As is known, lens fibers have not only various forms of protoplasmic processes at each edge but also wall spines on the surface of the lens fiber, and they interdigitate tightly with the adjacent lens fibers in fixed manners of interdigititation (Futagami, 1962; Tanaka and Iino, 1967; Matsuura and Watanabe, 1968).

Moreover, it has been suggested that each species has some similar characteristics in the forms of the processes and the wall spines, and also in the manner of interdigititation (Tanaka, 1968).

The junction systems of lens fibers of cyclostomes, elasmobranchs, and teleosts have already been investigated in detail (Tanaka, 1969), whereas those of reptiles and birds seem to have been neglected up to now.

It seems that such research is very important, interesting, and necessary in order to known the phylogenetic transitional process, or evolution, concerning the junction systems of lens fibers.

With this object, the author has observed the junction systems of lens fibers of reptiles and birds under an electron microscope by the filmy replica system method.

Materials and Methods

The species of reptiles examined were; Clemmys Japonica of the order Chelonia; Gekko japonicus, Eumeces laticutatus, Elaphe climacophora and Agkistrodon halys of the order Squamata; Alligator mississippiensis of the order Crocodilia.

Avian subjects were; Gallus g. var. domesticus of the order Galli; Anas platyrhynchos of the order Anseres; Strix uralensis of the order Striges; Corvus levaillantii, Hirundo rustica and Passer montanus of the order Passers.

Preparation for the replica system method

Freshly collected mature lenses were fixed for more than one week in 10% neutral formalin. The fixed lens was washed with water for about 30 min. After the water was excluded sufficiently, the lens fiber piece was peeled off like an onion skin by means of tweezers, and from this piece the replica was taken as follows:

1. First-step plastic replica making

The fiber piece was immersed in the solvent (methyl acetate) for about 3 min. Before volatilization of the solvent, the Acetylcellulose Replicating Film (Bioden R. F. C. Oken-Shoji Co., Ltd.) was laid over the sample and was pressed with sponge for 2 min. The replica was carefully stripped off the sample by picking it up with tweezers. Then, the following treatment was given.


In order to smooth out the creases in the film, it was placed between two plates of clean glass and fastened with cellophane-tape, and kept for about 30 min in an air
bath heated to 80°C. Then it was fixed with cellophane-tape on a glass plate with the reproduced surface upward. Then it was put into the vacuum evaporator, and shadow-casting in vacuum was performed at an angle of 45° from above with chromium. After that, a reinforcement film was made by evaporation of carbon from above, vertical to the surface.


After completing the vacuum evaporation treatment, the replica film was cut into pieces of suitable size to fit the specimen grids (about 2 mm square). Then a small piece of slide glass was prepared and heated to 55°C, and, for the purpose of making a protective film, paraffin was applied thinly on the glass. While the paraffin was in a melted state, the replica film was placed on the glass plate with the evaporated film side touching the melted paraffin.


After completing the coating treatment of the protective film, the replica film was placed on a glass plate, soaked in the solvent (mixed dichloromethane and methanol, 100:19/vol.), and placed in the air bath (conditioned to 60°C), where the paraffin and the Acetylcellulose Film dissolved.


After the dissolving treatment, the second-step evaporated replica film was picked out of the solvent and placed on the specimen grids.

The specimens thus obtained were examined and photographed under a Hitachi HU-IIP type electron microscope.

Observations

I. Class Reptilia

The junction system of lens fibers of this class varied greatly. Many kinds of protoplasmic processes and manners of their interdigitation were observed.

A. Order Squamata

This order, still alive, is divided into two suborders, i.e., the suborder Lacertilia, the suborder Ophidia; and their junction systems were entirely different from each other.

1. Suborder Lacertilia

In gekkoes, two kinds of protoplasmic processes, namely, the giant processes and the small processes, were observed. The giant processes (measuring 3-4 μ in length, about 2 μ in width), were very regularly arranged at the edges of the lens fiber, so that the overall aspect looked like sawteeth. The small processes which sprouted on a giant process, 18-20 in number, were 0.3-0.5 μ long, 0.2 μ wide, round-ended, and constricted at the roots. With these two kinds of processes, the lens fibers interdigitated one another tightly and complicatedly.

On the surface of the lens fiber, spherical wall spines of various sizes and hollows of their molds were observed (Fig. 1).

Though the lizard showed the same structure as the gekko in substance, neither of the two processes were so regular and highly developed as those of the gekko.
Fig. 1. Lens fiber junction system of Gekko. Complicated interdigitation with giant processes and small processes is seen. This junction system is peculiar to teleosts. ×9,000

Fig. 2. Elaphe lens cortex. Small protoplasmic processes grow thickly at each edge. These processes resemble the small processes of teleosts in size and form. ×4,800
2. Suborder Ophidia

At the edges of the lens fiber, fine protoplasmic processes grew luxuriantly, and interdigitated with the adjacent processes. The manner of junction was the same as that of bovines (Tanaka and Iino, 1967). These fine processes were uneven or irregular (0.3–0.8 μ in length, 0.1–0.3 μ in width). On the surface of the lens fiber, fine wall spines were observed (Fig. 2).

B. Order Crocodilia

The junction system of this order was the same as that of snakes in general, but the protoplasmic processes were a little larger than those of snakes (1.0–1.2 μ long, about 0.3 μ wide).

On the other hand, a fairly large number of broad, flat- or arc-ended protoplasmic processes existed among the small protoplasmic processes, and these types of processes resembled the characteristic forms of the protoplasmic processes of birds, which will be discussed later in this paper.

On the surface of the lens fiber, curious forms of wall spines, such as dovetailform and batonform, were observed in abundance (between 0.5–0.8 μ long). They were seldom seen in other reptiles except in just a few nucleus lens fibers of snakes, whereas, in lens fibers of birds, they were characteristically observed. In addition, the protoplasmic processes and their interdigitation, which were the same as in mammals, especially dogs, were occasionally observed in some parts of the lens cortex (Fig. 3).

C. Order Chelonia

Three kinds of junction systems were observed in this order.

First, about the equatorial zone of the lens cortex and intermediate layer (between the cortex and the nucleus), the boundary line between the adjacent fibers meandered generously, and protoplasmic processes as well as their interdigitation were quite irregular or obscure (Fig. 4).

Secondly, in the same layers, but a little apart from the equatorial zone and toward the poles, exactly the same junction system as that of the gecko was observed. The sizes of the two kinds of processes were the same as those of the gekko, too.

Thirdly, protoplasmic processes whose tops were round, with constricted roots, were observed about the poles. The manner of interdigitation was the same as that of bovines. On the surface of the lens fiber, many spherical wall spines like those of the gekko were distributed, but near the poles, none of them were observed (Fig. 4, Inset).

II. Class Aves

The junction systems of lens fibers in this class had common characteristics with few exceptions. They were as follows:

1. Protoplasmic processes were remarkably broad in comparison with their length. These processes showed various form, such as, a fan, a dovetail, a teaspoon, a seedleaf, a baton, etc. They were maximally developed in the intermediate layer of the lens (Fig. 5).

2. Remarkably developed wall spines were observed on the surface of the lens fiber. Though they were most developed at the equatorial zone of the lens cortex, they were gradually less so as they approached the lens nucleus or the poles (Fig. 6).
Fig. 3. *Alligator* lens, intermediate layer. Broad processes (P) and well-developed dovetailform wall spines (W) which are characteristically observed in birds, as noted. ×5,100

Fig. 4. *Clemmys* lens cortex, equatorial zone. Boundary lines meander generously. Protoplasmic processes and their junction manner are irregular and obscure. ×5,000. The inset shows a *Clemmys* lens, near the pole of the intermediate layer. This type of junction system is suggestive of mammals. ×5,800
3. The manner of interdigitation was the same as that of bovines.

A. Order Galli

In domestic fowls, protoplasmic processes were varied as mentioned above; for example, the smallest process was 0.5 μ long, 0.3 μ wide, batonform, while, on the contrary, the largest one was 1.5 μ long, 2.5 μ wide (about 1.3 μ wide at the root), and of dovetail or fanform (Fig. 5). Various forms of wall spines were observed on the lens fiber surface, but the spines of dovetailform or fanform were characteristic. The length sometimes measured 2 μ (Fig. 6a).

B. Order Anseres

Protoplasmic processes were generally a little smaller than those of the domestic fowl (0.6–1 μ in length, and varied in width). Their forms were commonly like a teaspoon or a fan. Wall spines which looked like rice stubbles were characteristic. However, they were seldom observed near the lens nucleus (Fig. 6b).

C. Order Passers

In case of crows, protoplasmic processes were like those of other birds in all lens fiber layers with the exception of the lens cortex, where the processes were so exceedingly short and flat that it seemed as if no interdigitation with the adjacent lens fibers existed. Plenty of wall spines of dovetailform or seedleafform were observed (Fig. 6c). Both the sparrow and the swallow were approximately the same as domestic fowls.

D. Order Striges

Though the birds of this order have a special ability, namely, they are able to see
Fig. 6 Various types of wall spines of birds. a. *Gallus.* ×5,500. b. *Anas.* ×8,400.
   c. *Corvus.* ×5,800  d. *Strix.* ×8,300
in the dark, there were no remarkable characteristics in the junction system of the lens fibers. However, protoplasmic processes were not so broad as those of the domestic fowl, and numerous relatively slender processes were observed. On the surface of the lens fiber, great and unfixed protuberances were observed in scattered locations (Fig. 6 d).

Discussion

As the class Reptilia is called a heterogenous group and considered to be a collection of a variety of kinds or types in respect to systemic zoology, the lens fiber junction system of this class also seems to be a mixture of various forms of processes or spines and manners of interdigitation. Therefore, no such fixed junction system as is peculiar to elasmobranchs, teleosts, birds, and mammals, exists. For instance, the lens fibers of lizards and some parts of the lens fibers of turtles show the same junction system as that of teleosts. Snakes show a junction system that is made up of fine protoplasmic processes only. Moreover, crocodiles have protoplasmic processes quite resembling those of birds.

From the viewpoint of the lens fiber junction system, it is strongly inferred from the results of my observation that reptiles and teleosts are closely related.

Fig. 7. a. Conger eel lens cortex. Giant processes have stretched here. ×5,500. b. Elaphe lens nucleus. Projections which resemble giant processes of teleosts are observed (arrows). ×7,300

Apparently, the lens fiber junction system of snakes is entirely different from that of teleosts, but the fine protoplasmic processes of snakes are similar to the small processes of teleosts in their forms and sizes. Therefore, it is suspected that if the
giant processes of teleosts stretch themselves and the small processes remain as they stand, the same manner of interdigititation as that of snakes is formed. Actually, plenty of transitional figures are observed here and there upon the lens fibers of conger eels among teleosts, where the giant processes stretch themselves gradually, and a manner of interdigititation similar to that of snakes is developed (Fig. 7 a).

In addition to that fact, in some parts of the lens fibers of snakes, there exist figures which seem to be the remainder of the giant processes (Fig. 7 b).

The above mentioned facts indicate that the lens fiber junction system of reptiles and that of teleosts are very closely related, and further reasoning suggests that common teleosts have developed into the order Squamata by way of the order Anguillida.

As for crocodiles, they have processes which resemble those of birds; whereas, the manner of interdigititation is the same as in mammals. These findings support the author’s assertion that this order is just at the turning point where reptiles developed into birds and mammals.

Lastly, birds have common and distinct characteristics as elasmobranchs and teleosts.

Their wall spines, especially, which are maximally developed among all vertebrates, seem to have reached the vertex, and it is suspected that these well-developed wall spines may have some sort of relation to the fact that lenses of birds have supreme ability of accomodation, and these wall spines prevent divergence from occurring at the time of accomodation.

These results, together with previous observations (TANAKA and IINO, 1967; TANAKA, 1969), suggest that the lens fiber junction systems, viewed from a phylogenetic angle, seem to have evolved directly from teleosts into reptiles, without passing through amphibians, and, furthermore, reptiles have branched off somewhere near crocodiles and have developed into birds or mammals.

**Summary**

Lens fiber junction systems of reptiles and birds were investigated from the phylogenetic point of view, mainly using the replica method. The results were as follows:

1. There were so many kinds of protoplasmic processes and their junction manners that no fixed form existed among reptiles; namely, gekkoes and turtles were provided with the same junction system as that of teleosts, snakes were composed of small processes only, and crocodiles had a characteristic junction system similar to that of birds.

2. Reptiles were more closely related to teleosts rather than to amphibians, and crocodiles seemed to be just at the turning point of developing into birds and mammals.
3. Birds had common characteristics in their junction system; i.e., broad protoplasmic processes, well-developed wall spines, and a junction manner which was the same as that of bovines.

Acknowledgement. The author wishes to show his sincere gratitude to Prof. K. Tanaka for his valuable advice and encouragement. The author is also grateful to Mr. Y. Kashima for his constant assistance.

References


Dr. Hiromi Yamasaki
Department of Anatomy
Tottori University
School of Medicine
683 Yonago, Japan

山崎 弘巳
〒683 米子市西町86
鳥取大学医学部第二解剖学教室