# FINE STRUCTURE OF THE CILIARY EPITHELIUM OF THE RABBIT, WITH PARTICULAR REFERENCE TO "INFOLDED MEMBRANES," "VESICLES," AND THE EFFECTS OF DIAMOX

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ABSTRACT

The structure of the ciliary epithelium of the adult albino rabbit has been studied by electron microscopy. Material was fixed in osmium tetroxide and embedded in epoxy resins. Two hitherto unappreciated features of the non-pigmented epithelial layer are described. First, the "infolded plasma membranes" described by previous workers are shown by serial sections to be projections or interdigitations from adjacent cells. Second, the "rows of vesicles" described by previous workers are shown by serial sections to be part of an unusual form of smooth-surfaced tubular endoplasmic reticulum. The tubules are highly convoluted and extensively interconnected. They are arranged in sheets, so that a cross-section through a sheet gives the appearance of a row of vesicles. The other structural features of the ciliary epithelium are also described. Previous workers have reported that Diamox, which inhibits the secretory activity of the epithelium, causes profound structural changes. An effort has been made to confirm these reports under carefully controlled experimental conditions. It was found that secretion could be inhibited by a maximally effective dose of Diamox without the occurrence of any detectable structural changes. The physiological significance of these findings is discussed.

## INTRODUCTION

The epithelium covering the ciliary body of the eye is generally believed to be involved in the formation of aqueous humor by an active transport process (1, 2). The exact manner in which the water and electrolytes which compose the aqueous humor are moved by these cells remains to be completely elucidated. It is known, however, that carbonic anhydrase inhibitors, such as acetazolamide (Diamox, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York), are potent inhibitors of aqueous humor formation (35, 36).

A number of reports on the fine structure of the rabbit ciliary epithelium has appeared in recent years (3–14). Most of these (5–13) have considered the effect of Diamox upon this structure.

The consensus of these reports is that several morphological features of the ciliary epithelium play a role in aqueous humor formation. (*a*) Infolded plasma membranes. Pronounced plasma membrane infoldings occur at the apical surface of the non-pigmented epithelial layer, and to a lesser extent at the basal surface of the pigmented layer. As was first pointed out by Pease (14), the

ciliary epithelium demonstrates infoldings in common with a number of other epithelia noted for transporting large quantities of water and electrolytes, such as kidney tubule cells, ependymal cells of the choroid plexus and cells of the serous portions of salivary glands. (*b*) Lateral interdigitations. The lateral borders between adjacent cells of the non-pigmented layer are highly convoluted, so that the cells interdigitate. (*c*) Vesicles. The cells contain many vesicular profiles, and at the apices of the non-pigmented cells these tend to be arranged in rows similar in pattern to the infolded membranes.

The principal morphological effect of Diamox has been reported to be a dramatic increase in the number of vesicles in the apical portion of the non-pigmented epithelium. Holmberg, for example, found a fourfold increase in these vesicles 15 minutes after Diamox administration (6). As a result of such experiments both Holmberg (7) and Pappas and Smelser (10, 13) strongly suggested that the vesicles were engaged in the formation of aqueous humor by a micropinocytosis mechanism, although they did not agree on the significance of this mechanism.

The present investigation was undertaken to elucidate further the morphological basis of aqueous humor formation, and in particular to examine the role of micropinocytosis. From this study three conclusions of particular interest seem to have emerged. (*a*) The infolded plasma membranes are interdigitations from adjacent cells. (*b*) The vesicular profiles seen in apex of the non-pigmented layer are not vesicles but part of an unusual form of tubular endoplasmic reticulum. (*c*) Diamox can inhibit aqueous humor secretion without producing demonstrable changes in the structure of the ciliary epithelium.

## MATERIALS AND METHODS

Most of the material was obtained during the course of experiments on the effect of Diamox upon the structure of the ciliary epithelium. Adult albino rabbits weighing 2.5 to 3.5 kg were anesthetized with urethan given intravenously. A No. 25 needle was inserted into the anterior chamber of the eye through the cornea in such a way that no aqueous humor escaped. The needle was connected through narrow gauge polyethylene tubing to a pressure transducer (Sanborn, model 267B) which in turn was connected to a continuous recording system (Sanborn carrier preamplifier, model 350-1100AS, and Sanborn recorder, model 320). When the intraocular pressure had stabilized, 20 mg/kg of either Diamox (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) or its inactive control compound (2-acetylamino-1,3,4-thiadiazole-5*N*-methyl sulfonamide) (34) was slowly injected intravenously. (The control compound was donated by Lederle Laboratories.) Only those animals which showed a clear cut fall of intraocular pressure of 3 to 5 mm Hg within a few minutes after Diamox administration or those which showed no substantial change after the control compound were carried further. Fifteen minutes after injection, a second needle was inserted into the anterior chamber, a few drops of aqueous humor collected through it, and the needle left in place. *Immediately* thereafter the recording needle was inserted through the pupil into the posterior chamber of the eye and infusion of fixative begun through the needle. The collected aqueous was treated with 8 per cent trichloroacetic acid, and, if there was indication of protein in the aqueous, the experiment was discarded.

The fixative was 1 per cent osmium tetroxide in Veronal buffer at pH 7.2 to 7.6, with balanced salts added (15). Fixation was carried out *in vivo* for 15 minutes by slowly injecting a total of 2 cc of cool fixative through the recording needle. The eye was then rapidly enucleated and opened, and pieces of the ciliary body were removed and placed in additional fixative. An effort was made to obtain all material from the same portion of the ciliary body, namely, the anterior two-thirds of the corona ciliaris. Fixation was carried out for an additional hour at room temperature. The tissue was then rapidly dehydrated through an ethanol series. Early experiments indicated that the tissue was badly preserved when embedded in prepolymerized methacrylate. The tissue was successfully embedded in both Epon 812 (16) and Araldite 6005 (17). Pale gold sections were obtained on a Porter-Blum microtome using both glass and diamond knives. Araldite was found much easier to section than Epon. Ribbons were mounted on carbon-coated 150 mesh grids. No special effort was made for mounting serial sections, but with patience it was often possible to trace portions of cells through five or more serial sections on the conventional grids. The sections were stained with a saturated aqueous solution of lead acetate, one-half hour for the Epon, two hours for the Araldite. An RCA EMU-3F was employed for microscopy.

In order to minimize sampling variation due to variability of fixation, material was used for careful study only if preliminary microscopic examination indicated good fixation. The relatively small amount of material that came up to acceptable standards was then studied systematically. Extensive strips of epithelium were located without reference to cytoplasmic details and then series of overlapping micrographs were made at 5,800 ×. Sets of micrographs

were gathered in this way to ensure random sampling without bias introduced by photographing only particularly interesting areas while neglecting other less spectacular but no less representative areas.

## OBSERVATIONS

Before the observations of the present author are considered, a few comments on the anatomical relations of this tissue are included for the sake of orientation. This seems especially desirable since its fine structure has never before been described in detail outside of the ophthalmological literature.

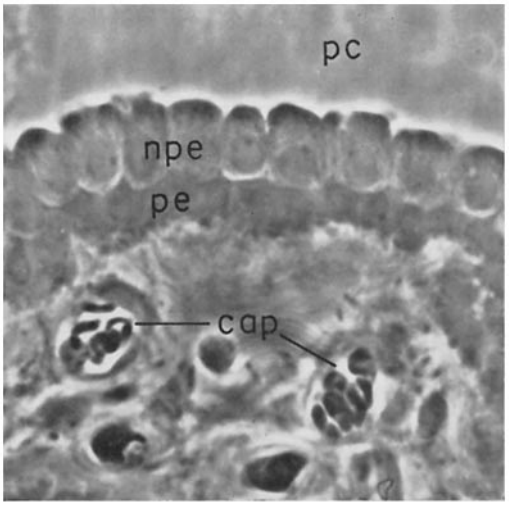



FIGURE 1

Phase micrograph of portion of a ciliary process. Note the wide capillaries (*cap*) in the stroma, the pigmented (*pe*) and non-pigmented (*npe*) layers of ciliary epithelium, and the posterior chamber of the eye (*pc*). Araldite. × 1,000.

The ciliary epithelium of higher vertebrate eyes is a double layer of epithelial cells, constituting the innermost layer of the ciliary body. The epithelium faces inward on the posterior chamber of the eye, which chamber is filled with aqueous humor. (The aqueous humor is a clear, non-viscous fluid with an ionic composition in many respects similar to an ultrafiltrate of plasma.) The epithelium rests upon a highly vascular stroma. In the more anterior portion of the ciliary body, the stromal core is thrown up into a number of meridionally disposed ridges, which are approximately ½ mm wide and rise a millimeter or so from the surface. These ciliary processes are remarkably vascular, containing numerous wide capillaries and small veins. Electron microscopy reveals the capillaries to be extremely thin walled, as thin as 300 A in places. Often a capillary is separated from the basal layer of the epithelium by only a scant amount of connective tissue (see Figs. 1 and 2).

The two layers of the ciliary epithelium are derived embryologically from the optic cup. The basal layer comes from the outer layer of the cup and the superficial layer comes from the inner layer (18). As a result of its embryogenesis, the ciliary epithelium possesses two basement membranes. Between the basal cell layer and the stroma lies the so called external limiting membrane, and between the superficial cell layer and the posterior chamber lies the internal limiting membrane. In the rabbit, each appears under the electron microscope as a dense 300 A thick layer in which it is difficult to resolve any finer structure and which is separated from the cell plasma membrane by a clear layer of about the same thickness.

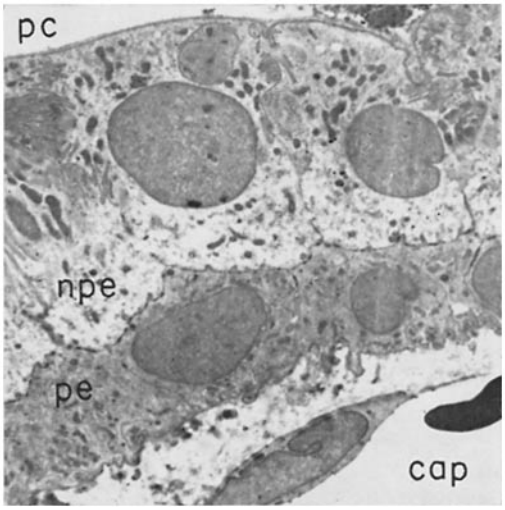



FIGURE 2

Low power electron micrograph of the ciliary epithelium. The columnar non-pigmented cells (*npe*) face directly on the posterior chamber (*pc*). The low cuboidal pigmented cells (*pe*) (which in the albino rabbit contain no pigment) rest on the stroma. The difference in cytoplasmic density of the two cell types is evident. Both types contain abundant mitochondria. Note the extremely thin walled capillary (*cap*), which is separated from the epithelium by only a small amount of stroma. Diamox. Araldite. × 3,000.

The basal cell layer is termed the pigmented epithelium and is continuous posteriorly with the pigmented epithelium of the retina. It contains many melanin pigment granules. The superficial cell layer

is termed the non-pigmented epithelium and is continuous posteriorly with the entire neural retina. It should be noted that the pole of the non-pigmented cells which is apical with respect to the stroma is embryologically the basal pole. As a result many of the structural features of the apical pole are more characteristic of the basal pole of the epithelia found elsewhere, *e.g.* the relation to a basement membrane, and the many "infolded plasma membranes."

Arising from the internal limiting membrane is a system of fibers approximately 80 A in diameter with a period of about 80 A (10). These are the zonular fibers which travel to the lens and constitute its "suspensory ligament." Through these fibers are transmitted the forces of accommodation generated by the ciliary muscle, which lies beneath the vascular stroma of the ciliary body.

The dimensions of the cells comprising the two epithelial layers vary somewhat from region to region of the ciliary body and also from species to species. The pigmented cells are generally low cuboidal, approximately 10 to 15 $\mu$ high and 15 $\mu$ wide in cross-section. The non-pigmented cells are columnar, approximately 30 $\mu$ high and 15 $\mu$ wide in cross-section.

Early investigators employing the light microscope described within the non-pigmented cells a basally located nucleus, numerous mitochondria, and sizeable vacuoles thought to contain incipient secretion. This early work has been well reviewed elsewhere (19).

### *Pigmented Epithelium*

The fine structure of the pigmented epithelium (see Fig. 3) can be summarized as follows. The basal surface is highly irregular with many projections into the stroma, so that the area of contact between stroma and cells is increased several fold. The basal plasma membrane is complexly "infolded" in the basal third of the cells. The "infoldings" resemble the apical interdigitations of the non-pigmented epithelium (*vide infra*), but they have not yet been analyzed by serial sections. The lateral cell surfaces are dotted by desmosomes. Though often irregular, the lateral surfaces do not form marked interdigitations of the type commonly found in the non-pigmented epithelium. Approximately three-fourths of the boundary between the pigmented and non-pigmented layers is relatively straight and dotted with desmosomes. The remainder of the boundary is more complex. Here many stubby, villous projections from the pigmented cells intermingle with similar projections from the non-pigmented cells (Fig. 4). Thus the area of contact between the two cell layers is increased. The endoplasmic reticulum is unremarkable. Numerous short tubular elements of both smooth and rough endoplasmic reticulum are scattered through the cytoplasm. Moderate

FIGURE 3

A survey of the pigmented epithelium. The external limiting membrane (*elm*) can be seen only at the basal surface at the far right. The basal surface is highly irregular and contains many complex membrane "infoldings." A typical Golgi apparatus (*ga*) is found above the nucleus (*n*). There are many mitochondria. Numerous, sizeable, single membrane-bounded bodies with granular content are seen (*mg*); these are identified as unpigmented melanin granules. The lateral cell border and the border with the non-pigmented epithelium (*npe*) are relatively uncomplex. Many vesicular and tubular profiles are scattered about the cytoplasm. Diamox. Araldite. × 26,000.

FIGURE 3 *a*

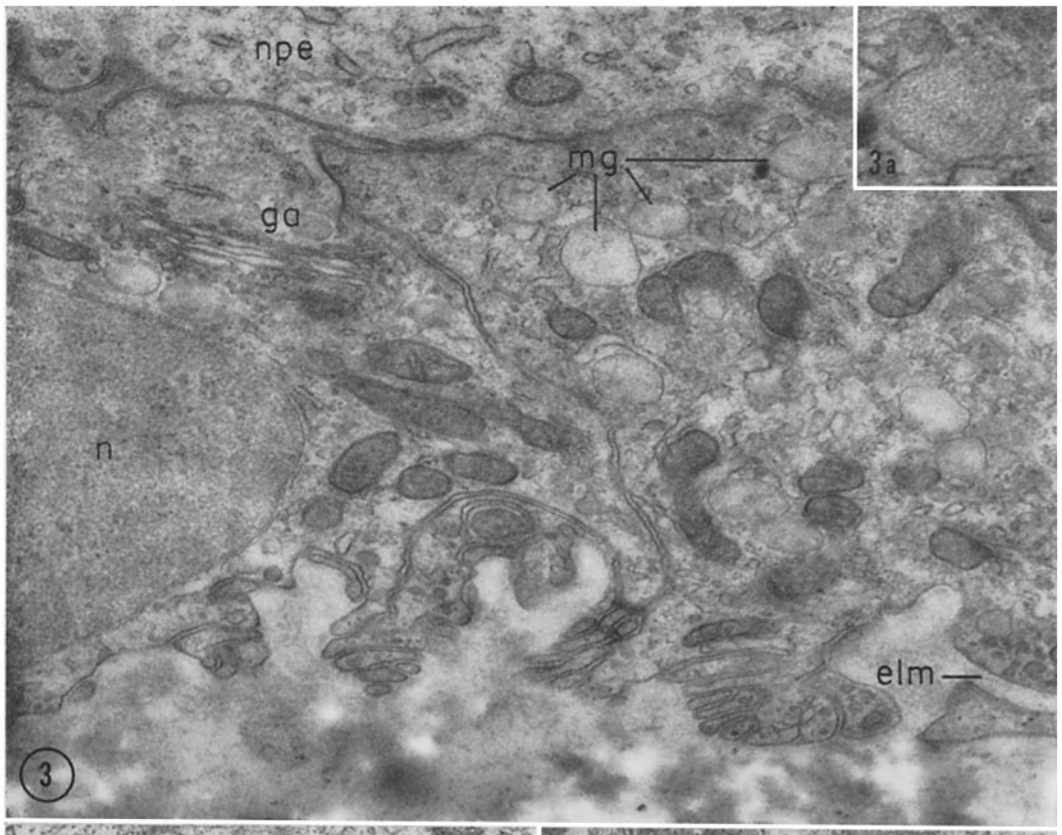
Enlargement of a portion of Fig. 3 to show an unpigmented melanin granule. Note the granular contents, which have a tendency to form a linear pattern. × 54,000.

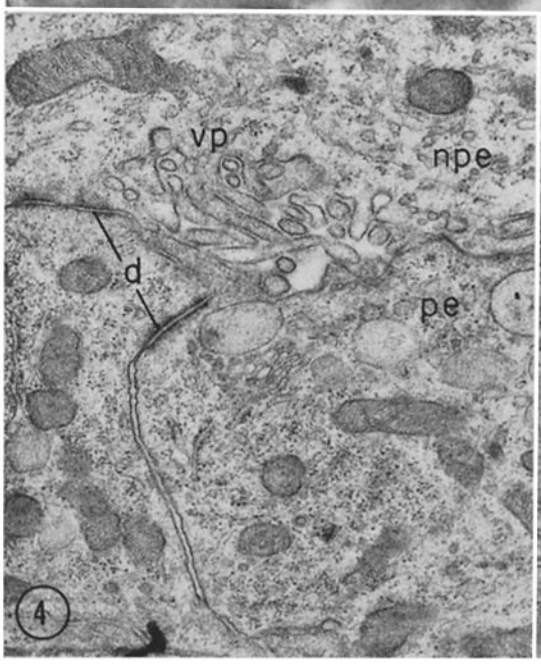
FIGURE 4

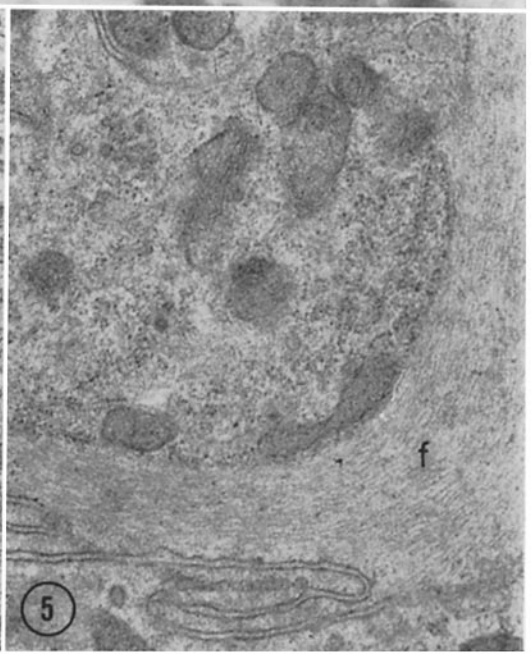
Micrograph showing border between two pigmented cells (*pe*) and a non-pigmented cell (*npe*). Most prominent here are stubby, villous projections (*vp*) between the two cell layers. Also well shown are desmosomes (*d*) and the difference in density of the two layers. Control. Araldite. × 26,000.

FIGURE 5

Micrograph of pigmented epithelium showing particularly large bundle (*f*) of fine fibrils of the type frequently found in this cell. Control. Araldite. × 32,000.


npe
mg
ga
n
elm
3
3a


vp
npe
d
pe
4


f
5

numbers of vesicles of variable size and density are seen throughout the cells, more notably near the basal surface. These vesicles show no tendency to line up in rows. Free RNP particles are abundant. The apical halves of the cells frequently contain well developed Golgi complexes, similar in appearance to the Golgi complexes of the non-pigmented cells. Also in the apical portion are oval bodies about the size of short mitochondria, bounded by a single membrane, containing fine granules which tend to be arranged in a linear pattern (Figs. 3 and 3 *a*). These are identified as unpigmented melanin granules (8, 20, 21). Mitochondria of typical morphology are numerous. The cytoplasm has a darker over-all appearance than that of the non-pigmented cells. In part this is due to more compact packing of the cytoplasmic elements and in part to numerous fine fibrous elements. These are similar in appearance to the fine fibers which have been identified as keratin or prekeratin fibers in epidermal cells. Occasionally the fibers are arranged in tight bundles (Fig. 5), reminiscent of the "figures of Eberth" described in the larval anuran epidermis (22), except that they bear no obvious relation to desmosomes. The nuclei tend to be somewhat flattened to correspond with the low cuboidal shape of the cells.

## *Non-Pigmented Epithelium*

### A. Plasma Membrane Specializations

The border between the non-pigmented and pigmented layers has been described above. In the basal portion of the non-pigmented cells the lateral membranes follow a relatively straight course and are studded with desmosomes. In the apical half of the non-pigmented cell the plasma membrane is often very complexly folded. As a result of these foldings, the cells interdigitate. These interdigitations have two forms, the lateral and the apical, which will now be described.

The lateral interdigitations are illustrated in Fig. 6. This picture shows the apical portions of two cells, designated *Cell I* and *Cell II*. In the central part of the picture, the paired lateral cell membranes execute a number of hairpin turns before rising to the free cell surface. As a result, slender fingers of cytoplasm (*li*) interdigitate between the two cells. Thus the area of contact between the adjacent cells is considerably increased. The plasma membrane pairs which convolute to form the lateral interdigitations are separated by an area of low electron opacity approximately 200 A wide.

Apical interdigitations are shown at the top of Fig. 6 (*ai*) and at higher magnification in Fig. 8 (*A* to *D*). Their appearance is shown diagrammatically in Fig. 7 *a*. It is seen that, like lateral interdigitations, apical interdigitations are demarcated by a pair of membranes which make a hairpin turn. The membrane pair is spaced at about 200 A. The outer of the two membranes (*om*, in Fig. 7 *a*) is usually continuous with the plasma membrane at the free cell surface. For this reason the apical interdigitations have often been described simply as "infolded plasma membranes"

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Figure 6

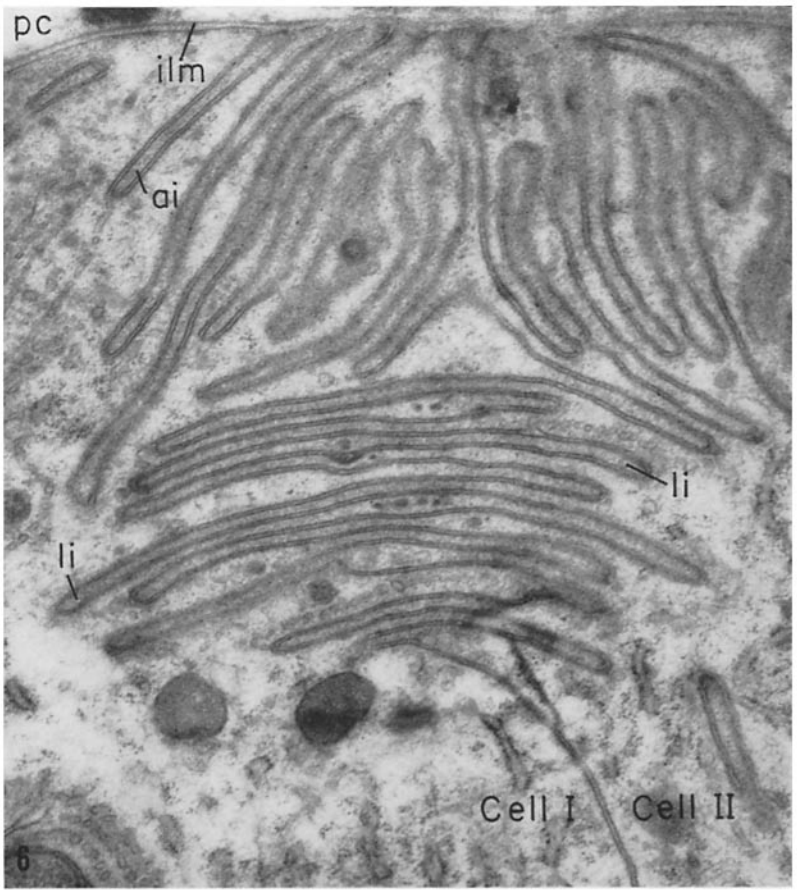
View of the apical portions of two non-pigmented cells (*Cell I*, *Cell II*). The posterior chamber (*pc*) and the internal limiting membrane (*ilm*) are visible at the top of the picture. Particularly well illustrated here are the lateral interdigitations (*li*), formed by the sharp convolutions of the lateral cell membranes, and the apical interdigitations (*ai*) at the free cell surface. Note the similarity between the lateral and apical interdigitations with respect to membrane spacing and general configuration. Diamox. Araldite. × 26,000.

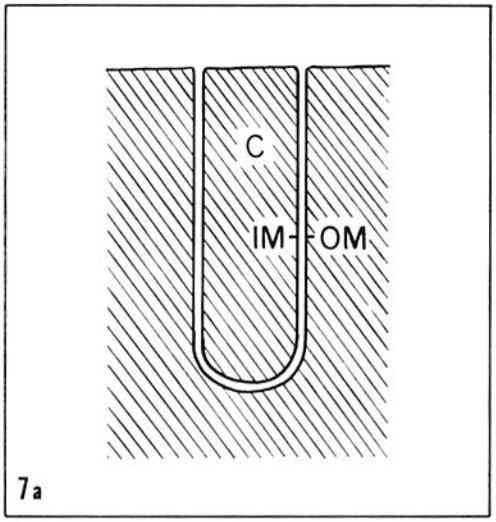
Figure 7 *a*

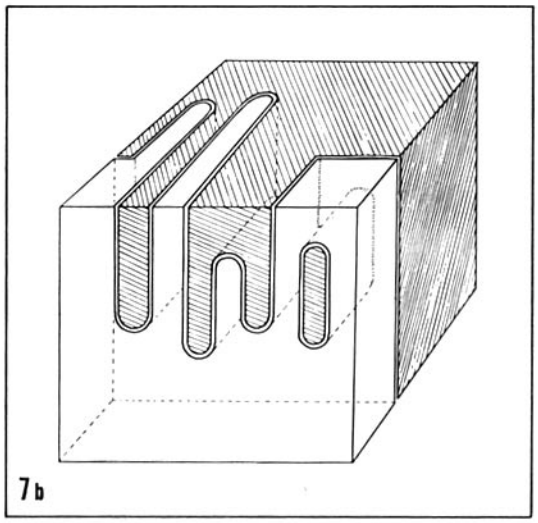
Diagram illustrating an apical interdigitation in two dimensions. The outer membrane (*om*) is continuous with the surface plasma membrane. The inner membrane (*im*) is the plasma membrane of a discrete area of cytoplasm (*c*) within the confines of the outer membrane.

Figure 7 *b*

Diagram to illustrate in three dimensions the geometric relations of the apical interdigitations.


pc
ilm
ai
li
li
Cell I
Cell II
6


C
IM
OM
7a


7b

(14, 24). The inner membrane (*im*, in Fig. 7 *a*) is seen to be the plasma membrane of a discrete area of cytoplasm (*c*, in Fig. 7 *a*) within the confines of the outer membrane. Since this area of cytoplasm is not in obvious contact with the cell within which it is found, there arises the possibility it might be an interdigitation from an adjacent cell, as is diagrammed in Fig. 7 *b*. To establish that this possibility is the actual situation, serial sections must be examined. Figs. 8 and 9 belong to a set of serial sections. The cytoplasm in the uppermost portion of each picture belongs to one cell (*Cell I*), whereas that of the lower four-fifths belongs to a second cell (*Cell II*). The cytoplasm of the interdigitations labeled *A* to *D* in Fig. 8 is not in obvious contact with either *Cell I* or *Cell II*. However, in Fig. 9 the cytoplasm of these interdigitations is revealed to be part of *Cell I*. Therefore, the apical interdigitations of *Cell II* are true interdigitations from *Cell I*. In effect, the apical interdigitations in one section have been transformed into lateral interdigitations in another section.

The demonstration that *all* so called apical interdigitations are true interdigitations is impossible. It is technically impossible to follow all or even most of these structures through serial sections to show their continuity with adjacent cells. However, in almost every set of serial sections studied, at least one and in some cases several apical interdigitations were seen to be transformed into lateral interdigitations. On the other hand, cytoplasmic continuity between an apical interdigitation and the cell in which it appeared has never been seen. Hence it can be stated with assurance that at least most and probably all the apical interdigitations are true interdigitations.

The apical and lateral interdigitations, therefore, are two aspects of the same basic plasma membrane specialization, and the distinction between them refers only to what is seen in an individual section. As a result of the interdigitations, the apical portions of the non-pigmented epithelial cells are interlocked in a complex manner. The area of contact between cells is greatly increased, as are the area of the plasma membrane, the volume of the intercellular space, and the area of the openings of the intercellular space at the free cell surface.

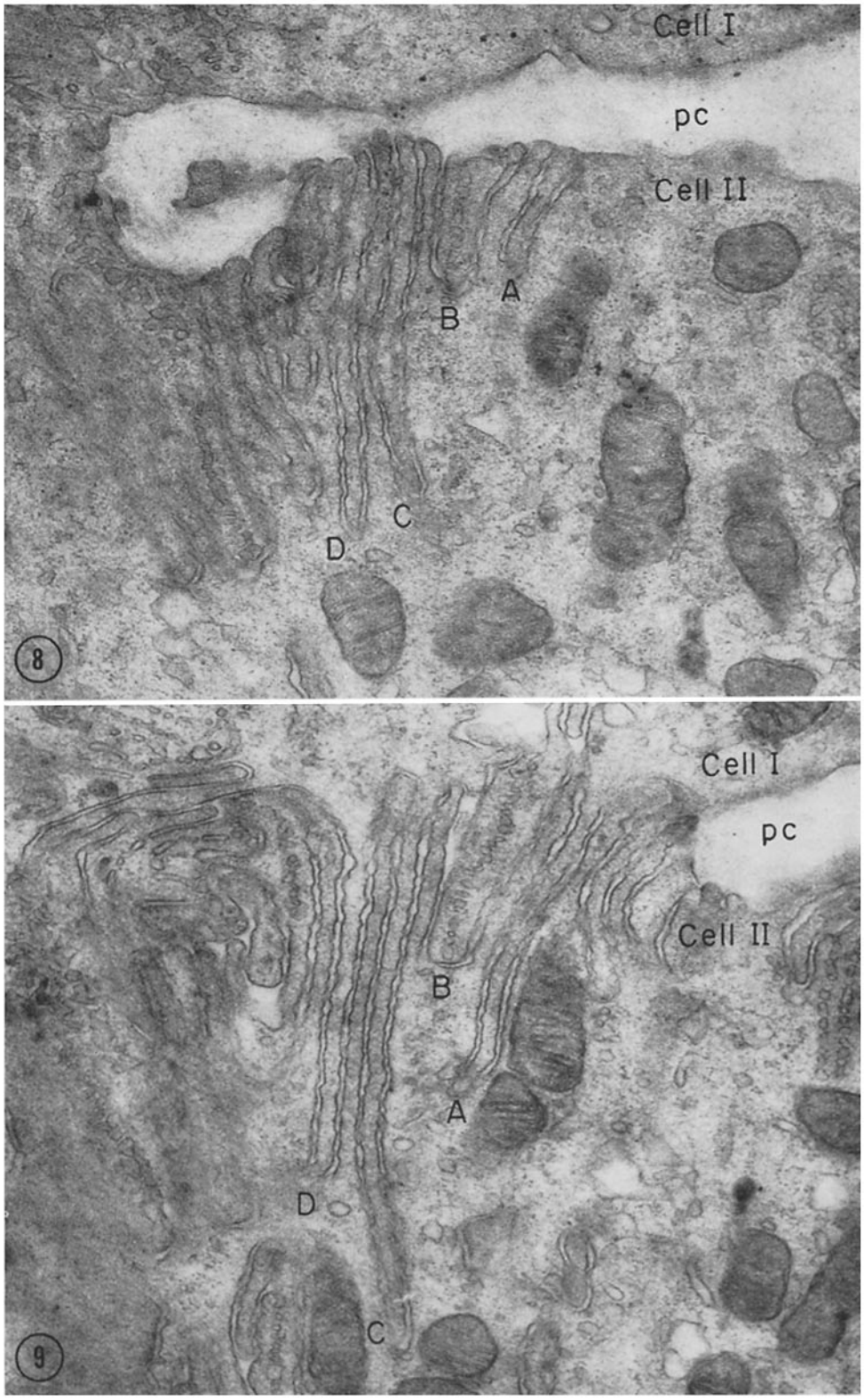
### B. Endoplasmic Reticulum

Highly ordered arrays of vesicular profiles often occur in the apical portion of the cells (Fig. 10). The width of these profiles is fairly uniform, approximately 300 A, but their length varies up to several times their width. They are arranged in rows. The rows tend to occur in pairs, although they also are seen singly and in triplicate. Commonly two rows of vesicles are joined to form a U-shaped loop with a configuration very similar to an apical interdigitation. The rows usually occur in the neighborhood of either the free cell surface or an apical or lateral interdigitation. When adjacent to the free cell surface, vesicles sometimes seem to be opening directly onto the surface, thus giving the impression they may be discharging their contents (Fig. 10). Similarly, when close to interdigitations, vesicles sometimes appear to be emptying directly into the intercellular space (Figs. 12 to 14, 18).

When rows of vesicular profiles are traced through serial sections, a remarkable constancy in their arrangement becomes apparent (Figs. 12 to 14). Some rows drop out after several sections and others appear, but most rows persist in essentially the same arrangement through many sections. For example, in one series of seventeen adjacent sections, one pair of rows of vesicles could be traced through the entire series. Since each section was about 800 A thick, the distance through which the rows persisted was some 1.4 microns. Therefore, vesicles are not simply strung out in

Figure 8 and 9

These are two of a set of serial sections. Two sections have been omitted between these two. A portion of the posterior chamber (*pc*) with poorly visualized internal limiting membrane is seen in each picture. The cytoplasm in the uppermost portion of each picture belongs to one cell (*Cell I*), whereas the cytoplasm in the bottom four-fifths belongs to another (*Cell II*). In Fig. 8, a set of presumptive apical interdigitations is labeled (*A* to *D*). Fig. 9 shows the same interdigitations similarly labeled. In Fig. 9, the cytoplasm of *Cell I* is shown to be continuous with that of the presumptive interdigitations. Therefore, the interdigitations of Fig. 8 are true interdigitations from *Cell I*. Diamox. Araldite. × 30,000.


Cell I
pc
Cell II
A
B
C
D
8
Cell I
pc
Cell II
B
A
D
C
9

rows like beads upon a necklace, but are actually arranged in sheets extending appreciable distances through the cells.

Since the vesicular profiles are arranged in sheets, one would expect to observe the sheets in oblique and tangential section about as often as in cross-section. When observing a sheet of vesicles tangentially, one would expect to see a sizeable area of closely packed vesicles without a linear arrangement. However, even after examination of hundreds of cells, such an appearance has never been encountered. Instead, closely packed smooth surfaced endoplasmic reticulum in the form of an extensive interconnecting system of tortuous tubules is found (Figs. 11 and 15). Hence it is concluded that the rows of vesicular profiles and the areas of closely packed endoplasmic reticulum are but two aspects of the same structure.

This view is confirmed by many pieces of evidence. Both the vesicular and tubular profiles are located only in the apical portion of the cell in the neighborhood of either the cell surface or the apical or lateral interdigitations. In cells where one form is abundant the other is similarly abundant. The diameter of the tubules is the same as the diameter of the vesicles. One frequently sees areas where vesicular and tubular profiles are inextricably intermingled (Fig. 11); such an appearance is readily explained as an oblique view of several sheets of tubular reticulum.

Here, then, is a new form of the smooth endoplasmic reticulum, which is made up of sheets of unusually compact and convoluted tubules. Such sheets viewed in cross-section give the appearance of rows of vesicles (see Fig. 19 for diagram).

It is not altogether surprising that earlier descriptions of the non-pigmented ciliary epithelium placed considerable emphasis on "vesicles" but did not mention the compact tubular endoplasmic reticulum. This is probably because the tubules have higher contrast in cross-section than in tangential section. When a tubule is in cross-section (long axis parallel to the electron beam) its walls are in a position to scatter electrons strongly; hence the walls have high contrast. But when a tubule is in tangential section (long axis perpendicular to the beam) there is relatively little membrane thickness to scatter electrons; hence the walls have relatively little contrast.

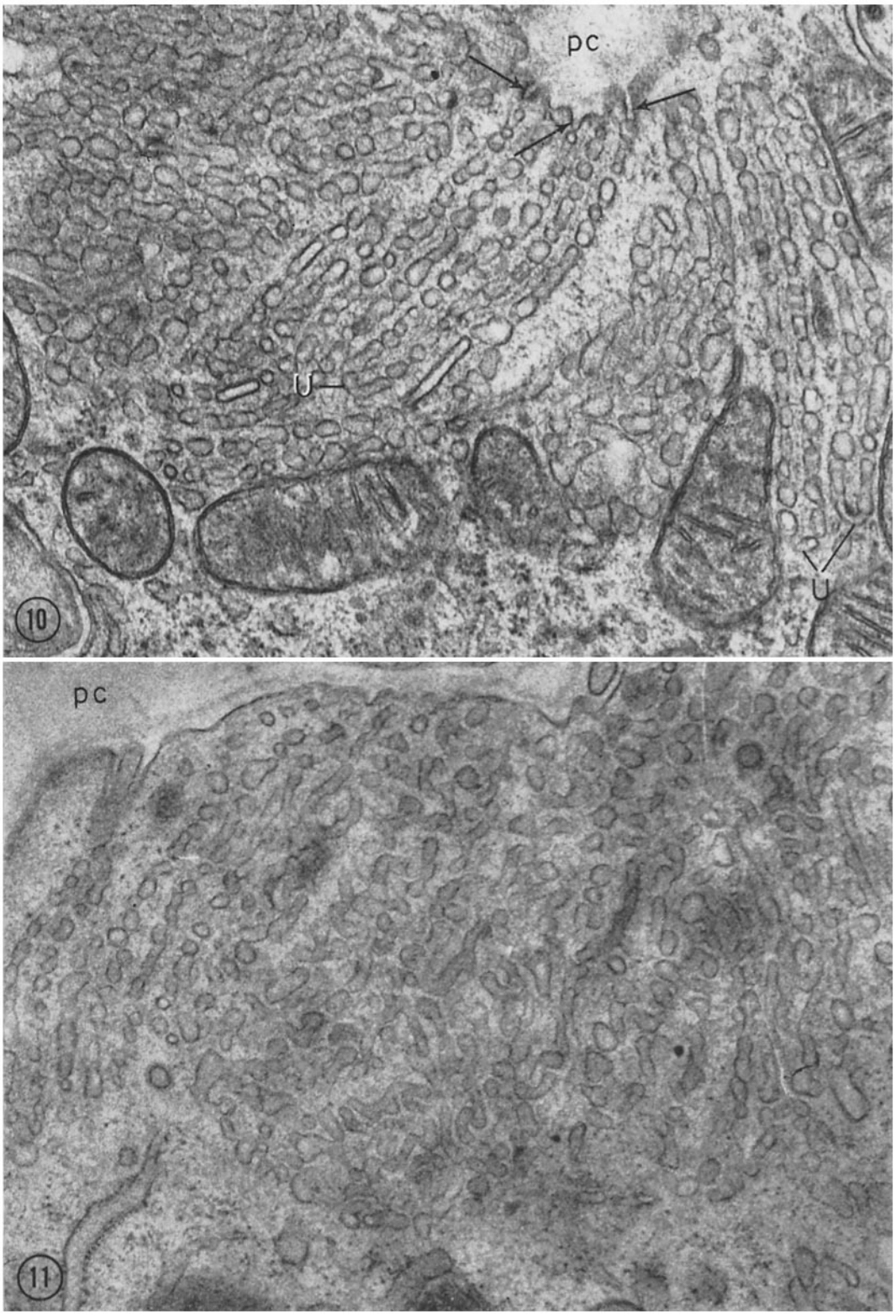
In addition to the sheeted form of tubular endoplasmic reticulum just described, many other elements of endoplasmic reticulum are present. There are many vesicular profiles which are not arranged in rows and which are distributed at random through the cytoplasm. Doubtless at least some of these represent true vesicles. Many smooth surfaced tubular profiles are seen (Fig. 17). Unlike the sheeted form of tubular reticulum, these elements are scattered throughout the cytoplasm, basally as well as apically. They give no evidence of being arranged in sheets. These tubules are relatively loosely arranged and are not particularly tortuous. Frequently their reticular arrangement is not obvious, but at times extensive interconnections are seen (particularly in the basal lateral part of the cell) and then the reticular pattern

FIGURE 10

Micrograph of apical portion of a non-pigmented cell. A small portion of the posterior chamber (*pc*) can be seen at the top. Most of the picture is filled with closely packed vesicular profiles. Their width is fairly constant at about 300 A but their length varies up to several times this figure. Most of the "vesicles" are arranged in a linear pattern and several pairs of rows are joined (*U*) to form U-shaped loops. The configuration of these loops is similar to the configuration of the apical interdigitations. In several places, indicated by arrows, the "vesicles" appear to be opening directly onto the cell surface. Diamox. Epon. × 45,000.

FIGURE 11

Another view at the apex of the non-pigmented epithelium. Here is seen a confusion of vesicular and tubular profiles. Several rows of discrete vesicular profiles similar to those of Fig. 10 are present on the left. Elsewhere, highly convoluted tubules predominate. These interconnect to a considerable extent and are inextricably intermingled with vesicular profiles. The diameters of the tubules and "vesicles" are approximately identical. This picture is interpreted as an oblique cut through an area of sheeted tubular endoplasmic reticulum which has caught a few sheets in approximately cross-section to give the appearance of vesicles. Control. Epon. × 40,000.


pc
U
U
10
pc
11

becomes apparent. Rough surfaced endoplasmic reticulum is present in small quantities (Fig. 16). Most often it is seen as isolated, relatively short, rough surfaced tubules in the apical half of the cell. Occasionally, several rough surfaced tubules appear lined up parallel to one another. Rarely, a rough surfaced tubule appears to be continuous with a smooth-surfaced one.

### C. Other Cellular Components

MITOCHONDRIA: The non-pigmented cells contain an abundance of mitochondria. Their morphology is typical of this organelle. They vary in shape from circular or oval forms to forms whose lengths are several times their widths. They are bounded by the usual double membrane and contain numerous transverse cristae. Occasionally, elongated mitochondria with cristae running axially rather than transversely are seen (Fig. 16). The mitochondria are distributed throughout the cell. Although large numbers are seen around the interdigitations and the sheeted tubular endoplasmic reticulum, they do not appear to have any marked affinity for the neighborhood of these structures and they do not stand in particularly intimate relation to them. (Compare the kidney tubular epithelium, whose basal infoldings are intimately associated with large, elongated mitochondria.)

GOLGI COMPLEX: The cells possess one or more discrete and (usually) small Golgi areas (Fig. 17). These are located in the basal half of the cells. Usually several closely spaced cisternae stand out distinctly in the midst of surrounding smooth endoplasmic reticulum. Vesicles are frequently seen in close association with the cisternae. Golgi vacuoles are few in number and relatively small in size.

ADDITIONAL CYTOPLASMIC ELEMENTS: The cytoplasm contains modest amounts of free RNP particles. Occasionally, small, dense bodies with pleomorphic content bounded by a single membrane are seen. These resemble lysosomes in appearance. Fine fibrillar elements are sometimes seen, but these are much less abundant here than in the pigmented epithelium.

NUCLEUS: The nucleus is located within the basal two-thirds of the cell, and is irregularly round or slightly oval. It frequently contains one or more deep incisurae. Its density is homogeneous with a slight increase at the margin. When seen, the nucleolus is single and located eccentrically. When cut in cross-section, the nuclear envelope reveals occasional "pores" bridged by what appears to be a diaphragm. When cut transversely, the nuclear envelope demonstrates circular profiles similar to those identified by Watson (23) as nuclear pores.

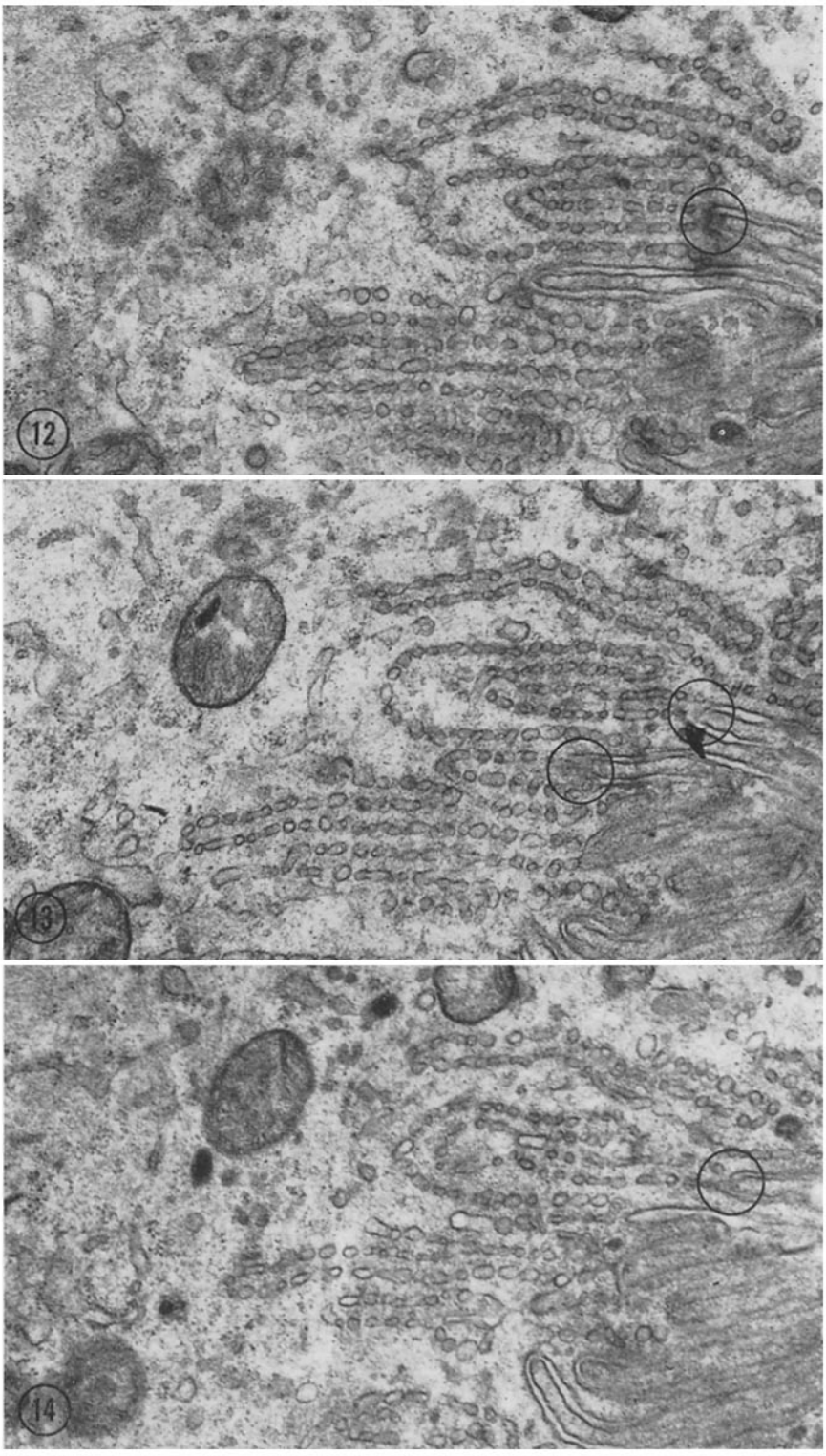
## *Effect of Diamox*

No difference was detected between the eyes which were treated with Diamox and those which were treated with the control compound. (Because of this, the foregoing description of "normal" structure is applicable to both control and Diamox-treated eyes, and the micrographs used to demonstrate normal structure have been taken from both groups of eyes.)

The most striking structural change reported by Holmberg was a fourfold increase in the number of vesicles in the apical portion of the non-pigmented epithelium 15 minutes after Diamox administration (6). This increase was of extremely high statistical significance (t value greater than 8). Pappas and Smelser (10) noted similar changes in vesicle formation but made no attempt to quantitate them. Holmberg (7, 8) noted no concurrent change in the infolded membranes, but Pappas and Smelser (10) claimed that as vesicles increased the infolded membranes shortened.

FIGURES 12 THROUGH 14

These are three of a set of serial sections. A section has been omitted between 12 and 13 and again between 13 and 14. The areas shown are just below the apical surface of the non-pigmented epithelium, and the solid membranes at the extreme right are convoluted lateral cell membranes forming lateral interdigitations. The vesicular profiles are arranged in linear fashion and the rows tend to occur in looped pairs. Note that through this series, which spans a distance through the cell of about 4,000 At the configuration of the vesicular profiles undergoes little change. This indicates that the vesicular profiles are arranged in sheets rather than simple rows or chains. In several locations, indicated by circles, there is an appearance suggestive of connections between vesicular profiles and the intercellular space. Diamox. Araldite. × 36,000.


12
13
14

Therefore, particular attention was paid in the present study to the "vesicles" and "infolded membranes" of the apical half of the non-pigmented layer. The observations in this portion of the cell extended to 168 micrographs of material from 18 tissue blocks representing 9 eyes in the control group, and 144 pictures of 15 blocks representing 9 eyes in the Diamox group. The remainder of both layers of the epithelium was also studied, but less extensively. In spite of the amount of material studied, no difference was apparent.

The accurate quantitation of the vesicular profiles was attempted and found quite difficult. This was largely because it was difficult to decide what was a vesicle and what was not. An attempt to count every more or less circular membrane profile led to difficulty, because some of the profiles were very distinct and many more were ambiguous. In this study, vesicle counts of selected areas of the same micrograph were found to vary by as much as 100 per cent on successive counts. Such counts were none the less attempted, but no differences could be demonstrated. Counting became much more objective when only "*rows* of vesicles" were considered. One semiquantitative approach employed was to pick an arbitrary number of "rows of vesicles" and to classify all micrographs having less than that number as not having a significant accumulation of "vesicles," and all above that number as having a significant accumulation. When six "rows of vesicles" were taken as a cut-off point, it was found that 41 per cent of the Diamox micrographs and 43 per cent of the control micrographs had six or more rows. This indicates that six was very close to the statistical median number of rows seen in a given micrograph, *whether or not* the animal had been treated with Diamox. Although the length of the rows has not been quantitated, it is doubtful that this would affect the conclusion that inhibition of aqueous humor formation by means of Diamox does not affect the number of vesicular profiles found within the ciliary epithelium 15 minutes after the drug is administered.

Holmberg (6) also noted a slight increase in the width of mitochondria, an increase in the number of Golgi vesicles, and a decrease in the length of the Golgi cisternae after Diamox. Although quantitation of these parameters has not been attempted, such changes were not observed in the present material.

## DISCUSSION

### *Interdigitations*

Previous workers did not regard the apical interdigitations of the ciliary epithelium as true

FIGURE 15

Micrograph of the apex of the ciliary epithelium. Here many highly convoluted tubules are seen to be interconnecting to form a dense endoplasmic reticulum. This picture is interpreted as a nearly tangential section of the sheeted endoplasmic reticulum. The posterior chamber (*pc*) is seen in the upper left corner. Control. Epon. × 40,000.

FIGURE 16

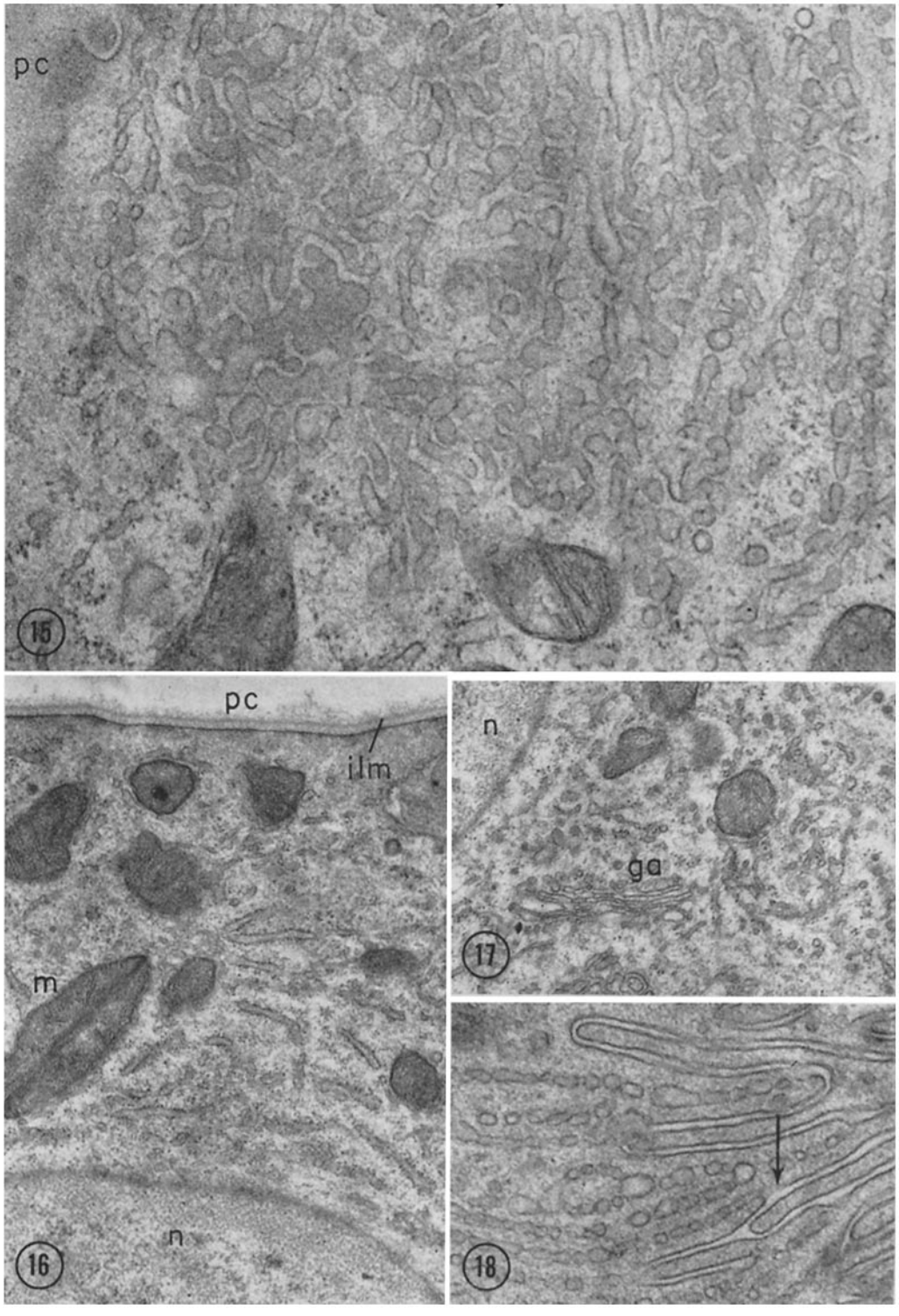
View of the apical portion of the non-pigmented epithelium. At the top is the posterior chamber (*pc*) with a distinct internal limiting membrane (*ilm*), and at the bottom is a nucleus (*n*). A number of relatively loosely arranged elements of both smooth and rough endoplasmic reticulum are evident. Also present is a mitochondrion (*m*) with an axially arranged crista. This view is typical of the apical cytoplasm of the many cells in which little sheeted endoplasmic reticulum is found. Control. Araldite. × 26,000.

FIGURE 17

Basal portion of a non-pigmented epithelial cell. Here a number of elements of smooth endoplasmic reticulum are arranged in the typically loose configuration of this part of the cell. A typical Golgi apparatus (*ga*) is seen. The nucleus (*n*) is in the upper left corner. Control. Araldite. × 26,000.

FIGURE 18

Micrograph of apical portion of non-pigmented epithelium. The paired membranes on the right side of the picture are convoluted lateral cell membranes forming lateral interdigitations. The arrow indicates a tubule of the sheeted endoplasmic reticulum in continuity with the intercellular space. Control. Epon. × 36,000.


pc
15
pc
ilm
m
n
16
n
ga
17
18

interdigitations. Instead, they described these structures simply as "plasma membrane infoldings" (11, 14) or β-cytomembranes (6). As a result the apical interdigitations were considered to be both morphologically and functionally separate from the lateral interdigitations. For example, one group proposed that the lateral interdigitations secrete the aqueous humor and that the "infoldings" selectively reabsorb certain components of the primary secretion (13).

The present study shows by means of serial sections that the apical interdigitations are true interdigitations. The apical and lateral interdigitations appear to be two aspects of the same structure. This will have to be borne in mind in any attempts to correlate the structure and function of the ciliary epithelium.

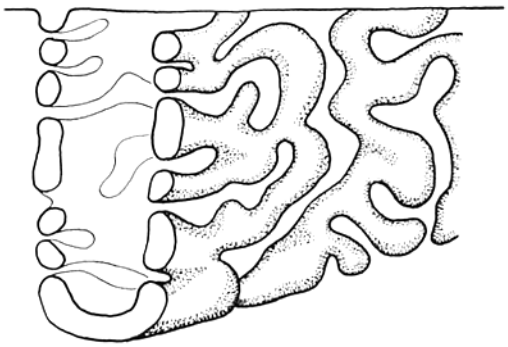


FIGURE 19

Diagram to represent in three dimensions the sheeted smooth-surfaced endoplasmic reticulum. When the closely packed, highly convoluted tubules are cut in cross-section, the appearance of a row of vesicles results.

Structures similar to the apical interdigitations have been described in a number of cell types. Commonly these structures have been described simply as "infoldings" (14, 24), and sometimes the possibility that they might be interdigitations has been denied (24). Rhodin (25), however, suggested that the "infoldings" of the kidney tubular epithelium might really be interdigitations from adjacent cells. Tandler (26) showed that the infolded membranes of the striated duct epithelium of the human submaxillary gland possess numerous desmosomes. The present study is the first demonstration by serial sections that, in one cell type at least, the "infoldings" are actually interdigitations. It remains to be seen whether the infolded membranes of other cell types are of the same nature.

The interdigitations of the ciliary epithelium possibly play an important role in aqueous humor formation. This is indicated by the fact that a number of other epithelia noted for their ability to transport water and electrolytes possess similar structures. Nevertheless, it is difficult to know what that role might be. It has been suggested that such structures facilitate secretion by increasing the area of the plasma membrane (14). The concept that the interdigitations considerably increase the efficiency of the cell surface in the transport of water and electrolytes needs to be viewed with some scepticism, however. It should be remembered that the space bounded by the membrane pairs of the interdigitations is intercellular space, that it is very narrow, and that it probably contains a gap substance with unknown permeability characteristics. Also, it is possible that the main role of the interdigitations might be the purely mechanical one of interlocking the cells.

### *Sheeted Form of Smooth Endoplasmic Reticulum*

Previous workers have been impressed by the numerous vesicular profiles aligned near the apex of the non-pigmented ciliary epithelium and have suggested that they are engaged in fluid transport by micropinocytosis (7, 10, 14). However, this study demonstrates that these vesicular profiles do not represent true vesicles but an unusual tubular form of smooth endoplasmic reticulum. These tubules are highly convoluted and arranged in sheets, so that a section at right angles to a sheet gives the appearance of a row of vesicles (Fig. 19). It is obvious that such a structure cannot be regarded as being directly engaged in micropinocytosis.

Now that micropinocytosis has been ruled out as an important mechanism in aqueous humor formation, it is tempting to speculate whether the tubular endoplasmic reticulum plays an important role. The structure of the reticulum is, in fact, compatible with such a role. The contents of the tubules have access to the exterior of the cell through numerous openings, which lead both directly onto the free cell surface and into the intercellular space of the interdigitations. Therefore, it is possible that the primary active transport could occur across the tubular membranes and that the

material thus accumulated in the tubular lumens could then leave the cell. However, there is no compelling evidence that this is actually the case.

A survey of the role of the smooth endoplasmic reticulum in other cell types neither confirms nor denies the above possibility. Unlike the rough surfaced endoplasmic reticulum, whose function seems to be mainly protein synthesis, the smooth-surfaced endoplasmic reticulum appears to have a wide variety of functions and forms. It has been implicated in lipid metabolism in testicular interstitial cells (27) and fetal zone cells of the adrenal (28), and in carbohydrate metabolism in the fasting liver (29). It assumes highly ordered configurations for special functions as in the sarcoplasmic reticulum of muscle and the myeloid body of the retinal pigment epithelium (30). As regards water and electrolyte secretion, the role of the endoplasmic reticulum is uncertain. No doubt this is in large part due to the simple fact that there is no way, at least at present, for visualizing water and electrolytes in the process of secretion in the same manner that it is sometimes possible to visualize protein, lipid, and carbohydrate products. That a well developed endoplasmic reticulum is not essential for active transport of fluids is clear from the example of the toad urinary bladder (31). Yet, on the other hand, such cells as the gastric oxyntic cell do possess well developed smooth endoplasmic reticulum (32, 33). Quite possibly, then, the smooth endoplasmic reticulum does play an important role in water and electrolyte secretion. The ciliary epithelium provides one more piece of evidence that this might be so. It also provides a striking example of the highly ordered forms this cytoplasmic component can adopt.

### *Effects of Diamox*

Carbonic anhydrase inhibitors, such as Diamox, are currently among the most effective pharmacological agents available for lowering intraocular pressure, and as such are widely employed in clinical ophthalmology. They act by reducing aqueous humor formation. In the case of the rabbit, this reduction has been variously estimated at between 25 and 60 per cent (35, 36). The precise manner in which carbonic anhydrase inhibition affects aqueous humor secretion is controversial, but it seems generally agreed that the site of the inhibitory action is the ciliary epithelium (1).

In the present experiments aqueous humor formation was inhibited by a maximally effective dose of Diamox (34) under carefully controlled conditions. The eyes were fixed 15 minutes after drug administration. No changes in the fine structure of the ciliary epithelium could be detected. Most notably, no changes in the sheeted endoplasmic reticulum were found.

The absence of changes in the vesicular profiles is not altogether surprising, since they are not micropinocytotic vesicles but tubular endoplasmic reticulum. This reticulum is highly organized and quite likely is relatively stable. Perhaps more chronic administration of inhibiting drugs might produce morphological changes.

The results reported by Holmberg (6) and by Pappas and Smelser (10) on the effects of Diamox on the "vesicles" and other structures of the ciliary epithelium seem to be incompatible with the present findings. There is no evident explanation for this difference. Among the points of similarity between this and previous studies are the use of medium-size albino rabbits, the use of a maximally effective dose of Diamox, and the fixing of the eyes at a time at which structural changes are supposedly maximal (6). The present study differed from the others in that the anesthesia was obtained with an anesthetic which has no effect on the intraocular pressure (37) and the embedding was carried out in epoxy resins rather than methacrylate. The test for protein in the aqueous humor, employed only in the present study, is particularly important. The blood-aqueous barrier is very easily broken down and then protein seeps into the aqueous. The morphological consequences of this are indicated by Pappas (12) and Pappas and Smelser (10), who showed that, when blood-aqueous barrier breakdown was achieved by a variety of techniques, the ciliary epithelium underwent changes virtually identical with those supposedly caused by Diamox.

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