STUDIES ON THE CORNEA. II. THE FINE STRUCTURE OF DESCEMET'S MEMBRANE*

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PLATES 82 AND 83

In a transverse section of the cornea, Descemet's membrane appears as a "glassy" layer between the stroma and the endothelium. It has been identified as the basement membrane of the endothelium (1, 2), and is described as a strong, structureless membrane, very resistant to chemical agents and to pathological processes, several micra in thickness, and normally in a state of tension (3).

During embryogenesis of the cornea, after the formation of the lens vesicle, mesodermal cells grow into the region between the lens and the surface ectoderm. They become arranged in a single layer, parallel to the surface, to form Descemet's endothelium. It is generally agreed that Descemet's membrane is a product of the endothelial cells, being either directly secreted by them or else formed extracellularly from precursors activated by enzymes or other agents liberated by these cells. If Descemet's membrane is injured, it is regenerated.

The contention that Descemet's membrane is the basement membrane of the endothelium is supported by its histochemical behavior. Day (1) reported that all basement membranes, among them Descemet's membrane, were stained intensely by the periodic acid-Schiff method. Wislocki (2) did not distinguish between the basement membrane of the corneal epithelium and Bowman's membrane, but he did observe a narrow linear zone beneath the basal cells which was stained as intensely, by this method, as was Descemet's membrane. Teng and Katzin (4) did describe a clearly defined epithelial basement membrane and found also that both it and Descemet's membrane were intensely periodic acid-Schiff-positive.

Conclusions about the composition of Descemet's membrane drawn from histochemical and polarization optical data have placed it in the collagen class of proteins. Wislocki (2) noted a similarity in the staining properties of Descemet's membrane and collagenous tissues, including the corneal stroma (the more intense periodic acid-

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Schiff reaction of Descemet's membrane may be due, he thinks, to a high proportion of mucopolysaccharide-containing ground substance).

Baud and Balavoine (5) investigated the behavior of Descemet's membrane in polarized light. They found it to be isotropic radially and to show, in sections immersed in water, birefringence which was positive with respect to the plane of the membrane. The magnitude of the birefringence decreased as the refractive index of the mounting medium was increased. When the refractive indices of medium and tissue were equal, Baud and Balavoine observed a reversal of sign, and concluded that Descemet's membrane was characterized by positive form and negative intrinsic birefringence, with respect to the plane of its surface. Citing similar results reported by Köhler and Tobgy on stroma, Bowman's membrane and Descemet's membrane (6), and by Jaeger on tendon (7), Baud and Balavoine felt that Descemet's membrane should be placed in the collagen group of proteins. They postulated a structure consisting of superposed very thin lamellae, lying parallel to the surface of the membrane and to each other. Because of the radial isotropy, they believed that the molecules in the plane of the lamellae have no preferential orientation.

Grignolo (8) confirmed most of the findings of Baud and Balavoine but did not observe a reversal of the sign of birefringence. He found that digestion with trypsin, hyaluronidase, or amylase caused little change in optical activity and suggested that Descemet's membrane might be a scleroprotein of collagen type.

On the basis of chemical analyses, Dohlman and Balazs (9) stated that the bulk of the organic material of beef Descemet's membrane is collagen. Comparing this tissue with beef corneal stroma, however, they found that the polysaccharides were different in type and were present in a lower concentration in Descemet's membrane, and that the solubility and swelling characteristics of the two tissues were different, Descemet's membrane being much more resistant than the stroma.

From the results obtained with polarization optical methods, it was hoped that in its fine structure Descemet's membrane would possess some discernible pattern of orientation. Thin sections of rat membrane, however, appeared to have no visible periodicity except that observed in scattered striated fibrils (10). Fortunately the investigation was continued on other species and results were obtained which made it obvious that the albino rat had been a poor first choice of experimental animal for this study. A preliminary account of the later investigation has already been presented (11).

Bairati and Grignolo (summarized in reference 8, with photographs) examined OsO_4-fixed, fragmented beef Descemet's membrane with the electron microscope and observed either clumps of fibrils, which they believe were not contaminating stroma collagen fibrils, or lamellar structures consisting chiefly of indistinct globular masses. The diameter they give for these structures is about 300 A.

Materials and Methods

Descemet's membranes of the following species have been examined: frog, turtle, fowl (chick embryo, chick, and capon), mouse, rat, beef (calf and steer), and human.

In the basic procedure, entire corneas were fixed in buffered 1 or 2 per cent OsO_4, at pH's

around 7.4 for adult tissue and 8.0 for embryonic tissue, for 1 to 3 hours, and dehydrated in either ethyl or methyl alcohol. During the dehydration the corneas were cut into strips, for transverse sectioning, or into squares, for tangential sectioning. The embedding medium was 10 or 15 per cent methyl methacrylate in *n*-butyl methacrylate, with inhibitor removed, catalyzed by benzoyl peroxide (about 2 per cent), and polymerized either under an ultraviolet lamp or in an oven at 46°C. Isolated Descemet's membrane, obtained by scraping it from fresh or from frozen and thawed corneas, was treated in the same way.

Contrast was increased by extracting the tissue, before fixation, with citrate buffer at pH about 4.8 for periods up to 2 days, in the cold, or by digesting it with 0.01 per cent trypsin at a slightly alkaline pH, at 37°C., followed by treatment with 0.1 to 2 per cent phosphotungstic acid added to the absolute alcohol during dehydration.

For examination with the polarizing microscope, water mounts were made of deparaffined sections of corneas, prepared by standard histological methods, or of fragments scraped from fresh or from frozen and thawed corneas.

The specimen for x-ray diffraction examination was prepared by stacking the larger pieces (up to 1 $cm.^2$) obtained by scraping the membrane from the inner surface of frozen and thawed beef corneas, from which the endothelium had been removed by gentle scraping. Each piece was rinsed in distilled water, placed momentarily on a porous plate to absorb the excess moisture, and then smoothed out rapidly with curved forceps, the first piece on a dry glass slide, and the others on top of the first, until a stack about 1 mm. in thickness was obtained. Another slide was then placed over the first and held in place with rubber bands until the specimen dried.

OBSERVATIONS

Isolated fragments and transverse sections of beef Descemet's membrane showed birefringence positive with respect to the plane of the membrane (Figs. 1 and 2) as had been observed by others. In the radial direction the fragments appeared to be isotropic (Fig. 1). The magnitude of the birefringence, in transverse sections, was less than that of the stroma collagen (Fig. 2).

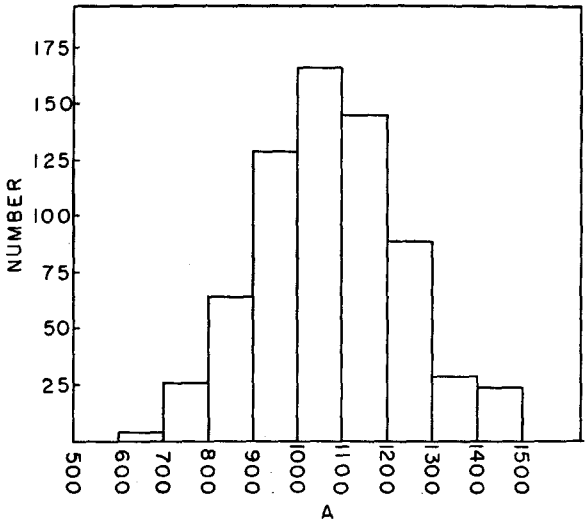
The first clear evidence of periodic structure in Descemet's membrane was observed in thin sections of beef tissue which had been isolated from the stroma and treated, during dehydration, with phosphotungstic acid. The periodicity took the form of narrow dark bands oriented roughly at right angles to the plane of the membrane and separated by wider light bands. Tissue which had been well fixed showed the banding throughout, *i.e.*, from the stroma surface to the endothelium surface. The same pattern could be seen without phosphotungstic acid treatment, but it showed much less contrast.

Descemet's membranes of other species were examined. Those of frog, turtle, and capon showed the same type of periodicity, extending from one surface of the membrane to the other, while in the human it was well defined only in the portion near the stroma and faded out toward the middle of the membrane. In the mouse the periodic structure appeared to be scattered throughout the membrane (Fig. 4); and even in the rat small organized regions were finally found in heavily phosphotungstic acid-stained tissue (Fig. 5).

Text-fig. 1 shows the distribution of measurements of the observed period

(the distance between dark bands). It represents 676 measurements made on six different species (human, turtle, mouse, capon, chick, beef, calf, and frog). Most of the measurements fell between 900 and 1200 A, and the average of all the values was found to be 1070 A. The distribution obtained for beef alone was almost identical with the composite plot; those for the other species had their maximal ranges shifted somewhat (e.g., human: 900 to 1100 A; capon: 1000 to 1250 A; chick: 1100 to 1300 A). However, since the numbers of measurements representing the other species were all less than the total for beef (201), these deviations only suggest a species, or age, difference.

The sections thus far described were all cut perpendicular to the plane of Descemet's membrane, but otherwise they were oriented at random. Yet,



TEXT-FIG. 1. Distribution of the spacing (distance between dark bands) in transverse sections of Descemet's membrane. Ordinates represent number of measurements of spacings shown by abscissae. The average spacing for this set of measurements is 1070 A.

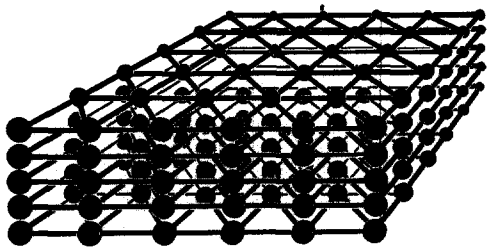
regardless of the orientation, the same structure was presented—an apparent cross-striation which seemed somewhat out of place in a membrane. It was obvious that cleaner preparations and tangential sections were required.

Extraction with citrate buffer or digestion with trypsin, followed by treatment with phosphotungstic acid, improved the preparations considerably. In transverse sections the dark bands now appeared to be made up of granules, while the light bands were traversed by fine filaments apparently forming connections between the dark granules (Fig. 3). In very thin sections, such as this one, the dark bands were often interrupted, as if they had run out of the plane of the section. It was the tangential section, however, that solved the puzzle. It showed a two-dimensional array of granular nodes connected by fine filaments, each node being equidistant from the six around it (Figs. 6

and 7). The distance between nodes was the same as the distance between dark bands in the transverse sections and now it could be seen why transverse sections cut through any portion of a given membrane had the same appearance.

In both transverse and tangential sections there appeared, in addition to the more obvious framework, two other components. One took the form of stray fibrils which seemed to wander into and out of the sections; they had a rather indistinct cross-striation which resembled the fine banding of collagen (Fig. 6). The other component was amorphous, almost cloud-like, in form but could frequently be resolved into extremely fine filaments. In tangential sections these fine filaments were sometimes arranged between the internodal filaments, in spider-web fashion.

The dark nodes, in both types of sections, were irregular in shape, ranging from a more or less distorted circle in outline to a star-shaped figure the arms of which seemed to run out into the internodal filaments. The filaments, too,



TEXT-FIG. 2. Diagrammatic representation of the structure of Descemet's membrane, based on its appearance in transverse and tangential sections.

were not uniform, either in width or in density. Most of them were less than 100 A in width, but they frequently appeared double, or double-edged, suggesting the possibility of their being ribbon-like rather than cylindrical in shape (Fig. 6). In both types of sections there could be seen occasionally what looked like a fine banding along the internodal filaments (Fig. 6). Measurements (141) of this dubious period fell into a very broad range, extending from 70 to 250 A, with rather low peaks about every 30 A.

A diagram of the probable structure of Descemet's membrane is shown in Text-fig. 2. The membrane is visualized as being made up of a series of two-dimensional grids, each consisting of nodes and connecting internodal filaments, superimposed in phase. The distance between nodes is about 1070 A, and the separation between filaments in transverse section, about 270 A. This figure also represents the thickness of the unit grid, and the thickness of the nodes. No attempt was made to show the amorphous material, the very fine filaments, the wandering striated fibrils, or the equivocal filament striation.

As has long been known, the embryological differentiation of Descemet's

membrane starts late and is slow. In chick embryos, Descemet's membrane has not been identified with certainty until about the tenth day of incubation. It appeared first as small, isolated pads between the stroma and the endothelial cells. The pads then seemed to grow laterally until they met and finally fused. Fig. 8 shows the Descemet's membrane of a 10-day (post-hatching) chick which, although continuous, is still very thin. It has a slight depression which probably represents the boundary between two adjacent primordia. Even after two months, the membrane is considerably thinner than that of the adult fowl. At all stages, however, the characteristic fine structure was observed (Figs. 7 and 8).

During the course of this investigation, Rougvie and Bear (12) kindly offered to examine some beef Descemet's membrane, and reported as follows:

"Specimens of beef Descemet's membrane were prepared for x-ray diffraction examination by cutting a dried stack of membranes into blocks. With the x-ray beam parallel to the membrane surfaces, the wide-angle patterns obtained were characteristic of poorly oriented, collagen-containing tissue. Arced reflections corresponding to spacings of 2.86 A (meridional) and 11.5 A (equatorial), plus a diffuse, unoriented halo at 4 to 5 A, were sufficient to establish the presence of an appreciable proportion of a member of the collagen class of fibrous proteins. Small-angle observations failed to discover any indication of axial reflections corresponding to a very large axial periodicity, but a pair of rather sharply oriented equatorial spots, related to transverse structure of size 36 A, were obtained. The origin of the latter spacing is not known. Although dried specimens of other collagens have not exhibited it, North, Cowan and Randall (13) have recently shown that physiological specimens have equatorial spacings of this magnitude."

DISCUSSION

Indirect but suggestive evidence led other investigators to classify Descemet's membrane as a type of collagen: the polarization optical data of Baud and Balavoine, and of Grignolo; and the histochemical findings of Wislocki. Much more positive evidence came from the chemical analysis of Dohlman and Balazs. With the addition of the x-ray diffraction results of Rougvie and Bear, little doubt remains about the identification of Descemet's membrane as a collagenous tissue.

The observations on the development of Descemet's membrane reported here support the thesis that it is a product of the endothelium and hence properly mesodermal in origin. Only these cells are in sufficiently close contact with the growing membrane to be directly implicated in its formation. Moreover, they appear to be actively metabolizing cells, having large nuclei with well developed nucleoli and a high concentration of cytoplasmic inclusions.

It is interesting to note that the repeating period of 1070 A observed in sections of Descemet's membrane is just twice the length of the repeating period seen in sectioned stroma collagen fibrils. (Isolated collagen from fragmented stroma, fixed or unfixed, has the usual collagen period, around 650 A,

but this shrinks down to 525 to 550 A during the remainder of the preparative procedure.) It is tempting to view this as a significant relationship, and to consider possible ways in which a single unit could be utilized to construct two structures so different architecturally. For example, two polarized collagen units could join in series to form the 1070 A Descemet's membrane unit, with either like ends or unlike ends together. If like ends were joined, simple lateral duplication of similar elements could be invoked to produce a filament of required width. If unlike ends were together, the elements might associate laterally out of phase and thus produce equivalent filament ends (this seems desirable, since the nodes appear to be equivalent). In order to bring about the observed state of tension of Descemet's membrane, part of each collagen unit could be extended to form its half of the internodal filament while the remainder, in collaboration with five other similar units, could form the node.

Whether the nodes and the internodal filaments are in fact composed of the same material cannot be said on the basis of available evidence. The difference in density (blackness in the photograph) appears to stem, at least in part, from a differential uptake of phosphotungstic acid; this, however, could result from either a chemical difference or simply a greater mass of material. Neither extraction with citrate buffer or weak acetic acid nor digestion with trypsin effected the removal of either nodes or filaments.

In all the species described, no endothelial basement membrane distinct from Descemet's membrane was observed. If Descemet's membrane is indeed a basement membrane, one might wonder why it is so thick and why it possesses such an elaborate fine structure. (So far as is known, this type of fine structure has not been previously described.) Could it be that it acts as a collimating device, held taut to be more effective? It is hoped that the examination of Descemet's membrane in animals with different visual characteristics may shed some light on its function.

SUMMARY

Descemet's membrane, previously thought to be "structureless," has been found to be characterized by a fine structure of great regularity. Sections perpendicular to the plane of the membrane surface appeared to be cross-striated, with narrow dark bands separated by wide light bands traversed by fine filaments. Tangential sections showed a two-dimensional array of dark nodes and thin internodal filaments which connected each node with the six others around it to form a hexagonal figure. The average distance between nodes, and between the dark bands in transverse sections, was about 1070 A; the width of the nodes was about 270 A; and the width of the connecting filaments was less than 100 A. This pattern has been found in all species of Descemet's membrane so far examined, although it appeared to be better developed in some forms than in others. So far as is known, it has not been observed in any other type of tissue.

The differentiation of Descemet's membrane has been followed in the chick embryo. It appeared late and developed slowly but showed the characteristic fine structure from the time it could be clearly identified.

The evidence for placing Descemet's membrane in the collagen class of proteins is strongly supplemented by the results obtained from x-ray diffraction examination by Rougvie and Bear.

The author wishes to express thanks to Dr. M. A. Rougvie and Dr. R. S. Bear for making the x-ray diffraction examination of Descemet's membrane, and for making available their unpublished results; and to Mr. W. J. Stenstrom for the schematic representation of Descemet's membrane.

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PLATES

EXPLANATION OF PLATES

PLATE 82

FIG. 1. Fragments of isolated beef Descemet's membrane mounted in water and photographed through a polarizing microscope with polarizer and analyzer perpendicular.

Radially the membrane is isotropic (dark), but tangentially, where it curls up at the edges, it shows strong birefringence. × 88.

FIG. 2. Deparaffined section of Zenker-fixed beef corneal stroma and Descemet's membrane mounted in water and photographed through a polarizing microscope with polarizer and analyzer perpendicular.

The birefringence of Descemet's membrane (*Dm*) is of the same sign but of smaller magnitude than that of the stroma collagen (*str*). × 88.

FIG. 3. Transverse section of beef Descemet's membrane digested with 0.01 per cent trypsin in 0.01 M $NaHCO_3$, at 37°C. for 24 hours, fixed in1 per cent OsO_4 buffered to pH 7.5, for 16 hours, and treated with 1 per cent phosphotungstic acid for 3 hours.

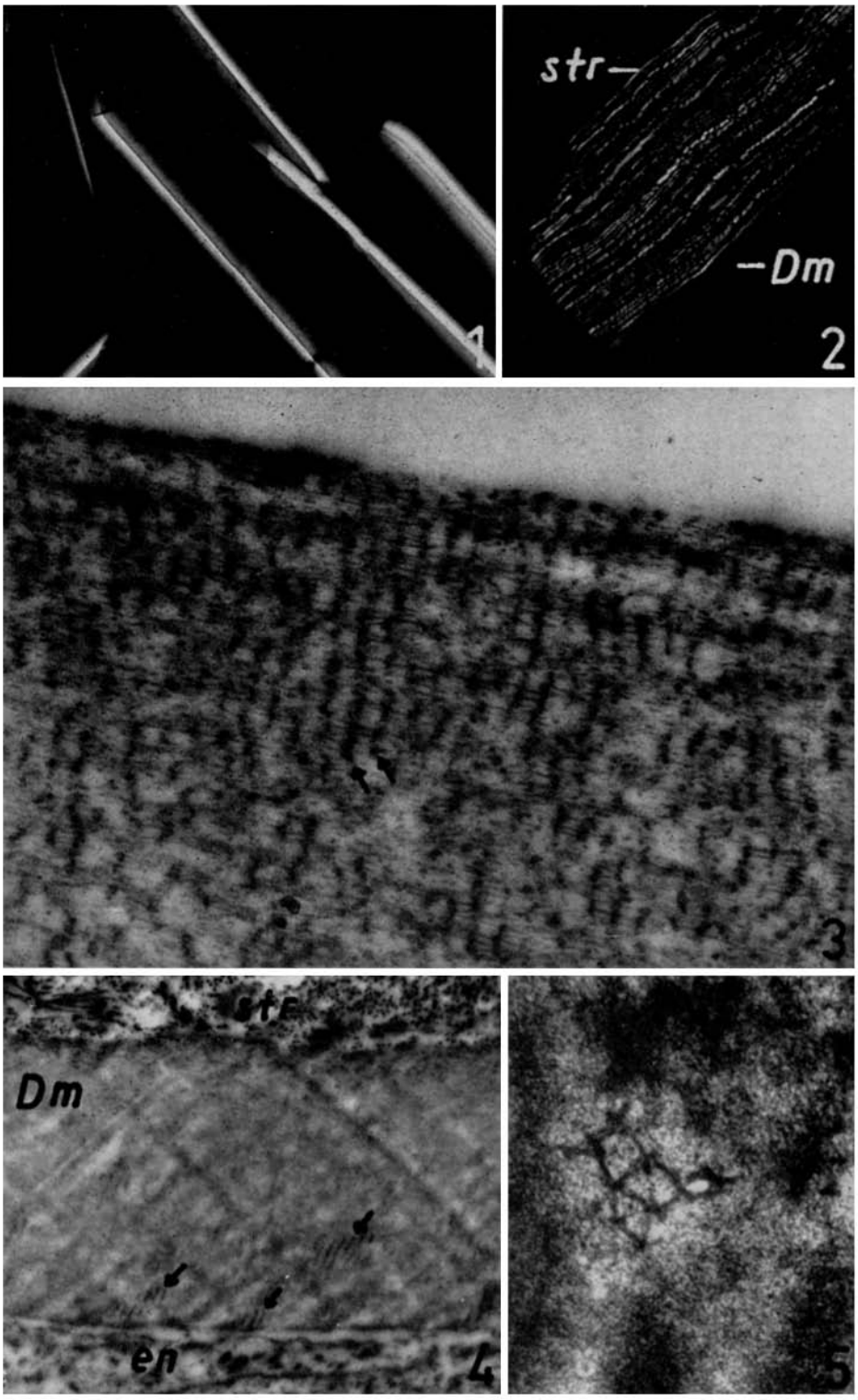
This very thin, heavily stained section shows the granularity of the narrow dark bands (arrows) and the fine filaments connecting them. × 38,300.

FIG. 4. Transverse section of adult mouse cornea fixed in 1 per cent OsO_4 buffered to pH 7.48 for 3 hours, and treated with 0.1 per cent phosphotungstic acid for 1 hour.

Between the stroma (*str*) and the endothelium (*en*) is Descemet's membrane (*Dm*), within which are seen scattered regions of oriented structure appearing as dark, thin, evenly spaced lines (arrows). The average period in this specimen is about 930 A. × 14,800.

FIG. 5. Section through Descemet's membrane of rat cornea fixed in 2 per cent OsO_4 buffered to pH 7.4 for 3 hours, dehydrated in methanol, and treated with 1 per cent phosphotungstic acid for 2 hours.

Portions of two incomplete, but quite symmetrical, hexagonal figures are seen. In rat Descemet's membrane the length of the period has been found to be small; in this specimen the average distance between nodes is about 640 A. × 83,600.



(Jakus: Fine structure of Descemet's membrane)

PLATE 83

FIG. 6. Tangential section of beef Descemet's membrane extracted with citrate buffer, pH 4.8, for 48 hours in the cold, fixed in 1 per cent OsO_4 buffered to pH 7.45 for 2½ hours, and treated with 1 per cent phosphotungstic acid for 3 hours.

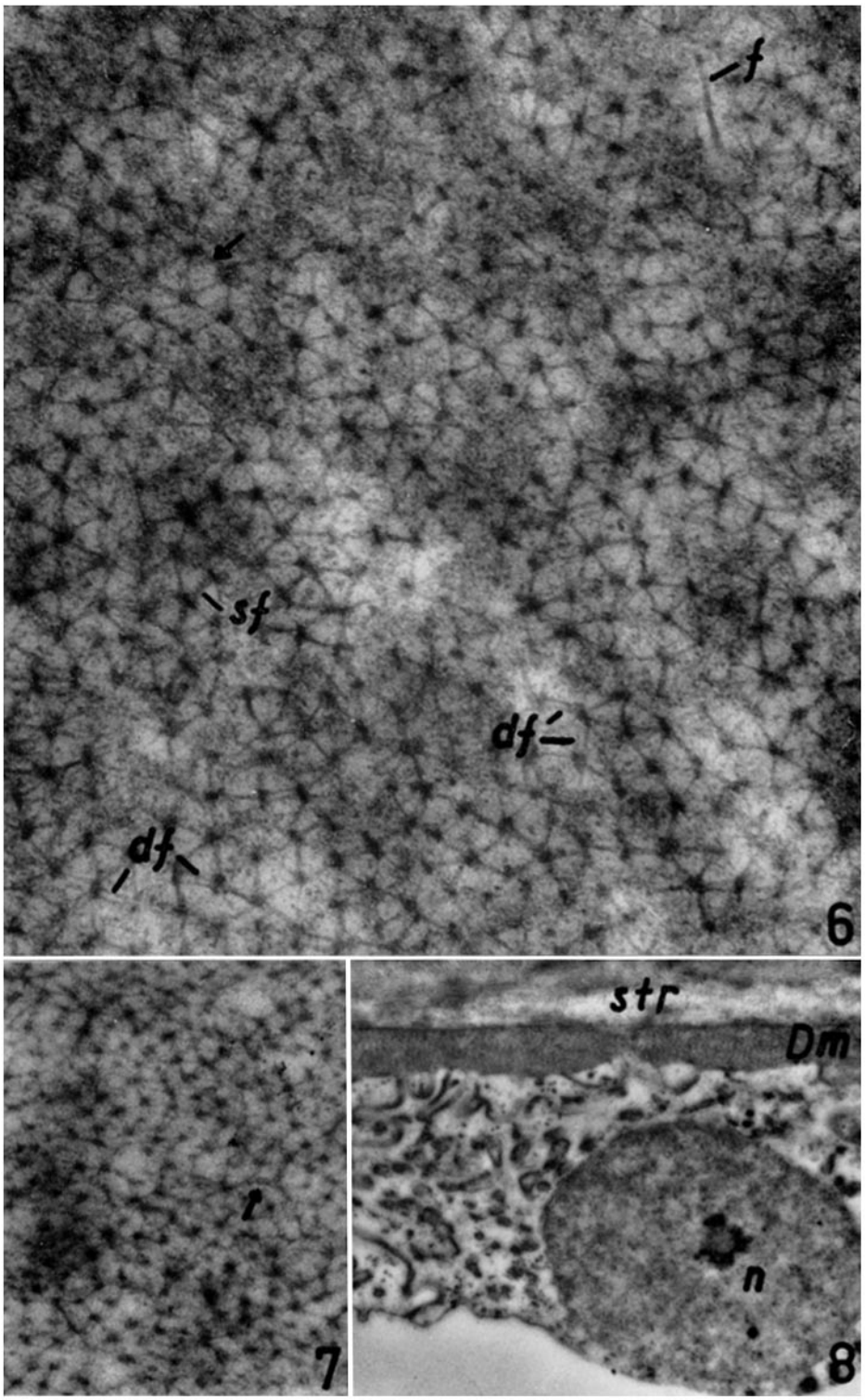
This section shows the hexagonal pattern characteristic of this plane. The arrow points to the edge of a symmetrical and complete figure. Shown also are a wandering fibril with a fine, not very distinct period (*f*), a number of double, or perhaps double-edged, filaments (*df*), an apparently striated internodal filament (*sf*), and considerable quantities of amorphous or filamentous material distributed somewhat unevenly in the background. × 66,000.

FIG. 7. Tangential section through Descemet's membrane of a 2-month chick cornea fixed in 1 per cent OsO_4 buffered to pH 7.48 for 3 hours at room temperature, 2 hours in the cold and 1½ hours again at room temperature, and treated with 0.1 per cent phosphotungstic acid for 1 hour.

Although the structure here appears less well organized than that shown in Fig. 6, it is essentially similar. The arrow points to one of several double-edged internodal filaments. × 33,800.

FIG. 8. Transverse section through a 10-day (post-hatching) chick cornea fixed in 1 per cent OsO_4 buffered to pH 8.57 for 4 hours, and treated with 0.1 per cent phosphotungstic acid for 30 minutes.

Beneath the stroma (*str*) is Descemet's membrane (*Dm*), showing the apparent cross-striation characteristic of transverse sections. Just to the right of its center is a depression which is thought to represent the junction of two adjacent primordia. Beneath the membrane is a portion of the endothelial layer. One nucleus (*n*) is seen, with its well-developed nucleolus (dark periphery, lighter center). The cytoplasm is rich in inclusions and infolded membranes. × 9,650.



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