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Fine Structural Changes Associated with the Onset of Calcium, Sodium and Water Transport by the Chick Chorioallantoic Membrane

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Summary. An attempt is made to correlate structure and transport function in the embryonic chorioallantoic membrane. The fine structure of the endoderm and ectoderm in the membrane was examined with particular attention given to the morphological changes that occur when transport is established, *in vivo*. Two distinctive cells, a granule-rich cell and a mitochondria-rich cell, appear in the endoderm at the time allantoic fluid sodium, chloride and water reabsorption commences. These are indistinguishable from the cells described in toad bladder epithelium. It is suggested that the granule-rich cell is responsible for bulk water movement and the mitochondria-rich cell is specifically engaged in active sodium transport. In the ectoderm, two distinctive cell types are also found to be associated with the onset of active calcium transport. These are referred to as the capillary-covering cell and the villus-cavity cell. The preponderate capillary-covering cell is most likely responsible for transcellular calcium transport. It is postulated that the function of the villus-cavity cell is to secrete hydrogen ions which are necessary, along with carbonic anhydrase, to mobilize Ca^{++} from the insoluble calcium carbonate of the eggshell.

The chorioallantoic (CA) membrane of the embryonic chick is composed of tissues derived from the ectoderm, mesoderm and endoderm. Well before 10 days of incubation, it is the outermost cellular layer of the chick and it is the inner lining of the non-cellular shell membranes.

The CA membrane has been generally considered to have two main functions: (1) to serve as a support for the extra-embryonic respiratory capillaries which lie directly below the shell, and (2) to form part of the wall of the allantoic sac which collects excretory products during embryonic development [19]. However, recent investigations have established that certain transport functions of this membrane are important to the water and electrolyte balance of the embryo [16, 21, 25, 26]. *In vivo* observations

of the volume and composition of allantoic fluid during development indicate that the reabsorption of water, sodium and chloride from the allantoic sac involves an active transport mechanism and that this transport capability is well developed by the 12th day of incubation [21]. Subsequent *in vitro* studies showed that, at about day 14, the CA membrane also gains the ability to actively transport Ca^{++} [26]. Since there is a very rapid increase in the Ca^{++} content of the embryo from incubation day 13–14 to hatching [14], this active transport mechanism operates to move Ca^{++} liberated from the eggshell into the embryonic circulation for ossification of the skeleton.

There are several advantages in using the CA membrane for the study of electrolyte transport. It is one of the few unambiguous cases of active transepithelial calcium transport; it is relatively simple in structure and function compared to other transporting epithelia; its transport functions appear at a defined stage of development, permitting investigations while transport is turned "off" or "on" by natural means, as well as by physical and chemical agents; finally, its ready availability and ease of handling make it an attractive experimental material. Thus, for further investigations, it would be a distinct advantage to know the cells responsible for these transport phenomena.

Although the histology and fine structure of this epithelium have received attention in the past [2, 8, 9, 15, 22], these studies were carried out without reference to its then undiscovered active transport functions. A prior light microscopic investigation suggested that distinctive changes in the morphology of the ectoderm and endoderm occurred simultaneously with the observed physiological changes [21]. This report extends these observations and deals with the fine structure of the CA membrane prior to, and just after, each type of active transport capability develops.

Materials and Methods

Fertile White Leghorn eggs obtained from Babcock Poultry Farms (Ithaca, New York) were used for this study. They were incubated at 99 to 100 °F, 80% relative humidity and turned automatically every 2 hr in a Jamesway Model-252 Incubator (Butler Manufacturing Company, Ft. Atkinson, Wisconsin). The staging technique of Hamilton [12] was used to determine the exact age of the embryos in the incubated eggs from which the membranes were obtained. The shell over the blunt end of the egg was cracked away and specimens of the CA membrane and attached inner shell membrane forming the floor of the airspace were removed. Membranes were either fixed immediately after removal or placed in a transport chamber long enough to insure that transport was occurring [25] and then fixed. Fixation was either in 3% glutaraldehyde in cacodylate

buffer, 0.5 to 1.0 hr at room temperature; or in 6% acrolein (provided by Dr. William Aldridge, Department of Anatomy, University of Rochester) in cacodylate buffer, 0.5 hr at room temperature. This was followed by post-fixation in osmium for 0.5 hr [20]. The fixed membranes were dehydrated with a graded series of ethanol and were embedded in Araldite (Ladd, Burlington, Vermont).

Thin (0.08 to 0.1 μ) and thick (0.5 to 2.0 μ) sections were cut with Porter-Blum MT-2 and Huxley-Cambridge microtomes using glass and diamond knives. Thin sections were mounted on carbon-coated copper grids and stained with lead citrate [18], uranyl acetate [28] or both [27] and examined in a Phillips EM-200 electron-microscope at 60 kV and 20 to 50×10^{-6} amp emission current. Images were recorded on Eastman Kodak Lantern Slides (contrast grade), developed in Dektol diluted 1:1 and printed on Kodabromide papers. Thick sections were stained at 60 °C with Paragon stain (Paragon C and C Company, Inc., 190 Willow Avenue, Bronx, New York), or 0.5% toluidine blue in 1% sodium borate at 60 °C and examined with light microscopes. Light microscope images were recorded on Polaroid 55 P/N film and printed on Kodabromide or Ektamatic papers.

Results

Fig. 1*a* is a low power light micrograph of a 14–15 day CA membrane with its attached inner shell membrane. The two electrolyte transport functions associated with these fully developed membranes are (1) the movement of sodium, chloride and water from the allantoic sac and (2) the transfer of Ca^{++} liberated from the eggshell into the circulation of the embryo. The endodermal layer of the tissue is in direct contact with the allantoic fluid. Some 80 μ or more away, and separated by the well defined mesoderm, is the ectoderm, which lies just beneath the non-cellular shell membrane. The extent of the morphological changes that take place in the two separate epithelial layers during incubation is suggested by comparing the mature membrane with its appearance at 9 days of incubation (Fig. 1*b*). This light micrograph is at a higher magnification and shows that both the ectoderm and endoderm begin as simple squamous cell layers. Also, at these early stages, the inner shell membrane does not appear to be directly attached to the ectodermal surface of the CA membrane. The mesoderm, in contrast to the epithelial layers, shows no major changes throughout development. This layer remains a loosely arranged areolar-type structure containing the blood vessels which supply the respiratory capillaries of the chorionic ectoderm, and is characterized by scattered fibroblasts and variable amounts of randomly oriented collagen fibers. As noted by other authors [8, 9, 15], changes in these elements from the 10th to 17th of incubation are relatively minor, being restricted to the enlargement of blood vessels and an increase in the overall thickness accompanied by a proliferation of collagen fibers.

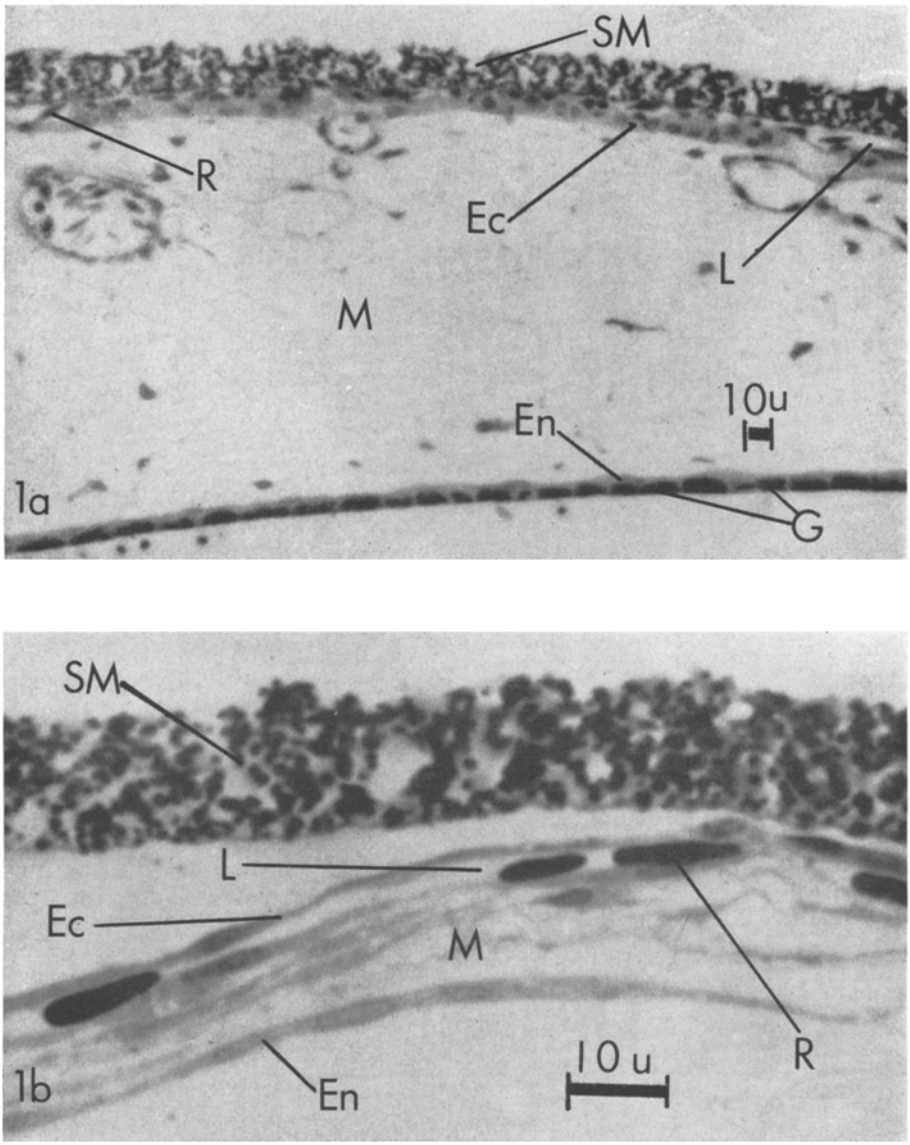

Fig. 1. Light micrographs of sections of embryonic chorioallantoic membrane stained with Toluidine Blue. (*a*) CA membrane from 14-day embryo. The ectoderm (Ec) is composed of a few cell layers. Respiratory capillaries, the lumina (L) of which contain erythrocytes (R), are visible between the ectoderm and inner shell membrane (SM). The mesoderm (M) is composed mostly of fibroblasts and loose connective tissue. The endoderm (En) is a single layer of cells most of which contain accumulations of darkly stained granules (G). Marker represents 10 μ. (*b*) CA membrane from 10-day embryo. The ectoderm (Ec) composed of one layer of squamous cells, is not attached to the shell membrane (SM). Erythrocytes are visible in the lumina (L) of capillaries in the relatively thin mesoderm (M). The endoderm (En) consists of one layer of squamous cells without dense staining granules; marker represents 10 μ

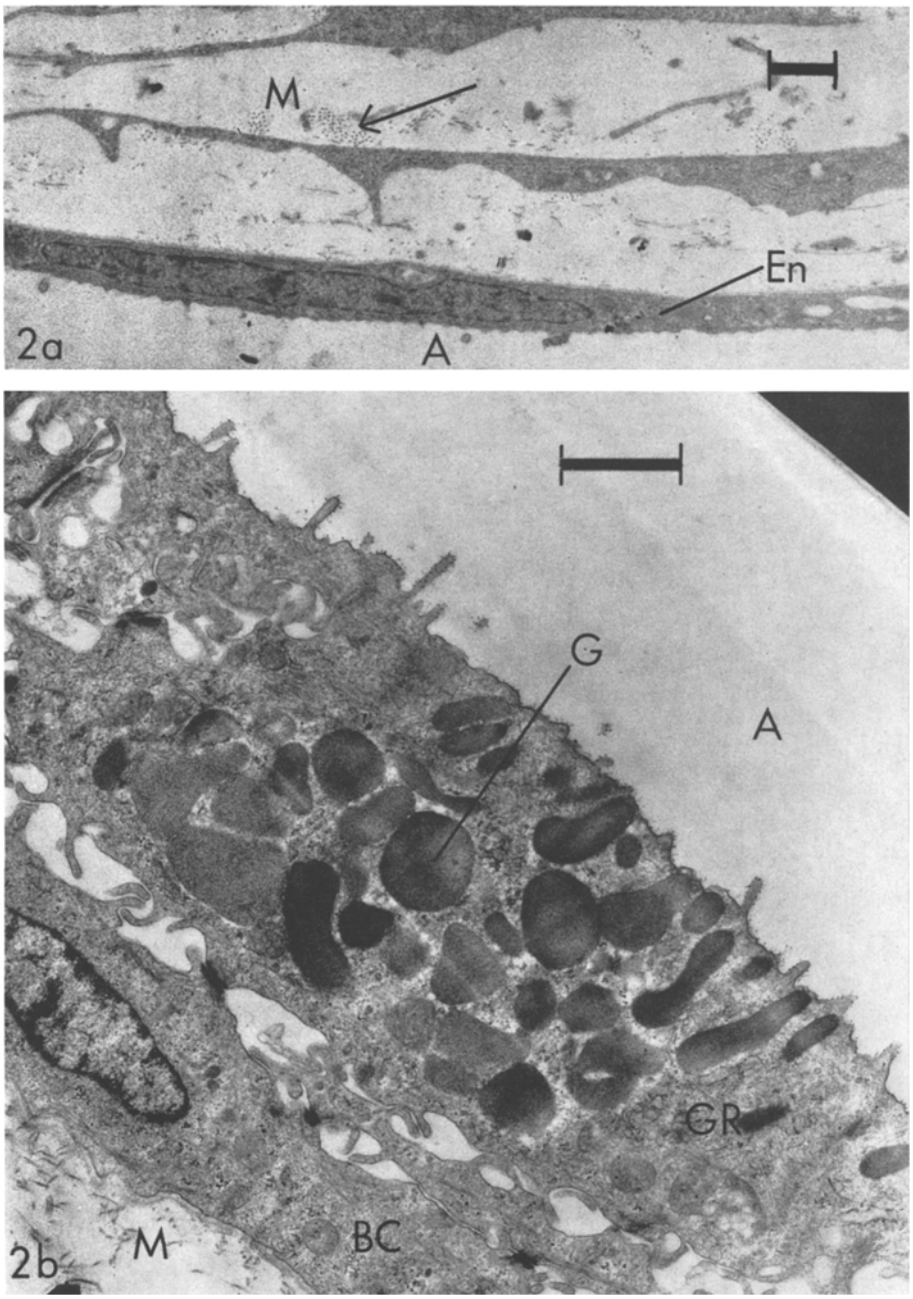

Fig. 2. (*a*) Electron micrograph of endoderm from 10-day CA membrane. A part of the mesoderm (M) consisting of collagen bundles (arrow) and the extended arms of fibroblasts is visible. The endoderm (En) at this stage is a single layer of thin squamous cells. The lumen of the allantoic sac is indicated by A. Marker represents 1 μ. (*b*) Electron micrograph of endoderm from 14-day CA membrane with a portion of a basal cell (BC) and most of a granule-rich cell (GR). The granule-rich cell contains many granules (G) of various shapes and densities and microvilli are seen on the cell surface facing the allantoic sac (A). Note the extensive interdigitation and prominent desmosomes in some areas of contact. M, mesoderm; marker represents 1 μ

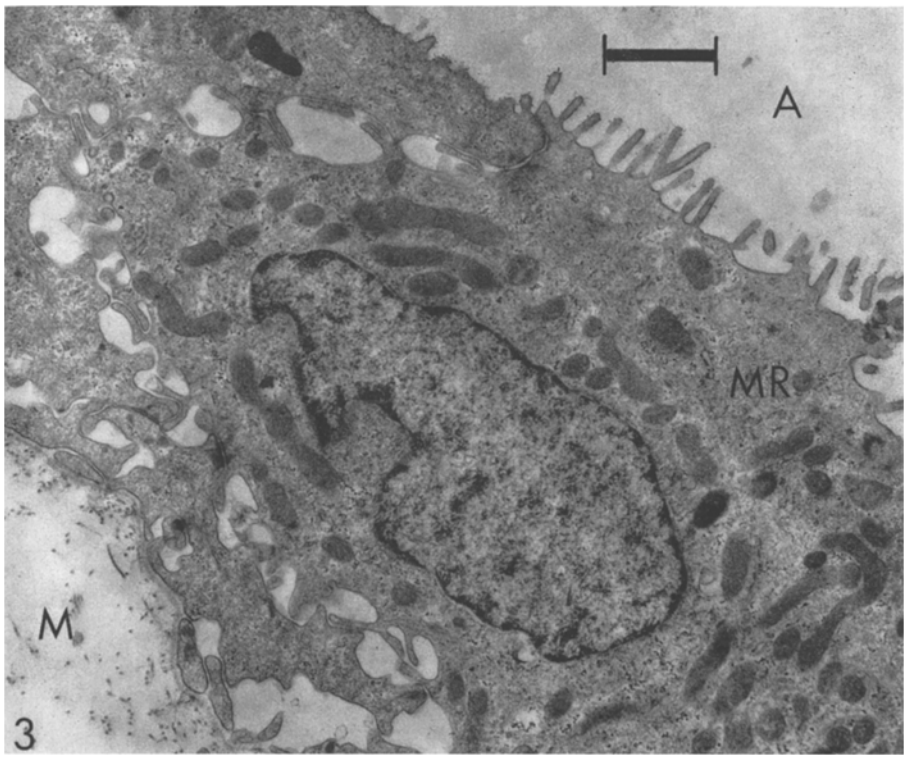

Fig. 3. Electron micrograph of mitochondria-rich cell (MR) from 14-day CA membrane. Numerous microvilli, some branched, are present on the surface of the cell facing the allantoic sac (A) and the cytoplasm contains many mitochondria clustered near the nucleus. M, mesoderm; marker represents 1 μ

Endoderm

The striking changes that occur in endodermal morphology are most apparent by electron microscopy. Fig. 2*a* shows the endoderm at 9 days of incubation. The squamous nature of the layer is evident, and there is a tendency for the allantoic fluid-facing surface to take on a corrugated appearance. These cells contain infrequent, small (approx. 0.1 μ), dense granules and some slightly larger, lighter ones. Somewhat later, at about 10 days, the cells become more cuboidal. Their intracellular spaces have smooth outlines and punctate-tight junctions can be identified. It is from 10 days onward that the very rapid series of developmental alterations of this cell layer takes place.

The irregular corrugations of the allantoic fluid-facing surface rapidly develop into distinct, separate microvilli and in most of the cells, there is a progressive increase in the number and size of the intracytoplasmic granules

(Fig. 2*b*). This increase continues until about day 17, when they seem to recede in size and decrease in number. From the time of their appearance, these granule-rich cells always constitute the bulk of the endodermal cell layer.

Certain cells do not exhibit this proliferation of granules. They assume a flask-like shape and produce more numerous surface microvilli that often tend to branch (Fig. 3). By day 13 or 14, these cells can be distinguished by their increased number of mitochondria. Because of their shape and scarcity, these mitochondria-rich cells constitute only a minor fraction of the endodermal surface. The differentiation of both cell types is accompanied by an extensive and irregular interdigitation of adjacent cell membranes. Not only are the lateral intercellular channels between all the endodermal cells extremely tortuous, but this tortuosity can be seen to include the base of the cells (Figs. 2*b* and 3) where a third type of cell occurs. However, this basally located cell (BC, Fig. 2*b*) does not materially change in appearance from day 10–11 onward. Thus, after day 12 and coincident with the development of sodium, chloride and water transport, the CA endoderm differentiates into a cell layer consisting primarily of granule-rich cells and fewer but distinct cells very rich in mitochondria. Except for the fact that it lacks mucous cells, it therefore strikingly resembles the amphibian bladder as described by Choi [3].

Ectoderm

Just as the allantoic endoderm undergoes extensive developmental changes at the time when active transport occurs, the chorionic ectoderm exhibits marked changes in organization as it gains the ability to transport Ca^{++} actively at about 14 days of incubation. At 9 and 10 days of incubation, the ectoderm is composed of two or so layers of relatively undifferentiated cells (Fig. 4*a*). The cells are seen to lie above the respiratory capillaries and have fairly smooth, regular surfaces with large, intercellular spaces in the interior of the ectoderm. They are attached to each other by desmosomes and punctate-tight junctions occur close to the shell membrane-facing surface. Subsequent changes are directed toward the development of two distinct cell types which form the entire surface just under the inner shell membrane. These cells will be referred to as the capillary-covering (CC) cell and the villus-cavity (VC) cell because of their unique structural features.

The CC cell shown in Fig. 4*b* is from a 14-day embryo. It is characterized by long, cytoplasmic processes which are interposed between the endothelium of capillaries and the shell membrane. These cytoplasmic arms are extremely thin, measuring 0.1 μ or less, and in some areas only 500 Å.

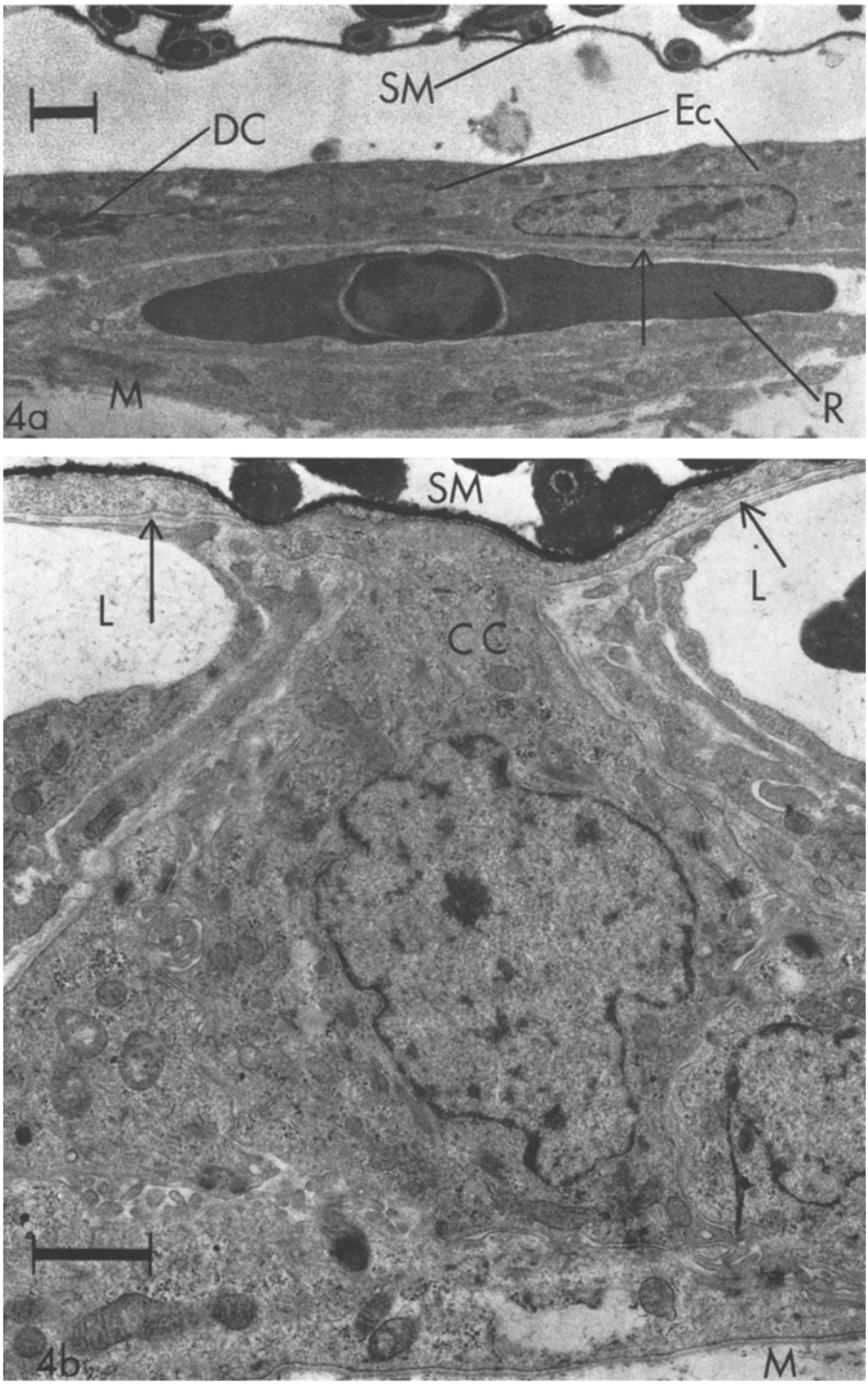

Fig. 4 a and b

They are bound tightly to the inner shell membrane externally (Fig. 5*a*) and are frequently seen to join over the respiratory capillaries (Fig. 5*b*). The processes are always separated from the capillary endothelium by a distinct basal lamina (Figs. 4*b*, 5*b*). The bond between the CC cell processes and the inner shell membrane is so strong that any attempt to separate these prior to fixation will tear open the CC cells, the cell body remaining with the CA membrane proper and the surface plasma membrane remaining with the shell membrane.

The surface of the CC cell that faces the inner shell membrane characteristically exhibits numerous infoldings, reminescent of cells carrying out endocytosis, and small vesicles are common in the apical region of the cell. Usually, no other cell organelles are present in the extended cytoplasmic arms. In the body of these cells, the nucleus tends to be centrally located and surrounded by a moderate number of mitochondria. Many small (200 to 250 Å) granules tentatively identified as glycogen by Leeson and Leeson [15] are scattered throughout the cytoplasm (Fig. 4*b*). Desmosomes are prominent along the lateral cell infoldings with fibers extending from them. In the cytoplasm, other fibers are present which occur in bundles that may branch and which tend to lie oriented in an apical to basal direction (Fig. 5*a*). Rough endoplasmic reticulum and Golgi membranes are virtually absent from these mature CC cells.

The transition between the undifferentiated state (Fig. 4*a*) and the mature CC cell (Fig. 4*b*) begins at about day 10 or 11. The outermost squamous cells become cuboidal and begin to attach to the shell membrane through an abundant array of fine fibrils. Capillaries proliferate within the ectoderm but these are always separated from the shell membrane by a cytoplasmic extension of the developing CC cell. By day 12 the attachment of the cells to the shell membrane is complete. At the same time, the cytoplasm fills with rough and smooth endoplasmic reticulum and, near the external plasmalemma, Golgi membranes and numerous small vesicles are frequently identified (Fig. 5*c*). Thus, at this stage, the CC cells have morphological characteristics usually associated with "secretory" cells.

Fig. 4. (*a*) Electron micrograph of ectoderm from 10-day CA membrane. The squamous cells of the ectoderm (Ec) have not yet attached to the inner shell membrane (SM). Capillaries containing erythrocytes (R) lie in the mesoderm (M) and are still separated from the ectoderm by a thin basal lamina (arrow). A portion of a dark cell (DC) is present. R, erythrocyte; marker represents 1 μ. (*b*) Electron micrograph of capillary-covering cell (CC) from ectoderm of a 14-day CA membrane. The CC cell is closely apposed to the inner shell membrane (SM) and extends long cytoplasmic processes over the basal lamina (arrows) of the capillaries (L). The nucleus is remote from the surface attached to the shell membrane. M, mesoderm; marker represents 1 μ

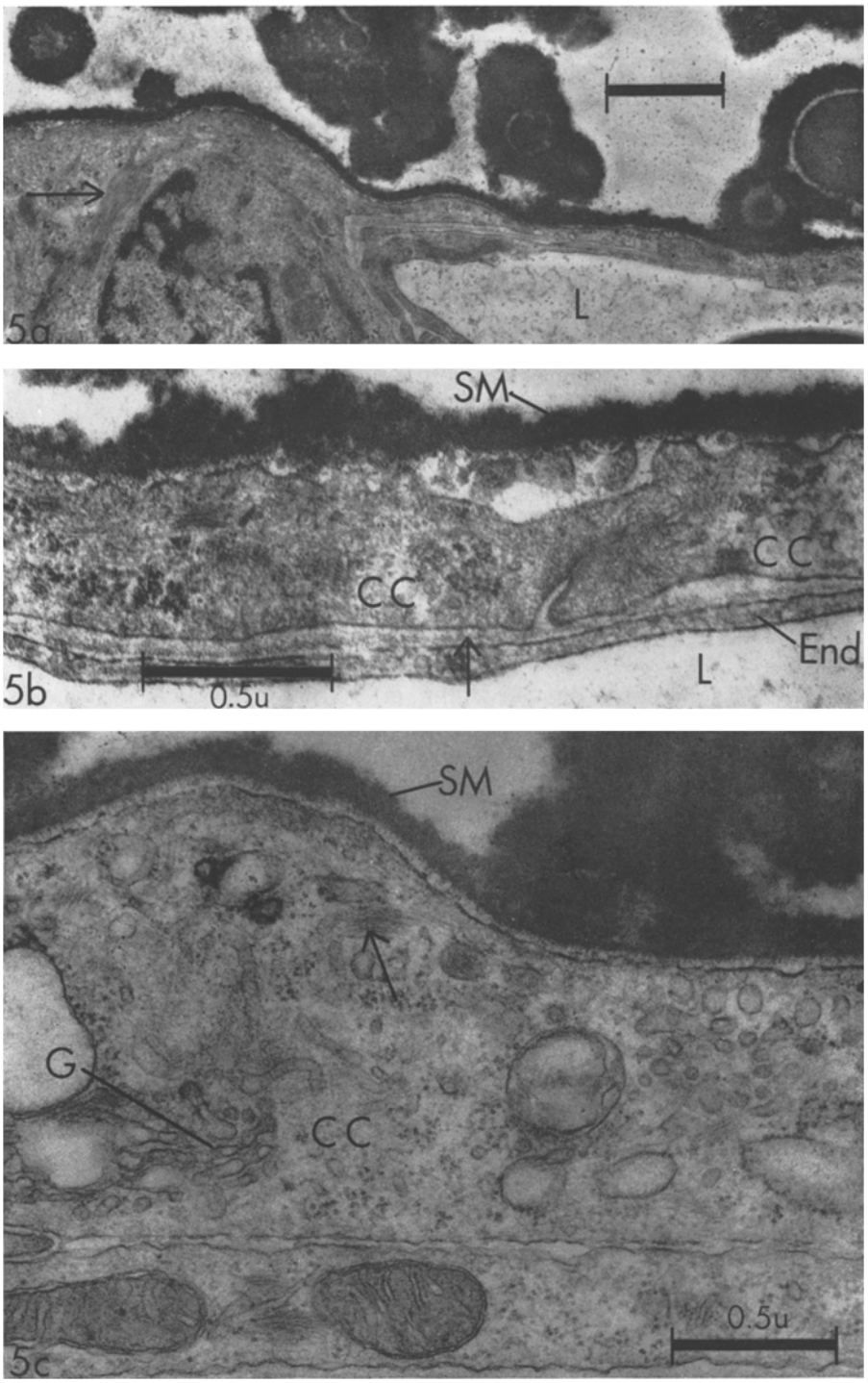

Fig. 5 a–c

Between day 12 and 14, two further changes occur. The cytoplasmic arms overlying the respiratory capillaries become thinner, permitting the capillary endothelium to move closer to the shell membrane. Concomitant with this thinning, the cells lose most of their complement of Golgi membranes and endoplasmic reticulum. These final changes result in the appearance of the definitive CC cell shown in Figs. 4*b*, 5*a* and 5*b*.

The other definitive cell type that forms along the surface of the ectoderm is the villus-cavity (VC) cell (Fig. 6*a*, 6*b*). At about 12 days of incubation, the VC cell develops a deep cavity between its cell body and the shell membrane. The apex of the cell in the cavity extends long villi toward the shell membrane. Some of these villi attach to the shell membrane by the fine fibers on their tips. The cavity of this cell is usually seen directly adjacent to the shell membrane, but many sections show that an arm of a CC cell may separate part of the cavity from the shell membrane (Fig. 6*a*). It is attached to the CC cells by punctate-tight junctions near the shell membrane. The cytoplasm is rich in mitochondria, usually clustered near the nucleus, and the region just beneath the apical microvilli is filled with vesicles of various sizes (Fig. 6*b*). Many desmosomes are prominent. This cell does not undergo major changes in organization as the ability to transport calcium appears, but remains much as described until at least day 17. Although the surface of the ectoderm that faces the shell membrane is composed almost exclusively of VC and CC cells, because of their shape and infrequent occurrence, the VC cells make up only a small fraction of this surface. Actually, the extended arms of the CC cells are the major constituent of the CA membrane surface.

Two other types of cells can be distinguished in the CA ectoderm at about day 11, but these undergo no major changes during further incubation. These cells are a basal or undifferentiated type of cell and a "dark" cell. The basal cell is similar in appearance to the undifferentiated ectodermal cells that occur at earlier stages. The fourth type of cell is unusually dark, containing a dense cytoplasm with distorted mitochondria and an extensively

Fig. 5. (*a*) Electron micrograph showing capillary-covering cell from 14-day CA membrane. Only a portion of the extension of the cell that separates the capillary (L) from the shell membrane is shown. This extension can be as thin as 0.1 μ. Arrow indicates bundle of fibers found in CC cells. Marker represents 1 μ. (*b*) Electron micrograph of portions of capillary-covering cells in 14-day CA membrane ectoderm, showing part of junctional complex between contiguous cells. Capillary lumen (L) separated from ectoderm by endothelial cell (End) and basal lamina (arrow). Marker represents 0.5 μ. (*c*) Electron micrograph showing portion of two capillary-covering cells in 12-day CA membrane. At this stage, the cytoplasmic extensions of the CC cell are still relatively thick and contain many membranous organelles as well as ribosomes and a few bundles of fibers (arrow). SM, inner shell membrane; G, Golgi region. Marker represents 0.5 μ

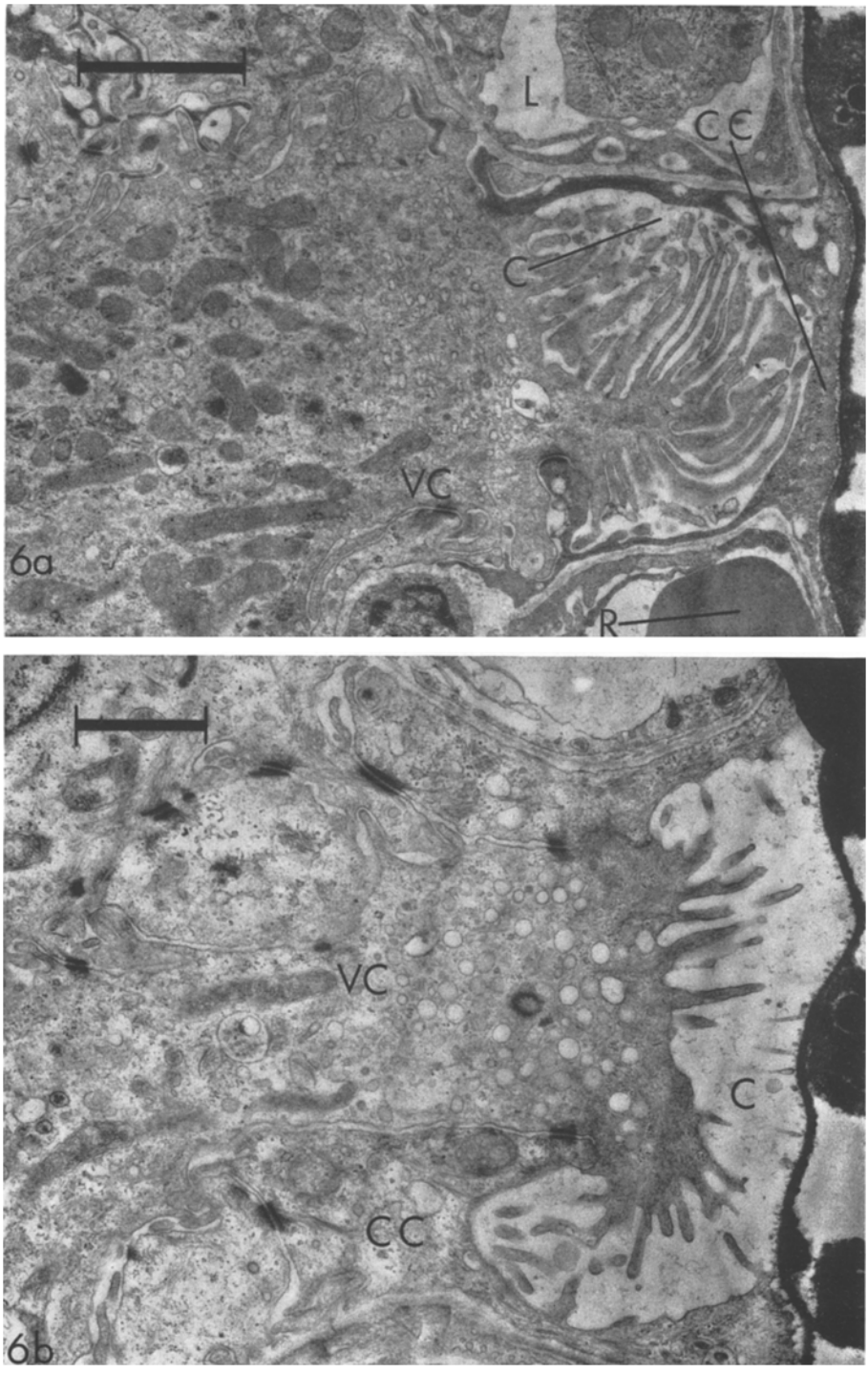

Fig. 6 a and b

convoluted cell membrane. The nucleus of these cells is unusually shrunken and quite dense. It occurs (independent of fixation methods) from 10 days onward, but whether this is a viable cell in the living membrane is not known.

Discussion

The distinctive changes noted in the fine structure of particular CA membrane cells as various transport functions become operative, although not compelling evidence by themselves, when considered with evidence from other sources, make it possible to suggest certain functions for specific cells, and to advance some reasonable hypotheses concerning the function of others. The endoderm is seen to develop two definitive cell types with the onset of allantoic fluid sodium, chloride and water transport. At this time, it assumes a morphology remarkably similar to the urinary bladder of the toad. This similarity in architecture is understandable when one considers that toad bladder and chick CA endoderm share a common derivation from embryonic hindgut, and each serve to store and selectively reabsorb urinary excretory products. Thus, both epithelia are closely related embryologically, structurally and functionally, and a comparative evaluation of experimental information on each tissue should be meaningful.

The mature toad bladder contains four types of cells: granule-rich cells, mitochondria-rich cells, mucous cells and basal cells [3]. The latter two cell types have not been considered by most investigators to be responsible for active transport in toad bladder. Our observations in the CA membrane support this opinion. The CA membrane carries out the same electrolyte transport functions as the toad bladder but is completely devoid of mucous cells. Whereas, the basal cell is common to both tissues, it is never in contact with the allantoic sac contents and, more importantly, remains morphologically unchanged when active transport begins, *in vivo*. Thus, the two remaining cell types which are present in both transporting epithelia (the granule-rich cell and the mitochondria-rich cell) must be considered to be the physiologically important cellular components.

Di Bona *et al.* [6], working with toad bladder, presented convincing evidence that the morphology of the granule-rich cells is selectively altered

Fig. 6. Electron micrographs of villus-cavity cells (VC) from ectoderm of a 14-day CA membrane. In (*a*) a portion of a capillary-covering (CC) cell is interposed between the cavity (C) of the VC cell while in (*b*) the cavity is open directly to the shell membrane. Just beneath the microvillus-rich apex of the cell, the cytoplasm is rich in vesicles. These are shown to advantage in 6*b* which was fixed in unbuffered 6% acrolein. Below the vesicle-containing region, the cells are usually rich in mitochondria, as in 6*a*. Desmosomes are frequent and prominent. R, erythrocyte; L, lumen of capillary; CC, capillary-covering cell; markers represent 1 μ

during vasopressin-induced bulk water flow. Significantly, granule-rich cells appear in the CA membrane just at the time rapid reabsorption of water becomes evident, and the fluid within the allantoic sac at this time is also hypotonic [21]. It seems reasonable, therefore, to conclude that the granule-rich cells are also responsible for bulk water flow in the CA endoderm. Whether vasopressin is the common hormonal mediator of water transport in both tissues remains to be examined.

The role of the mitochondria-rich cell in the CA endoderm, as in the toad bladder, is not clear. However, physiological data on the composition of blood and allantoic fluid in developing chick embryos [21] provide some insight into the possible function of this distinctive cell.

During development, the volume of fluid in the allantoic sac increases and reaches a maximum on day 12 or 13 of incubation. This fluid, containing nitrogeneous excretion products and a variety of electrolytes, is hypotonic (approximately 185 mosm) to chick plasma (approximately 270 mosm) and has a Na^+ concentration of about 90 mM. During the second half of incubation, from day 12 or 13 on, essentially all the water in the sac is reabsorbed. During this period, the allantoic fluid remains hypotonic relative to plasma so that there is always a favorable "down-hill" gradient for water reabsorption. To accomplish this, the allantoic endoderm selectively transports virtually all the sodium and chloride from the sac contents, while retaining and concentrating all other excretory products. By 15 or 16 days of incubation, allantoic fluid sodium and chloride have been reduced to about 15 and 4 mM, respectively, while the corresponding serum values are about 140 and 95 mM. Thus, the favorable "down-hill" water gradient is kept constant by creating a progressively steeper "up-hill" sodium gradient as a compensation for the increasing osmotic contributions of the retained excretory products. This must be an energy-consuming process and the mitochondria-rich cell, with its morphological characteristics indicating great potential for energy production, seems well suited for this role. The paucity of mitochondria-rich cells relative to granule-rich cells is not an inconsistancy when it is recalled that even in half-strength Ringer's solution, more than 500 moles of water must be reabsorbed for each mole of Na^+ transported.

These suggestions do not conflict with the proposals of Di Bona *et al.* [6] concerning toad bladder cells. They ascribed *vasopressin* induced net Na^+ transport seen in that tissue to the granular-rich cell, although on admittedly indirect evidence. On the other hand, they also felt that the mitochondria-rich cell served some important physiological function and, interestingly, speculated that these cells might be specifically responsive to the mineralocorticoid

effect of aldosterone. The pituitary and adrenal glands are both functional in the chick embryo by the 12th day of incubation [19]. It could be that the dramatic alterations in the physiological functions of the CA membrane endoderm noted in this communication are mediated by these hormones working in concert.

Both *in vivo* [14] and *in vitro* [26] investigations indicate that eggshell calcium is being transferred to the embryo's circulation by the 14th day of incubation. At this stage, tight junctions already exist near the shell membrane-facing surface of the ectoderm so that the movement of Ca^{++} must be through a cell which is in direct contact with the shell membrane. Only two of the four cell types seen in the ectoderm of the mature CA membrane form the outermost surface. These are the capillary-covering cell and the villus-cavity cell. Of these, all evidence points to the capillary-covering cells as being those directly involved in the active transport process. The most striking morphological changes associated with the onset of calcium transport occur in these cells. The changes involve a thinning of the cytoplasmic layer between the shell membrane and the associated capillary, the loss of Golgi membranes and rough endoplasmic reticulum from this region and the appearance of many plasma membrane invaginations and vesicles. These cells, with their long cytoplasmic processes, comprise virtually the entire outer surface of the ectoderm. The observation that only the capillary-covering cell is affected by "stripping" is significant since this procedure specifically abolishes *in vitro* active calcium transport [16, 26] and calcium-stimulated oxygen consumption [10]. In mature transporting membranes, the thin cytoplasmic layer overlying the capillaries is so firmly attached to the inner shell membrane that it tears away from the remainder of the cell when the CA membrane and shell membranes are mechanically separated. On the other hand, stripping does not appear to affect the villus-cavity cells.

The most direct evidence that the capillary-covering cells are responsible for the transport of Ca^{++} comes from recently published correlated electron-microprobe studies [4]. These studies revealed that in actively transporting membranes, calcium *X*-ray signals were localized to certain characteristic sites within the ectoderm and these sites were those occupied by the capillary-covering cells.

The villus-cavity cell also deserves comment. Although it also directly faces the inner shell membrane, it comprises only a very small part of the total surface of the ectoderm. However, its numerous mitochondria, elaborate microvillous apex, plentiful supply of vesicles and ribosomes mark it as an active cell. Indeed, these same features induced Leeson and Leeson [15] to compare it to a gastric parietal cell. While the two types of cells are

by no means identical morphologically, it is interesting to note that many secreting gastric cells have structural features in common with the villus-cavity cells [1, 13].

A secretory role for these cells in the calcium transport process can be visualized if one considers the fact that the Ca^{++} ions actively transported by the CA membrane must be mobilized from the ordinarily insoluble calcium carbonate (calcite) of the eggshell [23, 24]. To solubilize the Ca^{++} it is necessary to form bicarbonate, and for each mole of Ca^{++} liberated, 2 moles of H^+ ion are required. Dawes and Simkiss [5] studied the acid-base status of chick embryos throughout their development and concluded that some form of "metabolic acidosis" was necessary to explain how the embryo maintains a remarkably stable blood pH in the face of "the enormous source of base available from the reabsorbed eggshell". We would suggest, on the basis of its structural features, that the function of the villus-cavity cell is to supply hydrogen ions (and perhaps, carbonic anhydrase) so that a continuous supply of Ca^{++} can be transferred from the calcite eggshell to the calcium-transporting cells of the ectoderm.

Note Added in Proof: After submission of this paper, an electron microscopic study appeared by A. Owczarzak (*Exp. Cell Res.* **68**:113 (1971)), devoted to a detailed description of the cell identified here as the villus-cavity (VC) cell. He independently suggests that these cells secrete H^+. However, he also postulates that the same cell is solely responsible for Ca^{++} transport. This conclusion appears to be based on morphological analogies since, in his opinion, the VC cell (his CAC) resembles both gastric parietal cells and osteoclasts. We disagree. As outlined in the Discussion, our assignment of Ca^{++} transport to the CC cell (which he ignores) is based on extensive physiological, morphological and electron probe studies on this tissue.

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