Published February 22, 1981

The Golgi Apparatus (Complex)—(1954-1981)—from Artifact to Center Stage

MARILYN GIST FARQUHAR and GEORGE E. PALADE

To the cell biology student of the 1980s, it may come as a surprise to learn that until the late 1950s, the existence of the Golgi apparatus as a bona fide cell organelle was seriously questioned. Surprise would be in order on two accounts: first, the discovery of the Golgi apparatus by Camillo Golgi (1), for whom it is named, took place nearly a century ago; and, second, now no one questions that the Golgi apparatus is a distinct cell organelle, or is unaware of its participation in a wide variety of cellular activities. Indeed, the Golgi apparatus, or Golgi complex as it is often called, not only occupies the cell center, but it also has moved toward center stage, because it has been shown to be involved in so many cell activities. In this review we will describe the recent history of the Golgi apparatus—the developments that led from its position as a suspected artifact to the situation at present when it is rapidly becoming a main center of attention.

Brief Historical Perspective

The Light Microscope Era (Before 1954)

The period before the mid 1950s was characterized by controversy concerning the reality of the Golgi apparatus, with the scientific community divided into nonbelievers and believers. The acceptance of the status of the Golgi as a bona fide cell structure depended on whether one believed that the metallic impregnation methods (involving use of silver or OsO_4), which Golgi and others used to demonstrate the apparatus, were staining a common structure with variable form and distribution in different cell types, or alternatively, that these methods resulted in artifactual deposition of heavy metals on different cell structures in different cell types. The Golgi controversy lasted until the introduction of the electron microscope into biological research, in the early 1950s. Shortly thereafter, the believers began to outnumber the nonbelievers, and by 1963, even the most skeptical had become converts (see Whalley [2]) and Beams and Kessel (3) for details of the history of this period).

The Renaissance (1954-1963)

Electron microscope studies published before 1954 had verified the existence of a distinctive Golgi region in cells; but due to the technical limitations of the preparatory techniques at the time, the images obtained did not extend knowledge of its organization beyond what was known from studies with the light microscope. In 1954, however, the 'lamellar' nature of the Golgi was recognized and described in papers by Dalton and Felix (4), Sjöstrand and Hanzon (5), Rhodin (6), and Farquhar and Rinehart (7). It is Dalton and Felix who deserve the major credit for convincing the scientific public of the reality of the Golgi apparatus, and whose work (4, 8) had the greatest impact at the time. They established that the Golgi apparatus consists of several distinct fine structural components (lamellae, vesicles, and vacuoles), and, accordingly, introduced the term Golgi 'complex' for this organelle; they showed that variations in the form, amount, and disposition of these components occur in different cell types; and they demonstrated deposition of metallic osmium in its lamellar components, thereby relating the newly discovered fine structure to the light microscope studies of the classical Golgi literature which relied heavily on metallic impregnation methods.

The period that followed was characterized largely by detailed morphological descriptions of the fine structure of the Golgi apparatus (or complex) in everyone's favorite tissue. The electron micrographs and the details recorded improved with the introduction of better techniques for specimen preparation. Information on the function of the Golgi complex was limited, however, to noting the topographical association between this organelle and forming secretion granules. Attempts to use cytochemical techniques (other than heavy metal impregnation) or to isolate usable Golgi fractions were still to come. It is during this period that the ubiquity of the Golgi complex, its general structural characteristics, and detailed organization in a variety of cell types were established.

The Modern Period (1964-1973)

During the late 1960s and early 1970s, additional techniques were applied to the study of the Golgi apparatus which added new dimensions to our overall understanding of Golgi structure and function. These procedures included techniques for phosphatase cytochemistry, which yielded new information on the heterogeneity of Golgi elements; autoradiography, which provided the first information on the movement of secretory proteins through the Golgi complex and on the involvement of the organelle in glycoprotein synthesis and in sulfation; and

MARILYN GIST FARQUHAR and GEORGE E. PALADE Section of Cell Biology, Yale University School of Medicine, New Haven, Connecticut

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techniques for isolating Golgi fractions and (later) subfractions, which made possible biochemical analysis of Golgi components. The last development was greatly facilitated by the discovery of a reliable marker enzyme activity—galactosyltransferase—which is limited in its intracellular distribution to the Golgi apparatus and therefore could be used to monitor the effectiveness of fractionation procedures. Most of the known Golgi functions, which are summarized below, were established during these years.

The Current Period (1973 to the Present)

Currently all these procedures and approaches are being applied—usually in combination—to many different kinds of cells. The focus of current work is to determine the interactions between Golgi components and other cell compartments (ER, lysosomes, plasmalemma) in order to delineate the role of the Golgi complex in such basic and apparently diverse cell processes as secretion, membrane biogenesis, lysosome formation, membrane recycling, and hormone uptake.

Organization of the Golgi Complex

GENERAL DESCRIPTION: The collective electron microscope studies carried out over the past 25 years have established that the Golgi complex consists of a morphologically heterogeneous set of membrane-limited compartments that have common recognizable features and are interposed between the ER and the plasmalemma. Its constant and most characteristic structural component is a stack of smooth-surfaced cisternae (or saccules), which usually have flattened, platelike centers and more dilated rims (Figs. 1–6). Often the cisternae are slightly curved, with one side of the stack oriented toward the rough ER and the other facing the plasmalemma (Fig. 3) or the nucleus (Figs. 1 and 2). Typically, the former side is associated with small vesicles, and the latter with secretory granules or vacuoles in secretory cells (Figs. 1–4 and 7 and 8). Thus, the whole structure has a clearly recognizable polarity, and a number of terms have been introduced and are widely used in the literature to describe its polarity: (*a*) convex vs. concave side; (*b*) proximal vs. distal; (*c*) forming vs. mature; (*d*) entry vs. exit; and (*e*) cis vs. trans (9). We prefer and use the terms cis-trans because (*a*) and (*b*) are not always applicable (due to variations in shape and intracellular organization), and (*c*) and (*d*) assume more than we know at present about the function of both cis and trans Golgi elements.

It is now recognized that in addition to specific Golgi elements, the Golgi region is crowded with other cell structures, such as coated vesicles (Figs. 5, 8, and 9), lysosomes (Figs. 7 and 15), and, in many cases, centrioles with their associated satellites and microtubules (Fig. 2).

In their early studies on the organization of the Golgi complex, Dalton and Felix (8) recognized many of the main features that characterize this organelle: (*a*) its multiple components—flattened cisternal sacs (then referred to as lamellae)

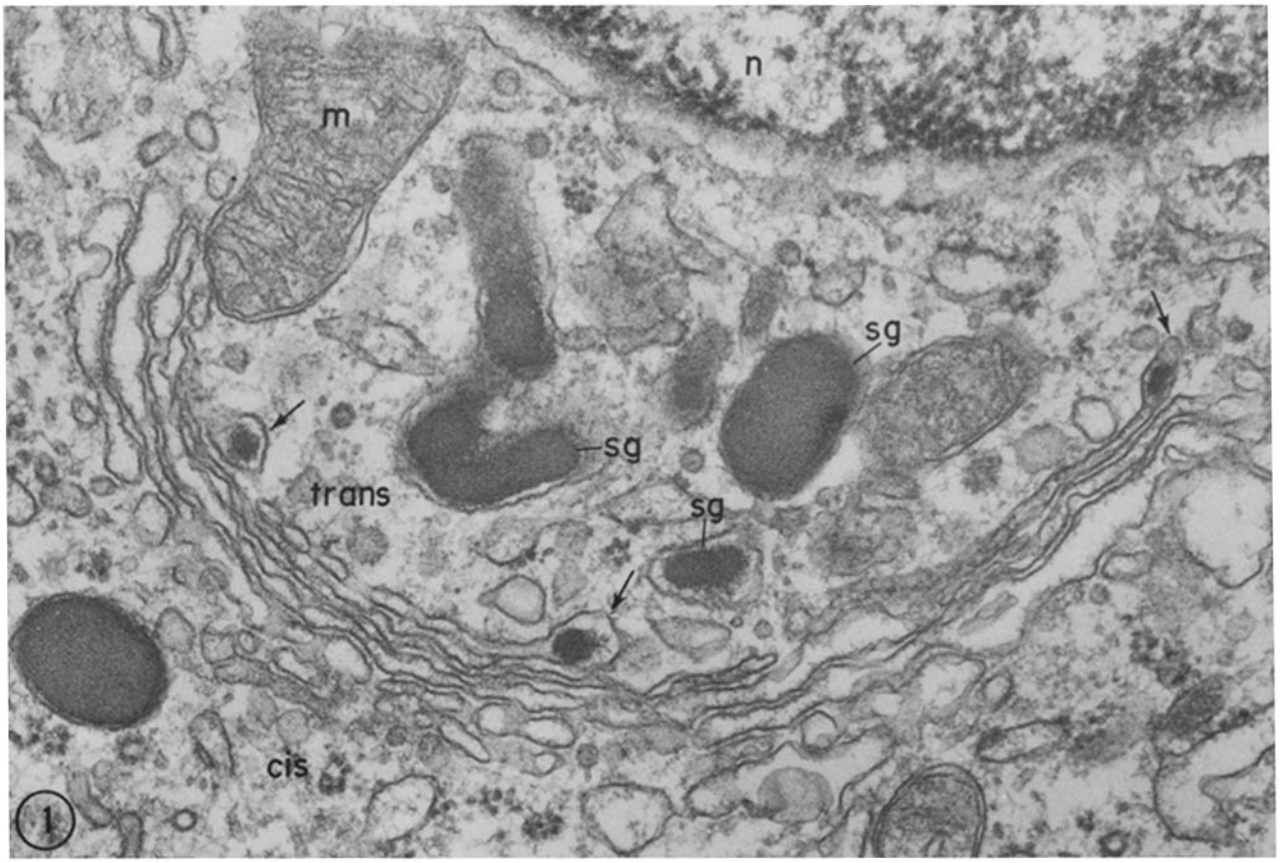

FIGURE 1 Golgi region of a mammotroph or prolactin-secreting cell from the anterior pituitary gland of a lactating rat. A stack of three to five slightly curved Golgi cisternae occupies the center of the field. The secretory granules (85% prolactin) arise within the trans cisternae along the concave face of the Golgi stack. In this field, three small (100–200 nm) prolactin granules are seen condensing within three of the transmost cisternae (arrows). The polymorphous secretion granules (sg) seen above result from the fusion and aggregation of several of the small Golgi-derived granules (as diagrammed in Fig. 26). n = nucleus; m = mitochondrion. × 67,000. From Farquhar (51).

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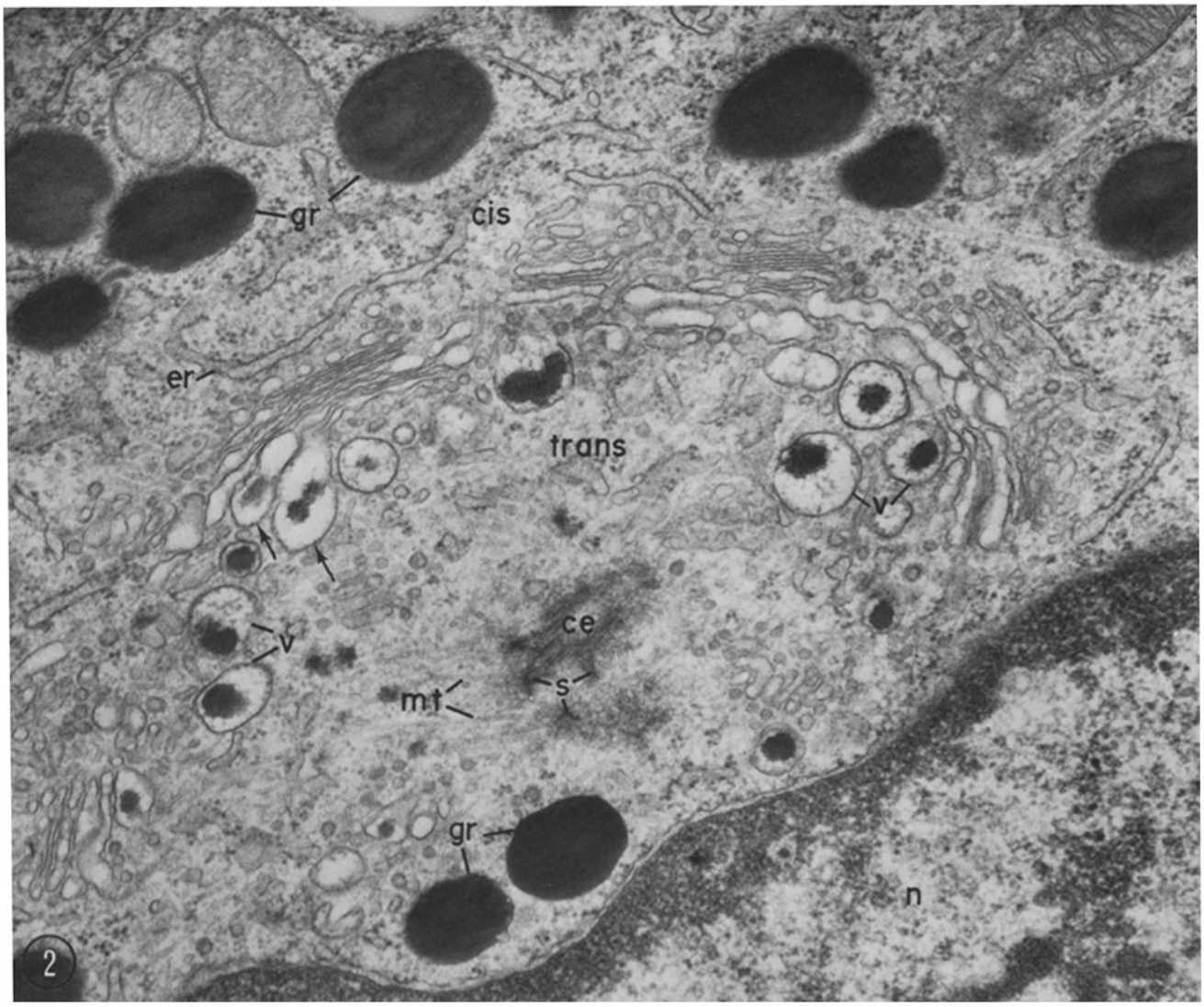

FIGURE 2 Golgi region of a developing PMN leukocyte (promyelocyte stage), illustrating the formation of azurophil or primary granules along the trans side of the Golgi complex. This complex consists of a stack of five to eight slightly curved Golgi cisternae which partially encircle a centriole (ce). The dilated ends of several of the transmost cisternae (arrows) are seen to contain condensing secretory products (i.e., lysosomal enzymes and peroxidase in these cells). Numerous dense-cored vacuoles (v), are also seen along the trans Golgi face; they are presumed to arise by budding from the dilated rims of the adjacent Golgi cisternae. Several of these vacuoles fuse, their contents aggregate and undergo further concentration, resulting in the formation of the compact and uniformly dense azurophil granules (gr). The assembly process is very similar to that involved in the formation of prolactin granules (see Figs. 1 and 26). s = centriolar satellites; mt = microtubules; n = nucleus. × 50,000. From Bainton and Farquhar (122).

as well as vacuoles and vesicles; (*b*) the high variability of the relative amounts of these elements in different cell types; (*c*) the frequent identity of vacuolar elements with dilated cisternae; (*d*) the absence of ribosomes (then referred to as small granules of Palade) from Golgi membranes; and (*e*) the fact that some of these membranes were thicker (8–10 nm) than the membranes of the rough ER (then called ergastoplasm).

Still other organelles, such as ribosomes, glycogen, mitochondria, peroxisomes, and rough and smooth ER, are found in the Golgi area but are usually excluded from the region where the stacks are located (Figs. 5 and 7); Mollenhauer and Morré (10) have referred to this region as the Golgi 'zone of exclusion'. They and others have noted that the matrix in which the Golgi complex is embedded is denser than the rest of the cytoplasmic matrix and has a fibrillar-granular appearance (Fig. 7). At present no information is available about the composition of this matrix material.

GOLGI STACKS OF ANIMAL CELLS: The early light microscope studies established that the intracellular distribution of the Golgi apparatus varies from one cell to another. In neurons, where it was originally discovered, the apparatus appears as a perinuclear network, whereas in exocrine glands it forms a ring-like structure between the nucleus and the apical cell surface. Electron microscopists have utilized cytochemical staining on thick (~ 1/2 μm) sections with tilting to study the three-dimensional interrelationships of Golgi cisternae in the cell. Using this approach, Novikoff et al. (11) and Rambourg et al. (12) have presented evidence that the cisternae in animal cells are extensively interconnected, and have suggested that the Golgi complex consists of a single set of stacked cisternae.

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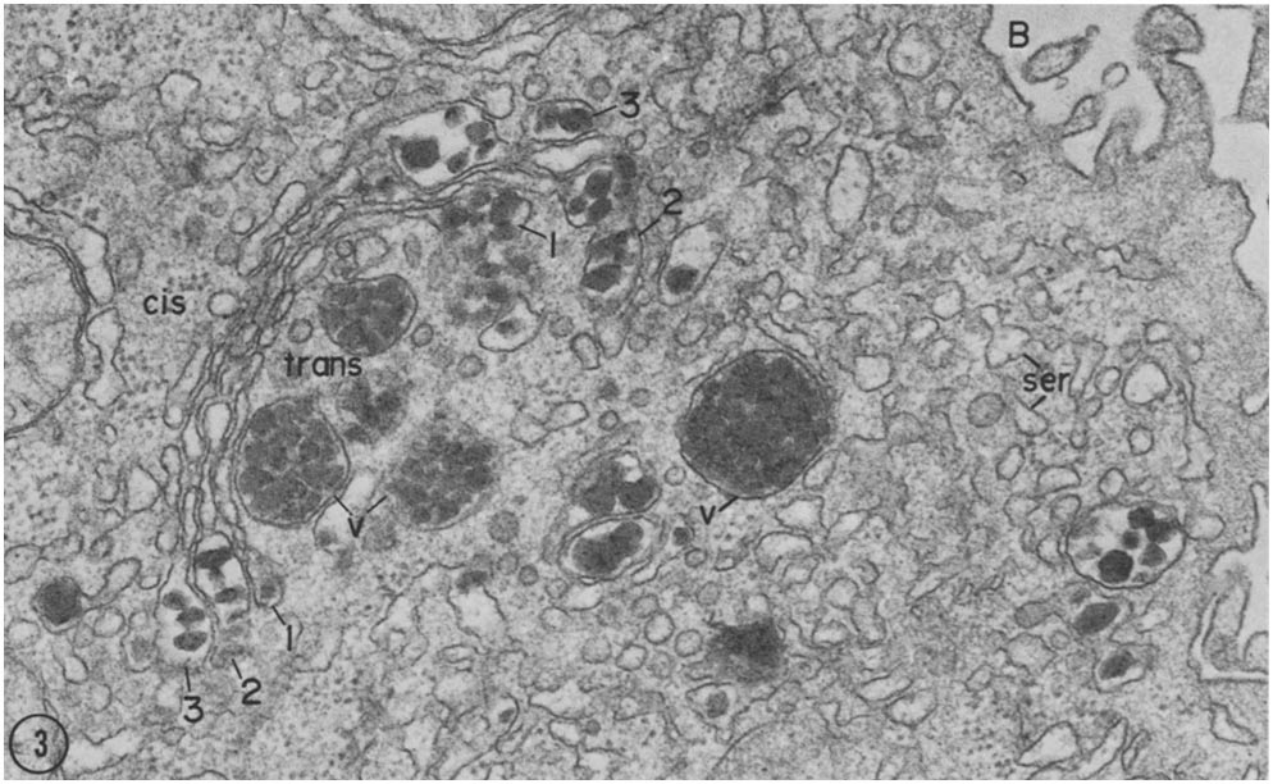

FIGURE 3 Golgi complex of a hepatocyte from an ethanol-treated rat. This complex consists of a stack of three to four slightly curved Golgi cisternae which face the bile canaliculus (B). Clusters of lipoprotein particles are seen in the dilated rims of three cisternae (1, 2, 3) in the trans part of the Golgi stack and in numerous secretory vacuoles (v) located on the trans side of the stack. The accumulation of lipoprotein particles in the rims of Golgi cisternae is a normal occurrence in the hepatocyte, but it is greatly increased following ethanol treatment. ser = smooth er. × 50,000.

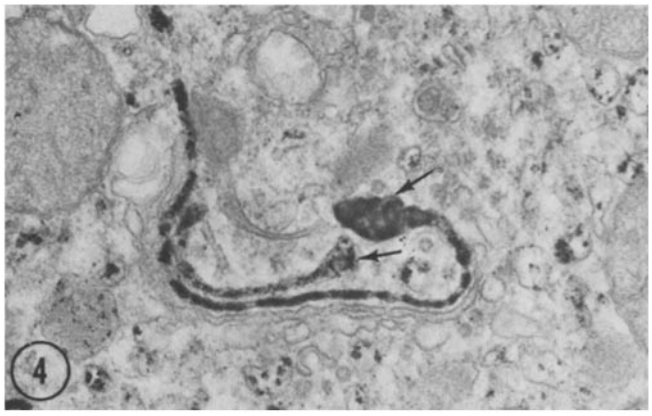

FIGURE 4 Golgi complex from a normal rat hepatocyte in a preparation reacted for TPPase. Reaction product (lead phosphate) is seen within two of the transmost Golgi cisternae, the dilated rims of which (arrows) also contain lipoprotein particles. × 30,000. From Farquhar et al. (20).

GOLGI STACKS OF PLANT CELLS (DICTYOSOMES): Structures which proved to be Golgi in nature had been studied for years in plant cells under the name of dictyosomes. Electron microscope studies during the 1950s established that each dictyosome is present in multiple copies and corresponds to an individual Golgi stack. The detailed organization of the Golgi in plant cells has been extensively studied, especially by Whalley, Northcote, Mollenhauer, Morré, and their associates (see Whalley [2] for a review). Some time ago, the latter two authors called attention to the similarities between Golgi complexes in animal and plant cells (13). In many (but not all) plant cells, there are distinct differences in the thickness of the membranes of the cisternae across the stack, with those on the cis side being thin (ER-like) and those on the trans side being noticeably thicker (plasmalemma-like), a feature first noted by Grove et al. (14). This led to the idea, proposed by Morré and co-workers (15), that a gradual increase in membrane thickness takes place as the cisternae move across the stack (see below).

OTHER MORPHOLOGIC FEATURES: Besides the general features that are applicable to most if not all Golgi complexes, other features have been described which occur less regularly. Examples are the rings of beads between the ER and Golgi cisternae in certain insects (16), and dense nodes of intercisternal material that occur in the cytoplasmic matrix between Golgi cisternae in some protozoans (17). After the discovery of coated vesicles, it was recognized that clathrin-coated vesicles are commonly seen in the Golgi region (18, 18a) and, in addition, that coated regions commonly occur on the rims of the Golgi cisternae and on condensing granules or vacuoles (Figs. 5, 8, and 9).

Finally, on the trans side of many (but not all) Golgi stacks, cisternae of characteristic morphology have been described; they are often separated from the stack (Figs. 10, 13, and 14), and their appearance varies from straight (rigid lamellae) to tubular and tortuous (Figs. 13 and 14). Novikoff and his coworkers, first described these cisternae in 1964, and, based on the observation that acid phosphatase activity is often associated with them, postulated that they constitute a link between

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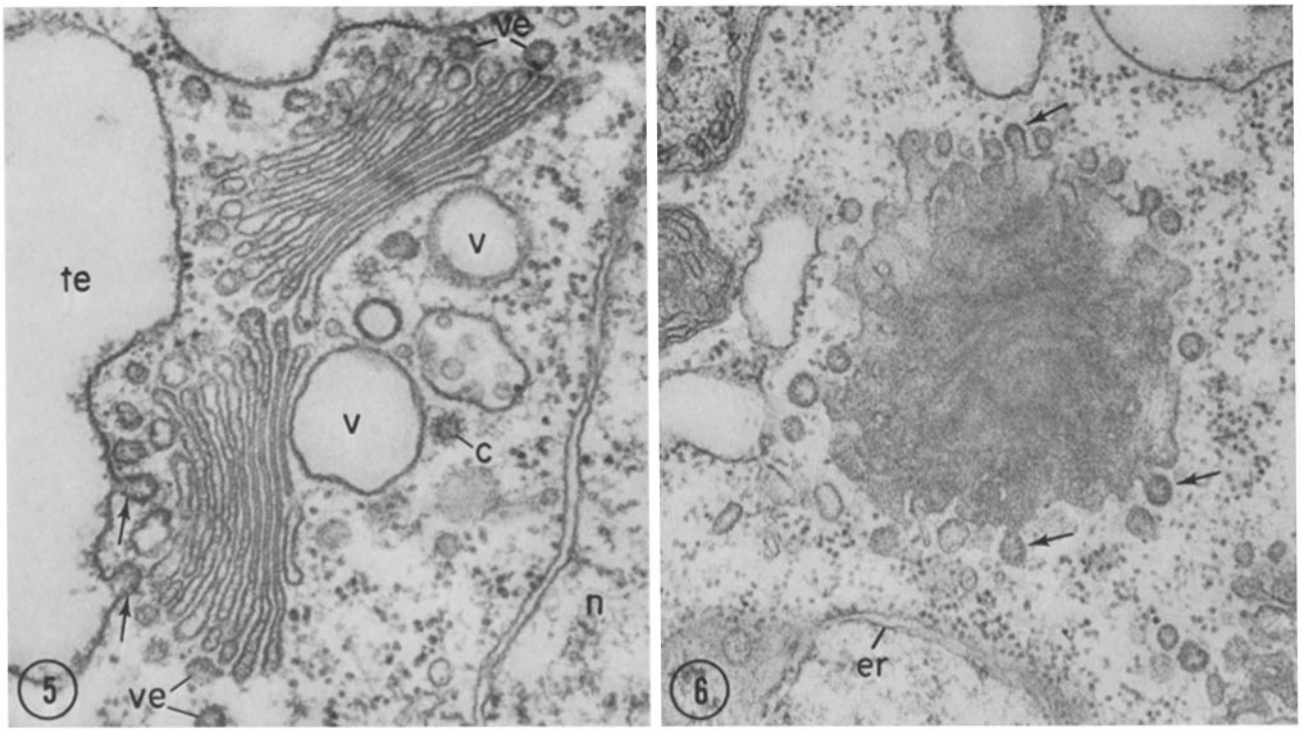

FIGURES 5 and 6 Features of dictyosomes or Golgi stacks found in plant cells (the green algae *Chlamydamonas rheinhardi*). Fig. 5 shows two dictyosomes each with 9 parallel cisternae cut in cross section. Characteristic features of these Golgi complexes are the presence of large vacuoles (v) and coated vesicles (c) associated with their trans side, transport vesicles with fuzzy-coats which bud (arrows) from transitional elements (te) of the rough ER on the cis side, and numerous vesicles (ve) associated with the dilated rims of the cisternae. Fig. 6 shows an obliquely sectioned Golgi stack seen *en face*. It illustrates the presence of numerous vesicles which appear to be in the process of fusing with, or budding from the cisternal rims (arrows). Fig. 5—× 80,000; Fig. 6—× 70,000.

the *G*olgi, and *ER*, and *L*ysosomes. Accordingly, Novikoff introduced the acronym GERL as their designation (see Novikoff et al. [11, 19]). The present status of the GERL concept is discussed further below.

Composition of the Golgi Complex

CYTOCHEMICAL STAINING: The first evidence of compositional heterogeneity among cisternae in the Golgi stacks came from the results of cytochemical staining procedures which demonstrated qualitative differences in staining for various enzymes and other components among Golgi cisternae (Figs. 9–12). These differences are best documented in the case of the hepatocyte (Table I), in which staining has been carried out both *in situ* and on Golgi fractions. The earliest studies of this type were those of Novikoff and Goldfischer (25), who demonstrated that thiamine pyrophosphatase (TPPase) and nucleoside diphosphatase activity (NDPase) represent cytochemical markers that could be used to study the form and distribution of the Golgi apparatus in many, but not all cells (in hepatocytes the ER also contains these enzymes [24]). In subsequent work, Novikoff and co-workers showed that these activities were restricted in their distribution to 1–2 cisternae on the trans side of the Golgi stack (Figs. 4 and 11), and that acid phosphatase (AcPase) was also restricted to one or two of the transmost cisternae (Figs. 10 and 15). Later, based on the study of thick (1/2 μm) sections as well as thin sections, they also demonstrated that AcPase and TPPase are present in different cisternae in many cell types (11, 19). Friend and Murray (23) showed that the classical osmium impregnation procedures preferentially stain one or two of the cismost cisternae in many cells (Fig. 12), and recently, Smith (26) found that intermediate cisternae selectively stain for nicotinamide adenine dinucleotide phosphatase (NADPase) in the ameloblast and several other cell types. In work from our laboratories, it was demonstrated that several other enzymes—5′-nucleotidase (20) and adenylate cyclase (21)—are present in virtually all cisternae, both cis and trans, within the stack. Rambourg and LeBlond (22) found that all Golgi cisternae stain with periodic acid-silver methenamine (PA-silver) (which stains complex carbohydrates), but staining is graded (increasing from cis to trans) across the stack.

Results of cytochemical staining also provided the first indication that, in addition to the differences in composition across the stack, there may be differences in the composition of the membrane of a given cisterna (20, 21, 27). Specifically, our finding that lead phosphate reaction product for both 5′-nucleotidase (20) and adenylate cyclase (21) was concentrated along the dilated rims of isolated Golgi elements (Figs. 16 and 17) and was missing or present in much lower concentration in the flattened centers of Golgi cisternae in liver fractions suggests that the dilated rims may have a composition different from that of the flattened centers. These findings also provide the first clear demonstration that these two plasmalemmal marker enzymes are indigenous to Golgi elements.

Based on the location of the reaction product (on either the inside or outside of Golgi membranes), cytochemical staining has provided suggestive evidence on the orientation or sidedness of the active site of several enzymes. For most enzymes studied (TPPase, AcPase), the lead phosphate reaction product was localized inside the cisternae where it was associated either

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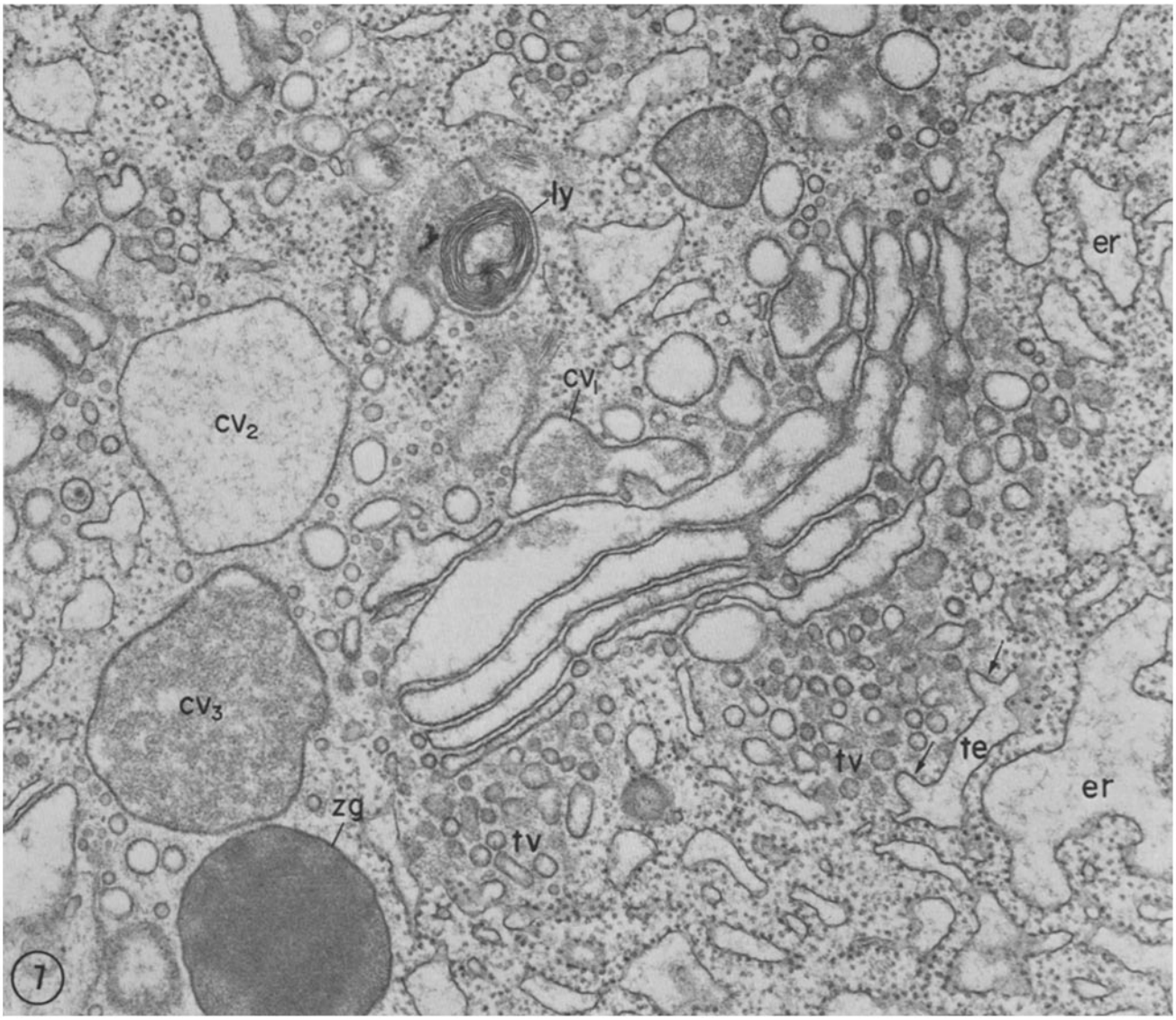

FIGURE 7 Golgi region from an exocrine pancreatic cell (guinea pig). Characteristic features of this Golgi complex are the presence of a stack of four to five slightly dilated Golgi cisternae associated with condensing vacuoles (cv_1) on its trans side, and a profusion of small peripheral Golgi vesicles, or transport vesicles (tv), along its cis side. These vesicles are assumed to bud (arrows) from the transitional elements (te) of the rough ER and to transport secretory products to the condensing vacuoles by a route still unknown. The condensing vacuoles gradually fill with secretory proteins (mostly pancreatic zymogens), undergo progressive concentration, thereby becoming increasingly dense (cv_{1-3}), and eventually become mature zymogen granules (zg). Note that there is a zone around the Golgi cisternae and transport vesicles in which the cytoplasmic matrix is denser than elsewhere in the cell, and from which ER elements (er) and ribosomes are excluded. ly = lysosome. × 38,000.

with the inside of the membranes or the cisternal content. In a few cases, however, 5′-nucleotidase (20) and adenylate cyclase (21), reaction product was found on the cytoplasmic side of the dilated rims of certain Golgi cisternae. It is of interest that the reaction product for 5′-nucleotidase was localized on the *outside* of cisternae and the *inside* of secretory vacuoles (20, 28). Thus far, biochemical assays on cell fractions have largely substantiated the cytochemical observations pertaining to sidedness; when reaction product was localized to the inside of Golgi membranes, the enzyme activity was latent and detergent treatment (to permeabilize the membranes) increased the activity, whereas when the reaction product was localized on the outside, addition of detergent had no effect (28) on the activity measured.

Among the components demonstrated cytochemically, most are enzymes that can be assumed to be associated with Golgi membranes; however, a few (such as AcPase and substances which stain with PA-silver) may also be associated with the cisternal contents. In addition to the localization of these presumptive Golgi components, there are also several examples of cells in which secretory products, primarily peroxidases, have been localized by cytochemical or immunocytochemical procedures.

To summarize, cytochemical findings have provided information on the existence of specialization among Golgi components and have indicated that differentiation exists across the stack, at least between the extreme cis and trans cisternae. In addition, the evidence has suggested that differentiation also exists within individual cisternae. The functional significance of these specific localizations remains largely unknown.

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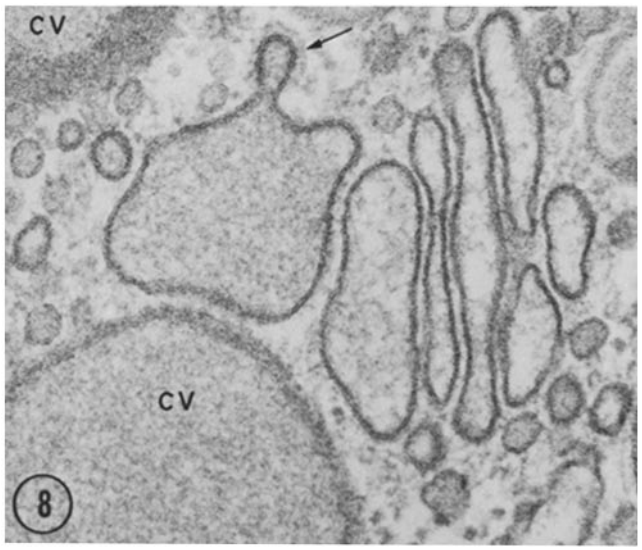
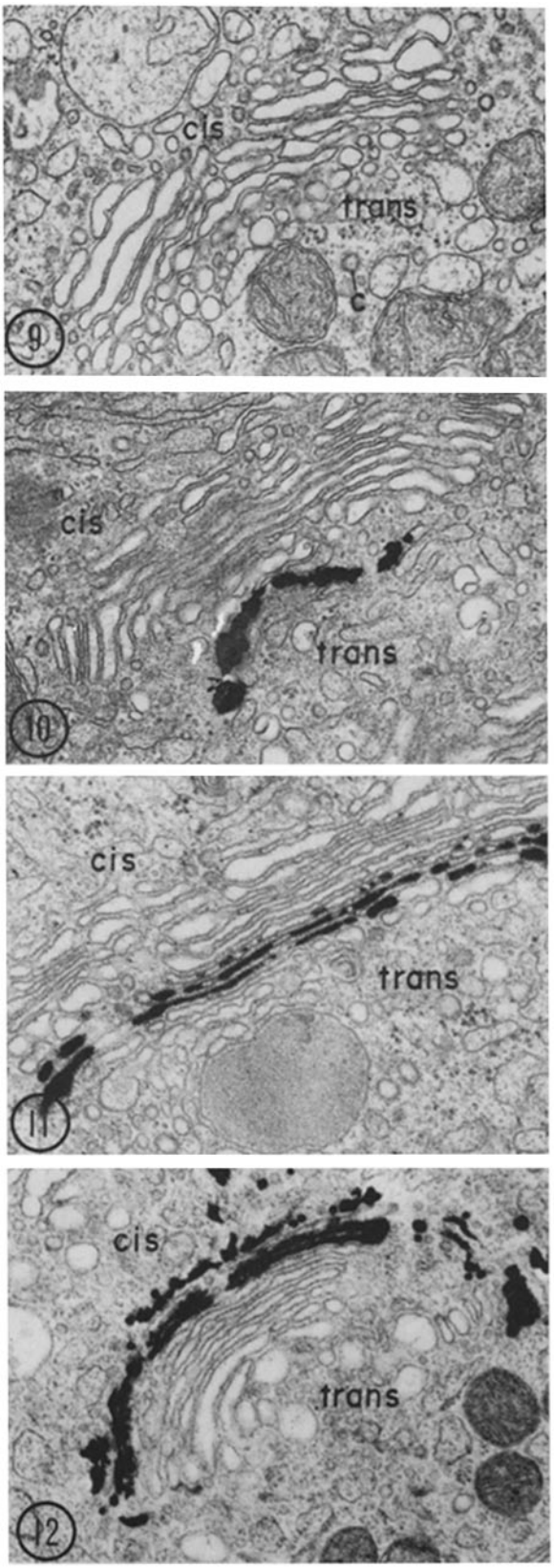


FIGURE 8 Another Golgi complex from an exocrine pancreatic cell showing 4–5 cisternae (to the right) and a condensing vacuole with a budding (or fusing) coated vesicle (arrow). Two other condensing vacuoles or granules (cv) are also present nearby. × 95,000.

METHODS FOR PREPARATION OF GOLGI FRACTIONS AND SUBFRACTIONS: The earliest attempts to isolate Golgi fractions can be attributed to Schneider and Kuff in 1954, who used the rat epididymis as starting material (29). The fractionation was monitored by light microscopy, and the results were puzzling because they seemed to indicate that there was DNA in the fractions. This unusual finding proved to be an artifact of the assay procedure created by the presence of carbohydrates in Golgi elements. Some time elapsed until Morré and his collaborators conducted a series of more fruitful fractionation attempts, first on plant cells (30) and later on rat liver (31, 32). They succeeded in isolating Golgi fractions from liver homogenates by a combination of differential and rate sedimentation procedures. The fractionation was monitored by electron microscopy (32), which revealed that many cisternae remained stacked, and the fractions were examined for a variety of enzymatic activities, mostly phosphatases (24, 33). Shortly after Morré's initial (30) work, B. Fleischer, S. Fleischer, and H. Ozawa (34) and Fleischer and Fleischer (35) simplified and improved the fractionation procedure and demonstrated the presence of a high concentration of galactosyltransferase activity in Golgi fractions from bovine and rat liver by using an exogenous acceptor (*N*-acetylglucosamine). The discovery of galactosyltransferase in Golgi fractions, and its apparent absence from other cell membranes, was an important development in Golgi history because it provided a much-needed marker enzyme for monitoring cell fractionation. Earlier attempts to prepare Golgi fractions had relied exclusively on morphological criteria for the identification of Golgi elements and had been hampered by the lack of a quantitative criterion



FIGURES 9–12 Golgi complexes from the epidydymis (rodent) in which the Golgi complex consists of 8–10, parallel stacked cisternae with numerous associated vacuoles and vesicles some of which are coated (c). Here they are seen either unstained (Fig. 9) or reacted for cytochemical procedures which stain the cisternae in the stack differentially. Fig. 10 shows reaction product for acid phosphatase (β-glycerophosphatase) in a single cisterna on the trans side of the stack which is set off from the rest, and which has the properties ascribed to GERL by Novikoff and co-workers (19). Fig. 11 shows reaction product for TPPase in two of three of the parallel transmost cisternae in the stack. No cisternae comparable to the AcPase-positive cisterna in Fig. 10 is seen. Fig. 12, from a preparation impregnated with OsO_4, shows osmium deposits in two of the cismost cisternae in the stack. From Friend (153). Fig. 9—× 40,000; Figs. 10 and 11—× 30,000; Fig. 12—× 24,000.

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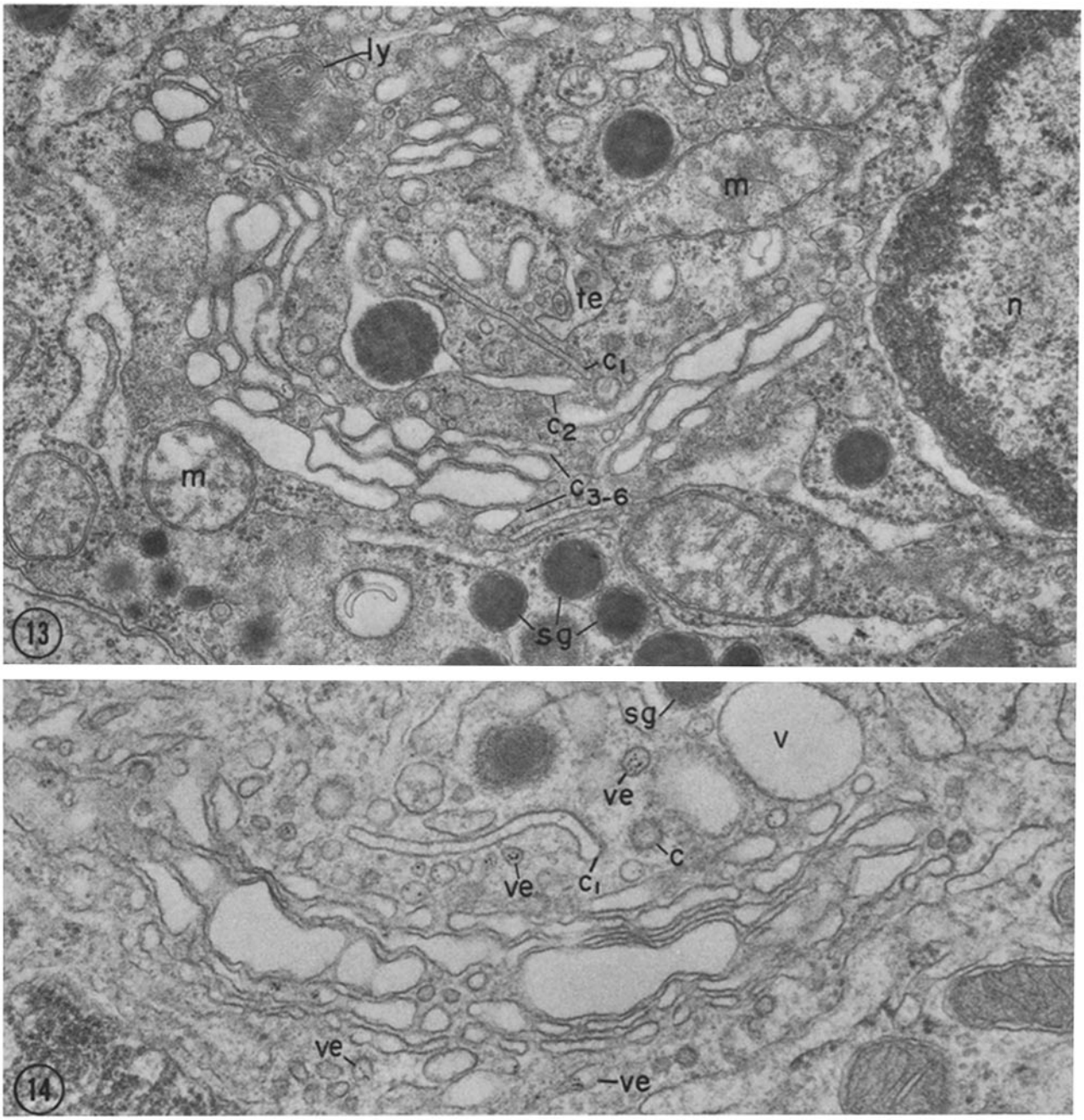

FIGURES 13 and 14 Golgi regions in two somatotrophs from the rat anterior pituitary, illustrating some of the variations encountered in the morphology of the cisternae present in the Golgi region. In Fig. 13, the first cisterna (c_1) on the trans side of the stack is straight rather than curved, and is set off from the rest. It has the morphology ("rigid lamella") ascribed to GERL by Novikoff and his associates; there is strict parallelism of the adjoining membranes which appear somewhat thicker than those of the rest of the cisternae. c_2, which is also set off slightly from the Golgi stack, contains a forming secretion granule. c_{3-6} are slightly curved and more dilated. Fig. 14 shows another Golgi stack with another cisterna (c_1) on the trans side set off from the rest with features similar to those of c_1 in Fig. 13. Numerous vesicles (ve) are present both on the cis and trans sides of the stack; some of these are coated vesicles (c). The cells were incubated with cationized ferritin prior to fixation, and many of the vesicles contain the tracer. sg = secretory granule. Fig. 13—× 36,000; Fig. 14—× 50,000. Fig. 13 is from Farquhar (51).

for yield and purity because no enzymes were known to be exclusively restricted to the Golgi complex. The work on glycosyltransferases was extended by Morré et al. (36) to demonstrate *N*-acetylglucosamine transfer to unspecified endogenous receptors, and by Schachter and co-workers (37, 38), who demonstrated the presence of other (sialyl and fucosyl) terminal glycosyltransferases by using appropriately prepared, natural glycoprotein acceptors for these glycosyltransferases.

Subsequently, a number of variants of either Morré's or the Fleischers' procedures have been published (39). Most of the fractions obtained retain stacked Golgi cisternae (Fig. 18).

The recovery of galactosyltransferase activity in Golgi fractions prepared by these procedures was no better than 30–40% (in reference to the homogenate). Hence, attempts were made to improve yield by overloading the Golgi elements with lipoprotein particles, thereby modifying their density (9, 40).

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Overloading was induced by acute ethanol intoxication of the animals (rats). At the beginning, the galactosyltransferase recovery appeared to be nearly complete (40), but later, better-controlled assays showed that the yield was no better than 50 to 60% (41). This procedure was capable of resolving (by flotation in a density gradient) two or three fractions of increasing density. The light Golgi fractions were enriched in trans vacuoles or secretory droplets filled with lipoprotein particles (Fig. 19), whereas the heavy Golgi fractions had a higher concentration of cis, predominantly cisternal elements (Fig. 20). These fractions have been used for a variety of enzymological (40, 41) and cytochemical (20, 21, 28) studies and for investigating the transport of secretory proteins within the Golgi complex (42).

TABLE I

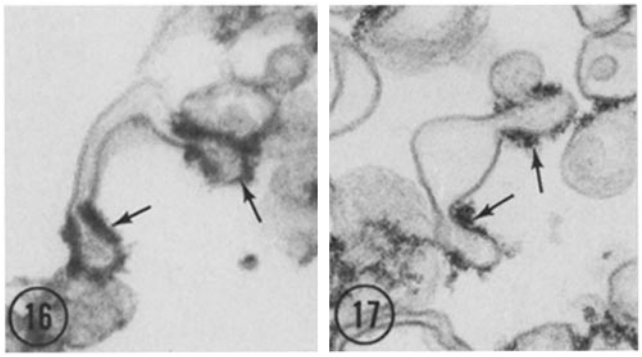
Cytochemical Reactions of Golgi Cisternae in the Hepatocyte

	Cis	Trans	Reference
5′-Nucleotidase	+	+*	20
Adenylate cyclase	+	+	21
Periodic acid-silver methenamine	+	+‡	22
OsO_4 impregnation	+	−	23
Acid phosphatase	−	+	19, 20
Thiamine pyrophosphatase	−	+	19, 20, 24
Glucose-6-phosphatase	−	−	20

* Both cis and trans elements were reactive, but a difference in sidedness of reaction product was detected: it was present on the outside of the membrane of cis elements (concentrated on the dilated rims) and on the inside of the membrane of trans elements.

‡ A gradient of increasing reactivity from the cis to the trans side was detected. From Farquhar (115).

In defining Golgi fractions, investigators have relied on galactosyltransferase as an accepted Golgi marker as well as on the absence (or low concentration) of microsomal (ER) markers, primarily glucose-6-phosphatase and NADPH-cytochrome P450 reductase. A complication arose when it was found (41) that assays carried out immediately upon fractionation showed the presence of microsomal enzyme activities in unexpectedly high concentrations. Further work indicated that the corresponding Golgi activities were lost rapidly during storage, presumably as a result of lipid peroxidation (41). This



FIGURES 16 and 17 Golgi cisternae from Golgi subfractions (GF_3) prepared by the method of Ehrenreich et al. (9) and reacted for 5′-nucleotidase (Fig. 16) or adenylate cyclase (Fig. 17) prior to fixation. Reaction product (lead phosphate) is concentrated on the dilated rims of the cisternae (arrows) and is absent from their central regions. × 85,000. Fig. 16 is from Farquhar et al. (20), and Fig. 17 is from Cheng and Farquhar (21).

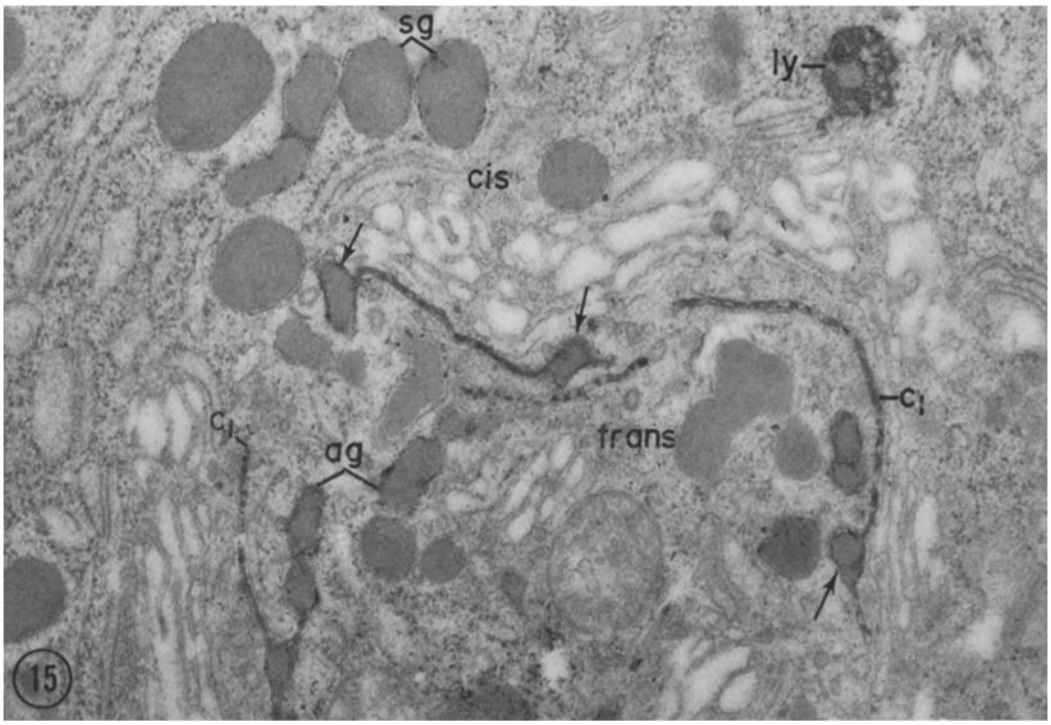


FIGURE 15 Golgi region of a prolactin-secreting cell from a lactating rat (similar to that in Fig. 1); preparation incubated for acid phosphatase. Condensing secretory granules (arrows) and reaction product for AcPase are present in the same Golgi cisterna—i.e., the innermost cisternae (c_1) along the trans side of the stack which is less dilated than the rest. In some places (to the right), the reactive cisterna seems to be included in the regular stack, and in other places (to the left) it appears to be set off from the stack. AcPase reaction product is also seen around some of the immature or aggregating granules (ag) found on the trans Golgi face, at the periphery of a few of the mature granules (sg) present on the cis Golgi face, and in a lysosomes (ly). × 30,000. From Smith and Farquhar (154).

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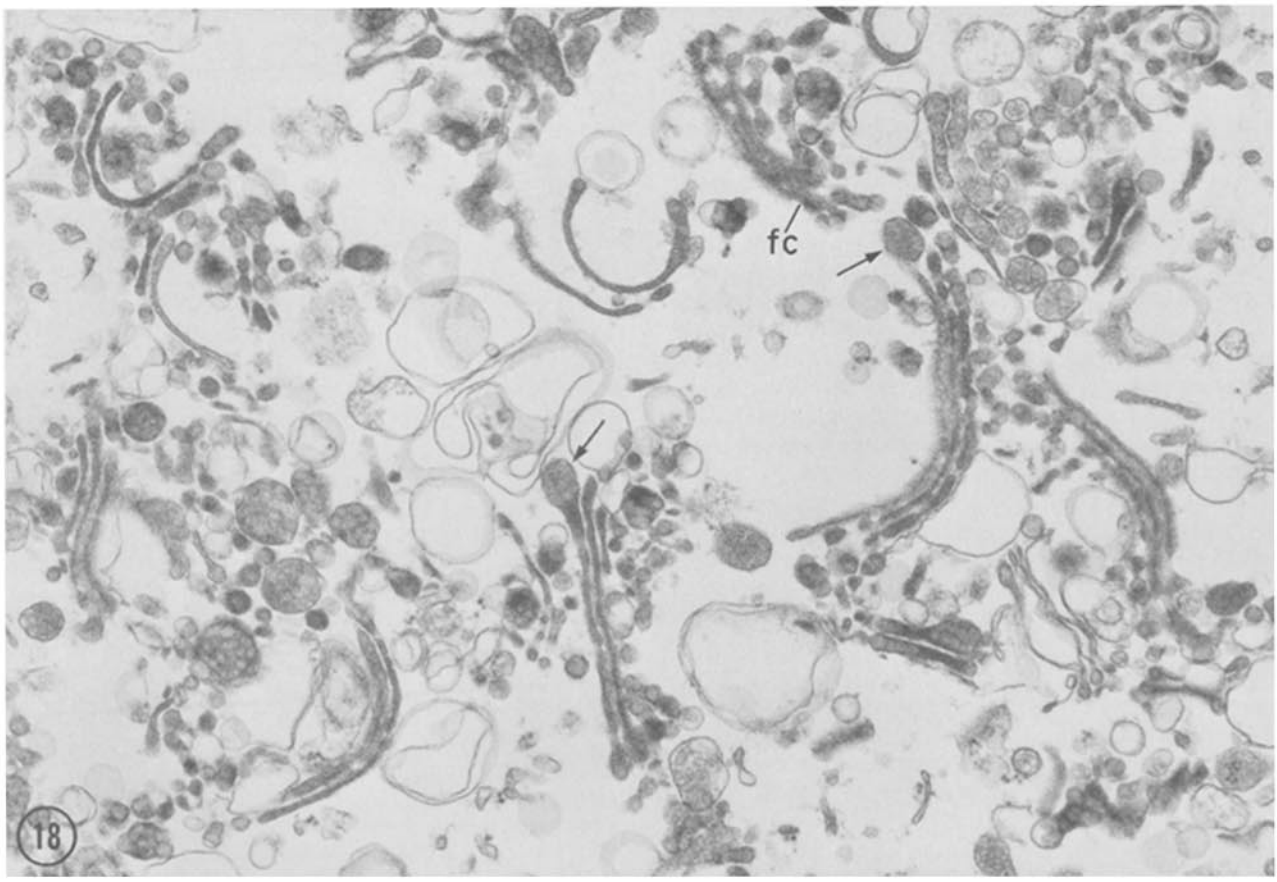

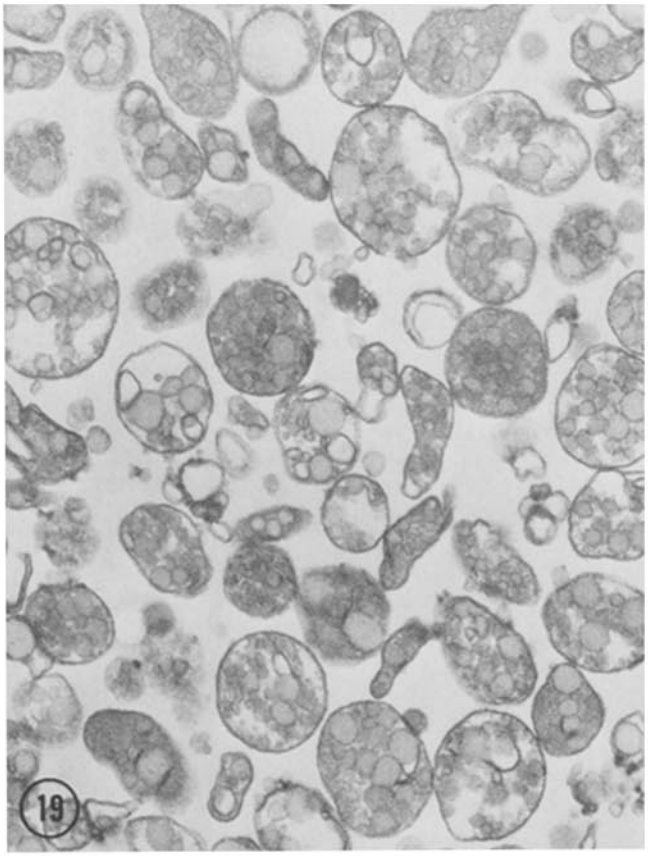

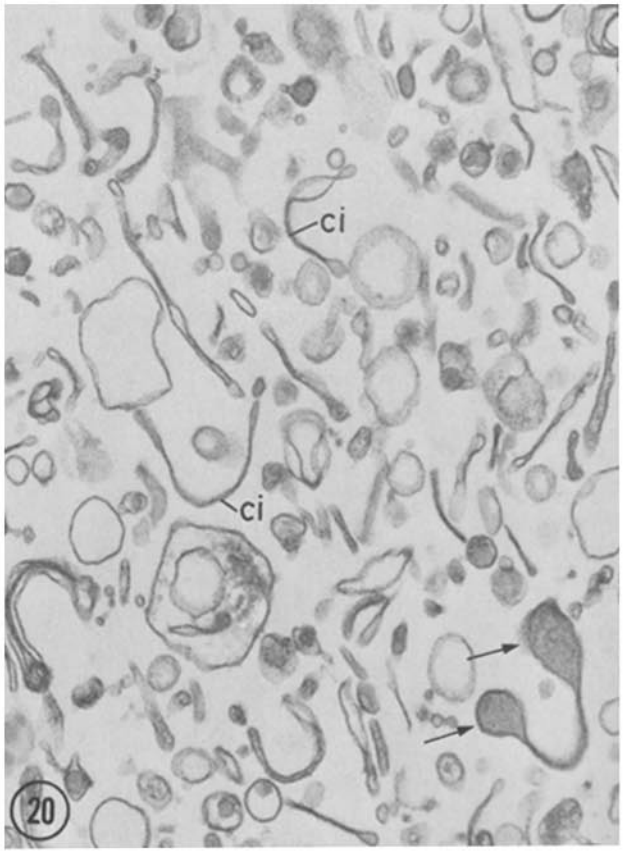

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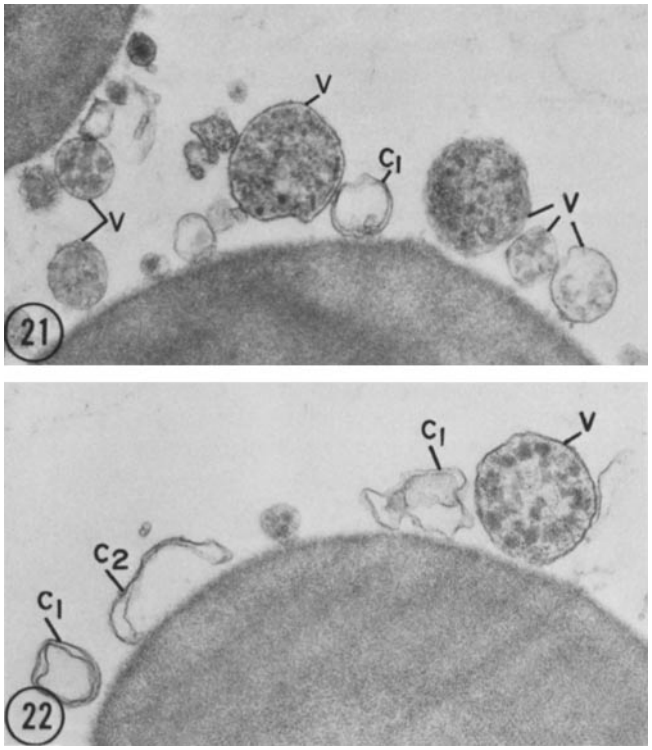
raised the question of whether these microsomal, marker-enzyme activities (like the plasmalemmal markers studied earlier [20, 21]), were indigenous to Golgi fractions, or instead represented contamination of the fractions with ER components.

To solve this problem, Ito and Palade (43) developed an affinity separation procedure. It uses an antibody to NADPH-cytochrome P450 reductase insolubilized to polyacrylamide beads, and allows biochemical assays as well as an electron-microscope survey of immunoadsorbed and nonadsorbed particles. When applied to a light Golgi fraction, the procedure revealed that bona fide Golgi elements—both lipoprotein-loaded secretory vacuoles (~ 58%) and cisternae (14%)—had the reductase in their membranes (Figs. 21 and 22). The affinity adsorption technique was extended to other enzymatic activities, and the results showed that a wide spectrum of microsomal enzymes was present in recognizable, immunoadsorbed Golgi vacuoles, whereas glycosyltransferase activities preferentially remained with the nonadsorbed vesicles. The tentative interpretation of these findings is that Golgi elements have distinct domains; the distended rims of at least some of the Golgi cisternae have 'ER-like' membranes, whereas the central part of the cisternae has an apparently 'Golgi-like' membrane rich in glycosyltransferase activities. It seems probable that the ER-like membrane represents the shuttle containers that transport secretory products from the ER to the Golgi complex. Current thinking (see below) assumes the existence of another membrane container (the equivalent of a secretion-granule membrane) recycling between the Golgi complex and the plasmalemma, but at present there is no information concerning its nature in hepatocytes.

Affinity separation techniques, based on insolubilized specific ligands, are expected to provide further information about the biochemical heterogeneity of Golgi elements and its functional implications. It should be pointed out that although galactosyltransferase activity is considered a marker for Golgi membranes, not only do some morphologically recognizable Golgi elements lack this activity (43), but also a substantial amount (40–50%) of it remains in a residual microsomal fraction in elements of still unknown morphology.

BIOCHEMISTRY OF GOLGI MEMBRANES: Data concerning the biochemistry of Golgi membranes are still limited, partly because of the difficulties encountered in the separation of bona fide Golgi elements from their membrane containers which shuttle between the complex and the ER or plasmalemma. The lipid composition of Golgi membranes appears to be quantitatively different from that of both the ER membrane (more sphingomyelin, less phosphatidylcholine) and the plasmalemma (less cholesterol, less sphingomyelin) (44–46). The electrophoretograms of Golgi membranes reveal a protein composition different qualitatively and quantitatively from that of ER and plasmalemma (35, 45), but the results are in need of extension and improvement.

Enzyme assays established the existence of compositonal overlap between ER and Golgi membranes (15, 45), at least in the case of the fatty acid desaturase system (NADH-cytochrome b_5 reductase); however, from the results of the affinity separation already mentioned (43), the overlap appears more extensive. It includes both the cytochrome P450 system and glucose-6-phosphatase.



FIGURES 21 and 22 Affinity technique for the separation of constituents of Golgi fractions on beads. Goat anti-rabbit IgG was covalently attached to polyacrylamide beads, rabbit anti-NADPH-cytochrome c reductase antibody was immunoabsorbed to the beads coated with the first antibody, and Golgi fractions (GF_{1+2}) were reacted with the beads. Recognizable Golgi elements immunoadsorbed to the antireductase-coated beads are secretory vacuoles (v) containing lipoproteins, and cisternae cut either in transverse section (c_1) or in perpendicular section (c_2). × 31,000. From Ito and Palade (43).

Enzymes involved in proximal glycosylation and translocation of nascent polypeptide chains remain unchallenged markers for ER membranes. The same may apply for enzymes involved in triacylglycerol and phospholipid synthesis as indicated by the work of van Golde et al. (46). As already mentioned, terminal glycosyltransferases as well as sulfotransferases (see below) are restricted to Golgi membranes.

Established Functions of the Golgi Complex

PACKAGING OF SECRETION GRANULES: The central role of the Golgi apparatus in secretion was recognized long ago by light microscopists (reviewed by Bowen [47]). Early electron microscopic studies carried out in the 1950s by Sjöstrand and Hanzon (5), Haguenau and Bernhard (48), and Farquhar and

FIGURES 18–20 Golgi fractions from rat liver. Fig. 18 illustrates a fraction prepared from the liver of a normal rat by the procedure of Leelavathi et al. (39), which yields Golgi elements that remain stacked. Lipoprotein particles can be recognized in the dilated rims of many of the cisternae (arrows). Figs. 19 and 20 are Golgi subfractions prepared by the method of Ehrenreich et al. (9) from livers of ethanol-treated rats. Fig. 19 shows a light Golgi fraction (GF_2), and consists mainly of secretory vacuoles filled with lipoprotein particles. Fig. 20, from the heaviest Golgi fraction (GF_3), consists either of whole cisternae or the central parts of collapsed cisternae (ci). A few cisternae contain lipoprotein particles in their dilated rims (arrows). Fig. 18—× 20,000; Fig. 19—× 27,000; Fig. 20—× 36,000. Figs. 19 and 20 are from Ehrenreich et al. (9).

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Rinehart (7) noted the close association between secretory granules and Golgi elements, and shortly thereafter several investigators (49, 50) published electron micrographs in which material resembling the contents of secretory granules was clearly recognized within Golgi elements. Subsequent morphological and autoradiographic studies (reviewed in 2, 3, and 51–53) established that in most cell types concentration and packaging of secretory products usually occurs in the dilated rims of the transmost cisternae (Figs. 1–4); however, in a few cell types (exocrine pancreas and parotid of some species), concentration takes place in specialized condensing vacuoles, which are separate from the stacked cisternae (Figs. 7 and 8). In either case, concentration results in the production of a storage granule with a condensed content and a membrane acquired in the Golgi complex. That concentration takes place in many (but not all) cell types has been corroborated by both autoradiographic (52, 54, 55) and cell fractionation (52) data demonstrating greatly increased specific activity of the content of forming and mature granules, as compared to that of the rough ER and Golgi cisternae (Figs. 23 and 24). Recent autoradiographic data obtained by high resolution autoradiographic analysis indicate that concentration up to 200 times that of the ER is achieved in granules of pituitary prolactins (55).

The basis of our current understanding of the overall route of intracellular transport taken by secretory products and the position of the Golgi complex along that route was provided by the combined morphological, autoradiographic, and cell fractionation studies that were initiated by Caro and Palade and further developed by Jamieson and Palade (reviewed in 52 and 57) on the exocrine cells of the guinea pig pancreas, which is diagramed in Fig. 25. With the in vitro systems used by Jamieson and Palade (52, 57, 58), temporal and spatial resolution were increased by using well-controlled, pulse-chase experiments. Moreover, the results of the experiments could be quantitated by autoradiography or by cell fractionation. Their work supports the following model: secretory proteins synthesized in the rough ER are transported to the Golgi region in small vesicular containers which are assumed to function as shuttles between the transitional elements of the ER (Fig. 7) and Golgi elements. Their studies did not establish the route taken by secretory products through the Golgi (see below), but their autoradiographic findings (54) demonstrated clearly that

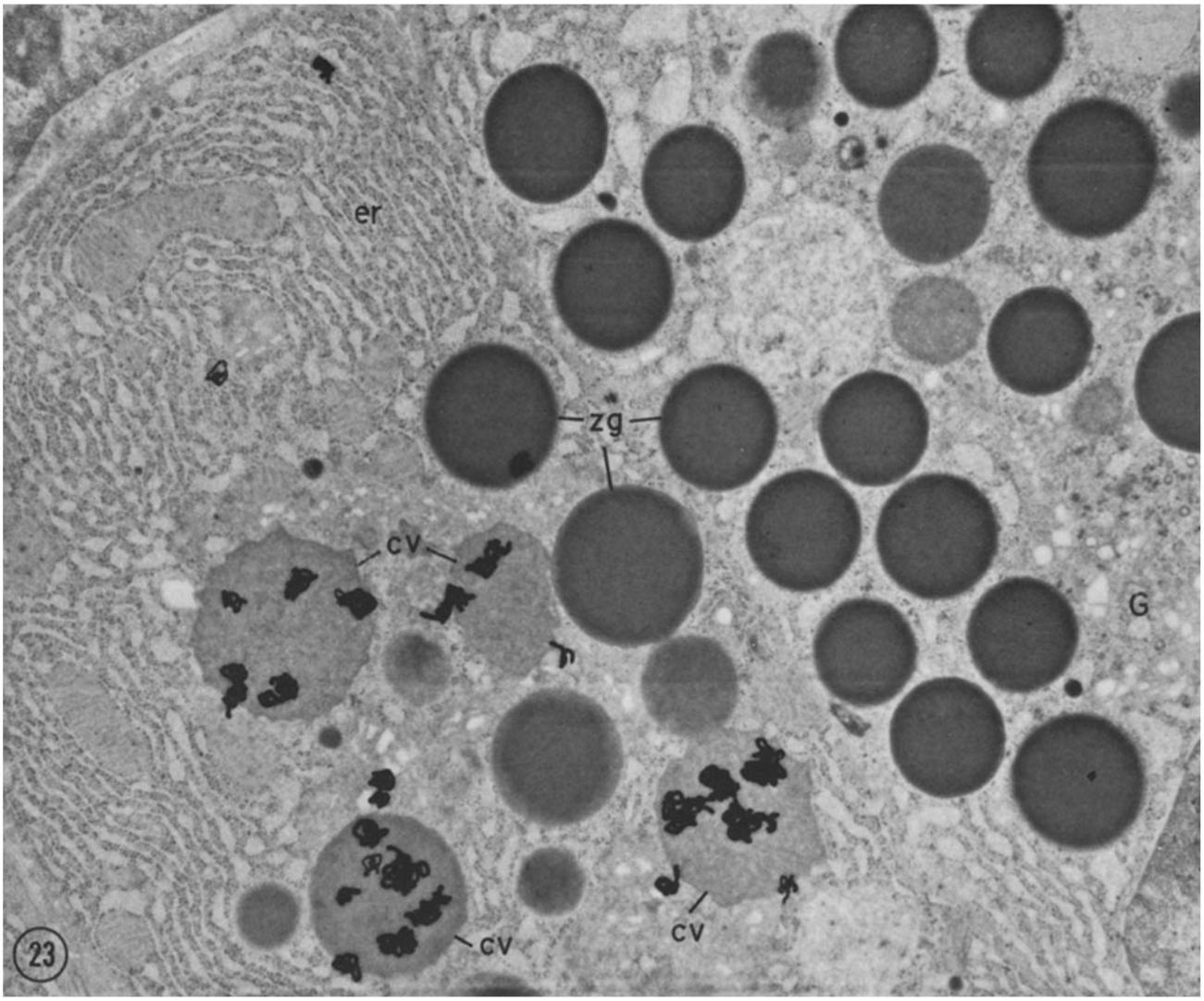

FIGURE 23 Autoradiogram of a pancreatic exocrine cell (guinea pig) pulse-labeled with [^{3}H]leucine in vitro and fixed at the end of a 20-min chase. Grains over condensing vacuoles (cv) are much more numerous than over the rough ER (er) or Golgi elements (G) at this time point. The mature zymogen granules (zg) are not labeled; their peak of radioactivity is reached later (60–80 min) postpulse. × 16,000. From Jamieson and Palade (54).

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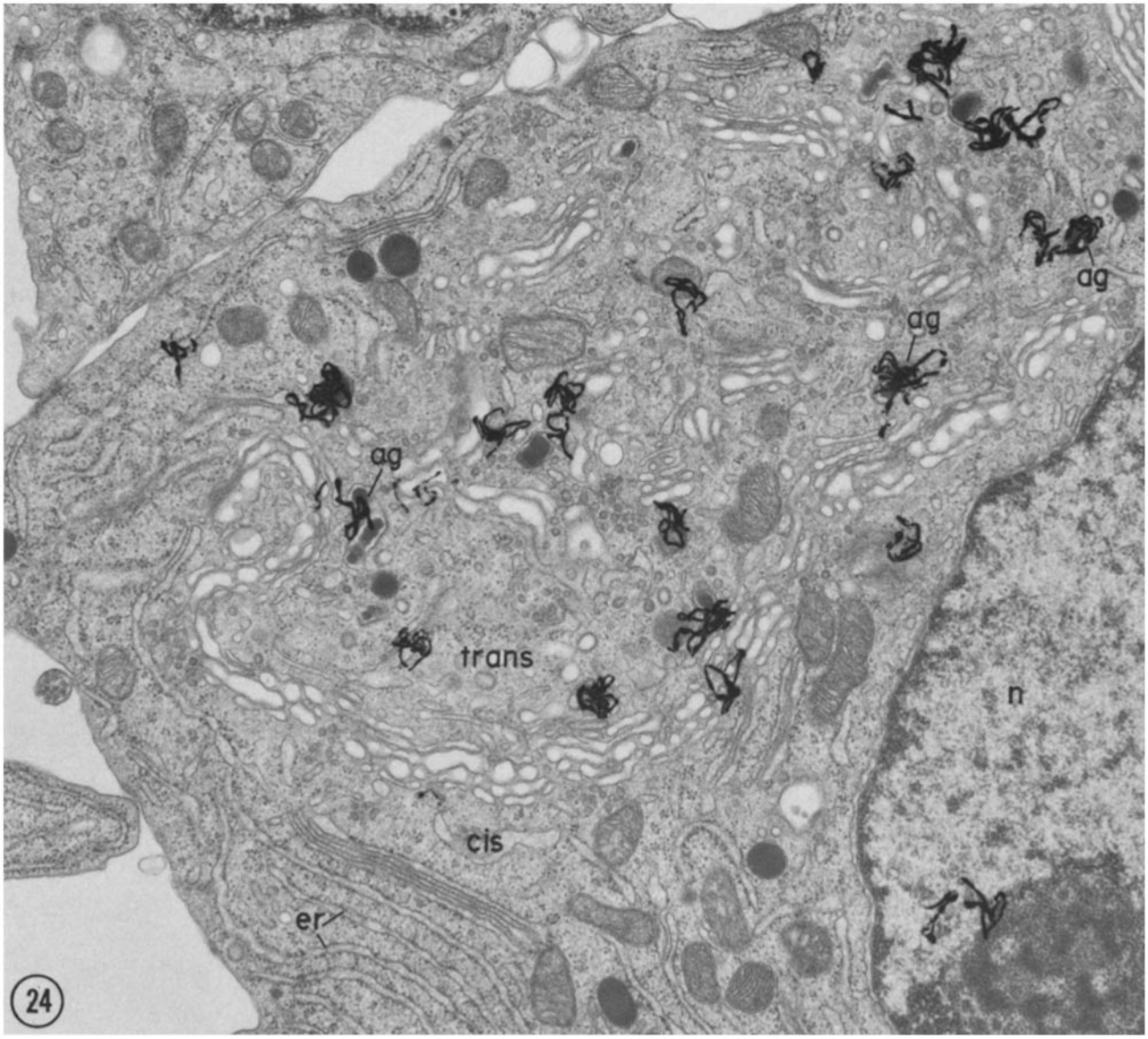

FIGURE 24 Autoradiogram of a prolactin cell (rat anterior pituitary) from a dissociated cell preparation pulsed in vitro for 5 min with [^{3}H]leucine and fixed after a 30-min chase. Grains are concentrated over immature or aggregating granules (ag) located on the trans side of Golgi stacks. When corrected for radiation spread, the grain density (grains/unit area) of the immature granules at peak labeling is 50–200 times that of the rough ER, indicating that the secretory product (> 85% prolactin) undergoes a ~ 200-fold concentration (56). × 24,000. From Farquhar et al. (55).

secretory products are transported to condensing vacuoles located on the trans side of the Golgi stacks (Fig. 23). As already mentioned, in most other cell types concentration normally takes place in the distended rims of the transmost cisternae (Fig. 24), which are the equivalent of condensing vacuoles. The same pattern was found in hyperstimulated pancreatic exocrine cells (58). Transport out of the ER to the Golgi was shown to be vectorial and energy-dependent, as it was arrested by inhibitors or uncouplers of oxidative phosphorylation (antimycin A, DNP). Subsequently it has become clear that, while in transit between the ER and forming granules, secretory proteins may undergo modifications such as glycosylation, sulfation, and proteolytic processing (described in subsequent sections), as well as concentration.

The general applicability of this model to a wide variety of cell types has been well documented and reviewed elsewhere (51, 52, 57). As far as Golgi involvement is concerned, the best studied cell types, are the parotid cell (52), the fibroblast (59 and Hay, this volume), the odontoblast (60), the β-cell of the pancreatic islets (61), the hepatocyte (42, 62), the thyroid cell (63), the mammotroph or prolactin cell of the anterior pituitary (64, 65) (Fig. 26), and leukocytes (53). There is no documented example of a cell in which the secretory product bypasses the Golgi. At one time it was suggested that collagen secretion by fibroblasts and immunoglobulin secretion by plasma cells might represent exceptions to the accepted scheme, and that in these cells at least part of the secretory product might be discharged directly from the ER, thus bypassing the Golgi. Autoradiographic studies carried out by several investigators (66, 67) were interpreted as supporting this contention. However, subsequent immunocytochemical results (Fig. 27 and Fig. 7 in Hay, this volume) have demonstrated the presence of the appropriate product (procollagen [59], and immunogloblins [68, 69]) in Golgi cisternae, thus confirming that in these cells

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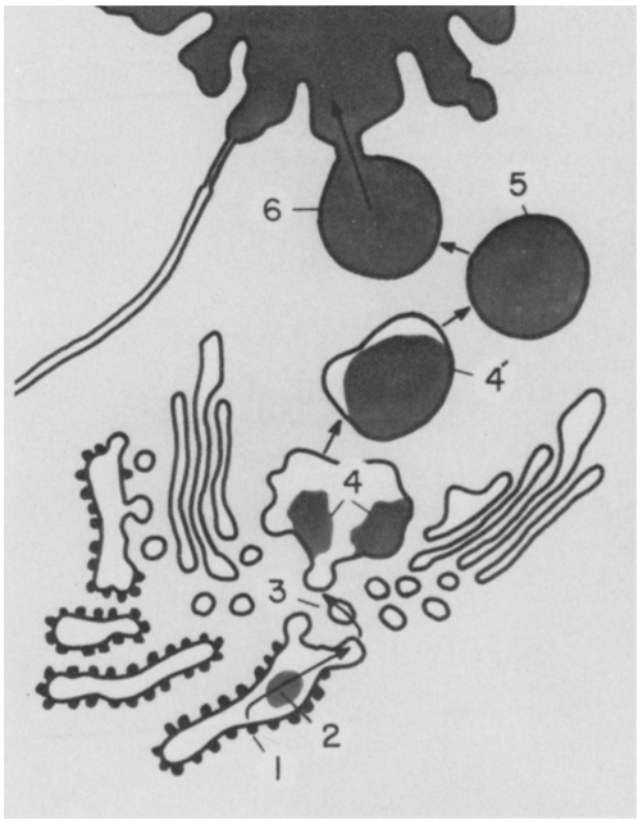

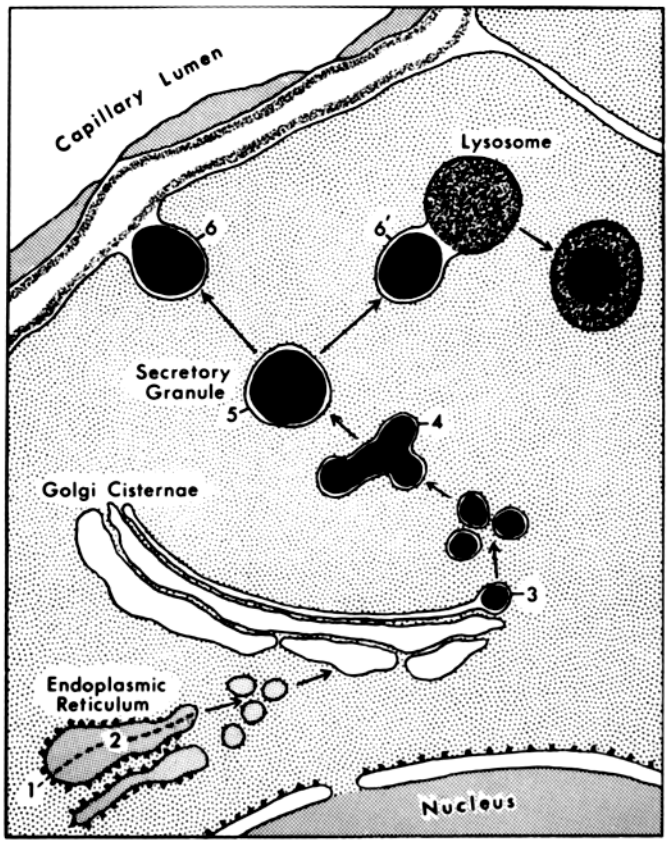

FIGURE 25 (left panel) Diagram of an exocrine pancreatic cell (guinea pig) showing the steps worked out by Jamieson and Palade for the synthesis and intracellular transport of digestive enzymes. The secretory proteins are (1) synthesized exclusively on polyribosomes which attach to the membranes of the rough ER, and are cotranslationally transferred across these membranes to be segregated (2) within the cisternal space of the rough ER. They are then transported (3) via small vesicles from the rough ER to condensing vacuoles located in the Golgi region where concentration (4) and (4′) takes place. The concentrated product is then stored (5) in secretion granules until discharged (6) by exocytosis, or fusion of the granule membrane with the plasmalemma at the apical cell surface.

FIGURE 26 (right panel) Diagram of events in the secretory process of the prolactin cell or mammotroph in the anterior pituitary of the rat from the work of Farquhar and co-workers. Prolactin is synthesized on attached ribosomes (1), segregated in the rough ER (2), transported to, and concentrated within granules in the Golgi complex. Small granules arising within the inner Golgi cisterna (3) aggregate (4) to form mature secretory granules (5). During active secretion, the latter fuse with the cell membrane (6) and are discharged into the perivascular spaces by exocytosis. When secretory activity is suppressed and the cell must dispose of excess stored hormone, some granules fuse with lysosomes (6′) and are degraded. This scheme is basically similar to that which takes place in the pancreatic exocrine cell (Fig. 25) except that (*a*) concentration begins in the stacked Golgi cisternae (instead of in specialized condensing vacuoles) and continues away from the complex in structures analogous to condensing vacuoles, and (*b*) there is a discharge option whereby the granules can be discharged either extracellularly (into perivascular spaces) or intracellularly into lysosomes by crinophagy. From Smith and Farquhar (154).

too, the secretory proteins follow the Golgi complex route. It is now clear that the earlier confusion and the inconclusive autoradiographic results can be explained by the fact that these cell types represent a special variant of the model in which the secretory products do not undergo concentration as a prerequisite for storage, and hence no secretion granules are formed. They are packaged in the Golgi complex in the usual manner and discharged by exocytosis in the usual manner, but the carrier consists of a fluid-filled vesicle instead of a dense granule (52, 69, 70).

The fact that concentration commonly takes place in the dilated ends of the Golgi cisternae raised the intriguing question of how concentration is brought about in the dilated ends of a continuous compartment. The first information on this problem came from the experiments of Jamieson and Palade (71), who showed that concentration in both condensing vacuoles and zymogen granules was maintained *in situ* in the absence of ATP synthesis. The findings led to the conclusion that concentration is *not* dependent on continuous expenditure of energy, as expected if the operation depended on an ion pumping mechanism. Instead, concentration apparently results from the formation of osmotically inactive aggregates, which is accomplished either by crystal formation (blood eosinophil [53] and pancreatic β-cell [52]), or by electrostatic interaction between secretory products and other molecules of opposite charge—especially protein-polysaccharide complexes: e.g., mast cell heparin with a basic polypeptide (72), cationic lysosomal enzymes (73) or cationic pancreatic proteins (74) with sulfated glycosaminoglycans (GAGs), prolactin with sulfated GAGs and glycopeptides (65). There is also evidence that calcium is present in certain secretion granules (i.e., those of pancreatic β-cells [75] and exocrine pancreatic cells [76]) where

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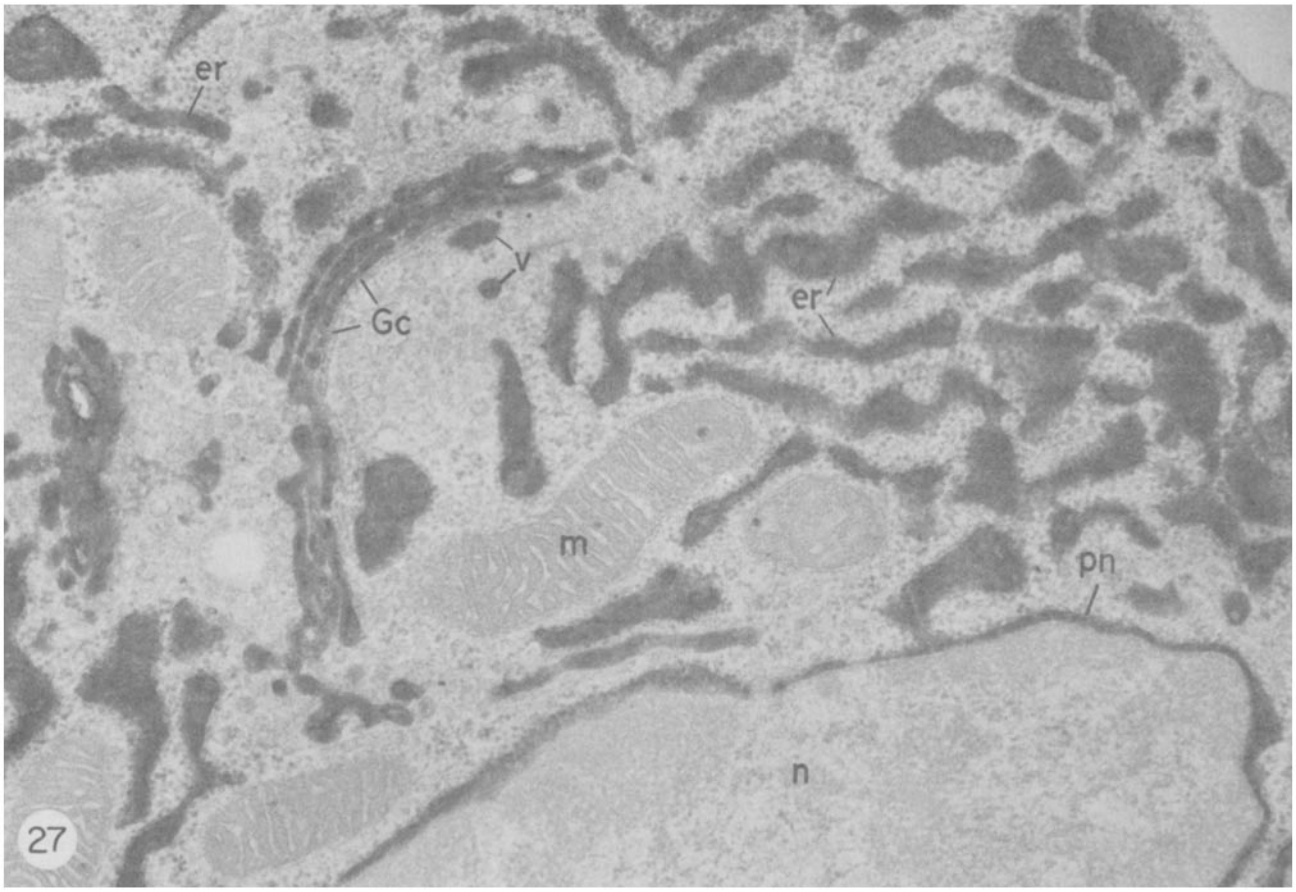

FIGURE 27 Immunocytochemical localization of immunoglobulins in the secretory compartments of a plasma cell from the spleen (rat). Spleen cells were harvested from a rat immunized against horseradish peroxidase (HRP), and lightly fixed; cryostat sections were incubated with HRP and subsequently reacted with diaminobenzine (DAB). Reaction product, indicating sites of localization of anti-HRP immunoglobulins, is seen throughout the rough ER (er), including the perinuclear cisterna (pn), and in the stacked Golgi cisternae (Gc) and associated secretory vesicles and vacuoles (v). × 22,000. From Ottosen et al. (69).

it is concentrated along the inner surface of their limiting membranes. This raises the possibility that calcium may participate in the ionic interactions that take place during concentration (76). In a few cases it has been shown that a constant ratio exists between the packaged products, e.g., in the adrenal medulla (ATP/catacholamines = 4/1) (77) and neurohypophysis (neurophysin/oxytocin or vasopressin) (78). In other cell types such as pancreatic acinar cells and prolactin cells of the anterior pituitary, the presumptive packaging molecules (sulfated polyanions) represent a relatively minor constituent of the contents and may serve to initiate aggregate formation (65, 74). Many secretory granules are insensitive to the osmolality of the medium even after isolation (58, 65), but are extremely sensitive to pH changes, presumably because the aggregates are stabile only at certain pHs.

Because one of the main functions of the Golgi complex in the packaging operation is to provide a membrane container that is competent for exocytosis of the secretory product, one would like to know the nature of this membrane and how its composition compares with that of membranes of other cell structures, especially those with which it interacts during intracellular transport. There are only a few cases in which the secretory granule membranes have been isolated in pure enough form (free from content proteins) to permit analysis of their protein composition. In such cases, e.g., the membranes of chromaffin granules, parotid granules, zymogen granules of the exocrine pancreas (reviewed in 79), it has been shown that the protein composition is different from, and generally simpler than that of the membranes of other cell compartments (ER, Golgi, plasmalemma).

In summary, it is clear that passage of secretory products through the Golgi complex is obligatory, and involves extensive modification and transfer to a membrane container which is competent to fuse with the plasmalemma at the time of exocytosis. It is in this Golgi-derived membrane container that concentration of secretory products is accomplished, but concentration is not an obligatory operation. When it does occur, which is in the majority of secretory cells, it often involves the complexing of secretory products leading to the formation of macromolecular aggregates which are insoluble under *in situ* conditions. Further details about the nature of the membrane containers and the factors that affect or control concentration mechanisms need to be obtained.

GLYCOSYLATION OF GLYCOPROTEINS: It is now clear that one of the major functions of the Golgi apparatus pertains to the posttranslational modification of glycoproteins. The apparatus is exclusively responsible for the attachment of terminal or capping sugars (*N*-acetylglucosamine, galactose, fucose and sialic acid) to the oligosaccharide chains that are *N*-glycosidically-linked to glycoproteins in the rough ER (63, 80, 81). Less is known about the site of addition of oligosaccharide chains O-glycosidically linked to serine, threonine, and tyrosine resi-

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dues of mucin-type glycoproteins, but the biochemical information available (81) and the autoradiographic findings summarized below suggest that it also takes place in the Golgi complex.

Progress in understanding the biochemical events in glycoprotein synthesis and the intracellular localization of these sequential biosynthetic steps has been so rapid that one must pause and recall that the first evidence of a role for the Golgi apparatus in glycoprotein synthesis was obtained barely 15 years ago. That evidence was provided by the findings of Neutra and Leblond (80); they showed, by autoradiography in animals sacrificed 5–15 min after administration in vivo of radiolabeled hexose ([^{3}H]glucose and [^{3}H]galactose), that the vast majority of the autoradiographic grains were localized over the Golgi region of intestinal goblet cells and many other cell types (80). The grains were localized directly over Golgi cisternae by electron-microscope (EM) autoradiography. A few years later, using a similar LM and EM autoradiographic approach to study glycoprotein synthesis in the thyroid epithelial cell, Leblond and co-workers (63, 82, 83) demonstrated that the addition of core sugars ([^{3}H]mannose) to the peptide backbone of thyroglobulin takes place in the rough ER, whereas the addition of terminal sugars (galactose, fucose, and, more recently, sialic acid [63]) takes place in the Golgi apparatus. Thus, autoradiography proved to be very useful for identifying the initial cellular site of incorporation of various monosaccharide precursors. As used by Leblond and his associates, it has not only provided the first indication of the role of the Golgi complex in glycoprotein synthesis, but also has yielded the first evidence for intracellular separation of labor—between the rough ER and the Golgi complex—in the proximal and distal glycosylation of complex glycoproteins.

The localization of hexose incorporation to the Golgi complex by autoradiography took place well before the discovery that the glycosyltransferases responsible for the addition of terminal hexoses are associated exclusively within Golgi fractions. The next key event in the development of the glycoprotein story was the discovery in 1969, by B. Fleischer, S. Fleischer, and H. Ozawa (34), that a galactosyltransferase activity with the ability to transfer radioactive galactose to exogenous receptors (from UDP-gal to *N*-acetylglucosamine) was concentrated (80×) in Golgi fractions from bovine liver. Subsequent studies by the Fleischers and others, notably Morré (36), and Schachter and Roseman and their co-workers (37, 38), confirmed the presence of galactosyltransferase activity in Golgi fractions. This provided the first biochemical evidence for the involvement of Golgi membranes in the addition of terminal hexose residues to glycoproteins. Subsequently, fucosyl and sialyl transferases were also shown to be characteristic Golgi enzymes (84), but to this day, galactosyltransferase remains the main marker enzyme for the Golgi complex. Recently, B. Fleischer (85) has established that both galactosyltransferases and sialyltransferases are membrane proteins with active sites located on the luminal side of the Golgi cisternae.

It should be noted that, although galactosyltransferase activities are found inside most cells bound to Golgi membranes, they also occur in soluble form (84) (e.g., in milk, serum, and epididymal [86] fluids), and in milk globule membranes (usually assumed to be derivatives of the plasmalemma of the mammary epithelium [87]).

As information increased about the existence of different types of oligosaccharide chains in glycoproteins and the steps involved in their biosynthesis, it became apparent that many secretory and membrane proteins contain *N*-glycosidically-linked, complex-type oligosaccharides which are first synthesized (from dolichol intermediates [88]) in the ER as mannose-rich precursors with extra glucose and mannose residues. These residues are subsequently trimmed, with removal of all of the glucose and some (six) of the mannose residues, before addition of the terminal hexoses (89). The trimming of mannosyl residues was localized indirectly to the Golgi apparatus by the discovery in Golgi fractions of an α-D-mannosidase activity, which is capable of processing asparagine-linked oligosaccharides and is distinct from the mannosidases of the cytosol and lysosomes (89, 90).

Recently, Kornfeld and his associates (91, 92) have delineated a role for the Golgi complex in the trimming and glycosylation of lysosomal enzymes. They found that the biosynthesis of lysosomal enzymes involves the transfer of an *N*-acetylglucosamine phosphate to mannose residues of the enzymes. These glucosamine residues are then removed to expose the mannose-6-phosphate, which is believed to be the recognition marker for lysosomal enzymes (see Bainton, this volume). Kornfeld's group has shown also that both the transferase activity (*N*-acetylglucosamine 1-phosphotransferase) and the trimming enzyme (α-*N*-acetyl glucosaminyl phosphodiesterase) are concentrated in Golgi fractions.[1]

An important but still unresolved question is where, in the heterogeneous Golgi complex, do glycosylation and trimming take place? The question has not yet been answered because the transferases were found to be equally distributed in Golgi subfractions (93). However, that there may be a restricted or specialized distribution is suggested by the results obtained by a new affinity separation technique, which showed that galactosyltransferase and NADPH-cytochrome P-450 reductase are associated with different, morphologically recognizable Golgi elements (43).

Two other important questions are the subject of current research by B. Fleischer and her associates: How are the nucleotide sugars that serve as substrates for the transferases (and which are synthesized elsewhere in the cell) transported across the Golgi membranes? And how are the products of the transferase reaction (UDP and CMP) removed? Regarding the latter, Brandon and Fleischer (94) have shown that UDP formed in intact Golgi vesicles during galactosylation is rapidly broken down by nucleoside diphosphatases (NDPases) present in the lumen of Golgi vesicles. It is tempting to suggest that the neutral NDPase activity, as well as the acid phosphatase activity (which can be demonstrated using a variety of substrates including CMP) found by cytochemical localization in certain Golgi membranes, may be involved in these operations. To address the first question, Fleischer (95) recently has studied the nucleotide profile of rat liver Golgi by high-pressure liquid chromatography and found major peaks associated with several nucleotides: UDP, AMP, UMP, and CMP. The fact that there is a selective distribution of nucleotides, together with the finding that UDP is selectively retained after osmotic shock, led Fleischer to suggest (96) that the Golgi is not freely permeable to these molecules, and that a selective transport system or binding protein exists for the uptake or exclusion of specific nucleotides from this organelle.

GLYCOSYLATION OF GLYCOLIPDS: There is also evidence (97, 98) that, in addition to glycosylation of glycoproteins, the Golgi apparatus is involved in glycosylation of at least some glycolipids, especially those that contain terminal galactose and

[1] Kornfeld, S. Personal communication.

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sialic acid residues, i.e., cerebrosides and gangliosides. As far as is known, the glycolipids are present in tissues exclusively as membrane constituents, but their concentration differs from one tissue to another (i.e., high in brain and kidney and low in liver). B. Fleischer (95) has shown that a number of glycosyltransferases (as well as a sulfotransferase) (98) which function in the addition of hexose residues to glycolipids are localized in Golgi fractions isolated from kidney homogenates, and Richardson et al. (97) have found the same in Golgi fractions from rat liver.

SULFATION: As in the case of glycosylation, the first indication that the Golgi complex functions in sulfation was obtained by autoradiography. In 1964, Lane et al. (99) and Godman and Lane (100), working with L. Caro, who, with R. van Tuburgen, had just introduced techniques for EM autoradiography a few years before, demonstrated that immediately after administration of radioactive sulfate in vivo, exposed grains were concentrated over Golgi cisternae and vacuoles in goblet cells and cartilage cells. Much later, Young (101) surveyed a variety of cells and found uptake of radioactive sulfate by the Golgi complex in 14 additional cell types, e.g., leukocytes, Schwann cells, endothelial cells, keratinocytes, fibroblasts, and follicular cells of the ovary. The uptake of sulfate by cartilage and goblet cells was to be expected, because these cells are known to produce high levels of sulfated proteoglycans (chondroitin sulfate) and sulfated glycoproteins (mucins), respectively. More surprising at the time was the finding of sulfate incorporation into the other cell types mentioned. Since then, however, it has become clear that many cells (leukocytes, endothelial cells, fibroblasts, and ovarian cells) synthesize sulfated proteoglycans which can either be deposited in the extracellular matrix, retained intracellularly (e.g., in secretion granules), or remain associated with the cell surface. It has also become clear that many other classes of molecules, such as glycolipids (98), glycoproteins (99), and steroid hormones can be sulfated (101). Apparently all these reactions occur in the Golgi, for in all cases initial incorporation has been localized to this organelle by autoradiography. However, retention of sulfated steroids in such experiments remains to be proven.

In sulfation, as in glycosylation, the sulfate is activated by binding to a nucleotide from which it is transferred to an appropriate receptor molecule by a specific sulfotransferase (98, 101). Less is known about the location of the enzymes involved in sulfation than about that of glycosyltransferases, but the information available indicates that the a number of sulfotransferases are Golgi-associated enzymes (98, 102–104). The first sulfotransferase to be localized in Golgi fractions and to be solubilized and characterized (103), is a cerebroside sulfotransferase, present in rat kidney, which converts cerebroside to sulfatide (a sulfated glycosphingolipid). This enzyme, like the glycosyltransferases, appears to be an intrinsic membrane protein (103). Sulfotransferase activity has also been localized in Golgi-enriched fractions from liver (102) and mast cells (104), but the enzymes involved have not yet been characterized. Evidence has been presented that the mast cell enzyme is involved in proteoglycan synthesis (104).

In summary, autoradiographic findings and information obtained on Golgi fractions indicate that sulfation, like terminal glycosylation, is exclusively a Golgi function, but supporting biochemical data derived from cell fractionation are still quite limited.

PROTEOLYTIC PROCESSING OF PROPROTEINS: Over the past few years it has become evident that most secretory and membrane proteins undergo one or more intracellular proteolytic processing steps during biosynthesis. Examples are the cleavage of presecretory and prosecretory proteins, and cleavages that occur during the assembly of macromolecular structures such as virus capsids and membrane associated enzyme complexes (see Steiner et al. [105] for a recent review). The processing event that usually occurs in the Golgi complex involves the conversion of proproteins to secretory proteins. Many small peptide hormones (proinsulin, proparathormone, proopicocortin) as well as other secretory proteins (proalbumin) undergo processing of this type to yield their mature discharged form. The association between proprotein processing and the Golgi complex was made initially by Steiner and his collaborators (106) shortly after the discovery of proinsulin, and was based on the finding that when intracellular transport from the ER to the Golgi complex was stopped (by treatment with inhibitors of ATP synthesis such as antimycin A), no processing of proinsulin to insulin occurred. This finding demonstrated that transport out of the ER to the Golgi area was necessary for the processing of proinsulin to occur. The kinetics of the processing, which revealed an initial delay of 10–20 min followed by continued activity for up to 1 h, supported that conclusion. Similar findings were also obtained for the conversion of proparathormone to parathormone (107). The fact that conversion continued for up to 1 h, whereas in most systems, transport to the Golgi is assumed to be virtually completed by 30 min, suggested that processing might continue in secretion granules after packaging (105). Findings by Gainer and his associates (78) on the kinetics of processing of propressophysin (the common precursor of neurophysin and vasopressin) in neurosecretory neurons indicated that this is the case. In these cells, the precursor is packaged in the usual manner into neurosecretory granules in the Golgi complexes of the neuronal cell bodies, which are located in the supraoptic nuclei of the hypothalamus. After packaging, the granules migrate (by axonal flow) down the axons in the pituitary stalk to reach the posterior lobe of the pituitary, where storage takes place. When the products obtained from the hypothalamus, the stalk, and the posterior lobe were compared, it became evident that processing was more complete in the stalk and posterior lobe than in the hypothalamus. Gainer et al. concluded (78) that progressive processing takes place in the granules while they are in transit down the stalk. By implication, the granules must contain the enzyme(s) involved in processing.

This brings us to a consideration of what is known concerning the enzymes involved in proteolytic processing within the Golgi complex and/or secretion granules. Work from Steiner's laboratory (105) demonstrated that conversion of proinsulin to insulin can be accomplished in vitro by the combined action of an endopeptidase (pancreatic cationic trypsin) and an exopeptidase (carboxypeptidase B). Habener and associates (107) found the same situation to apply to the processing of proparathormone to parathormone. However, the nature of the endogenous activity that accomplishes the conversion is still problematical. Over the years, there have been claims that the zymogen forms of trypsin and chymotrypsin, cathepsins, kalikreins, or plasminogen activator (among others) are the proprotein processing enzymes (105). According to Steiner (personal communication), all of these alternatives have been challenged, and the actual identity of the proteolytic activity remains an open question. It does appear that there is a fundamentally similar processing enzyme for all proproteins, for they all contain paired basic residues at the sites the enzyme recognizes for cleavage; however, Golgi proteases have not

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been purified and characterized, and their precise intra-Golgi location is entirely unknown.

In short, the available evidence indicates that the proteolytic processing of proproteins is a post-ER step which requires transport to the Golgi complex and continues after the secretory product is packaged into granules. The precise nature of the processing enzyme(s) is unknown, as it has not yet been isolated and characterized. Indirect evidence suggests that it may be acquired at the time of formation of the secretion granules. Its mode of delivery to the granules—whether it is acquired with the Golgi membrane during packaging or by membrane fusion after packaging—is also unknown.

LIPOPROTEIN PACKAGING: It has been assumed that the Golgi complex plays a role in lipid metabolism since the electron microscope studies in the 1950s of Palay and Karlin describing the presence of lipid droplets in the Golgi cisternae of intestinal absorptive cells (108). Observations were soon extended to physiologically defined conditions in an attempt to correlate the presence of lipid droplets within the Golgi complex either to lipid absorption in the intestinal epithelium (109) or to lipoprotein secretion in hepatocytes (110–112). Moreover, lipoprotein particles were isolated from Golgi fractions and found to contain particles comparable to serum VLDL (111, 112). More recent work on this topic has been extensively reviewed (113).

At present it is assumed that the ER is the site of synthesis of both the apoproteins and lipids (triacylglycerols, cholesteryl esters, and phospholipids) of hepatic lipoproteins. The assembly of these different components is thought to take place in the cisternal space of the ER as suggested by the appearance of osmiophilic (lipid) droplets of appropriate dimensions in that space, especially within the smooth ER (110). The pathway taken thereafter is the same as for other secretory products, that is, ER → Golgi cisternae → condensing secretory vacuoles, which are discharged by exocytosis at either the vascular (hepatocyte) or lateral (enterocyte) front of the cell. Thus far, the only functions established for the Golgi complex in lipoprotein secretion are terminal glycosylation of the appropriate apoproteins, all of which are glycoproteins (113), and packaging. Evidence obtained over the last few years indicates that the hepatocytes produce only VLDL and HDL; however, recent work by Howell and Palade (114) on lipoprotein particles isolated from hepatic Golgi fractions revealed extensive heterogeneity in particle size and biochemical composition. These findings suggest that most Golgi lipoprotein particles are immature products that require extensive modification in their lipid composition before release by exocytosis as either VLDL or HDL.

Traffic Through the Golgi Complex

At present it is clear that there is extensive traffic from more than one direction into and through the Golgi complex. This traffic is connected with membrane biogenesis, discharge of secretory proteins, membrane recycling, and uptake (interiorization) of informational molecules. In this section we will review the available evidence on the nature and direction of that traffic, as well as the ways in which the evidence was obtained.

TRAFFIC OF SECRETORY PRODUCTS: The general route taken by secretory proteins through the cell—from rough ER → transitional elements at the periphery of the Golgi complex → condensing vacuoles → secretion granules → discharge by exocytosis—was established as a result of the work on the exocrine pancreatic cell by Palade and his associates, primarily Jamieson and Palade. Still uncertain, however, is the route taken by secretory products through the Golgi complex itself as they move from the transitional elements of the rough ER to condensing granules or vacuoles (reviewed in 115).

For more than 20 years, the prevailing idea has been that secretory products move sequentially across the Golgi stack from the cis to the trans side, traverse the cisternae one by one, and undergo packaging on the trans face (see references 15, 38, and 63). The Golgi cisternae were thought to be formed on the cis face and used up in packaging on the trans face. The origin of this concept can be traced to Grassé, who in 1957, based on EM findings, proposed that the continuous formation of peripheral (cis) Golgi saccules (cisternae) balances the conversion of central (trans) saccules into secretion granules (116). Inherent in this formulation was the idea that membrane and contents move in synchrony from one side to the other of the stack, the products remaining in the same cisterna throughout the process. Subsequent morphologic, autoradiographic, and cell fractionation data were, for the most part, interpreted as supporting this scheme. In this section evidence that pertains directly to the pathway taken by secretory products is considered.

In their early autoradiographic studies, which involved the use of [^{3}H]hexose labeling, Neutra and Leblond (80) found grains associated at early time points with Golgi cisternae and at later time points with mucous granules of intestinal goblet cells. They interpreted these findings as support for the cis-to-trans flow diagram, and they and others estimated a turnover time for a cisterna of ~2 min. Jamieson and Palade (54), using autoradiography to investigate the route taken by secretory proteins in the pancreas, found no evidence for the direct involvement of the stacked cisternae in transport, as indicated by the absence or low density of grains over the Golgi stacks after a pulse-chase experiment with [^{3}H]leucine. However, they and others subsequently found autoradiographic grains over Golgi cisternae in other cell types, i.e., parotid cells (117) and pituitary prolactin cells (55), as well as in hyperstimulated pancreatic acinar cells (58). In none of these autoradiographic studies was the route and direction of movement of label *within* the stacks studied in detail.

These morphological findings, primarily the autoradiographic data of Neutra and Leblond, are the basis for the widespread belief that secretory products enter the Golgi at the cis side and emerge on the trans side. Indeed, this traffic pattern is implied in the naming of the two faces of the Golgi: i.e., the entry or immature face vs. the exit or mature face. Other work, e.g., the study of Bergeron et al. (42), on Golgi subfractions, in which it was shown that [^{3}H]leucine-labeled secretory proteins peak first in heavy Golgi fractions (believed to be derived primarily from cis Golgi cisternae) and a few minutes later in light Golgi fractions (believed to consist largely of secretory vacuoles from the trans side), was in keeping with this view. Moreover, EM studies on the assembly of scales in certain algae (118) supported the view that individual cisternae, in which scales are progressively assembled, move in the trans direction across Golgi stacks, while new scale components are added at each 'station.' As a result, the concept of cis-to-trans flow across the Golgi stacks became almost a dogma, and was the framework in which most investigators interpreted their findings without questioning the validity of the 'dogma.'

Studies in which secretory products have been localized within Golgi cisternae by cytochemical procedures also have contributed information concerning traffic through the Golgi

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complex. In the first of these studies, endogenous peroxidases were localized in eosinophils (119) and parotid cells (120), and were found to be present in all the cisternae in the Golgi stacks (Fig. 28). Similar but less striking findings have also been obtained when secretory products were localized by immunocytochemistry in other cell types, i.e., pancreatic enzymes in pancreatic exocrine cells (121), procollagen in fibroblasts (59; see Fig. 7 in Hay, this volume), and IgG in plasma cells (68, 69; Fig. 27). These observations suggested that all the stacked cisternae are involved in the transport and processing of secretory products, and hence, were compatible with the cis-to-trans flow diagram. However, they did not give any *direct* information on the route followed.

Thus, the evidence available is compatible with the assumption that all Golgi cisternae are involved in the transport and/or processing of secretory products. The data are also compatible with the view that secretory products move sequentially across the Golgi in the direction cis-to-trans, but with the evidence at hand, other possibilities cannot be ruled out (115).

SEGREGATION OF MULTIPLE SECRETORY PRODUCTS: Because the Golgi complex is responsible for concentration of secretory products, the question arises, how does the organelle handle the processing and packaging of multiple secretory products? In many if not most cases (e.g., the exocrine pancreas), the problem is resolved by avoiding segregation, and the Golgi complex packages the secretory proteins as a mixed cocktail in the same container (121). In one case, that of the PMN leukocyte which has two granule populations of different composition (one lysosomal, one not) (53), the problem is solved by making the two sets of granules in two waves of protein synthesis, which are well separated in time in the maturation process of the cells; one set is completed before the other is started. Interestingly enough, opposite sides of the Golgi complex are used for the packaging of the two sets of products (122).

LYSOSOMAL ENZYMES: The major problem in segregation faced by all secretory cells concerns the handling of lysosomal enzymes and their separation from secretory proteins. There is now large body of circumstantial evidence (see Bainton, this volume) which supports the hypothesis that mannosyl phosphate residues (mannose-6-phosphate) on lysosomal enzymes constitute a special recognition marker that serves to direct lysosomal enzymes to lysosomes. There is also evidence that coated vesicles are involved in the transport of lysosomal

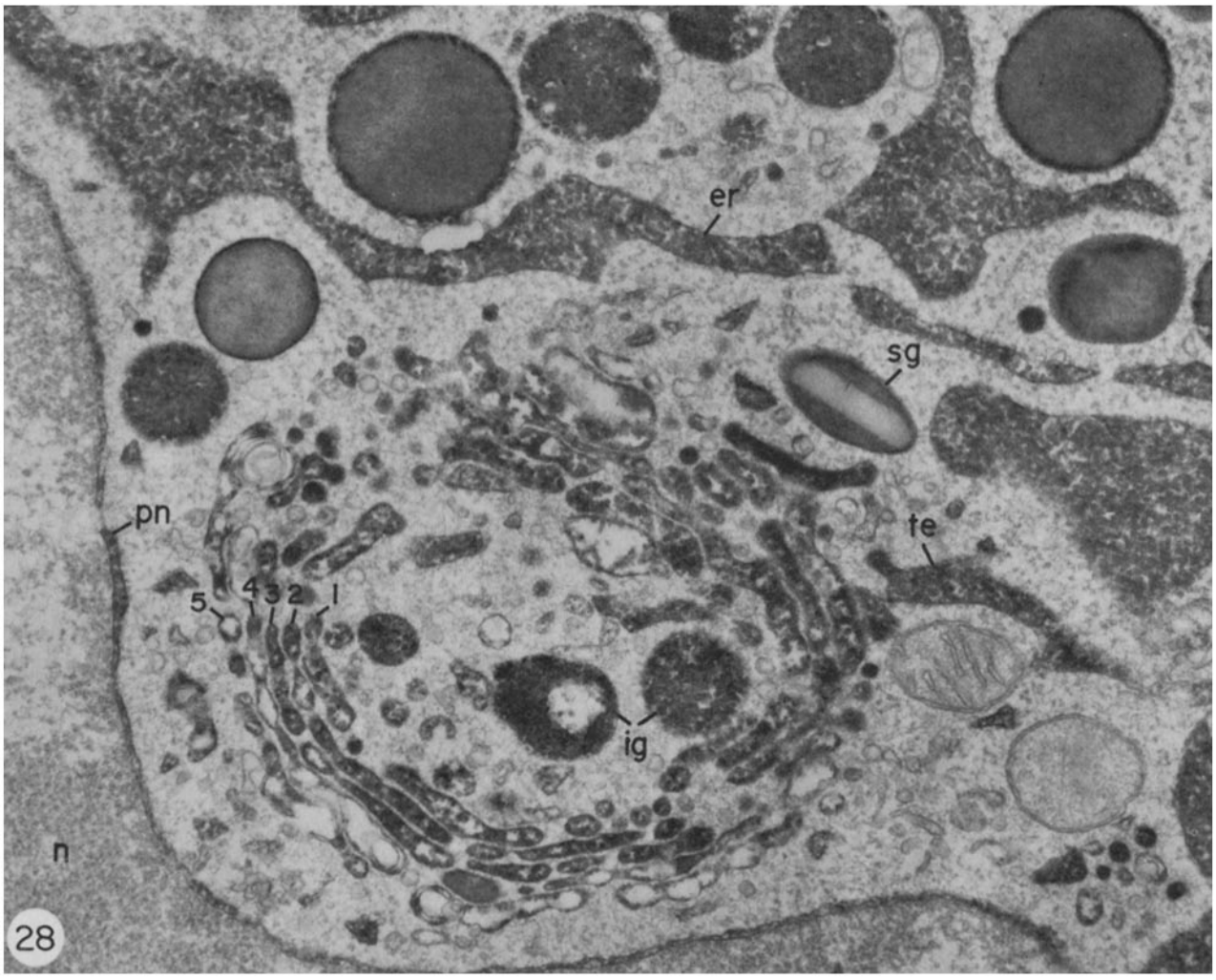

FIGURE 28 Developing eosinophilic leukocyte from rat bone marrow (myelocyte stage) incubated for endogenous peroxidase activity. During this stage, peroxidase is synthesized and packaged into eosinophil secretion granules. Here the peroxidase reaction product is seen throughout all the secretory compartments—rough ER (er), transitional elements of the ER (te), Golgi cisternae (1–5) and associated vesicles, and immature granules (ig). Note that all five cisternae in the Golgi stack are reactive. sg = mature secretion granule with crystalline inclusion. × 50,000. From Bainton and Farquhar (119).

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enzymes from the Golgi complex to lysosomes (18, 18a). However, it is not known where in the Golgi complex the sorting of lysosomal enzymes and secretory proteins takes place.

In 1964, Novikoff and co-workers presented evidence (reviewed earlier) that in nerve cells acid phosphatase (AcPase) was present in a special ER cisterna, which Novikoff called GERL. Based on this finding, he proposed that lysosomal enzymes were synthesized in the associated ER and packaged in GERL, thus bypassing the stacked Golgi cisternae. Later, to explain the presence of AcPase and secretory granules in the same cisterna (see Fig. 15), he expanded the GERL concept to include condensing granules and condensing vacuoles (11, 19). Inherent in the earlier formulation was the assumption that lysosomal enzymes and secretory products remained separated from one another; however in the revised concept, the idea is that lysosomal enzymes and secretory products are segregated together and sorted out within the same (GERL) cisterna or condensing vacuole by an unknown mechanism. The revised concept also assumes that both secretory products and lysosomal enzymes move from the rough ER to the GERL, *without* passing through the rest of the Golgi complex, an assumption that does not fit with the autoradiographic findings (referred to earlier) in which exposed grains were located over the stacked Golgi cisternae in several secretory cell types.

At present there is no clear understanding of the GERL concept; some investigators consider GERL as a separate entity, distinct from Golgi cisternae, whereas others consider it part—often the granule forming part—of the Golgi complex. Several of the reasons for this situation were discussed in the preceding paragraph; another reason is the variability in the morphological properties, cytochemical staining, and detectable functions of the cisternae on the trans side of the Golgi stack. In many cells concentration of secretory products can be seen not only in cisternae that correspond morphologically to Novikoff's description of GERL (rigid cisternae, often separated from the stack, limited by thick membranes), but also in one or more of the transmost cisternae in the regular Golgi stack of the same cell type (See Figs. 1–4, 13, 15). Another problem is that sometimes AcPase is found in the transmost cisterna of the Golgi stack, rather than exclusively in structures which correspond morphologically to GERL (122a). Also, since multiple AcPases have been found in several cell types (see 115) AcPase may not be exclusively a lysosomal marker enzyme.

In summary, in view of the absence of criteria other than morphological ones for distinguishing GERL from Golgi cisternae, the frequent overlap between their cytochemical straining properties and functions, and the absence of supporting data for the assumption that traffic from the ER is directed to GERL bypassing the Golgi complex, there seems to be little justification for setting GERL aside as a distinct entity from the rest of the Golgi complex. It is clear that a major function of the Golgi complex is to sort secretory and certain membrane (see below) proteins and to direct them to their correct intracellular and extracellular distinations. It is also clear that the trans side of the Golgi complex is where a great deal of the biosynthetic and other traffic converges. Certainly, however, much more work is needed in order to disentangle the traffic lanes, to identify the site of sorting of lysosomal enzymes and secretory products, and to understand "fractionation" mechanisms in the Golgi complex.

MEMBRANE BIOGENESIS: The earliest concept of biogenesis of membrane constituents, proposed in the late 1950s, was that the traffic of cell membranes was accomplished by the movement in concert of the membrane and content (secretory) proteins from the rough ER (or nuclear envelope) → cis Golgi face → trans Golgi face → plasmalemma, with delivery occurring by exocytosis upon fusion of the granule membranes with the plasmalemma. What was envisaged was a movement of membranes in bulk. The term 'membrane flow' was introduced in 1956 by Bennett (123); he placed the main emphasis on what was then the new concept of incoming vesicle flow (pinocytosis), but he also clearly envisaged an outbound flow, at least from the nuclear envelope to the rough ER. Bennett did not consider the Golgi complex in his formulation, for its existence was still questioned at the time. The membrane flow concept lay dormant until 1971, when it was introduced again by Franke, Morré, and their co-workers (124) to designate the physical transfer of membrane from one compartment to another in sequential fashion. The concept was based on radiolabeling experiments in which they found sequential labeling of ER, Golgi, and plasmalemma. Subsequently, Morré and his associates (13, 15) developed this concept further and coupled membrane flow and membrane differentiation to account for the origin of the complex system of internal membranes or 'endomembranes' in eukaryotic cells. Inherent in this concept was the idea that membrane flow is *undirectional* and *coupled* to the flow of secretory products. The route taken through the Golgi was also addressed; and in the case of the hepatocyte three pathways were envisaged and depicted in diagrammatic form: (1) New membrane was believed to arise as primary vesicles which, as in the case of the pancreatic secretory model, pinch off transitional elements and fuse with Golgi cisternae on the cis side of the complex, after which they were assumed to undergo progressive transformation across the stack to be used in packaging at the forming face and eventually reach the plasmalemma. The secretory products (lipoproteins in this case) were believed to be transported either (2) by direct connections at the periphery of the Golgi, called the 'boulevard periphérique,' or (3) by a direct pathway from the smooth ER to the cell surface thus bypassing the Golgi. The last alternative can be seriously questioned and essentially ruled out because, as stated before, there is no evidence supporting direct discharge of secretory products from the ER, in general, and for lipoprotein particles, in particular. In the latter case, obligatory passage through the Golgi is expected by most workers, because the apolipoproteins are glycoproteins (113). The second pathway—i.e., the existence of direct connections between ER and Golgi—has also been questioned on the grounds that because such connections are visualized primarily in unfixed, negatively stained preparations, are not seen with comparable frequency in fixed, negatively stained preparations, and are rarely seen in thin sections (see, however, Claude [125]), they may arise from artifactual fusions that occur during negative staining procedures. The first pathway, as already stated, was generally accepted among the proponents of the membrane flow theory.

In the last few years a number of new developments have forced a reconsideration of this concept which, as originally proposed (15) and usually understood (38, 63, 87), oversimplifies intracellular conditions. (*a*) First, it is now clear (see reference 79) that membrane proteins turn over at much slower rates than the transit time of secretory proteins; hence, concurrent synthesis of membrane and content is no longer tenable. (*b*) Additional findings (summarized below), difficult to reconcile with the membrane flow diagrams, concern the extensive, membrane reutilization or membrane recycling that takes

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place in most cells—i.e., recycling of membranes of secretory granules, synaptic vesicles, and transitional and endocytic vesicles (see below). Therefore, the traffic of recycling membrane must be considered together with the biogenetic traffic. (*c*) It has also been established that although some membrane proteins, especially glycoproteins, follow the postulated membrane flow route, others, namely peripheral membrane proteins and their viral equivalents (such as the 'M' protein of the vesicular stomatitis virus [VSV]), are inserted directly from the cytoplasmic matrix without the involvement of either the ER or the Golgi complex (126). (*d*) Furthermore, the existence of cytochemical specialization among Golgi cisternae and within the same cisterna is difficult to reconcile with the concept of progressive transformation across the stack. (*e*) Finally, the realization that membranes on different domains of the cell surface differ in their composition and that secretory granules fuse preferentially with one domain (usually the apical domain in exocrine cells), leads to the conclusion that secretory granule membranes cannot serve as vehicles for all the domains of the plasmalemma.

Current Information on Involvement of Golgi in Biogenesis of Membrane Components

At present it is assumed that the Golgi complex is involved in the biogenesis of at least some membrane components, primarily intrinsic transmembrane proteins, which are found not only in the plasmalemma but also in other membranes of the secretory (and perhaps the endocytic) pathway. The rationale is that all transmembrane proteins studied so far have oligosaccharide chains of the type that is produced or completed in the Golgi complex. Examples of terminal glycosylation occurring at the cell surface are limited (84), and their general applicability is questioned. Hence, transit through the Golgi is expected to effect terminal glycosylation. A similar situation applies to glycosylation and sulfation in the case of membrane glycolipids (96–98).

The information available is compatible with the involvement of the Golgi complex in biosynthesis of membrane glycoproteins, but the data are still limited. Leblond and coworkers (63, 127) have obtained autoradiographic evidence that in intestinal epithelial cells (considered nonsecretory), labeled fucose and sialic aid precursors (*N*-acetylmannosamine) are first incorporated into macromolecules in stacked Golgi cisternae. Later, the cognate autoradiographic grains are found over small vesicles, and later still, over the plasmalemma. These findings have been interpreted as indicating that plasmalemmal components (in this case, mainly fucosylated and sialated glycoproteins) are modified (terminally glycosylated) in the Golgi and then ferried to the cell surface via small vesicles. The data are certainly compatible with this interpretation, but due to the limitations of resolution of the autoradiographic method, transport by alternate routes cannot be ruled out. Moreover, although it is likely, in view of the biochemical data (128), that the labeled species are membrane proteins, their identity was not established.

The involvement of Golgi cisternae in plasma membrane biogenesis is also suggested by the demonstration of adenylate cyclase (21) and insulin receptors (129) in Golgi fractions and of acetylcholine receptors in the Golgi apparatus of chick skeletal muscle cells in culture (130). In the work on insulin receptors and adenylate cyclase, no distinction could be made between recycling and biogenetic traffic to explain the presence of these proteins in the Golgi complex, for there was no way of knowing whether they appear first in the plasma membrane or the Golgi complex. In the case of chick muscle, suggestive evidence was obtained for the appearance of at least some (~10%) of the acetylcholine receptors in the Golgi complex prior to their insertion into the plasma membrane, but interpretation of the data was complicated by the presence of a large number of receptors in structures other than the Golgi complex.

The best and only direct evidence on the involvement of the Golgi complex in biogenesis of membrane proteins comes from work on the viral envelope spike or 'G' protein of VSV. Biosynthetic studies have established that this glycoprotein is synthesized on membrane-bound polyribosomes and partially glycosylated in the ER, and then further glycosylated as it is transported within a smooth membrane fraction before it is delivered to the plasma membrane (126), apparently via coated vesicles (131).

Indirect evidence for glycosylation of the VSV-'G' protein by Golgi membranes was recently brought forward by Rothman and Fries (132) in experiments in vitro in which they mixed a crude extract obtained from a CHO cell mutant incapable of carrying out terminal glycosylation with either a crude membrane fraction from wild-type CHO cells or a Golgi fraction prepared from rat liver. In this heterogeneous, reconstituted system, they obtained terminal glycosylation of the G protein. More direct evidence for the participation of the Golgi in the biogenesis of the G protein was obtained recently by Bergman, Tokuyasu, and Singer (133). They used immunochemical procedures to demonstrate the sequential appearance of the G protein in ER, Golgi, and plasmalemma, and took advantage of the availability of a temperature-sensitive mutant and a shift-down step to synchronize the release of the protein from the ER. The authors also observed that initially (before 11 min) only cis cisternae were labeled, but within 11 min after the temperature shift, labeling was seen throughout the Golgi complex. These findings represent the first *direct* evidence for the involvement of the Golgi complex in the biogenesis of a membrane protein. The authors plan to use this approach to study the route taken through the Golgi complex and to the plasmalemma.

The existing evidence is compatible with the view that transmembrane proteins are transported to their site of final function already assembled in a membrane carrier. The assumption is in keeping with the asymmetry of their assembly and with the progressive glycosylation of their cisternal domains, which would make other ways of transport highly improbable thermodynamically. Retention of membrane specificity implies that the traffic of these carriers is regulated so as to allow each one to arrive (and be accepted) at the appropriate destination.

MEMBRANE RETRIEVAL AND RECYCLING OF GRANULE MEMBRANES: The concept of membrane *retrieval* was actually suggested as early as 1959 (134), when exocytosis in secretory cells was first described. At that time, it was recognized that membrane must be removed from the cell surface to compensate for that added during exocytosis, in order to maintain a constant cell size. It was also suggested that the 'pile of cisternae in the centrosphere region' may represent the membrane depot of the cell. Thus, from the beginning it was suggested that endocytosis and exocytosis are coupled, and that the Golgi cisternae may be involved in these phenomena. Membrane reutilization or recycling was not mentioned.

A long period followed in which the recycling of granule

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membranes was questioned and generally not favored (see 135, 136). This situation lasted until a few years ago. Evidence on the fate of granule membranes came from two sources: biochemical experiments in which the turnover rates of membrane proteins and content proteins were compared in secretion granule fractions, and experiments in which the fate of the membrane was followed using electron dense tracers. The results of early turnover experiments, which were carried out on membranes heavily contaminated with content proteins, revealed no differences in the turnover of proteins for the two sources; hence, it was erroneously concluded that the membranes are not reutilized but are destroyed after each exocytotic event.

The results of early tracer experiments that used content markers, mainly horseradish peroxidase and native ferritin, were also misleading. They clearly demonstrated that, after exocytosis, membrane is recovered intact by endocytosis; but they suggested that the membranes were subsequently destroyed, rather than reutilized, because, in the majority of these studies, the tracers were found early in endocytic vesicles and later in lysosomes.[2] More recent experiments have shown, however, that the content and the membranes of recycling vesicles do not necessarily follow the same pathway. Thus, both the early tracer and turnover data were erroneous and led to the conclusion that, after exocytosis, membrane is recovered by endocytosis and destroyed in lysosomes. This view was proposed in several reviews (87, 135, 136) that were published just a few years ago (1978–1979).

Three recent developments (reviewed in 137) have changed the situation and have led to a gradual acceptance of granule membrane recycling. The first was the publication of more reliable turnover data, based on the preparation of granule membranes free from content proteins, which demonstrated that the proteins of granule membranes turn over at a much slower rate than do content proteins. The second development came from new tracer experiments, primarily with dextrans and cationized ferritin, in which transport of exogenous tracers to multiple stacked Golgi cisternae was demonstrated in a variety of cell types: parotid and lacrimal gland cells (138); anterior pituitary cells (139; Figs. 29–31); thyroid epithelium (140); pancreatic endocrine (61) and exocrine (141) cells; plasma cells and myeloma cells (69), and macrophages (142). The demonstration of endogenous secretory product and exogenous tracer segregated together in newly formed secretion granules (or vesicles) was possible in a few instances—i.e., anterior pituitary (139; Figs. 29–31), exocrine pancreatic (141), and plasma cells (69). The third development was the demonstration of extensive membrane reutilization in other systems, especially in neurons and macrophages (see 115 and 135), which, together with the data on granule membranes, led to a gradual realization of the widespread occurrence and (often) surprising magnitude of membrane recycling and its importance for a wide variety of cell processes.

[2] In a few of these early studies, (e.g., those by Mata and David-Ferreira on the seminal vesicle, Pelletier and Farquhar and co-workers on anterior pituitary cells, Orci and associates on the β-cells of the endocrine pancreas and Gonatas on cultured neurones), tracers were also detected in Golgi elements or GERL (see reference 137). The amount of reaction product was either small or limited to certain Golgi elements. Much more attention was given to the presence of tracer in lysosomes so that at that time the latter were assumed to be the primary terminus of the incoming vesicular traffic involving membrane recovered from the cell surface.

RECYCLING OF OTHER GOLGI MEMBRANES: Secretion granule membranes, as well as synaptic and endocytic vesicle membranes, represent particularly favorable objects for following the fate of retrieved and recycled membrane, thanks to the large quantities involved and the fact that, at least in the first two cases, relocation of membrane can be controlled experimentally. In many other cases in which membrane is relocated intracellularly—e.g., vesicles that transport secretory products from ER to Golgi, or lysosomal enzymes from Golgi to lysosomes—a similar recycling mechanism seems likely, but pertinent data are much more difficult to obtain in the absence of appropriate tracers and well-established membrane markers.

USE OF AGENTS WHICH PERTURB GOLGI TRAFFIC: Various agents, including uncouplers of oxidative phosphorylation, inhibitors of tubulin polymerization into microtubules, amines, and ionophores have been used in attempts to gain information on Golgi traffic and functions; some of these agents disrupt the architecture of the Golgi complex (see [143] for a recent review). In the few cases in which specific agents were available, and their effects were investigated by combined morphological and biochemical procedures (e.g., intracellular transport of secretory proteins [52]), this approach has provided useful and clearly interpretable information. In other cases, however, the use of traffic perturbants has produced results that are difficult to interpret, primarily because these agents have multiple effects that are not limited to the Golgi complex. For example, at appropriate concentrations inhibitors of tubulin polymerization prevent microtubule assembly anywhere in the cell, not just in the Golgi region. Acidic ionophores (nigericin, X537A, and monensin) as well as weak bases (e.g., methylamine, ethylamine, and chloroquine) are "lysosomotropic agents," so-called because they accumulate in lysosomes, thereby causing an increase in the intralysosomal pH (from 4.5 to 6.0) (144), which prevents or retards intralysosomal digestion. Amines also have been shown to block endocytosis (145, 146). Many of these agents cause an increase in size of the lysosomal compartments, probably by interfering with membrane recycling along the endocytic pathway. However, they may also disturb membrane traffic at other intracellular sites. Of particular interest is the recent finding (147) that monesin as well as nigeracin, now commonly used as Golgi-perturbing agents, perturb recycling of LDL receptors and inhibit lysosomal digestion of LDL (presumably by raising the intralysosomal pH).

The use of agents that perturb Golgi traffic is potentially an interesting and promising approach for unraveling traffic patterns into and out of the Golgi complex, but the interpretation of the results obtained require caution (as well as additional work) because of the problems outlined above and the inherent and still poorly understood complexity of the traffic through the Golgi apparatus.

IMPLICATIONS OF MEMBRANE TRAFFIC FROM THE CELL SURFACE TO THE GOLGI COMPLEX: The existence of a pathway along which membrane from the cell surface can reach Golgi cisternae has broad implications: it provides a means by which cell surface molecules can be brought back to a biosynthetic compartment. Thus, a mechanism exists whereby—in principle—surface membrane components such as receptors, enzymes, and other membrane proteins could be modified or repaired (e.g., reglycosylated, sulfated, phosphorylated) while in transit through the Golgi complex during recycling. To date no specific examples of this type of phenomenon are available, but there is no reason why it could not take

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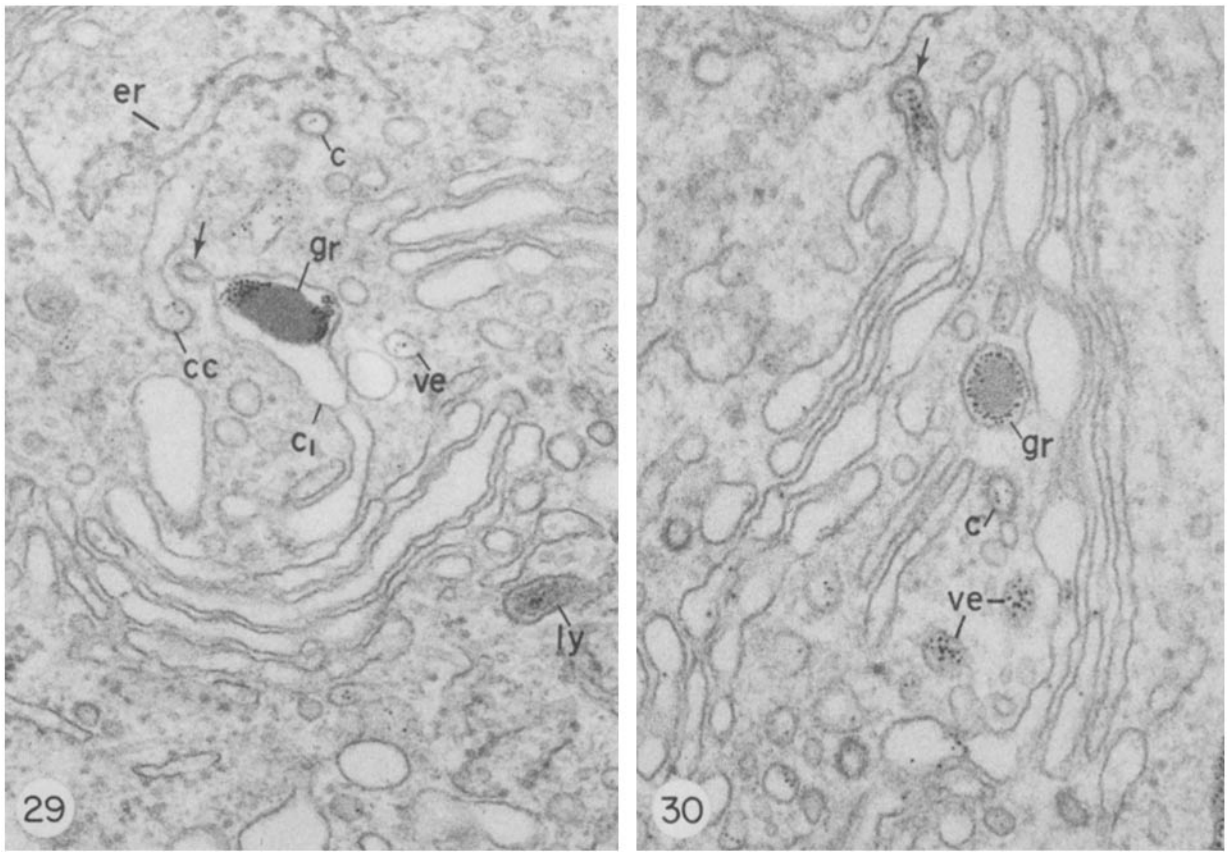

FIGURES 29 and 30 Golgi complexes from pituitary prolactin cells incubated with cationized ferritin (CF) for 60 min to trace the fate of membrane internalized at the cell surface. Fig. 29 shows that, when the CF binds to the cell surface, it is taken up by endocytosis and the incoming vesicles carrying CF fuse preferentially with the trans Golgi elements. Here the tracer is particularly concentrated around a forming secretory granule (gr) within the transmost Golgi cisterna (C_1). Note that the CF is concentrated at the periphery of the forming granule adhering to its dense content. An empty coated vesicle appears to be in the process of budding from (or fusing with) the cisterna (arrow). CF is also seen within several vesicles (ve) one of which is coated (c) in the Golgi region, within another cisterna with a coated rim (cc), and within a lysosome (ly). Fig. 30 shows CF within multiple (4–5) stacked Golgi cisternae and around a forming granule (gr). One of the CF-marked cisternae has a coated tip (arrow), suggesting that a coated vesicle loaded with CF has just fused with it. CF is also seen within numerous vesicles (ve) adjacent to the stacks; some of these vesicles are coated (c). Fig. 29—× 70,000; Fig. 30—× 87,000. From Farquhar (139).

place if the molecules are brought into contact with the proper Golgi compartments or subcompartments. The route from the cell surface to the Golgi complex also provides a pathway whereby various informational molecules from the extracellular environment (peptide hormones, catecholamines and other agents) can reach the Golgi complex where they may undergo modifications as described for surface components and from where they may influence certain intracellular events. It is now quite clear that many peptide hormones are internalized by endocytosis and can reach lysosomes (148). Work primarily by Bergeron, Posner, and their associates demonstrates that several hormones (prolactin, insulin) reach bona fide Golgi elements (149, 150) upon internalization.

It is not yet known whether this uptake is connected with specific modifications of metabolic events, with the removal and degradation of hormones, with the regulation of receptor distribution, or simply with constituitive (continuous) membrane recycling. The physiological meaning of hormone internalization into Golgi elements, lysosomes, and perhaps other cell compartments is a most intriguing problem yet to be resolved.

Summary and Conclusions

We have related herein the major developments of the last 25 years that have brought us to our current level of understanding of the structure and function of the Golgi apparatus. Thirty years ago, when only light microscopes were used in cell research, the very existence of the Golgi apparatus was questioned, but electron microscope observations demonstrated that the complex is a cytological reality and its acceptance was rapid and general. The conclusion was based primarily on the reproducible demonstration by relatively simple procedures of an ubiquitous, characteristic structure.

Initially, our concepts of the functions of the Golgi complex and traffic within it were rather simplistic. The organelle was seen as a kind of 'bottling station', which existed primarily or solely for the packaging of secretory products. The cisternae were believed to move undirectionally from one face to another across the stack, as on a conveyor-belt in a factory assembly line, being used up for granule packaging on the trans (then called mature) face and reformed by new membrane assembly on the cis (immature) face. As information accumulated, it became apparent that the complex had the exclusive capability to modify secretory products by terminal glycosylation, sulfation, and proteolytic processing of proproteins. Similar modifying activities were detected for membrane proteins. The conveyor-belt or assembly line concept was retained, and new data concerning the role of the Golgi complex in the production and

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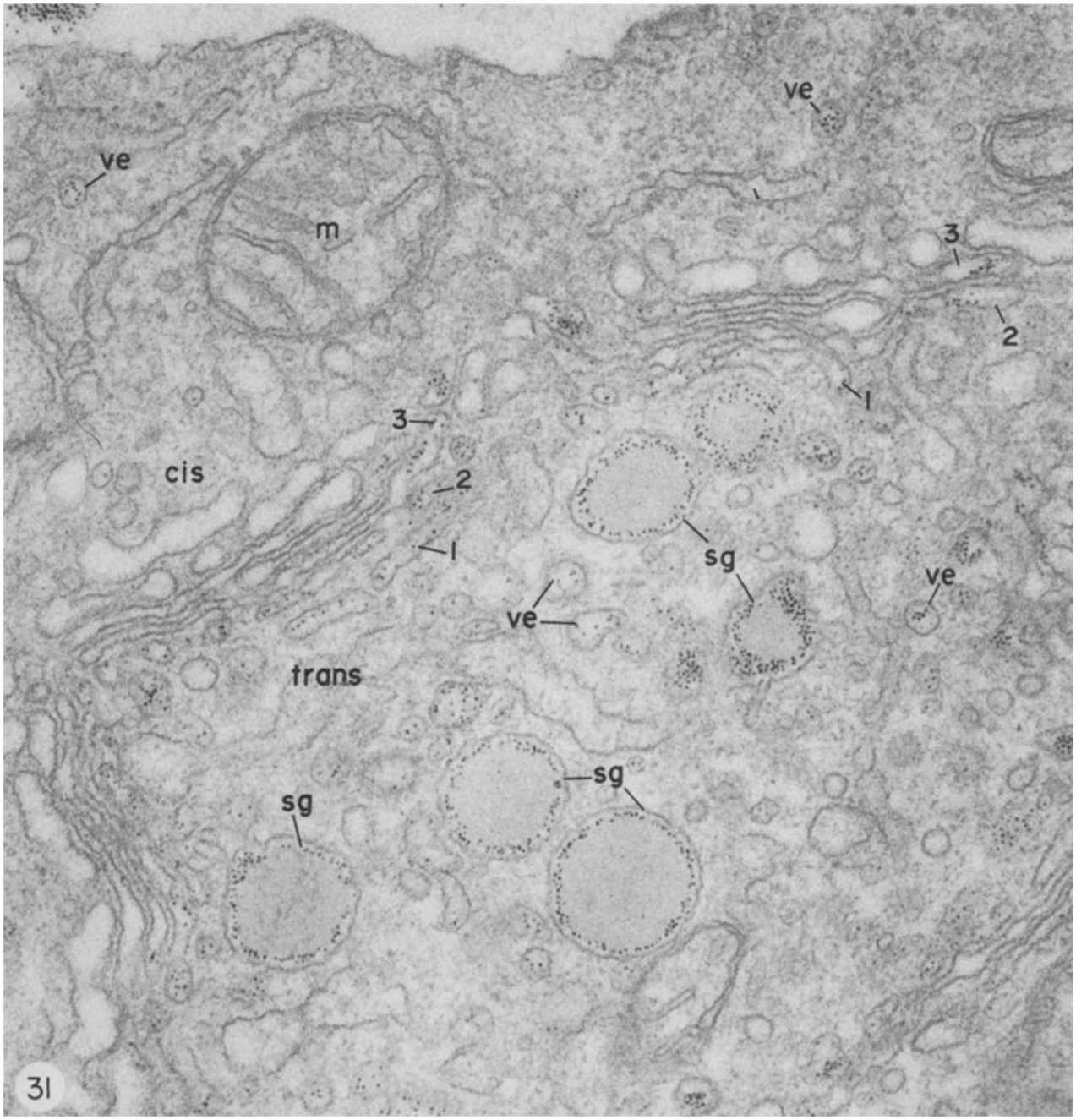

FIGURE 31 Somatotroph or growth hormone secreting cell from a male rat incubated 60 min in CF (0.05 mg/ml), illustrating uptake of CF into multiple Golgi cisternae and secretion granules (sg). CF molecules are also present within multiple vesicles (ve) in the Golgi region. Note that the CF is most abundant in the three cisternae (1-3) on the trans side of the Golgi stack. The incoming vesicles carrying the CF appear to fuse preferentially with the trans cisternae. The tracer is packaged along with growth hormone into the forming granules where it is located between the granule membrane and its dense contents. × 85,000. From Farquhar (139).

processing of secretory products or membrane components were interpreted within the framework of this concept. Diagrams that reflected this concept of the flow of secretory products and membranes to and through the Golgi complex were published repeatedly (13, 15, 38, 63).

Data acquired more recently, however, are not compatible with these ideas about traffic through the Golgi complex, and especially do not support a simple cis-to-trans flow diagram.[3] One set of data concerns the composition of the Golgi membranes themselves. Originally the membrane flow hypothesis proposed that each Golgi cisterna is immature as it enters the stack and is progressively modified (matures) in transit across the stack. However, cytochemical and biochemical data now available attest to the existence of considerable heterogeneity among Golgi membranes, and documentation has been pro-

[3] At present the only case in which a cis-trans movement of Golgi cisternae appears to be established is that of scale-producing algae (118). However, this may represent a rare formula connected with the unusual geometry and size of the product: a whole cisterna is needed to accommodate each scale under construction.

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vided for the existence of specific compositional differences between adjacent cisternae within a given stack and within the same cisterna. Also, data on the recycling of secretion granule membranes (137) (diagramed in Fig. 32) are not in agreement with the old flow diagrams. These diagrams should be revised to take into account the multiple formulae that are now known to be involved in biogenesis and transport of membrane constituents, as well as recent data concerning the mechanisms and pathways for transport of secretory products and lysosomal enzymes. Although the new data are not compatible with a simple cis-to-trans flow diagram, they are in agreement with the following conclusions:

(*a*) Individual Golgi cisternae, like other cell components, retain the specificity of their membranes.

(*b*) Transport of both secretory products and membrane components is largely effected by vesicular carriers, which interact (fuse) preferentially with the dilated rims of the cisternae.

(*c*) Each Golgi cisterna is a mosaic in which differentiated domains are maintained in the plane of the membrane by means so far unknown.

(*d*) The dilated rims of the Golgi cisternae represent a special subcategory of Golgi membranes that differ from the rest in their protein composition and enzymic activities.

(*e*) The main flow of both the secretory products from the rough ER and the secretion granule membrane recycled from the cell surface is to the dilated rims of multiple Golgi cisternae.

(*f*) In cells that concentrate their secretory products, traffic from the cell surface is heaviest to the transmost Golgi cisternae where concentration takes place.

These conclusions (137), which are based on information about the three major types of traffic on which we have some, albeit limited, knowledge—i.e., secretory proteins, recycled granule membranes, and intrinsic membrane proteins—*are* accommodated by the diagram depicted in Fig. 33. The pathway taken by other types of membranes and products that are known to pass through the Golgi complex, such as lysosomal enzymes and lysosomal shuttles, cannot yet be drawn with certainty.

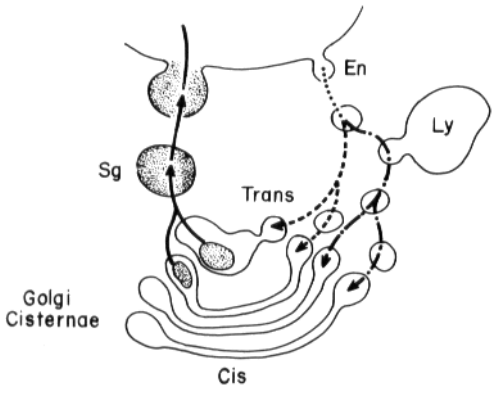

FIGURE 32 Diagram showing routes which can be taken by surface membrane to reach the stacked Golgi cisternae in secretory cells. Following exocytosis of secretory granules (——), patches of surface membrane are recovered by endocytosis (· · ·) and fuse with the dilated rims of multiple stacked Golgi cisternae or with lysosomes. The recovered membrane may fuse first with the membrane of lysosomes (—·—) and then with that of Golgi cisternae or may reach the latter directly (– – –). The available evidence suggests that both routes are used in different cell types. From Farquhar (137).

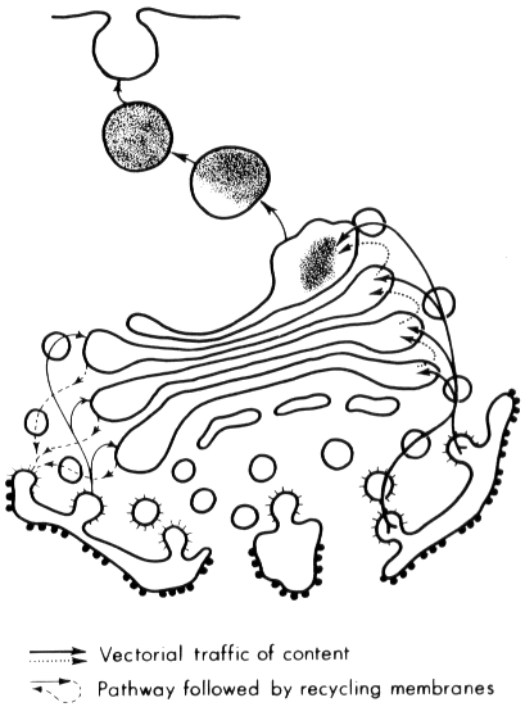

FIGURE 33 Flow diagram illustrating the proposed routes taken by membranes (left) and secretory products (right) to and through the Golgi complex. The available evidence suggests that small transport vesicles which bud from the transitional elements of the rough ER and fuse with the dilated ends of multiple Golgi cisternae are involved in this operation. The secretory proteins (shown to the right) move vectorially and become concentrated in the trans Golgi cisternae where they are packaged into granules. The dotted line indicates that secretory proteins may be moved sequentially from cisterna to cisterna. The membrane vesicles (shown to the left) which serve to ferry secretory proteins, pinch off and return—i.e., recycle, back to the transitional elements of the rough ER.

It is clear that a major function of the Golgi complex is to sort secretory, lysosomal, and certain membrane proteins and to direct them to their correct intracellular or extracellular destinations. Fig. 34 is a tentative interpretation of sorting mechanisms in a Golgi cisterna seen *en face*. The position of the sorting devices in the Golgi stack is unknown. They may be present in different or in the same cisternae.

In revising the membrane flow-differentiation hypothesis the most important point to take into account is that each cell inherits at birth a complete set of differentiated membranes from its mother (151)—it does not have to differentiate ER membrane into either Golgi membrane or plasmalemma, but it must retain the biochemical specificity of each of these membranes. This appears to be achieved, not by converting one type of membrane into another, but by controlling the traffic from the sites of synthesis to the sites of final assembly for either protein molecules or membrane vesicles, so that only appropriate components are assembled in each membrane (152). Retention of biochemical specificity also requires nonrandom removal of membrane carriers from at least one of the interacting compartments. The only 'differentiation' established so far for membrane proteins that move down the secretory pathway does not exceed the usual posttranslational modifications—i.e., terminal glycosylation and partial proteolysis undergone by many proteins and most glycoproteins that pass through the Golgi complex.

Although the proposed formulations accommodate our cur-

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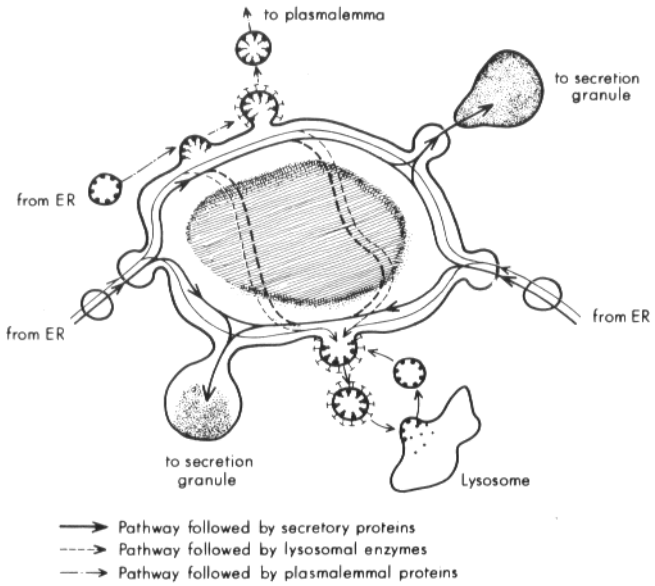

FIGURE 34 Diagram of a Golgi cisterna viewed *en face* showing the presumed routing of the biosynthetic traffic of membranes and secretory products along its dilated rims. Four types of traffic are depicted: (1) ER → Golgi; (2) Golgi → lysosomes; (3) Golgi → condensing granules or vacuoles; and (4) Golgi → plasmalemma. In all cases, transport is assumed to be effected by vesicular carriers which must possess specific receptors for transported species on their inner (cisternal) surfaces and appropriate recognition signals for the receiving compartment on their outer surfaces. In two cases (types 2 and 3) there is evidence that coated vesicles are involved. In only one case (type 2) is the specific recognition marker (mannose-6-phosphate) known. The large dots attached to the membrane represent the receptor and the small dots the lysosomal enzymes. Most of the traffic is assumed to move along the dilated periphery of the cisterna (solid lines) rather than through its flattened central region (dotted lines, shaded area).

rent knowledge, it can be safely anticipated that in another 25 years—or even sooner—they, too, will prove to be far too simplistic. The reasons should be clear: first, the biological sciences, especially cell biology, are advancing at a remarkably rapid rate. In addition, information on the complexity of the Golgi complex, which appears to be the hub of intracellular traffic, and the multiplicity of its connections continues to accumulate as a result of new findings. In the meantime, however, these new formulations may provide a useful framework to be tested and validated or modified by further experiments.

ACKNOWLEDGMENTS

The original research summarized herein was supported by research grants AM-17780 (to MGF) and GM-27303 (to GEP) from the National Institutes of Health. The authors gratefully acknowledge the assistance of Pam Ossorio in the preparation of the figures and Lynne Wootton in the preparation of the manuscript.

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