fetal iridocorneal angle: a light and scanning electron microscopic study

P G McMENAMIN

From the Department of Anatomy and Human Biology, University of Western Australia, Nedlands, Perth 6009, Western Australia

SUMMARY  The iridocorneal angle and inner layers of the trabecular meshwork in human fetal eyes were studied by scanning electron microscopy. Tissue from 32 eyes of 17 fetuses with a gestational age from 12 to 22 weeks were investigated in order to determine the morphological changes in the cellular lining of the anterior chamber angle recess during development. The findings indicate that, although hexagonal corneal endothelial profiles extend almost to the angle apex in a few of the younger eyes examined (12–14 weeks), the lining is always perforated by a few discrete intercellular gaps (2–6 μm diameter). As development progresses it becomes clearer that the maturing meshwork is lined by uveal trabecular endothelial cells which are morphologically distinguishable from corneal endothelium. The frequency and size of the gaps between the inner uveal trabecular endothelial cells increase and are well developed by 18–20 weeks, clearly providing a route of communication between the fetal anterior chamber and the developing intercellular spaces in the primitive trabecular tissue. The implications of these observations on the ‘Barkan’s membrane’ theory of congenital glaucoma are discussed.

There have been several hypotheses on the cause of congenital or primary infantile glaucoma. The suggestion that obstruction to aqueous humour drainage from the anterior chamber was due to a semitransparent cellular membrane which had failed to regress normally from the iridocorneal angle was proposed by Barkan. The concept of ‘Barkan’s membrane,’ which was based on gonioscopic observations, found favour among surgeons. However, histopathological studies could not confirm its presence. Further support for the membrane theory was the notion that the human fetal chamber angle is normally lined by a complete layer of cuboidal corneal endothelial cells until late in development. The timing of the normal perforation or ‘regression’ of this membrane, which has been suggested as 28–32 weeks, or in the last few weeks before birth, appears to have found general acceptance and is often quoted in textbooks and reviews. However, some authors believe that such a continuous lining exists neither in the normal fetal eye nor in cases of congenital glaucoma.

Although it is now over 30 years since Barkan first made his proposal, confusion still exists in this area. This is due partly to the restricted number of ultrastructural studies which have specifically addressed the nature and developmental changes in the lining of the primitive trabecular meshwork in the normal human chamber angle. Previous investigations have often been limited in the number of fetal eyes available for study. To the author’s knowledge there has been only one previous scanning electron microscopic (SEM) study of the lining of the chamber angle in human fetal eyes, in a previous light microscopic and ultrastructural investigation the present author has described the pre- and postnatal development of the lining of the human anterior chamber angle. It was shown that in fetuses as young as 12–14 weeks old the cellular covering of the trabecular anlage is incomplete and intercellular gaps become larger and more frequent as development progresses. The aim of this paper is to present convincing SEM evidence which supports the findings of the previous study. Obtaining an unobstructed view of the angle recess, which is not normally possible, proved to be crucial to the aims of this investigation.

Correspondence to P G McMenamin.
**Materials and methods**

The fetuses used in this investigation included 15 normal and two in which neural tube defects were present. Details of the 17 cases (32 eyes) are provided in Table 1.

Gestational age was calculated from the date of last menstruation, ultrasonographic data, and morphometric data obtained at the time of termination, which was prostaglandin-induced. The fetuses were stored at 4°C until the time of enucleation, which was generally less than two hours. The eyes were immersed in 4% cacodylate buffered glutaraldehyde fixative (pH 7.4). After a minimum of 24 hours' fixation the eyes were carefully divided at the equator and the lens gently freed from the anterior segment without damage to the optic cup. Each anterior segment was cut into meridional wedges 1 or 2 mm in thickness. In small eyes it was possible to obtain only four or five pieces of tissue, while in older eyes up to 15 wedges of limbal tissue could be obtained. One or two pieces of tissue from each eye were reserved for scanning electron microscopy (SEM), while the others were embedded in Araldite for light microscopy (LM) and transmission electron microscopy (TEM). Tissue for SEM was osmicated, dehydrated, critical point dried, sputter coated with gold, and examined in a JEOL JSM T300 (Department of Anatomy, Glasgow University, Scotland) or a Philips 505 (Department of Anatomy and Human Biology, University of Western Australia, Perth, Western Australia). After initial examination of intact wedges of limbal tissue it was apparent that to obtain a clear view of large areas of the angle recess further dissection would be necessary. Therefore the wedges were either further divided in the meridional plane, cut tangentially, or the primitive iris/ciliary body and
neural retina were peeled gently from the corneoscleral tissue as illustrated in Fig. 1. This last procedure made a thorough examination of the transitional zone between corneal endothelium and the cells covering the angle recess possible.

**Results**

The anterior segment tissues of the eyes including those with neural tube defects appeared normal on macroscopic and microscopic examination. The eyes were divided into three groups for ease of description.

**12-14 week fetal period**

Primitive ciliary folds were not discernible by SEM on the inner surface of the optic cup at 12 or 13 weeks but became barely discernible by 14 weeks. No true 'iridocorneal angle' existed at 12 or 13 weeks, since the iris had not yet begun development. Therefore the primitive anterior chamber was limited posteriorly by loose vascular mesenchymal tissue which passed over the anterior or outer aspect of the optic cup margin to form the pupillary membrane of the tunica vasculosa lentis (Fig. 2a). The angle apex lay in an anterior position relative to the optic cup margin, while the trabecular anlage was approximately level with it at this stage in development (Fig. 2a). The collapse of the loose mesenchyme of the pupillary membrane on to the cornea made the examination of the apex of the recess difficult in undissected specimens. However, once the anterior portion of the optic cup (including primordial ciliary body and iris) was carefully removed from the wedges of anterior segment tissue as shown in Fig. 1 the true nature of the cellular lining of the angle recess became apparent.

SEM examination of the inner corneal surface distant from the angle demonstrated the character-

---

**Fig. 2**  *Micrographs of the anterior chamber angle tissues in a 13-week-old human fetal eye.* (a) Low power LM to illustrate the general configuration of the anterior segment tissues at this stage in development. c=cornea, s=sclera, ocm=optic cup margin, TA=trabecular anlage, arrow= apex of anterior chamber angle recess. (b) SEM of corneal endothelial surface showing general topography of the polyhedral cells. Note the two globular cells, probably macrophages, which are commonly associated with the tunica vasculosa lentis during development. (c) SEM of the transition zone between corneal endothelial cells (ce) and uveal trabecular endothelial cells (te). An artefactual crack (large arrow) can easily be distinguished from the intercellular gaps. The point where the tissue has separated during dissection, approximately at the angle apex, is indicated (small arrows). The gaps are shown at higher magnification in (d). Magnifications: (a) ×100, (b) ×1200, (c) ×820, (d) ×1600.
istic arrangement and surface topography of the endothelial cells, namely, hexagonal shape, slightly elevated cell borders (Fig. 2b), and often a centrally located single cilium. Within 40–50 μm of the apex of the primitive chamber angle recess the arrangement of the cells became less regular, borders were less discrete, and cilia were less obvious (Fig. 2c). These cells will be referred to as uveal trabecular endothelial cells (TECs). The thickness of corneal endothelial cells, as seen in views of the lateral cut surface, decreased near the angle apex, where they merged with the uveal TECs and ultimately with the more squamous pleomorphic cells which covered the vascular mesenchymal tissue of the iris stromal anlage. Numerous rounded or ovoid intercellular gaps with maximal diameter of 2–8 μm were usually identifiable within 20–30 μm of the apex. The openings were occasionally bridged by delicate cytoplasmic processes or filopodia (Fig. 2d). On inspection of the lateral cut surface it was evident that these gaps overlay the loose wedges of mesenchyme of the trabecular anlage.

15–17TH WEEK FETAL PERIOD

By 15 weeks of gestational age the optic cup margin, representing the primitive iris, had grown forward from the ciliary folds. The optic cup margin therefore now lay anterior relative to the apex of the iridocorneal angle (Fig. 3a,b). SEM examination of the inner surface of the optic cup (Fig. 3b) revealed smooth-surfaced simple ciliary folds, 300–500 μm long and =100 μm in width, orientated in the anteroposterior plane. A smooth-surfaced area (100–150 μm long) representing the primitive iris projected anteriorly from these folds. A distinct angle now existed between the developing iris and posterior corneal surface. The vessels passing over the optic cup margin were clearly discernible (Fig. 3b).

The typical hexagonal appearance of corneal endothelial cells (Fig. 3c) was still conspicuous to within =50 μm of the angle apex, where the transition occurred to thinner cells with a more pleomorphic arrangement and less distinct cell margins (Fig. 3d,e). In the transitional region near the angle apex intercellular gaps were easily identified in dissected specimens. These gaps were of similar dimensions and frequency to those in the 12–14 week age group, that is, 2–8 μm in diameter. On close examination it is evident that many gaps appeared subdivided or occurred in clusters and were only partly bound by a sharp cellular border (Fig. 3e). Fine cytoplasmic filopodia often traversed the gaps. On examination of the cut surface the mesenchyme of the trabecular anlage appeared more loosely arranged.

Examination of the uncovered trabecular anlage in dissected specimens revealed bundles of circumferentially orientated collagen fibres loosely arranged among the irregular shaped mesenchymal cells.

18–22 WEEK FETAL PERIOD

Light microscopic examination of tissue from this period showed a more highly developed ciliary body and iris including the appearance of the sphincter pupillae and ciliary muscle primordia (Fig. 4a). A clear external and internal scleral sulcus were identifiable at the corneoscleral junction. SEM revealed larger ciliary folds with a more undulating epithelial surface than in younger eyes (Fig. 4b). Minor folds were now evident between the major folds. SEM examination of the angle revealed that typical corneal endothelial cells terminated more anteriorly in this age group, giving way to the more irregular uveal TECs. Intercellular gaps were now more common than in younger eyes and occasionally could be identified in undissected specimens viewed from the lateral aspect (Fig. 4c). Their shape and size range (2–15 μm in diameter) was more variable than in the younger eyes. They were regularly spaced along the inner surface of the trabecular anlage (Fig. 4d) and would obviously allow communication between the anterior chamber and the now well developed intercellular or intertrabecular spaces.

Fig. 3 Micrographs of the anterior chamber angle structures in 15-week old human fetal eyes. (a) Low power LM to illustrate the general arrangement of the tissues. Note the developing iris (compare with Fig. 2a), ciliary folds (cf) and the trabecular anlage (TA) at the apex of the angle recess (arrow). (b) SEM of a large wedge of limbal tissue in which the neural layers of the optic cup have not been removed. The difficulty of viewing the lining of the angle recess (arrow) from the lateral cut surface is clearly illustrated. (c) SEM of normal corneal endothelial cells a short distance from the chamber angle recess which demonstrates the prominent cell borders, cuboidal morphology and the primitive Descemet’s membrane (dm) on their basal aspect. (d) SEM of corneoscleral tissue after removal of the neural tissue. The plane of cleavage between the neural layers and the corneoscleral tissue corresponds approximately to the arrow in (a). The line where separation occurred is clearly discernible anterior to the trabecular anlage (TA). The transition zone between corneal endothelial cells (cc) and the uveal TECs (ut) is clearly demonstrated. The intercellular gaps between the TECs are shown at higher magnification (boxed area) in (e). Note that some gaps have only one sharply delineated margin (arrow). Abbreviations (a)–(c): c=cornea; oc=optic cup margin; cc=corneal endothelium; cf=ciliary folds; TA=trabecular anlage; pm=vessels of pupillary membrane (tunica vasculosa lentis). Magnifications: (a) ×430, (b) ×140, (c) ×2300, (d) ×220, (c) ×870.
Human fetal iridocorneal angle: a light and scanning electron microscopic study

Fig. 3
Fine cytoplasmic filopodia were a more prominent feature of the uveal TECs than in the younger eyes. Only occasionally were single rounded cells with membrane ruffles, which may represent macrophages, identified close to the inner surface of the trabecular anlage.

Discussion

The present scanning electron microscopic study of the cellular lining of the anterior chamber angle is part of a comprehensive investigation of the normal prenatal development of the human aqueous outflow pathways. Earlier observations made by the present author at the LM and TEM level refute the suggestion of previous investigators that the inner surface of the meshwork is lined by a continuous layer of endothelial cells until late in development. The aim of the present study was to provide supplementary evidence by an additional more appropriate method (SEM) of examining the uveal meshwork inner surface. However, during examination of the lateral cut surface of meridional slices of limbal tissue difficulty was experienced in obtaining a clear view of large enough areas of the cellular covering of the chamber angle recess. Furthermore the view was often totally obscured by the tendency of the loose vascular iris mesenchyme and pupillary membrane to collapse over the angle. With appropriate care the inner neural tissues of the optic cup (retina, ciliary folds and iris) and some mesenchyme could be gently separated from the underlying corneoscleral tissue in the dried specimens (Fig. 1). Fortunately the tissue usually split at the angle apex exposing the inner surface of the trabecular anlage. As previously pointed out by Kupfer, the weak forces between the inner neural tissues and the outer corneoscleral layers which facilitated such damage free separation could easily be misinterpreted as a plane of 'cleavage', as indeed occurred in earlier histological studies. In the present study dissection or shrinkage artefacts, almost unavoidable hazards of SEM preparation, were easily differentiated from the smooth edged ovoid or round intercellular gaps in the lining of the trabecular anlage. The advantages of SEM examination of tissues dissected in this manner exceeded the limitations described, since it allowed a true appreciation of the size, shape, and frequency of the intercellular gaps over a large area of tissue. This information could otherwise be accrued only by tedious serial section studies.

SEM observations of tissue prepared in the manner described provide convincing corroboratory evidence that there are extensive channels of communications between the fetal anterior chamber and the developing intertrabecular spaces via gaps in the cover of uveal trabecular endothelial cells as noted in a previous study. Intertrabecular gaps, while becoming more common by 18–22 weeks, were a consistent feature even in 12–14 week old fetal eyes. One limitation of the present investigation was the unavailability of material older than 22 gestational weeks. Presumably, however, the gaps would continue to increase in size and frequency in this period to reach the infant configuration.

The only previous SEM investigation of the inner surface of the human fetal aqueous outflow system claimed that the angle was covered by a continuous monolayer of endothelial cells until the eighth month of development, though no evidence was provided, making comparison with the present study difficult. Intercellular gaps deep in the angle recess, only truly apparent in the present study after dissection, may have been hidden in the specimens examined by Hansson and Jerndal. Surprisingly, no details of the numbers or ages of specimens were provided by these authors. It is difficult to reconcile why previous ultrastructural and light microscopic studies have claimed that the trabecular surface has a complete endothelial covering until the seventh or eighth month of development when clearly the present SEM and previous LM and TEM evidence show large numbers of sizeable intercellular fenestrations or gaps. Some previous investigators, more in accordance with the present study, have considered the inner surface of the trabecular anlage to be covered by loosely organised layers of mesenchymal or trabecular cells, though these studies have either not included younger eyes or have failed to emphasise the nature and extent of the intercellular gaps in early fetal eyes.

In an SEM investigation of the iridocorneal angle...
Human fetal iridocorneal angle: a light and scanning electron microscopic study

Fig. 4
development in macaques Van Buskirk identified small intercellular gaps in the lining in the first trimester, whereas by the second trimester only the anterior half to two-thirds of the trabecular meshwork was covered by intact endothelium. Despite evidence of gaps, as in the present study, this was interpreted as further support of late regression of a pretrabecular membrane in the normal fetal eye. Since many species of non-human primates retain an operculum and lamellate uveal trabeculae into adult life" one would perhaps expect different remodelling events in development. Therefore the appearance in the human of large and numerous gaps may be an important species difference of functional significance.

The presence of openings in the chamber angle lining early in gestation correlates with physiological evidence that some degree of aqueous drainage is effective by 17-18 weeks of fetal life and gradually increases during development. Indeed channels of communication between the anterior chamber and the trabecular anlage may be vital to the normal formation or cavitation of intertrabecular spaces, which were evident in the uveal layers as early as 12-14 weeks in the present study. In a study of the developing cat angle Richardson et al. correlated increased volume of the intertrabecular spaces with the appearance of perforations in the 'membrane' which temporarily covers the meshwork.

The concept of an uninterrupted endothelial membrane (Barkan's membrane) lining the trabecular anlage during normal development has found support among clinicians who envisage an abnormal persistence or failure of perforation of this membrane to be a likely cause of obstruction to aqueous outflow and therefore of congenital glaucoma. Disruption of such a membrane during goniotomy or trabeculotomy appeared to correlate with a clinically demonstrable increase in aqueous outflow. In contrast, histological evidence of a membrane continued to elude pathologists. This was considered by supporters of the membrane theory to be due to difficulties in obtaining suitable eyes at the early stages of the disease or to disruption of the membrane during processing. There are many theories on the aetiology and pathogenesis of congenital glaucoma including failure of atrophy or cleavage; defective neural crest cell migration; and excessive formation of collagen which prevents the normal posterior sliding of the ciliary body. On the basis of the present evidence it is clear that these explanations should no longer include failed regression of a complete endothelial pretrabecular membrane, since even in the early fetal eyes openings were present which appear of sufficient frequency and size to allow unpimpeled egress of aqueous from the anterior chamber. Alternative explanations of Barkan's membrane may include condensed pectinate ligaments, excess proteoglycans on the inner surface of the meshwork (W R Lee, personal communication), or the formation of secondary pretrabecular membranes due to corneal endothelial downgrowth.

Theories on the aetiology and pathogenesis of primary congenital glaucoma and other developmental abnormalities of the anterior segment, such as the iridocorneal endothelialisation (ICE) syndromes, should take the common neural crest origin of the ocular mesenchyme into account. These cells give rise to corneal stroma, corneal endothelium, iris stroma, ciliary muscle, ciliary body connective tissue, and trabecular endothelial cells. Vascular endothelial cells, and therefore presumably the endothelium of Schlemm's canal, arise independently from mesoderm. This information has been elucidated in birds by means of chick/quail chimera and autoradiographic studies (see Noden for review). Although definitive proof in mammals of a comparable ontogeny is still not available, it seems likely especially since craniofacial abnormalities are often associated with anterior segment dysgenesis, including congenital glaucoma. Theories of delayed maturation as the cause of congenital glaucoma need not be restricted to the inner or uveal portion of the meshwork. Equally important morphogenetic and remodelling events are occurring throughout the aqueous humour outflow system which if disturbed could have serious functional consequences. These developmental changes will form the basis of future reports.

The author acknowledges the help of Dr Jeanne Bell and coworkers for the collection of the human fetal eyes and Karen McLachlan for technical assistance. This research was partly carried out in the Department of Anatomy, Glasgow University. Funding for this research was obtained from the W H Ross Foundation (Scotland) for the Study of Prevention of Blindness and the Medical Faculty Research Fund, the University of Western Australia.

References

Human fetal iridocorneal angle: a light and scanning electron microscopic study


Accepted for publication 18 May 1989.
Human fetal iridocorneal angle: a light and scanning electron microscopic study.
P G McMenamin

doi: 10.1136/bjo.73.11.871

Updated information and services can be found at: [http://bjo.bmj.com/content/73/11/871](http://bjo.bmj.com/content/73/11/871)

**Email alerting service**

*These include:*

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to: [http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to: [http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to: [http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)