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Three-Dimensional Ultrastructure of the Golgi Apparatus in Bovine Mammary Epithelial Cells during Lactation

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The three-dimensional ultrastructure of the Golgi apparatus in milk secreting epithelial cells of bovine mammary gland was explored. From computer-aided reconstructions of serial thin sections, it was determined that the Golgi apparatus was composed of a single set of stacked cisternae. The three-dimensional shape of the dictyosome varied from cell to cell, but the overall shape was that of a hollow cone, cylinder, or bowl. The *cis* and *trans* surfaces of the dictyosome were arranged in three-dimensional space such that the *cis* face was located on the outer surface of the hollow structure and the *trans* face on the inner surface. The cytoplasmic channel (secretory channel) that traversed the longitudinal axis of the hollow dictyosome contained secretory vesicles. Densely stacked cisternae of rough endoplasmic reticulum surrounded the dictyosome, and microvesicles appeared to fuse with, or bud from, cisternae of both organelles. These findings suggest that Golgi apparatus of the lactating epithelial cell is highly organized and that the Golgi apparatus and secretory channel are essentially an independent compartment within the cell.

The Golgi apparatus in mammary epithelial cells during lactation consists of a morphologically heterogeneous set of membrane-limited compartments which appear to function in synthesis, postsynthetic modification, and compartmentation of the nonfat phase of milk (Jeffers, 1935; Feldman, 1961; Sekhri *et al.,* 1967; Helminen and Ericsson, 1968; Keenan *et al.,* 1970, 1979; Linzell and Peaker, 1971a,b,c; Beery *et al.,* 1971; Hollmann, 1974; Baumrucker and Keenan, 1975; Franke *et al.,* 1976; Sasaki *et al.,* 1978; Pitelka and Hamamoto, 1983). The ultrastructure of Golgi apparatus in the mammary epithelium has been studied extensively, and much is known about its basic features, the structural similarities among mammalian species, and the effects of various alkaloids and other drugs on structure (for reviews, see Linzell and Peaker, 1971a; Keenan *et al.,* 1974; Patton and Jensen, 1975). Little is known, however, about how the ultrastructural features (i.e., *cis* and *trans* faces) of the Golgi apparatus are arranged three-dimensionally within the cell. Speculation concerning the three-dimensional architecture of the Golgi apparatus has come through extrapolation of information from randomly cut, individual thin sections. There have been no reports describing three-dimensional reconstructions of entire mammary epithelial cell Golgi complexes, by serial thin sectioning or any other method. Reconstructions of Golgi complexes are needed, however, because they (a) can resolve a number of uncertain issues with regard to Golgi apparatus ultrastructure, and (b) may lead to a better understanding of structure–function relationship in the mammary epithelial cell.

High-resolution, three-dimensional reconstructions of cell organelles or complete cells have been relatively rare because, until recently, the techniques needed for completing such reconstructions were time-consuming and cumbersome. However, three-dimensional reconstructions have been facilitated by the recent development of computer programs designed to collect information from serial sections and then display it in three-dimensional form (LoPresti *et al.,* 1973; Levinthal *et al.,* 1974;

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Ware and LoPresti, 1975; Veen and Peachey, 1977; Crang and Pechak, 1978; Macagno *et al.*, 1979; Sobel *et al.*, 1980; Moens and Moens, 1981; Nierzwicki-Bauer *et al.*, 1983).

The purpose of the present study was to elucidate the three-dimensional architecture of the Golgi apparatus in mammary epithelial cells. The mammary Golgi apparatus was chosen for this study because its basic ultrastructural features are well documented, and because this complex appears to play a critical role in milk formation.

MATERIALS AND METHODS

Preparation of Samples for Electron Microscopy

Primi- and multiparous Holstein cows, between 2 and 7 years of age, were slaughtered between the 2nd and 12th months of lactation. About 1 hr before slaughter each animal was milked by hand. Small samples of mammary tissue, approximately 1 mm^3, were removed within 5 min of slaughter and fixed by immersion by one of the following two methods: (a) the first fixative (Franke *et al.*, 1969) consisted of a 1:1 mixture of a 2% solution of osmium tetroxide and a 4% solution of glutaraldehyde in cacodylate buffer (0.2 *M*, pH 7.0). The two solutions were mixed immediately before use. Tissue samples were fixed for 1 hr, rinsed briefly in cacodylate buffer (pH 7.0), and then postosmicated in 2% osmium tetroxide in 0.1 *M* cacodylate buffer (pH 7.0) for 3 hr, all at 4°C. (b) Fixation by the method of Karnovsky (1971) wherein tissue specimens were fixed initially in a 2.5% solution of glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.4) for 1½ hr at 4°C. Tissue samples were washed briefly in 0.1 *M* cacodylate buffer and were then postfixed for 1–2 hr in a 1:1 mixture of a 2% solution of osmium tetroxide in 0.1 *M* cacodylate buffer (pH 7.4) and a 3% solution of potassium ferrocyanide in 0.1 *M* cacodylate buffer (pH 7.4) all at 4°C. The two solutions were mixed immediately before use.

Specimens were washed in cold distilled water, soaked overnight in aqueous 1% uranyl acetate at 4°C, dehydrated in an ethanol and acetone series, and embedded in a mixture of Epon and Araldite (Poolswat, 1973). Serial thin sections were cut with a diamond knife on a Sorvall–Blum MT-2B ultramicrotome. Serial sections were retrieved on carbon–Formvar-coated copper slot grids and stained in aqueous uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965). Sections were examined and photographed with a Philips EM300 electron microscope operated at an accelerating potential of 60 kV.

Computer-Aided Reconstruction of Serial Thin Sections

Serial thin sections of over 20 mammary epithelial cells from three different cows were examined. These cells included representative examples of cells sectioned in different planes (i.e., some had been serially longitudinally sectioned, others had been serially cross-sectioned, etc.). These differing sectioning directions were used as an internal control, since reconstructed Golgi structures would be expected to appear similar regardless of how they had been sectioned. The average section thickness was estimated at about 120 nm based on the gold color of sections floating in the collecting trough. Serial sections through each of the cells were photographed at magnifications sufficient to resolve important features of the Golgi apparatus (× 5000 to 7000). These series of micrographs (100 to 140 sections for each cell) were printed at a final magnification of 12 000. The nucleus, plasma membrane and outlines of the Golgi apparatus profiles were traced from micrographs onto 8 × 10-in. sheets of acetate. The acetate "overlays" were placed in proper alignment with respect to the *X* and *Y* axes (in three-dimensional space) and a pair of corresponding fiducial marks was placed on each overlay of a series.

Tracings of Golgi apparatus outlines were entered into a VAX 11/780 minicomputer with the aid of a VICOM digital image processor. The computer was then instructed to display reconstructions of the Golgi apparatus on a video display system. These reconstructions were studied visually while they were rotated about the *X*, *Y*, and *Z* axes. Selected views were photographed directly from the display screen with a Matrix Instruments color graphic camera using Polaroid SX-70 film.

The stereo pairs were generated by taking the *y*-coordinate for corresponding points to be identical. Given an image with a boundary point having *x*-coordinate x_1 and 3-D-depth *z*, the corresponding point on the second image has *x*-coordinate x_2, where

$$x2 = x_1 \cos(A)/\cos(B)$$
$$B = \tan^{-1} z/x_1$$
$$A = B + \Delta\theta.$$

The angle $\Delta\theta$ was set to 7°. Stereo pairs were photographed to facilitate three-dimensional visualization of the Golgi apparatus.

RESULTS

Three-Dimensional Architecture of the Golgi Apparatus

The Golgi apparatus was identified as a finely reticulate cytoplasmic structure which could be distinguished readily from rough endoplasmic reticulum, secretory vesicles,

and other cellular organelles (Figs. 1–4). From a two-dimensional perspective, the Golgi complex appeared to form a large, pleomorphic network throughout the supra- and juxtanuclear regions of the cytoplasm (Figs. 1–4).

Three-dimensional reconstructions of serial thin sections showed that, in most cells, the Golgi apparatus consisted of a single mass of cisternae in which individual dictyosomes were not discernible (Figs. 5–7). From this evidence, we interpreted the Golgi apparatus to consist of a single dictyosome. By definition, a dictyosome consists of a number of cisternae, usually five or six, stacked together (Dalton, 1961). Although most cells contained one continuous dictyosome, 6 of the 20 cells examined contained one or two small, peripheral masses of stacked Golgi cisternae which were not continuous with the main dictyosome (not shown in figures).

The three-dimensional shape of reconstructed Golgi apparatus was variable, ranging from pleomorphic masses which lacked symmetry properties to hollow cones, cylinders, and bowls (Figs. 5–7). When the Golgi apparatus occupied both juxta- and supranuclear regions, it had the overall shape of a wrinked, hollow cone or cylinder with

FIGS. 1–4. Four sections of a serial series showing the Golgi apparatus in the apical region of an epithelial cell. The four sections were not printed in angular register. The plane of section is oblique with respect to the longitudinal axis of the cell. The positions of the four sections within the series are indicated in the reconstructions shown in Figs. 5, 6. The Golgi apparatus in each figure was delineated by the same contours as were used in the three-dimensional models. Specimen fixed by method (a) described under Materials and Methods.

FIG. 1. Section 27 of series. The Golgi apparatus (*cis* face, large arrows; *trans* face, small arrows) appears to form a nearly continuous ring of stacked cisternae. Note that most secretory vesicles (arrowheads) are located within the ring; nucleus (n), rough endoplasmic reticulum (er), intracellular lipid droplet (ild), mitochondria (m), alveolar lumen (l), and portion of *cis*-most cisterna (cis). × 8000. Scale bar = 2.0 μm.

FIG. 2. Section 64 of series. The Golgi apparatus (*cis* face, large arrows; *trans* face, small arrows) is W shaped in this sectional plane. In side view, the dictyosome consists of a stack of five to seven cisternae. Note that the polarity of the Golgi apparatus is such that the *cis* side of the stack is located on the outer surface of the complex, and the *trans* side on the inner surface; secretory vesicles (arrowheads), alveolar lumen (l), nucleus (n), and rough endoplasmic reticulum (er). × 8000. Scale bar = 2.0 μm.

FIG. 3. Section 80 of series. The Golgi apparatus (*cis* face, large arrows; *trans* face, small arrows) appears U shaped. Note that secretory vesicles (arrowheads) are the predominate cytoplasmic structures on the *trans* side of the Golgi complex and that rough endoplasmic reticulum (er) predominates on the *cis* side; nucleus (n), intracellular lipid droplet (ild), and alveolar lumen (l). × 8000. Scale bar = 2.0 μm.

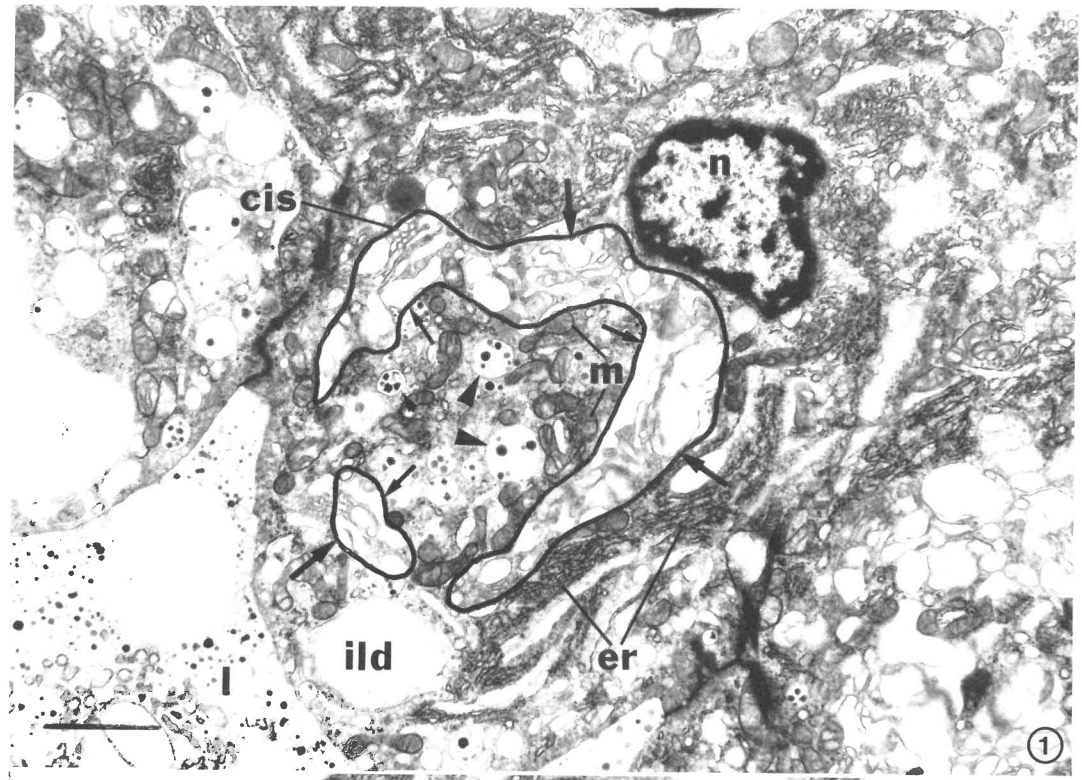
FIG. 4. Section 92 of series. Cisternae of rough endoplasmic reticulum (er) are parallel with the *cis* side of the Golgi complex (*cis* face, large arrows; *trans* face, small arrows); secretory vesicle (arrowhead), and alveolar lumen (l). × 8000. Scale bar = 2.0 μm.

FIGS. 5–7. Stereo pairs of Golgi apparatus reconstructions, illustrating the secretory channel of the complete apparatus from different rotational views. The two members of each pair are photographs of the same field but taken at different angles (±7°) from the original 0° position. Every fourth section of the serial series is displayed in the reconstructions. The magnifications are approximately × 4000.

FIG. 5. Boundary line reconstruction of Golgi apparatus from the original plane of section. The image is displayed in continuous tones from white to gray, where white is closest to the viewer and gray is farthest. The apparatus is viewed obliquely along the longitudinal axis of the cell in an apical to basal direction. The Golgi apparatus is conical and has a centrally located secretory channel (c) that traverses the entire longitudinal axis of the structure; region occupied by the nucleus (n) and sections 80 (arrow) and 92 (arrowhead) of serial series.

FIG. 6. Boundary line reconstruction in black and white rotated 35° on the *Z* axis from the original plane of section. Boundary lines located beneath areas occupied by preceding sections have been removed from the image. The secretory channel (c) of the Golgi apparatus is viewed along its central longitudinal axis in an apical to basal direction. This axis is approximately 25° from parallel with the longitudinal axis of the cell; region occupied by the nucleus (n) and sections 27 (arrow) and 64 (arrowhead) of serial series.

FIG. 7. Solid image reconstruction in continuous tones with the same rotation as that in Fig. 6; secretory channel (c), and region occupied by nucleus (n).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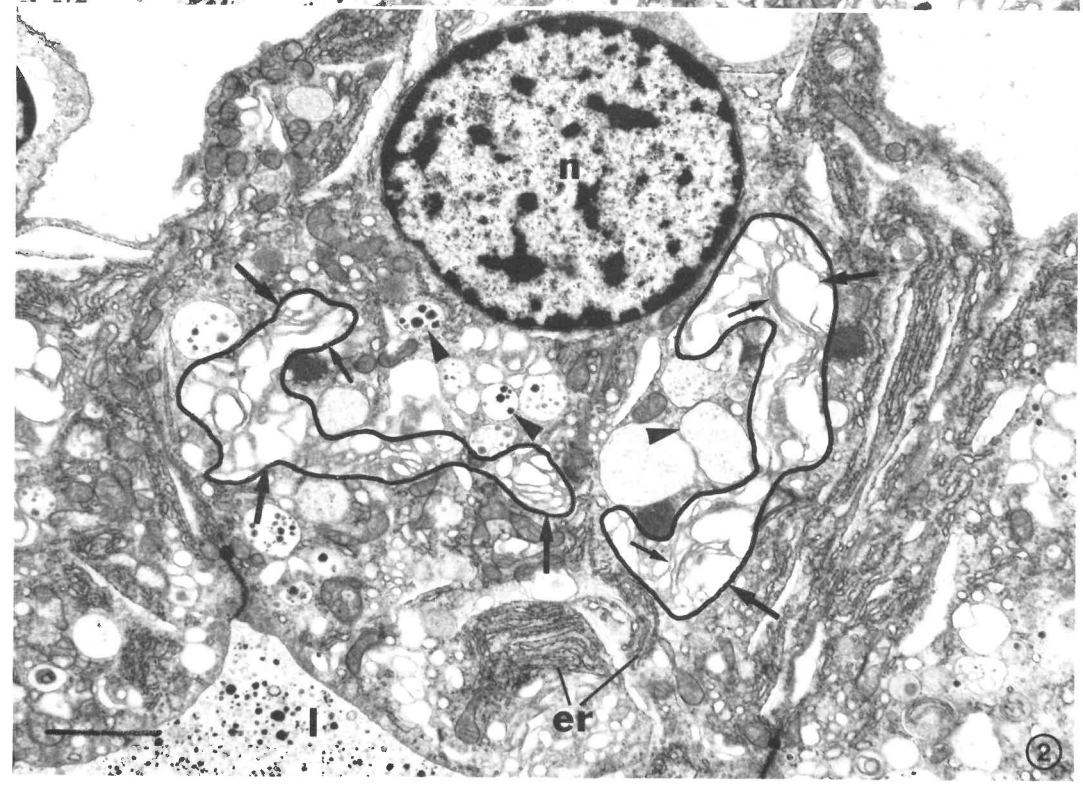
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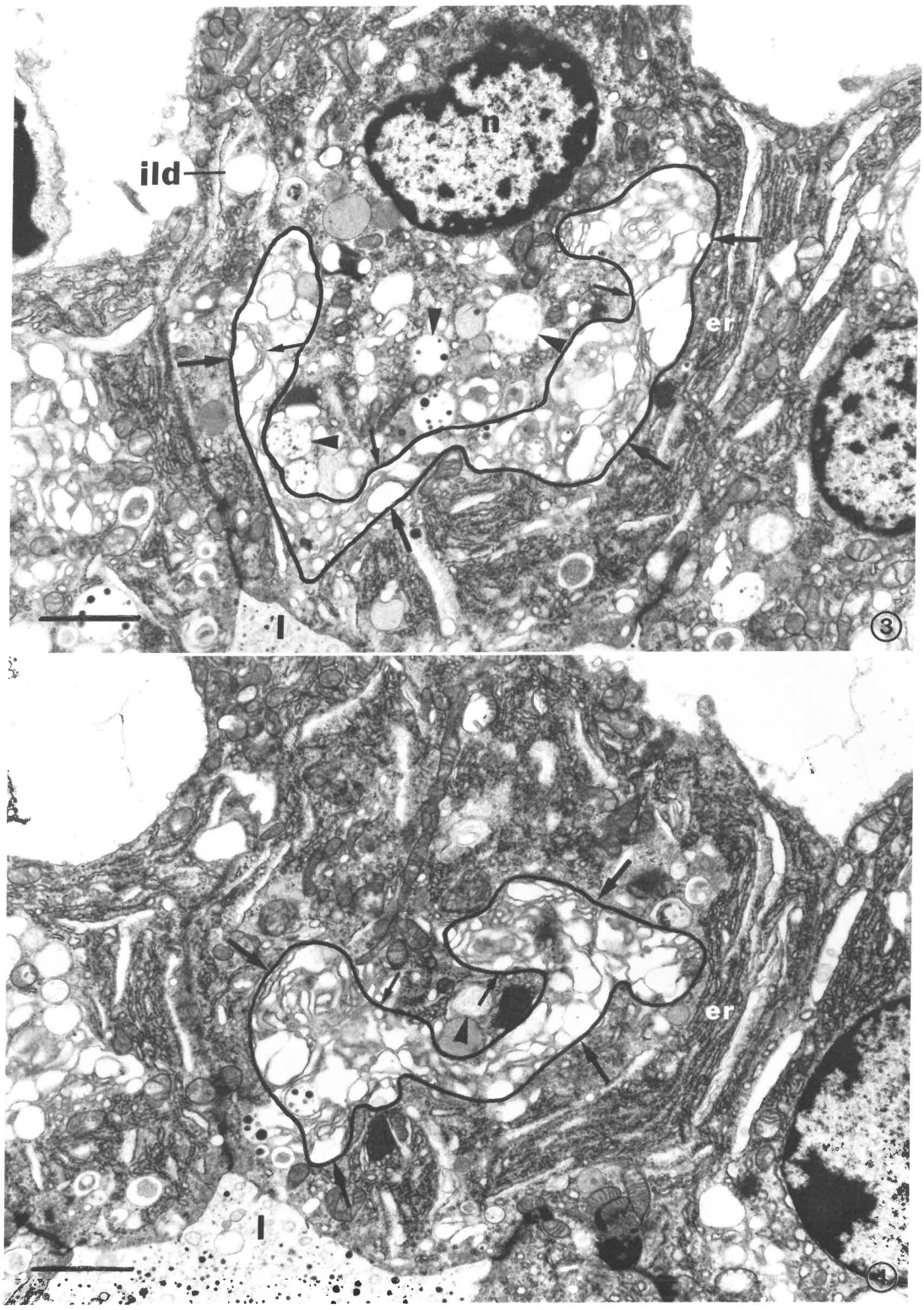
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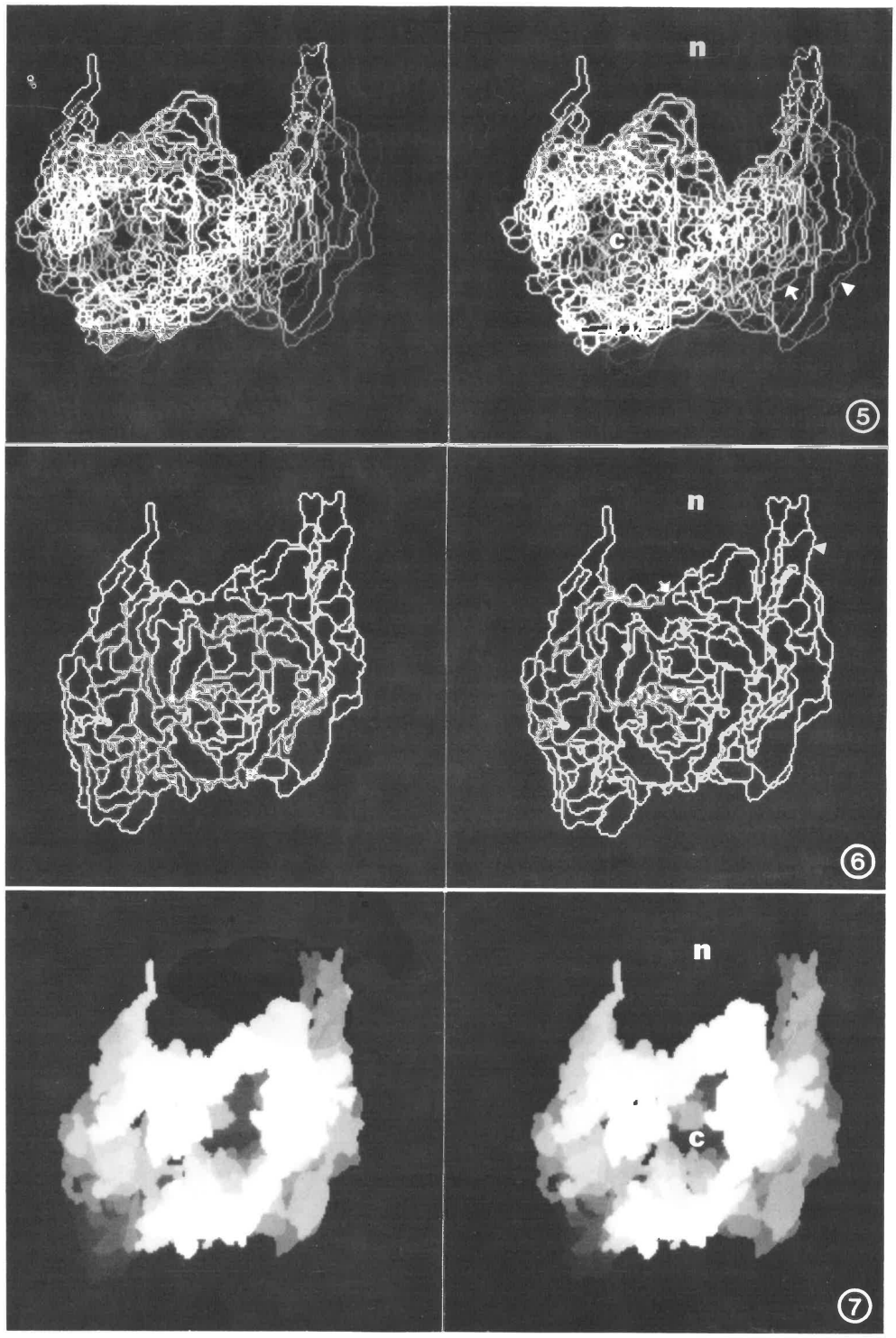
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an apical, basal, and several lateral openings or pores (Figs. 5–7). The longitudinal axis of the cone was 9.5 to 15.6 ($\bar{x}$ = 13.2) μm in length. The diameter of the cone was 3.8 to 5.8 ($\bar{x}$ = 4.5) μm at the apical end and 6.1 to 10.8 ($\bar{x}$ = 8.2) μm at the basal end. When the Golgi apparatus occupied only the supranuclear region of the cell, it was usually bowl shaped and confined laterally by the endoplasmic reticulum and basally by the nucleus (not shown in figures).

Bowl-, cone-, and cylindrical-shaped dictyosomes, with respect to *cis* and *trans* sides in three-dimensional space, appeared to be arranged such that the *cis* side was located on the outer surface of the structure and the *trans* side was located on the inner surface (Figs. 1–4). In side view, the dictyosome consisted of a stack of five to seven cisternae with an average width of 1.5 μm (Figs. 1–4). The *cis*-most cisternae of the stack was composed of a reticulum of anastomosing tubules (Fig. 1). Cisternae on the *trans* face were dilated and appeared to contain condensing secretory products (Figs. 2–4). Numerous secretory vesicles were located within the central cytoplasmic space (henceforth referred to as the "secretory channel") that traversed the longitudinal axis of the Golgi apparatus (Figs. 1–7). Secretory vesicles were presumed to arise by budding from dilated cisternae on the *trans* side of the stack (Figs. 1–4). Other cytoplasmic organelles and structures found within the secretory channel included ribosomes and mitochondria (Figs. 1–4). Rough endoplasmic reticulum and intracellular lipid droplets were never observed within the channel.

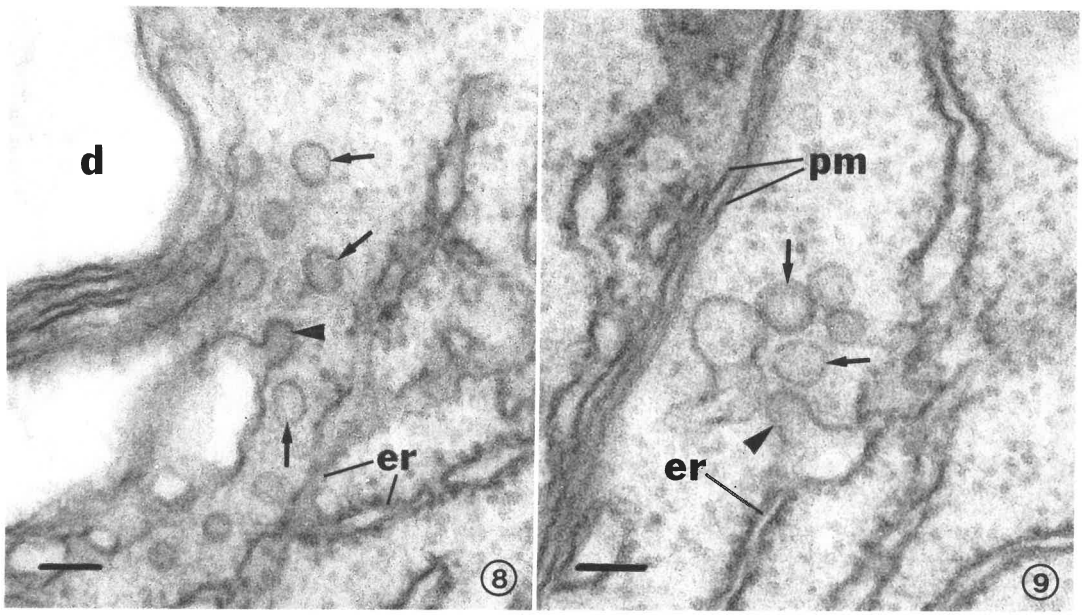
Three-Dimensional Arrangement of the Apical Zone of the Cell

Transverse sections through the apical zone of epithelial cells revealed that Golgi apparatus occupied the central region of the cell (Figs. 1–4). The longitudinal axes of the Golgi apparatus secretory channel and the epithelial cell were never perpendicular and were often nearly parallel (e.g., ± 25°) to each other (Figs. 5–7). Stacked cisternae of rough endoplasmic reticulum appeared to be parallel with the *cis* surface of the dictyosome (Figs. 1–4). In some cells, rough endoplasmic reticulum completely surrounded the Golgi apparatus.

In the cytoplasmic space between the *cis* face of the dictyosome and the surrounding cisternae of rough endoplasmic reticulum, numerous microvesicles were observed (Figs. 8, 9). These vesicles were membrane-bounded, and 0.041 to 0.084 ($\bar{x}$ = 0.065) μm in diameter (Figs. 8, 9). Profiles of microvesicles which appeared to be in the process of fusing with, or budding from Golgi apparatus cisternae (Fig. 8), and cisternae of rough endoplasmic reticulum (Fig. 9) were observed frequently. Although microvesicles were observed in tissue specimens fixed by both methods, they were much easier to distinguish in material fixed by the method of Karnovsky (1971) than by the method of Franke *et al.* (1969).

DISCUSSION

Since the work of Bargmann and Knoop (1959), electron micrographs of randomly cut, individual sections of mammary epithelial cells have been interpreted to imply that the Golgi apparatus is pleomorphic and composed of numerous dictyosomes (Wellings and Philp, 1964; Helminen and Ericsson, 1968; Pitelka and Hamamoto, 1976; Tedman, 1983). Through serial section analysis and computer reconstructions the structural organization of this organelle could be determined (Fig. 10). Our findings suggest that the Golgi apparatus in most bovine mammary epithelial cells consists of a single set of stacked cisternae with coordinated polarity in three-dimensional space. Variations in the three-dimensional shape (i.e., cylindrical, conical, and bowl-like) of the dictyosome did not appear to influence the polar arrangement of the stacked cisternae. The *cis* face of the stack was always located on the outer surface of the hollow structure and the *trans* face on the inner surface. The cytoplasmic composition of the secretory channel appeared to be rigidly seg-



FIGS. 8 AND 9. Microvesicular interactions with cisternae of Golgi apparatus and rough endoplasmic reticulum. Specimens fixed by method (b) described under Materials and Methods.

FIG. 8. Microvesicles (arrows) on *cis* side of dictyosome (d). Note profile of one microvesicle (arrowhead) which appears to be in the process of fusing with, or budding from a Golgi apparatus cisterna; rough endoplasmic reticulum (er). × 81 000. Scale bar = 0.1 μm.

FIG. 9. Cluster of microvesicles (arrows) near rough endoplasmic reticulum (er). Note profile of one microvesicle (arrowhead) which appears to be in the process of fusing with, or budding from a cisterna of rough endoplasmic reticulum; lateral plasma membranes (pm) of adjacent epithelial cells. × 95 000. Scale bar = 0.1 μm.

regated, whereby precursors of milk lipid globules (i.e., intracellular lipid droplets) were excluded from this region, and structures associated with the biosynthesis and compartmentation of milk serum were predominate. Therefore, the secretory channel and surrounding dictyosome appeared to be essentially an independent compartment within the cell. We interpret this compartment to be more functional than structural (Figs. 11, 12). Because tissue samples were not prepared for electron microscopy by methods which preserve microtubules (Warchol *et al.,* 1974) it is not known whether the apical migration of vesicles through the secretory channel is aided by the orientation of these structures. The arrangement of microtubules in the apical zone of the lactating epithelial cell has been shown to be associated with the direction of secretory vesicle migration (Nickerson and Keenan, 1979).

Numerous, smooth-surface microvesicles were located between the Golgi apparatus and stacked rough endoplasmic reticulum. We assume that these microvesicles bud from cisternae of rough endoplasmic reticulum and transport secretory products and membrane to the *cis* face of the Golgi apparatus. Vesicles of this description have been observed in many cell types (Caro and Palade, 1964; Friend, 1965; Manton, 1961; Flickinger, 1969; Susi *et al.,* 1971; Weinstock and Leblond, 1971; Chrétien, 1972; Farquhar and Palade, 1981), and are presumed to function as vehicles for the transportation of secretory products between the two organelles (Zeigel and Dalton, 1962; Kessel, 1971; Palade, 1975; Farquhar and Palade, 1981).

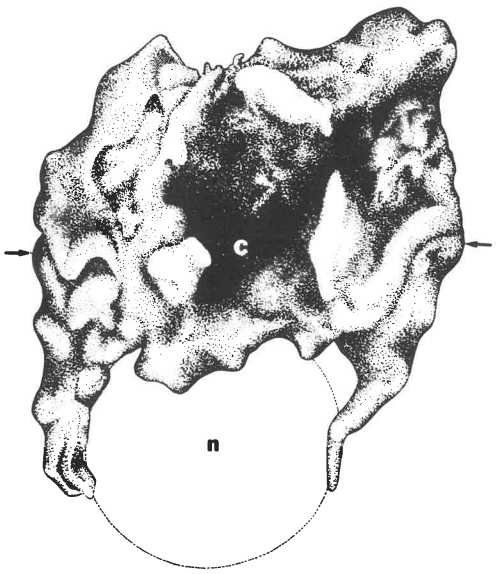
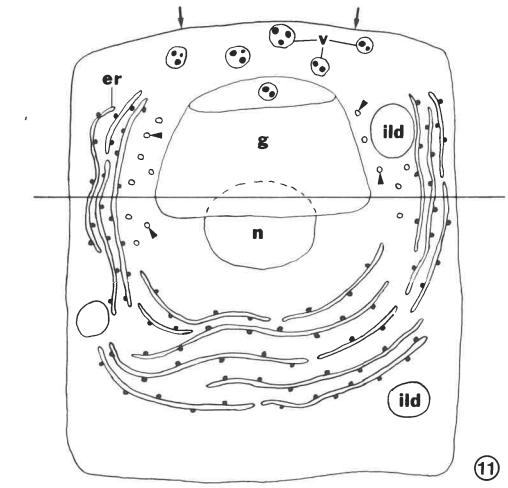
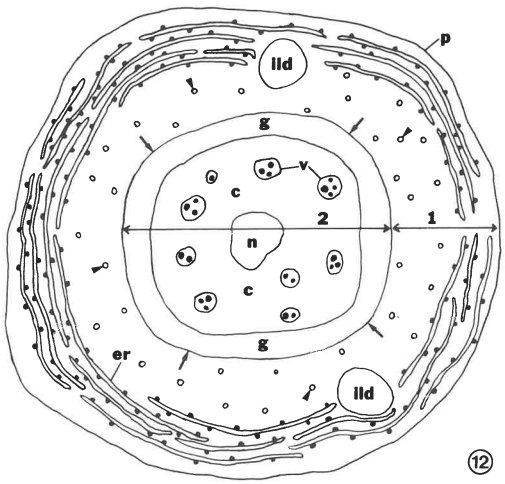


FIG. 10. Diagrammatic representation of the overall three-dimensional architecture of the bovine Golgi apparatus in mammary epithelial cell during lactation; nucleus (n), secretory channel (c), and *cis* face of Golgi stack (arrows).

Apart from the work of Rambourg *et al.* (1979) and Novikoff *et al.* (1971), who studied the interrelationships of Golgi apparatus cisternae in Sertoli cells of the rat, there have been no detailed three-dimensional reconstructions of Golgi apparatus reported for plant or animal cells. Through the utilization of cytochemical staining of thick sections, with tilting, Rambourg *et al.* (1979) presented evidence that the Golgi cisternae in Sertoli cells are extensively interconnected, and he interpreted the Golgi apparatus to consist of a single set of stacked cisternae.

Apparently, the presence of one unit per cell of a cytoplasmic organelle is not uncommon, and appears to be unrelated to cell function or phylogeny. Unlike Golgi apparatus, there have been several three-dimensional reconstructions of mitochondria (Belcher, 1968; Schötz, 1972; Schötz *et al.*, 1972; Arnold *et al.*, 1972; John *et al.*, 1973; Atkinson *et al.*, 1974). The majority of this work has been conducted on unicellular plants and microflagellates. Although most





FIGS. 11 AND 12. Diagrammatic representations of the spatial arrangement of organelles in the epithelial cell during lactation.

FIG. 11. Medial longitudinal section of epithelial cell showing the hollow, conical juxtanuclear Golgi apparatus (g) surrounded by rough endoplasmic reticulum (er); intracellular lipid droplets (ild), nucleus (n), microvesicles (arrowheads), secretory vesicles (v), and apical plasma membrane (arrows). The line which traverses the cell indicates the level of section in Fig. 12.

FIG. 12. Medial transverse section of epithelial cell illustrating the separation of organelles into two intracellular compartments or regions. Region 1 extends from the lateral plasma membrane (p) to the *cis* side (arrows) of the juxtanuclear Golgi complex (g) and includes rough endoplasmic reticulum (er), microvesicles (arrowheads), and intracellular lipid droplets (ild). Region 2 includes the Golgi apparatus (g), the secretory channel (c), and secretory vesicles (v) within the channel. Grazed nucleus (n).

of these organisms were found to contain more than one mitochondrion per cell, some contained a single, simple mitochondrion (Manton, 1959, 1960, 1961), and others contained a single, extensive mitochondrial reticulum (Arnold *et al.,* 1972; Schötz, 1972; Schötz *et al.,* 1972; John *et al.,* 1973; Atkinson *et al.,* 1974).

Perhaps the most intriguing general aspect of the Golgi apparatus in mammary epithelial cells is the fact that the three-dimensional architecture was so consistent from one cell to another. Such an intracellular organization must be the result of accurate control mechanisms or it would not be reproduced so consistently in each cell. It should be realized that although this structural arrangement appears to be fairly constant in Holstein cows, complete three-dimensional analyses of Golgi complexes from other mammalian species would be needed to determine if this is a universal feature of all mammary epithelial cells.

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