

AN ELECTRON MICROSCOPE STUDY OF THE EPITHELIUM IN
THE NORMAL MATURE AND IMMATURE
MOUSE CORNEA

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PLATES 49 TO 53

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Preliminary to investigating experimentally induced changes in the epithelium of the mouse cornea with the electron microscope, a study of the normal cytoarchitecture was undertaken. While this work was in progress, Jakus published a study of the rat cornea in which she described interdigitating cell processes, cytoplasmic filaments and granules, nuclear membrane indentations in basal cells, and a fibrous basement membrane (1). The present communication concerns additional findings obtained at high resolution regarding the structure and arrangement of the cell surface membrane, constituents of the cytoplasm and cytoplasmic organelles, and the appearance of the basement membrane.

Materials and Methods

Mature white Swiss mice weighing 15 to 25 gm. were decapitated and 1 per cent isotonic osmium tetroxide in veronal-acetate buffer, pH 7.2-7.4 (2), was immediately instilled into their eyes with a pipette. The eyes were enucleated within 90 seconds of decapitation.

All eyeballs were bisected while in fixative and embedded, after graded alcohol dehydration lasting 6 hours, in a 16:1 mixture of *n*-butyl-methyl methacrylate which was polymerized at 45°C. (3). 1 per cent benzoyl peroxide was used as catalyst. 0.1 per cent phospho-12-tungstic acid was added to the last change of absolute alcohol of some specimens.

Thin sections were cut with a specially sharpened steel knife in a Sjöstrand microtome (4) and picked up from 20 per cent ethyl alcohol solution on formvar¹-coated copper grids. An RCA EMU 2c electron microscope with compensated objective pole piece, an objective aperture of about 50 microns, three apertures in the projector lens, and small condenser lens aperture was used. Magnification was calibrated by means of a diffraction grating and Dow latex spheres (4).

OBSERVATIONS

Sections from the corneas of eighteen mature and seven immature white Swiss mice showed the same general arrangement of the tissue as demonstrated by light microscopy and other electron microscopic studies (1, 5).

¹ Shawinigan Products Corporation, New York City.

The most superficial cells of the epithelium were flattened, non-keratinized, and contained elongate nuclei. Deeper or intermediate cells were oriented parallel to the surface of the cornea. The deepest or basal cells, apparently resting on the basement membrane, were cuboidal, having rounded nuclei lying near the flattened base of the cell. In the immature cornea the intermediate cells were less numerous, the epithelium often being only three cells thick, and the extracellular spaces appeared greater between the cells of all layers. A survey electron micrograph illustrates the three layers of the epithelium, the basement membrane, and a portion of the substantia propria from the cornea of a 3 day old mouse (Fig. 1).

Outer Cells

The free or outer border of the cornea in all sections from both mature and immature corneas appeared irregular. The irregularities took the form of small, finger-like projections similar to the microvilli described at the surface of the chorioallantoic membrane (6, 7). At high magnification where the cell surface membrane was oriented perpendicular to the plane of section, the membrane appeared as two parallel thin dense lines separated by a less dense area, the thickness of the whole membrane being about 80 A. The same arrangement was seen in cells of both immature and mature animals (Figs. 2 and 3).

In sections from four of the eighteen mature animals, round bodies were seen lying on the surface of the cornea and between microvilli. Serial sections showed these bodies were round or elliptical. These round bodies had a relatively dense outer substance limited by a membrane similar to that described as the surface membrane of the epithelial cells, and a central portion which varied in appearance, most commonly appearing somewhat reticular. Occasionally such round bodies were seen within the cytoplasm of cells at the surface of the cornea. The bodies were never seen in sections from immature (unopened) eyes.²

Within the outermost cells elongate nuclei oriented parallel to the surface of the cornea were sometimes seen. The nucleoplasm appeared to consist of granules superimposed on a less dense background but the nucleoplasm was never so dense as that of the basal cells. The nucleus was bounded by a double-contoured membrane such as that described by other authors (10, 27), but any discontinuities in the membrane were not obvious. Near the nucleus in sections from most cells a system of membranes similar to that described by Dalton and Felix, Sjöstrand and Hanzon, and others (11-13) was seen.

These dense membranes were about 60 A wide, arranged in pairs and superimposed on an homogeneous ground substance that appeared somewhat

² These round bodies closely resemble some bacteria seen in thin section (8, 9), and are believed to represent the normal flora of the eye.

denser than the surrounding cytoplasm, thus answering to previous descriptions of the Golgi complex. However, these juxtannuclear organelles of the epithelial cells of all layers of both the mature and immature cornea appeared most often as a closely packed system of membranes without the large vacuolar spaces that are such a prominent part of the Golgi complex in cells of the epididymis, pancreas, and intestine (Fig. 4). Mitochondria were often seen in close topographic association with the Golgi complex.

The mitochondria of the outer epithelial cells in both the mature and immature cornea appeared as small membranous structures enclosing an homogeneous ground substance more dense than the surrounding cytoplasm as described in cells of other tissues (14, 15). The membranes of the mitochondria appeared as two dense lines separated by an intervening less dense space of slightly varying dimensions. However, unlike the mitochondria of the proximal convoluted tubule, no dense granules were present in the mitochondrial ground substance. The mitochondria of the cornea were less densely aggregated than in certain areas of the retina, kidney, pancreas, or intestine (16-19), but were of the same order of magnitude as those described in the rat cornea, being less than 1 micron long and 0.4 micron wide, their dimensions depending of course on the plane of section and configuration of the organelle. In some sections of all epithelial layers the mitochondria as well as areas of the cytoplasm seemed poorly preserved and did not present the "normal" appearance as seen in other specimens. It was assumed that some artifact of fixation or embedding caused this distortion. From the better preserved specimens studied, we had the impression that the mitochondria in the outer epithelial cells of the mature mouse cornea differ from those of deeper cells, particularly those of the immature epithelial cells. The cells of the immature mouse epithelium frequently showed more loosely organized mitochondria with a less dense ground substance while the mitochondria of the outer cells of the mature cornea appeared quite dense and compact. Quite apart from any obvious artifacts, the mitochondria of the corneal epithelium did not appear identical in all sections, and the differences were most marked between mitochondria of immature epithelial cells and those of mature cells (Figs. 5 and 6).

The cytoplasm of the superficial cells appeared less dense than the cytoplasm of the basal cells. On closer examination, the cytoplasm of all cells appeared to include several components.

An homogeneous or non-particulate background that varied in density within a given cell and varied from specimen to specimen was common to all cells. This homogeneous background was more dense at the cell borders of all cells and in deeper cells.

A second ubiquitous component of the cytoplasm was small dense particles about 150 A in diameter which were seen singly, in groups, and often ar-

ranged in "rosettes" (20). Dense particles of a similar size also were seen attached to thin dense membranes arranged with their smooth sides apposed and their granular surfaces outward. While these membranes were of approximately the same dimension as the Golgi membranes, they did not appear so dense or clearly delineated. This arrangement of granules on membranes has been so frequently described in other cells that the designation " α -cytomembrane" was suggested by Sjöstrand as a term without connotation to be used until the functional and biochemical significance of this structure was known (21). In the epithelial cells of the cornea the α -cytomembranes did not constitute a major portion of the cytoplasm as they do in some tissues, but small strands were seen in most cells.

Thin membranes were often seen to border areas of varying size and density, appearing in some places as vacuoles and in others as granules. In some parts of the cytoplasm such membrane-bounded areas certainly represented sections through cellular projections or invaginations, in other places they were more difficult to interpret.

Filamentous structures arranged in strands or bundles within the cytoplasm of intermediate and superficial cells of the rat cornea as described by Jakus were seen in osmium tetroxide-fixed tissue but appeared more obvious at lower magnifications in this study. Apparently, without treatment by phosphotungstic acid, these filaments were not sufficiently dense to be clearly delineated at high magnification, and unless phosphotungstic acid was added to the last change of alcohol the filaments often escaped recognition among other components of the cytoplasm. There is no doubt of their existence, however, and where they are sectioned at right angles to their long axis they must contribute to the slightly particulate nature of the cytoplasm in those areas which are not clearly homogeneous, granulated, membranous, or vacuolated. In tissues treated with phosphotungstic acid the cytoplasm presented quite a different appearance; the now prominent filaments were often arranged in groups and constituted a large proportion of the cytoplasm, being easily seen in cells of all layers. These filaments were about 50 A wide and of indeterminate length (Fig. 7).

Intermediate Cells

The cells of the intermediate layers were separated from adjoining cells by extracellular spaces of variable dimensions but appeared to adjoin neighboring cells both by foot-like processes and "mortise and tenon-like attachments" similar to those described by Fawcett in the liver (22). Where the foot-like processes were approximated a less dense space wider than the less dense component of either cell surface membrane was seen. There was no suggestion of fibrils or precipitates extruding from cells that might be interpreted as "intercellular bridges." Occasionally, however, where foot-like processes were approximated, the homogeneous ground substance appeared

more dense than at other parts of the cell surface, a structure similar to that described earlier in other epidermal tissue (23-25). The extracellular spaces were more infrequent and smaller between cells nearer the basal cells, the intercellular interdigitations becoming progressively more complex and pronounced nearer the basement membrane in the epithelium from the mature mouse. However, the extracellular spaces were much greater between cells of all layers in the immature cornea, and intercellular interdigitations were rare. Occasional mononuclear leucocytes were seen between cells of the mature cornea.

The elongate nuclei of intermediate cells frequently contained massed dense granules which were interpreted as elements of the nucleolus. The cytoplasm of intermediate cells appeared generally less dense than that of the basal cells but it was difficult to determine the exact cytoplasmic component responsible for this difference in density. In the basal cells the granular component of the cytoplasm was a much more prominent feature than in more superficial cells while the filamentous structures were not easily seen without phosphotungstic acid treatment. Mitochondria were often seen in close topographic association to the Golgi complex, and in a few sections from both mature and immature animals, where the denser background substance of the Golgi complex appeared to include the mitochondria, the section passed through suggestions of connecting membranes (Fig. 5).

Basal Cells

As stated above, in sections where the cytoplasm appeared "normal," the basal cells from both immature and adult animals were considerably denser than more superficial cells. The nuclei were generally round but the nuclear membrane often appeared invaginated at some place on its circumference. In some immature cells it was most difficult to distinguish the granular nucleoplasm from the densely granular cytoplasm. Nuclear membrane discontinuities were seen in occasional sections. Membranous structures, apparently mitochondria, appeared in most sections of basal cells of immature corneas. The anatomy of many mitochondria from the basal cells of the immature cornea departed from earlier descriptions of mitochondria and from mitochondria seen in the adult mouse cornea, appearing sometimes as loosely arranged aggregates of membranes, often lacking any obvious pattern, and clearly differing from the regularly arranged Golgi complex. The appearance of these mitochondria (Figs. 6 and 7) suggested that the space within the dense components of the transverse mitochondrial membranes was wider than the space within the dense components of transverse membranes of mitochondria from the outer epithelial cells in the mature animal (Fig. 5). This appearance was similarly observed in specimens from many animals and was seen in tissues treated with phosphotungstic acid (Figs. 6 and 7).

In the adult mouse cornea the intercellular interdigitations of the basal cells

were complex, but the base of these cells was flattened and presented a somewhat corrugated appearance where it adjoined the substantia propria.

Basement Membrane

In the cornea of the mouse as in that of the rat there is no Bowman's membrane. Light microscopy shows epithelial cells are separated from the substantia propria by a thin refractile boundary referred to as the basement membrane. In electron micrographs of osmium tetroxide-fixed tissue the inferior aspect of the basal cell surface membrane was separated from the substantia propria by two distinct entities. A non-dense area of varying dimensions lay between the inferior limiting membrane of the basal cells and a dense band at the surface of the substantia propria. In osmium tetroxide-fixed tissue this band appeared uniformly dense and did not present a fibrous appearance; it was not sharply delineated from the underlying substantia and there was no sharp boundary separating the non-dense area between it and the basal cells.

In phosphotungstic acid-treated specimens increased contrast made the basement membrane more clearly defined at higher magnifications. The only indication of a fibrous structure was the suggestion of periodic banding perpendicular to the plane of the basement membrane, a structure similar to that described by Bargmann (26) (Fig. 8). There was no evidence for any fibrils or granules lying within the dense band. The exact width of the basement membrane was difficult to determine due to absence of any precise end-point but was approximately 600 Å. Occasional dense extensions from the basement membrane into the substantia propria were seen; in some cases these appeared to be collagen fibers, in others their nature was not apparent. No projections into the epithelium were noted.

DISCUSSION

The interpretation of electron micrographs is complicated both by artifacts of technique and by a lack of knowledge as to what dense and non-dense areas represent in biochemical terms. Obviously dense areas in osmium tetroxide-fixed specimens may represent either concentrations of reducing substances or concentrations of elements in general. Interpretation becomes more complex when we consider that non-dense areas may represent non-reactive substances or substances removed at some time during preparation such as the alcohol-soluble portion of the Golgi complex (11) or the fluorescent granules of kidney tubule cells (27). Within the framework of present techniques there are, however, reproducible, experimentally induced alterations in sub-microscopic morphology; *e.g.*, the postmortem changes in mitochondria (17,

19). It hardly seems necessary to add that the age of a cell as well as its metabolic state and function may contribute to its appearance.

Since extensive work has been done on the mitotic rate of basal epithelial cells of cornea (28, 29) and it is believed that they are younger than those of more superficial layers, it seems possible that some of the observed submicroscopic morphologic differences between basal and superficial cells (*e.g.* the appearance of mitochondria) could be correlated with a difference in cellular age.

The round bodies seen at the corneal surface in some mature animals but never in the unopened eye of the newborn most closely resemble bacteria seen in section. Since free epidermal surfaces are well known to be contaminated by bacteria, it is suggested these bodies represent the normal bacterial flora of the eye.

The structure and arrangement of the cell surface membrane are of interest owing to its importance as a diffusion barrier and because it is an area with particular configuration and function in different cell types.

The observation of the microvillous arrangement of the surface membrane of corneal epithelial cells is in keeping with other electron microscope observations and the rare modifications of corneal cell surface membranes where they are approximated have been previously described as common structures in invertebrate, amphibian, and mammalian epidermis (23-25). The function and biochemical nature of this specialized zone in epithelial tissue remain in question. In cardiac muscle a similar zone has been described as the structure of the intercalated disc where it could function both as an adhesive plate and as a transmitter of action potentials (30).

While it remains to conclusively demonstrate that the dense and non-dense components observed in electron micrographs as the structure of the cell surface membrane represent a protein-lipide complex, both the appearance and the dimensions of this structure are in keeping with earlier suggestions (31).

The importance of the basement membrane as a zone of metabolic interchange has been stressed by studies from the Wilmer Institute of Ophthalmology (32). Other experiments have indicated the adhesive property of the basement membrane (33, 1) identified here as the opaque 600 A band bounding the superior margin of the substantia propria. A similar structure has been identified at the interface between most epithelial cells and underlying connective tissue (19, 24, 26, 34). It has been suggested that the basement membrane consists of both a polysaccharide and a protein-lipide component (33). The difficulty in obtaining a clear structural pattern of this important diffusion barrier suggests techniques other than osmium tetroxide and phosphotungstic acid impregnation are needed to elucidate its nature. It is hoped

that further developments will permit a more complete functional and biochemical understanding of such problems.

SUMMARY

1. An electron microscope study at high resolution of the corneal epithelium of the normal mature and immature mouse revealed new information regarding the submicroscopic appearance of these cells.

2. Two thin dense lines separated by a less dense area constituted the structure of the limiting surface membrane of epithelial cells; the thickness of this membrane was about 80 A.

3. Some differences in the appearance of the cytoplasm and mitochondria of cells from the immature mouse cornea and the appearance of the cytoplasm and mitochondria of cells from the adult mouse cornea were observed.

4. The basement membrane appeared as a dense band about 600 A wide separating the basal epithelial cells from the substantia propria. Suggestions of periodicity were seen in some phosphotungstic acid-treated specimens.

5. Round bodies believed to be bacteria were seen on the surface of the outer epithelial cells in the adult mouse cornea but not in the immature, unopened eye.

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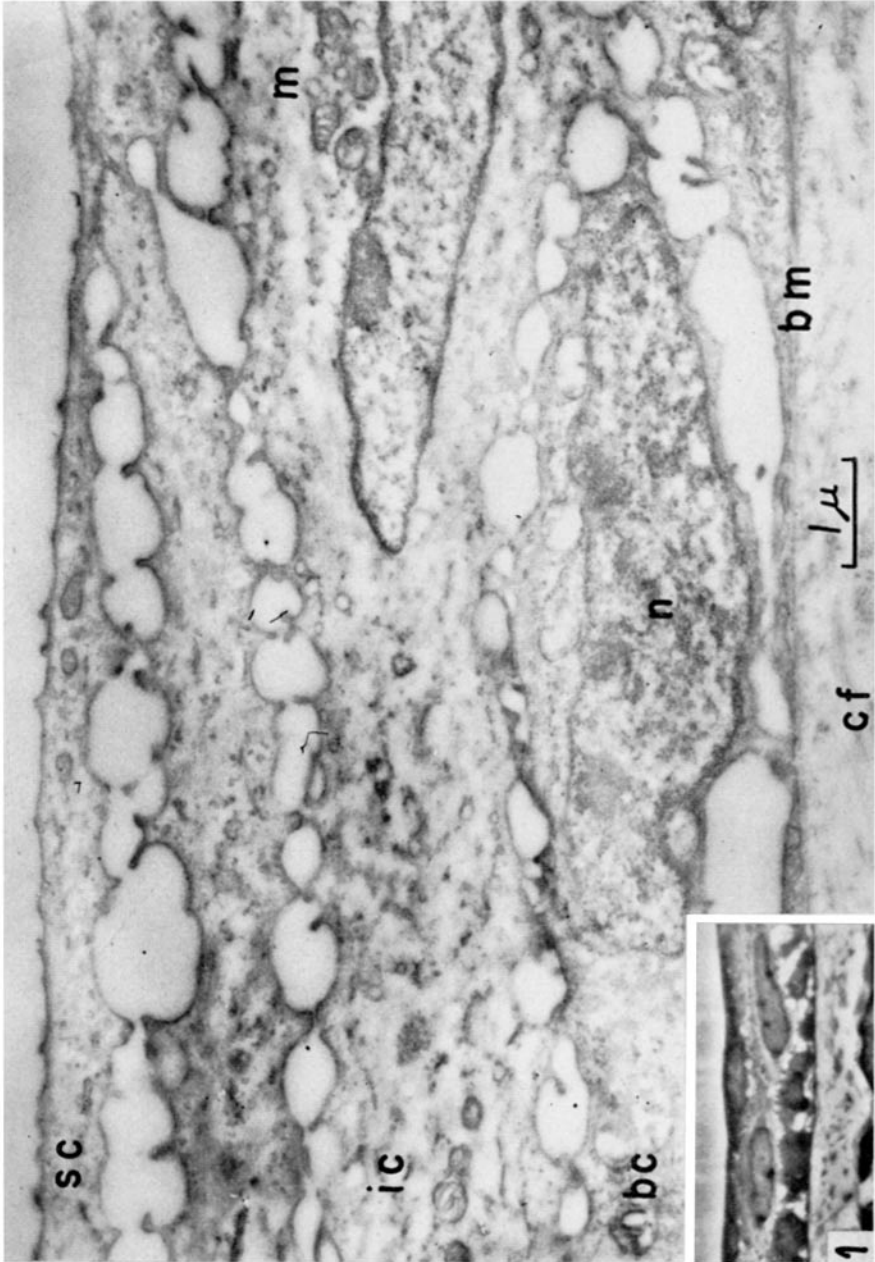
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EXPLANATION OF PLATES

PLATE 49

FIG. 1. Survey electron micrograph of the epithelium showing superficial cells (*sc*), intermediate cells (*ic*), basal cells (*bc*), basement membrane (*bm*), and a portion of the substantia propria from a 3 day old mouse cornea. Mitochondria (*m*) and nuclei (*n*) may be seen within the cells and collagen fibrils (*cf*) in the substantia propria. $\times 14,000$. Inset: phase contrast light micrograph of section from same specimen.

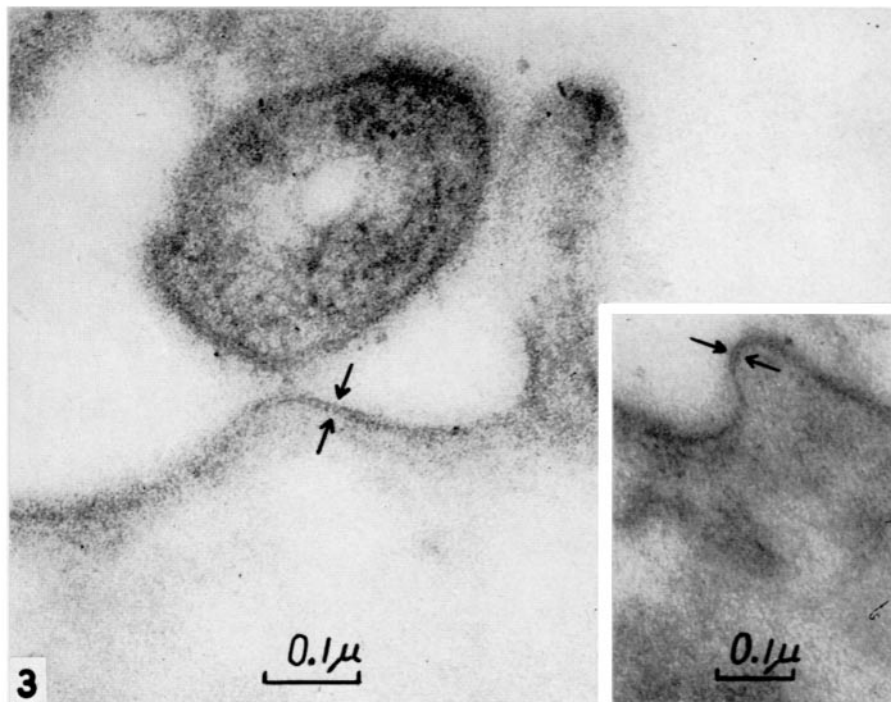
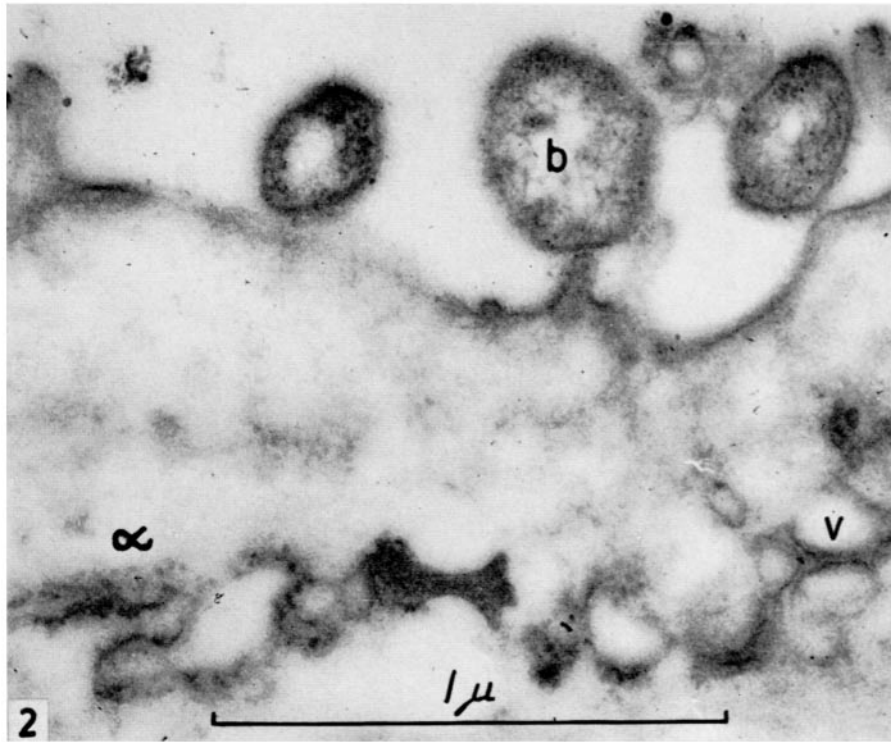


(Sheldon: Epithelium in mature and immature mouse cornea)

PLATE 50

FIG. 2. Electron micrograph showing portion of outer surface of epithelial cell from adult mouse cornea. Cell surface possesses microvilli and there are round bodies (*b*) believed to be bacteria at the surface. Vacuoles (*v*) and α -cytomembranes (*a*) may be seen in the cytoplasm. $\times 70,000$.

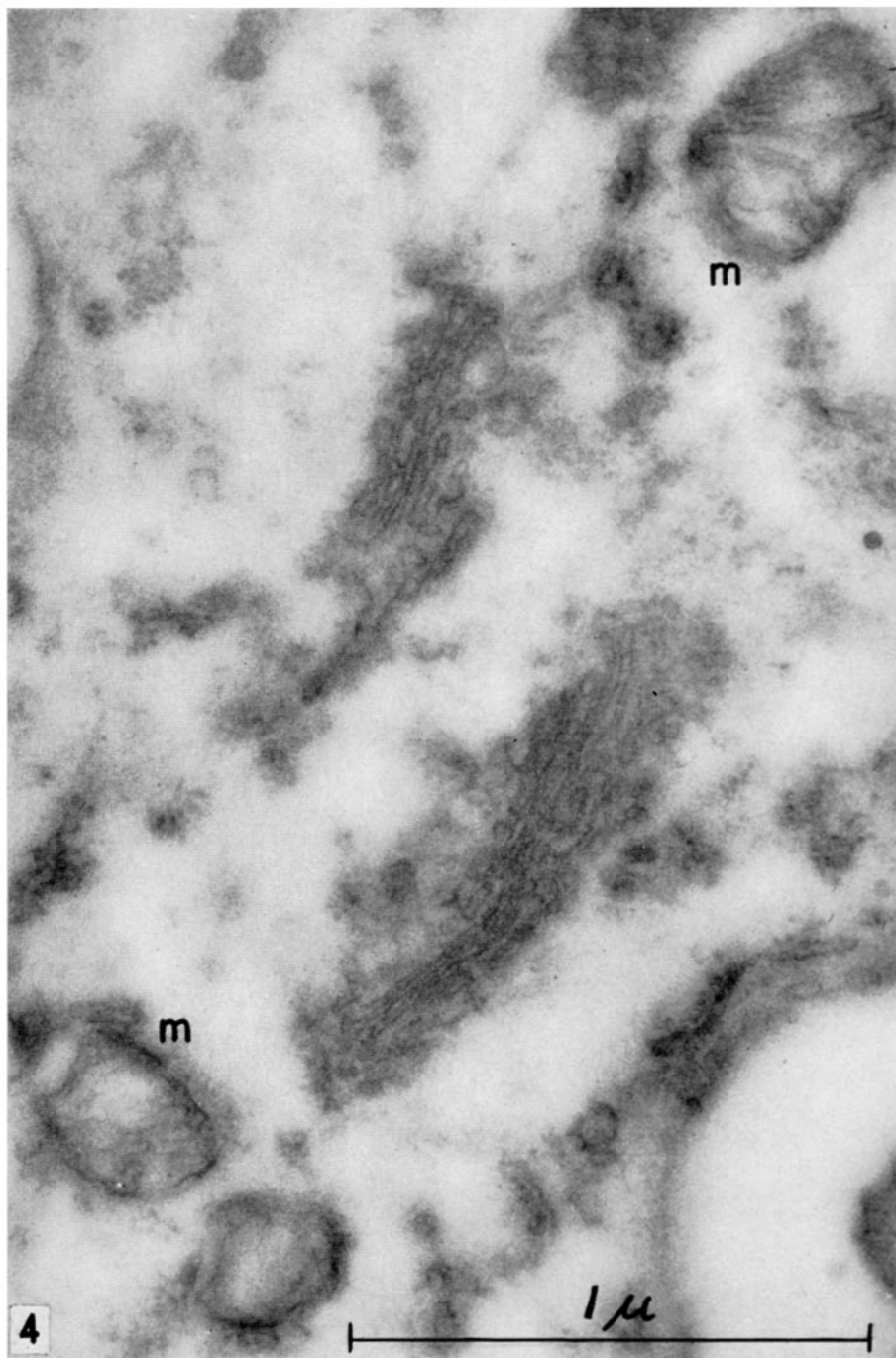
FIG. 3. Enlargement of portion of above micrograph to illustrate the ultrastructure of the cell surface membrane (see arrows). $\times 125,000$. Inset: similar area from portion of outer cell surface membrane of 3 day old mouse. $\times 104,000$.



(Sheldon: Epithelium in mature and immature mouse cornea)

PLATE 51

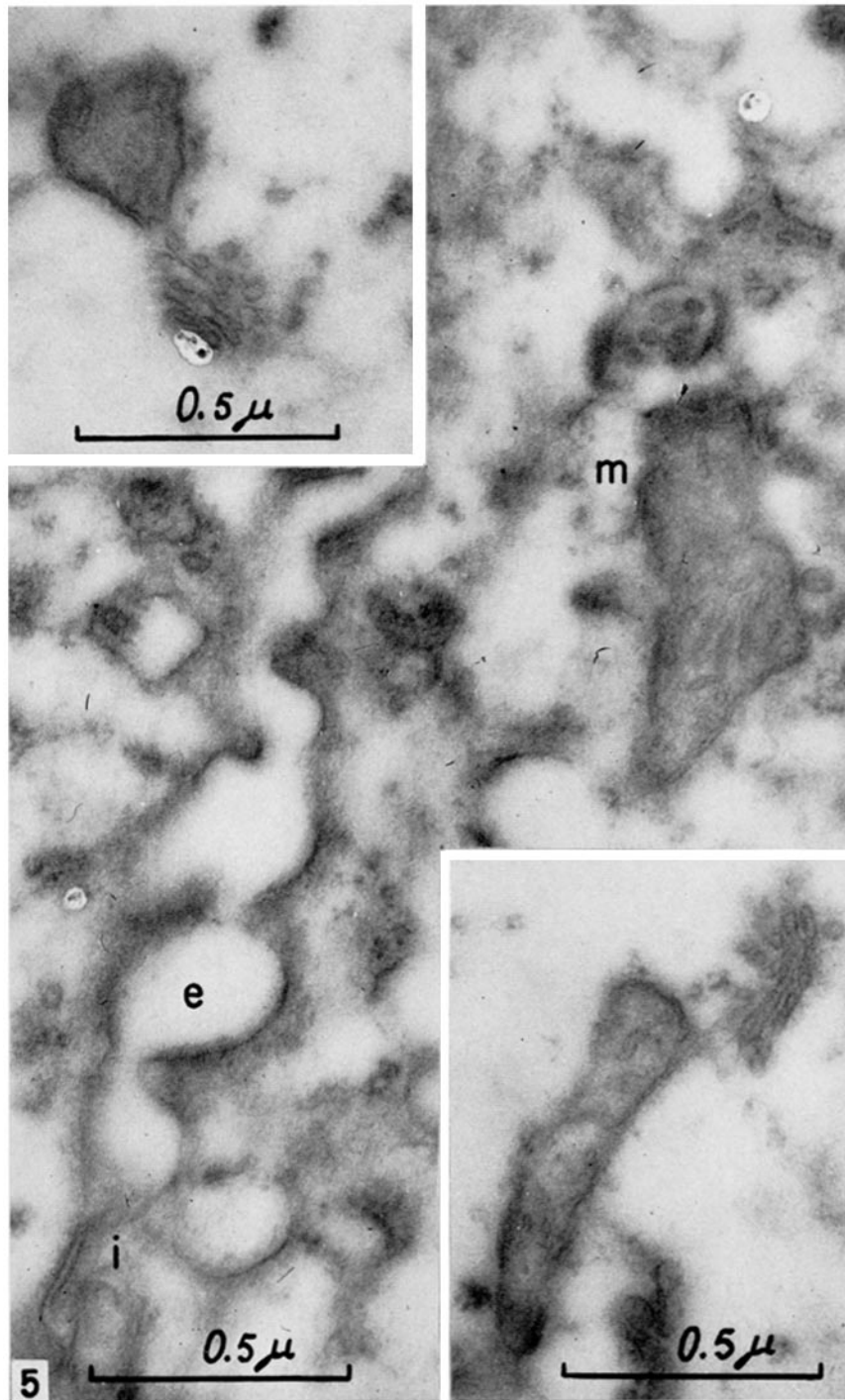
FIG. 4. Electron micrograph from juxtannuclear zone of intermediate cell from 3 day old mouse cornea illustrating compact, membranous, Golgi complex and mitochondria (*m*). $\times 70,000$.



(Sheldon: Epithelium in mature and immature mouse cornea)

PLATE 52

FIG. 5. Electron micrographs from portions of intermediate epithelial cells from adult mouse cornea showing mitochondria (*m*), extracellular space (*e*), and in the lower left hand corner, intercellular interdigitations (*i*). Insets: micrographs of mitochondria in juxtannuclear region showing adjacent portions of Golgi complex, adult mouse cornea. $\times 70,000$.



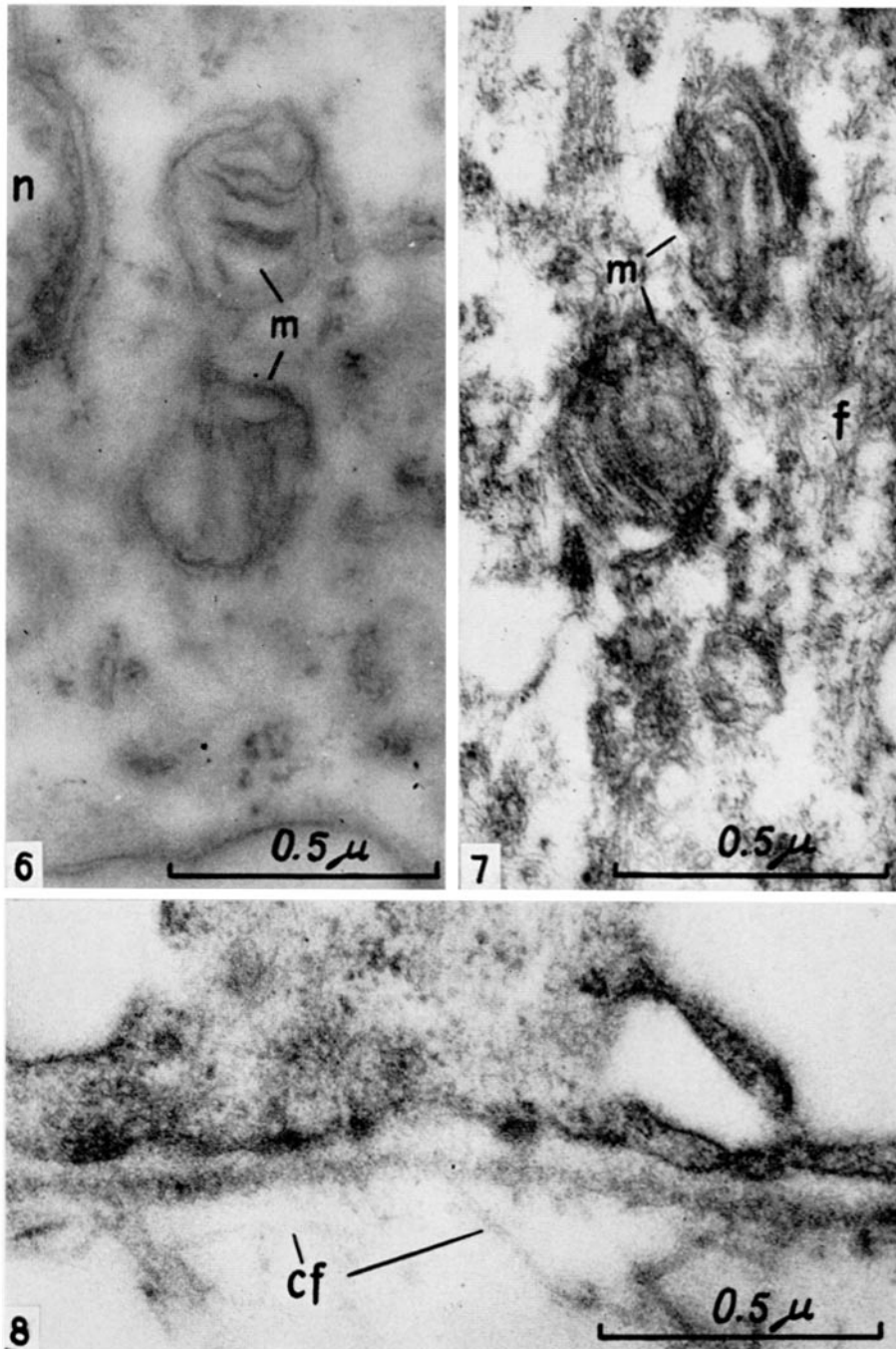
(Sheldon: Epithelium in mature and immature mouse cornea)

PLATE 53

FIG. 6. Electron micrograph showing portion of nucleus (*n*) and mitochondria (*m*) from intermediate epithelial cell of 2 day old mouse cornea fixed 2 hours in osmium tetroxide. $\times 71,000$.

FIG. 7. Electron micrograph showing mitochondria (*m*) and fibrils (*f*) from intermediate epithelial cell of 2 day old mouse cornea fixed 2 hours in osmium tetroxide and treated with 0.1 per cent phospho-12-tungstic acid for 30 minutes. Compare appearance of cytoplasm with that of Fig. 6. $\times 71,000$.

FIG. 8. Electron micrograph of juncture between basal epithelial cell and substantia propria of 3 day old mouse cornea demonstrating the basement membrane, which appears as a dense band running across the middle of the micrograph. Suggestions of periodic banding perpendicular to the plane of the basement membrane may be occasionally seen. Collagen fibers (*cf*) are seen in the substantia propria of this specimen, which was treated with 0.1 per cent phosphotungstic acid for 30 minutes. $\times 75,000$.



(Sheldon: Epithelium in mature and immature mouse cornea)