A Reappraisal of the Development of the Eyelids

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Summary

The eyelids develop from ectoderm and from mesoderm. This evolution passes through distinct phases of maturation. Contemporaneously, the anterior segment of the eye takes form. The role of the amniotic fluid in the development of the eyelids is considered.

The purpose of this report is to describe the development of the eyelids. The evolution of the eyelids from early embryonic life through different stages of development to term is examined.

Materials and Methods

Sixty-four human eyes (Fig. 1) from embryos and fetuses ranging in size from 13.6 mm to term were examined.^{1,2} Between 60 and 110 microscopic sections were examined, respectively, from each embryo and fetus. The normal development of the embryo/fetus was an essential prerequisite, so the embryo/fetus from septic abortion was not accepted for investigation. If the mother had been exposed to teratogenic drugs during pregnancy, the embryo or fetus was excluded from the survey. Where salpingectomy was done (for ectopic pregnancy), the fallopian tubes were dissected with the aid of a dissecting microscope and the embryos retrieved. Usually these embryos were found within blood clots.

Age of Embryo and Fetus

The maternal history, with reference to duration of pregnancy, was found to be unreliable. A more satisfactory correlation was found between the clinical size of the uterus and the age of the embryo/fetus. Where possible, however, a correlation was attempted between the clinical age of the embryo/fetus and its crown-rump length. A micrometre gauge was used for measuring the length of

the fetus up to 170 mm; thereafter a centimeter metal rule was used. Development of the eye was also correlated with development of other body organs. It was then possible to assess this interrelationship, and therefore assign an age to the embryo-fetus.^{3,4} Specimens were examined microscopically with a dissecting microscope. Small specimens (less than 80 mm crown/rump length) were fixed for at least one week and larger specimens (larger than 80 mm crown/rump length) for at least two weeks.

All specimens were serially sectioned in conventional anatomic planes, either frontal (coronal) or transverse (horizontal). Stains used on the sections were heamatoxylin-eosin, Masson trichrome, Mallory's phosphotungstic acid heamatoxylin, Verhoeff's van Gieson elastic, Bodian's silver nitrate and Luxol fast blue.

Results

It was noted that the eyelids pass through five stages of development:

- (1) A stage of eyelid folds (15 mm to 35 mm—6 weeks to 8 weeks);
- (2) a stage of eyelid fusion (35 mm to 175 mm—8 weeks to 5 months);

Fig. 1. Crown-rump length (mm) and age (weeks) of embryos and fetuses examined.

- (3) a stage of development of specialised structures of the eyelids (35 mm to 250 mm—8 weeks to 7 months);
- (4) a stage of eyelid separation (175 mm—250 mm—5 months to 7 months);
- (5) a stage of eyelid maturation (250 mm to term).

Up to the size of approximately 15 mm, the optic vesicles are bathed in amniotic fluid. The cornea prior to the lid fusing is covered by three to four layers of epithelium, which are continuous with the surface ectoderm.

Stage of lid folds (15 mm to 35 mm, 6 weeks to 8 weeks).

At about 15 mm in size, the upper lid folds develop superiorly from a proliferation of mesodermal cells from the medial and lateral protuberances extending from the frontal nasal process (Fig. 2). The lower lid develops from a proliferation of mesodermal cells arising from the maxillary process (visceral

mesoderm). These folds grow towards one another. They are covered by epithelium on their respective anterior and posterior surfaces. There are two layers of epithelium on the anterior surface and a single layer of epithelium on the posterior surface of the lid fold. The posterior single layer of epithelium becomes modified to form the tarsal conjunctiva and merges at the fornices with the bulbar conjunctiva.

Stage of eyelid fusion 35 mm to 175 mm, 8 weeks to 5 months (Fig. 3).

The superior and inferior lid folds elongate and the epithelial margins fuse just anterior to the horizontal meridian of the eye (Fig. 4). A rather shallow conjunctival cavity results. At the site of fusion there are four opposing layers of epithelium; two layers of superficial epithelium from the upper lid and two from the lower lid. Contemporaneously with the fusion of the lids, an orchestrated development of morphological changes is triggered in the anterior segment of the eye.

Stage of development of specialised structures of the lids (35 mm to 250 mm—8 weeks to 7 months).

It is during the stage of lid fusion that the specialised structures of the lid develop. These include:

- (1) the orbicularis oculi muscle (Fig. 5);
- (2) the tarsal plates and meibomian glands;
- (3) the lacrimal puncta and canaliculi;
- (4) the skin appendages;
- (5) the conjunctiva.

The orbicularis oculi muscle develops from local mesenchymal tissue of the second vis-

Fig. 2. Eyelid fold (arrow) developing from frontal nasal process (30 mm, Hematoxylin-eosin $\times 3$).

Fig. 3. Lids fused in 4 month fetus.

Fig. 4. Fusion (arrow) of upper and lower eyelid folds (80 mm, Heamatoxylin-eosin $\times 3$).

ceral arch and develops in a similar manner to that of the extraocular muscles. The stages of muscle development include the stage of mesenchymal cell (up to 36 mm size embryo), stage of early myoblast formation (13.6 mm size embryo), the myoblast stage (from 38.2 mm size embryo), the fusion of myoblast stage (from 54 mm size embryo), the myotube stage (from 54 mm size embryo), and mature

Fig. 5. Orbicularis oculi muscle (arrow) develops in lid and surrounds canaliculi (open arrow) (180 mm Heamatoxylin-eosin $\times 3.5$).

muscle stage (from 80.0 mm size fetus.)¹ The sphincter-like orbicularis oculi muscle appears as a sheet covering the entire anterior surface of the lids during the fusional stage of development. The pretarsal, preseptal and orbital parts of the orbicularis oculi muscles are noted more distinctly at the 250 mm stage of development. With separation of the upper and the lower lids this sheet-like muscle is divided. The muscle extends medially to be attached to the medial palpebral ligament and also to the lateral palpebral ligament (*vide infra*).

The anlage of the tarsal plate is first noted at about the 40 mm stage of development. It develops from local mesenchymal tissue in the lid which becomes thickened and more compact. The tarsal plate lies posterior to the developing orbicularis oculi muscle. This compacted mesenchymal tissue extends from the eyelid margin to the site where the levator palpebrae superioris muscle develops at the superior margin of the lid (40 mm). The levator palpebrae superioris muscle passes through similar phases of development to those of the extra ocular muscles.¹ The levator palpebrae superioris muscle, the superior rectus muscle, and the superior oblique muscles, develop from the same mesodermal complex, i.e., the superior mesodermal complex (Figs 6, 7).^{1,6,7} By a process of differential growth and degeneration, these muscles separate.

Fig. 6. Levator palpebrae superioris (LPS), superior rectus (SR), superior oblique (SO), part of lateral rectus (LR) and part of medial rectus (MR) muscles arise from the superior mesodermal complex (light stipple); inferior rectus (IR), part of medial rectus (MR), and part of lateral rectus (LR) muscles arise from the inferior mesodermal complex (dark stipple).

Fig. 7. The levator palpebrae superioris tendon (arrow) differentiates from the superior rectus (double arrow) and the superior oblique (open arrow) tendons. All three tendons are derived from the superior mesodermal complex (60 mm, *Heamatoxylin-eosin* $\times 2.5$).

With growth and maturation, the superior tarsal plate becomes D-shaped, the horizontal inferior border becoming the inferior lid margin. The levator palpebrae superioris muscle, merges with the convex superior border, with the anterior surface of the tarsal plate (60 mm) and with the skin anteriorly via septae which pass through the substance of the orbicularis oculi muscle and fuse with the superficial epidermal layer (80 mm) forming the superior tarsal sulcus.

The inferior tarsal plate develops *pari passu* with that of the upper lid (40 mm), however, with maturation it remains sausage-shaped. In man, there is no muscle attached to the inferior border of the tarsal plate. The mesodermal tissue at the inferior border thickens and develops into fibrous tissue and extends to the inferior orbital margin as the septum orbitale. The medial and lateral collateral ligaments develop from the mesodermal tissue adjacent to the medial and lateral aspects of the tarsal plate (80 mm) and fuse with the adjacent periorbita of the medial and lateral tubercles respectively.

The anlage of the meibomian glands are not noted until 40 mm. At this stage of development, epithelial buds from the lid margin develop in the flattened fibrous tissue of the tarsal plate. By a process of differential degeneration in the centre of this epithelial core, ducts develop from the lid margins and extend the length of the tarsal plate. By the 60 mm stage of development a central tube is present within the tarsal plate. By 100 mm,

the meibomian glands are lined by cuboidal-like secreting epithelial.

The skin appendages develop from the two-layered epithelium lining the anterior surface of the lids. During the 35 mm stage of development, there are two layers of epithelium. The superficial layer is flattened and the deeper layer is cuboidal. During the 60 mm stage of development, three layers of cells develop from the basal cuboidal cells (the future stratum germinativum). Hair develops at this time. Keratin is present in the superficial layer of the epithelium (150 mm). The first crop of the lanugo hair covers the face and lids of the fetus (160 mm) (Fig. 8). This is shed just before and after birth. The lanugo hair in the region of the eyebrow, however, persists throughout life. Eyelashes are derived from the ingrowth of surface epithelium while the lids are fused (35 mm to 175 mm). A solid core of epithelium grows into the anterior margin of the fused lid margins (40 mm) and later hair shafts are noted in the follicles (90 mm). The glands of Moll and Zeis also develop from epithelial ingrowths (110 mm).

The tarsal conjunctiva develops from a single layer of epithelium on the posterior surface of the lids and becomes mucous membrane (50 mm). Goblet cells develop in the conjunctiva after the lids have separated (200 mm). The accessory lacrimal glands of Krause are developed by epithelial ingrowths of the conjunctiva at about the 50 to 60 mm stage of development. These epithelial cores canalise by 110 mm.

The puncta and canaliculi develop from a core of superficial epithelium that becomes (50 mm) buried below the surface of the upper

Fig. 8. Lanugo covering face, 5 months fetus.

and the lower lids occupying the medial 1/6 of these structures (Fig. 9).⁸ The trailing epithelial cells between the surface epithelium and the anlage of the future canaliculus atrophy. There is rapid growth of the maxilla compared to that of the frontal bone and the inferior canaliculus becomes stretched laterally.⁹ The inferior punctum therefore lies lateral to the superior punctum. Canalisation of the puncta and the canaliculi occurs between the 60 and 65 mm stage of development.

Prior to the lids forming, the optic vesicle and therefore the cornea are exposed to the amniotic fluid. In embryos ranging in length from 13.6 mm to 28.0 mm, the corneal epithelium, which is 3 to 4 cells depth, is noted to be continuous with the surface ectoderm. Once the lids have fused (35.0 mm), the epithelial layer is reduced to a two layer structure. Only in the 210 mm fetus does the corneal epithelium become a 4 to 5 layered structure as is recognised in the term fetus.

Fig. 9. Core of buried epithelium (arrow) forms from the superior and inferior canaliculi (open arrow) (60 mm, *Heamatoxylin-eosin* $\times 20$).

Fig. 10. Diagram showing separation of lids occurring on the anterior and posterior surfaces of the epithelial plug.

Stage of Eyelid Separation (175 mm–250 mm, 5 months to 7 months). (Fig. 10).

The lids start separating at about the 175 mm stage of development beginning nasally and extending temporally. The epithelial plug (formed by two epithelial layers of the upper lid and two layers of epithelium from the lower lid) commence to keratinise first on the epithelial surface and then on the conjunctival surface (Fig. 11). This causes erosion of the epithelial plug. In addition areas of degeneration occur in epithelial cells in the plug. This is evidenced by fragmentation of the nuclei eventually forming dust-like particles. The cytoplasm changes from an eosinophilic to a heamatoxylin stain and by 300 mm stage of development vesicles are noted in the epithelial plug. With this constant erosion, and cellular degeneration, separation of the lids

Fig. 11. Separation of lids (arrow) commences with keratinisation of epithelial surface (200 mm, *Heamatoxylin-eosin* $\times 3$).

occurs. Fine elongated epithelial strands remain between the lids to term.

Stage of Eyelid Maturation (250 mm to term).

Eyelid movement occurs about the 250 mm stage of development when nerve fibres in the orbicularis oculi and levator palpebrae superioris muscles are noted to be mature. The lanugo hair degenerates except for the region of the eyebrows. The lid margins, at the site of the remaining interpalpebral strands, become smooth. The epithelial plugs in the meibomian ducts disintegrate and meibomian secretions are noted just before term.

Discussion

Amniotic fluid may play a role in the development of the lids, although this has not been proved scientifically. Up to about the 35 mm stage of development the optic vesicle is exposed to the amniotic fluid in which there is a high concentration of sodium chloride. However, at about 12 weeks (70 mm),¹⁰ the kidney starts functioning and while the concentration of sodium chloride commences to decrease, the urea, uric acid and creatinine content of the amniotic fluid increases.¹¹ The lids fuse (35 mm) prior to renal function commencing (70 mm). It is during the phase of lid fusion that the cornea matures developing five layers of epithelium, Bowman's membrane and Descemet's membrane so that by the time the lids separate the cornea is well protected from the renal excretory contents within the amniotic fluid. (Fig. 12).

Thyroxine (T₄) and cortisone may play a role in lid closure. Juriloff¹² noted that in rats, the injection of thyroxine (T₄) prevented the eyelid closure defect in Ig^{m1}/Ig^{m1} fetal mice. Watney and Miller¹³ and Harris¹⁴ noted that cortisone administered to pregnant dams prevented expression of the open eye defect in Ig^{m1}/Ig^{m1} fetuses. These authors (Juriloff,¹² Watney and Miller¹³ and Harris,¹⁴) are of the opinion that thyroxine or cortisone causes growth of the eye lids across the cornea and may result in 'premature maturing of the epidermis.' There is no clinical evidence in the human being to show that there is an increase of thyroxine or cortisone in amniotic fluid

during the stages of the formation of the eye lid fold (15 mm–35 mm) or during the stage of eye lid fusion (35 mm–175 mm). During the phase of lid closure, the cornea matures in terms of developing five layers of epithelium a Bowman's membrane, thickened stroma, Descemet's membrane, and an endothelial layer.

Separation of lids occurs over a protracted period of time [175 mm (5th month) to 250 mm (7th month)]. The mechanism of eye lid separation is not well understood. During this period of separation, keratinisation of the epithelium at the junction of the upper and lower lids commences. The process is noted initially at the medial canthal region. Triangular defects of keratinisation and degeneration occur at the fusional site both on the epithelial and on the conjunctival surfaces. As the process continues, the lid margins separate. It is difficult to assess the role of the levator palpebrae superioris muscle in the separation of

Fig. 12. A diagrammatic representation of lid development correlated with substances in the amniotic fluid.

the lids. While the muscle is distinguishable in its development as early as 54 mms, cross striations are not noted until the 80 mm sized fetus. The inverted D-shaped tarsal plate becomes discernable about the same time. This may account for the levator palpebrae superioris muscle contracting, 'elongating' and therefore moulding the malleable tarsal plate to which it is adherant not only to the upper convex border but to its anterior surface as well. During the period of fusion of the lids, the meibomian glands develop and by the time the lids are separated (250 mm) acini are present in the glands. While no lytic substances have been demonstrated in meibomian secretions, which would aid lid separation, its oily consistency would certainly assist in maintaining the separation of the lids.

Cohen,^{15,16} having isolated a protein (epidermal growth factor) from the submaxillary gland of the rat, noted that it caused premature opening of the lids in the rat as early as seven days instead of the normal twelve to fourteen days. This protein appeared to enhance keratinisation of epithelium and caused a breakdown in the epithelial plug between the upper and lower lids. Orth¹⁷ has found that epidermal growth factor is present in human amniotic fluid from at least 20 weeks of development. It is tempting to suggest that the epidermal growth factor plays a role in the separation of the lids by causing keratinisation of the epithelial cells at the junction of the upper and lower lids.

References

- ¹ Sevel D: The origins and insertion of the extraocular muscle. Development, Histological features, and clinical significance. *Tr Am Ophthalmol Soc* 1986, **84**: 488-526.
- ² Sevel D: A reappraisal of the origin of human extraocular muscles. *Ophthalmology* 1981, **88**: 1330-8.
- ³ Arey LB: Developmental Anatomy. Philadelphia: WB Saunders 1942: 129-133.
- ⁴ Tuchmann-duPlessis H, David G, Haegel P: Embryogenesis Illustrated Human Embryology. London: Chapman and Hall 1972: 88-91.
- ⁵ Sevel D: Development of the nerves of the extraocular muscles. In Reinecke RD, ed. Strabismus. New York: Grune and Stratton 1984: 645-57.
- ⁶ Sevel D: Ptosis and underaction of the superior rectus muscle. *Ophthalmology* 1983, **91**: 1080-5.
- ⁷ Sevel D: Brown's Syndrome. A possible etiology explained embryologically. *Ophthalmology* 1981, **18**: 26-31.
- ⁸ Sevel D: Development and congenital abnormalities of the nasolacrimal apparatus. *J Ped Ophthalmol and Strab* 1981, **18**: 13-9.
- ⁹ Sevel D: Insufflation treatment of occluded nasolacrimal apparatus. *J Ped Ophthalmol and Strab* 1982, **89**: 329-34.
- ¹⁰ Hewer EE: Secretion by the human foetal kidney. *Qart J Exp Physiol* 1924, **14**: 1924.
- ¹¹ Seeds AE: Water metabolism of the foetus. *Am J Obstet Gynecol* 1965, **92**: 727.
- ¹² Juriloff DM: Prevention of the eye closure defect in Ig^{m1}/Ig^{m1} *J Teratol* 1985, **32**: 3-86.
- ¹³ Watney MJ and Miller JR: Prevention of a genetically determined congenital eye anomaly in the mouse by the administration of cortisone during pregnancy. *Nature* 1964, **202**: 1029-31.
- ¹⁴ Harris MJ, Juriloff DM, Biddle B: Cortisone cure of the lidgap defect in fetal mice. A dose-response and time-response study. *Teratology* 1984, **29**: 287-95.
- ¹⁵ Cohen S: Isolation of a mouse submaxillary gland, protein accelerating incisor eruption and eyelid opening in the newborn animal. *J Biol Chem* 1962, **237**: 1555-62.
- ¹⁶ Cohen S and Elliott GA: The stimulation of epidermal keratinisation by a protein isolated from the submaxillary gland of the mouse. *J Invest Derm* 1963, **40**: 1-5.
- ¹⁷ Orth D: Personal communication 1987.