DEVELOPMENTAL STUDIES ON THE FUSION AND THE
DISJUNCTION OF THE EYELIDS IN THE
WHITE MOUSE

BY

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INTRODUCTION

Fusion and disjunction of epithelial tissues are common occurrence in the
development of various kinds of organs, e.g. closure of the operculum or the
amniotic folds, and has been already subject of investigations by several workers
from the cytological or histological view points.

The formation of the eyelids and the fusion of them at their margins occur
during fetal life for any mammal, while the disjunction of the fused lids takes
place either during later fetal life in some species or after birth in the others.

Contino (1907), Addison and How (1921), studied the processes of forma-
tion and fusion of the eyelids in mammalian fetus. Contino, who studied this
process in the human fetus, reviewed mostly the mechanism of the disjunction.
Addison and How studied this in the rat.

However, much remains still unknown in the histo-cytological points. For
example, Addison and How based the mechanism of the disjunction of the
eyelids on the keratinization of the fused epithelium. It would be unreasonable
to ascribe the only reason of disjunction to the keratinization. More precise
cytological investigation might be desirable to clarify the nature of this event.

For this purpose, the developmental studies of the eyelids on the white
mouse were undertaken.

MATERIALS AND METHODS

For the histological studies sections were prepared of fetuses ranging from
the 12th to 21st day of gestation, as well as of young animals from birth to the
15th day after birth. They were fixed in 10% neutral formalin, Helly’s
Zenker-Formol, Carnoy’s and Heidenhain’s Susa solution, embedded in paraffin,
and sectioned serially in 5–7 μ thickness. Also cellloidin sections of 10–15 μ
thickness were made. These sections were stained by hematoxylin-eosin, Unna’s
water blue-orcein-eosin, Hg- brom-phenol-blue, Heidenhain’s Azan, Heiden-
hain’s iron alum hematoxylin, methyl green-pyronin, and some of these serial
sections were used for graphical reconstruction. Some other fetuses fixed with
formalin were stained with Böhmer’s hematoxylin in toto and the arrangement
of superficial cells on and near the eyelids was observed under lower magnification.

**General Description of Observations**

On the eleventh day of the fetal life, there appear protruding ridges like embankment in the area where the lids are expected, and the front surface of the eyeball is wholly exposed between them (Fig. 1). The epithelium on the protruding ridges of the lids consists of one to three layers of cubiform cells (Fig. 5). These cubiform cells are taller than those of other portion, and mitotic figures are often found in the superficial cell layer (Fig. 6). On about the 13th day of gestation, the margins of the upper and lower lids come in contact. In the majority of cases the contact of the lids starts at the outer canthus and proceeds toward the inner one: the junction of the lids thus occurs at the outer canthus. On the other hand, the same occurs successively on the inner canthus, too, and this proceeds outwards. Consequently nearly the middle point of the slit of lids joins lastly. As the lower lid grows up dorsally and proceeds over the center of the cornea, the complete fusion of both lids occurs dorsally of the axis of the eyeball. Only the epithelial tissue joins at the margin of the lids, and builds the thin epithelial covering in front of the cornea.

The tonofibrils in the epidermal cells on the outer surface of the lids run distinctly at right angle to the edge of lid-slit and at the same time parallel to the outer surface of the epidermis. At this stage of development, striated muscle fibres appear in the mesenchym of the upper eyelid. They represent the early stage of M. levator palpebrae superioris. Gradually the junctional epithelial tissue increases in its thickness, and the mesenchymal tissues in the upper and lower eyelids approach each other. In other words, the mesenchymal tissue enter into the thin junctional epithelium which overspreads the surface of the cornea.

Fig. 2 shows the arrangement of the epithelial cells on the surface of the eyelids which were stained as a whole with BÖHMER’s hematoxylin in the same stage as Fig. 5.

If we compare the lid slit to a magnet, lines of arrangement of the epithelial cells remind us the magnet lines of force. The distance between the outer and inner canthi remains nearly constant through all the stages of the fusing process. When the junction epithelium was sectioned after completion of the fusion at right angle to the lid-slit, it was revealed that the flattened epithelial cells cross the junction epithelium obliquely from the outer surface of the lower eyelid to the conjunctiva of the upper lid.

Before the closure of the lid-slit, cilia-like structure was observed on the epithelium of the palpebral conjunctiva (Fig. 9). When the fusion finished completely, this structure went out of sight. Observation of the living material showed an active ciliatory movement of this structure. The epithelium of the palpebral conjunctiva consists of two to four layers of flattened cells. The nucleus is of ellipsoid form and fills the minimum diameter of the cells, the maximal diameter of this nucleus being about half or one thrice of the cell
length. One or two layers facing the cornea are eosinophile. The cytoplasm of their cells is not granular, and their nuclei are distinct. The cells are stained brown by water blue-orcein-eosin, and are not stained strongly blue by Hg-brom-phenol-blue staining. Though, at first sight, their cells look keratinized, it is far from true keratinization as their nuclei are distinctly visible.

The junction between the lids takes place only in the epithelium, the mesenchymal tissue in the lids does not join together. The time of complete fusion of the eyelids is a definite one, occurring on the fifteenth day of fetal life. The intervening cells, immediately after the fusion of the lids, have lighter cytoplasm than any other epithelial cells, and show vesicular formation in the cytoplasm (Fig. 10). There are one or several vesicular structures in a cell. When the structures become larger, the nucleus was sometimes pushed against the margin of the cell.

The cells adjacent to the mesenchymal tissue in the junction epithelium are cylindrical and dark in cytoplasm. The nuclei are somewhat large, and poor in chromatin.

In the deeper region of the intervening epithelium, the spiny cells have smaller nuclei provided with rich chromatin.

The intercellular clefs in the intervening epithelium are a little wider than in other parts of the lining epithelium of the lids, their width being inconstant. Crossing the intercellular spaces, fine conflicting fibrous structures are seen connecting the cells each other in a complicated manner. Usual intercellular bridges are sparse.

The sections on the nineteenth day of the fetal life show the initial formation of keratohyaline granules in the middle part of the junction epithelium (Fig. 11). The mesenchym of the lids approach farther to each other, accompanied with this change of the junction epithelium. The junction epithelium becomes a thin connecting membrane. In the new born animal, the adjacent epidermis to the junction epithelium begins to differentiate the cornified layer. The epithelium of the lids in this stage grows some hair buds (sometimes hair shales). The already mentioned vacuoles in the junction epithelial cells increase in number before the cells keratinize.

In about 24 hours after birth, the epithelial cells adjacent to the mesenchymal tissue become lower and lower and acquire roundish shape, provided with globular nuclei.

The intercellular bridges of these cells become distinct as the cells change their shape, while those of the intermediate cells are not obvious.

The fine fibrous structures which appeared to be interwound in about fifteen days of fetal life are replaced by the straight intercellular bridges.

In three or four days after birth, the ducts of the palpebral glands (tarsal glands) can be seen to open to the body surface passing through the junctional epithelium.

The keratinization begins to appear around these ducts, the keratohyaline granules being distinctly visible there. These ducts are well differentiated, in the eight- or nine-day-old animal (Fig. 12).

After this stage the cells of the intervening epithelium develope spinelde shape,
and the cytoplasm of the cells become darker, and three layers, i.e. Str. basale, spinosum, and granulosum, differentiate into the stratified flattened epithelium.

The intercellular bridges are visible between the cells of Str. granulosum. At the beginning of the 13th day, it is observed that cracking of the junction epithelium appears along the ducts of palpebral gland.

It is visible that the vacuoles in the innermost cells in the junction epithelium become larger and larger, to lead the cells at last to the plasmalemma (Fig. 13).

The width of the intercellular clefs in the junction epithelium remains generally constant. Many a intercellular bridges are there visible. And the separation of the lids is finished completely during 3 days ranging from the 13th to the 15th day after birth (Fig. 14, 15).

**Discussion**

The period of the gestation is 22 days on an average in the mouse, and the eyeball is not covered with the lids during the first 15 days of fetal life.

In about 13 days of fetal life, the primordium of the eyelids appears as folds of the integument on the upper and lower margin of the optic area to grow downward and upward respectively, and finally extends to cover the front surface of the eyeball within nearly 24 hours. **Addison & How (1921)** showed that in the rat fetus the eyeball was not covered by the lids in 18 days of fetal life. On the 17th day of gestation, the folds appeared on the upper and lower region adjacent to the eyeball, and spread to cover the eyeball within a day. This chronological discrepancy between both animals might be due to the difference of morphogenetic processes, which is slower in the rat. In man the integument folds are indicated at the end of seventh week, and their edges meet and fuse two weeks later. Thus formation of the eyelid belongs to a relatively rapid occurrence. The period of gestation in man reaches 40 weeks, while that of these animals only 3 weeks, being the ratio 40:3=13.3. This is nearly equal to the ratio of the length of time necessary for the closure of the lids 14:1.

In the epithelium of the free edge of the lids cell proliferation appears more active in the superficial layer than in the deeper ones in contrast to ordinary predominance of mitotic activity in the basal layer of the common epidermis. This fact may show a special character of the marginal epithelium of the embryonic eyelid ready for fusion. The fusion of the lids are finished completely by the 15th day of the fetal life. At this time the tonofibrils run distinctly in the direction of the longer axes of the cells. The cells on the surface of the lid-edge stand perpendicularly to the surface itself and send out protruding cytoplasmic processes towards the opposing lid (Fig. 7). It would not be unnatural to think that these processes would have been moving in the living state, just like undulating membranes of the cells at the margin of the epithelial sheet in tissue culture.

**Heidenhain (1911)** is of opinion that the growth of tissue causes dynamic tension in the tissue concerned, and this tension, for example, affects the appearance and direction of the tonofibrils in the epidermis. **Höpke (1927)** also
pointed out that the tonofibrils were formed by the pressure in the tissues, and were strengthened by the external powers. The perpendicular arrangement of the tonofibrils to the surface in the margin of the fusing lids shows existence of a tension caused by the movement or active transformation of the epithelial cells in the region.

Morii (1958) considered that the tonofibrils run mainly in the cytoplasm perpendicularly to the surface of the skin. For instance, in the epithelium grown on the margin of the lids, the perpendicular direction to the surface of the epithelium coincides with the direction of the protrusion of the marginal cells of the lids. In the developmental stage the marginal cells of the lids move in this direction and divide themselves. When the junction epithelium after the fusion of the eyelids was sectioned perpendicularly to the future lid-slit, flattened epithelial cells are seen crossing the junction epithelium obliquely from the surface of the lower eyelids to the upper palpebral conjunctiva. And when the epithelial cells on the surface of the lids and the vicinity were stained with Bürker's hematoxylin as a whole, it was observed that the cells were flattened and become to spindle form whose long axis were directed in the direction perpendicular to the lid-slit. When cells are thrown into movement as a group, they usually transform into slender shape in the direction of their movement. Flattening of the epithelial cells in the marginal region of the lids could be explained in the following manner: as already described, the distance between both canthi remains constant during the lid formation, so that free edge of the lids must be shortened during the fusing process, because curved edge changes to a nearly straight line which connects the both canthi with each other.

Sections of the palpebral region prepared soon after fusion of the lids show an upheaval of the epithelial tissue over the surface of the joined epidermis, as a result of excess collision of the opposing tissues (Fig. 8). This mechanism resembles the case in the regeneration of the epidermal tissue observed in amphibia by Nuzima et al. (1958).

Now the mechanism of the fusion of both margins of the palpebrae will be discussed here. There are principally two kinds of mechanisms in closure of an opening in animal body. Katsumaya (1935) observed the process of development of the operculum of Rana nigromaculata and Bufo vulgaris, applying the local vital method and found that the formation of the spiracle bases on convergence of the free margin of the operculum. He also ascribed the closure of the amnion folds to the manner of pursing a bag by draw-strings. However, the closure of the eyelids is different from that of above mentioned matters. Margins of both lids meet together at every corresponding point and unite with each other in the way of adhesion. There can be no pursing at all, since even after completion of the closure, the epithelial cells on the margin are situated perpendicular to the union line and parallel to each other.

Judging from the fact that the distance between the outer and the inner canthi remains constant during the fusing process, the mechanism of the fusion of the eyelids must be an adhesion, and should be distinguished from the process of formation of the operculum in the amphibian larvae or fusion of the amnion folds. If the fusion of the lids were a kind of pursing, mesenchymal
tissue should accompany the reducing slit. But the fact is not so. The fact that surface cells of the marginal epithelium all show cytoplasmic protrusion would prove equivalency of those cells in fusion.

Next comes the problem on the mechanism of separation of the fused lids. Following factors might be considered as agencies concerning the separation of the lids: intercellular spaces in the intervening epithelium, situation of intercellular bridges, cornification of the epithelial cells, development of the tarsal gland, and development of the hair.

After the fusion the intercellular spaces in the intervening epithelium are slightly wider than those of the adjacent epidermis. They vary according to individual spaces. Intercellular bridges are few in number, and instead of them finer fibrillar structures are visible crossing the intercellular spaces. In a last few days of gestation ordinary intercellular bridges increase in number and replace the fibrillar structures. The latter, therefore, may be the precursor of the intercellular bridges.

In the newborn animal some hair follicles or hair shafts in the epidermis of the lid are found.

According to Addison and How (1921), no hair shaft was visible before the 8th day after birth.

Contino's investigation showed that lanugoes were found in the 5th-month human fetus, and the disjunction of the eyelids occurred in the 6th month of fetal life, while according to Ask, P. (1907) the disjunction of the lids occurred at the beginning of the 7th month of the fetal life.

About 3 or 4 days after birth it is visible that the ducts of the tarsal glands open to the surface of the skin through the junction epithelium.

Schweiger-Seidel (1866) claimed the secretion of the tarsal glands as the chief agency of the separation. But this reveals to be not so operative in the mouse. Because the duct passes in spiral through the junction epithelium, and does not open within the latter, but reaches the surface of the skin. Keratinization of the junction epithelium seems to occur intensely around the ducts. He regarded as the chief factors in the separation of the lids:

1). the keratinization within the hair follicles developing from the junction epithelium.

2). the formation of the spaces between the cells of the junction epithelium by the secretion from the tarsal glands.

Contino (1907) considered that the separation of the corners of the lid margins was due to the secretion from the tarsal glands and that the separation of the intern marginal epithelium was due to the cornification of the central layers of the cells in the human fetus.

Seiler (1890) made much of the cornification of the cells of the intervening epithelium as a factor to separate the lids, studying the eyelids of young puppies at one, four, eight, and nine days after birth.

Nussbaum (1908), in the course of his résumé of the development in human eye, referring to the results of the observation at two and ten days after birth in mice, said that at ten-day animal the process of cornification had advanced into the lid fissure, and the epithelium split.
ASK (1908) said that the cornification process in the junction epithelium between two lids was the most important factor in separation of the eyelids in kittens, and that the cornification process was derived from the hair follicles into the junction epithelium.

In my study also the cornification in the junction epithelial cells became visible distinctly around the ducts of the tarsal glands and the hair follicles, and then the junction epithelium separated in fissures presumably owing to the cornification. Moreover the separation was aided by the fact that the cells of the central layer in the junction epithelium fell into degeneration.

**Summary**

1. Formation and fusion of the eyelids occurs in the mouse on the 13th and 14th day of the fetal life.
2. Two lids fuse each other on the upper half of the eyeball.
3. The junction of the palpebral epithelium begins by formation of a thin epithelial layer covering the cornea on the margin of the lids.
4. The junction epithelial cells are replenished not only from the marginal cells of the lids, but from the remote epithelial cells around the eye by way of migration.
5. In the process of the fusion of the epithelial cells of the lids resemblance is seen to the regeneration of the epidermis in the fact that cytoplasmic processes are sent out from the superficial cells of the margin of the lids.
6. Before the fusion of the lids is completed, the ciliary structures are visible on the epithelium of the palpebral conjunctiva. They disappear after completion of the fusion.
7. The innermost cells in the junction epithelium and the cells of palpebral conjunctiva become eosinophile soon after the fusion. (The author wants to advocate this phenomenon as “pseudokeratinization”.)
8. The separation of the fused lids occurs in the 13th to 15th day after birth.
9. The separation seems to be the result of the cornification and the degeneration of the intervening cells. Active contraction of the M. levator palpebrae superioris will help the separation.
10. Separating role of the secretion of the tarsal glands cannot be highly evaluated.

**Acknowledgement**

The author is indebted to Prof. Mitio Nuzima of Tokyo Medical and Dental University for his kind guidance and encouragement in this study, and also to Prof. Kiyoshi Takewaki of Tokyo University for his kind advice.

**References**

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EXPLANATION OF FIGURES.

PLATE I.

All the figures show the arrangement of the epithelial cells on the surface of the eyelids which were stained as a whole with Bohmer's hematoxylin.

Fig. 1. 11-day-fetus
The front surface of the eyeball is wholly exposed.

Fig. 2. Diagram showing cell arrangement of the lid epidermis of the same stage as Fig. 1.

PLATE II.

Fig. 3. 14-day-fetus
Fig. 4. 16-day-fetus

PLATE III.

Fig. 5. Section cut dorso-ventrally parallel to the axis of the eyeball.
Hematoxylin-Eosin staining. 40x

Fig. 6. 13-day-fetus
Mitotic figures seen in the superficial layer of the epithelium at the protruding ridge of the expected lid. Arrows indicate mitosis. H-E staining. 1000x

Fig. 7. 13-day-fetus
Cytoplasmic processes (arrow) sent out from the free edge of the developing lid. H-E staining. 400x

Fig. 8. 13-day-fetus
An upheaval of the epithelial cells at the fused edges of both lids soon after fusion. H-E staining. 400x

PLATE IV.

Fig. 9. Cilia like structure (arrow) on the epithelium of the palpebral conjunctiva. 14-day-fetus. H-E staining. 1000x

Fig. 10. Intervening epithelium sectioned parallel to the surface of the lids. 13-day-fetus. 1000x Heidenhain's iron hematoxylin staining.

I: intervening epithelium M: mesenchymal tissue V: vesicular formation in the cytoplasm

Fig. 11. Initial formation of keratothyline granules (arrow) in the middle part of the intervening epithelium. 19-day-fetus. H-E staining 1000x

Fig. 12. Keratinization around the duct of the palpebral gland. 8 days after birth. H-E staining. 1000x

PLATE V.

Fig. 13. Plasmarexis in the innermost epithelial cells of the fused area. 14 days after birth. Methyl green-Pyronin staining. 1000x

Fig. 14. 14 days after birth. Separation of the lids mainly as a result of plasmarexis. H-E staining. 1000x

Fig. 15. 14 days after birth. Separation of the lids mainly as a result of keratinization. Heidenhain's iron hematoxylin staining. 1000x
PLATE II.

Fig. 3

Fig. 4
PLATE V.

Fig. 13

Fig. 14

Fig. 15