ON THE DEVELOPMENT OF CONNECTIVE TISSUE FIBERS
OF THE SCLERA IN THE DOMESTIC FOWL

BY

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INTRODUCTION

Ischreyt (1899) studied fibrillar structure of the human sclera and obtained the result that in the anterior half of the eyeball the connective tissue fibers running in the meridional and latitudinal direction are superior in strength to those in other directions, especially at the limbus of the cornea; in the posterior half there exists no regularity. On the eyeball of the ox, we have works of Ischreyt and E. Fischer (1933). There is no study on the development of connective tissue fibers themselves in the sclera except for the description on the development of the clefline system of the sclera (Abe. 1959). We have also yet no description on connective tissue structure of the sclera which includes bony tissue in the avian eyes. The function of the sclera lies in the fact that it maintains proper form of the eyeball and protects its contents. This mechanical function depends entirely upon the architecture of the connective tissue fibers in it.

In case of avian eyes which are provided with bony tissue in the sclera histological relationship between the bone and connective tissue must be taken into consideration from the standpoint of dynamics.

The fact that avian eyes contain bony tissue has been known since as early as 1250, and its histological structure was clarified, but the relationship between the bony and the fibrous tissue remained still obscure. The aim of the present study is directed to make clear the process of formation of the fibrous wall of the eyeball and also the histological or structural difference of the sclera between birds and mammals.

MATERIALS AND METHODS

Eyeballs of the domestic fowl in various stages of development ranging from 7-day-old embryo to the adult were used. Before the 7th day of incubation connective tissue fibers are not found in the organ concerned. Both sections and exteired membranous preparations were employed for histological observation. Observation of section preparations only does not suffice the reconstruction of the fibrous architecture of the tissue, as stated by E. Fischer.

Sometimes, therefore, observation of eyeball under the falling light was necessary.
In order to prepare section preparation, an eyeball was dissected out, fixed in 10% formalin and divided into anterior and posterior halves with a pair of scissors.

Each half was placed under a binocular dissection microscope, and the inner components of the wall of the eyeball, the retina and the chorioidea were stripped off. Then the remained membrane, the sclera, was treated with silver salts. Until the 8th day of incubation there is no difficulty in observing the preparation, for the sclera remains one layered. After the 9th day of incubation the sclera can be distinguished in two layers: inner cartilagenous and outer fibrous. So that one can observe the structure of the fibrous layer more clearly, the cartilage layer was sometimes removed at various regions of the ball. In order to keep in sight general topographical relation of one region to the others, each half of the eyeball was not cut off any more, and was mounted as a whole on a slide glass as membrane preparation. Several cut had, then, to be made in a radial direction in order to avoid of folding of the membranous preparation. Up to the 10th day of incubation, there was no difficulty in preparing the specimens, as the tissue remained still thin. But after that time the tissue increases in thickness and acquires elasticity, so we often met somewhat difficulty in preparing and observing the object by ordinary microscopy. In these cases falling light was helpful in the later stage of development of the eyeball.

Section-preparations were referred to the membranous ones of the same stage of development. For silver-impregnation of the connective tissue fibres a modification of Bielschowsky method was employed.

**Findings**

7-day-old embryo. No trace of fiber formation was discerned in the outer layer of the eyeball. In the section preparation of this stadium the eyeball is surrounded by a mesenchymal tissue, the border line of the scleral capsule being obscure. The sclera is not yet differentiated (fig. 1, 2).

8-day-old embryo. In a membrane preparation the initial sign of connective tissue fibers becomes discernible. In the section of the same stage the sclera appears as an independent formation (fig. 3).

9-day-old embryo. In the section cartilage is seen apparently in the sclera. Outside the latter a thin layer of connective tissue is seen (fig. 4, 5). Distinct argyrophile fibers are recognizable there in this stage. In the membrane preparation one can see silver impregnated fibers running undulately in all the directions (fig. 11).

10-day-old embryo. Silver impregnated membranous preparations show that the fibers are on the way of transformations from wavy to straight form (fig. 12).

They form in the margin of the cornea a network with regular arrangement, while they changes there arrangement gradually and run parallel to each other in the meridional direction. As they approach the posterior pole of the eyeball, they become to cross with each other in an acute angle. At the area,
where the optic nerve leaves the eyeball, fibers radiate from the junction of
the nerve in all directions (fig. 13). They come to join another system men-
tioned above. In birds the optic nerve leaves not in the vicinity of the posterior
pole, but rather near the equator of the eyeball, and the shape of the junction
area appears in the form of ellipsoid, different from mammals, whose optic
nerve leaves eyeball in a rounded area. This is based upon the fact that when
the choroid fissure closes, the closure does not reach the base of the optic nerve,
but stops on the way, so that the junction remains elongated, resulting conse-
quently complicated arrangement of the connective tissue fibers in the sclera
near the junction of the optic nerve.

As for the argyrophily of the fibers in the sclera, fibers between the cor-
neal limbus and the anterior margin of the scleral cartilage keep strong argy-
rophily up to the adult stage (fig. 20), while the fibers in the other parts of the
eyeball lose their affinity to silver sooner or later. Argyrophily of the fibers in the posterior pole reduces gradually in 10 days of the embryonal life. At
the same time the eyeball itself begins to transform.

This phenomenon is in closer relation to a change in thickness of the
sclera (fig. 8, 9).

The shape of the fowl eyeball is approximately round up to the 9th day of
incubation. After the 10th day the eyeball begins to protrude externally at the
region a little bit anterior to the equator (fig. 21). This protrusion is caused
by that of the cartilage. Sections of this stage show that on the protruded
area of the cartilage the fibrous tissue becomes thinner and thinner as the car-
tilage grows in this direction (fig. 9). Thus the growth of the cartilage results
transformation of the eyeball and simultaneously unequalness of the fibrous
layer on the cartilage. The fibrous layer of the sclera becomes thickest at the
posterior pole as well as at the anterior margin of the cartilage and thinnest
on the equator of the eyeball.

According to the preparation of the 11- and 12-day-old embryo, reduction
of argyrophily is evident (fig. 14), with the exception of the anterior region
mentioned above. In this region the arrangement of the fibers are changing
at this time: parallel fibers to the corneal limbus become predominant. This
transitional region where the scleral fibers are connected to the corneal ones (fig.
6) develops an osseous tissue about the 13th embryonic day. This is apparent
in sections. It becomes considerably distinct on the 15th day of embryonic life
(fig. 10). This hard tissue corresponds to the scleral ring in the adult. The
relation of osseous ring to the surrounding connective tissue fibers in the adult
is demonstrated in fig. 22 diagramatically.

The outer and the inner surfaces of the ring are covered respectively by
a thin and thick layer of the fibers running concentrically. The meridional
fibers, which are running parallel in the equatorial region, meet each other
near the anterior margin of the scleral cartilage in an angel (fig. 24). Parts of the deeper fibers come here to attach the posterior edge of the cartilaginous
ring, while the greater majority becomes transitional fibers noted above. They
form regular networks (fig. 24). The fibers in the posterior half of the eyeball
does not change their arrangement during more advanced stages of develop-
ment. Their argyrophily, however, reduces gradually, thus acquiring characteristics of collagenous fibers. Hand in hand with the progress of collagenization, the fiber bundles become coarser.

**Discussion**

As stated above, the eyeball of the domestic fowl exhibits a remarkable change of shape during its embryonic development.

In the later stages the cartilage in the sclera takes predominance in growth, so that the connective tissue proper cannot avoid of secondary changes in architecture.

This is the point of difference from mammalian eyes. In the mouse, for example, the equatorial region of the sclera consists of meridionally running, parallel fibers in the earlier stages of development like in the chick. But, later, fibers running in various directions are added and their arrangement becomes more complicated as the development proceeds. On the contrary, in the chick, development of the connective tissue fibers here is suppressed by that of the cartilage. They remain in the initial state and run constantly in the longitudinal direction up to the adult stage. Another prominent difference between birds and mammals in the fiber arrangement is found in the behavior of the fibers at the peripheral region of the cornea. In the mouse there is no parallel fiber to the corneal margine in the transitional region. Further difference is seen near the junction of the optic nerve and the eyeball. This area is situated near the equator in the chick, so that the meridional fibers are gathered at the posterior pole, whereas in the mouse some fibers are gathered around the junction of the optic nerve and flow into the sheath of the nerve, since the junction is situated near the posterior pole. At the insertion of the ocular muscles the scleral fibers are in disorder.

**Concluding Remarks**

Arrangement of the scleral connective tissue fibers was studied in various stages of development in the domestic fowl mainly by the aid of silver impregnation of sections as well as membrane preparations of the eyeball. The primordium of the sclera appears as the mesenchymal condensation around the optic vesicle, and connective tissue fibers become visible in the mesenchym on the 8th day of incubation as wavy minute fibrils. On the next day the cartilage begins to form in the sclera. The wavy fibrils are gradually straightened with the progress of the growth of the eyeball.

The connective tissue fibers are arranged in the meridional direction as a whole, but in the anterior margine of the sclera, where they are in connection with the corneal fibers, form a network with regular shaped meshes. As the development proceeds, the fibers here tend to run parallel to the margine of the cornea. The fibers are concentrated at the posterior pole of the eyeball, where complicated crossing networks are seen. Thickness of the fibrous layer
of the sclera is at first uniform in various parts of the eyeball, but later it diminishes in the equatorial region.

Further development of the fibers in the sclera seems to be suppressed by the presence of the cartilage, which does not exist in the mammalian eye. In the latter the fibers develop in accordance with the development of the eye. The fibers appear to be argyrophilic in the initial stages of development, but gradually they acquire collagenous nature with the exception of the region around the cornea. The latter remains argyrophilic further.

References

EXPLANATION OF FIGURES

Fig. 1. Section through the corneal margin. 7-day-old chick embryo. Hematoxylin-eosin stain. ×170.

Fig. 2. Section through the equator of the eyeball. 7-day-old embryo. The sclera does not appear as an independent layer yet. H-E stain. ×170.

Fig. 3. Section through the equatorial region. 8-day-old embryo. The cartilaginous tissue does not exist yet. H-E stain. ×170.

Fig. 4. Section through the corneal margin. 9-day-old embryo. H-E stain. Cartilage is not visible. ×340.

Fig. 5. Section through the equator. 9-day-old embryo. H-E stain. The sclera consists of fibrous and cartilagenous layers. ×170.

Fig. 6. Section through the corneal margin. 11-day-old embryo. H-E stain. Future scleral ring is expected here. ×340.

Fig. 7. Section through the anterior part of the cartilage. 11-day-old embryo. H-E stain. ×170.

Fig. 8. The same portion as Fig. 7. 13-day-old embryo. H-E stain. The fibrous tissue is thick here. ×170.

Fig. 9. Section through the equator. The fibrous tissue is thin here. 15-day-old embryo. H-E stain. ×170.

Fig. 10. Section through the corneal margin. 15-day-old embryo. H-E stain. The scleral ring is visible. ×170.

Fig. 11-19. are all membranous preparations stained with silver.

Fig. 11. Equatorial region. 9-day-old embryo. Fibers run wavy. Black spots represent cartilage. ×170.

Fig. 12. Posterior half of the sclera. 10-day-old embryo. Fibers are straightened already. ×170.

Fig. 13. Junctional area of the optic nerve and the eyeball. 10-day-old embryo. ×170.

Fig. 14. Posterior pole of the eyeball. 11-day-old embryo. Fibers are straightened and crossing each other. Argyrophilic diminished. ×170.

Fig. 15. Equatorial region. 12-day-old embryo. Fibers run parallel to each other in the meridional direction. ×170.

Fig. 16. Posterior pole. 13-day-old embryo. ×170.

Fig. 17. A little bit anterior to the equator. 16-day-old embryo. Crossing in acute angles is recognizable. ×660.

Fig. 18. Equatorial region. 16-day-old embryo. Note parallel running fibers. ×660.

Fig. 19. Posterior pole. 16-day-old embryo. Note complicated crossing arrangement of the fibers. ×660.

Fig. 20. Transitional portion of the sclera to the cornea. Blackened area means strong argyrophilic of fibers.

Fig. 21. Comparison of the fibrous layer of the sclera of the mouse (top) and the chick (bottom).

Fig. 22. Diagram showing arrangement of the fibrous component around the scleral ring in the adult.

Fig. 23. Posterior view of the sclera showing fiber arrangement. Black area means the junction of the optic nerve and the eye.

Fig. 24. Side view of the eyeball showing fiber arrangement of the sclera.
LIST OF ABBREVIATIONS

C.: Cornea.
Ca.: Cartilage in the sclera.
RE.: Retina
Sc.: Sclera.
Sr.: Scleral ring.
Tr.: Transitional region between the sclera and the cornea.
Fig. 20

Fig. 21