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Inter- and Intracisternal Elements of the Golgi Apparatus

A System of Membrane-to-Membrane Cross-Links*

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Summary. Electron opaque cross-bridge structures span the inter- and intracisternal spaces and provide membrane-to-membrane connections between adjacent cisternae of dictyosomes of pollen tubes of *Clivia* and *Lilium*. Additionally, the classic intercisternal rods, characteristic of intercisternal regions near the maturing face of dictyosomes, are connected with the adjacent membranes through similar cross-bridge elements. We suggest that these structural links are responsible for maintaining the flattened appearance of the central parts of Golgi apparatus cisternae as well as for the coherence of cisternae within the stack. Observations on other plant (e.g. microsporocytes of *Canna*) and animal cells (e.g. rodent liver and hepatoma cells, newt spermatocytes) show that such an array of membrane cross-links is a universal feature of Golgi apparatus architecture. The cross-bridges appear as part of the complex "zone of exclusion" which surrounds dictyosomes, entire Golgi apparatus and Golgi apparatus equivalents in a variety of cell types.

Key words: Golgi apparatus — Membranes — Cross-bridges — Electron microscopy.

Introduction

Dictyosomes are characteristically recognized as stacks of cisternae which in their central regions are conspicuously parallel with a relatively regular separation. This ultrastructure of the Golgi apparatus raises some basic structural questions to which hitherto no answer has been found. For instance, first, what keeps the cisternae so flattened in their central region? And secondly, what binds the cisternae together within the stack to guarantee the parallel arrangement and to prevent their separation due to the action of random particle movements or cytoplasmic streaming or during isolation from the cell (Morré and Mollenhauer, 1964)? There have been only two types of non-membraneous elements so far described in the Golgi apparatus. These are the intercisternal elements (for literature see Mollenhauer and Morré, 1966) which appear to be of a fibrillar nature, and distinct fibrillo-granular aggregates which can be associated with both the maturing (Kartenbeck and Franke, 1971) and the forming pole (Franke *et al.*, 1971) of the dictyosomes. Recently, we have demonstrated (Franke *et al.*, 1971 b) that parallel membrane associations of quite different types of membranes

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are connected with electron opaque membrane-to-membrane cross-links, and we have hypothesized that membrane-to-membrane arrangements generally might be stabilized by such distinct intermembrane structures. The present study supports this concept and shows the existence of inter- as well as intracisternal elements in the Golgi apparatus of both plant and animal cells. Combining these observations we propose a general model of the dictyosome in which inter- and intra-cisternal membrane-to-membrane bridges are visualized as integrating parts which establish the characteristic morphology of the central region of the dictyosome. Intercisternal elements as first described by Mollenhauer (1965) and Turner and Whaley (1965) are envisaged as a variation in the arrangement of such bridging materials which is characteristic of regions of the maturing face of some plant dictyosomes.

Materials and Methods

Lilium longiflorum pollen tubes were obtained as previously described (VanderWoude *et al.*, 1971). Anthers of *Clivia miniata* were collected from open flowers of room grown plants. The *Clivia* pollen grains were germinated immediately after collection on the surface of 2 ml of a 10% sucrose plus 10 p.p.m. boric acid sulution and were allowed to grow for about 2 h at room temperature.

Earlier stages of microsporogenesis and pollen development were prepared from anthers of *Canna generalis* Bailey as described elsewhere (Scheer and Franke, 1972). Pieces of rat and mouse liver were taken from the animals immediately after decapitation. Morris hepatoma pieces were obtained from the leg musculature of Buffalo rats and were prepared as previously described (Franke *et al.*, 1971 b). Testes were obtained from the Alpine newt (*Triturus alpestris* Laur.) captured in the Black Forest ponds during springtime.

Fixation and washes of the pollen tubes were carried out using a multiple suction funnel device made by Hölzel-Technik, Dorfen, Germany. In this apparatus the specimen are brought onto supporting millipore filters, and the specific suspension media can be removed by suction. Fixation was performed for 30 min in a cold solution containing 2% glutaraldehyde and 0.05 M sodium cacodylate buffer, pH 7.2. The fixed specimens were then thoroughly washed with ice-cold buffer, post-fixed for 90 min in 2% osmium tetroxide in cacodylate buffer, washed several times with distilled water, then soaked for 12 h with 1% aqueous uranyl acetate and finally dehydrated in a graded ethanol series. Specimens were then transferred through propylene oxide and propylene oxide: epon (1:1) and embedded in Epon. Thin sections were cut using a Reichert OmU2 ultramicrotome, poststained with lead citrate and uranyl acetate, and examined with the Siemens Elmiskop 1 A.

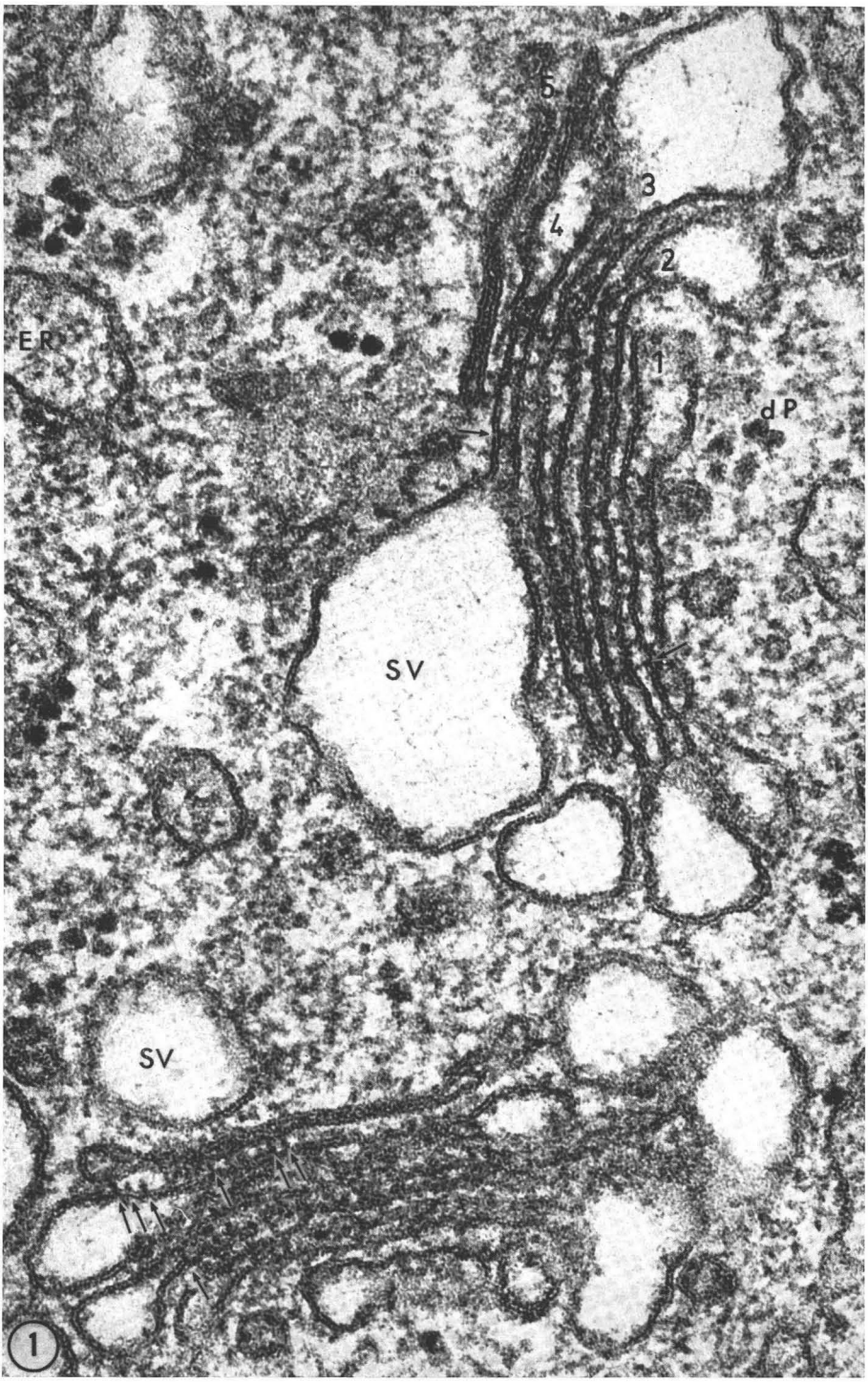
The rodent liver and hepatoma specimens as well as the newt testis material were fixed with glutaraldehyde and osmium tetroxide, either in sequential or simultaneous use, as described elsewhere (Franke, 1970; Franke *et al.*, 1971 b) and were then processed in the same way as indicated above for the pollen tubes.

Observations

Pollen Tubes of Lilium and Clivia

The dictyosomes of pollen tubes consist of 4 to 7 cisternae [see the Figs.; 65% with 6, 30% with 5, and very few have either 7 (e.g. Fig. 8) or 4]. Polarity of dictyosomes is indicated by the presence of a highly characteristic dictyosome-

Fig. 1. Dictyosomes of the *Clivia* pollen tube as seen in cross (above) and oblique (lower part) section. Cisternae are numbered in the direction from the forming to the maturing face. A "dictyosome associated polyribosome" (*dP*) is present in the cytoplasm adjacent to the forming face. Arrows denote membrane-to-membrane cross-bridges. *ER* endoplasmic reticulum; *SV* secretory vesicle. Magnification, ×155000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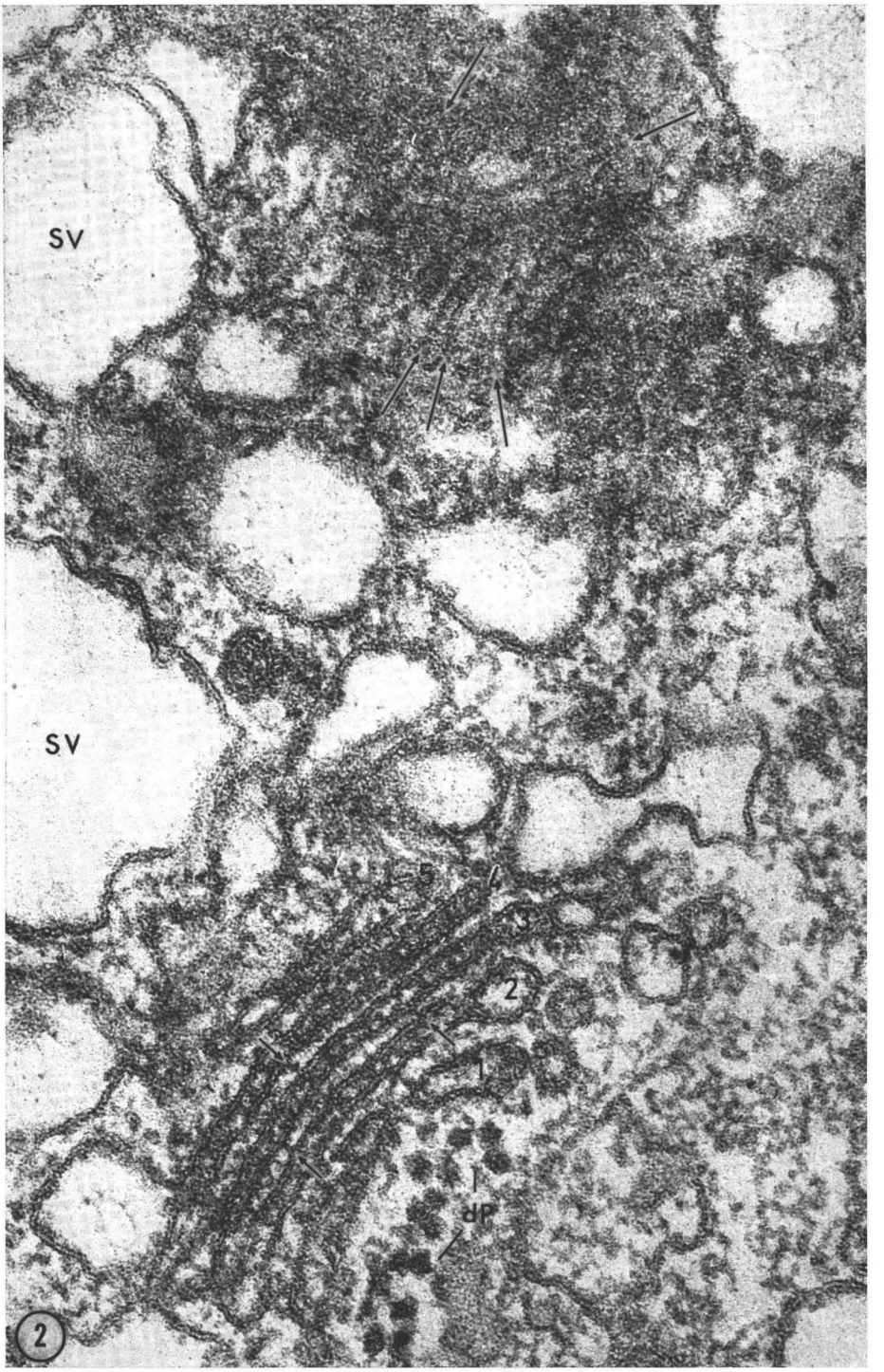
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associated polysome which obviously is non-membrane-bound and located in the cytoplasm at the forming pole (Fig. 1, 2, 6—8). Additionally, the central portions of the forming face cisternae are more highly fenestrated and a gradual decrease in the luminal width of the intracisternal space is observed from the forming toward the maturing face. The fenestrations at the forming pole show significantly more intraporous structures such as "central dots" and radiating filaments (c.f. Franke and Scheer, 1972). Frequently, membranes of mature dictyosome cisternae are slightly thicker than those of forming cisternae and show the dark-light-dark pattern more clearly (c.f. Grove *et al.*, 1968). Marginal formation of secretory vesicles is also dramatically pronounced on the mature pole although not totally restricted to it. Yet, changes in the thickness of intercisternal space across the stack were not observed in our material.

In cross-sections, electron-opaque "cross-linking" elements, oriented parallel to the dictyosomal axis, are observed to span both the intercisternal and intracisternal spaces (Figs. 1—3). Such membrane connecting elements are also recognized in oblique sections (Figs. 1 and 2). The "classic" intercisternal elements as mentioned in the Introduction are also recognized in the intercisternal regions between some cisternae of almost every dictyosome. Intercisternal elements are preferentially located in between the more mature cisternae (e.g. Figs. 3—6). In some, however, intercisternal elements were identified in between almost all the cisternae of a dictyosome (Fig. 8).

Intercisternal Connections. Such materials are recognized as somewhat irregularly shaped "granules" of a diameter of 75–100 Å (Fig. 2) or as connecting threads (ca. 50–110 Å in diameter, e.g. Figs. 1—3, 6—8) or as thin filaments (ca. 30 Å, e.g. Figs. 6, 7). The angle of these bridge-elements with the membranes is mostly perpendicular. Their length, usually coincident with the intercisternal space, was approximately 140 Å, but in a few cases as low as 65 Å. Intercisternal bridges are more prominent among the forming face cisternae but can also be identified to span the space between central cisternal surfaces, between cisternal surfaces and secretory vesicles and are occasionally seen in the interspace between membrane faces of adjacent vesicles. Sometimes the intercisternal bridges are so frequent, in particular at cisternae 2–4 within a stack, that they display a quite regular arrangement (e.g. Fig. 3). At many sites the bridging elements appear composed of granular subunits.

Intracisternal Connections. Intracisternal dense elements of variable size distribution are seen throughout the dictyosomes. They appear more frequently and are sharper in outline within cisternae near the maturing face (Figs. 3, 4, 5). Here they also are shorter as a consequence of the decrease in cisternal width characteristic of cisternae of this face. Interestingly, the intracisternal bridging elements are lacking at the, possibly in parts artificially, dilated parts, be they centrally ("central dilations", Franke 1970) or peripherally located, the latter probably representing secretory vesicles *in statu nascendi*.

Fig. 2. *Clivia* pollen tube dictyosomes in cross-section show numerous intercisternal bridges (stack in the lower part, arrows). Sections tangential to cisternae of the mature pole reveal rod-like structures (arrows in the upper right). Note the "free" polyribosome at the forming face of the lower dictyosome. Magnification, ×155000

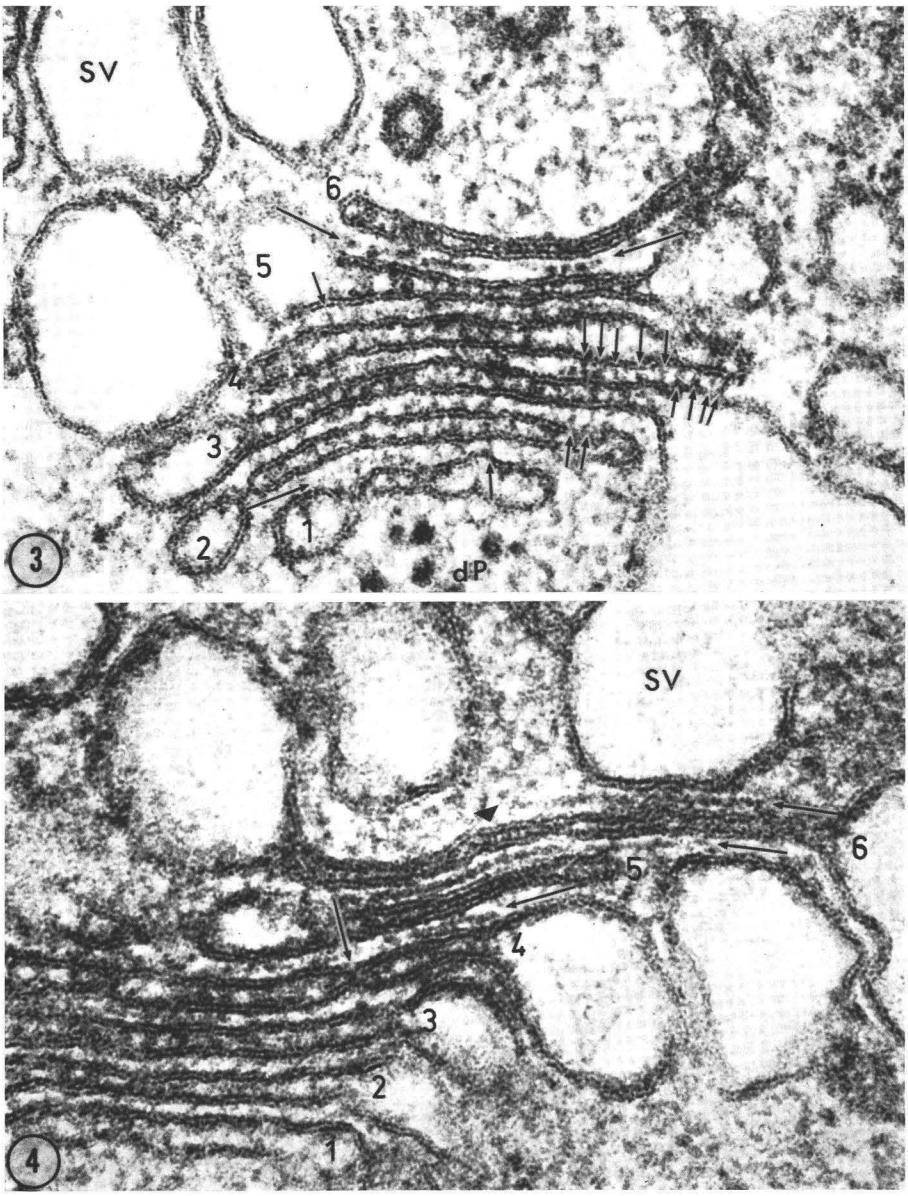
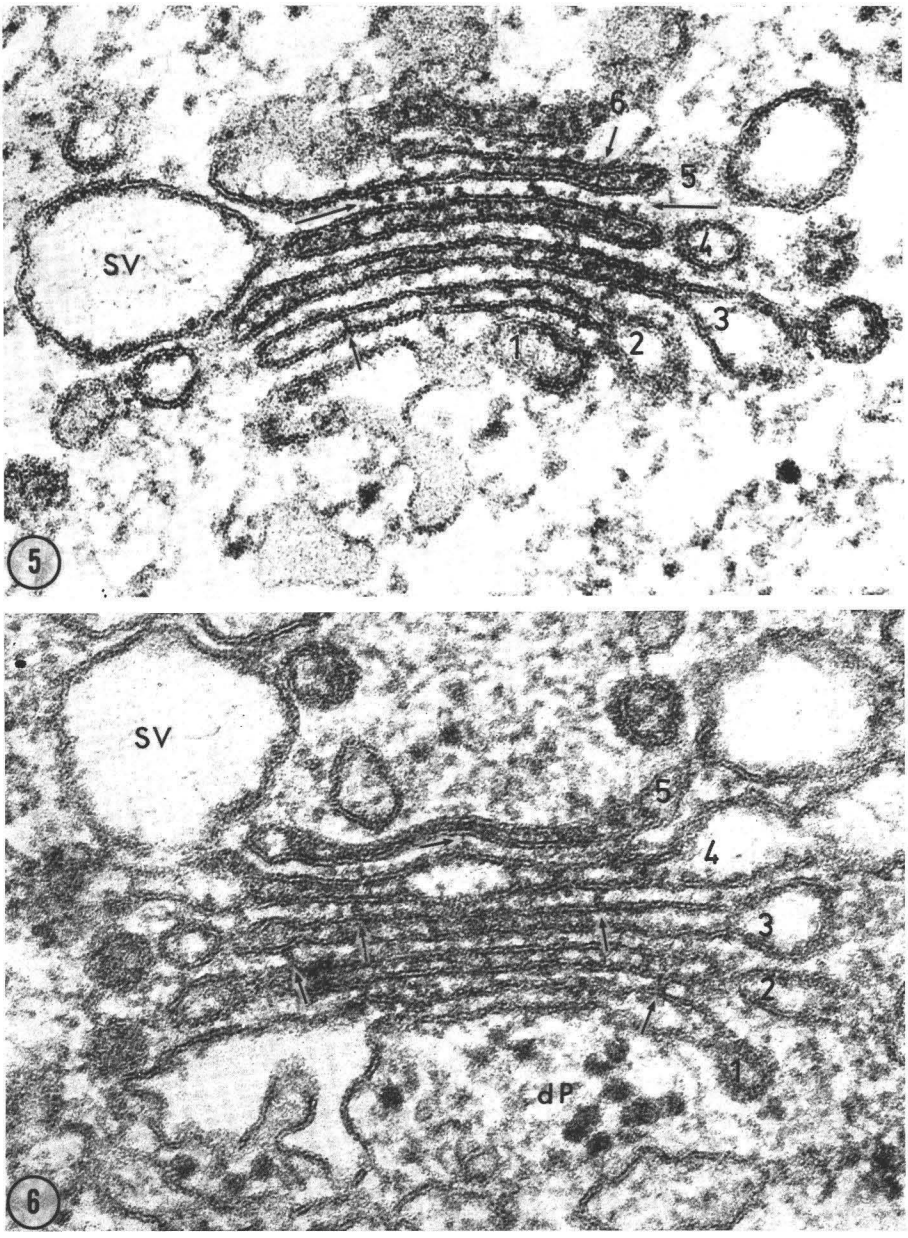


Fig. 3. Dictyosome in a lily pollen tube. Intercisternal linking elements are especially numerous (denoted by arrows) and regularly spaced. They occur within intercisternal spaces throughout the stack. Classic intercisternal elements are most conspicuous and regularly spaced at the mature pole (arrows between cisternae No. *5* and *6*) but are also occasionally recognized between cisternae at the forming face (horizontal arrow between cisternae No. *1* and *2*). Magnification, ×165000

Fig. 4. As in the previous Fig. 3 showing intercisternal rods (horizontal arrows). Short intracisternal bridges are, e.g., recognized in cisterna No. *6* (denoted by the triangle). The upright arrow indicates a site where the two membranes of cisterna No. *4* seem to converge and are in local contact, possibly the margin of a fenestration. Magnification, ×170000



Figs. 5 and 6. The intercisternal elements sometimes appear as a row of dots (left horizontal arrow between cisternae No. *4* and *5* in Fig. 5) which lie in between the cisternae or are associated with the membrane surface (at the right horizontal arrow between cisternae No. *4* and *5* of Fig. 5). Upright arrows in Fig. 5 point to intracisternal bridges whereas the upright arrows of Fig. 6 indicate intercisternal ones. Note that the membranes of the central dilation of cisterna No. *4* in Fig. 6 do not show intracisternal elements but are bridged to the adjacent cisternae with intercisternal linkers (e.g., at the horizontal arrow between No. *4* and *5*). Both, $\times 155000$

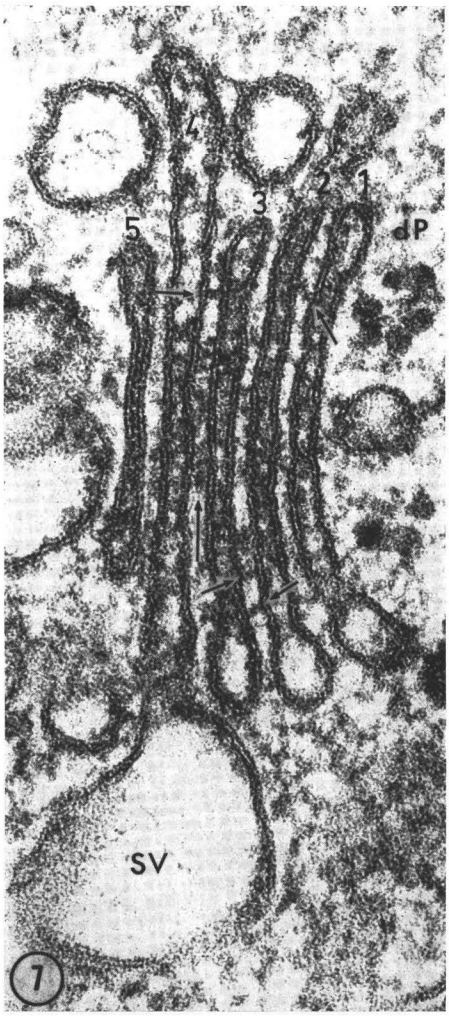


Fig. 7. Details of the structural appearance of the intercisternal bridges (arrows). The bridging elements are variable in width. Some appear as thin threads (e.g., at the upper right arrow) whereas others are granular (e.g., between cisternae No. *3* and *4*, upright arrow) or represent ca. 70 Å broad columellae. Magnification, ×160000

"*Intercisternal Element*"—*Dictyosomal Membrane Connections.* The appearance of the intercisternal elements is similar to what has been described in the literature from a variety of lower and higher plant cells (for references see the introductory chapter). They appear either as a dense line 55–80 Å in width (e.g. Figs. 3, 4, 8) or as a series of more or less equidistant and electron dense dots (e.g. Figs. 3, 5). These two views represent the two perpendicular section planes through a regular planar arrangement of rod-like structures as envisaged in the model of Mollenhauer and Morré (1966). The rod-like nature is also well recognized in grazing sections (Fig. 2). Occasionally, an intercisternal dense line

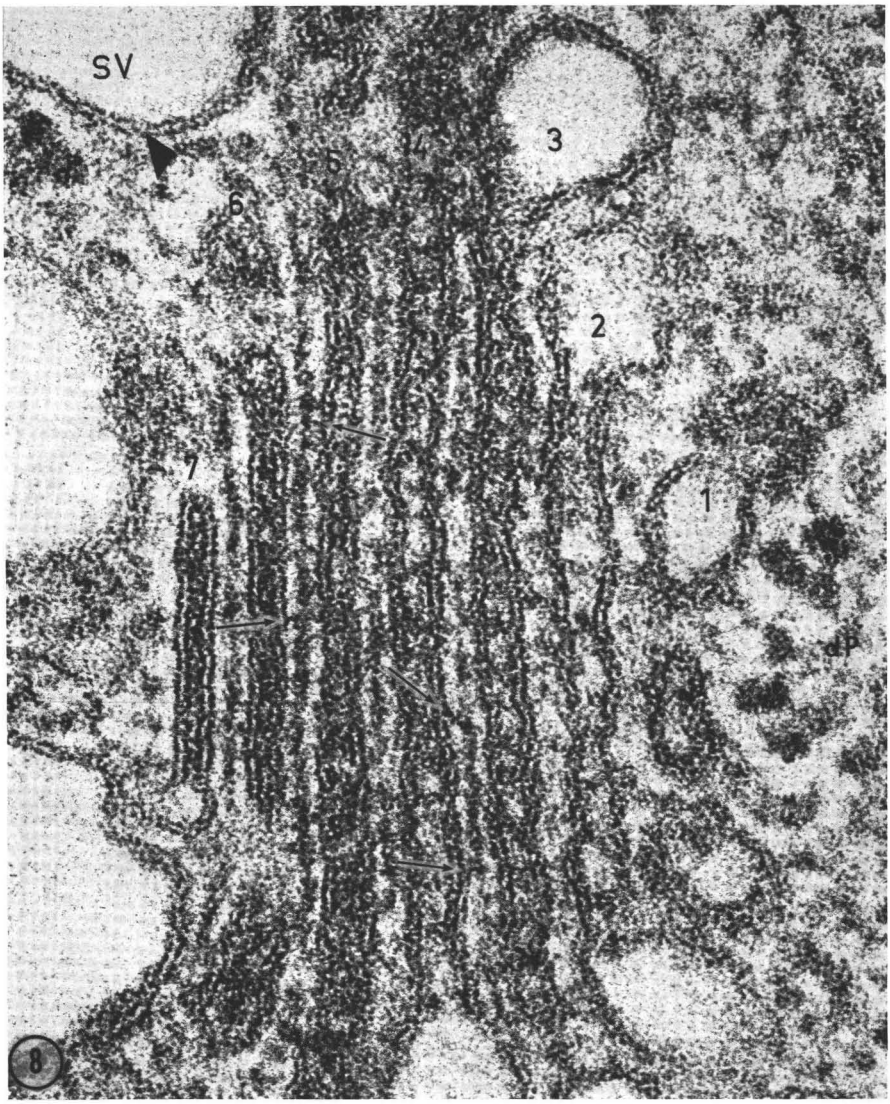


Fig. 8. Dictyosome of *Lilium* in cross-section in which the rod-like character of the intercisternal material predominates throughout the entire stack. Sites of lateral bridges between the intercisternal rods and the adjacent cisternal membrane are shown at the arrows. The triangle in the upper left points to intercisternal rod-like material which curves around the cisterna No. *6*. Magnification, ×260000

may extend slightly beyond the margin of a cisterna when adjacent to a secretory vesicle (e.g. Fig. 8). Many of the micrographs give the impression that the intercisternal rods are made up of linearly arranged globular subunits (e.g. in Figs. 3, 6). At higher magnification some of the 40–65 Å bridges are seen to connect the intercisternal rod-like elements with the adjacent membranes (e.g. Fig. 8).

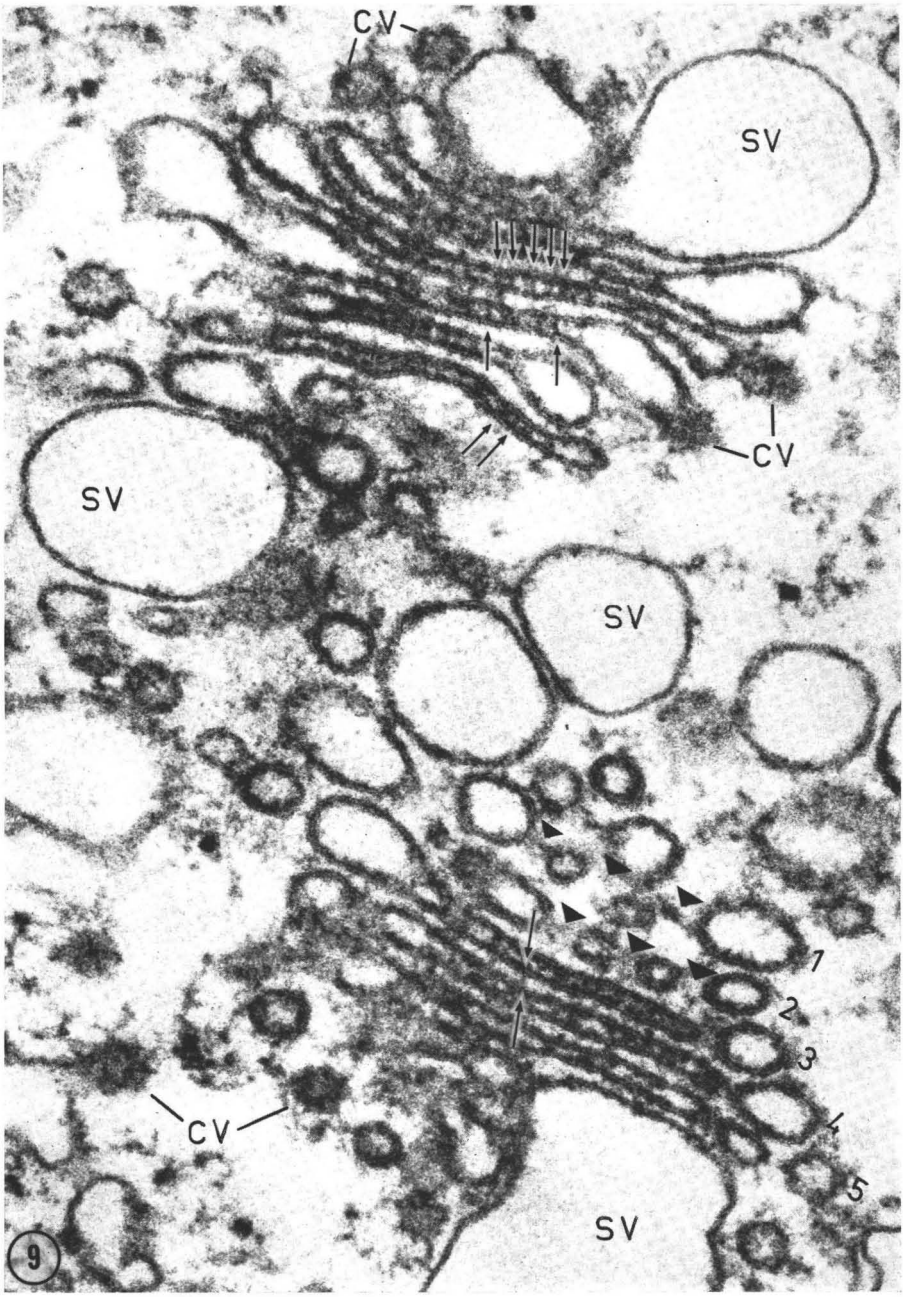


Fig. 9. Dictyosomes of a pollen mother cell of *Canna generalis*. Inter- and intracisternal cross-bridges are indicated by arrows. Note the numerous cisternal fenestrations at the forming face (e.g. at the triangles). *SV* secretory vesicles; *CV* coated vesicles. Magnification, ×136000

Other Plant Cells

The inter- and intracisternal membrane-to-membrane cross-link elements of dictyosomes, which have been described above in detail for vegetative pollen tube cells, were noted in our laboratories in almost every plant cell type that had been looked at in the electron microscope. Examples include, for instance, meristematic and differentiated onion and cress root tip cells, bean mesophyll and leaf epidermal cells, and the green alga, *Acetabularia mediterranea*. Fig. 9 presents one example showing such cross-links in dictyosomes of pollen mother cells of *Canna generalis*.

Animal Cells

The membrane-to-membrane cross-linking threads are commonly found in dictyosomes of animal cells whereas, interestingly, the classic intercisternal rods (Mollenhauer and Morré, 1966) described in the pollen tube chapter apparently are confined to some plant cell types. Fig. 10 presents examples of such electron dense elements linking membrane faces in the Golgi apparatus of rat and mouse liver. Here it is especially noteworthy that the inflations which are thought to become secretory vesicles, as identified by their characteristic lipoproteinaceous aggregate contents, are in manifold thread connections with other adjacent membrane faces (Figs. 10 b—d). The thickness of the cross-linking elements again is somewhat variable. While one category is about 100 Å in width (Fig. 10 c) another type is much thinner (30–40 Å in width, e. g. Fig. 10 d).

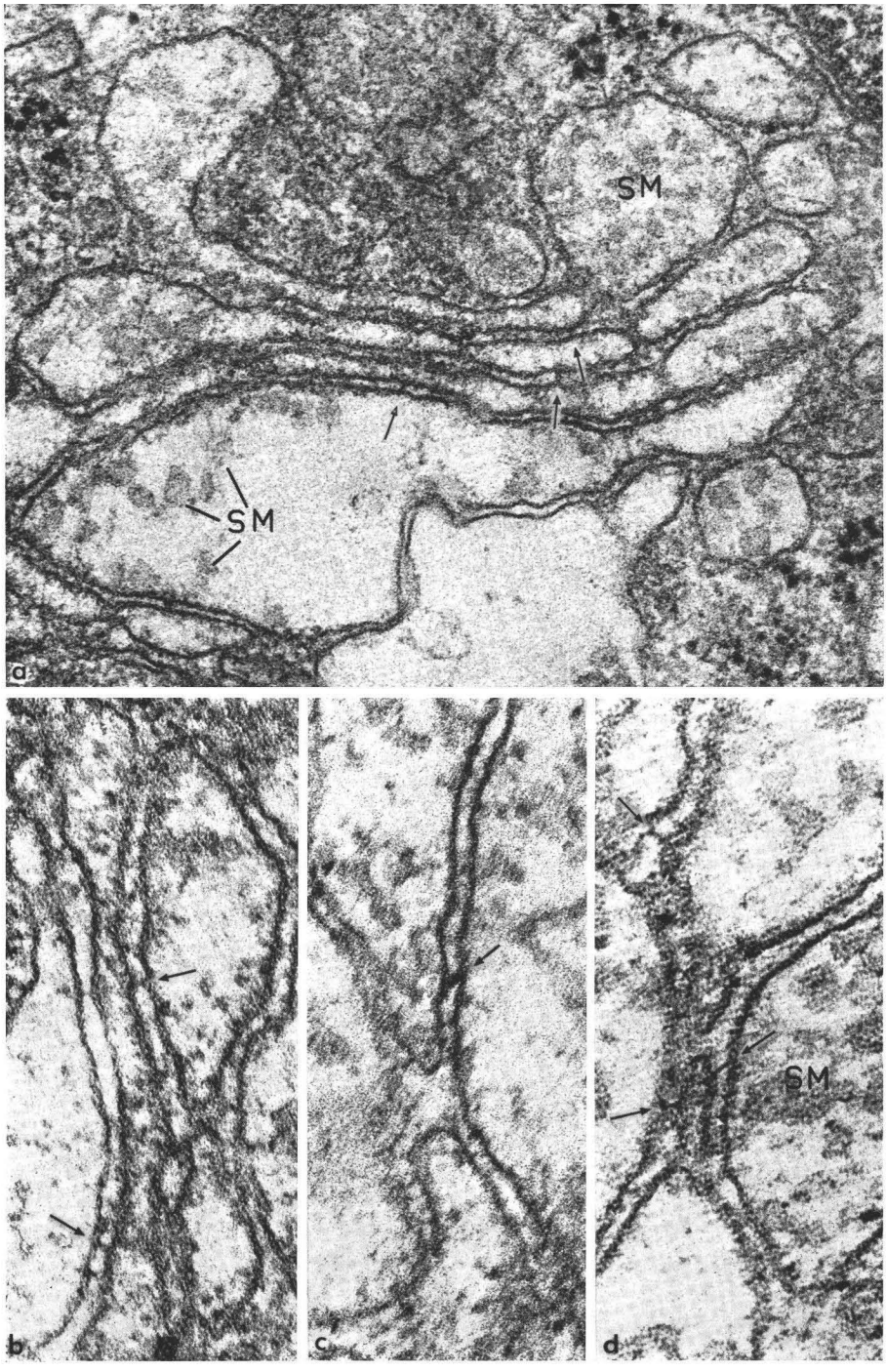
Fig. 10 documents that the principle of membrane-to-membrane cross-linking of dictyosomal membranes is maintained in minimal deviation tumors such as the Morris hepatoma cells. Intercisternal elements are commonly seen between cisternal faces as well as between cisternal sacs and adjacent vesicles, presumably secretory ones (Fig. 11 b, c). Intracisternal bridges are demonstrated in Fig. 11 a.

Some other examples of the occurence of dictyosomal membrane-to-membrane bridges in animal cells observed in our laboratories are spermatocytes of newts, rodents (compare also Franke *et al.*, 1971 b), and snails, of growing amphibian oocytes and intestinal goblet cells.

Discussion

Since dictyosomes can be isolated as intact stacks of cisternae (Mollenhauer and Morré, 1964; for review see Morré, 1971), something must function to hold the cisternae together and to maintain their characteristic spacing. Membranes of adjacent cisternae are continuous infrequently (Bracker *et al.*, 1971), if at all, in most species (Morré and Mollenhauer, 1964). Yet, there is usually a uniform minimal distance between adjacent cisternae along the flattened plate-like portions of the cisternae and centrally positioned fibrous elements are visible within the intercisternal region in some plant cells (Mollenhauer, 1965; Turner and Whaley, 1965; Cunningham *et al.*, 1966; Mollenhauer and Morré, 1966).

Our observations on dictyosomes of pollen tubes lead to a model (Fig. 12) in which the intra- and intercisternal spaces are visualized as being bridged by intermembrane cross-links. This concept includes the classic intercisternal rods as a component of the bridging material. The latter appear connected with the surfaces of the cisternal membranes through lateral bridges. We propose that these inter-



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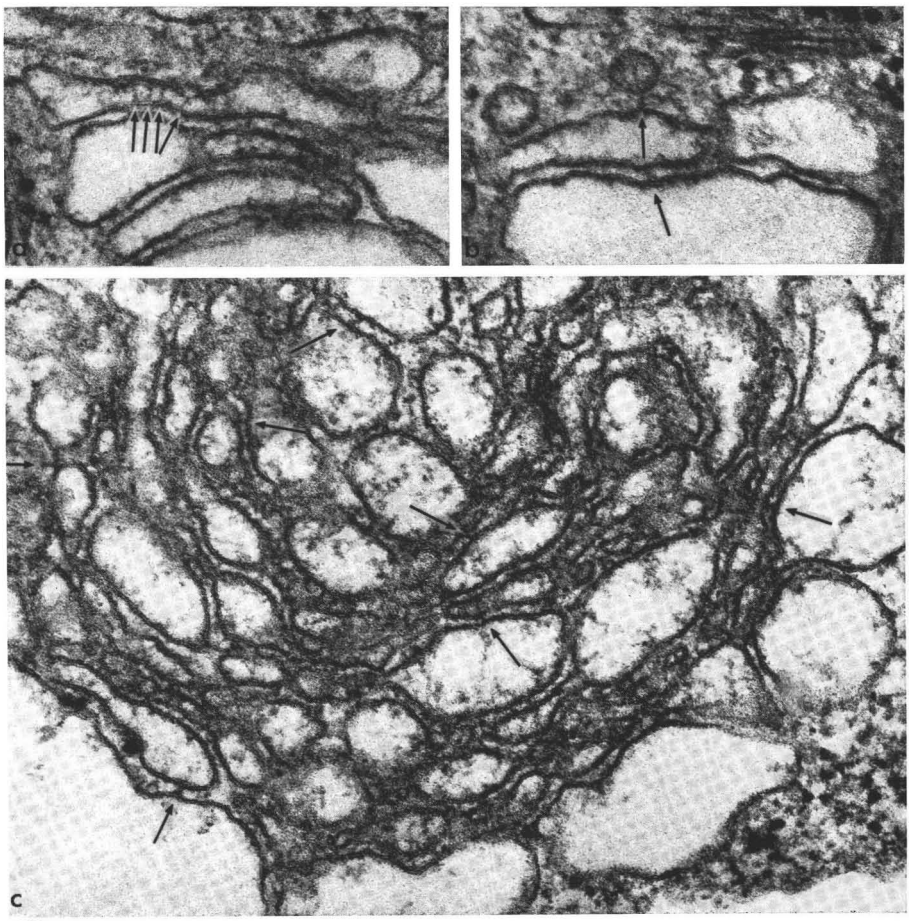

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Fig. 11a—c. Dictyosomes of Morris hepatoma cells showing intracisternal (a, arrows) and intercisternal (e.g. b and c, arrows) membrane-to-membrane cross-bridges. Similar bridges are also recognized between the membranes of cisternae and adjacent small vesicles (upper arrow in b). Magnification, a and b, ×100000; c, ×90000

Fig. 10a—d. Membrane-to-membrane cross-links in dictyosomes of rat (a) and mouse (b—d) hepatocytes. Intercisternal bridges (arrows) connect plate-like as well as vesicular parts. The sacs which probably are secretory vesicles in statu nascendi are characterized by their aggregate lipoproteinaceous contents (secretory material *SM*). Note that the widths of the intercisternal bridges are variable: Fig. c gives an example of an especially thick one (85 Å) whereas Fig. d shows a very thin thread connection (35 Å). Magnification, a, ×80000; b—d, ×175000

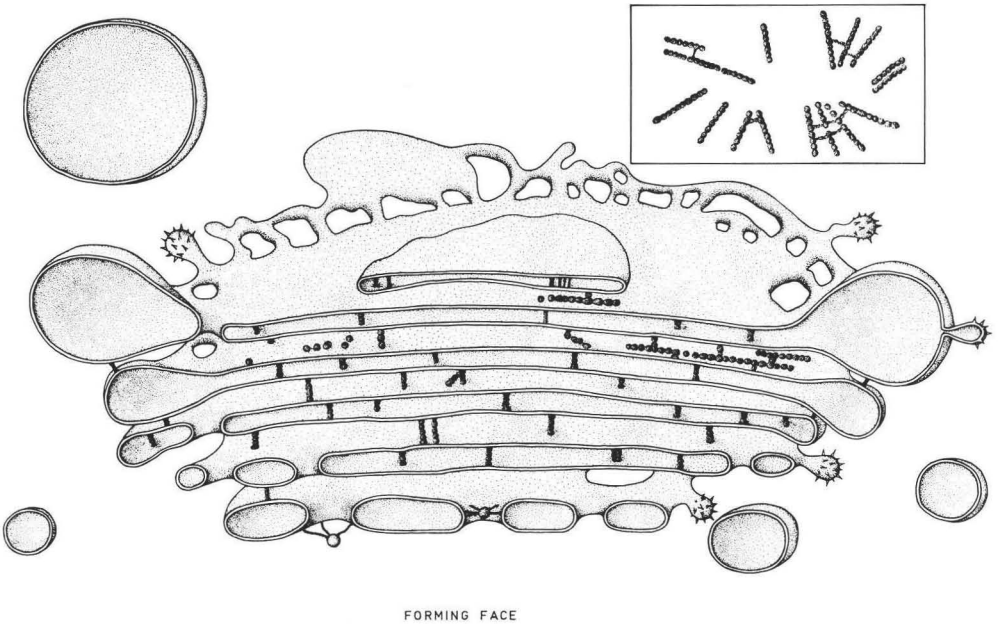

Fig. 12. Schematic drawing of a pollen tube dictyosome which includes the structural principle of membrane-to-membrane cross-linking. The insert represents the pattern of arrangement of intercisternal elements (a top view onto an interspace) as is characteristic for cisternae at the mature pole. In principle this view of structural organization holds for dictyosomes in general

membrane bridge structures maintain the flattened state of the central regions of the cisternae and contribute to the binding of the cisternae in the stacks. Breakdown of intercisternal bridges, on the other hand, would be a necessary condition for vesicles or cisternae to be released from the dictyosome. Since we have found the intra- and intercisternal bridges in a variety of widely different plant and animal cells we ascribe universality to this model concept of dictyosomal structure.

The chemical nature of the bridging elements is unknown. They appear to be susceptible to attack by proteolytic enzymes and it seems reasonable that proteins are a major constituent.

Franke *et al.* (1971 b) have discussed the relationship of intermembrane bridges to membrane-microtubule and to the microtubule-microtubule cross-bridges. Intermembrane cross-bridges emerge as universal cell components of cellular regions where a stable but not necessarily permanent association[1] between conjoined cell components may be essential to a specific cellular function (see also Bracker and Grove, 1971, and Franke *et al.*, 1971 a).

1 An especially conspicuous example of parallel membrane associations which is maintained through ca. 200 Å long cross-links is apparently demonstrated in the micrographs of G. Corbière-Tichané (1971). Regularly stacked plasma membrane invaginations occur in dendrite parts of neuronal cells in the larval antenna of the cave Coleopteran, *Speophyes lucidulus* Delar., and show local inflations and vesiculations at sites where the cross-bridge structures appear to have broken down.

Whether the bridges are structured as part of the cisternal membrane or exist as part of the cytoplasm remains problematic. Yet, as emphasized by Morré *et al.* (1971), dictyosome cisternae are not just flattened sacs but represent a highly complex system of subcellular compartments. The bridges may play an integrating role within this system but probably only as one of several components of the intercisternal region which occupies some 20–40% of the dictyosome volume (Mollenhauer *et al.*, in preparation). Additionally, dictyosomes are surrounded in the cytoplasm by a differentiated cytoplasmic region or "zone of exclusion" in which ribosomes, glycogen, and organelles are scarce or absent. Morré *et al.* (1971) have advanced the hypothesis that zones of exclusion function in the origin and continuity of cell components by providing a suitable milieu for the multiplication of cellular structures. Kartenbeck and Franke (1971) favor the notion that constituents of the zone of exclusion provide a "pool" of intracellular precursors to be utilized in the formation and/or transformation of dictyosome cisternae. In this regard, zones of exclusion might serve as repositories for constituents removed from the membranes during their transformation from ER-like to plasma membrane-like (Morré *et al.*, 1971) or provide a local milieu in which membrane transformation and assembly takes place. Some structural evidence for the latter function of the zone of exclusion is also provided by the present study in that each dictyosome of *Clivia* or *Lilium* pollen tubes is associated with *one* highly characteristic polyribosome located within the cytoplasmic zone of exclusion just adjacent to the forming pole of the stacked cisternae. The constancy of this association suggests a fundamental role of these polyribosomes in dictyosome functioning and adds the new dimension of protein synthesis to considerations of the role of the Golgi apparatus in biosynthesis and membrane differentiation.

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