# STRUCTURE OF ISOLATED PLANT GOLGI APPARATUS REVEALED BY NEGATIVE STAINING

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## ABSTRACT

Sucrose-gradient–purified dictyosomes of plant Golgi apparatus appear, after glutaraldehyde stabilization, as stacks of highly fenestrate and tubate cisternae when negatively stained with phosphotungstic acid, shadowed with heavy metal, or $OsO_4$-stained in thin section. The tubular proliferations (diameter 200 to 400 A) extend for several microns from the central region and are united at intervals into an anastomosing network. Associated with the tubules are two kinds of vesicles which are distinguishable on the basis of texture, size, shape, and staining characteristics. One vesicle type is rough-surfaced, nearly spherical, and of uniform dimensions (diameter approximately 600 A). Metal shadowing shows that these vesicles remain spherical after drying. The other vesicle type is smooth-surfaced and varies in both size and shape. Intercisternal elements are revealed, by negative staining, on the surface of internal cisternae after fragmentation of the dictyosome. The progressive differentiation of cisternae from the forming face to the maturing face is observed in thin sections of these isolated preparations. The morphological characteristics observed in negatively stained dictyosomes indicate regions of functional specialization within the dictyosome cisternae and reveal a dictyosome structure more extensive than that envisioned from sections.

## INTRODUCTION

The Golgi apparatus of plant and animal cells has been visualized as consisting of stacks of flattened cisternae (dictyosomes)[1] and associated vesicles (1). An element has recently been described bisecting the intercisternal space after glutaraldehyde-$OsO_4$ fixation (2, 3). Although a secreting face and a forming face of the Golgi apparatus as well as different vesicle types have been recognized (4), regions of structural specialization are usually difficult to detect in sectioned and stained material.

The isolation of dictyosomes from plant cells (5, 6) has facilitated the analysis of the morphology of the Golgi apparatus. Structural transformations (5) and fenestrated cisternae with tubular interconnections between groups of dictyosomes have been reported (6). Intercisternal bonding is evidenced by the fact that Golgi apparatus can be isolated intact. More recently, structural components of the intercisternal region have also been observed in isolated Golgi apparatus from

[1] The following definitions will be used in this paper. A cisterna is a single platelike membranous structure along with its attached tubules and vesicles. A number of cisternae usually five or six, stacked together constitutes a dictyosome. A group of interassociated dictyosomes forms a Golgi apparatus.

cauliflower inflorescences (2) stabilized with glutaraldehyde. In the present study, the technique of negative staining has been used to study glutaraldehyde-stabilized, isolated dictyosomes. This technique has proven useful in an analysis of the morphology of the dictyosome and has revealed some details not previously described.

## MATERIALS AND METHODS

Dictyosomes were isolated from onion (*Allium cepa*) stem and attached leaf bases by the methods of Morré and Mollenhauer (5, 6) with the aid of glutaraldehyde stabilization. Cells were broken by means of chopping the tissue with a motor-driven, cam-operated chopping device fitted with ten double-edged razor blades spaced 5 mm apart and operated at ca. 30 chops per sec. The chopping surface was a Plexiglas trough.

Dictyosomes, purified by sucrose gradients, were washed by centrifugation in homogenizing medium. One half of each pellet was prepared for sectioning by overnight postfixation in cold 1% osmium tetroxide (buffered to pH 7.3 with 0.1 M phosphate). The other half of the pellet was resuspended in a 0.05% bovine serum albumin solution, mixed with an equal volume of 2% phosphotungstate (PTA) (neutralized with KOH to pH 6.8), spread on carbon-stabilized, collodion-coated grids, and air-dried. Similar samples were dried on grids without added PTA and were shadowed with palladium at a 12° angle. Whole tissue was fixed with 2.5% glutaraldehyde at pH 7.3 for 1 hr at 0°C and postfixed in 1% $OsO_4$ for 1 hr. Materials to be sectioned were embedded in the Epon-Araldite mixture of Mollenhauer (9). Specimens were observed and photographed using a Philips EM 200 or a Siemens Elmiskop I.

## RESULTS

Isolated dictyosomes which have been stabilized with glutaraldehyde are composed of stacks of highly fenestrate, tubate cisternae (Figs. 1–3). Negative staining shows that the tubular proliferations of the cisternae may extend more than 1 $\mu$ beyond the compact central region, uniting at intervals into an anastomosing network. The diameter of the tubules varies from 200 to 400 A. Although contrast is very low without the addition of some electron-opaque material, these isolated dictyosomes can be dried on a grid and observed to be tubular in the absence of stain.

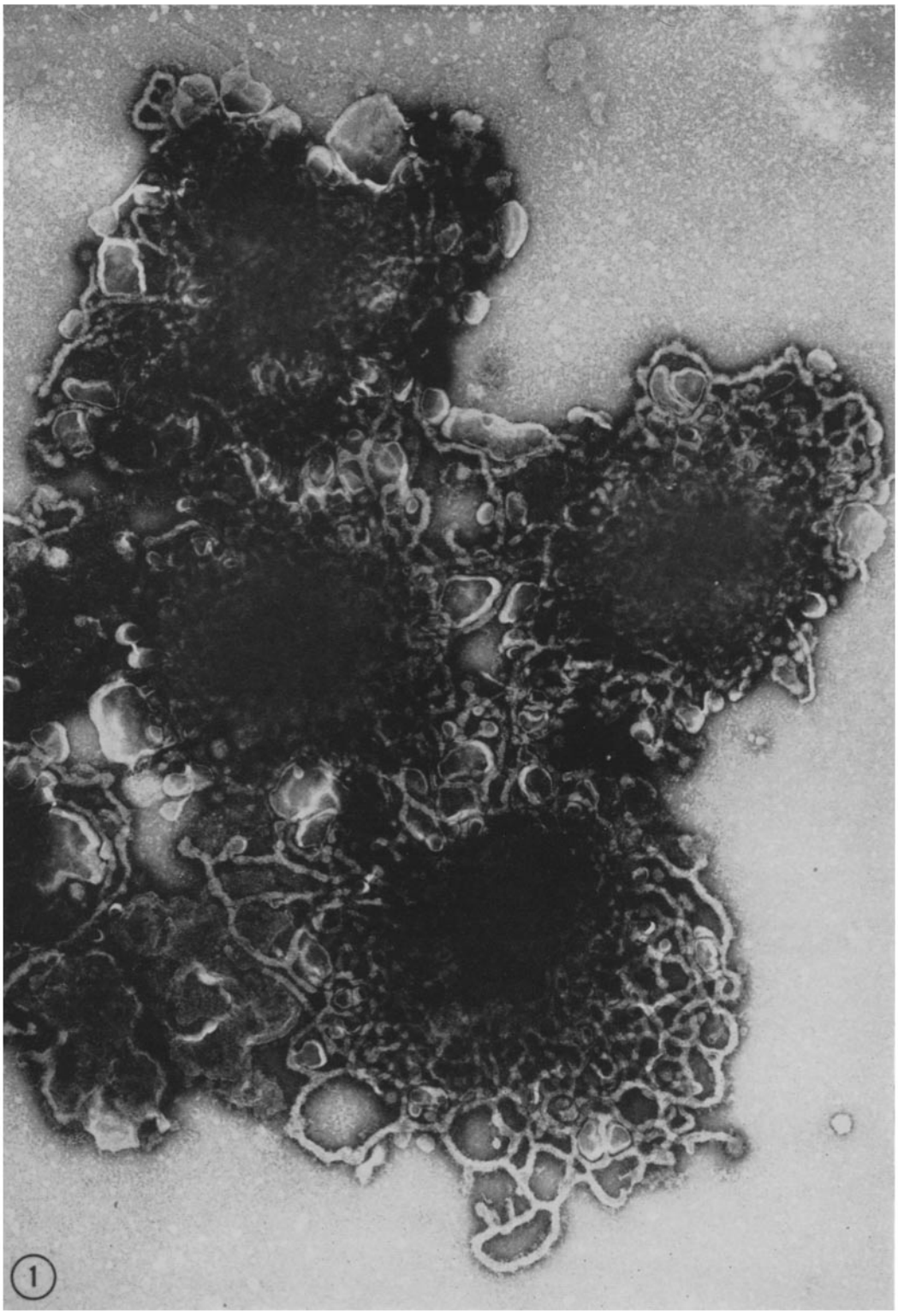
When the isolated glutaraldehyde-stabilized dictyosomes are postfixed in $OsO_4$, embedded and sectioned, typical profiles of stacked cisternae are observed (Fig. 2). Sections tangential to the surface of a cisterna show an anastomosing tubular system similar to that seen with PTA. In both negatively stained material and sectioned material, the compact central region appears to be composed of discs less than 1 $\mu$ across. Because of the tubular complex, however, the entire dictyosome often extends for more than 3 $\mu$. The network is clearly revealed in the surface views of negatively stained dictyosomes (cf. Figs. 1 and 2 *b*).

The differentiation of cisternae from the forming face to the maturing face is preserved in the isolated, sectioned dictyosomes. The cisterna of the maturing face appears compressed (Fig. 2 *b*, arrow; see also Fig. 1 of reference 3). The cisterna at the forming face is often swollen (the degree being influenced by preparative conditions).

Many vesicles are associated with isolated dictyosomes (Figs. 3 and 4), and the majority of them is clearly attached to the tubular cisternal extensions. After PTA staining, two vesicle types are distinguishable on the basis of morphology and staining characteristics (see single cisterna of Fig. 4). One type of vesicle is nearly spherical. It is of uniform size (diameter ca. 500 A) and has a rough surface which attracts stain. The other type of vesicle varies widely in size and form and has a smooth surface which does not attract stain (Fig. 4). Smooth vesicles appear flattened, round to oblong, with a diameter of 200 to 800 A. PTA accumulates in the centers of this vesicle type, leaving a thick electron-transparent border which sometimes has a multilayered appearance. Small blebs extending from the tubule walls and large sheets of membrane which frequently sediment with the isolated dictyosomes also exhibit this multilayered appearance.

Attachment of the two vesicle types to the cisternae differs. Rough vesicles are found at the end of tubules or are attached by a short stalk to the periphery of the tubular network. Smooth vesicles

FIGURE 1 Several dictyosomes with stacked, cisternal elements characteristic of these preparations. Groups of dictyosomes often appear together, a fact which suggests that tubes may entwine or are interconnected. Stained with phosphotungstic acid. × 42,000.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are either attached directly to the side of a tubule or interposed between two segments of a tubule. Smooth vesicles are usually located within the tubular network or, occasionally, at the center of a cisterna.

Fragmentation of the Golgi apparatus sometimes occurs during isolation, and one or two of the smallest cisternae separate from the dictyosome. Both single cisternae (Fig. 4) and incomplete cisternal stacks (Fig. 5) can be recognized in negative stain. Heavy accumulations of PTA usually occur in the dictyosome centers and obscure detail. This region can be clearly observed only with single cisternae. The cisterna shown in Fig. 4 has a rather small nonperforate central region. The extent of this central region seems to vary, possibly depending on the position of the cisterna within the dictyosome. At the present time we have not observed enough single cisternae to determine whether there is a strict correlation between the compact central region seen in negative stain and the cisternal profiles seen in section. Rough-surfaced vesicles predominate on the smaller cisternae, while larger cisternae (Fig. 4) have a mixture of rough- and smooth-surfaced vesicles.

Intercisternal elements are revealed on the surface of dictyosomes from which some of the outer cisternae have been removed (Fig. 5). The elements appear to be arranged in a single layer of parallel fibers extending from one side of the cisterna to the other but rarely covering the entire cisternal surface. Groups of fibers often extend beyond the dictyosome or lie adjacent to it on the substratum.

After palladium shadowing (Fig. 6), the tubules seem to be considerably flattened and disrupted. Spherical vesicles about 600 A in diameter occur around the periphery of the cisternae but surface details are not revealed. The shadows of these vesicles show that they remain spherical even after drying.

Many vesicles are associated with the dictyosomes in sections (Figs. 2 *a* and 2 *b*). Most of the vesicles are round and range from 200 to 800 A in diameter. After postfixation in $OsO_4$, the smaller vesicles appear electron-opaque. These vesicles may be the type that remain spherical after shadowing, as well as the type that are rough-surfaced in PTA-stained preparations. The tubate cisternae are observed in sections of glutaraldehyde-fixed cells as well as in isolated preparations. The similarity of the longitudinal and tangential sections of dictyosomes in both whole cells and isolated preparations (cf. Fig. 7 *a* and *b* with Fig. 2 *a* and *b*) suggests that the tubate structure revealed by negative staining is not an artifact of the isolation procedure.

Chemical evidence suggests that glutarladehyde stabilization occurs primarily by cross-linking of the free amino groups of adjacent polypeptide chains (7). Studies in progress comparing various cell organelles (chloroplasts, mitochondria, proplastids, endoplasmic reticulum, and nuclei) indicate that glutaraldehyde preserves organelle structure to an extent not currently obtainable with other methods. Chloroplasts, isolated by the same methods employed here for dictyosomes, retain the outer limiting membrane, closely appressed lamellae, and dense stroma characteristic of plastids observed within the cell after glutaraldehyde-$OsO_4$ fixation.

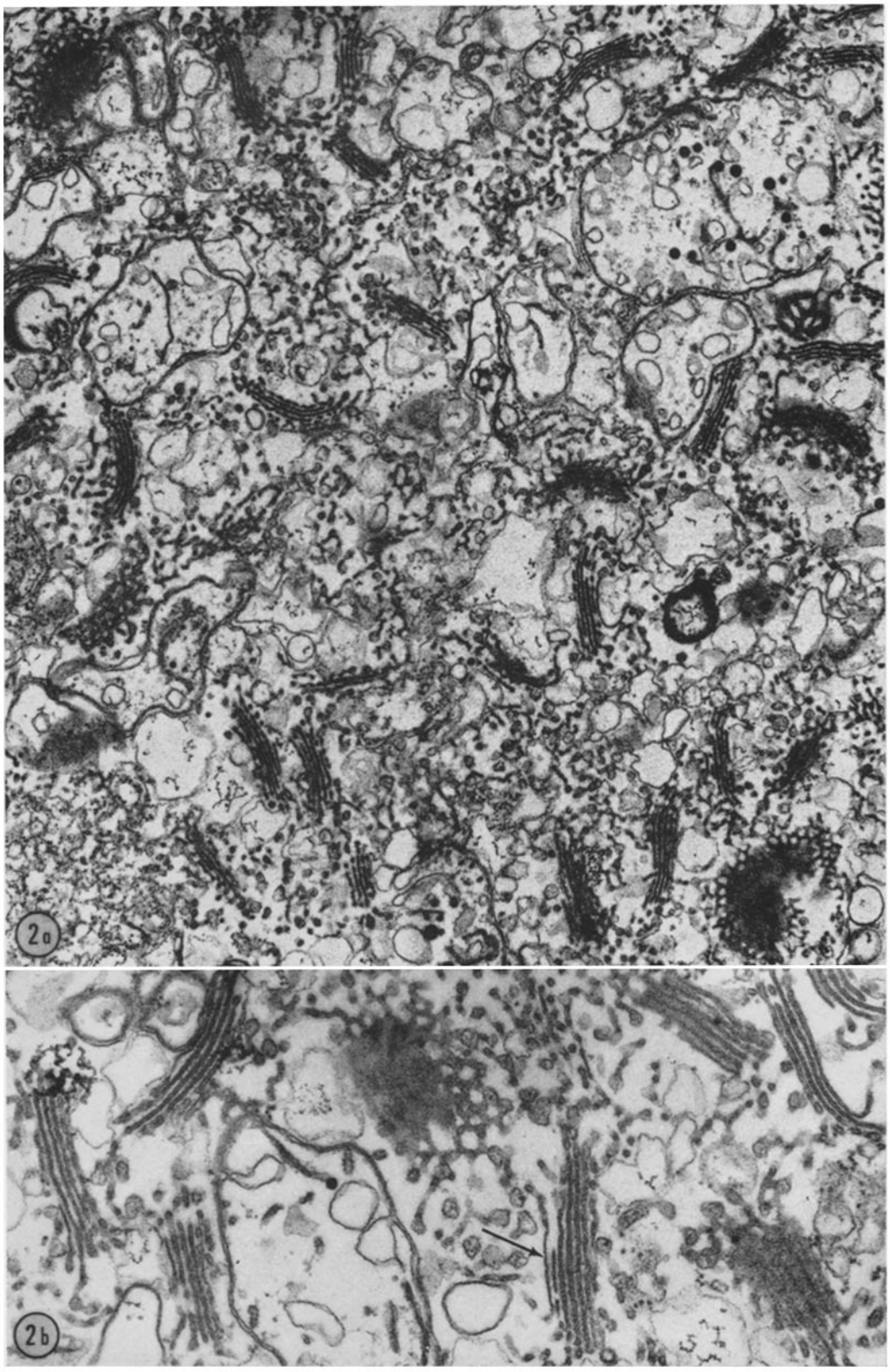
## DISCUSSION

The cisternae of glutaraldehyde-stabilized dictyosomes are not flattened sacs. Negative staining reveals that they are composed of plates and a highly branched system of tubules. The tubules are concentrated around the nonperforate central region and give rise to the form of the cisterna seen in sectioned material. The over-all structure, including the tubular extensions, possibly corresponds to the Golgi region (cisternae plus associated vesicles) of sectioned material.

The cisternal membranes are continuous with two kinds of vesicle membranes. Studies of nega-

FIGURE 2*a* A thin section from the same preparation shown in Fig. 1 stained with $OsO_4$. These preparations are highly enriched with respect to dictyosomes, which aided in the positive identification of the negatively stained image. Poststained with uranyl acetate and lead citrate. × 20,000.

FIGURE 2*b* A thin section from same pellet, enlarged to show constricted cisternae of the maturing face (arrow) of dictyosome. × 42,000.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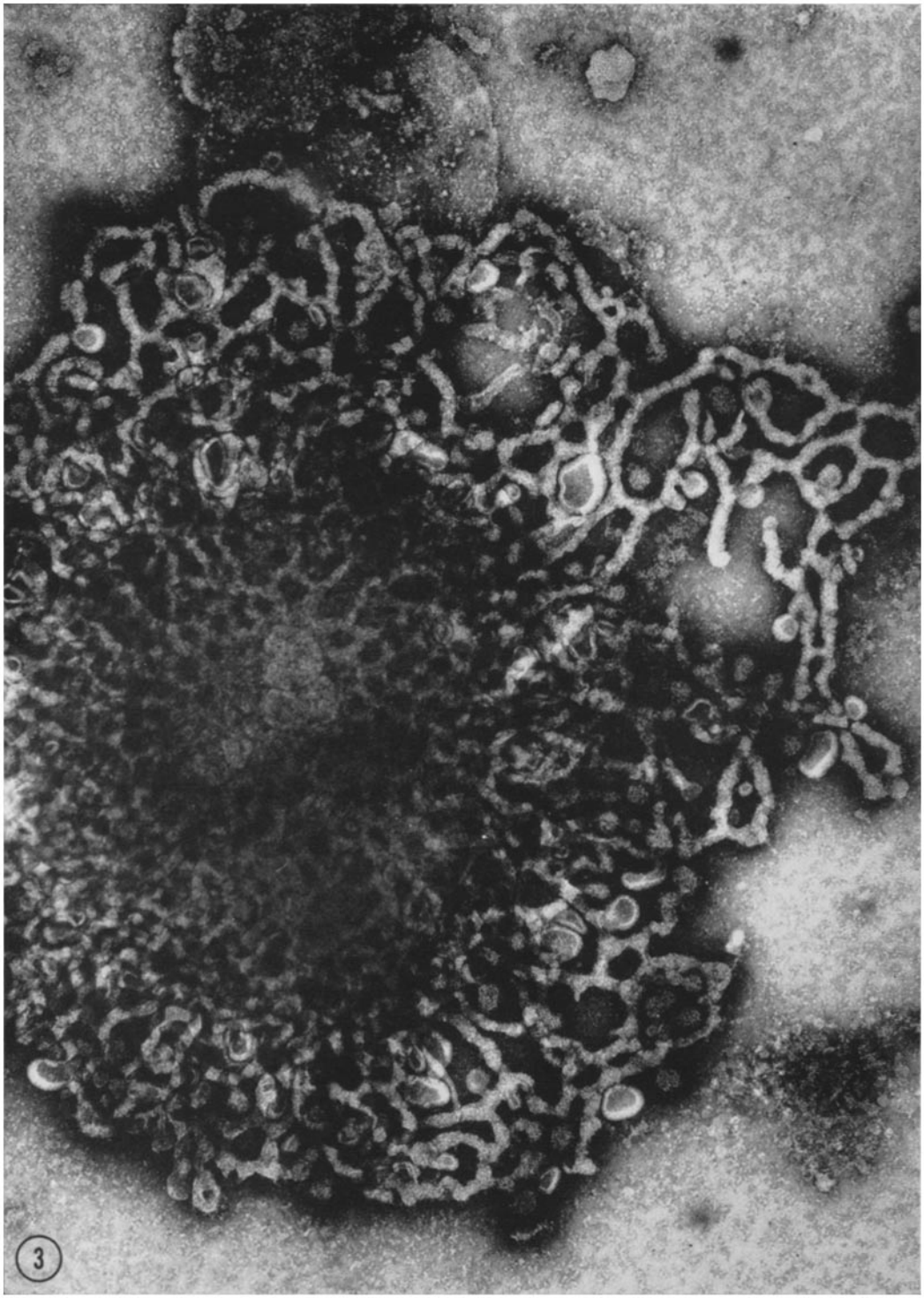



FIGURE 3 Enlarged view of a single dictyosome stained with phosphotungstic acid. × 71,000.

tively stained microsomal fractions from plants and animals (Cunningham and Morré, unpublished) suggest that the rough-surfaced vesicles of the dictyosomes stain the same as does the granular endoplasmic reticulum. The appearance of the smooth vesicles observed in negative staining, on the other hand, resembles that of the cell membrane (Cunningham and Crane, in preparation). A wide variety in the number, size, and shape of these smooth vesicles has been observed. This may indicate that they are in various stages of formation or incorporation. It may also indicate that they are artifacts of the preparatory procedure. Secretory vesicles of plant dictyosomes have been identified only in sections of secretory cells where the large size and distinctive staining characteristics of the vesicles make possible their recognition.

Apparently, the component cisternae of a dictyosome are not equivalent. They differ structurally and probably have different functional capabilities. The differences were described by Grassé (10) and Grimstone (11) in protozoa as distal and proximal poles of the dictyosome, and by Mollenhauer and Whaley (4) in higher plants as a progressive maturation of cisternae, beginning at a forming face and terminating with a maturing face. Cisternal maturation is accompanied by progressive formation of intercisternal elements (2, 3) and by constriction of the intracisternal lumen. As a result, the forming and maturing faces of plant dictyosomes can be distinguished in the absence of secretory vesicles or after isolation from the cell.

The concept of membrane transformations in the Golgi apparatus can be derived indirectly from the evidence for a forming face of the dictyosome and progressive formation of secretory vesicles along the cisternal stack. Cisternal components ultimately separate from the dictyosome, and maintenance of the dictyosome depends upon the formation of new cisternae on the opposite or forming face. A similar inference regarding membrane transformations can be drawn from membrane dimensions reported by Sjöstrand (14) and Yamamoto (15) and from the observations of Novikoff (16) and of Daniels (17). Experimental evidence for continuous renewal of dictyosome cisternae has been reported by Grimstone (11).

That there is some bonding constituent between the cisternae and within the intercisternal matrix is indicated by the fact that dictyosomes can be isolated and by the relatively constant minimal spacing of about 115 A which exists between adjacent cisternae, both within the cell and in isolated preparations. The existence of an intercisternal element (dense line) has been demonstrated (2, 3) using glutaraldehyde-stabilized plant material, postfixed with osmium tetroxide. As with microtubules (12), these elements are not often preserved in the absence of glutaraldehyde stabilization. In cross-section, these lines appear discontinuous and suggest a repeating pattern (3). In isolated preparations, what is probably this same structure appears in face view as a system of more or less parallel fibrous elements extending over part of the cisternal face. The center-to-center spacing of the fibrous elements is about 100 A. In sectioned material, the elements appear to have the same orientation throughout the dictyosome (2, 3).

The nature of the intercisternal region may vary depending upon its position within the cisternal stack (2, 3). Although principally localized within the central portion of the dictyosome, groups of fibers or material resembling these fibers may extend to, and even beyond, the periphery of the dictyosome in isolated, negatively stained preparations. This demonstrates their structural reality. Associations between microtubules and the Golgi apparatus have been reported (13), but a similarity between microtubules and the intercisternal elements has not yet been established. The common and repeating orientation of intercisternal fibers suggests a structural anisotropy orthogonal to the functional polarity of the dictyosome.

Thus, negative staining techniques have revealed a complex dictyosome structure with clearly recognizable regions of morphological specialization. A tubular network extending through the cytoplasm would account for the fragility of the Golgi apparatus as well as provide a possible means of intercommunication among dictyosomes (6, 18). According to current concepts (1, 4, 16), the Golgi apparatus participate in compartmentalization of secretory products and transformation of cytomembranes. In many respects, the tubate model seems to offer a wider range of functional capability than would be expected from a Golgi apparatus constructed from flattened saclike structures.

This work was supported in part by the National Science Foundation (GB 1084) and by the American

Heart Association (Grant No. 6264). Journal paper No. 2610. We wish to thank Dr. F. L. Crane for his support of a portion of this study and for critical discussions. The assistance of Mr. R. Gamble in designing the tissue chopping device and the technical assistance of Mr. N. C. Force are gratefully acknowledged.

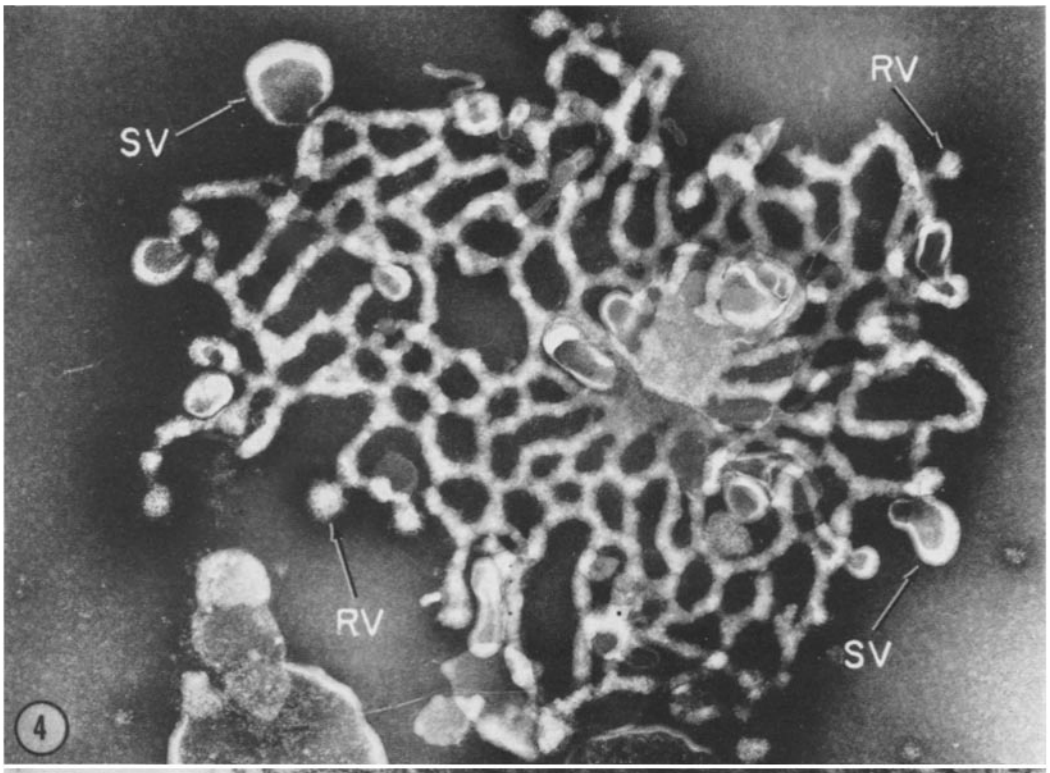
*Received for publication 30 July 1965.*

## REFERENCES

1. DALTON, A. J., Golgi apparatus and secretion granules, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press Inc., 1961, **2**, 603.
2. MOLLENHAUER, H. H., An intercisternal structure in the Golgi apparatus, *J. Cell Biol.*, 1965, **24**, 504.
3. TURNER, F. R., and WHALEY, W. G., Intercisternal elements of the Golgi apparatus, *Science*, 1965, **147**, 1303.
4. MOLLENHAUER, H. H., and WHALEY, W. G., An observation of the functioning of the Golgi apparatus, *J. Cell Biol.*, 1963, **17**, 222.
5. MORRÉ, D. J., and MOLLENHAUER, H. H., Isolation of the Golgi apparatus from plant cells, *J. Cell Biol.*, 1964, **23**, 295.
6. MORRÉ, D. J., MOLLENHAUER, H. H., and CHAMBERS, J. E., Glutaraldehyde stabilization as an aid to Golgi apparatus isolation, *Exp. Cell Research*, 1965, **38**, 672.
7. MORRÉ, D. J., MOLLENHAUER, H. H., and CUNNINGHAM, W. P., Chemical and structural analysis of dialdehyde stabilized Golgi apparatus, *Plant Physiol.*, 1965, **40**, suppl., 39.
8. BRENNER, S., and HORNE, R. W., A negative staining method for high resolution electron microscopy of viruses, *Biochem. et Biophysica Acta*, 1959, **34**, 103.
9. MOLLENHAUER, H. H., Plastic embedding mixtures for use in electron microscopy, *J. Stain Technol.*, 1964, **39**, 111.
10. GRASSÉ, P. P., Ultrastructure, polarité et reproduction de l'appareil de Golge, *Compt. rend. Acad. Sc.*, 1957, **245**, 1278.
11. GRIMSTONE, A. V., Cytoplasmic membranes and the nuclear membrane in the flagellate *Trichonympha*, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 369.
12. LEDBETTER, M. C., and PORTER, K. R., A "microtubule" in plant cell fine structure, *J. Cell Biol.*, 1963, **19**, 239.
13. SLAUTTERBACK, D. B., Cytoplasmic microtubules. I. Hydra, *J. Cell Biol.*, 1963, **18**, 367.
14. SJÖSTRAND, F. S., A comparison of plasma membranes, cytomembranes and mitochondrial membranes with respect to ultrastructural features, *J. Ultrastruct. Research*, 1963, **9**, 561.
15. YAMAMOTO, T., On the thickness of the unit membrane, *J. Cell Biol.*, 1963, **17**, 413.
16. NOVIKOFF, A. B., ESSNER, E., GOLDFISCHER, S., and HEUS, M., Nucleoside-phosphatase activities of cytomembranes, *in* The Interpretation of Ultrastructure, (R. J. C. Harris, editor), New York, Academic Press Inc., 1962, 149.
17. DANIELS, E. W., Origin of the Golgi system in amoebae, *Z. Zellforsch. u. Mikr. Anat.*, 1964, **64**, 38.
18. MOLLENHAUER, H. H., Transition forms of Golgi apparatus secretion vesicles, *J. Ultrastruct. Research*, 1965, **12**, 439.

FIGURE 4 Single cisterna showing two kinds of attached vesicles and the nonfenestrated central region. *SV*, smooth vesicle; *RV*, rough vesicle. Stained with phosphotungstic acid. × 90,000.

FIGURE 5 Surface of an internal cisterna after dictyosome fragmentation, revealing the intercisternal elements (parallel fibers). These structures are seen to extend beyond the periphery of the dictyosome (arrows). Stained with phosphotungstic acid. × 45,000.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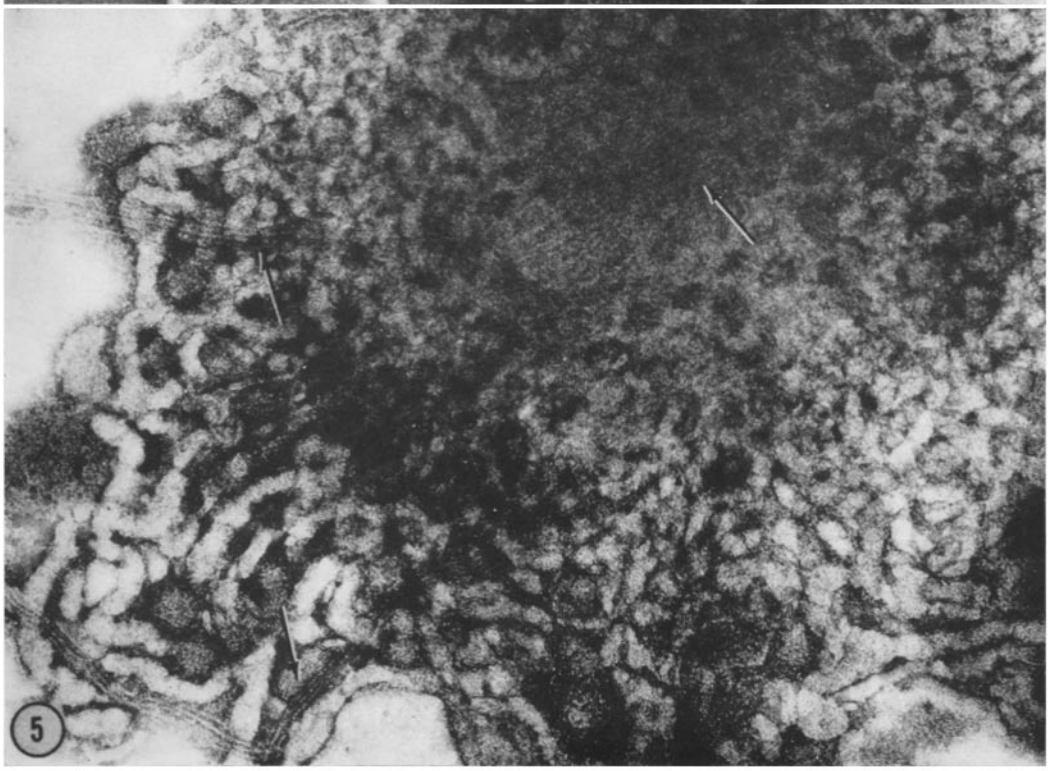
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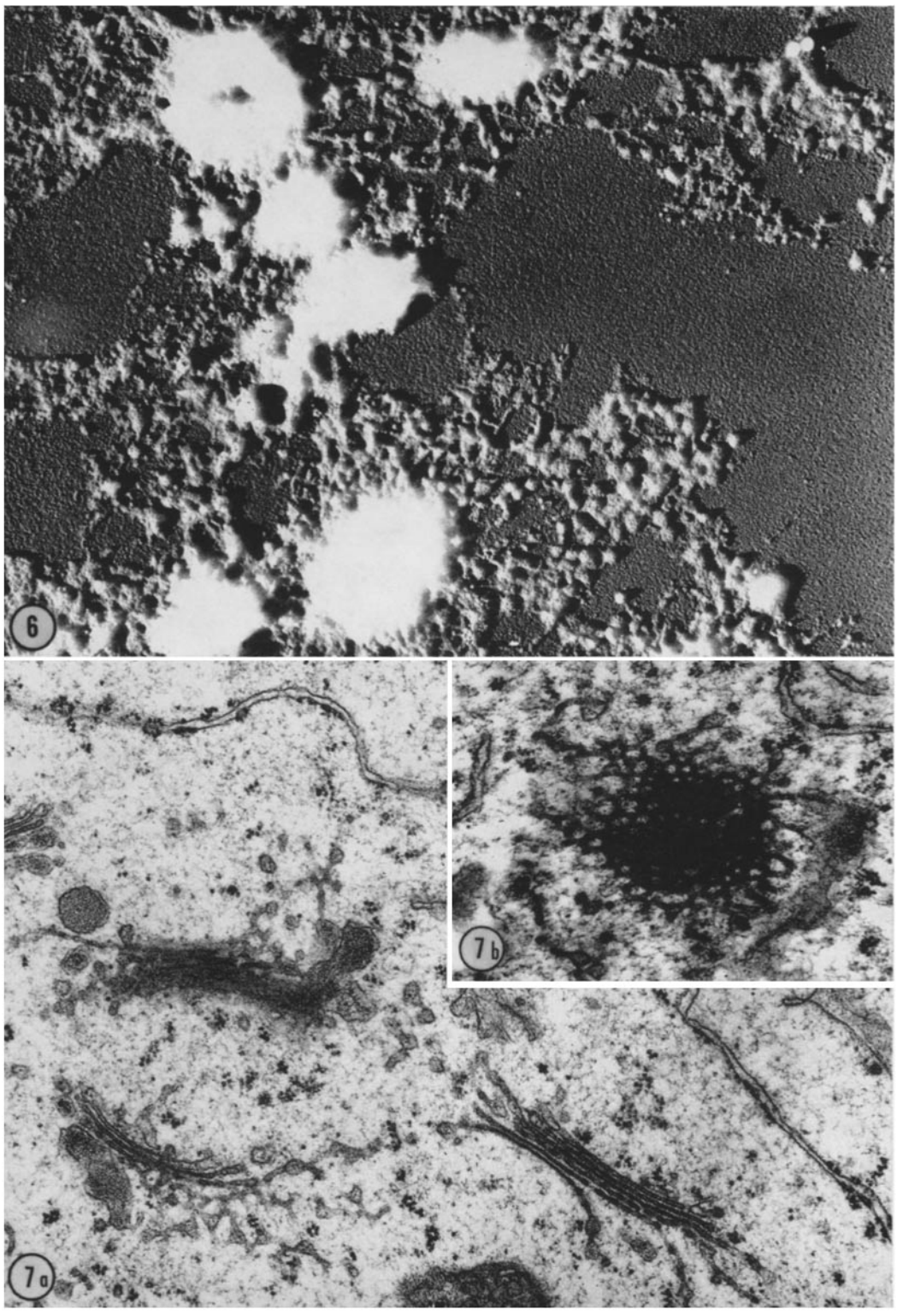
FIGURE 6 Micrograph of palladium-shadowed dictyosomes from a preparation similar to that of Figs. 1 and 2. Surface tension caused by drying may have caused flattening and some disruption of the tubules. The small spherical vesicles do not flatten, indicating noncompressible contents. × 37,000.

FIGURE 7 Glutaraldehyde-$OsO_4$ images of dictyosomes in thin sections of maize root tip. Fig. 7 *a* shows side view and Fig. 7 *b* shows face view of dictyosomes. Note that the highly fenestrated, tubate character of the cisterna may also be preserved by glutaraldehyde stabilization of whole tissue. × 42,000.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