THE GOLGI APPARATUS IN CHICK CORNEAL EPITHELIUM: CHANGES IN INTRACELLULAR POSITION DURING DEVELOPMENT

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ABSTRACT

The intracellular position of the Golgi apparatuses in the basal cell layer of the corneal epithelium in embryonic and hatched chicks has been studied in the light microscope by impregnating the Golgi apparatus with silver. During two distinct periods in development the Golgi apparatuses in the basal cells shift from an apical to basal position. Each of these periods correlates in time with the appearance of an acellular collagenous matrix beneath the epithelium. Examination of the basal epithelial cells in the electron microscope confirms the intracellular shifts in position of the Golgi apparatus. The results suggest that the Golgi apparatus shifts to the basal cell pole of the corneal epithelium in order to excrete connective tissue materials into the developing corneal stroma.

INTRODUCTION

In most cells in a simple epithelium the Golgi apparatus is located in the apical half of the cell between the nucleus and free surface of the cell (Cajal, 1914). Tissues have been described, however, in which the Golgi apparatus occupies the basal half of the cell (see Duesberg, 1914, and Kirkman and Severinghaus, 1938, for reviews). Occasionally the Golgi apparatus undergoes a change in intracellular position, shifting from one pole of the cell to another. Such changes in intracellular position of the Golgi apparatus have been suggested by some authors to reflect a change in the polarity of a cell function such as excretion[1] (Cowdry, 1922; Nassonov, 1927; Litwer, 1928; Ludford and Cramer, 1928; Beams and King, 1933; Rosof, 1934; Fischer, 1938; McManus, 1944; Wimsatt, 1948; Brown, 1969) whereas other authors have considered such changes in Golgi apparatus location as simply the result of mechanical displacement (Giroud, 1928; Okkels, 1934; Hibbard, 1942) or histological artifact (Gillman, 1934).

In the corneal epithelium of the embryonic chick the Golgi apparatuses in the basal layer of cells change their intracellular position from apical to basal during early development (Hay and Revel, 1969). The period during which the Golgi apparatuses are basally located coincides with the appearance beneath the epithelial surface of an acellular collagenous matrix called the post-epithelial layer (Meyer and O'Rahilly, 1959) or primary corneal stroma (Hay and Revel, 1969). A

[1] The terminology used by Bowen (1929) is employed here. Secretion refers to the intracellular processes involved in the synthesis of secretory materials. Excretion refers to the processes involved in their discharge from the cell.

number of investigators have suggested that the corneal epithelium produces the primary corneal stroma (Kessler, 1877; Laguesse, 1926; Redslob, 1935; Coulombre, 1965), and Hay and Revel (1969) have suggested that the basal Golgi apparatus is directly involved in the excretion of this collagenous structure.

The present study of the embryonic chick corneal epithelium provides a quantitative evaluation of the intracellular position of the Golgi apparatus in the basal cell layer at successive stages of development. The results show that there are two distinct periods during development when the Golgi apparatus changes position within the basal cell, each period correlating in time with the appearance of a collagenous matrix beneath the epithelium.

MATERIALS AND METHODS

Embryos of white Leghorn chickens were incubated at 38°C and staged according to Hamburger and Hamilton (1951). The Golgi apparatus was impregnated with silver by modifications of the method developed by Lascano (1959) and McDonald (1964). All procedures were conducted at room temperature. Tissues were fixed for 4 to 6 hr in 6% formaldehyde buffered with 0.28 M glycine at pH 3.2 (adjusted with HNO_3). Following fixation the tissues were rinsed for several seconds in aqueous 1.5% $AgNO_3$ under subdued lighting and then placed in a fresh solution of 1.5% $AgNO_3$ for 4 hr in the dark. Following silver impregnation, the tissues were rinsed for several seconds in the reducing solution, 1.5% hydroquinone in 7% aqueous formaldehyde, and then placed in fresh reducing solution in the dark for a minimum of 2 hr. The tissues were usually left overnight in the reducing solution and then dehydrated in ethanol and embedded in Araldite (6005, R. P. Cargille Laboratories, Inc., Cedar Grove, N.J.). Sections 1.5 μ in thickness were cut on a Porter-Blum MT-2 microtome (Ivan Sorvall, Norwalk, Conn.), stained with 0.25% toluidine blue in 0.25% sodium borate, and coverslipped with glycerin.

To quantitate the change in position of the Golgi apparatus within the cell, camera lucida drawings were prepared of the silver-impregnated corneas in which the position of the cell nucleus and Golgi apparatus were recorded. Each cell was subdivided into quadrants by two perpendicular lines which intersected in the center of the nucleus, each line forming a 45° angle with the surface of the epithelium. The position of the Golgi apparatus was then scored as in the quadrant apical, lateral or basal to the position of the nucleus of the same cell. The two lateral positions were scored together. From 500 to 1000 cells were counted from each animal and the percent of the Golgi apparatuses in the three positions, apical, lateral, and basal to the cell nucleus, was determined. Animals were studied from 3 days of development to 15 months after hatching. From one to six animals were sampled from each stage. Measurements of corneal stroma thickness were made on Araldite-embedded sections with an ocular micrometer.

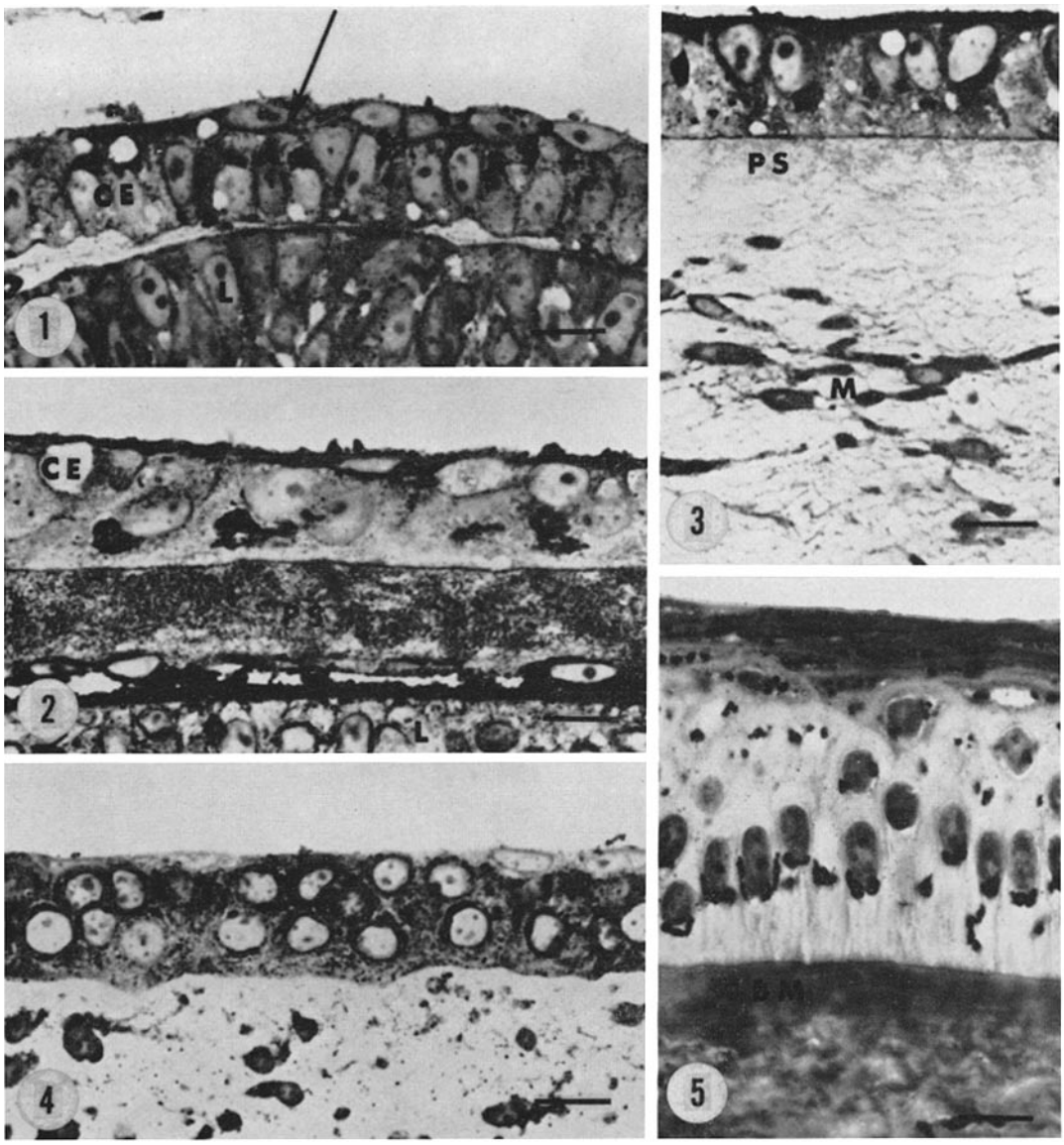
Tissues for electron microscopy were fixed at room temperature for 15 to 45 min in 2.5% glutaraldehyde and 4.0% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 (Karnovsky, 1965), washed briefly in 0.1 M sodium cacodylate and postfixed at 4°C in 1.3% osmium tetroxide in collidine buffer at pH 7.1. Following osmication, the tissues were stained en bloc with 2.0% uranyl acetate in collidine buffer at pH 5.1. The tissues were dehydrated in ethanol and embedded in Araldite (6005). Sections with silver interference colors were cut on a Porter-Blum MT-2 microtome, mounted on naked 200 mesh copper grids, and stained with lead citrate. Sections were examined in an RCA-3E or AEI EM6 electron microscope.

RESULTS

From day 2½ (stage 17) to day 10 (stage 34) the corneal epithelium consists of two cell layers, an inner columnar layer and an outer squamous layer (Figs. 1–4). Beginning at about day 10 the number of cell layers increases and by day 18 (stage 44) reaches the adult number of six cell layers (Fig. 5). The quantitative data on the intracellular position of the Golgi apparatus presented here pertain only to the basal cell layer of the epithelium whether at the early two-layered stage or at later multilayered stages. The data represent the intracellular position of silver deposits as observed in the light microscope, but it can be assumed that the position of the silver deposits is the same as that of the Golgi apparatus on the basis of ultrastructural observations to be presented later in the paper.

Light Microscopic Observations

At day 2½ (stage 17) the Golgi apparatuses in the basal layer of cells are predominantly apical to the cell nucleus (Fig. 1). Beginning on day 3 (stage 18) an infrequent cell is found in which the Golgi apparatus is located basal to the cell nucleus, and by the end of day 3 (stage 23) about 20% of the cells possess a basal Golgi apparatus (Fig. 6; Table I). During day 4 an increasing percentage of cells contain basally located Golgi apparatuses, and by late in day 5 (stage 28) the Golgi apparatuses are present in the basal cell pole of nearly 70% of the



FIGURES 1–5 Silver impregnations of the Golgi apparatus in the corneal epithelium at different stages of development. Mark: 10 μ. × 1080.

FIGURE 1 Day 2½ (stage 17). The Golgi apparatuses (arrow) in the basal layer of the corneal epithelium (*CE*) are located apical to the cell nucleus. The primary corneal stroma has not begun to accumulate beneath the epithelium. The anterior surface of the lens vesicle (*L*) is apposed to the basal surface of the corneal epithelium.

FIGURE 2 Day 5 (stage 28). The Golgi apparatuses are prominent and located basal to the cell nucleus in all basal cells illustrated in the corneal epithelium (*CE*). The primary stroma (*PS*) has begun to accumulate beneath the epithelium. The collagen fibrils in the primary stroma are moderately packed and the entire stroma is 10–15 μ thick. Mesenchyme cells have not yet invaded the primary stroma. (*L*), lens.

FIGURE 3 Day 7 (stage 31). The Golgi apparatuses in the basal layer of the corneal epithelium continue to be located in the basal cell pole. The primary stroma (*PS*) has swelled and has been invaded throughout by mesenchyme (*M*) except for a zone about 10 μ thick beneath the epithelium.

FIGURE 4 Day 12 (stage 38). The Golgi apparatuses in a majority of the basal cells are either apical or lateral to the cell nucleus. The size of the Golgi apparatus is smaller than in the preceding stages. The uninvaded portion of the primary stroma is now only a narrow zone about 1–2 μ thick beneath the epithelium.

FIGURE 5 Hatched chick. The Golgi apparatuses in the basal cell layer are predominantly basal to the cell nucleus. A 10 μ thick acellular matrix of collagen which constitutes Bowman's membrane (*BM*) is present beneath the epithelium.

TABLE I

Position of the Golgi Apparatus in Respect to the Nucleus in Per Cent of Basal Corneal Epithelial Cells During Development and Posthatching Growth

Stage	Apical	Lateral	Basal	Age	Apical	Lateral	Basal	Apical & basal	Perinuclear
16	90	10	0	1 wk	1	0	85	7	7
17	82	17	1	2 wk	1	1	65	8	25
18	91	9	0	3 month	0	1	77	8	14
20	82	13	5	7 month	1	1	66	24	8
21	55	42	3	11 month	0	0	82	3	15
22	81	14	5	15 month	5	1	30	49	15
23	43	37	20						
24	40	33	27						
25	30	43	27						
27	23	27	50						
28	8	24	68						
29	9	43	48						
30	17	42	41						
31	23	45	32						
32	17	49	34						
33	25	52	23						
34	19	45	36						
35	20	59	21						
36	30	55	15						
37	35	54	11						
38	41	49	10						
39	41	49	10						
40	45	48	7						
42	15	43	42						
43	14	58	28						

cells (Figs. 2 and 6; Table I). From day 5 (stage 28) through day 14 (stage 40) there is a decrease in the percentage of cells with basally located Golgi apparatuses, and by day 14 less than 10% of the cells possess a basal Golgi apparatus (Figs. 4 and 6; Table I). Beginning on day 15 (stage 41) the Golgi apparatuses in the basal layer of cells begin to reappear in the basal cell pole with increasing frequency, and by hatching nearly 85% of the cells possess a basal Golgi apparatus (Figs. 5 and 6; Table I). The Golgi apparatus remains in the basal cell pole of about 80% of the basal cells for at least the first 15 months of adult life (Fig. 6; Table I.)

The percentage of cells with Golgi apparatuses either apical or lateral to the cell nucleus is constantly changing once the Golgi apparatus begins to appear in the basal cell pole, which is to be expected if an actual movement of the organelle is taking place (Table I).

There is no apparent relationship between the position of the Golgi apparatus in one cell and that of the Golgi apparatuses of the adjacent cells, nor between the position of the Golgi apparatus in the basal cell and the location of the basal cell in the corneal epithelium.

The location of the Golgi apparatus in the cells of the other layers of the corneal epithelium seems not to change very much. In the squamous layer of the early two-layered epithelium the Golgi apparatuses are located predominantly lateral to the nucleus (Fig. 1). Later in development, the wing cells which lie immediately above the basal cells often possess a circumnuclear Golgi apparatus (Fig. 5). The outer squamous layers have a pattern similar to that described for the early squamous layer.

During the early stages of development the conjunctival epithelium is two layered and distinctly thinner than the adjacent corneal epithelium. The Golgi apparatus in the basal layer of cells in the conjunctiva is not prominent and is almost invariably apical or lateral to the cell nucleus. Less than 1.0% of all basal cells of the conjunctival epithelium examined from day 3 of development to

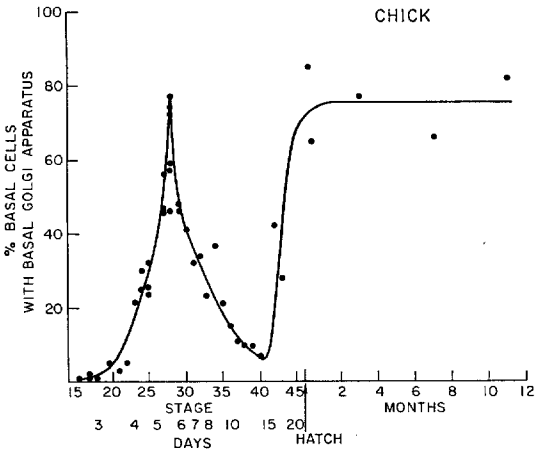


FIGURE 6 Percentage of basal cells (from the corneal epithelium) with basal Golgi apparatuses as a function of time. Corneas at the indicated stages of development were impregnated with silver to stain the Golgi apparatus. Approximately 1000 cells in the basal cell layer of the epithelium were examined at each stage in tissue sections in the light microscope, and the percentage of cells with a Golgi apparatus in the basal cell pole was recorded. The shift of the Golgi apparatuses to the basal cell pole which occurs between day 3 and day 10 correlates with the period when the primary corneal stroma is deposited beneath the corneal epithelium. The second shift of the Golgi apparatus which begins on day 15 correlates with the appearance of Bowman's membrane beneath the corneal epithelium.

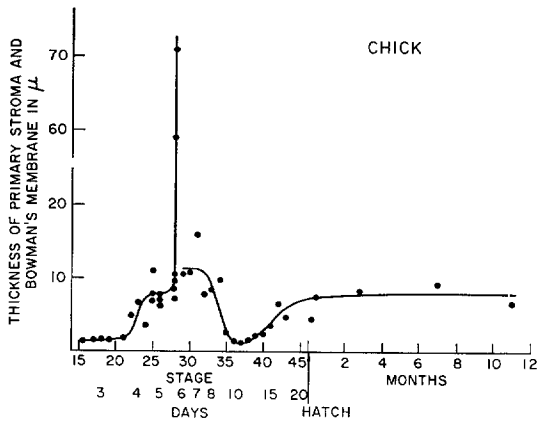


FIGURE 7 Thickness of the acellular collagenous matrix beneath the corneal epithelium as a function of time. Corneas at the indicated stages of development were fixed and sectioned, and the thickness of the acellular stroma beneath the epithelium was measured in the light microscope. The acellular stroma present between day 3 and day 10 is the primary corneal stroma; that present after day 15 is Bowman's membrane. The primary corneal stroma begins to accumulate on day 3, and late in day 5 it rapidly swells to a thickness of 50–70 μ. Immediately upon this swelling the mesenchyme invades the primary stroma except for a 10 μ thick zone beneath the epithelium which is progressively invaded during the subsequent 4 days so that by day 10 only a 1–2 μ thick remnant of the acellular primary stroma is present. Beginning on day 15 the subepithelial collagenous matrix rethickens, and by hatching it has become the 8 μ thick Bowman's membrane.

15 months after hatching contained a basal Golgi apparatus.

Electron Microscopic Observations

Basal epithelial cells of the cornea examined in the electron microscope at successive stages of development from day 3 to 3 months posthatching show the Golgi apparatus to be present in different intracellular positions in a pattern consistent with the observations obtained with the silver impregnations (Fig. 8). During the first reversal of intracellular position, the Golgi apparatus is accompanied by the centrioles (Fig. 8). At some later time in development, however, these two organelles separate so that in the adult the Golgi apparatus is present in the basal cell pole and the centrioles in the apical cell pole. The nucleus is generally in the cell pole opposite the pole in which the Golgi apparatus lies. The endoplasmic reticulum and mitochondria appear to be uniformly distributed throughout the cytoplasm.

Corneal Stromal Development

The development of the primary corneal stroma and Bowman's membrane correlates closely in time with the changes in intracellular position of the Golgi apparatus (Figs. 6 and 7). Shortly after the formation of the corneal epithelium on day 3 (stage 18), the acellular collagenous primary stroma begins to form beneath the epithelium (Fig. 8). The primary stroma consists, in great part, of layers of orthogonally disposed collagen fibrils (Fig. 8). The fibrils are uniformly about 250 A in diameter and have a periodicity which averages about 600 A. The primary stroma increases in thickness and density during the subsequent 2½ days of development, and then shortly before mesenchymal invasion it swells to a thickness of 50 to 70 μ (Fig. 7). The mesenchyme then invades the swollen primary stroma and begins producing the connective tissue of the secondary or cellular

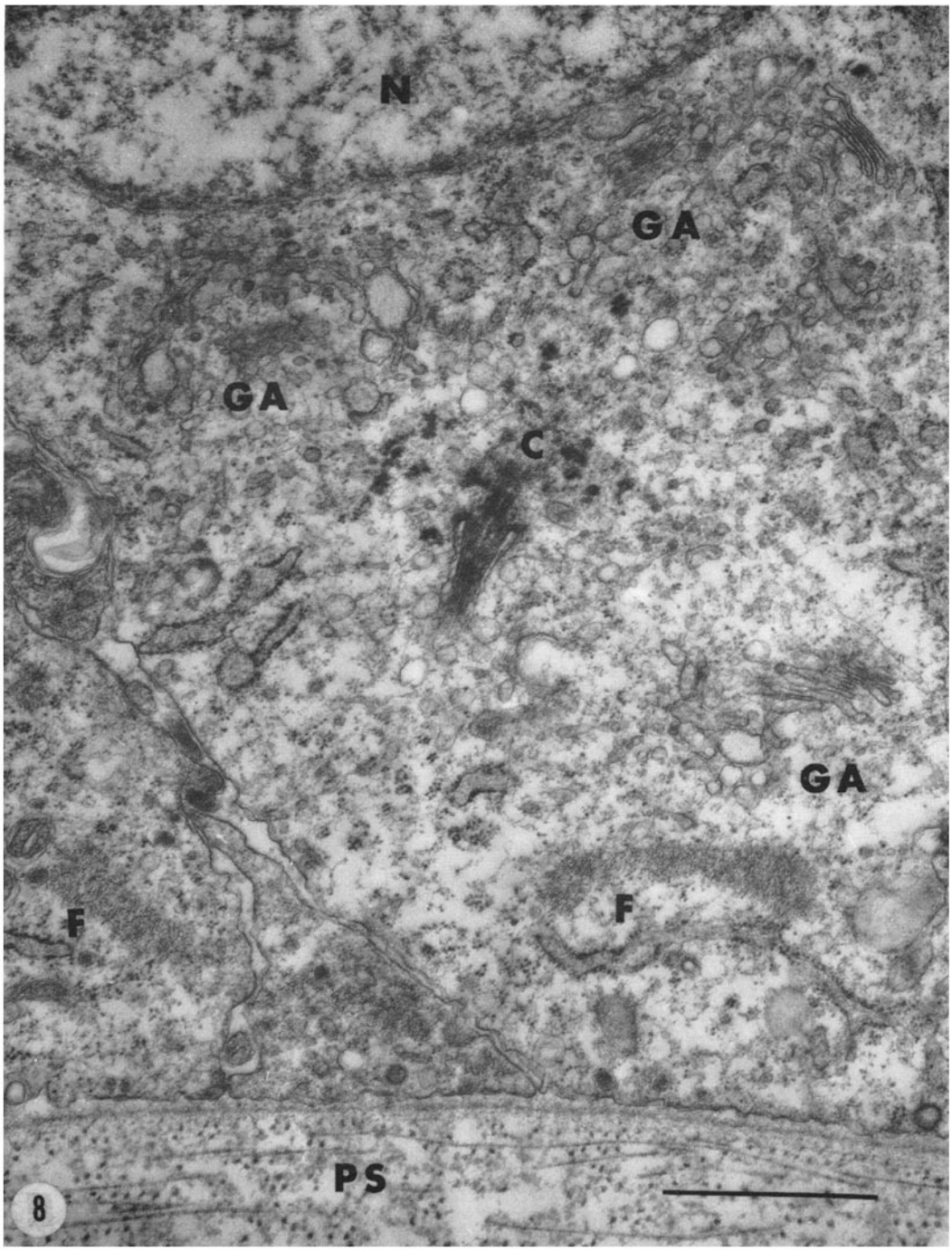


FIGURE 8 Electron micrograph of the basal pole of a basal epithelial cell at stage 28. The Golgi apparatus (*GA*) consists of several groups of cisternae, vesicles and vacuoles all located basal to the cell nucleus (*N*). The centriole (*C*) forms the basal body of a cilium. Numerous centriolar satellites from which microtubules appear to emanate surround the centriole. Bundles of filaments (*F*) are frequently seen in the basal cell region. The basal surface of the cell is covered by a continuous basement membrane. The primary corneal stroma (*PS*) is organized into orthogonally disposed collagen fibrils. Mark: 1.0 μ × 31,000.

corneal stroma. An uninvaded portion of the primary stroma remains, however, beneath the corneal epithelium until about day 10 (Fig. 3). Between day 10 (stage 36) and day 14 (stage 40) this uninvaded portion of the primary stroma decreases to less than 1.5 μ in thickness (Figs. 4 and 7). On about day 15 (stage 41) another acellular collagenous structure which will become Bowman's membrane begins to form beneath the basal surface of the outer epithelium, and by hatching it has thickened to 6–8 μ (Fig. 5). The thickness of Bowman's membrane remains constant for at least the first 12 months of life (Fig. 7).

DISCUSSION

The present study demonstrates that the Golgi apparatuses in the basal cell layer of the embryonic chick cornea shift from an apical to basal location during two separate periods in development. One possible explanation of these two separate basal relocations of the Golgi apparatus is suggested by the fact that the first occurs when the primary corneal stroma is forming beneath the epithelium and the second when Bowman's membrane is forming beneath the epithelium. Based on what is known of the role of the Golgi apparatus in the synthesis and excretion of secretory materials from cells (see Beams and Kessel, 1968, for review), it would seem possible that the Golgi apparatuses in the corneal epithelium shift to the basal cell pole to play a role in the production and discharge of the primary corneal stroma and Bowman's membrane from the epithelium. Support for this suggestion derives both from studies which indicate that the embryonic corneal epithelium produces connective tissue materials and also from studies which indicate that the direction in which materials are excreted from a secretory epithelium is related to the intracellular position of the Golgi apparatus.

Evidence that the embryonic corneal epithelium in the chick produces connective tissue material comes from both morphological and biochemical studies. Early investigators concluded, on morphological grounds, that the primary corneal stroma was derived from the corneal epithelium principally because it first begins to accumulate beneath the epithelium before mesenchyme cells are present (Kessler, 1877; Laguesse, 1926; Redslob, 1935). Ultrastructural studies provide additional evidence to support this view by showing that the embryonic chick corneal epithelium has a well-developed endoplasmic reticulum and Golgi apparatus, two cytological characteristics of a secretory epithelium (Brini et al., 1966; Pouliquen et al., 1966; Hay and Revel, 1969). More direct evidence for corneal epithelial production of connective tissue materials comes from in vitro studies of isolated embryonic chick corneal epithelia in which synthesis of a hydroxyproline-rich protein, probably collagen, has been demonstrated (Goodfellow et al., 1969; G. W. Conrad, manuscript in preparation). Several lines of evidence thus indicate that the corneal epithelium in the embryo, and possibly also in the adult (Herrmann, 1958; Herrmann and Love, 1959), excrete connective tissue materials including collagen into the subepithelial stroma.

The correlation between the direction in which materials are excreted from a cell and the intracellular position of the Golgi apparatus derives from observations on a variety of secretory tissues. It is particularly evident in exocrine glands in which the Golgi apparatus is almost invariably in the cell pole (apex) from which secretory materials are discharged (see Kirkman and Severinghaus, 1938, for review). In endocrine glands a similar correlation has been suggested (Cowdry, 1922; Courrier and Reiss, 1922; Reiss, 1922; Ludford and Cramer, 1928; Rosof, 1934). One of the most convincing demonstrations of the correlation between the direction of excretion and the intracellular position of the Golgi apparatus in an epithelium comes from studies of the enamel organ of the developing rat tooth. The ameloblasts in the epithelial enamel organ excrete enamel matrix from their basal pole only after the Golgi apparatuses have shifted from the apical to basal cell pole (Jasswoin, 1924; Beams and King, 1933). Such studies suggest that the intracellular position of the Golgi apparatus is regulated in some way by the cell and that the direction in which secretory materials are discharged from the cell is related to the intracellular position of the Golgi apparatus. Although the possibility remains that in some secretory epithelia the intracellular position of the Golgi apparatus results from passive displacement (Giroud, 1928; Okkels, 1934; Hibbard, 1942), the present observations are most consistent with the view that the shift of the Golgi apparatuses to the basal cell pole of the corneal epithelium represents an active change in cellular organization and is related to the basal excretion of the primary corneal stroma.

The nature of the materials excreted by the Golgi apparatuses into the corneal stroma is not

known. Because the primary corneal stroma contains predominantly collagen (Hay and Revel, 1969; G. W. Conrad, manuscript in preparation) and probably mucopolysaccharides (Conrad, manuscript in preparation), it would seem likely that it is one or both of these materials which is excreted by the basal Golgi apparatuses. The Golgi apparatus is known to play a major role in the synthesis and excretion of mucopolysaccharides (Neutra and Leblond, 1966). The role of the Golgi apparatus in collagen excretion, however, is not as well defined. Most investigators agree that collagen is synthesized in the endoplasmic reticulum (Lowther et al., 1961; Revel and Hay, 1963; Ross and Benditt, 1965; Salpeter, 1968), but the manner in which the collagen is excreted from the cell remains unsettled. Some authors have suggested that the collagen must pass through the Golgi apparatus during the process of excretion (Revel and Hay, 1963; Hay and Revel, 1969), whereas other authors have proposed routes of collagen excretion which do not involve the Golgi apparatus (Ross and Benditt, 1965; Cooper and Prockop, 1968; Salpeter, 1968). The present study provides no additional data directly bearing on this point, but the corneal epithelium does appear to be a particularly good tissue in which to study this question because of the polarized excretion of the collagen and the polarized arrangement of the Golgi apparatus.

The manner in which the cell might regulate the intracellular position of the Golgi apparatus is unknown. Whatever the mechanism, the centrioles appear to be similarly affected as they change their intracellular position along with the Golgi apparatus during at least the early stages of development. It is conceivable that the centrioles and their associated microtubules may play a causal role in the movements of the Golgi apparatus, for in sea urchin blastulae solubilization of microtubules randomizes the intracellular position of the normally polarized Golgi apparatus (Tilney and Gibbins, 1969) and in other tissues microtubules have been associated with intracellular movement or positioning of a variety of organelles (Porter, 1966; Bikle et al., 1966; Inoué and Sato, 1967; Green, 1968; Holmes and Choppin, 1968).

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