AN INTERCISTERNAL STRUCTURE IN THE GOLGI APPARATUS

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The Golgi apparatus in plant and animal cells consists of a stack of flattened sacs and associated vesicles (2, 4, 13). In addition, there may also be a matrix, differentiated from the cytoplasmic ground substance, which encompasses all or part of the Golgi apparatus (12). The Golgi apparatus, at least in some cells, is a dynamic system in which cisternal elements form, mature, and ultimately are discharged in a pattern which can be traced across the cisternal stack (1, 7, 10). Observations in this laboratory indicate that the number, size, and form of the cisternae may be changed materially when the cell divides or matures, at times of Golgi apparatus multiplication, or when the cell is subjected to adverse conditions. The cisternae of the Golgi apparatus may appear flat or curled in transverse section but, in all cases, the cisternal elements remain separated from each other by a relatively constant minimal distance. So far as is known, no information regarding the structural

aspects of this intercisternal space has been reported.

MATERIALS AND METHODS

Apical segments of the primary roots of maize seedlings were fixed by a modification of the method of Sabatini *et al.* (9). The procedure utilized 0.0025 per cent, 0.5 per cent, or 2.5 per cent glutaraldehyde (1 hour), a buffer rinse (½ hour), and a postfixation in 1.0 per cent OsO_4 (1 hour). All of the solutions were buffered using either 0.05 M phosphate (3, 5) or cacodylate (3) buffer at pH 6.8 or 7.4. The temperature was maintained at 2 to 4°C throughout fixation. Specimens were dehydrated in ethanol and acetone and embedded in No. 1 Epon-Araldite epoxy resin mixture according to Mollenhauer (6).

RESULTS AND DISCUSSION

Certain aspects of the ultrastructural image, in the maize root tip at least, are dependent upon the concentration of glutaraldehyde in the above fixation procedures. It has been found that low concentrations of glutaraldehyde are particularly beneficial, producing in this material an excellent fixation image. For tissue blocks a glutaraldehyde concentration of 0.5 per cent has proven useful; for individual cells or cell components a concentration of 0.0025 per cent is satisfactory and apparently represents the lowest level at which a good fixation might be expected.

Using the glutaraldehyde-OsO_4 fixation procedures outlined here there is a discernible difference in the form and density of the cisternae of the Golgi apparatus in relation to their positions within the cisternal stack. In epidermal cells wherein this pattern can be clearly visualized, the cisterna, on what is believed to be the forming face of the Golgi apparatus, is highly vesiculate; it is apparently formed from a series of relatively large, nearly transparent, spheroidal vesicles (Figs. 1 to 3). As the cisterna matures, it becomes more uniform, assuming the flattened shape of most Golgi cisternae and changing in both intracisternal spacing and membrane density (Fig. 1).

Concomitant with the progressive change in cisternal form is an alteration in the appearance of the intercisternal region of the Golgi apparatus. This is clearly seen in the Golgi apparatus of epidermal and cap cells after fixation in low concentrations of glutaraldehyde and postfixation in OsO_4.

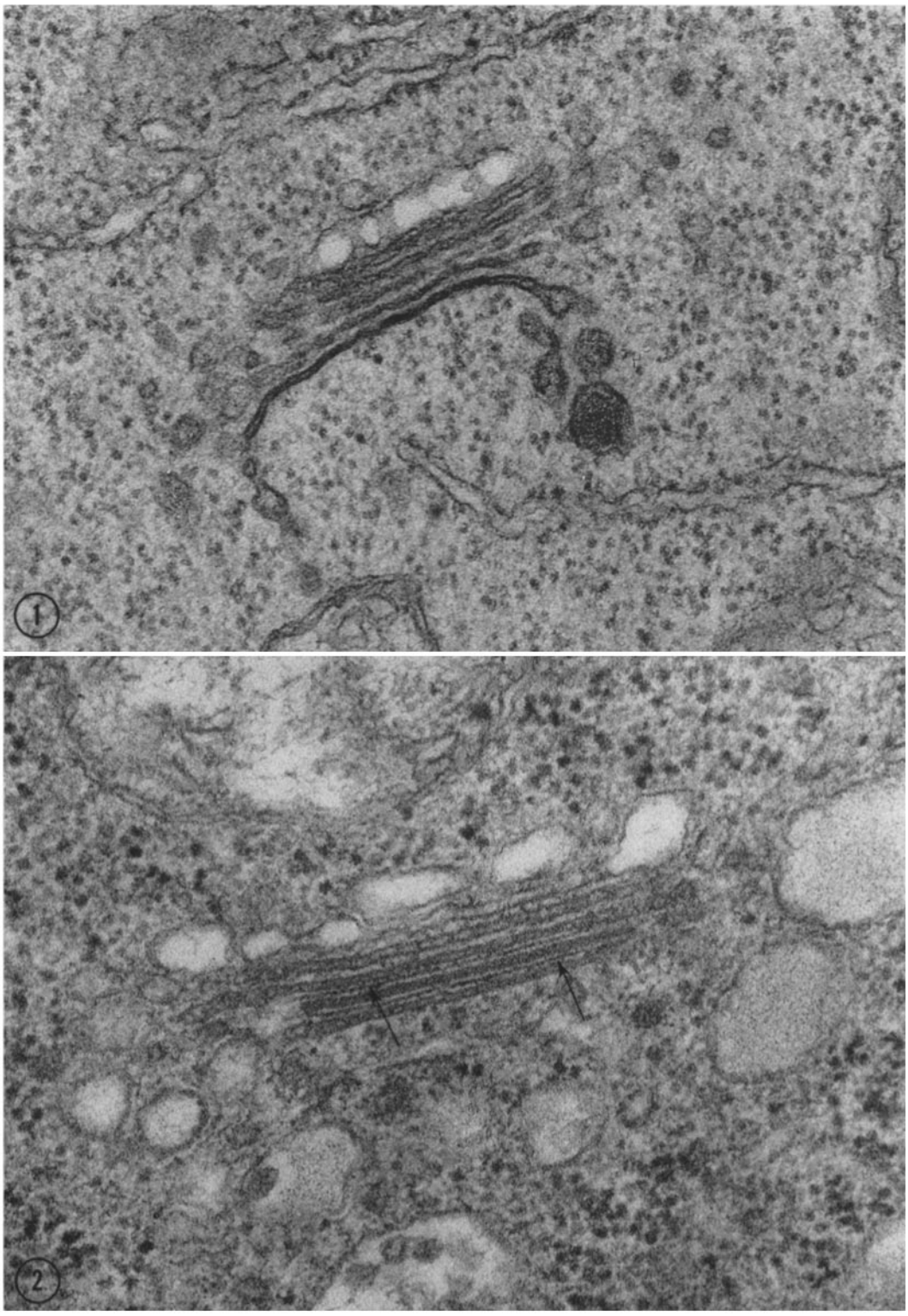
Primarily, this change involves the appearance of a dense line (see arrows, Figs. 2 and 5) in those regions of the Golgi apparatus wherein the spacings of the cisternae are relatively uniform. This dense line (or opaque layer) may appear continuous or discontinuous possibly depending on the condition of the interface region, the fixation procedure, or because of some additional substructure not yet totally apparent. The visualization of the opaque layer is dependent upon position within the Golgi apparatus. It is seldom seen between the newly forming cisterna and the second cisterna of the stack; it is clearly seen between cisternae of the mature side of the Golgi apparatus (Figs. 2 to 4, and 6) and between forming Golgi vesicles that are still closely appressed to each other (Fig. 5).

The dimensions of the intercisternal region of the Golgi apparatus of this system are relatively constant. The minimal distance between the outer surfaces of adjacent cisternae is about 115 A. This region contains three easily differentiated zones: two electron translucent zones, each about 35 A thick, next to the cisternal surfaces; and a centrally located, often discontinuous (See Figs. 3 and 4), opaque layer about 45 A thick. These observations lead to the suggestion that the electron translucent zones may each be composed of a double layer of lipid molecules, and that the centrally located, opaque layer may be protein. This is in keeping with the dimensions and fixation responses of other ordered membrane systems such as the plasma membrane of the Schwann cell and the myelin figures it produces (8, 11). Furthermore, a somewhat similar configuration was hypothesized by Sjöstrand (10) in a diagrammatic depiction of the Golgi apparatus of pancreatic secretory cells.

The intercisternal detail described here has thus far been seen in the Golgi apparatus of the epidermis and cap cells of the maize root tip and in certain vegetative cells of *Nitella* (F. R. Turner, personal communication). It has also been found in isolated Golgi apparatus prepared by the fractionation and differential centrifugation of cells of cauliflower inflorescence (Fig. 7) and in the Golgi apparatus of pollen mother cells (D. A. Larson, personal communication). Though the number of observations utilizing tissues fixed in low concentrations of glutaraldehyde have been limited, the frequent visualization of the intercisternal structure suggests that this may be a universal feature of Golgi apparatus.

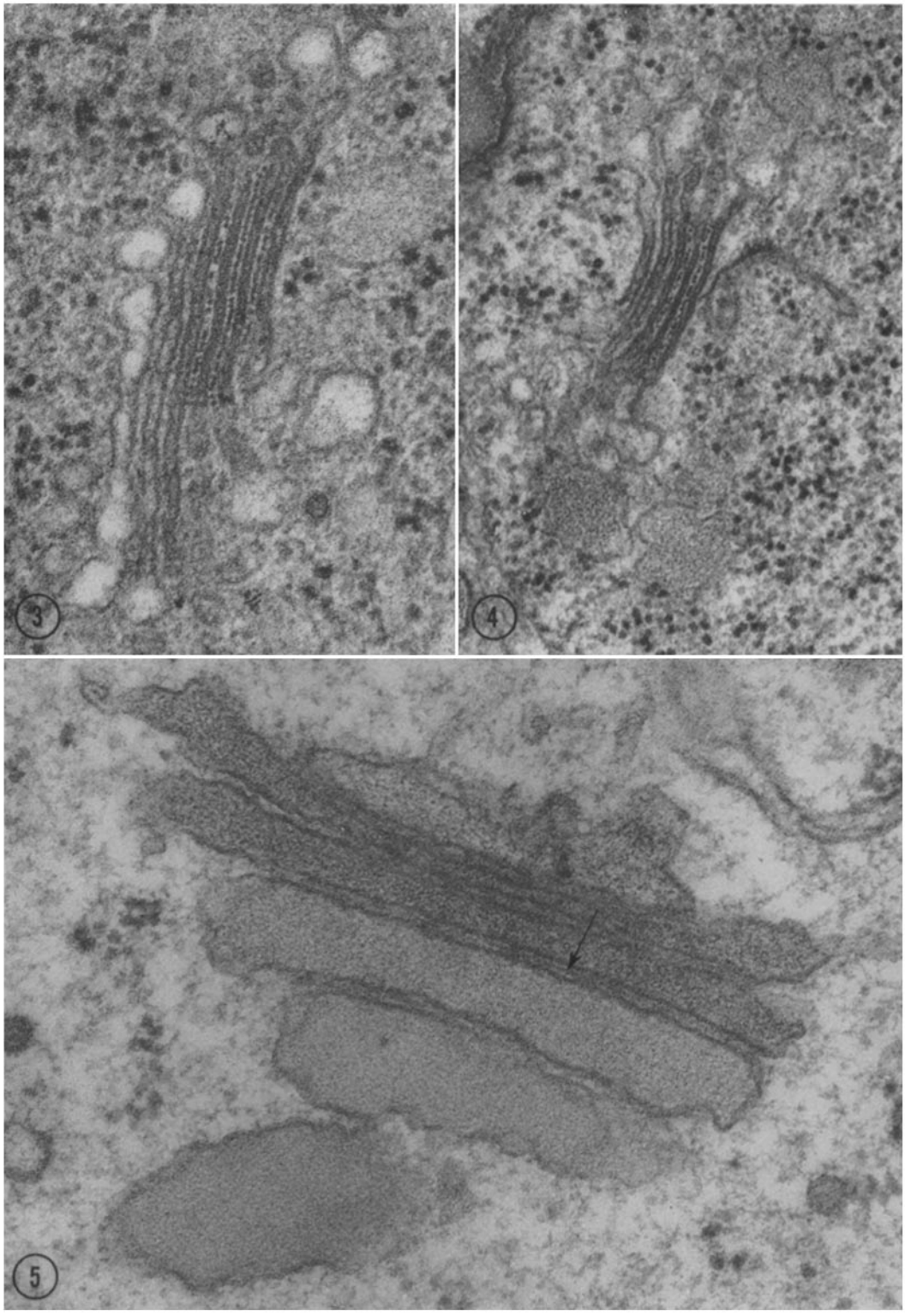
FIGURE 1 A Golgi apparatus from an epidermal cell of the maize root tip which shows some of the progressive changes in appearance of the cisternae as they mature and produce secretion vesicles. The forming face of the Golgi apparatus is seen in the upper part of the micrograph. In this material, when fixed by the prescribed methods, the forming cisterna has an open vesiculate appearance. As the cisterna matures, it changes dimensionally and in opacity as illustrated in the micrograph. Fixation in 2.5 per cent glutaraldehyde, postfixation in 1.0 per cent OsO_4. The interface image is not apparent after this fixation procedure. × 86,000.

FIGURE 2 A Golgi apparatus from an epidermal cell of the maize root tip after fixation in 0.5 per cent glutaraldehyde and postfixation in 1.0 per cent OsO_4 showing the interface structure (arrows) between cisternae of the maturing side of the Golgi apparatus. × 116,000.



FIGURES 3 and 4 Golgi apparatus from epidermal cells of maize root tip in which the interface structure appears discontinuous. Fixation in 0.5 per cent glutaraldehyde, postfixation in 1.0 per cent OsO_4. Fig. 3, × 115,000; Fig. 4, × 77,000.

FIGURE 5 A peripheral section through maturing secretion vesicles of a Golgi apparatus in the outer mantle of maize root cap showing the existence of the interface structure (arrow) between some of the hypertrophied Golgi cisternae. Fixation in 0.5 per cent glutaraldehyde, postfixation in 1.0 per cent OsO_4. × 120,000.



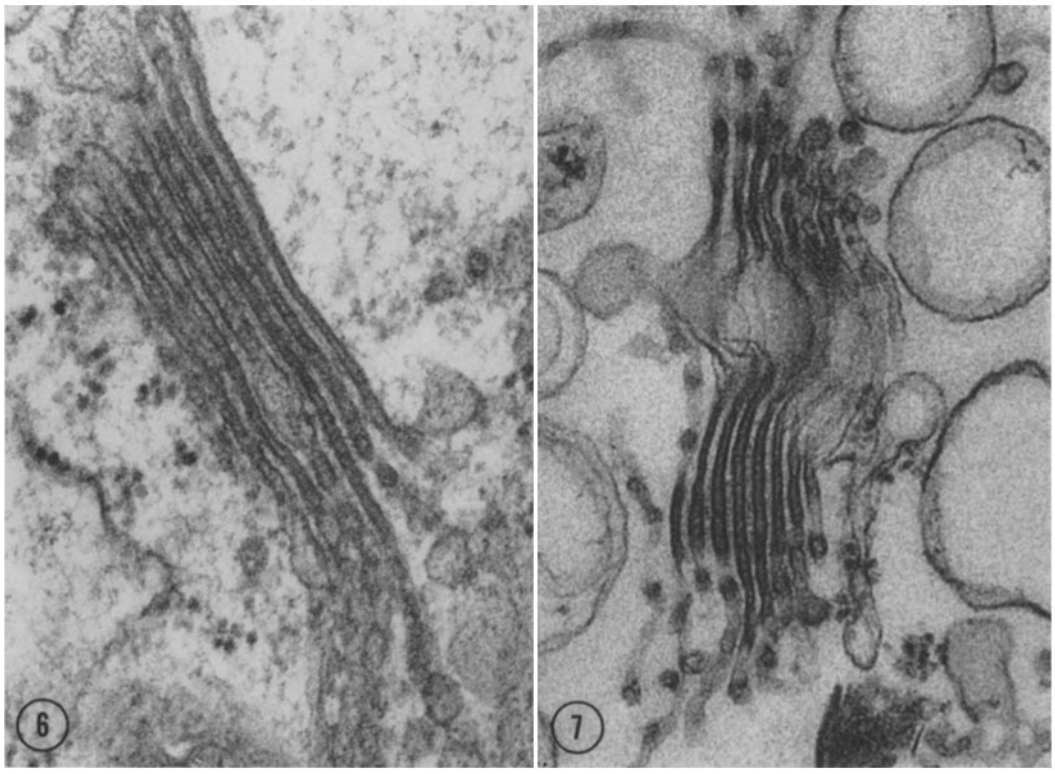


FIGURE 6 A Golgi apparatus from the central region of the maize root cap showing the interface structure. Fixation in 0.5 per cent glutaraldehyde, postfixation in 1.0 per cent OsO_4. × 92,000.

FIGURE 7 A Golgi apparatus from cauliflower inflorescence, isolated by differential centrifugation and showing the interface structure between some of the cisternae. Fixation in 0.0025 per cent glutaraldehyde, postfixation in 1.0 per cent OsO_4. × 87,000.

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