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ON THE PRESENCE OF ELASTIC FIBRES IN THE CORNEA.

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The present piece of work was undertaken with the view of demonstrating the development of the cornea in its relation to cutaneous structures, in order that some light might be thrown on the pathology of the corneal tissue.

The subject has been treated from a comparative point of view, and therefore, in addition to the human being, the corneæ of lower animals, when possible, were examined, developmentally and otherwise.

I must gratefully acknowledge my indebtedness to Dr A. Maitland Ramsay for his kind supervision in this work, and for giving me free access to all the material of the pathological laboratory of the Ophthalmic Institution.

The exact condition of matters in the development of the human cornea has always been much disputed. In the lower animals, where the chance of obtaining a continuous series of sections is much more favourable, a great deal of work has been done, and for many species there is a fairly complete description of the development; but in the human species, where the possibility of obtaining examples of all the different stages in early fœtal life is much more scanty, one can understand that great difficulty has been found in making a dogmatic statement as to what actually occurs. In the case of several very young human fœtuses which I obtained for examination, although fixed with all promptitude, yet, when looked at microscopically, they were found to be slightly disorganised and therefore of little use for cellular investigation. In some cases I imagine that the disorganisation was due to the condition which gave rise to abortion, and that the fœtus was born after death had taken place. I had examples of fœtuses from six weeks onwards, with some in the middle term of pregnancy and some in the later months.

Of the examples of the lower animals, those that gave me best results were those of the foal and calf.

Development of the Eye.

The adult cornea consists externally of a layer of epithelium which is a derivative of the epiblast, directly continuous with the conjunctiva,

and somewhat modified for the refraction of light. Little doubt exists as to the origin of this layer; but when it comes to the corneal tissue proper —the substantia propria—we are on disputed ground.

A ring-shaped space is formed between the epithelium and its derived primitive lens follicle, and into this there passes a process of mesoblast. This is at first a structureless layer, and extends right across the front of the lens. According to some authorities, this homogeneous layer is of the nature of an exudation from the corneal epithelium, and therefore epiblastic. It gradually becomes invaded by the corneal corpuscles, which are of connective-tissue origin and are mesoblastic. At the same time a layer of endothelioid cells advances on the inner aspect of this corneal layer, and they constitute the endothelial layer of Descemet's membrane. The cornea grows in thickness through the acquisition of these invading connective-tissue cells, and gradually it becomes more compressed, and changes from a rather loosely knit structure to a finely striated fibrous structure. The compression takes place from behind forwards, so that the posterior layers are more closely packed at first than the anterior. Fig. 14, Pl. XXVI., shows this in a fœtus about the fifth month.

The original homogeneous layer, in front, persists as Bowman's membrane, or "anterior elastic lamina." This is not a proper designation, since the membrane does not stain deeply by stains that pick out elastic tissue. It is thicker at the periphery than centrally, and increases in thickness with advance of life. It is easily seen by the sixth month.

There is a "posterior elastic lamina" or membrane of Descemet which, according to some, is the remnant of the original structureless layer posteriorly; according to others it arises as an exudation from the endothelium covering it posteriorly. It is stained by elastic tissue stains, and decreases in thickness with age. The endothelium covering it is mesoblastic and is continuous with that of the ligamentum pectinatum iridis and the iris.

In early fœtal life there is no aqueous chamber, and the lens remains closely applied to the cornea. It is not till nearly full time that the anterior chamber becomes dilated with fluid (fig. 10, Pl. XXV.)

Analogy of the Cornea in Structure to the Skin.

The analogy of the cornea to the skin, both in development and adult structure, opens up a field whereby the relations of pathological conditions in each might be compared, and it was with this in view that I began my investigation.

The development, structure, and pathology of the skin have received

much attention, and are well established. But I very soon found that, considering the scope of the work, the material I could command for developmental research was too unsatisfactory to be of general use, and I confined my attention in the meantime to the one point of analogy, namely, the presence or absence of elastic fibres in the cornea.

The presence of elastic fibres in the substantia propria has been the subject of much difference of opinion. That they do occur in the human cornea has been affirmed by several, notably Kiribuchi, Prokopenko, and Tartuferi. Their presence has been denied by Stutzer and Sattler.

Kiribuchi showed fine fibrils by the resorcin-fuchsin method, and this was confirmed by Prokopenko by the acid-orcein method. It is generally accepted that they occur in the cornea of the calf. In the skin they occur widely distributed throughout the corium and subcutaneous tissue, in which they form a scaffolding or supporting structure. The fibres may be of a very fine nature, or may be of considerable thickness. In the skin they are said to vary from fibrils of very great delicacy to fibres of 11μ in breadth. They branch and anastomose in an irregular way. They have ends which are clean cut across and often curl up. In the skin their chief function seems to be that of supporting the various structures rather than of giving elasticity.

Elastic fibres resist the action of weak acids, and the other structures do not, so that the fibres are differentiated by prolonged use of weak acids, or it may be a short exposure to the action of strong acids.

With regard to the development of these fibres there has been much dispute. According to some, they are produced by the activity of certain embryonic cells. Others think they are elongated and modified cell-processes. The most likely view seems to be that they arise from growth of nuclei longitudinally. Fig. 11, Pl. XXV., shows the appearance of an elongated nucleus in the midst of young elastic fibres.

Methods of Preparation.

The fixative solution I have found of most value for detail in cell-work is a mixture containing in 100 parts of water 0·25 part chromic acid and 1 part glacial acetic acid. If the eyeball be intact, it is necessary to allow it to remain in this from 10 to 14 days. If the eyeball be divided, 24 hours is a sufficient immersion. It is then necessary to wash in running water for 12-24 hours. After this the tissue is placed in alcohols of constantly increasing strength where it is desired to cut in paraffin or celloidin. But I found that freezing gave least distortion of parts—especially in cases of tangential sections. I consider that while paraffin

and celloidin produce the most picturesque sections, yet one misses much of the natural arrangement of parts, as this may be altered by such histological processes.

For elastic fibres the method of partially macerating the cornea in dilute acetic acid for about three weeks, and making sections by freezing in a tangential direction, was the one that was most satisfactory. Mounting in glycerine was resorted to as a rule in the freezing method, as the treatment necessary to prepare for mounting in Canada balsam also causes shrinkage in the tissues.

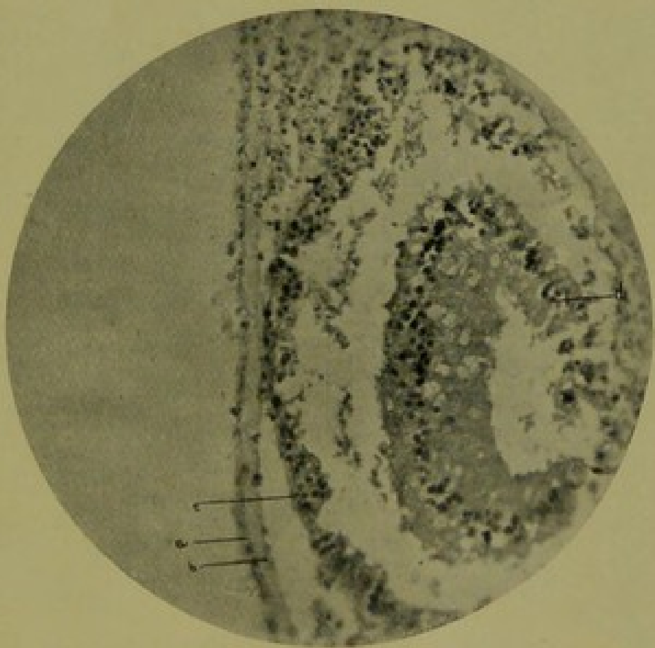

FIG. 1.—Human fœtus of about six weeks. Vertical section of the cornea and lens. Low power.

a, Corneal epithelium showing loosely scattered cells. *b*, Substantia propria of cornea still homogeneous, but becoming invaded at margins. *c*, Epithelium of lens constriction complete, but lens lies close to cornea. *d*, Posterior segment of lens.

The earliest period of fœtal development of which material for examination in the human fœtus was obtained, was between five and six weeks. (Fig. 1 is from a photograph of vertical section through the front of this eye.) At this stage the cells are still embryonic in character, and have not become differentiated into fibrous tissue, etc. Staining for elastin was entirely negative. The corneal epithelium is seen as a fairly narrow band which passes along in front of the cornea, the nuclei being scattered throughout its extent in a single layer. The lens with its epithelial covering is seen close to the corneal epithelium, but quite distinctly separated from it. The first appearance of the substantia propria of the cornea is seen in the narrow, homogeneous layer which separates the epithelium and the lens.

The connective-tissue cells are beginning to invade it from the side, but as yet have not reached very far towards the centre.

The lens is seen to consist of two segments. The figure gives a fairly clear demonstration of the relation of parts, but the demonstration of minute cellular detail was not possible in the specimen, which, although apparently quite fresh to the naked eye, and fixed with all speed, must have suffered slightly from degeneration.

The next stage at which I examined fœtal material was that of a three to four months fœtus.

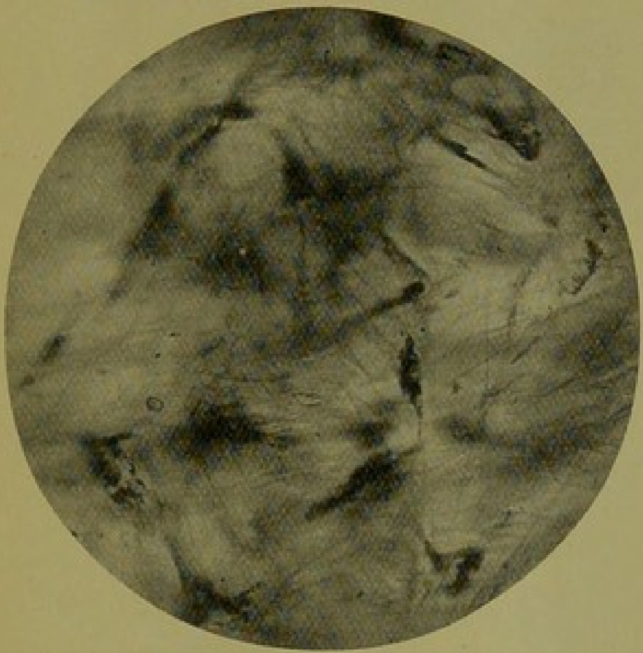

Fig. 2.—Cornea of calf. Semi-macerated in dilute acetic acid. Stained by Weigert's method. High power.

Elastic fibres are seen lying in the ground-tissue of the limbus.

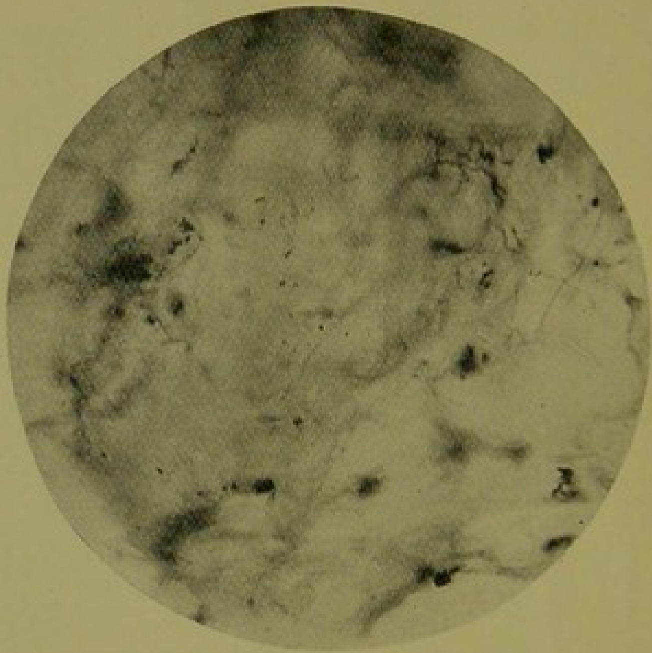

Fig. 3.—Cornea of calf. Semi-macerated in acetic acid. Highly magnified.

The specimen is taken from the limbus. Tangential section. Weigert's stain. The elastic fibres are distinct, especially towards upper part of figure. The large black masses are the pigment cells of the ciliary body, and locate the site which is at the limbus in its deeper layers.

When I began my investigation, I did not realise that it was not possible to obtain a good demonstration of the presence of elastic fibres from the ordinary vertical sections of the cornea. It was only when I began to macerate slightly in acetic acid, and make frozen sections in a tangential direction, that I could obtain a view of the fibres to my satisfaction. In a lamellar structure such as the cornea, when the fibres are compressed between the lamellæ, they may readily escape notice, especially if the differentiating process, after staining, is not completely carried out. This is only one illustration of how the component parts of a section may be missed, especially in one which has undergone shrinkage from any cause, such as is met with in the paraffin or celloidin methods.

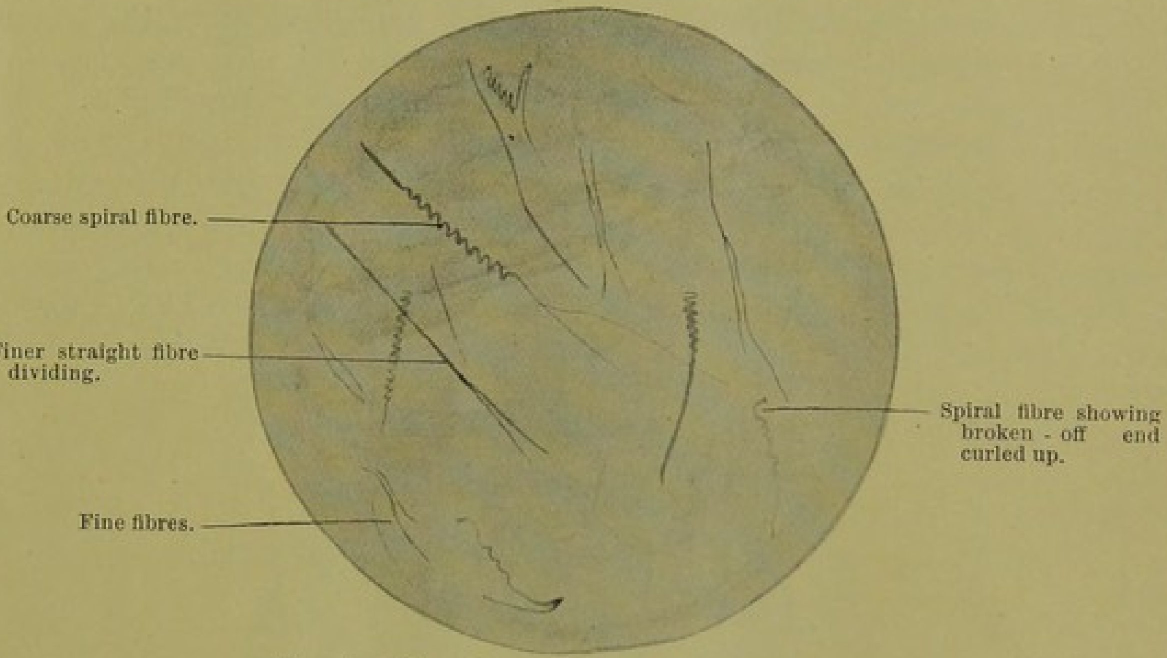

FIG. 4.—Piece of macerated cornea from the calf.

Piece of cornea which had been macerated for four to six weeks in dilute acetic acid, teased out on slide, and then stained by Weigert's stain.

Some of the elastic fibres are distinctly wavy in outline and of considerable magnitude; others are quite straight for a considerable part of their length. Fine fibrils pass from one fibre to another at places.

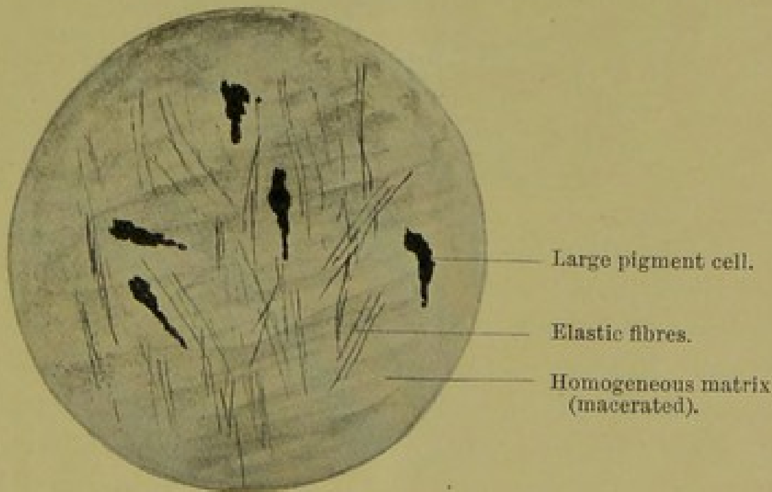

FIG. 5.—Cornea of young calf. Semi-macerated in acetic acid. Tangential section.

Cornea at limbus.—Frozen tangential section stained by Weigert's elastic method.

Fine fibrillar network of elastic fibres. The homogeneous matrix represents the disintegrated tissue which constitutes the rest of the cornea at this part.

As early as the third or fourth month the fibrous nature of the human cornea is well established. Here and there amidst the wavy fibrous tissue one can make out the sharply cut end of a more deeply stained fibre. The posterior layers are more compressed than the anterior, and it is at this region that the cornea takes up the elastic stain more or less uniformly.

At the third month Bowman's membrane is not seen as in the adult cornea, but is represented by a matrix with some more or less loosely scattered corpuscles lying in its substance. Fig. 16, Pl. XXVI., shows a specimen of this age in which the cornea is stained by Weigert's elastic

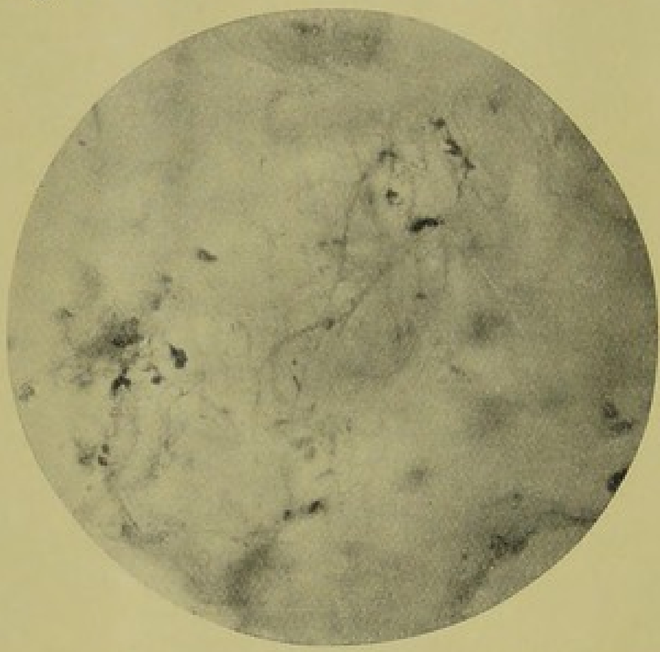

FIG. 6.—Cornea of calf. Semi-macerated in acetic acid. Tangential view. Weigert's stain.

Coarse, spirally twisted fibres can be seen lying in homogeneous matrix at limbus. The pigment corpuscles indicate that the region is the deeper zone over the ciliary area.

stain. The difficulty of differentiating elastic from other tissue is shown in this specimen.

It is stated as a fact beyond dispute, that elastic fibres occur in the cornea of the *calf*. I therefore took this as a basis of comparison, and used it as a method of control for the other material examined. In all my preparations I found a freshly prepared Weigert's elastic stain was the most satisfactory, although in some cases I got quite good results from the orcein method. Figs. 2 and 3 show the presence of elastic fibres in the calf's cornea. The preparations were macerated for three to four weeks in dilute acetic acid. By this treatment the cornea became swelled and the cells somewhat indistinct in outline. Frozen sections were prepared, or portions of the cornea at the various parts were excised and pressed out

between cover-glass and slide. In this latter way a film preparation was produced, and this was stained as mentioned in the description appended to the figures.

In the superficial layers (see fig. 4) which comprise the loose areolar tissue just beneath the conjunctiva outside the limbus, elastic fibres are very abundant. In this region they are broad and wavy, and form a more or less thick feltwork. In the deeper layers (fig. 5) and more internal to the limbus, the fibres are somewhat finer and more delicate.

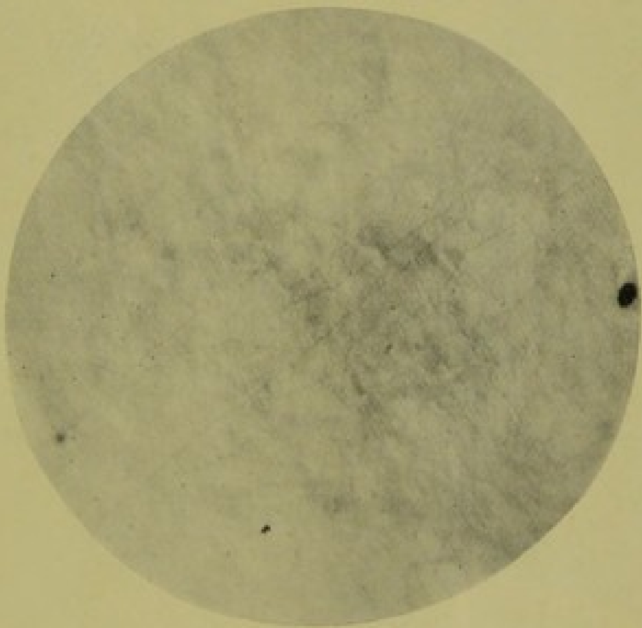

FIG. 7.—Human fœtus about eighth month. Limbus corneæ. Semi-macerated in acetic acid. Tangential section.

The very long, fine elastic fibres are in some places slightly waved, in others almost straight. The fibrous background seen in the right-hand corner is the sclerotic tissue. The corneal tissue lies on the opposite side.

They are more isolated, and the anastomosis with one another is more obvious. The fibres become more scanty towards the centre of the cornea, and in the most central part, they are absent altogether. The large pigmented masses which appear in some of the sections are the pigmentary cells of the ciliary process, and are seen in those sections which have passed through the deeper layers of the limbus.

We therefore see that the elastic fibres are found chiefly in the deeper layers of the cornea, near Descemet's membrane, and are confined to the peripheral portion of the cornea.

In the human cornea the condition is found to be practically the same. The specimens which showed the presence of the fibres most clearly were

those of the later months of pregnancy. In fig. 12, Pl. XXV., the limbus area in a tangential section is seen under a low power of magnification. The stain was Weigert's resorcin-fuchsin. The densely fibrous nature of the sclerotic merging into the somewhat hyaline (when macerated) region of the cornea through the limbus is indicated. In fig. 7 the same appears under high magnification. The fibres are seen passing across the limbus into the peripheral part of the cornea. In this region they are of an extremely fine and delicate nature even when highly magnified. This

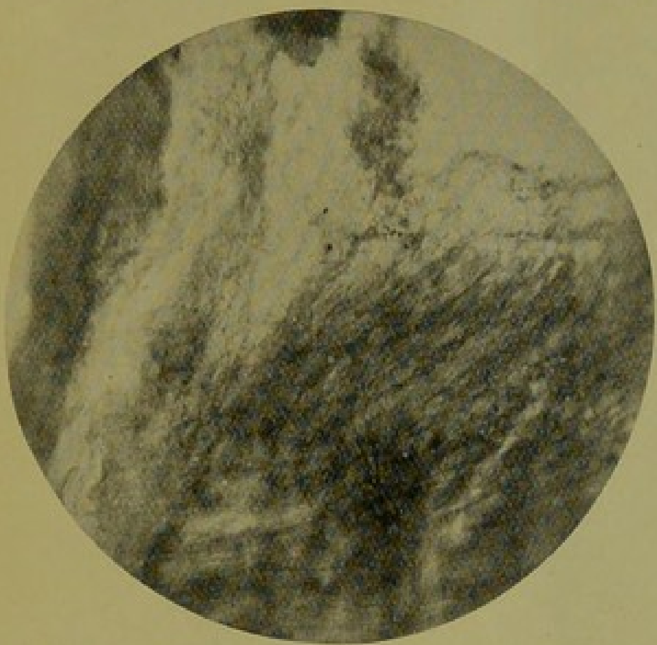

Fig. 8.—The cornea of foal. Tangential section. Superficial layers. Semi-macerated in acetic acid. Low power view. Weigert's stain. Taken from region of limbus.

Note.—The dense mass of elastic fibres at lower part of diagram. The fibres are very coarse in character.

Fig. 9.—Cornea of foal. Semi-macerated in acetic acid. At limbus.

Showing fine network of elastic fibres under moderately high power of magnification. The section is tangential and frozen. The dark areas at lower part are the pigment cells of the ciliary body.

specimen is from a fœtus about the seventh or eighth month of pregnancy. Fig. 7 is from a photograph of superficial layers at the limbus. In this specimen long, isolated fibres are found in abundance in the superficial layers just beneath the conjunctiva at the limbus, but these are lost when the epithelium becomes definitely corneal in character. Fig. 13, Pl. XXV., shows the condition of matters in the vertical section of the same specimen.

The difficulty of demonstrating the elastic fibres is apparent here. Whereas they do not appear in the midst of closely packed lamellæ, yet they are readily seen in the loose conjunctival region just beyond the limbus, even under a low power of magnification.

In the more advanced specimens, and in adult corneæ, fibres can be shown to exist at the limbus, in the deeper layers.

Similar results were obtained in the cornea of the foal which I have examined. Figs. 8 and 9 show the presence of elastic fibres in great abundance. In fig. 8 the coarse, wavy fibres occupy the superficial layers just under the conjunctiva, and illustrate the felt-work condition referred to already. In fig. 9 the fibres exhibited are in the deeper layers and are more delicate in character.

Conclusion.

Although denied by many authors, I have proved to my satisfaction that elastic fibres occur in the cornea. They are chiefly restricted to a zone round the periphery of the cornea, and become lost to view as the central part of the cornea is reached. They are demonstrable in almost equal distinctness, by the Weigert method, in the eye of the human being, in that of the calf, and young horse. In the early weeks of human fœtal life the tissues are still embryonic, and do not take up elastic staining. Definite fibres appear about the third or fourth month. The fibres in the cornea proper are perhaps more abundant in the middle term of pregnancy, but can be demonstrated at the limbus in the later months of extra-uterine life, and in early adolescence.

Preparations, to be satisfactory, must be sections made in a tangential direction, and should be stained by the resorcin-fuchsin method of Weigert.

I have to point out that in a piece of work of this kind it is difficult to obtain good illustrations. I had to make repeated attempts, as the stains do not lend themselves readily to photographic reproduction.

The distinctness of outline necessary for illustration in this way is absent from these semi-macerated specimens, and the elastic fibres are not sufficiently obvious in many instances to make them stand out clearly against the background.

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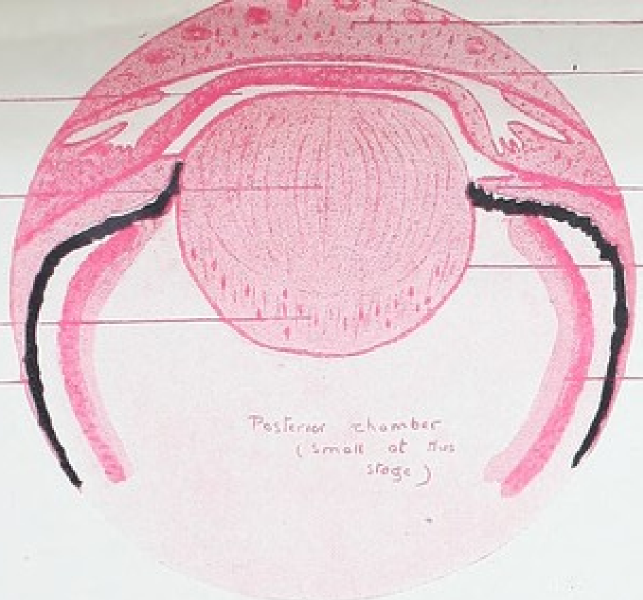

FIG. 10.—Human fœtus. About fourth month. Section of front of eye *in situ*.

FIG. 11.—Elastic fibres. Fœtus about eighth month.

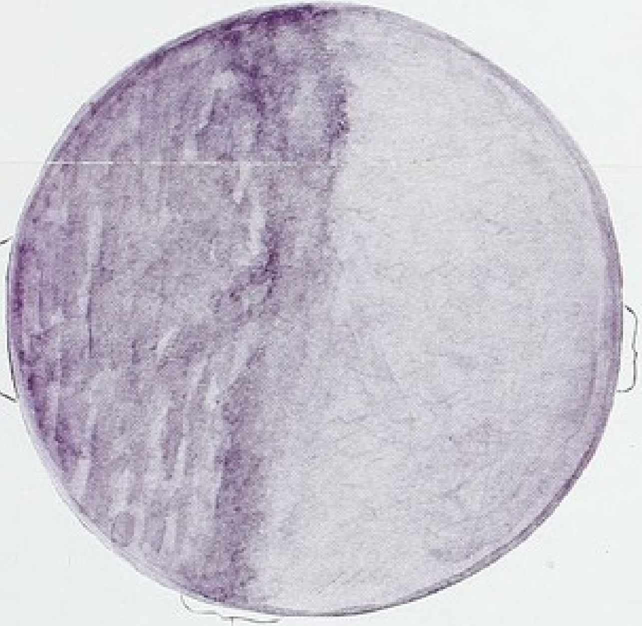

FIG. 12.—Human fœtus. Celloidin. Weigert stain. Tangential section. Low power. Obj. 3. Oc. 3. Leitz.

FIG. 13.—Human fœtus. About eighth month. Semi-diagrammatic. Vertical section.

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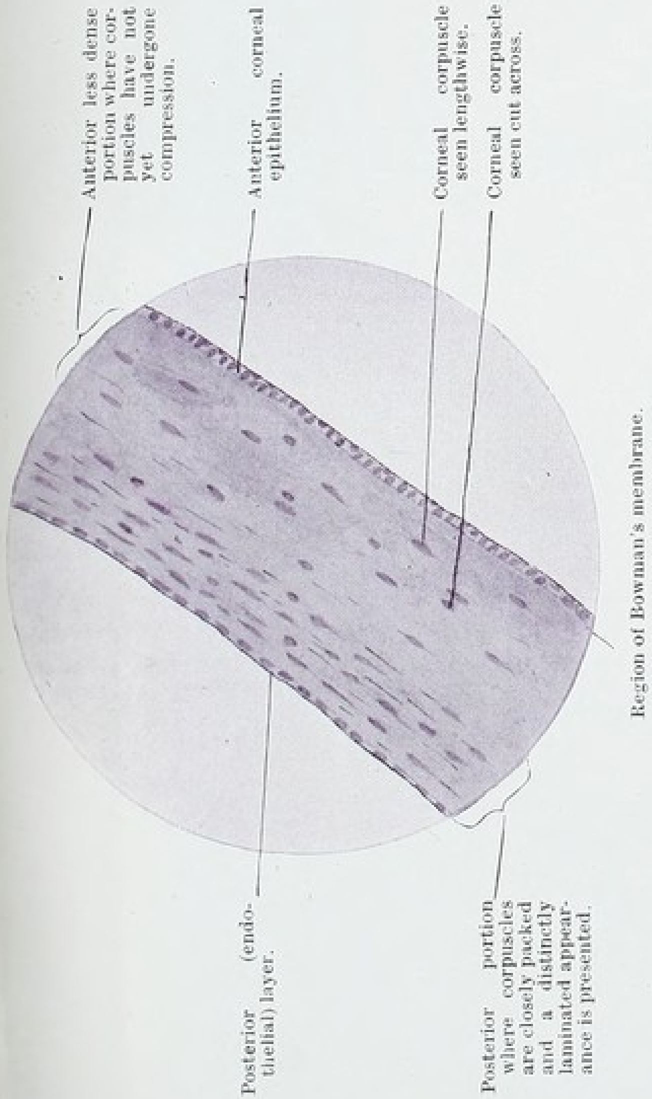

FIG. 14.—Human fœtus. About fifth month. Cornea near the middle. Vertical section.

FIG. 15.—Foal's cornea. Frozen tangential section. Middle zone. Weigert stain. Oil immersion $\frac{1}{12}$. Leitz. Oc. 3.

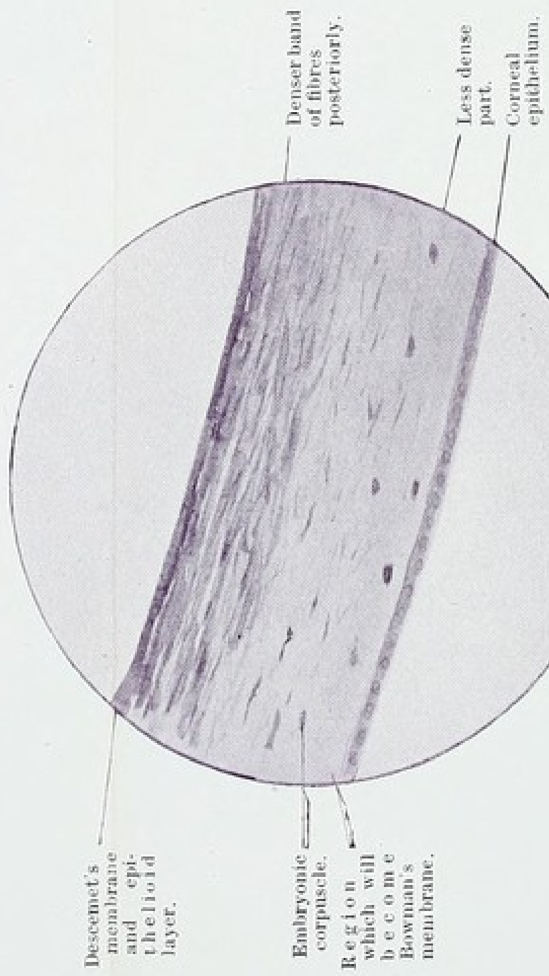

FIG. 16.—Human fœtus. Three to four months. Weigert stain.

FIG. 17.—Human fœtus. Celloidin. Weigert stain. Tangential section. Oil immersion $\frac{1}{12}$. Oc. 3. Leitz. Semi-diagrammatic. Limbus corneæ. Showing elastic fibres.

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