STUDIES ON THE CORNEA

III. The Fine Structure of the Frog Cornea and the Uptake and Transport of Colloidal Particles by the Cornea *in vivo*

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ABSTRACT

The fine structure of the frog cornea has been studied and compared with that of the rabbit cornea (1, 2) particularly in relation to the uptake and transport of colloidal particles. The frog corneal endothelium does not possess a terminal bar and the fluid space of the intercellular space is apparently continuous with that of the anterior chamber. Colloidal markers (ThO_2, Fe_2O_3) placed in the anterior chamber pass down the intercellular space into the cornea. Markers injected intrastromally diffuse freely in the stroma and Descemet's membrane but pass across the endothelium only via membrane-bounded vesicles. These results are compared with those of similar experiments in the rabbit and it is concluded that the primary pathway for the passage of materials into the cornea is intercellular and that the pinocytotic pathway of the rabbit corneal endothelium (Kaye and Pappas; Kaye *et al.*) is an adaptation to the presence of a terminal bar. The significance of the separation of inward and outward pathways in terms of corneal metabolism is considered.

INTRODUCTION

Previous reports from this laboratory have described the fine structure of the rabbit cornea with particular emphasis on the structure of the corneal endothelium (Descemet's mesothelium) in relation to the uptake and transport of colloidal particles *in vivo* and *in vitro* (1, 2).

Preliminary studies of the cornea of *Rana pipiens* (2) demonstrated certain morphological differences between the frog and rabbit corneas which might affect the transport pathways. An experimental series similar to that carried out on the rabbit cornea (1) was, therefore, performed on corneas of *Rana pipiens* and *Rana catesbeiana* in order to investigate the corneal transport pathways in these animals and compare them with those in the rabbit.

MATERIALS AND METHODS

Methods for the Study of the Normal Cornea

Adult female *Rana pipiens* were anesthetized by cooling overnight to 4–6°C. Adult *Rana catesbeiana* which had been stored in a tank of cold water were used without anesthesia. Animals of either species were immobilized by tightly wrapping all but their heads in damp towels. A fixative solution containing 1 per cent OsO_4 in M/14 veronal-acetate buffer was injected into the posterior and anterior chambers of the eye. The eye was then enucleated in the case of *R. pipiens*; or the anterior segment was removed in the case of *R. catesbeiana*. The anterior segments of the eyes of both species were fixed for 30 to 90 minutes in fresh chilled 1 per cent OsO_4 in M/14 veronal-acetate buffer at pH 8.2. After approximately ⅔ of

the fixation time had elapsed, the lens and iris were removed and the central cornea was cut into strips up to 0.5 mm wide by 1 to 2 mm long. The strips were then fixed in fresh solution for the remainder of the particular fixation period used. The tissue was dehydrated in cold graded ethanol solutions and subsequently embedded in methacrylate containing 0.75 per cent UNO_3 (3) or in Epon 812 (4). In embedding the corneas, care was taken to orient the block in the capsules so that the face of the block which was seen at the bottom of the capsule contained epithelium, stroma, Descemet's membrane and endothelium.

Methods for the Study of the Uptake and Transport of Colloidal Particles by the Cornea in vivo

Test solutions (0.1 to 0.3 ml) of thorium dioxide, saccharated iron oxide, hemoglobin, or ferritin were injected into the anterior and posterior chambers of the eyes of adult female *R. pipiens* and adult *R. catesbeiana*. The test solutions in *R. pipiens* were left 30 to 60 minutes while those in *R. catesbeiana* were left for approximately 18 hours. The thorium dioxide solutions (Thorotrast, Testagar & Co., Detroit, Michigan) were either dialyzed against ion-free water or were used directly from the manufacturer. Other test solutions contained suspensions of saccharated iron oxide (approximately 50 per cent Fe_2O_3), hemoglobin (10 or 20 per cent Hb) or ferritin (10 per cent) in a solution similar in composition to aqueous humor (5) or in ion-free water.

The cornea of *R. pipiens* is approximately 0.1 mm thick. It was impossible, therefore, in this species to inject a small amount of the marker solution into the stroma to produce a bleb without the use of micro-manipulator techniques. For this reason, corneas from *R. catesbeiana* were used for the intrastromal injection series. This cornea is approximately 0.2 mm thick and it is possible, with a No. 30 hypo-

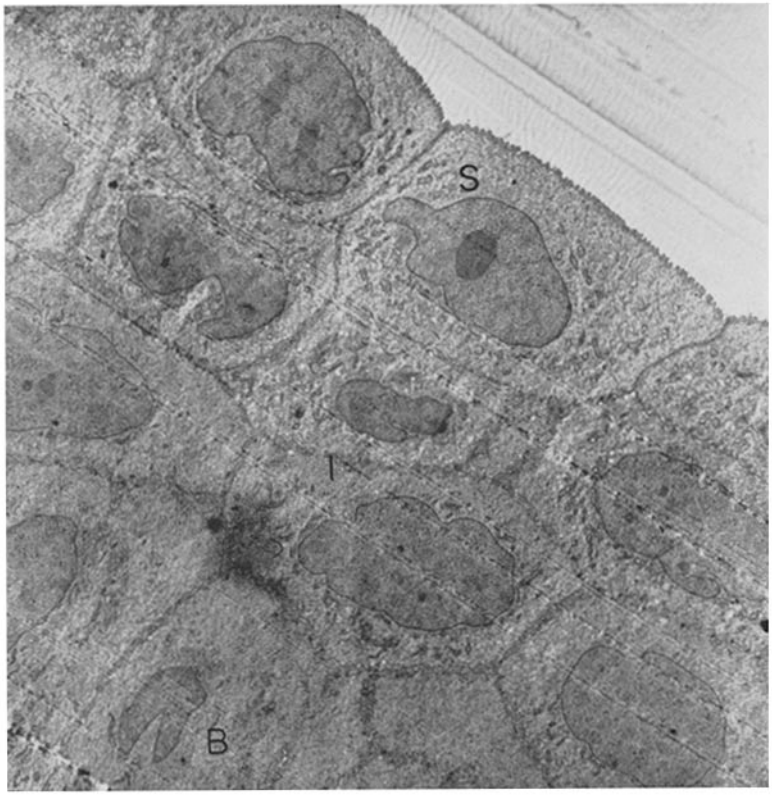


FIGURE 1

The corneal epithelium of *Rana catesbeiana* showing the basal (*B*), intermediate (*I*), and surface (*S*) cell layers. The surface layer is not so flattened as that of the mammalian cornea (1). Low power micrograph. × 2,800.

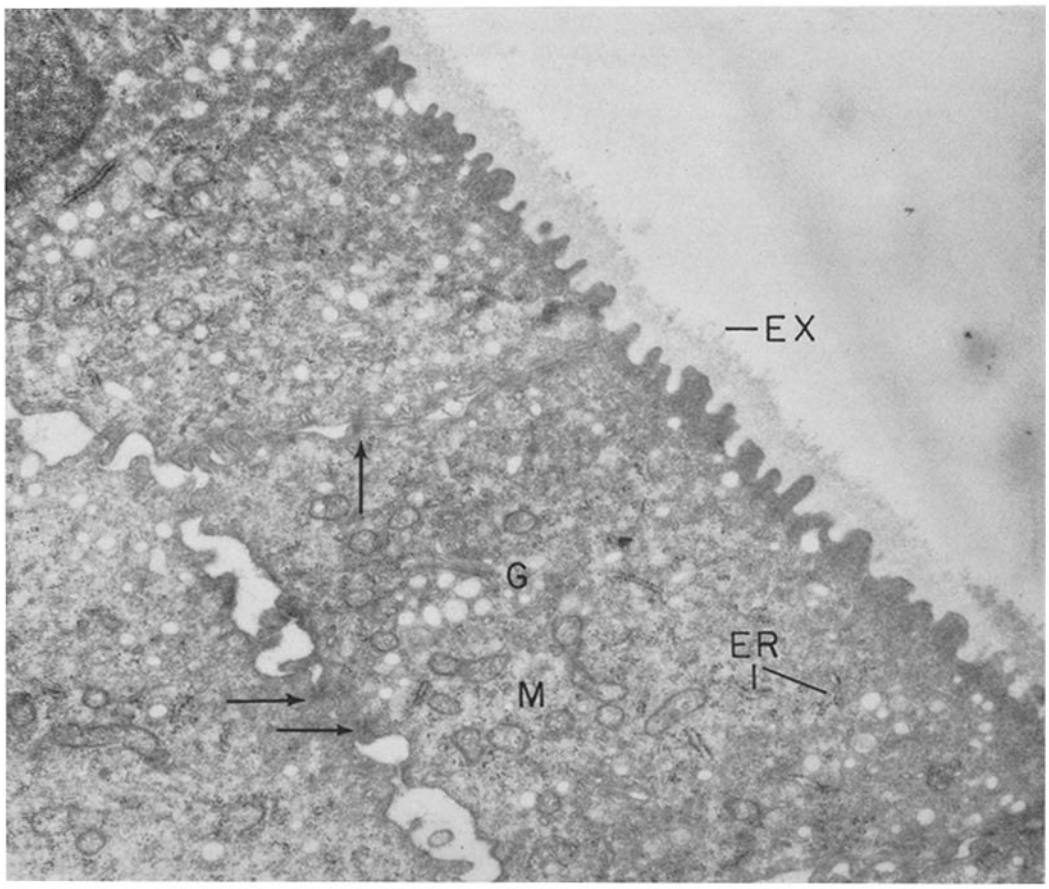


FIGURE 2

A part of the surface layer of the corneal epithelium of *R. catesbeiana*. Numerous mitochondria (*M*), elements of both the granular and vesicular endoplasmic reticulum (*ER*), and several Golgi zones (*G*) may be seen in the cytoplasm of these cells. The presence of these cytoplasmic organelles is in sharp contrast with the paucity of organelles in the rabbit cornea (1). A thick extracellular coating (*EX*) of moderate density is seen at the surface. Interdigitations and small desmosomes (arrows) may be seen at the cell margins. × 22,000.

dermic needle, to inject a small amount of test solution (approximately 0.05 ml) into the stroma where it remains as a reservoir of colloidal material.

At the end of the experimental period the corneas were fixed, dehydrated, and embedded in the manner described previously under Methods for the study of the normal cornea.

OBSERVATIONS

The Structure of the Frog Cornea

The cornea of *Rana pipiens* measures approximately 0.1 mm (100 microns) in thickness when embedded in methacrylate blocks. The cornea of *Rana catesbeiana* is approximately twice this thickness. The external surface of the cornea of each species is covered with a stratified squamous epithelium which measures approximately 35 to 40 microns in thickness. The underlying stroma is about 55 to 60 microns thick in *R. pipiens* and about 150 microns thick in *R. catesbeiana*, whereas the thin Descemet's membrane and much flattened endothelium measure no more than 2 to 4 microns each in both species.

The epithelium of the frog cornea (Fig. 1) is similar to that of most mammalian corneas (1, 6–9) since it can be divided into three layers: a

basal layer of columnar cells (one cell thick), an intermediate zone of polygonal and slightly flattened cuboidal cells (2 to 3 cells thick), and a surface layer of squamous cells (1 to 2 cells thick). The epithelial cells of the frog cornea (Fig. 2) contain a much greater number of cytoplasmic organelles than do those of the rabbit (1), rat (6), mouse (8) or human (7) corneas. It is often difficult to distinguish a true squamous layer at the surface because the cells at the surface are rarely more flattened than those of the immediately subjacent layer. The fibrous component of the epithelial cells is less regularly disposed in the frog cornea (Fig. 2) than in the mammalian corneas (1, 6–9) and is often (when distinguishable) randomly distributed among the vesicular components of the endoplasmic reticulum.

The external surface of the lining cells of the frog corneal epithelium is coated with a thick, relatively homogeneous extraneous coat of moderate density (Fig. 2). This coat is thicker and more dense than that of the rabbit cornea. The significance of this coating in terms of transport phenomena or protection is unclear in these amphibia.

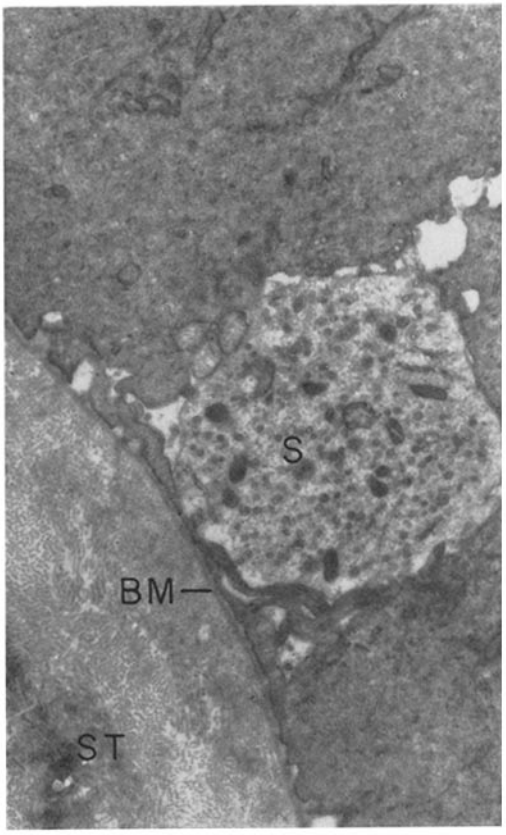


FIGURE 3

The basal portion of the epithelium showing parts of three epithelial cells, the basement membrane (*BM*) and part of the stroma (*ST*). Part of the cytoplasm of a Schwann cell (*S*) which has apparently penetrated through the basement membrane is seen between the basal-lateral margins of the epithelial cells. This Schwann cell is presumably associated with the non-myelinated nerves (Fig. 4) which are seen at this same site in other sections. × 13,000.

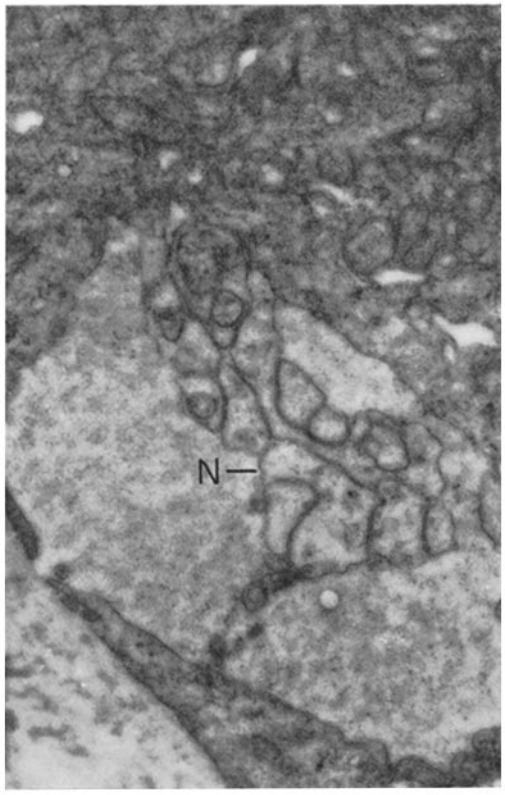


FIGURE 4

An area in the basal portion of the epithelium similar to that seen in Fig. 3 showing a small bundle of non-myelinated nerves (*N*). × 19,000.

Interdigitations of adjacent cell membranes (Fig. 1), intercellular desmosomes (Figs. 1 and 2), and half desmosomes attaching the basal cell layer to the underlying basement membrane (Fig. 3) are found in the frog and are similar to those in the mammalian cornea.

The epithelium of the frog cornea appears

extremely well innervated when compared with that of the rabbit (1) or mouse cornea (8). It was possible to find unmyelinated fibers, Schwann cells, or axon bundles in the basal portion of the intercellular space separating adjacent basal cells in almost every specimen examined (Figs. 3, 4). In these instances the basement membrane appears continuous over the opening of the intercellular space. Whitear (8) reported a similar disposition of the basement membrane in the mouse. It is not possible to tell in either the present case or in Whitear's (8) report whether some basement membrane material has preceded or followed the penetrating bundle or whether the basement membrane is split by the nerve as it enters the epithelium.

No distinguishable Bowman's membrane is found underlying the epithelium of the frog cornea.

The stroma of the anuran cornea is similar in architectural organization to that of mammalian cornea (Fig. 5). The lamellae lie in a plane parallel to the surface of the cornea, and the collagen fibers in a given lamella are parallel to each other, and at right angles to those in the adjacent lamellae. The lamellae nearest the epithelial side are thinner than those near the endothelial side. This difference probably reflects the dual (dermal and scleral) origin of the anuran cornea.

The stromal cells which are found between the lamellae are greatly flattened and have extensive attenuated processes extending along the surfaces of the lamellae. An apparently fibrous material, of a density greater than that of the collagen, is usually found surrounding the stromal cells and their processes and may often be seen outlining the collagen lamellae even when no cell processes are visible (Fig. 5). Strands of this dense material often pass from one lamella through the next, usually in a direction normal to the long axis of the collagen fibers.

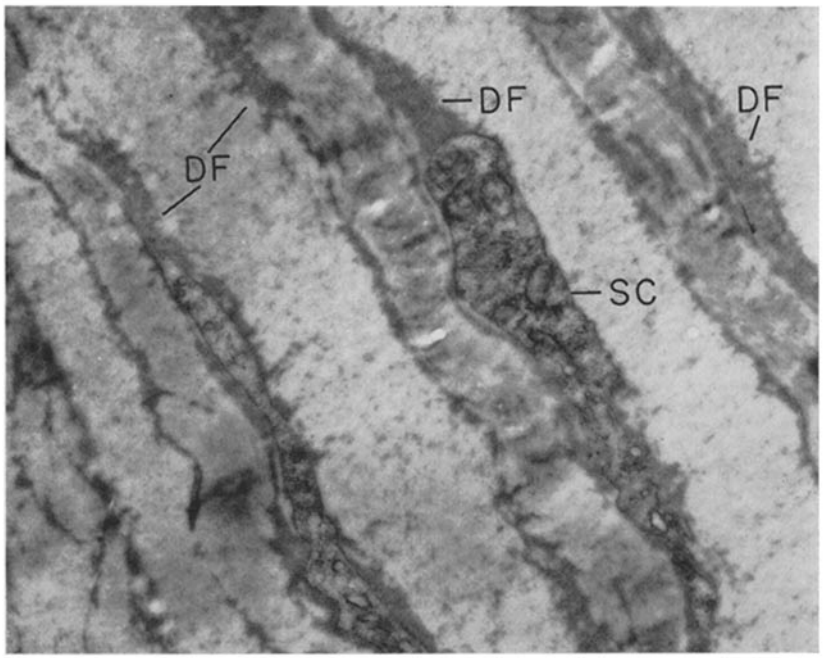


FIGURE 5

Part of the stroma of the cornea of *R. pipiens*. Stromal cells (*SC*) are seen between the alternating collagen lamellae. A dense fine fibrillar material (*DF*) surrounds the stromal cells and their processes and is seen separating the collagen lamellae even when no stromal cell process is identifiable in the section. Low power micrograph. × 12,000.

Nerve bundles (Fig. 6) are found running in a plane parallel to the lamellae and are most often seen slightly below Descemet's membrane. These bundles consist of a Schwann cell which partially or completely envelops numerous non-myelinated fibers. The Schwann cell in turn is surrounded by

a basement membrane which resembles the dense fibrillar material found between collagen lamellae. It is probably from these bundles that the nerve fibers which enter the basal portion of the epithelium are derived. This relationship has been demonstrated in mammals by light microscopic techniques (10) and in the mouse by electron microscopic techniques (8).

Descemet's membrane in the frog cornea (Figs. 7 and 8) is thinner but the fibrillar make-up is (Fig. 8) more apparent than in the rabbit cornea (1).

The endothelium of the frog cornea (Figs. 7 and 8) is much more flattened than that of the rabbit cornea (1), and more nearly resembles that of the human cornea (7). The lateral margins of the cells are highly convoluted and interleaved, producing a tortuous intercellular space which contrasts with the relatively straight intercellular spaces of the rabbit corneal endothelium (1). However, the width of the intercellular space (Figs. 7 and 8) is more regular than in the rabbit. This may reflect a stronger adherence of the more convoluted lateral margins of the frog endothelium. No terminal bars or "tight" junctions have been observed in the frog corneal endothelium.

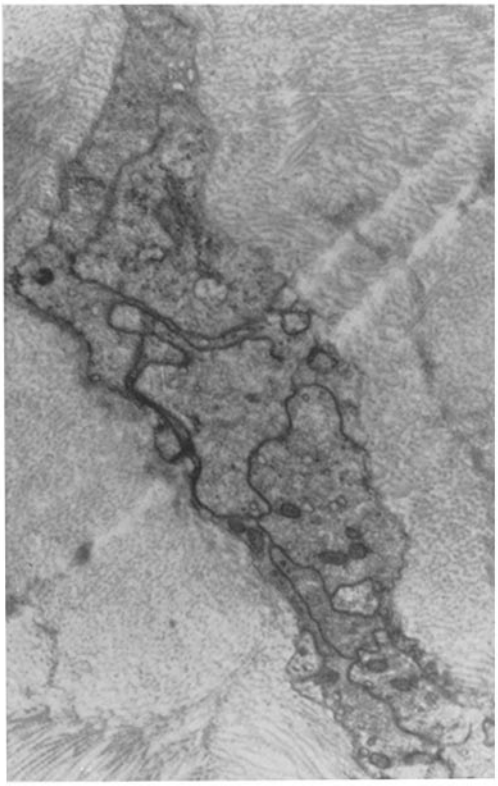


FIGURE 6

A bundle of non-myelinated nerve fibers found in the stroma near Descemet's membrane. It is from these bundles that the nerves seen in the epithelium are derived. × 19,000.

The nuclei of the endothelial cells are greatly flattened. Numerous mitochondria, some granular endoplasmic reticulum, some typical Golgi zones, and numerous small vesicles suggestive of pinocytotic vesicles are found in the cytoplasm of the endothelial cells (Figs. 7 and 8). The external surface of the apical cell membrane is covered with a thin, relatively homogeneous extraneous coat of intermediate density similar to that described in the rabbit corneal endothelium (1).

The Uptake and Transport of Colloidal Particles by the Cornea in Vivo

The injection of electron-opaque colloidal particles (ThO_2, Fe_2O_3, ferritin or hemoglobin) into the anterior chambers of eyes of *R. pipiens* and *R. catesbeiana* produced results similar to those previously reported in the rabbit (1).

In corneas of *R. pipiens* fixed 30 to 60 minutes after the injection of approximately 0.1 ml of ThO_2 into the anterior chamber, the marker is found attached to the apical surface of the endothelial cell as well as within the intercellular space throughout its tortuous course from the apical to basal surfaces and piling up at and diffusing into Descemet's membrane (Fig. 9). In addition, a few particles are found within vesicles located primarily in the apical cytoplasm (Fig. 9).

The corneas of *R. catesbeiana* were fixed approximately 18 hours after injection of the marker into the anterior chamber. By this time a state of equilibrium appears to be established (Fig. 10). While few, if any, particles of ThO_2 are found at the apical surface of the endothelial cell, the marker is found in the intercellular space at various levels and accumulated at the margin of Descemet's membrane and the endothelium as well as within vesicles and vacuoles of varying sizes located in the basal and medial cytoplasm. The size and content of the latter vesicles are typical of vesicles carrying marker out of the cornea in the rabbit (1) as well as in the frog (Figs. 17 and 20).

Similar results are found when saccharated iron oxide is used as the marker in either *R. pipiens* or *R. catesbeiana*. Fig. 11 shows a part of the corneal endothelium of *R. pipiens* fixed approximately 40

minutes after exposure to saccharated iron oxide in the anterior chamber. Marker particles are found in the intercellular space at all levels. Fig. 12 shows part of a cornea of *R. catesbeiana* fixed 18 hours after injection of the marker. In this instance the marker is found almost exclusively within membrane-bounded vesicles and vacuoles at all levels of the cytoplasm. In addition some marker and the stroma were invaded by lymphocytes. In the bleb experiments it seemed that the movement of these two markers out of the cornea was so rapid that the piling up of the marker at the margin of Descemet's membrane and the endothelium was so extreme as to effectively lift the endothelium off Descemet's membrane.

Thorotrast injected into the stroma of *R.*

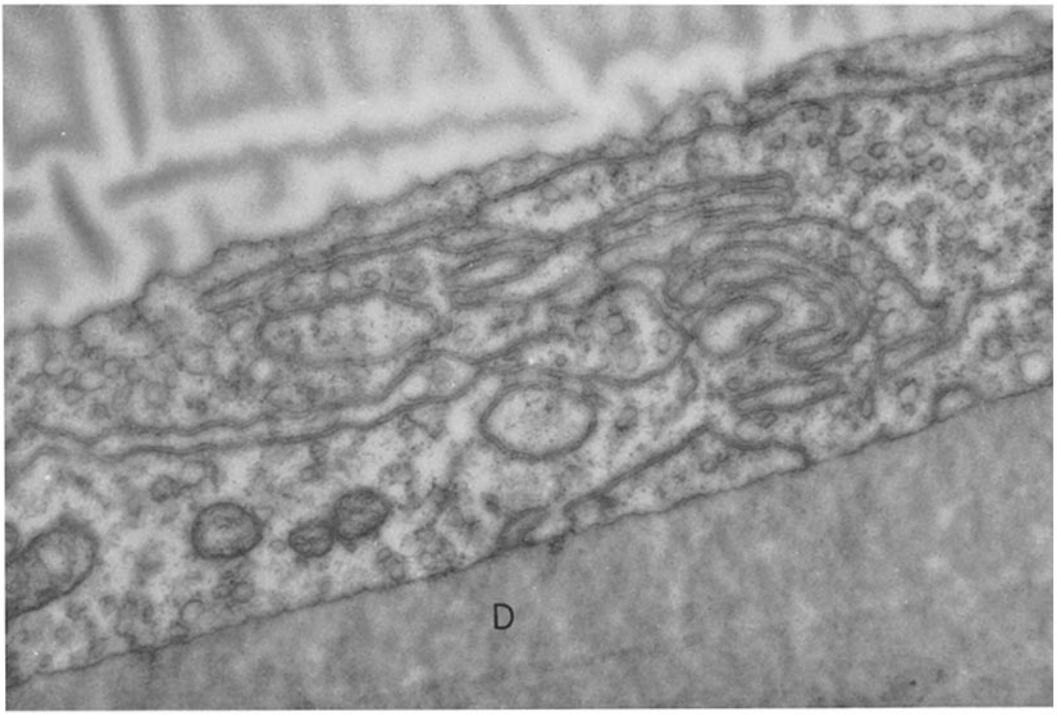


FIGURE 7

Parts of two endothelial cells in the cornea of *R. pipiens*. The cytoplasm contains mitochondria and numerous elements of the vesicular component of the endoplasmic reticulum. The lateral margins of the cells exhibit a complex interleaving. The width of the intercellular space is regular and no terminar bar or "tight" junction is visible. *D* = Descemet's membrane. × 28,000.

is found accumulated at the junction of Descemet's membrane and the endothelium. It is probable that this represents an equilibrium situation and that the marker has been transported across the endothelium since particles are readily found in the stroma of the cornea (Fig. 13) 18 hours after injection into the anterior chamber.

The concentrations of ferritin and hemoglobin used in both the anterior chamber and stromal injection experiments in *R. catesbeiana* were so high that foreign body reactions were produced in these corneas, thereby masking the normal transport pathways. Both the anterior chamber *catesbeiana* diffuses freely from the injection site and piles up at the lamellar borders (Fig. 14). This may involve the fibrous material surrounding stromal cell processes which was described in an earlier section (Fig. 15). Figure 15 shows a portion of the corneal stroma of *R. catesbeiana* in which a stromal cell process contains several large and small vacuoles and vesicles which contain dense accumulations of particles of thorium dioxide. The reaction of the stromal cells of the frog cornea to colloidal markers is identical to that of the rabbit cornea (1).

Fig. 16 shows part of the endothelium and

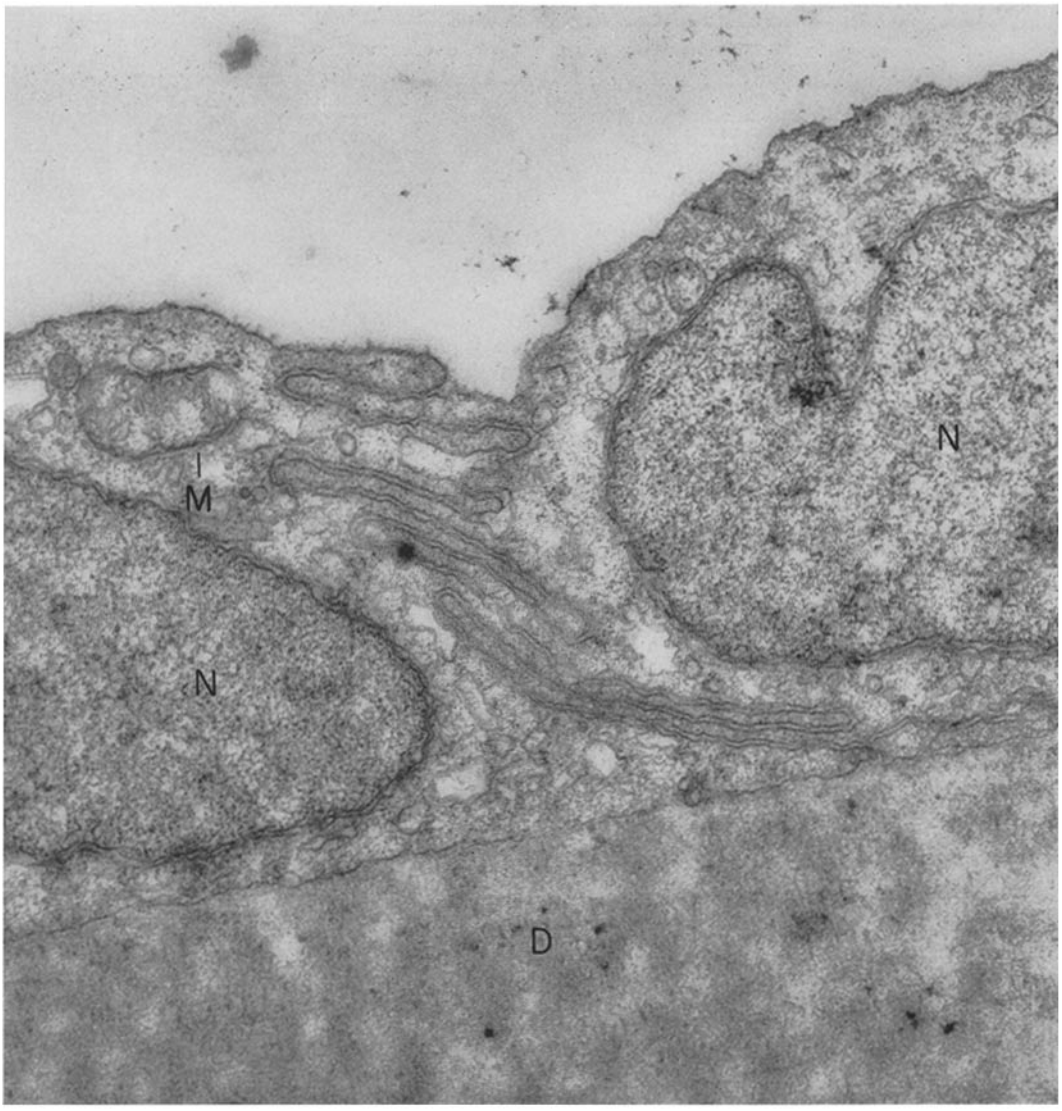


FIGURE 8

Part of the corneal endothelium of *R. catesbeiana* showing the interleaving of the lateral cell margins and the absence of terminal bar or haptomere (11). *M* = Mitochondria, *N* = Nucleus, *D* = Descemet's membrane. × 32,000.

underlying Descemet's membrane of a frog cornea in which a stromal injection of ThO_2 had been made about 18 hours prior to fixation. Particles are found accumulated at the junction of Descemet's membrane and the endothelium as well as within several membrane-bounded vacuoles and vesicles in the endothelial cell cytoplasm. In experiments in which the marker is placed intrastromally and allowed to travel out of the cornea the marker does not appear in the intercellular spaces of the endothelium but solely within vesicles in the cytoplasm of the endothelial cells (Fig. 17). In the frog it is also possible to demonstrate that the colloidal markers can pass from the stroma across the basement membrane of the epithelium and can be found in vesicles in the basal epithelial cells (Fig. 18). This phenomenon was not clearly demonstrated in the rabbit (2)

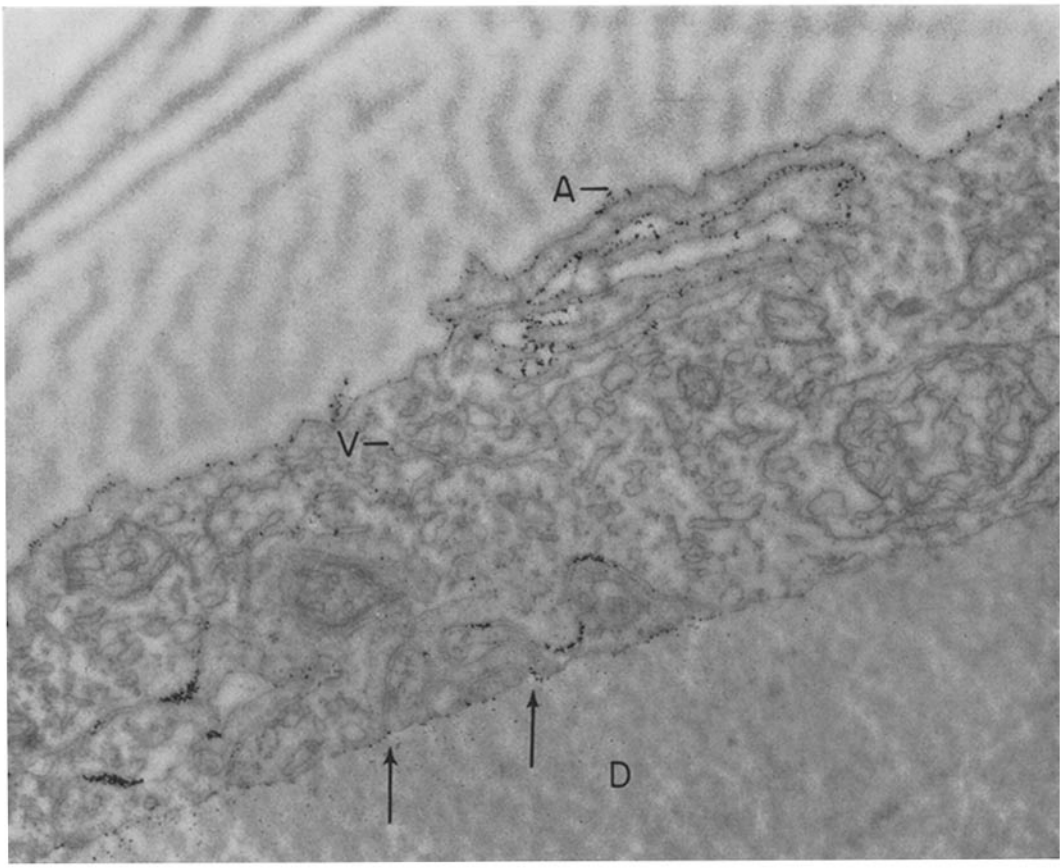


FIGURE 9

Part of the corneal endothelium of *R. pipiens* which had been exposed for approximately 1 hour to a suspension of thorium dioxide injected into the anterior chamber. The marker is found at the apical surface (*A*) and in the intercellular space throughout its length. Particles (at arrows) are also found diffusing into Descemet's membrane (*D*). Only a single vesicle (*V*) is seen to contain the marker. × 28,000.

although there was some evidence that it might occur.

Injection of saccharated iron oxide into the corneal stroma of *R. catesbeiana* produces similar results. Marker particles are found to diffuse throughout the stroma and to accumulate in large amounts within stromal cells (Fig. 19). In addition the marker is found accumulated at the junction of Descemet's membrane and the endothelium as well as in membrane-bounded vesicles in the cytoplasm of the endothelial cells (Fig. 20).

DISCUSSION

The cornea of the normal adult frog closely resembles that of other higher vertebrates. A significant difference, however, between the frog and rabbit corneas (1) is the total absence of terminal bars or haptomeres (11) in the endothelium of the frog cornea. Because of this fact, as well as the complex interleaving of the lateral margins of the endothelial cells of the frog cornea, this layer in frogs most nearly resembles that in the human (7).

Previous reports from this laboratory (1, 2) have described transport pathways across the rabbit corneal endothelium. The transport of materials inward across the endothelium depended on the pinocytosis of colloidal particles at the cell surface and their transport around the terminal bar in membrane-bounded vesicles, the subsequent fusion of the vesicles with the lateral cell margin

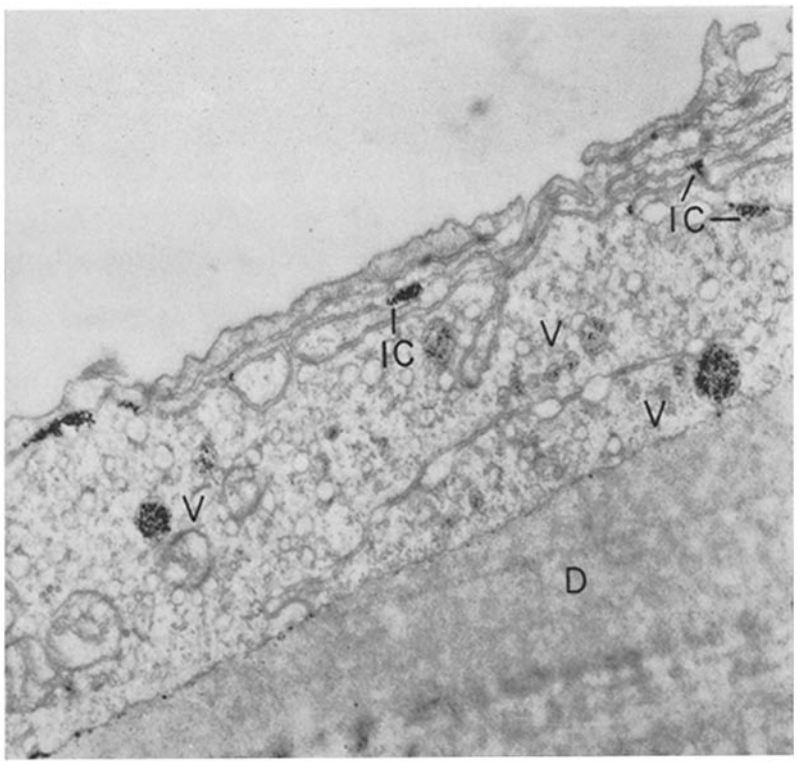


FIGURE 10

Part of the corneal endothelium of *R. catesbeiana* which had been exposed for approximately 18 hours to a suspension of ThO_2 injected into the anterior chamber. In this instance an equilibrium appears to be established between influx and efflux pathways. While no marker is seen at the surface, particles are found in the intercellular space (*IC*) at several levels as well as in large vesicles and vacuoles (*V*) primarily found in the basal cytoplasm. Marker is also seen accumulated at the junction of Descemet's membrane and the endothelium. The large vacuoles resemble those found when marker is known to be travelling out of the cornea across the endothelium (see Figs. 16, 17 and 20, as well as reference 1, Figs. 12 and 13). It is likely that the long exposure has allowed the marker to trace both the outward and inward pathways. × 20,000.

basal to the terminal bar, and the flow of these particles down the intercellular space and into Descemet's membrane. Particles injected intrastromally diffused freely through the stroma and Descemet's membrane, accumulated at the junction of Descemet's membrane and the endothelium, and passed out across the endothelium via membrane-bounded vacuoles.

It was concluded (2) that in the rabbit cornea the primary pathway of inward transport was intercellular while transport out of the cornea was primarily by an intracellular route. The presence of a terminal bar which apparently separated the fluid space of the intercellular space from that of the anterior chamber probably led to the adaptation of the generalized cell process of pinocytosis to the specific task of by-passing the terminal bar.

The conclusion that the primary pathway of transport into the cornea across the endothelium is intercellular is confirmed by the present observations on the frog cornea. In all short-term exposures of frog corneas to marker particles injected into the anterior chamber the marker is found absorbed at the cell surface and filling the tortuous intercellular space. Occasional pinocytotic vesicles containing colloidal particles are seen in the apical cytoplasm but pinocytosis does not appear to play a significant role in the transport of colloidal particles into the frog cornea.

In long-term experiments (18 hours) the marker

is found in the intercellular space and in membrane-bounded vesicles and vacuoles in the basal and medial cytoplasm of the endothelial cells (Figs. 10 and 12). It is likely in these instances that an equilibrium situation has been established in which the colloidal particles are behaving as tracers of the pathways both into and out of the cornea. The large particle-filled vacuoles seen in these instances appear the same as those found when marker is injected intrastromally in both the rabbit (1) and the frog (Figs. 17 and 20). In addition, the large amounts of marker found in the stroma in these experiments (Fig. 13) suggest that after 18 hours the colloidal markers are moving in both directions.

Injection of colloidal particles intrastromally into the frog cornea produces a situation like that found under similar experimental conditions in the rabbit cornea (1). The marker diffuses freely in the stroma and Descemet's membrane, is taken up in large amounts by the stromal cells, and piles up at the junction of Descemet's membrane and the endothelium. Despite this pile-up, the marker does not appear in the intercellular space but is carried out across the endothelial cell within membrane-bounded vesicles and vacuoles.

The use of the relatively thin frog cornea has allowed the demonstration of the movement of the marker particles across the basement membrane of the epithelium and the uptake of tracer into vesicles in the basal epithelial cells (Fig. 18). While this had been suggested in the study of transport in the rabbit cornea *in vitro* (2), it remained for the present experiments on the frog cornea to demonstrate this phenomenon *in vivo*.

Fig. 21 summarizes the comparative aspects of the transport pathways of the rabbit and frog corneal endothelia. It is obvious from this figure that there are two separate pathways for the transport of materials into or out of the cornea. Transport into the cornea across the endothelium occurs via the intercellular space. The pinocytotic system described in the rabbit cornea (1, 2) is apparently a mechanism adapted to by-passing the terminal bar. In the frog, where no terminal bar is found, colloidal markers pass "unimpeded" down the intercellular space to Descemet's membrane. In both species colloidal markers placed intrastromally trace a pathway out of the cornea which is exclusively intracellular. The marker diffuses freely in the stroma and Descemet's membrane, piles up at the junction of Descemet's membrane and the endothelium, and passes across the cell solely within membrane-bounded vesicles. The marker rarely, if ever, appears in the intercellular space. This observation, in addition to the flow pattern seen as marker enters Descemet's membrane (2, Fig. 9), indicates that there is probably a one-way flow of fluid in the intercellular space.

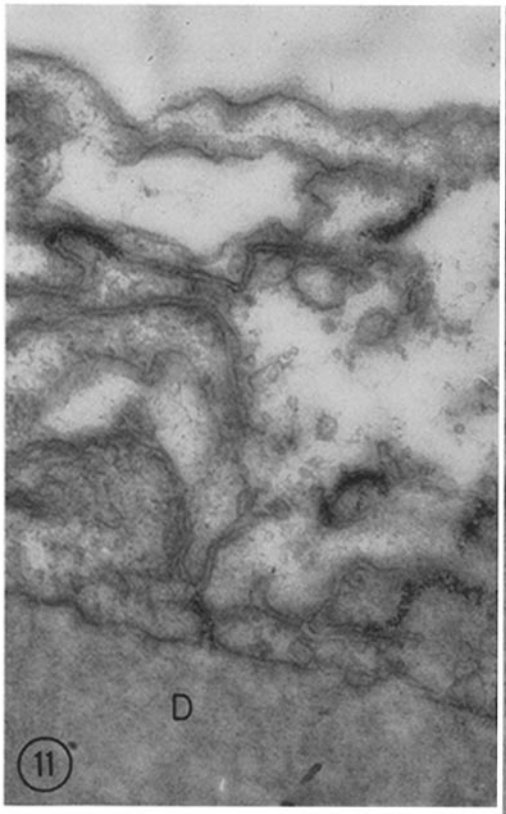
With the exception of gaseous exchange (12, 13), the cornea derives most of its nutrients from, and excretes most of its wastes into, the aqueous humor (14). It would be reasonable to suppose, therefore, that the pathway of inward fluid movement would be relatively non-selective and that the hydrostatic pressure of the aqueous humor would, in most instances, be sufficient to drive fluid containing substrates into the cornea across the endothelium. The removal of wastes would necessarily be more selective and, although there is evidence for free diffusion within the stroma and Descemet's membrane, indeed for a flow towards Descemet's membrane (the pile-up of marker at the junction of Descemet's membrane and the endothelium indicates that the marker is arriving at the site more rapidly than it can be removed from that site), the pathway of movement of the marker outward across the endothelium is entirely intracellular (*via* membrane-bounded vesicles). This would serve to segregate wastes during their passage across the endothelium.

The central cornea, being avascular, is essentially an "ideal" system for the study of such transport phenomena. There are, however, indications that similar processes as well as similar separation of transport pathways may occur at other sites.

Palade (15) and Wissig (16) have described the transport of colloidal markers in pinocytotic vesicles from the capillary lumen to the pericapillary space. Majno and Palade (17, 18), on the other hand, have demonstrated that the primary pathway for large volumes of fluid leaving the vascular system in inflammation appears to be the enlarged intercellular space of the endothelium.

Pappas and Tennyson (19) believe that the intercellular space of the endothelium may be a pathway for fluid flow from the vascular system even under normal conditions. The terminal bar in the vascular endothelium may not form a complete gasket around each cell so that a combination of free flow and by-pass pathways may exist.

Brandt has recently speculated (20) that the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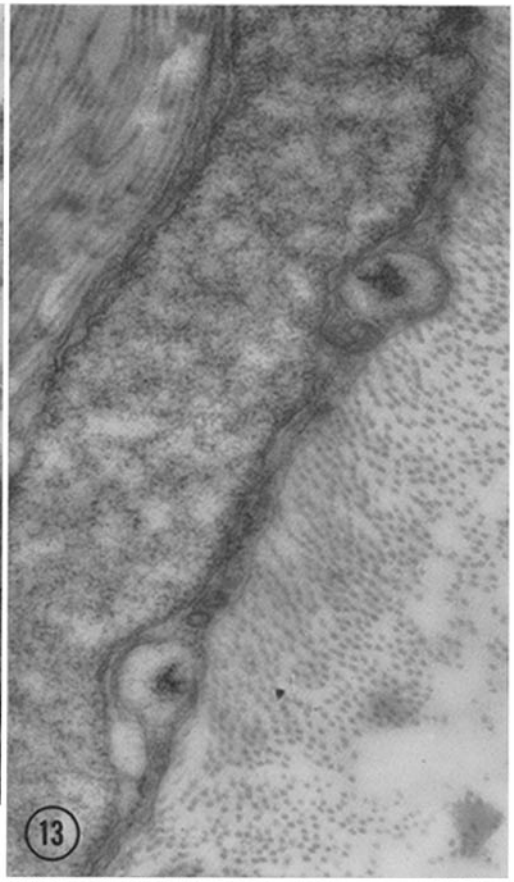
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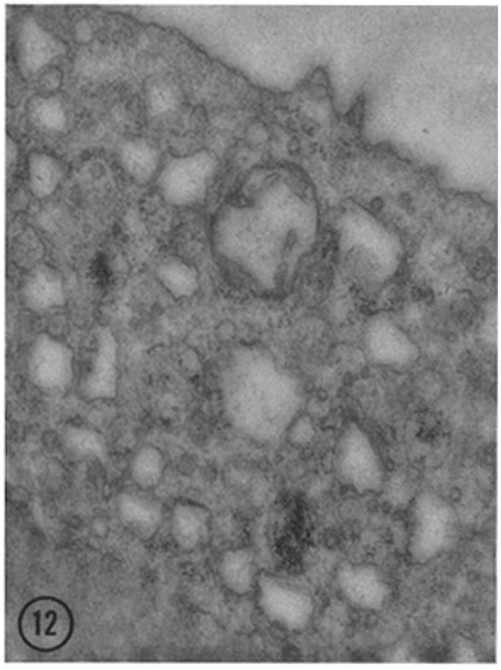
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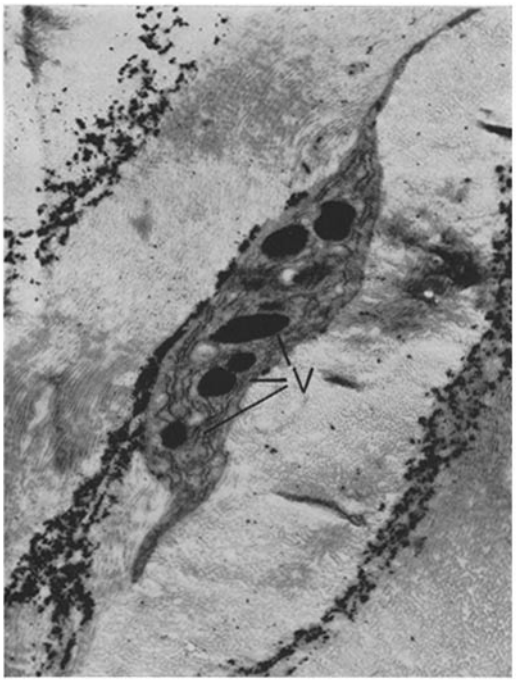

FIGURE 15

Part of the stroma and a stromal cell from an 18-hour preparation similar to that shown in Fig. 14. Note the many large, densely filled vacuoles (*V*) in the stromal cell cytoplasm as well as the pile-up of marker in the fine fibrillar material at the lamellar margins. × 16,000.

pinocytotic vesicles which Palade (15) described as a major cytoplasmic component of muscle capillaries may function primarily in the transport of materials from the tissular to the luminal surface of the capillary.

Palay and Karlin (21) have described the uptake of fat droplets at the apical surface of the jejunal epithelium and their transport *via* a membrane-bounded system (they implicate the endoplasmic reticulum) to the lateral cell margin at which place the fat droplets are expelled into the intercellular space. Fawcett (22) has reported the apparent transport of ThO_2 around the terminal bar of the epithelium of the proximal convoluted tubule of the frog kidney by a system which appears identical with that of the rabbit corneal endothelium.

A common denominator in many of these systems appears to be the presence of a physiological fluid under some degree of hydrostatic pressure. It is found in the cornea and appears possible in the capillaries that the pathway which this fluid under pressure takes is primarily intercellular. Materials moved against this pressure into the fluid appear to travel by an intracellular pathway which is probably energy-dependent. The formation of the pinocytotic vesicles in the by-pass of the terminal bar in the rabbit corneal endothelium is temperature-dependent (2), suggesting a

FIGURE 11

Part of the corneal endothelium of *R. pipiens* which had been exposed for approximately 1 hour to a suspension of saccharated iron oxide injected into the anterior chamber. The marker is seen at all levels in the intercellular space and apparently entering Descemet's membrane (*D*). × 43,000.

FIGURE 12

Part of the corneal endothelium of *R. catesbeiana* which had been exposed for 18 hours to a suspension of saccharated iron oxide injected into the anterior chamber. The marker is located almost exclusively in vacuoles suggesting that the marker is coming out of the stroma. This is also suggested by the information in Fig. 13. × 35,000.

FIGURE 13

The deep stroma from the cornea shown in Fig. 12. A portion of a stromal cell has two large vacuoles containing the saccharated iron oxide marker. It is obvious that the marker has diffused through the stroma and that Fig. 12 probably represents material coming out of the cornea across the endothelium. × 35,000.

FIGURE 14

The injection site of a ThO_2 injection into the corneal stroma of *R. catesbeiana*. At 18 hours after injection the marker is seen to have diffused from the site in all directions and appears to have accumulated at the margins of the lamellae. This pile-up may be against the stromal cells and the fine fibrous material found between the lamellae (see Fig. 5). Low power micrograph. × 4,400.

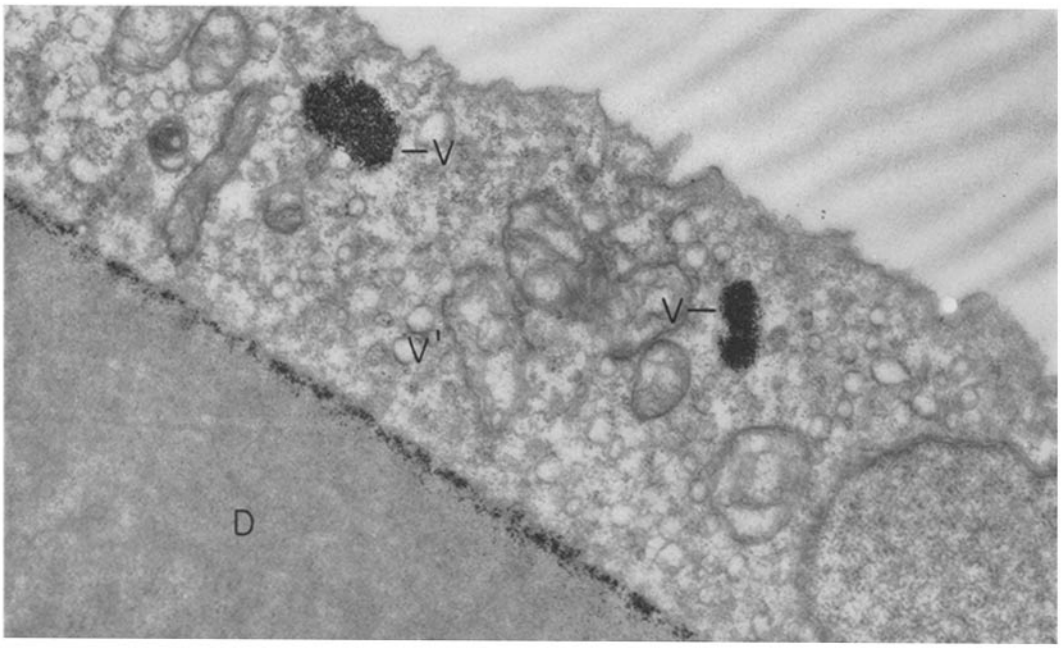

FIGURE 16

Part of the corneal endothelium of *R. catesbeiana* after an 18-hour exposure to an intrastromal injection of ThO_2. Marker is seen accumulated at the margin of Descemet's membrane (*D*) and the endothelium as well as in large vacuoles (*V*) and small vesicles (*V'*) in the endothelial cytoplasm. In this instance it is known that the marker is passing out of the cornea across the endothelium. × 23,000.

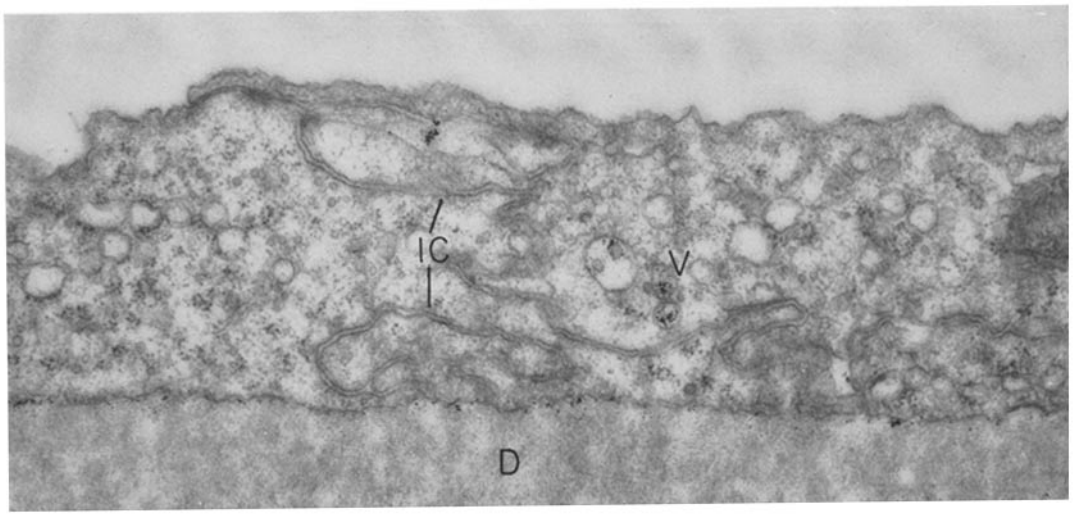

FIGURE 17

Another example of an 18-hour intrastromal injection of ThO_2. In this instance it is seen that, although marker is found at the margin of Descemet's membrane (*D*) and the endothelium as well as in vacuoles (*V*) in the endothelial cytoplasm, no marker is found in the intercellular space (*IC*). × 32,000.

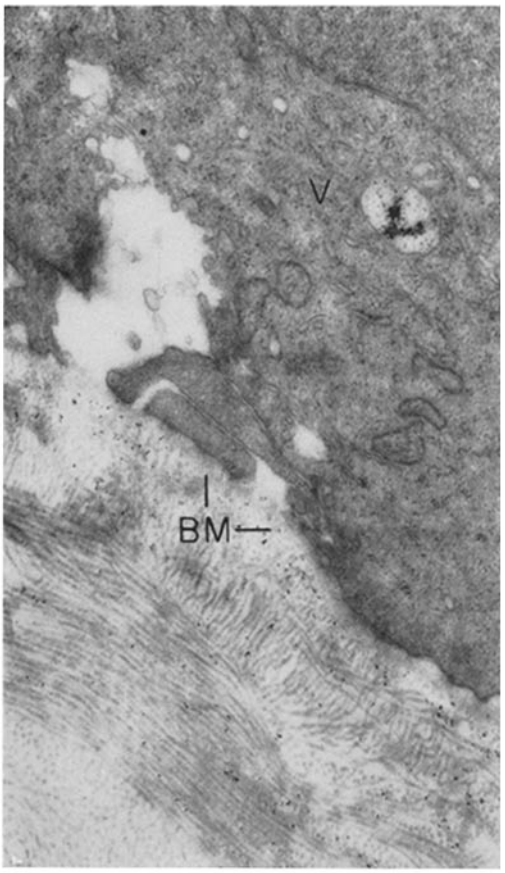

FIGURE 18

From an 18-hour intrastromal injection experiment. Part of the stroma and the basal portion of the epithelium. It is clear that some of the marker seen in the stroma has passed across the basement membrane (*BM*) of the epithelium and has been accumulated in a membrane-bounded vacuole (*V*) in the basal cell cytoplasm. × 37,000.

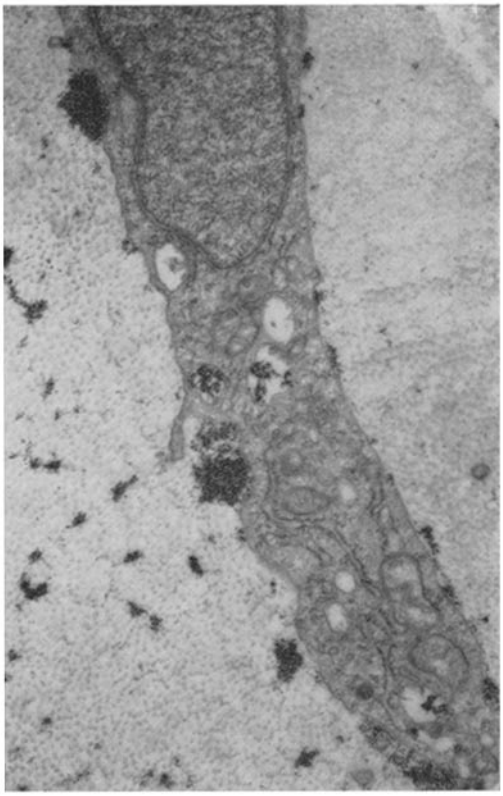

FIGURE 19

Part of the corneal stroma of a frog which had received an intrastromal injection of saccharated iron oxide 18 hours prior to fixation. The iron oxide particles exhibit their characteristic clumping in the stroma (1) but numerous large vacuoles containing the marker are seen in a stromal cell on the surface of which the marker has accumulated. × 20,000.

metabolic dependence. In the frog, on the other hand, most of the studies were conducted at 4–6°C, the temperature at which the frogs are stored, without apparently reducing the flow of colloidal marker down the intercellular space.

If further investigation of the capillary endothelium and other epithelia support the above views, the morphological separation of transport pathways relative to the direction of transport which has been demonstrated in the corneal endothelium may prove to be a general phenomenon in the movement of materials across cellular barriers.

This investigation was supported in part by Grants B-2314, and B-3448 from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service, and by a grant from the Life Insurance Medical Research Fund.

The author wishes to express his appreciation to Dr. George D. Pappas for aid and advice during the course of this study.

Received for publication, June 18, 1962.

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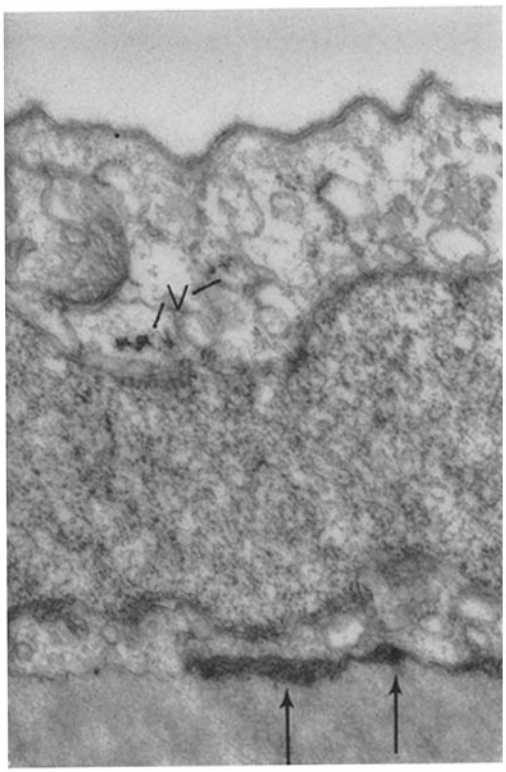

FIGURE 20

Part of the endothelium from the cornea shown in Fig. 19. Saccharated iron oxide may be seen at the margin of Descemet's membrane and the endothelium (arrows). The marker is also seen in two vacuoles (*V*) in the supranuclear cytoplasm. × 36,000.

REFERENCES

1. KAYE, G. I., and PAPPAS, G. D., Studies on the cornea. I. The fine structure of the rabbit cornea and the uptake and transport of colloidal particles by the cornea *in vivo*, *J. Cell Biol.*, 1962, **12**, 457.
2. KAYE, G. I., PAPPAS, G. D., DONN, A., and MALLETT, N., Studies on the cornea. II. The uptake and transport of colloidal particles by the living rabbit cornea *in vitro*, *J. Cell Biol.*, 1962, **12**, 481.
3. WARD, R. T., Prevention of polymerization damage in methacrylate embedding media, *J. Histochem. and Cytochem.*, 1958, **6**, 398.
4. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
5. SCHWARTZ, B., DANES, B., and LEINFELDER, P. J., The role of metabolism in the hydration of the isolated lens and cornea, *Am. J. Ophth.*, 1954, **38**, 182.
6. JAKUS, M. A., Studies on the cornea. I. The fine structure of the rat cornea, *Am. J. Ophth.*, 1954, **38**, 40.
7. JAKUS, M. A., The fine structure of the human cornea, *in* The Structure of the Eye, (G. K. Smelser, editor), New York, Academic Press, 1961, 343.
8. WHITEAR, M., An electron microscope study of the cornea in mice, with special reference to the innervation, *J. Anat.*, 1960, **94**, 387.
9. SHELDON, H., and ZETTERQVIST, H., An electron microscope study of the corneal epithelium in

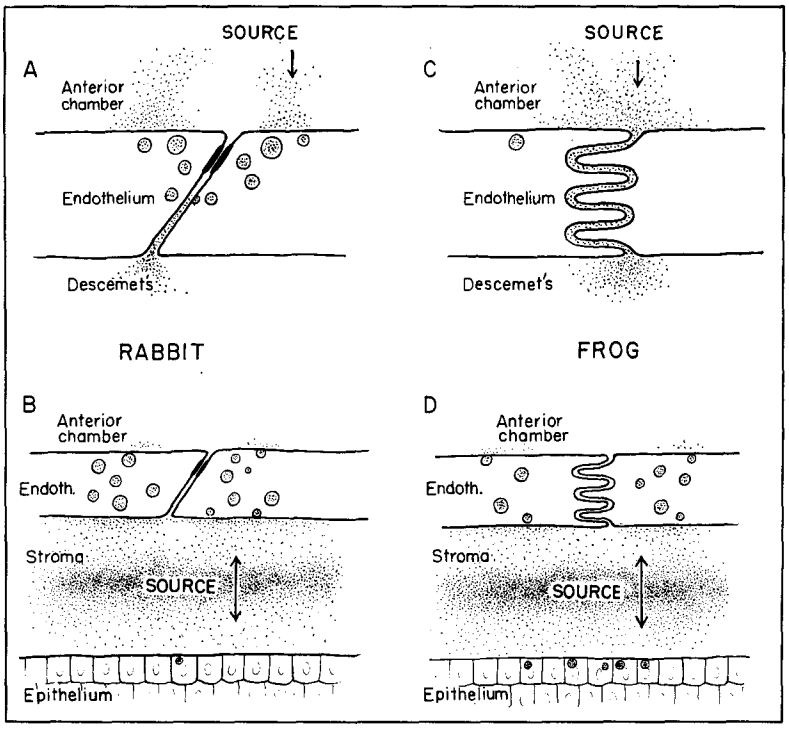

FIGURE 21

This figure schematically summarizes the comparative aspects of the transport pathways described in the corneas of the rabbit (1, 2) and frog. In the rabbit, colloidal markers are adsorbed at the apical surface of the endothelial cell and transported around the terminal bar region by pinocytosis (*A*). They are subsequently expelled into the intercellular space from which they apparently flow into Descemet's membrane. Particles diffuse freely in Descemet's membrane and the stroma so that when marker is placed in the stroma (*B*) the particles accumulate at the margin of Descemet's membrane and the endothelium. These pass out across the endothelium only within membrane-bounded vacuoles, rarely appearing in the intercellular space. Some marker may pass across the basement membrane of the epithelium and be taken up by the basal epithelial cells.

In the frog (*C*) there is no terminal bar or other specialization of the apposing marginal membranes of endothelial cells and the marker passes freely down the intercellular space. Only an occasional pinocytotic vesicle is found in the apical cytoplasm of these cells. If marker is placed in the stroma (*D*) the pattern of particle transport is identical with that seen in the rabbit (*B*), except that more marker appears to cross the basement membrane of the corneal epithelium in the frog than in the rabbit.

the vitamin A deficient mouse, *Bull. Johns Hopkins Hosp.*, 1956, **98**, 372.

10. ZANDER, E., and WEDDELL, G., Observations of the innervation of the cornea, *J. Anat.*, 1951, **85**, 68.
11. BRIGHTMAN, M. W., An electron microscopic study of ferritin uptake from the cerebral ventricles of rats, *Anat. Rec.*, 1962, **142**, 219.
12. LANGHAM, M. E., Utilization of oxygen by the component layers of the living cornea, *J. Physiol.*, 1952, **117**, 461.
13. LANGHAM, M. E., Glycolysis in the cornea of the rabbit, *J. Physiol.*, 1954, **126**, 396.
14. PIRIE, A., and VAN HEYNINGEN, R., Biochemistry of the Eye, Oxford, Blackwell, 1956.
15. PALADE, G. E., Transport in quanta across the endothelium of blood capillaries, *Anat. Rec.*, 1960, **136**, 254.

16. WISSIG, S. L., An electron microscope study of the permeability of capillaries in muscle, *Anat. Rec.*, 1958, **130**, 467.
17. MAJNO, G., and PALADE, G. E., Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: An electron microscopic study, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 571.
18. MAJNO, G., PALADE, G. E., and SCHOEFL, G .I., Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: A topographic study, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 607.
19. PAPPAS, G. D., and TENNYSON, V. M., An electron microscopic study of the passage of colloidal particles from the blood vessels of the ciliary process and choroid plexus of the rabbit, *J. Cell Biol.*, 1962, **15**, 227.
20. BRANDT, P. W., A consideration of the extraneous coats on the plasma membrane, *Circulation*, Suppl. The Plasma Membrane, 1962, in press.
21. PALAY, S. L., and KARLIN, L. J., An electron microscopic study of the intestinal villus. II. The pathway of fat absorption, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 373.
22. FAWCETT, D. W., The fine structure of capillaries, arterioles and small arteries, *in* The Microcirculation, (S. R. M. Reynolds and B. W. Zweifach, editors), Urbana, University of Illinois Press, 1959, 1.