The Development of the Pecten Oculi in the Chick

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ABSTRACT. The development of the pecten oculi, a structure peculiar to the avian eye, was studied by scanning electron microscopy (SEM) correlated with light microscopy (LM) in embryonic and adult chickens. The development of the chick pecten was divided into 4 phases: (1) formation of the primordial pecten (Hamburger-Hamilton's stages 27 to 29), (2) formation of the plate-like pecten (stages 30 to 34), (3) pleat formation and pigmentation (stages 35 to 37), and (4) bridge formation and high-vascularization (stage 38 to adult). The primordial pecten is formed entirely from the ectoderm by fusion of the inwardly-projecting edges of the optic fissure. The primordial pecten grows into a tall, thin plate rising from a broad base. The pecten begins to fold slightly at stage 35. The number of pleats increases rapidly, from 7 at stage 35, to 16 at stage 36, 18 at stage 37, and 19 to 20 at stage 40. The bridge begins to form at stage 38 by a swelling of the apical edge of the pecten and completes its development by the twentieth post-hatch day. Blood vessels appear first in the broad base of the plate-like pecten, then become more numerous and gradually extend into the pleats. The pecten becomes more vascular than cellular at stage 43, and it is highly vascularized in the adult. The pleat surface becomes conspicuously irregular with increased vascularization. The periplectinate cells, located on the pecten, are already present at stage 27—KEY WORDS: chick, development, eye, pecten oculi, scanning electron microscopy (SEM).


The pecten oculi, a structure peculiar to the avian eye, arises from the optic disc and projects a variable distance into the vitreous body. Based on its morphology, the pecten has been characterized as the vaned type, found in most ratites, and the pleated type, occurring in carinates [24]. The latter type is the most common in birds.

Despite its relative simplicity, numerous studies have been made on the pecten oculi by transmission electron microscopy (TEM) [1, 3-6, 10, 15, 17-19, 21, 25]. More recently, the surface morphology and microvascularature of the pecten have been observed with the scanning electron microscope (SEM) [1, 9, 14, 20, 22].

According to a detailed review by Hodges [8], the pleats of the pecten are attached basally at intervals along the entire length of the linear portion of the optic disc, i.e., along a line corresponding to the optic head proper. Apically, the pleats are attached to the bridge of the pecten.

The early embryological studies on the pecten have been reviewed by Lillie [11] and Romanoff [16]. Thereafter, there is a paucity of more recent embryological studies [12, 26]. Although several investigators have studied pecten development by two dimensional, serial section reconstructions, the development of the three dimensional structure of the pecten oculi is poorly understood.

The pecten of the adult chick is not damaged by mechanical removal of the vitreous body. However, the pecten of the chick embryo is a fragile structure and easily damaged by mechanical removal of the vitreous body anchored to its apical margin. For this reason, previous SEM studies have found it difficult to visualize the embryonic pecten. Recently, the observation of the
embryonic pecten by SEM has become feasible by the use of chemical digestion methods exposing previously hidden surfaces of tissues by removing the otherwise obstructive components. The present study utilizes such a method, and attempts to elucidate the three dimensional structure of the pecten oculi in the developing chick by using SEM correlated with light microscopy (LM).

MATERIALS AND METHODS

Chicken embryos (White Leghorn) ranging in age from Hamburger-Hamilton's [7] stages 27 to 46 (HH 27 to 46) were used in this study. In addition, 10- and 20-day-old chicks and adult chickens were used as well. Fertile eggs were incubated in a humidified egg incubator maintained at 38°C.

Processing for SEM: The heads removed from embryos at stages HH27 to 34 and the eyes isolated from older embryos were fixed in a mixture containing 2% glutaraldehyde-2% paraformaldehyde in 0.1M Sorensen's phosphate buffer (pH 7.4). Then, the specimens were successively hydrolyzed with 8N HCl at 40°C for 1–4 hr to accomplish the removal of the vitreous body. Following a rinse with the above buffer, the specimens were treated by a conductive staining method [23] to confer electrical conductivity and harden the tissue. The specimens were immersed in a mixture containing 2% glycine, 2% sodium glutamate and 2% sucrose transferred to a solution of 2% tannic acid for 2 hr, then rinsed for at least 30 min in distilled water, and returned to a solution of 2% tannic acid for another 2 hr. Then the specimens were washed thoroughly in distilled water in advance of postfixation and later processes.

Following deep ether anesthesia, post-hatch chicks were perfused through the heart with the above fixative. The eyes were removed and immersed in the same fixative.

The vitreous body was easily removed from the eye of the post-hatch chick with a forceps. Tissue samples from both embryonic and post-hatch chicks were postfixed in 1% osmium tetroxide for 3 hr, dehydrated through a graded series of ethanol, and dried by the critical point method. The specimens were then sputtercoated with platinum, and observed under a Hitachi X-650 SEM.

Processing for LM: The head or eyes were immersed in Bouin's fixative and embedded in paraffin by standard procedures. Serial sections were cut transversely at 5μm and were stained with hematoxylin and eosin. Some embryos were embedded in a mixture containing n-butyl methacrylate and methyl methacrylate. Plastic transverse sections were stained with hematoxylin and eosin.

RESULTS

Formation of the Primordial Pecten (HH 27 to 29): The pecten oculi developed in association with the closure of the optic fissure. The fissure was closed distally from its proximal end to almost half of its entire length by stage 27 and to about three-quarters by stage 29. The primordial pecten projected into the vitreous humor as a low ridge along the line of the fusion of the fissure (Figs. 1, 3). The edges of the proximal portion of the open fissure projected inwardly to form a double ridge on the inner surface of the optic cup by the cell proliferation throughout the entire region of the edges of the fissure. This development contrasts with that of the retinal cells proliferating only in the outermost layer (Fig. 2). The double ridge then fused to form a single ridge, i.e., the primordial pecten (Fig. 3). Most distally, the edges of the fissure simply came into apposition and became progressively shorter (Fig. 4). The mesenchymal tissue containing blood vessels, the
Fig. 1. The primordial pecten observed as a low ridge between the small arrows. The mesenchymal tissue (large arrow) protrudes from the open optic fissure as a low ridge. The peripunctate cells, observed as dots on the pecten, are widely distributed throughout the pecten. HH 28. ×80.

Fig. 2. Inward projection of edges of the optic fissure showing mitotic cells (arrow head) distributed randomly within the edges. HH 28. ×130.

Fig. 3. The distal pecten primordium forming a tunnel enclosing the mesenchymal tissue. HH 28. ×220.

Fig. 4. The edges of the distal optic fissure coming into apposition. HH 28. ×220.

arteriae cuplæ opticae, filled the entire open portion of the fissure.

With subsequent closure of the optic fissure (Fig. 5), the cauda of the optic nerve formed a low ridge along the primordial pecten on the ventral surface of the eye. The primordial pecten joined ventrally with the cauda or head of the optic nerve. The pectinate cells, which are precursors of the pigment cells, were similar in appearance to cells in the optic nerve (Fig. 5). Since the ridge of the cauda was shorter than the pecten, the most distal portion of the pecten formed a tunnel-like structure enclosing the mesenchymal tissues (Fig. 3).

The peripunctate cells were already found on the pecten and the edges of the optic fissure by HH 27 (Figs. 2, 6). The pecten was avascular during these developmental stages.

Plate-like Pecten and Vasculogenesis (HH...
30 to 34): The pecten became taller during these stages and developed into a thin plate rising from a broad base (Figs. 7, 8). Superficial cells of the pecten were regularly arranged, and the deeper cells located in the broad base were more loosely arranged (Fig. 9). A few blood vessels appeared in the triangular base by HH 30 (Fig. 9).

The progressive closure of the fissure was completed by HH 34. Following complete

Fig. 5. Continuation of the proximal pecten primordium with the cauda of the optic nerve (asterisk). The pectinate cells, precursor of the pigment cells of the pecten, can not be distinguished from the cells of the optic nerve. HH 29. ×100.

Fig. 6. The peripectinate cells on the pecten. HH 27. ×900.

Fig. 7. A thin, plate-like pecten. The peripectinate cells are distributed on the pecten. The mesenchymal tissue (arrow) is seen on the distal portion of the pecten. HH 34. ×55.

Fig. 8. Ventral continuation of a thin, plate-like pecten with the cauda of the optic nerve (asterisk). HH 34. ×54.

Fig. 9. The broad base of plate-like pecten consisting of superficial epithelial cells, more centrally located, irregularly arranged cells, and a few blood vessels. HH 34. ×260.
Fig. 10. The pecten showing the folding. The apical margin of the pecten is bent in this micrograph. HH 35. ×30.

Fig. 11. The pecten at HH 37. ×30.

Fig. 12. The apical portion of the pecten as thick as the pleats. HH 37. ×40.

Fig. 13. The pleats of the pecten showing blood vessels, pigment granules and the peripertinate cells (arrows). The pecten is rich in cellular components than vascular ones. HH 37. ×250.

closure of the optic fissure, the mesenchymal tissue began to fuse to the apical end of the distal portion of the pecten (Fig. 7). The cauda of the optic nerve elongated farther than the distal end of the pecten adjoined with the optic nerve along its entire length.

_Pleat Formation and Pigmentation (HH 35 to 37):_ The pecten began to fold slightly at HH 35 (Fig. 10). The number of pleats increased rapidly to about 7 at HH 35, about 16 at HH 36, and about 18 at HH 37 (Figs. 11, 12). The pleats appeared initially about midway between the two ends, and then in both the distal and proximal portions.

Although the blood vessels of the pecten became more numerous and extended into the pleats of the pecten, the pecten was still markedly rich in cellular components than vascular ones (Fig. 13).

The pigment within the pecten first appeared at HH 35 (Fig. 13). The degree of pigmentation was more prominent in the apical portion than in the basal portion, and more so in the distal portion than in the proximal portion.

The mesenchymal tissue was found on the apical end of the most distal portion of the pecten, despite the complete occlusion of the optic fissure.

_Peak Formation and High Vascularization (HH 38 to the adult):_ The pleat formation was completed by HH 40. The number of the pleats varied from 19–22, but general-
ly ranged between 19 and 20 (Fig. 14). The pleats began to be constricted at their bases by HH 38. These constrictions became more prominent during subsequent developmental stages (Figs. 15, 20).

The bridge of the pecten began to develop as a swelling of the apical margin (Fig. 16) with the extension of the grooves of the pleats onto the bridge by HH 38 (Fig. 14). The grooves were still found on the bridge in the 10-day-old chick (Fig. 17), but disappeared in the 20-day-old. The pecten of the 20-day-old chick appeared to be completed except for its size (Fig. 18), which was about 6.0 mm long and 3.2 mm tall in the 20-day-old chick, and about 8.0 mm long and 4.2 mm tall in the adult chicken.

The blood vessels of the pecten increased gradually from HH 38 onward. By HH 43,
the pecten was much richer in vascular components than cellular ones (Fig. 19). As the number of blood vessels increased, the surface of the pleat became conspicuously irregular by reflecting the tortuous arrangement of the vascular network (Fig. 20). The pecten was relatively richer in vascularization in the adult chicken than in the 20-day-old chick (Figs. 21, 22). Because the vascular network of the bridge was less developed and more centrally located than that of the pleats, the surface of the bridge remained relatively smooth (Figs. 18, 23).

The peritectinate cells were scattered within the undulating folds of the surface of
pecten by HH 40.

DISCUSSION

The pecten oculi of the chicken consists of thin, darkly pigmented pleats attached apically to the bridge. Histologically, the pecten is divided into two types of tissue components; a complex vascular network and a highly pigmented intervascular tissue. The intervascular tissue is composed of polymorphic pigmented cells and fills the spaces among the predominant blood vessels. The whole structure of the pecten is covered with a continuation of the inner limiting membrane of the retina. Occasional peripatinate cells are found on the external aspect of this covering membrane [8].

Previous morphological studies of the pecten oculi by LM and TEM provided information about its internal structures, but could not visualize its structures in three dimensions. The present investigation is the first one to analyze the development of the three dimensional structure of the chick pecten oculi by SEM.

The development of the chicken pecten oculi was divided into 4 stages in the present study: (1) formation of the primordial pecten, (2) formation of the pleat-like pecten, (3) pleat formation and pigmentation, and (4) bridge formation and high-vascularization.

The development of the pecten is closely related with the closure of the optic fissure. The primordial pecten developed as a single ridge formed by fusion of the double ridges, i.e., the inward projection of the edges of the optic fissure. Initially, the optic fissure occluded rapidly from proximal to distal, but its most peripheral part remained open, as described by Lillie [11], until considerably later stages.

The origin of the pigmented, intervascular cells is still controversial [2, 11, 15, 16, 26]. Although many authors consider the pigmented cells to be glial in origin, many others refute this view. The primordial pecten was formed by the fusion of the inward projection of edges of the optic fissure. Such ingrowth was due to cell proliferation throughout the entire regions of the edges. The glial cells of the optic nerve adjoining the proximal pecten were similar to the cells of the primitive pecten in size, shape, and stainability. In general, the neurons proliferated only in the ventricular zone, while the glial cells proliferated throughout the central nervous system. Thus, the pectinate cells, i.e., the precursors of the pigmented cells, are thought to be glial in origin.

According to Romanoff [16] and O’Rahilly and Meyer [13], the pleats begin to be formed at HH 35 or 36. The number of pleats is 7 at HH 37, 15 at HH 36 to 38, and 17 at HH 39. In the present study, the pecten began to fold at HH 35, and the number of pleats was 7 at HH 35, 16 at HH 36, 13 at HH 37, and 19 to 20 by HH 40, the same number as in the adult. The result of the present study that the plate-like, primordial pecten began to fold at HH 35 agrees well with previous reports. However, the present study showed that the folding of the pecten was more rapid than previously reported from serial section observations.
This difference probably is owed to the superiority of the SEM method currently employed in detecting more subtle changes in surface structures.

The number of pleats in the chicken pecten is variably reported [8]. However, recent SEM studies show the approximate number of pleats to be 18–20 [1, 5]. In the present study, the number ranged from 18 to 22, especially 19 or 20 in the majority of cases.

There is almost no information on the development of apical bridge in the pecten. In the present study, that the bridge was formed by the swelling of the apical portion of the pecten at HH 38, and the grooves of the pleats extended to the bridge. The bridge swelled gradually, and the grooves on the bridge eventually disappeared by 20 days post-hatching. After HH 38, the bridge increased in size, and retained a relatively smooth surface. However, the pleat surface became gradually covered by tortuous vascular ridges to make evident a sharp demarcation between pleats and the bridge.

In the present study, dispersed cells, termed perirectinate cells by Fischlschweiger and O’Rahilly [6], were always found on the external aspect of the pecten but not on the surface of the adjoining retina. Although there is little information about these cells [6, 19], they are observed as regular elements of the pecten. Morphologically, these cells resemble macrophages. Since the perirectinate cells are also found on the edges of the optic fissure, they may represent mesodermal cells having migrated into the eyeball through the optic fissure from extraocular areas.

The morphological features of the pecten suggest that it has an active physiological role. Indeed, a number of investigators have suggested that the pecten plays some nutritional role in retinal support [1, 2, 4, 5, 9, 14, 19, 20, 24, 25]. Furthermore, the avascular retina may be protected in birds against infection with blood-borne pathogens by the perirectinate cells found exclusively on the pecten oculi.

REFERENCES


要 約

鶏における網膜機能の発生学的研究：上原正人・大森佐和・北川浩・上嶋俊彦（鳥取大学農学部獣医学科家畜解剖学講座）—鶏の眼内に見られる網膜機能の発生を走査型電子顕微鏡と光学顕微鏡を用いて観察した。材料はHamburger—Hamiltonのステージ27（HH27）から成鶏までである。網膜機能は鳥類特有の構造で、19から20枚のヒダ及びその先端を結ぶ「橋」からなり、組織的には主に豊富な血管とその間を埋める色素細胞からなる。鶏の網膜機能の発生は4期に分けられた。すなわち、（1）網膜機能の形成（HH27–29）、（2）薄板状の網膜機能の形成（HH30–34）、（3）ヒダの形成と色素の出現（HH35–37）、（4）「橋」の形成と血管の急速な増加。網膜機能は眼杯膜の後端が内方に突き出して2列のヒダを形成し、ついでそれらのヒダが中心側から融合することによって形成される。原基は急速に高さを増し、比較的幅が広い基部を持った薄板状になる。ヒダはHH35で初めて網膜機能の中央部に出現し、その数は急速に増し、HH40までに成鶏なみの数になる。「橋」はHH38で薄板状の遊離部が肥厚することにより形成され、その完成は遅くとも孵化後20日までかかる。血管は最初薄板状の網膜機能の基部に出現し、増加しながら網膜機能の先端に向かって侵入する。HH43になると網膜機能は血管の方が優勢になり、以後血管の占める割合は増しに増加する。網膜機能の表面に分布する網膜機能周囲細胞は今回観察したHH27で既に眼杯裂の縁に出現していた。