VACUOLES IN THE EMBRYONIC CHICK CORNEAL EPITHELIUM, AN EPITHELIUM WHICH PRODUCES COLLAGEN

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INTRODUCTION

Studies on a variety of cells which excrete protein have established that synthesis of the protein occurs in the endoplasmic reticulum and that subsequent concentration, storage, and excretion occur via the Golgi apparatus (see Beams and Kessel, 1968 for review). Ultrastructural radioautographic studies on the mode of collagen excretion, however, have been variously interpreted in respect to the role of the Golgi apparatus, with some investigators suggesting that collagen is processed by this organelle (Revel and Hay, 1963; Hay and Revel, 1969) and others suggesting that it is not (Ross and Benditt, 1965; Cooper and Prockop, 1968; Salpeter, 1968). An issue central to these studies has been the frequency of radioautographic detection of secretory vacuoles which contain radioactive protein. Despite the reported quantitative differences in the frequency at which labeled secretory vacuoles have been found, it should be emphasized that vacuoles containing ultrastructurally recognizable collagen, as well as dense vacuoles of unknown content, have been described in a variety of collagen-producing cells (Stearns, 1940; Fitton-Jackson, 1960; Sheldon and Kimball, 1962; Movat and Fernando, 1962; Fernando and Movat, 1963; Revel and Hay, 1963; Voelz, 1964; Welsh, 1966; Welsh and Meyer, 1967; Reith, 1968; Hay and Revel, 1969). The purpose of the present report is to describe two distinct types of vacuoles in an epithelium which produces collagen, namely the embryonic chick corneal epithelium. One type of vacuole contains cross-striated aggregates like collagen, and the other type has an elongated shape and contains a dense, slightly fibrillar material.

MATERIALS AND METHODS

Chick embryos at different stages of development (Hamburger and Hamilton, 1951) were fixed in 2.5% acid-stabilized, purified glutaraldehyde (Trelstad, 1969), 4.0% paraformaldehyde in 0.1 M sodium cacodylate at pH 7.5 (Karnovsky, 1965) for 15–30 min at room temperature. The tissues were then washed briefly in 0.1 M cacodylate buffer and fixed for 1 hr at 4°C in 1.3% osmium tetroxide buffered with 0.2 M collidine at pH 7.5. After osmication the tissues were washed in 0.2 M collidine at pH 6.1 for 20 min at room temperature, and then stained with 2.0% uranyl acetate in 0.2 M collidine at pH 5.1 for 1½ hr at room temperature. After staining, the tissues were washed for 20 min with 0.2 M collidine at pH 6.1, dehydrated in ethanol, and embedded in Araldite (6005, Ciba Products Co., Summit, N.J., supplied by R. P. Cargille Laboratories, Inc., Cedar Grove, N.J.). Sections were cut on a Porter-Blum MT 2 Ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.), stained with lead citrate, and examined in an AEI 6B electron microscope.

RESULTS

The corneal epithelium during the period studied (stage 24, day 4–stage 34, day 8) consists of two layers: an outer layer or periderm, and an inner or basal layer. All of the present observations pertain only to the basal cell layer. For a complete description of the ultrastructure of corneal development, see Hay and Revel (1969).

The vacuoles which contain cross-striated aggregates like collagen are located near the basal cell surface of the basal cells. The vacuoles range from 0.3 to 0.7 μ in greatest diameter and are surrounded by a 55 A trilaminar membrane (Figs. 1 *a* and 2). The vacuole content consists of a fluffy, amorphous material and the aggregates. Fragments of cell organelles such as are seen in lysosomes have not been seen in these vacuoles. The aggregates generally lie in the center of the vacuole and may have a slightly curved profile. They have imprecisely defined lateral margins and range from 200 to 600 A in width and from 3000 to 6000 A in length. The pattern of cross-striations in the aggregates is very similar to that found in the native collagen fibrils in the corneal stroma beneath the epithelium (Figs. 1 *b* and 1 *c*).

Another distinct type of vacuole is also found in the basal region of the cell (Fig. 3). It has an elongated shape measuring an average of 1000 A

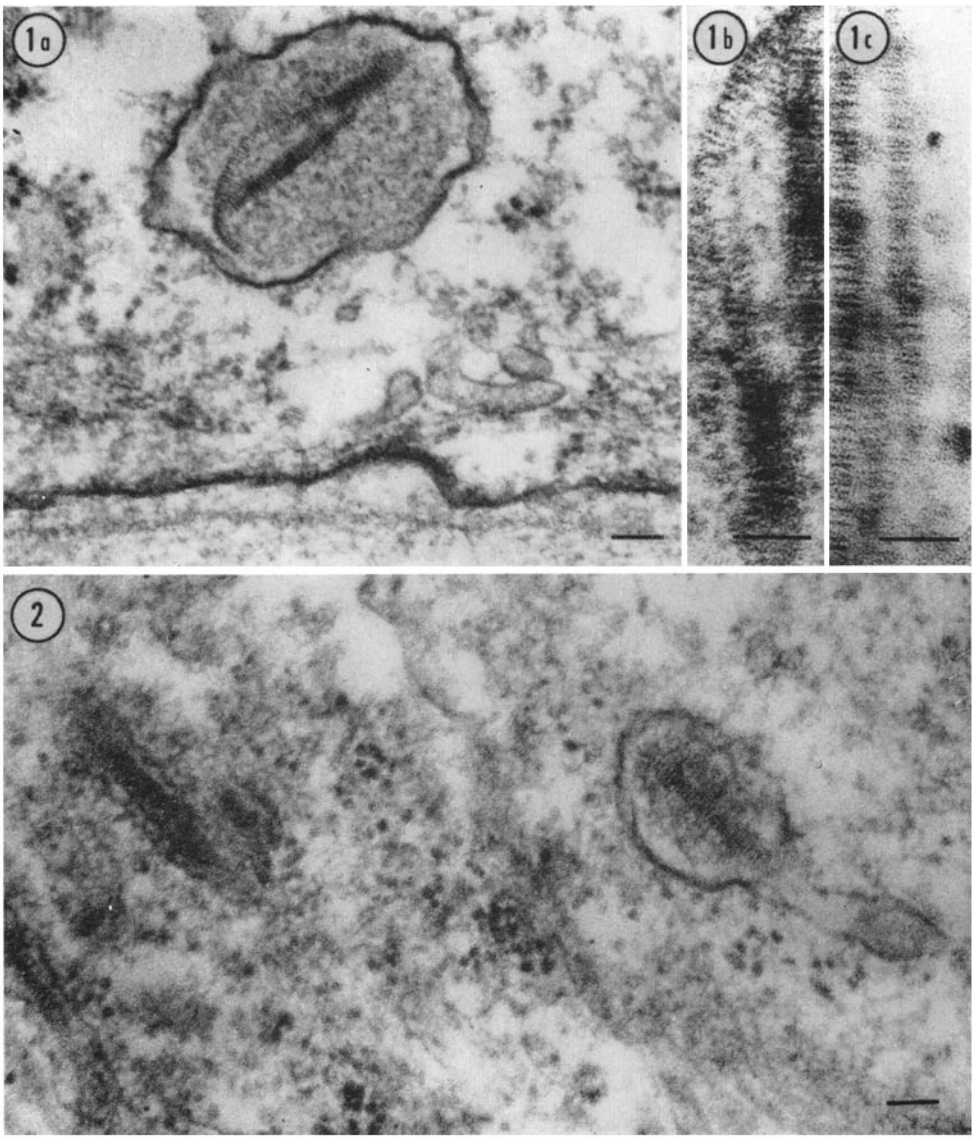


FIGURE 1 *a* Stage 28 (5½ days). Basal portion of a cell from the basal layer of the corneal epithelium. The plane of the section is perpendicular to the corneal surface. A vacuole containing cross-striated aggregates is present near the basal cell surface. The collagen-like aggregates are surrounded by a fluffy amorphous material. The basal cell surface is covered by a continuous basement membrane. Mark, 0.1 μ. × 69,600.

FIGURE 1 *b* Higher magnification of the cross-striated aggregates in Fig. 1 A. The pattern of cross-striations in the aggregates is nearly identical to that of the extracellular collagen fibrils shown in Fig. 1 C. Mark, 600 A. × 172,000.

FIGURE 1 *c* Collagen fibrils from the subepithelial primary corneal stroma mounted to facilitate comparison of the banding pattern with that of the aggregates shown in Fig. 1 B. Mark, 600 A. × 172,000.

FIGURE 2 Stage 34 (8 days). Basal portion of a cell from the basal layer of the corneal epithelium. The plane of the section is parallel to the corneal surface. A vacuole containing cross-striated aggregates is present in the cell on the right. In the cell on the left, several elongated vacuoles with dense contents are present. The shape and orientation of all vacuoles illustrated are quite similar. Mark, 0.1 μ. × 69,600.

in diameter and 6000 A in length, and is surrounded by a trilaminar membrane 55 A in thickness. The appearance of the vacuole in various planes of section confirms that it is cylindrical and not discoid in shape (Fig. 3). Its content consists of a dense, slightly fibrillar material which lies in the center and a fluffy, amorphous material which lies around the periphery. Cross-striations have not been observed in the condensed central material. Elongated vacuoles have been found in close proximity to the vacuoles which contain collagen-like aggregates (Fig. 2). Near the basal cell surface the elongated vacuoles often become aligned parallel to the adjacent cell membrane (Figs. 3 *a* and 3 *d*). In addition, they are oriented within the cell such that the projection of their long axes onto the plane of the basal cell surface is in register with the axes of the orthogonal collagenous matrix which lies beneath the epithelium (Fig. 3).

The possibility that one or both of the vacuoles are sectioning artifacts, and actually represent either finger-like invaginations of the basal cell surface or the intercellular space, can be eliminated. The basal cell surface is remarkably flat in sections cut perpendicular to the corneal surface, and large invaginations into the basal region of the epithelial cells have never been observed. In sections cut parallel to the corneal surface where the intercellular spaces can be clearly identified, the intracellular location of both types of vacuoles can be positively determined.

DISCUSSION

At issue in the present report is what role, if any, the two types of vacuoles described here play in the excretion of collagen from the epithelial cells. It is possible that the vacuoles with collagen-like aggregates contain phagocytosed material and not secretory products destined for excretion. In circumstances where collagen is being phagocytosed, however, there are usually numerous lysosomes within the cells containing other cellular debris (see Woessner, 1968, for review). Such structures are not present in the cornea. In addition, the low frequency with which the vacuoles containing cross-striated aggregates are found (approximately 1/100 cells) is unusual for a phagocytic tissue. There is little evidence, therefore, that the corneal epithelium is involved in the breakdown and phagocytosis of collagen. There is good evidence, on the other hand, that the corneal epithelium is producing collagen and excreting it into the subepithelial space (Goodfellow et al., 1969; Hay and Revel, 1969). From a consideration of several factors heretofore ignored in discussions of collagen excretion, it is suggested that the vacuoles described in the present report contain collagen being concentrated and excreted from the cell.

The marked propensity of collagen molecules to interact even at very low concentrations (Flory, 1956) places certain restrictions on the manner in which a cell might excrete collagen. In the extracellular space, collagen molecules readily precipitate to form aggregates which may have one of several different patterns of cross-striation (Gross, 1956). It might be expected that intracellular collagen, destined for excretion, could also precipitate to form aggregates, especially if it were concentrated in a specific cytoplasmic compartment such as a vacuole. Such aggregates might not show cross-striations simply because the conditions during concentration (ionic strength, ionic composition, pH, etc.) might favor the formation of an amorphous or nonstriated form which is commonly produced in vitro (Gross, 1956). On the other hand, if the vacuole is in the process of concentrating the collagen at the time of fixation for electron microscopy, it is conceivable that conditions might be favorable for precipitation in a cross-striated form.

Support for this hypothetical aggregation of collagen within the cell can be found in the vacuoles described in the present report. The large vacuoles with cross-striated aggregates appear to contain collagen. These vacuoles are only infrequently observed, are relatively large in size, and could be in the process of concentrating their contents, at the moment of fixation, into the dense aggregates found in the elongated vacuoles. The length and shape of the elongated vacuoles suggest that they contain an asymmetric, relatively rigid material. The collagen molecule is asymmetric, measuring 3000 A in length and 15 A in diameter, and behaves in solution like a relatively rigid rod (Boedtker and Doty, 1956). The similarity between the length of the elongated vacuoles (3000–6000 A) and the length of the collagen molecule is consistent with the suggestion that the elongated vacuoles contain condensed amorphous aggregates of collagen.

A consideration of the polarity of collagen excretion by the corneal epithelial cell provides

additional support for involvement of the elongated vacuoles in collagen excretion. In most fibroblasts it is assumed that collagen excretion is not polarized (Ross, 1968). From such an assumption, it follows that the entire cell surface is involved in the excretory process. This is not the case in the corneal epithelium. The collagen is excreted by the cell in a highly polarized manner, being deposited solely beneath the basal surface of the epithelium. It is likely, therefore, that the basal cell surface is the principal surface across which collagen excretion occurs. This somewhat unusual feature of the corneal epithelial cells is of highly practical importance when one is attempting to observe the excretory surface of the cell by electron microscopy. Whereas any thin section through a fibroblast will sample less than 1.0% of the total excretory surface of the cell, a thin section parallel to the basal surface of the corneal epithelial cell can sample nearly the entire excretory surface. Moreover, if the elongated vacuoles in the fibroblast are parallel to the cell surface, but otherwise randomly oriented, only a small fraction would be visualized in an elongated profile; the majority would be cut in cross- or oblique-section and would appear in circular or slightly ellipsoid profile. Since many of the elongated vacuoles in the corneal epithelium lie parallel to the basal cell surface and in a preferred orientation, they can be readily observed by sectioning in the appropriate plane. Thus, in most ultrastructural studies of collagen-excreting cells, the elongated vacuoles could have been overlooked for both sampling and stereographic reasons. It should be emphasized, nonetheless, that elongated vacuoles have been described in a variety of different collagen-excreting cells (Movat and Fernando, 1962; Voelz, 1964; Welsh, 1966; Reith, 1968; Hay and Revel, 1969), and have been considered by several investigators to be involved in the process of collagen excretion (Movat and Fernando, 1962; Welsh, 1966).

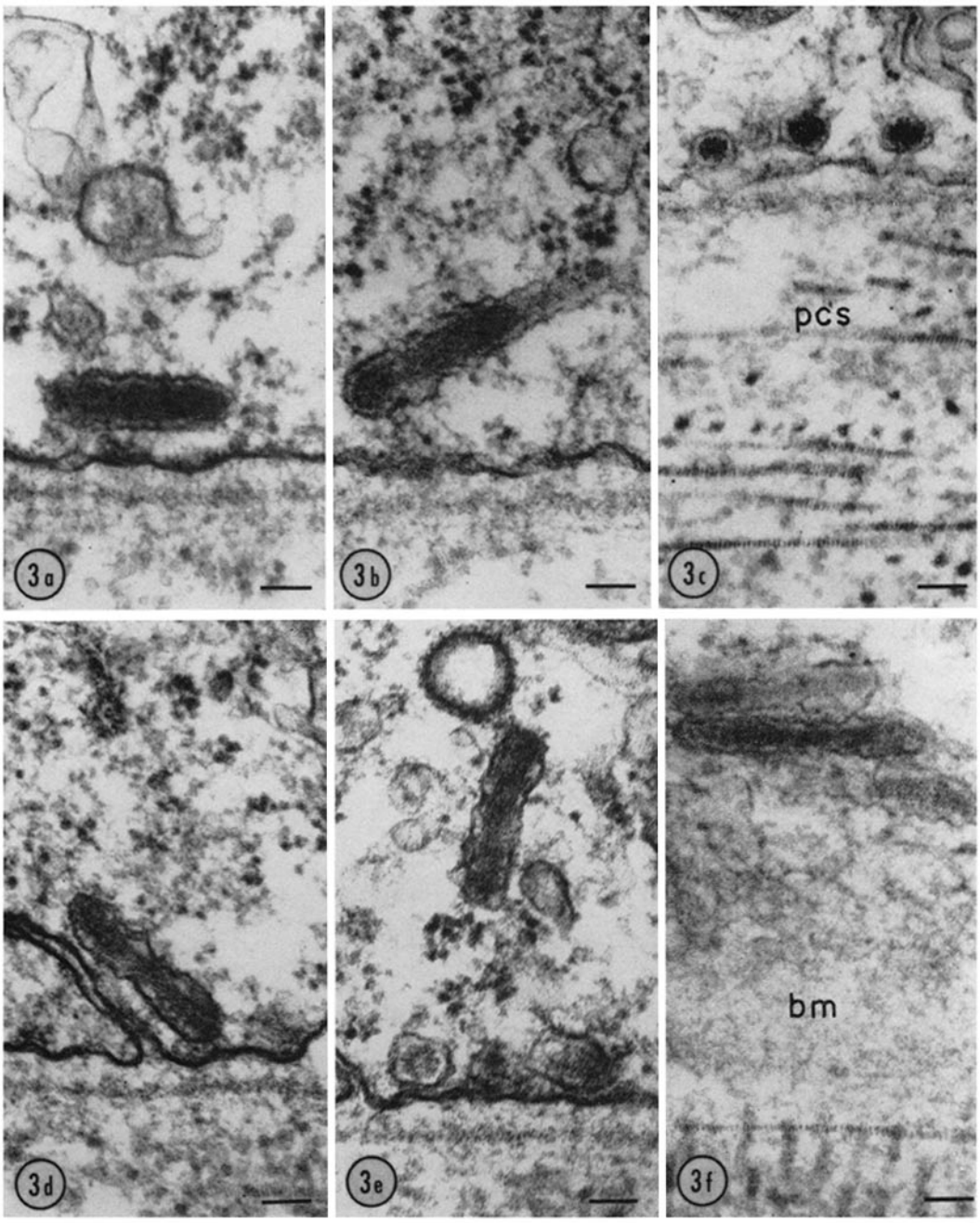
If the elongated vacuoles are involved in excretion, the question arises as to their mode of formation within the cell. From what is known about the intracellular route of other secretory proteins (Beams and Kessel, 1968), it seems likely that they form by fusion of small vesicles derived from the endoplasmic reticulum. Whether this fusion occurs within the Golgi apparatus or elsewhere in the cell cannot be determined from the present data. However, the Golgi apparatus in the basal epithelial cell is prominent (Hay and Revel, 1969) and is optimally positioned in the basal pole of the cell precisely during the period when collagen is being excreted by the epithelium (Trelstad, 1970). It is possible, therefore, that the cells of the embryonic chick corneal epithelium are similar to other protein-excreting cells in that they synthesize the product (in this case collagen) in the endoplasmic reticulum, transport it to the Golgi apparatus via small vesicles, package and condense it in vacuoles, and excrete it by fusion of the vacuole with the cell surface membrane.

FIGURE 3. Examples of elongated vacuoles from corneas cut in several different planes of section at different stages of embryonic development. Mark, 0.1 μ. × 69,600.

FIGURE 3 *a*, *b*, *d*, and *e* Stage 28 (5½ days). Plane of section is perpendicular to the corneal surface. Four elongated vacuoles in a lateral profile are illustrated. The dense central content of the vacuoles is slightly fibrillar. The width of the dense content is two-to-four times greater than the width of the collagen fibrils in the primary corneal stroma. Near the basal cell surface the vacuoles generally become aligned parallel to the cell membrane (*a*, *b*, and *d*), but examples of vacuoles oriented perpendicular to the cell membrane (*e*) are not unusual.

FIGURE 3 *c* Stage 28 (5½ days). Plane of section is perpendicular to the corneal surface and perpendicular to the plane illustrated in *a*, *b*, *d*, and *e*. Examples of three elongated vacuoles cut in cross-section. It is apparent that vacuoles cut in this plane will not be prominent. The orthogonal arrangement of the subepithelial primary corneal stroma (*pcs*) is illustrated. The orientation of the elongated vacuoles parallels that of the set of collagen fibrils which lie perpendicular to the plane of the page.

FIGURE 3 *f* Stage 34 (8 days). Plane of section is parallel to the corneal surface. Elongated vacuoles near the basal cell surface are illustrated. The basement membrane (*bm*) is obliquely sectioned. The orientation of the elongated vacuoles parallels the orientation of one set of the subepithelial orthogonal collagen fibrils.


pcs
bm
3a
3b
3c
3d
3e
3f

Note added in proof: In a recently published ultrastructural radioautographic study of dentinogenesis, Frank (1970) has shown that dense elongated vacuoles in the odontoblast are involved in the excretion of the collagenous dentin matrix.

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