# The Golgi Apparatus

# The Golgi is What?

**Hilton H. Mollenhauer**

In days long gone
the Golgi was what?

Why, a stringy thing,
or so Camillo said.

Oh no, said some,
just dots – not strings.

You are wrong said others,
not real at all.

But, now we have worked and now we all know
that the Golgi is, well, you know.

A bunch of flat plates
stacked one on another.

With vesicles close by
all positioned in space.

And a pile of small tubules
that spread out and about.

And with buds of small tubules
important no doubt.

But how do we know that all is now clear?
What have we done to bring this to fore?

Why, we have seen it you dummy
with EM's galore.

We have chopped it to bits
to see what's inside.

We fed it some markers
to make it abide.

So now we know all, there's no reason to doubt.
That what we have now is a Golgi for sure.

But, somehow, I am still not so sure,
that all is clear as some might suppose.

Many still fret about this and about that,
about strings, about dots, and about tubules galore.

Perhaps it is time to look once again
to consider the past and look on ahead.

Perhaps this time we might actually know.
The Golgi is what? – and be reasonably sure.

# The Golgi Apparatus

## The First 100 Years

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ISBN: 978-0-387-74346-2 e-ISBN: 978-0-387-74347-9

Library of Congress Control Number: 2008933895



Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

# Preface

*The Golgi Apparatus: The First 100 Years* traces the first 100 years of Golgi apparatus discovery from the first published accounts from Pavia, Italy in 1898 to the Centenary Celebration in Pavia, Italy in 1998 and into the decade beyond. It is not intended, however, to be a comprehensive survey but rather to present the perspectives of the authors to summarize their contributions over the past 50 years in parallel with the modern era of Golgi apparatus discovery initiated in 1954 and made possible by the advent of the electron microscope. Included are methods of cell fractionation and biochemical analysis leading up to the present where efforts focus heavily on molecular biology.

Topics where the authors and their colleagues have made substantial and/or pioneering contributions are emphasized including Golgi apparatus morphology and structural organization and function (especially in plants), the existence and importance of cisternal tubules, development of methods of plant and animal Golgi apparatus isolation and subfractionation, biochemical analyses of highly purified plant and animal Golgi apparatus fractions in comparison to equally highly purified reference fractions. The use of such fractions in cell-free system analyses of membrane trafficking, the concept of Golgi apparatus function as part of an integrated system of internal endomembranes (the Endomembrane System), evidence for differentiation of membranes across the stacks of Golgi apparatus cisternae, and flux of membrane constituents along the polarity gradient defined by membrane differentiation all culminating in the membrane maturation or flow-differentiation model of Golgi apparatus function. More recent contributions to Golgi apparatus in cell growth (enlargement) and to cancer are summarized in the final chapters.

The authors' view of the dynamic working of the Golgi apparatus were based initially on static electron micrographs of the maize root tip generated by Hilton Mollenhauer in the Cell Research laboratory of the University of Texas in Austin, then under the direction of the late W. Gordon Whaley. Subsequent quantitative studies in the laboratory of D. James Morré at Purdue University suggested that massive amounts of membrane were moved to the plasma membrane at the cell surface in the discharge of secretory products in the outer cap cells of the maize root. The logical source of this membrane was the endoplasmic reticulum. The concept was further fueled by observations of Stanley Grove, then a graduate student in the laboratory of Charles Bracker at Purdue University. Grove's investigations with the Golgi apparatus of a fungus clearly demonstrated

a gradient of membrane morphology across the stacked cisternae from endoplasmic reticulum-like on one face to plasma membrane-like on the opposite face. A membrane composition of Golgi apparatus intermediate between that of the endoplasmic reticulum and the plasma membrane was determined using isolated cell fractions from both rat liver and mammary gland in collaboration with Thomas W. Keenan also from Purdue University. The actual concept of the dynamic passage of membrane material from the endoplasmic reticulum to the plasma membrane (i.e., membrane flow) was first tested experimentally in the laboratory of Werner Franke in the Department of Peter Sitte at the University of Freiburg. With the assistance of Barbara Deumling, Ernst Jarash, Jürgen Kartenbeck, Ronald Cheetham, and Hans-Walter Zentgraf, rats were pulse-labeled with $^{14}$C-leucine, the livers were excised, purified fractions of endoplasmic reticulum, Golgi apparatus, and plasma membrane were isolated and stripped to remove extrinsic and sectretory proteins, and the residual intrinsic membrane proteins were analyzed for specific radioactivity. The pulse-chase kinetics were consistent with passage of membrane proteins from the endoplasmic reticulum to the plasma membrane via the Golgi apparatus. The membrane flow concept seemed secure. Unfortunately, alternative views based on recycling models soon prevailed and dominated the literature for more than two decades nearly up until the centenary year of Golgi apparatus discovery in 1998. That year marked a renewed appreciation for the dynamic, cisternal maturation model of Golgi apparatus function. The new resurgence of interest in the potential for membrane flux or flow across the stacked cisternae of the Golgi apparatus has provided the impetus to complete this monograph on the Golgi apparatus with emphasis on the dynamic aspects of Golgi apparatus underrepresented in all but the most recent Golgi apparatus literature.

We express our appreciation to the many colleagues, postdoctorals, graduate students, undergraduate assistants, and technicians whose invaluable assistance made possible the experimental studies and the even greater numbers who challenged and criticized the work to force us to work even more diligently to distinguish among possible interpretations of the findings. We thank Janet Sweet, Sarah Craw, Keri Safranski, and Peggy Runck for assistance with manuscript preparation covering at least four different versions over several years and Matthew Miner for assistance with preparation of the figures. We are especially indebted to the unwavering support of Dorothy Morré, Barbara Mollenhauer, the Morré children, Connie, Jeffrey, and Suzanne, and the Mollenhauer children, Paul, John, and David, some of whom still sport electron micrographs of negatively stained Golgi apparatus on their refrigerator doors.

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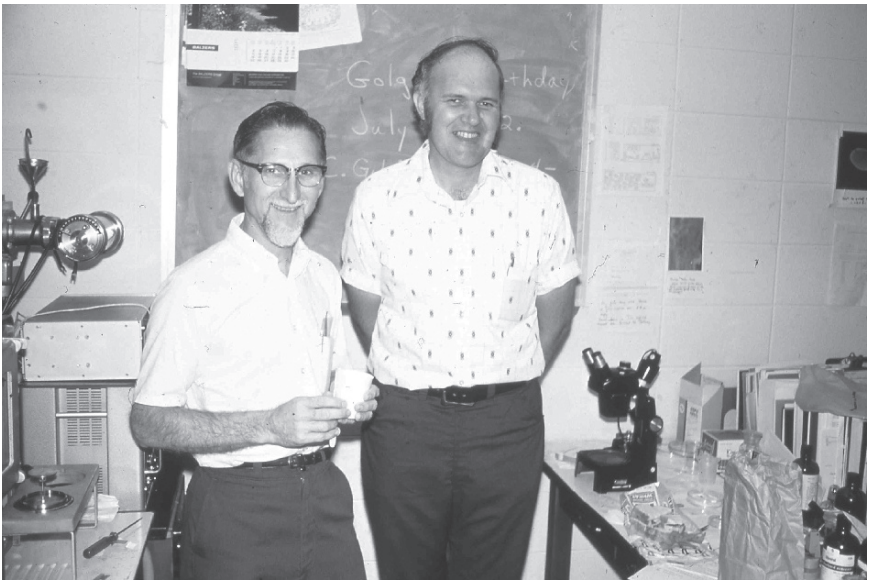

The authors, Hilton H. Mollenhauer (left) and D. James "Jim" Morré (right) celebrating Camillo Golgi's birthday on July 8, 1972. Electron Microscope Laboratory of the Cell Research Institute, The University of Texas, Austin.

# Contents

# 1

# Discovery and Rediscovery

The era of Golgi apparatus discovery may be divided conveniently into three phases – the initial discovery (1865–1925), the controversy (1925–1955), and the modern rediscovery (1955–1963) (see chronology of events, Appendix Table 1 p. 257). Although doubtless seen by others earlier, the discovery of the Golgi apparatus is ascribed to the Italian cytologist Camillo Golgi (1898) who described an *apparato reticolare interno* (internal reticular apparatus) in his now famous "Sur la structure des cellules nerveuses." The description was based on light microscopy of nerve cells of the barn owl and cat made possible through experiments in specimen preparation, where tissues were placed in a silver nitrate bath after preliminary fixation in a solution of bichromate. This method, in modified form, persists today as the "Golgi–Cox" method of preparing nervous tissue for light microscopic examination. Various elements of the nervous system (i.e., Purkinje cells) are rendered dark brown or black against an almost clear background. The modern equivalent of what Golgi initially described is still not clear. It was a darkly staining internal reticular apparatus (Fig. 1). There is little doubt that the region of the cell presently equated with the Golgi apparatus was included in Golgi's observation but the possibility remains that other cell components, such as portions of the endoplasmic reticulum which may be darkly stained by the method, also were included.

Many workers, in the period 1915–1945 and beyond, applied Golgi's methods or variations thought to stain an equivalent region of the cell. They equated a variety of structures with the apparatus of Golgi but not in all cell types and tissues. Various misidentifications were major contributions to an element of doubt as to the reality, generality, and function of the Golgi apparatus. This era between 1925 and 1955 is often referred to as the era of *Golgi apparatus controversy*. It was not until the advent of the electron microscope and the publications of Dalton and Felix in 1953 and of Sjöstrand and Hanzon in 1954 that the modern era of Golgi apparatus discovery would begin.

## 1.1. The Discovery of the Golgi Apparatus

Camillo Golgi, for whom the Golgi apparatus is named, was born on July 9 (July 8 by some accounts), 1843 in the Italian town of Corteno in the province of Lombardy. The son of a physician, he studied medicine, and accepted a position at the University of Pavia where he worked, with two interruptions, from 1865

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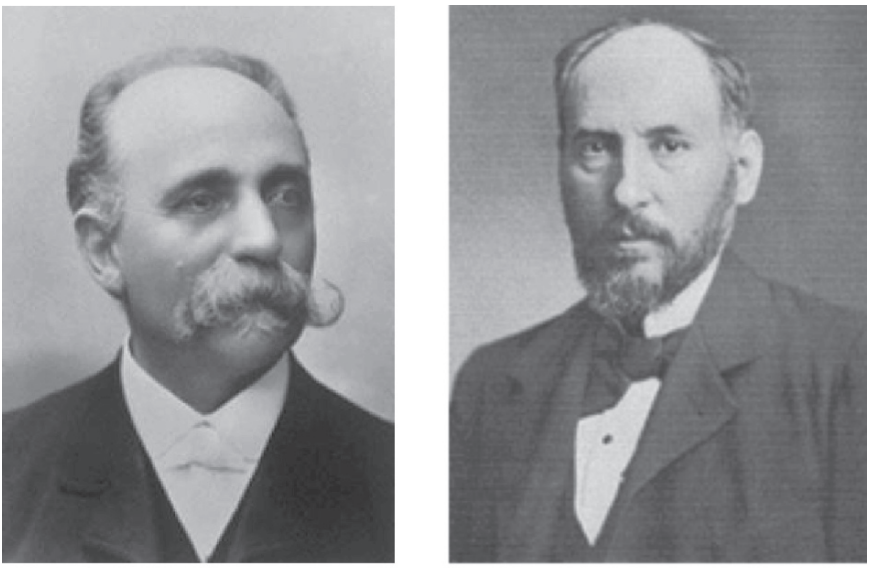


**Fig. 1.1.** Camillo Golgi and Santiago Ramon y Cajal.

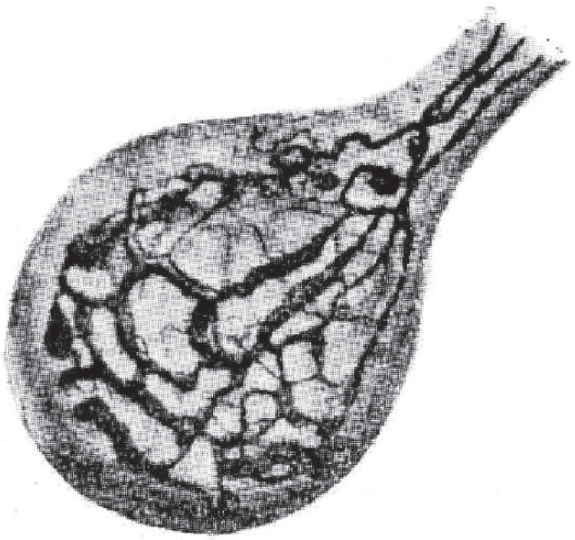

**Fig. 1.2.** An original drawing from the work of Golgi (1898) of the internal reticular apparatus as seen in a Purkinje cell of a barn owl.

until shortly before his death in 1926. It was on one of his absences from Pavia (1872–1875) while resident physician at the Home for Incurables at Abbiategrosso near Pavia that he discovered his chromate of silver method (*la reazione nera)* that was to revolutionize the study of the nervous system and eventually made possible the observation of the *apparato reticolare interno*.

Much is known about events in Golgi's life preceding the discovery of the apparatus and a modern account is given in the book by Whaley (1975). Most Golgi followers find their first reading of the original account a disappointment. What is presented is a brief description of what Golgi observed along with a pen and ink drawing. This is entirely in character with Golgi's style of writing which was to limit himself to a concise description of the morphology of the nervous system. It is generally agreed that Golgi and his followers considered that they had discovered a new cell component and were aware of its potential importance to secretion but most was left for contemporaries to sort out.

One such contemporary of Golgi was Santiago Ramón y Cajal (1852–1934), a Spanish physician, who, with Golgi, shared a Nobel prize in 1906 for work on the nervous system (Cajal, 1923). The views of Cajal differed from those of Golgi. Cajal viewed each nerve cell as a separate entity whereas Golgi's view of the nervous system was that of a continuous network. As to the internal reticular apparatus, Cajal accepted Golgi's view, refined Golgi's impregnation methods, established the generality of the structure, and contributed substantially to the beginnings of a functional understanding. A major account, published in 1914, marks the real beginning of early Golgi apparatus discovery so much so that Cajal might be justly credited as a co-discoverer. It is in Cajal's writings that subsequent generations found the impetus to probe deeper. Cajal, perhaps more than Golgi, established that the internal reticular apparatus was a new cell component, that it existed in diverse cell types, and that its form and appearance changed during differentiation of the cell and with changes in metabolism. He noted the relationship between the apparatus and the region of the cells that contain the centrioles, and its changes with activity of secretory cells. Basically, he proposed that the apparatus enclosed materials that were consumed during periods of activity and that accumulated during the quiescent phases. He was aware of the appearance of the apparatus and its changes under many conditions and came very close to deducing its correct role as a component of the cell's secretory apparatus. Cajal's papers might be profitably read even today in terms of certain aspects of Golgi apparatus dynamics and susceptibility to postmortem change often overlooked in the design of modern biochemical and molecular investigations.

A role of the Golgi apparatus in secretion was implicit in most of the early accounts. The work of Nassonov (1923, 1924) following that of Negri on Golgi apparatus function in the parotid and pancreas (see Whaley, 1975) was important in that a consistent association between secretory products and Golgi apparatus was noted in addition to staining reactions common to the two (Bowen, 1929). Also, important was the paper by Fuchs (1902) concerning the epididymal epithelium of the mouse. He cautioned correctly, for example, that in certain instances the Golgi apparatus might serve only to function as an intermediate between synthesis and final discharge of secretory materials.

The value of Nassonov's contributions were heightened by the fact that he was able to reach certain conclusions bearing correctly on Golgi apparatus function. He noted that secretory granules made their initial appearance within the Golgi apparatus meshwork. As a second step, he concluded that the granules upon reaching a certain size were released from the apparatus and collected near the luminal surface of the cell. He also noted that in different cells different materials

were processed through the apparatus. Finally, he concluded that formation of the granules containing secretory materials and the eventual discharge via the granules of the secretory materials from the cell were separable events. Granule formation was ascribed to an immediate activity of the Golgi apparatus per se, while the exteriorization process was considered to proceed without further Golgi apparatus involvement.

Much of the early work leading to concepts of considerable importance would be virtually inaccessible to the hurried contemporary cell biologist were it not for Bowen's epic review "The Cytology of Glandular Secretion" published in 1929. Bowen concluded both from his own work and his extensive familiarity with the literature that secretion was a cellular process whereby products destined for export were collected or built up in the Golgi apparatus and then separated from it. As these materials were being discharged to the cell's exterior, new secretory granules would take their place through continued activity of the Golgi apparatus. The Golgi apparatus always appeared to remain intact during successive cycles of secretion and details of the process varied from one cell type to another. Bowen was also to set the stage for the concept of Golgi apparatus function as an integral part of a complex system of internal cytomembranes (the Endomembrane System of Chapter 2). This is found in his comment, "apparently the Golgi apparatus plays some immediate role in the process of accumulation and final synthesis of the secretion products, but the concomitant changes in other cellular structures suggest that all parts of the cell contribute in some way."

## 1.2. The Controversy

Much of what was to be learned in the light microscopy era [see book by Wilson (1925), for example] was discovered by 1925 and can be found in Bowen's (1929) review. Subsequent investigations only served to complicate the problem to the extent that were it not for the coming of modern electron microscopy techniques, the Golgi apparatus might have been relegated to the category of artefact. To appreciate this, one must realize that study of the Golgi apparatus in all of its variations from cell to cell and even within a single cell type using techniques then available to light microscopists was a formidable task. Only with information derived using much more adequate instrumentation was it possible to move ahead. Some of the controversial literature arising in the 1930s and 1940s became so involved as to defy resolution even with modern techniques.

Exemplary in this regard was the analogy between the Golgi apparatus and the Canals of Holmgren (1902) which led to assumptions concerning the equivalence of Golgi apparatus and elements of the vacuome (vacuolar apparatus/vacuoles). An extreme view elaborated by Parat and Painlevé (1924a, b) was that all animal and plant cells have two fundamental and independent morphological elements, the vacuome and the chondriome (mitochondria). The vacuome was regarded as an aqueous phase stained specifically with neutral red, while the chondriome was

a lipid phase stained by lipid-soluble dyes. The reticular apparatus of Golgi and the Canals of Holmgren were initially regarded as artefacts produced by precipitation of silver or osmium at the surface of, inside, or between the vacuoles. The whole subject of neutral red cytology was attacked by Gatenby in 1931 but some aspects of the arguments of Parat may have been correct. Elements of the chondriome are now equated with mitochondria and the possibility remains that the Holmgren canals were endoplasmic reticulum.

Yet another aspect of the controversy was contributed over the period 1944 to1963 by Baker and colleagues at Oxford who denied the existence of the apparatus and explained their observations and those of others to modification of spherical bodies which were stained with Sudan black, a lipid stain. Mostly they were unable to find any signs of a network using standard impregnation techniques and phase contrast microscopy. The situation was further clouded by the inability of various workers to visualize the typical network of fixed preparations in unfixed living cells. No matter that Hirsch (1939) pointed out that the inability to see the apparatus in living cells was no indication that it was not present since it had a refractive index identical with that of the cytoplasm. In fact, Ludford (1925) published photographs of living tissue culture cells stained in vitro with methylene blue in which a distinct Golgi apparatus area was visible near the nucleus. The area appeared somewhat reticular. Ludford also presented images of cells photographed in ultraviolet light where the Golgi apparatus region occupied a half-moon area around the nucleus and appeared reticular. In the same year, Strangeways and Canti (1927) denied categorically that the Golgi apparatus existed.

Doubtless much artefact contributed to the controversies of the light microscope era. Palade and Claude (1949a, b) unequivocally showed that Golgi apparatus-like structures could be created in cells by the fixatives used in the Golgi impregnation techniques. These were actually myelin figures probably produced from phospholipids.

The situation in plant cells became hopelessly complex. Bowen first developed an idea that the plastids might equate to the plant Golgi apparatus. He later returned to the question and correctly described structures, termed osmiophilic platelets, which he equated to the animal Golgi apparatus. Strong evidence in support of this concept was provided by Beams and King (1935), who used centrifugations of whole tissues to demonstrate that the Golgi apparatus of animal cells and the osmiophilic platelets of plant cells were displaced to the same level. However, the majority of plant papers were concerned with other approaches. In the absence of definitive correlative information Nahm (1940) concluded, based on a thorough review of the literature, that a Golgi apparatus equivalent did not exist in plant cells.

It is impractical to mention all of the various aspects of the period of Golgi apparatus controversy much less analyze the vast literature dealing with the subject. This brief summary may provide some indication of the level of confusion that existed prior to the electron microscope era. An excellent review is that of Bourne and Tewari (1964).

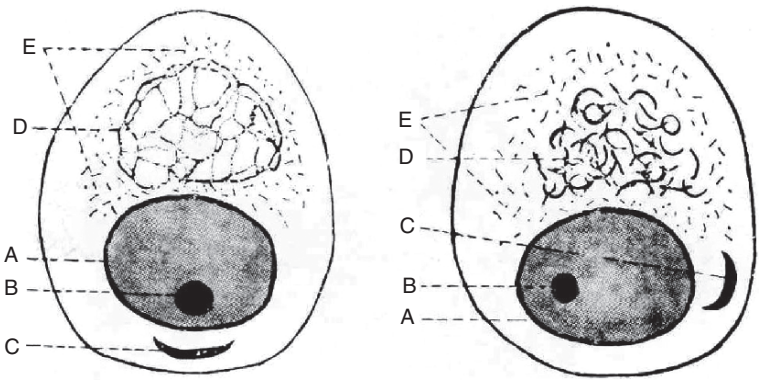


**Fig. 1.3.** Original diagram of Perroncito (1910) illustrating dictyokinesis and the elements to which he applied the term dictyosome. *Left*: A cell before dictyokinesis begins. A = nucleus, B = nucleolus, C = centrosome, D = Golgi apparatus, E = chondrosomes of Meves (mitochondria). *Right*: Dictyokinesis in progress. A = nucleus, B – nucleolus, C = centrosome, D = dictyosomes, E = chondrosomes of Meves (mitochondria).

## 1.3. Modern Rediscovery

The first micrographs correctly identifying the Golgi apparatus in the electron microscope were published by Dalton and Felix (1953). These micrographs were available to Gatenby for his 1955 review and were accompanied in 1954 by papers by Sjöstrand and Hanzon describing the Golgi apparatus of mouse pancreas by electron microscopy. The structures took the form of a mixture of large vacuoles, flattened sacs, and groups of vesicles. In 1956, Dalton and Felix showed that all these parts of the Golgi apparatus reduced osmium tetroxide and helped to form the classic reticulum of Golgi. The Golgi apparatus equivalents in invertebrates, the osmiophilic platelets or dictyosomes, were also shown by electron microscopy to be homologous with the Golgi apparatus of vertebrate cells (Dalton and Felix, 1956; Beams and colleagues, 1956). Similar structures were subsequently found in a variety of plant cells (Porter, 1957; Buvat, 1957a, b; Heitz, 1957a, b, c; Perner, 1957, 1958; Sitte, 1958; Dalton and Felix, 1957; Charder and Rouiller, 1957; Sager and Palade, 1957). A portion of a Golgi apparatus stack (dictyosome) is illustrated but not identified in a 1956 electron microscope study by Hodge et al. (1956) with *Nitella*.

Thus, within a span of about 2 to 4 years, the advanced technology afforded by the electron microscope erased nearly 40 years of controversy generated from light microscopy. There were some who resisted. Kanwar (1961–1962) argued that the so-called “Golgi apparatus” of electron microscopy was not homologous to the light microscope “apparato reticolare interno” of Golgi and not until 1963 did Baker reluctantly become a follower.

## 1.4. Summary

The "apparato reticolare interno" discovered by Camillo Golgi and published in the late 1800's resulted from work with silver impregnation techniques developed to visualize neuronal networks. The 50 years following the first report were years of controversy. Even the reality of the structure was openly questioned by some while others proceeded with structural and functional studies. It remained for electron microscopy to end the "Golgi controversy" at the beginning of the 1950s with the description of a morphological entity with the requisite characteristics to be called the Golgi apparatus. In the 50 years that followed the modern rediscovery, rapid progress was made in the cytochemical differentiation of the cisternal stack, and elucidation of its role in secretion and in post-translational modifications including demonstrations by autoradiography and by classical biochemical analyses applied to isolated Golgi apparatus. The roles of the Golgi apparatus in secretion were firmly established. Development of cell-free systems and of immunochemical and molecular probes of Golgi apparatus function provided the beginnings for the modern era.

# 2

# Structure

With no cell compartment or organelle has morphology served such a pivotal role in its discovery and investigation as with the apparatus of Golgi. The original description of the "apparato reticulo interno" (internal reticular apparatus) now known as the apparatus of Golgi or Golgi apparatus was based on light microscopy (Chapter 1). Both classical Golgi apparatus study prior to 1953 and the modern rediscovery due to the advent of the electron microscope all were based on morphology. An understanding of Golgi apparatus architecture was one of the more important early developments resulting from electron microscopy.

Morphology was the sole basis to guide early attempts at Golgi apparatus isolation and, until its isolation in the mid to late 1960s and early 1970s, and the introduction of autoradiography (Peterson and LeBlond, 1964), morphology was the only basis for investigation of this complex cellular component. Even today, morphology remains as a major criterion by which Golgi apparatus are defined.

Biochemical definitions of Golgi apparatus are complicated by the fact that Golgi apparatus (either singular or plural) are transitional cell components sharing many biochemical characteristics with either the endoplasmic reticulum or the plasma membrane or both. Unlike the situation with true organelles such as chloroplasts or mitochondria, there have been virtually no biochemical markers common to all cell types and not shared with either endoplasmic reticulum or plasma membranes. Additionally, the functioning of Golgi apparatus in secretion and other activities is highly dependent upon the co-participation of endoplasmic reticulum, secretory vesicles, and other structures. Thus, the Golgi apparatus exists in the cell as a component within a highly integrated endomembrane system (Morré and Mollenhauer, 1974) often with biochemical characteristics intermediate between those of the endoplasmic reticulum and those of the plasma membrane, but with a characteristic and easily recognized pattern or morphology that unambiguously distinguishes the Golgi apparatus from all other cell components.

The most common form of the Golgi apparatus, exemplified by most mammalian cells, consists of side by side piles or stacks of smooth membrane cisternae lacking ribosomes (Fig. 2.1). The stacks are seen to be distributed and located within a special region of cytoplasm called the Golgi apparatus zone or Golgi apparatus matrix (Fig. 2.2). In plants, and in many animal cells as well, individual stacks of cisternae may be spread more or less evenly, through the entire cytoplasm. These stacks, even though dispersed, function synchronously indicating that they are functionally and perhaps structurally interconnected.

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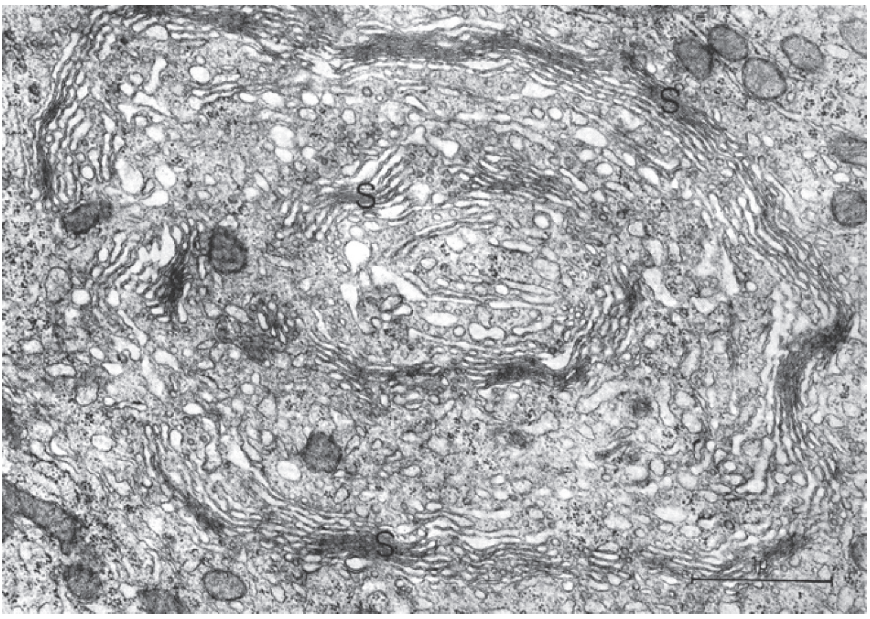


**Fig. 2.1.** Portion of the Golgi apparatus of rat epididymis showing numerous closely-spaced stacks (S). Karnovsky's fixative. From Flickinger (1969b). Electron micrograph courtesy of Dr. C. J. Flickinger, University of Virginia Medical School, Charlottesville. Reprinted by permission of Academic Press, Inc., New York.

Vesicles and tubules either may be attached to or associated with various parts of the Golgi apparatus. Structural features at each level of organization will vary depending on cell type, method of fixation, and of specimen preparation and the physiological state of the cell. However, those emphasized may be observed as consistent features of most electron microscope preparations.

## 2.1. Cisternae

By definition, a cisterna (plural = cisternae) is a sac or cavity within a cell or organism, usually filled with fluid (i.e., a cistern). The term was used originally in electron microscope morphology to describe one of the interconnected vesicles, lamellae or tubules comprising the endoplasmic reticulum but has served the same purpose for the Golgi apparatus. Each cisterna of the Golgi apparatus consists of a lumen or central cavity surrounded by a membrane lacking ribosomes (one of the several so-called "smooth" membranes that frequently contribute to the smooth microsome fraction of cell homogenates). Cisternae will differ in architectural detail depending on cell type and their position in the stack (Figs. 2.3 to 2.7). Perhaps no two are exactly alike. Many cisternae especially from the mid-region of the stack (i.e., the intercalary cisternae) often have

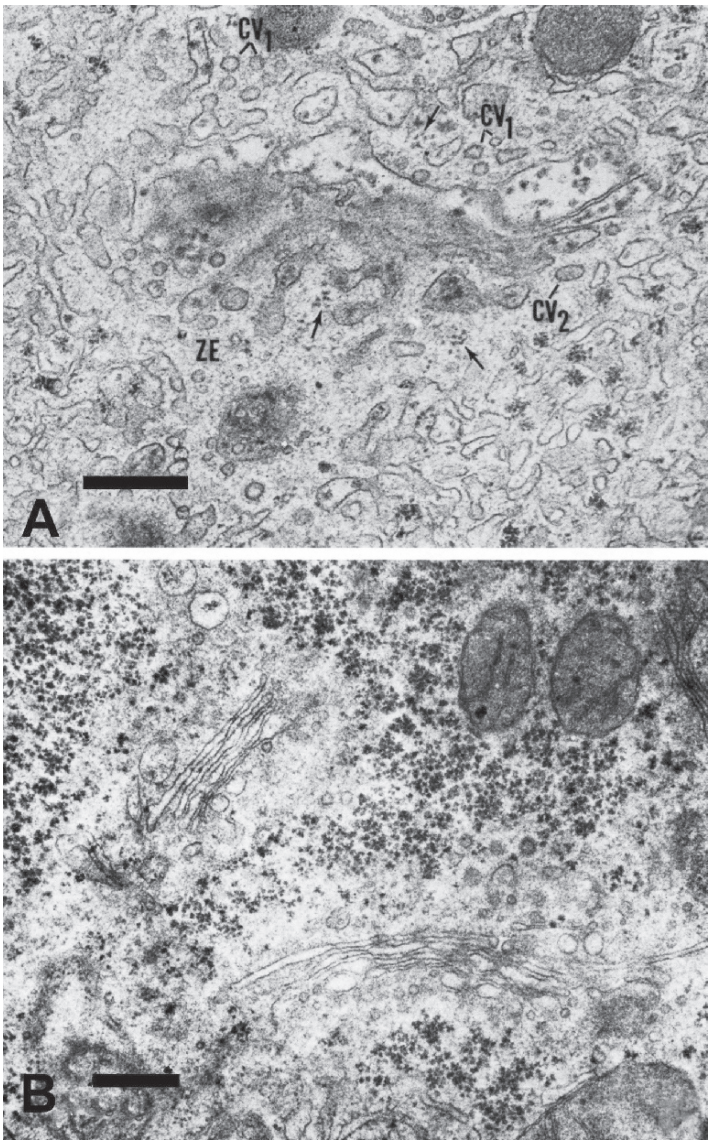


**Fig. 2.2.** Golgi apparatus regions showing the zone of exclusion or Golgi matrix. Coated vesicles (CV) of the Golgi apparatus regions are restricted to this zone. Elements of endoplasmic reticulum entering the zone are smooth (lacking attached ribosomes). Free polyribosomes (Golgi apparatus polyribosomes) are frequently observed within the zone. (A). Normal rat liver. (B). Rat hepatomas induced by N-2-fluroenylacetamide (FAA). The hepatoma cells contain a Golgi apparatus with dispersed stacks but still each stack is surrounded by a zone of exclusion. Reproduced from Mollenhauer and Morré, 1978b with permission from Springer Science + Business Media. Scale bar = 5 μm.

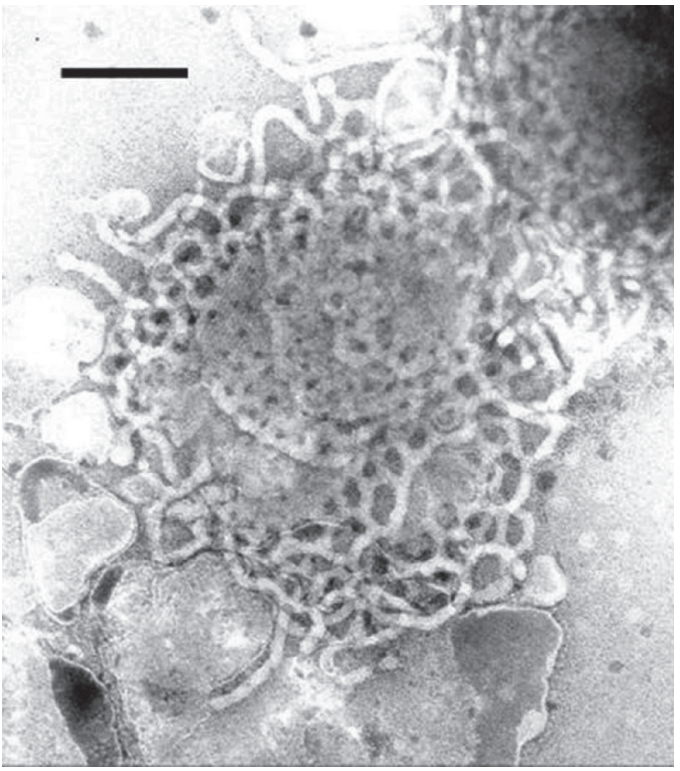

**Fig. 2.3.** A Golgi apparatus stack from radish root isolated and viewed in negative contrast directly on an electron microscope grid. The stack has been partially unstacked to reveal a progression change diagrammed in Figure 2-4 from the cis (top) to trans (bottom) face of the stack. Reproduced from Mollenhauer and Morré (1966) from the Journal of Cell Biology, 1966, 29:373-376. Copyright 1966b The Rockefeller University Press. Scale bar = 0.2 μm.

small perforations or fenestrae at the margins. This central portion of the cisternal stack is referred to also as the central *saccule* or simply as the saccule. Normally, 4 to 6 such saccules are arranged one on top of the other, in parallel array, to yield the characteristic "stack" or dictyosome as one of the more obvious features used to identify Golgi apparatus both in situ and isolated in cell fractions (Chapter 3). The plate-like regions, up to the fenestrated margins, are typically 0.5 to 1 μm in diameter (Fig. 2.5).

Continuous with the central plate like region is a complex system of tubules and secretory or coated vesicles (Fig. 2.9). Secretory vesicles lack coats or are partially coated over their surfaces with spiny clathrin coats. Vesicles of the Golgi apparatus zone coated either with clathrin or coatomer proteins (COP) may be either free or attached to tubule ends (Mollenhauer and Morré, 1966b). The connecting tubules that attach secretory vesicles to saccules are short (Fig. 2.7) and begin as narrow partitions extending beyond the fenestrated peripheries of the central plates. Longer tubules may continue for several microns and follow

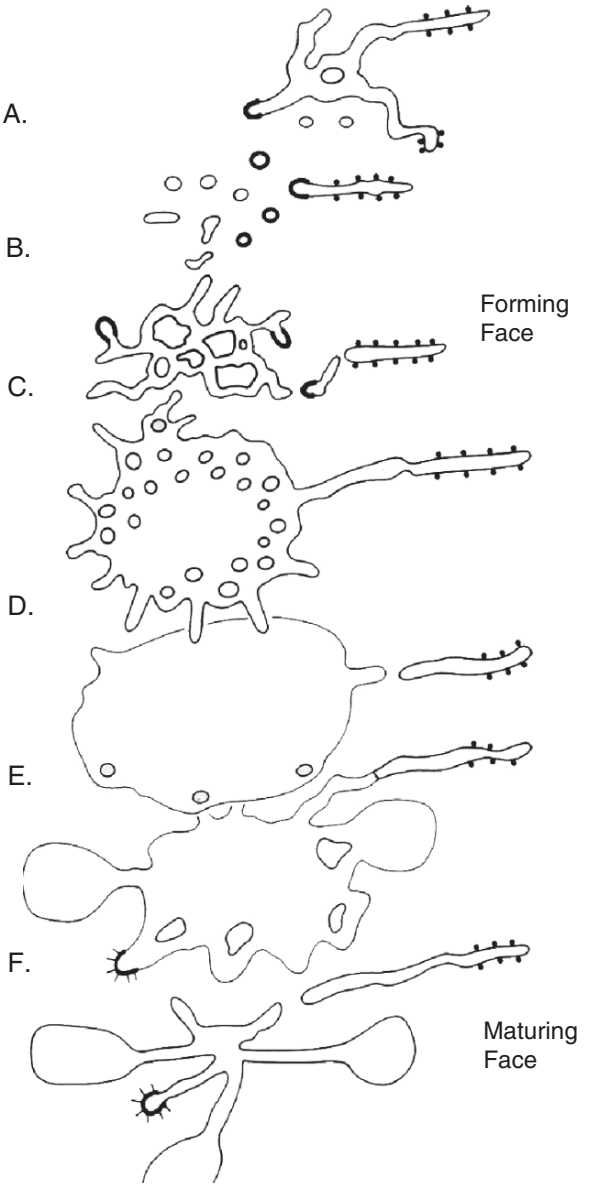


**Fig. 2.4.** Diagrammatic representation of successive cisternae within a single stack as partially revealed in Figure 2-3. (A). Part rough (with ribosomes) -part smooth (lacking ribosomes) transitional endoplasmic reticulum with (B). COPII—coated buds and vesicles which (C). coalese to form the largely tubular first Golgi apparatus cisternae of the forming face. (D). Continued delivery of ER-derived material results in fenestrated cisternae with a central plate-like region and tubular peripheries. (E). As cisternal maturation proceeds, plate-like regions dominate. (F). Secretory vesicles connected to plate-like regions by tubules characterize the maturing face. (G). The post Golgi structures or trans Golgi network = cisternal remnants with clathrin-coated membrane and vesicles represent the final stage of cisternal maturation and utilization in secretory vesicle formation.

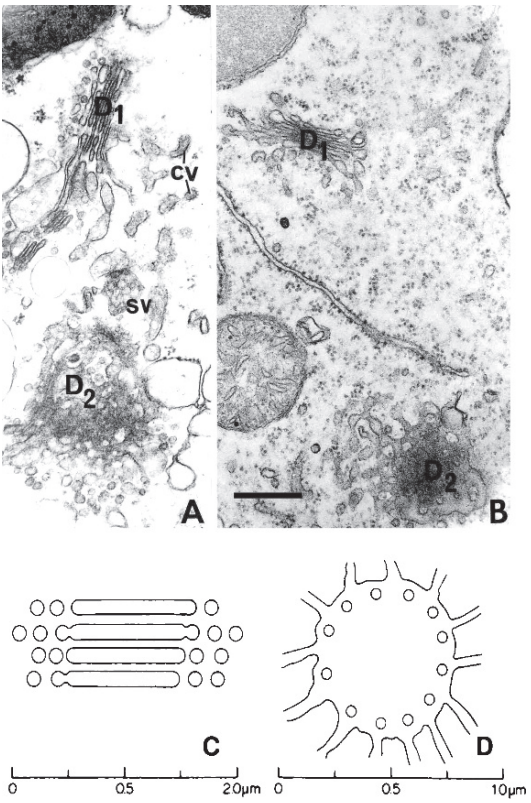


**Fig. 2.5.** Golgi apparatus stacks isolated (A) and in situ (B) from soybean illustrating the complementary cross sectional ($D_1$) and face ($D_2$) views. SV = Secretory vesicle. CV = clathrin-coated vesicle. Reproduced from Morré, 1977a with permission from the author. Scale bar = 0.5 µm. C, D. Diagram illustrating the correspondence of the cross-sectional and face view images of stacked Golgi apparatus cisternae. The fenestrated (with openings or holes) and/or tubular peripheries seen in face view (D) are represented by small vesicles or interruptions in the flattened saccules when viewed in cross section (C). Reprinted from Morré and Ovtracht, 1981, Copyright 1981 with permission form Elsevier.

an irregular course through the cytoplasm. The full extent of such tubules, or whether they interconnect to distant stacks, has not been determined.

In intercalary cisternae, the solid plate like regions seem to dominate (Figs. 2.4 and 2.5). Exterior cisternae tend to be more fenestrated with a dominance of tubular elements. These differences in morphology observed within a single stack contribute to the polarity of the dictyosomal stacks discussed in the next section. The interconnected system of plates, tubules, and vesicles that constitute Golgi apparatus cisternae allows for considerable subcompartmentation and restriction of functional activities to specific regions even within a single cisterna.

In the diagram of Fig. 2.4 based on analysis of negatively stained partially unstacked plant (Fig. 2.3) and animal (Fig. 2.10) preparations as well as serial section analysis (e.g., Brown and Arnott, 1971; Fig. 2.8), predominantly tubular cisternae are present at the pole proximal to the nuclear envelope (cis face) while

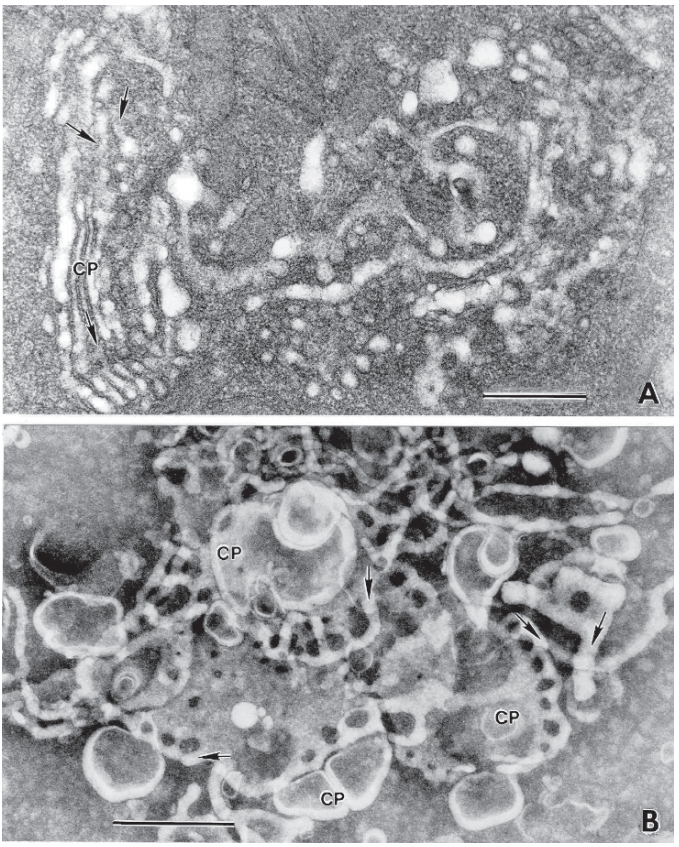


**Fig. 2.6.** Tubular peripheries of Golgi apparatus cisternae (arrows) of cat trachael epithelia fixed in situ with a tannic acid-containing fixative (A) and seen by negative staining in an isolated Golgi apparatus preparation (B). CP = central plate-like portion of cisternae. Reproduced from: Tandler and Morré, 1983 with permission from Springer-Wien. Scale bar = 0.5 µm.

plate-like regions become dominant toward the center of the stack. Cisternae at the pole most distal from the nuclear envelope (trans face) may again present a more tubular aspect although the form of the tubules is quite distinct from those at the cis face.

The extent of the system of peripheral tubules associated with the Golgi apparatus first became evident from preparations of plant Golgi apparatus stabilized with aldehyde fixatives during isolation and visualized by negative staining with phosphotungstic acid (Mollenhauer and Morré, 1966a; Cunningham et al., 1966; Fig. 2.6B) and later extended to animal cells (Mollenhauer et al., 1967; Morré and Ovtracht, 1981; Fig. 2.10). The tubules, which are 300–500 Å in diameter, may serve to connect cisternae of adjacent dictyosomes (Fig. 2.11), function as attachment sites for secretory vesicles (Figs. 2.7 and 2.12), and seem to facilitate direct connections between Golgi apparatus cisternae and smooth portions of the endoplasmic reticulum (Fig. 2.13). Some authors have argued that the tubules

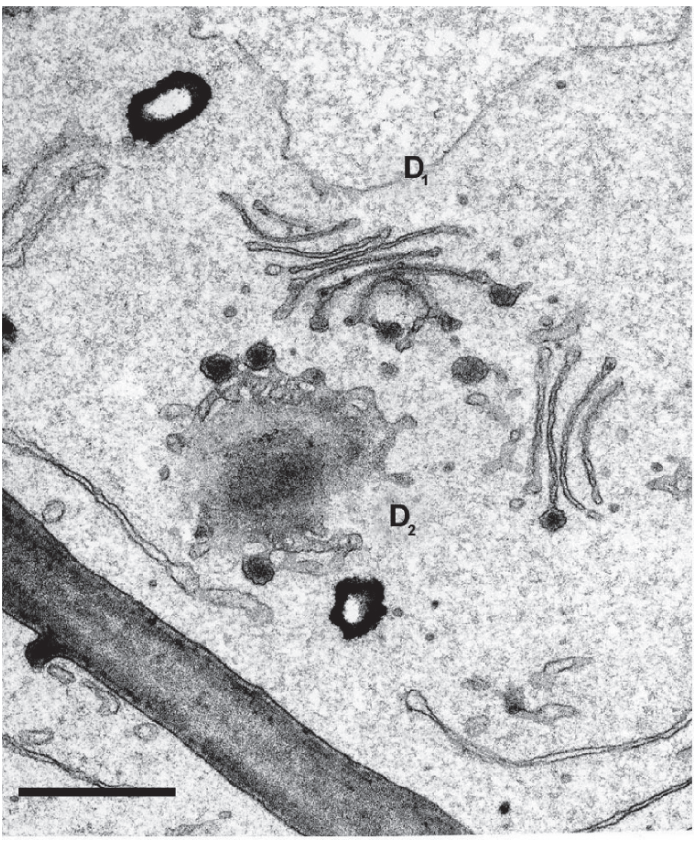


**Fig. 2.7.** Dictyosomes (cisternal stacks) from epidermal cells of maize root fixed with potassium permanganate and contrasting cross-sectional ($D_1$) and face ($D_2$) views. In face view, the cisternal peripheries are seen to exhibit the tubular structures characteristic of other fixatives. Reproduced from Mollenhauer and Morré, 1994 with permission from Springer-Wien. Scale bar = 0.5 µm.

and fenestrae may be artefacts due to extraction of proteins from the Golgi apparatus membranes during preparation of the specimens for staining (Cunningham et al., 1974). Clearly, prolonged contact between Golgi apparatus and any concentrated salt solution will result in extraction of lipids and proteins which may result in morphological modifications. However, cisternae tubules are observed *in situ* following fixation with glutaraldehyde–osmium tetroxide (Fig. 2.5), glutaraldehyde–tannic acid–osmium tetroxide (Fig. 2.6), osmium tetroxide alone (not shown), or potassium permanganate (Fig. 2.7). They are present in freeze–fracture–etch preparations in which no fixatives are involved (Fig. 2.14). While care must be exercised in the interpretation of negatively stained images, especially of small or fragmented structures, Golgi apparatus tubules in situ and in isolated preparation, negatively stained, however, are similar in extent and appearance (Tandler and Morré, 1983; Fig. 2.6) which argues against them being solely artifactual.

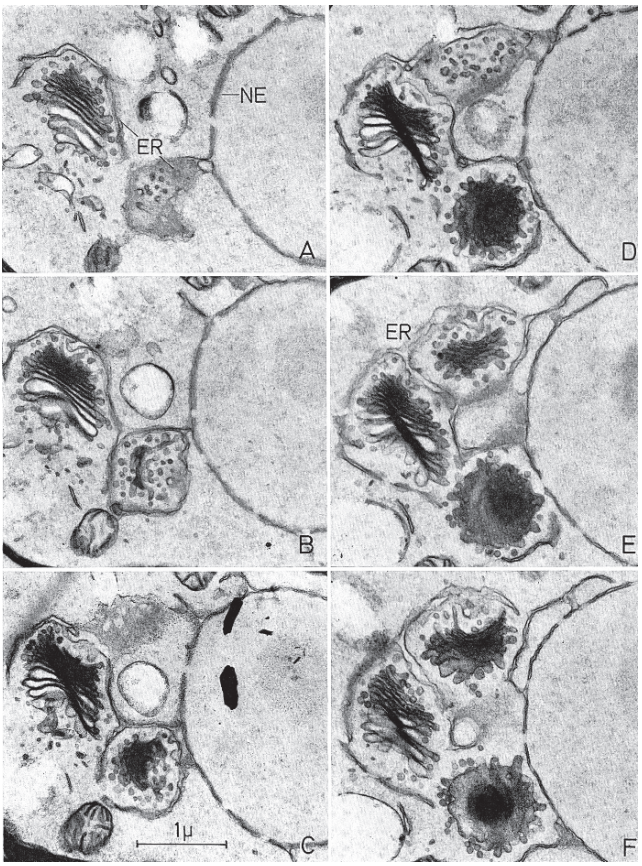


**Fig. 2.8.** Serial sections through a portion of the endomembrane system of the alga *Tetracystis excentrica* including three adjacent stacks. Sheets of endoplasmic reticulum (ER) continuous with the nuclear envelope (NE) and known as the amplexus, surround each stack, except for a region at the maturing face through which secretory vesicles are discharged. The stack at the lower center is sectioned tangentially and shows successive cisternae in face view beginning with the endoplasmic reticulum at the forming face in A. To the left, a second stack is sectioned transversely and shows the entire stack in cross section. A third stack becomes evident in D–F at upper center and C provides a tangential view of the sheet of endoplasmic reticulum associated with the proximal face of the stack. $KMnO_4$ fixation. Unpublished electron micrographs courtesy of Drs. R. Malcolm Brown, Jr. and Howard J. Arnott, Cell Research Institute, The University of Texas, Austin. From: Morré et al., 1971c. Reproduced by permission of Elsevier. Scale bar = 1 μm.

## 2.2. The Cisternal Stack or Dictyosome

Golgi apparatus cisternae are usually organized into stacks of five to eight cisternae (Table 2.1). The stacks also are known as *dictyosom*es (Mollenhauer and Morré, 1966a). Twenty or more cisternae per dictyosomal stack are not unusual among lower organisms such as *Euglena gracilis* (Mollenhauer, 1974).

The term dictyosome (from the Greek word *dictyos* meaning net or network = net body) was used originally by Perroncito (1910, see Whaley, 1975, Chapter 1)

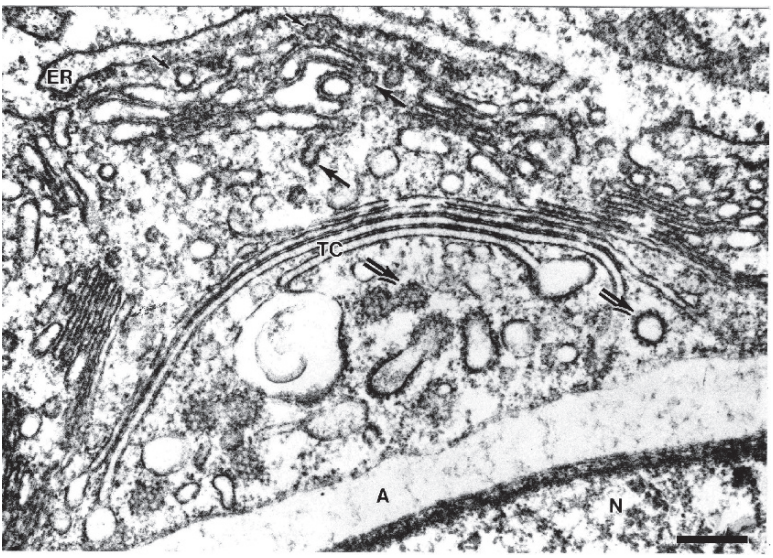


**Fig. 2.9.** Golgi apparatus region of a rat spermatid adjacent to the forming acrosome (A) fixed with 37% glutaraldehyde – 2% paraformaldahyde – 10% saturated picric acid containing 1% tannic acid to enhance vesicle coats. Illustrated are COP-coated endoplasmic reticulum-derived transition vesicles at the forming face (single arrows) and clathrin-coated membranes and vesicles of the maturing face (double arrows). Also present is an elaborate post Golgi structure consisting of elongated cisternae with thick membranes (TC). N = nucleus. Reproduced from Mollenhauer, Hass, and Morré, 1976 with permission from the Société Française de Microscopie Electronique. Scale bar = 0.5 µm.

to designate a component of the Golgi apparatus that was visible following cell division and that had a definite pattern of distribution in the daughter cells. The term was also used to indicate a form of Golgi apparatus characteristic of invertebrates which appeared more as discrete units than the complex reticular apparatus first described by Camillo Golgi. The modern usage of the term dictyosome differs little from the historical, and "net body" accurately describes the modern concept of Golgi apparatus structure.

A major difference among species and cell types in regard to Golgi apparatus organization is the distance by which the individual stacks or dictyosomes are separated. There are approximately 500 such stacks in a typical plant or animal cell. These may be arranged side by side as an almost continuous ribbon as seen in many mammalian cells (Figs. 2.1 and 2.15) or they may be so widely separated as to appear as discrete units as in most plants and invertebrates. Generally, the dispersed arrangement is more characteristic of undifferentiated cells and tissues or differentiated cells not involved in protein secretion whereas the compact arrangement becomes most evident in cells specialized for protein secretion.

Dictyosomes are polarized structures in that cisternae at one pole or face of the cisternal stack differ from those at the opposite pole or face (Fig. 6.2). In many cells of animals, algae, and fungi, one pole of each dictyosome is associated with the nuclear envelope or endoplasmic reticulum in a characteristic manner. This pole or "face" of the Golgi apparatus stack and of the Golgi apparatus per se

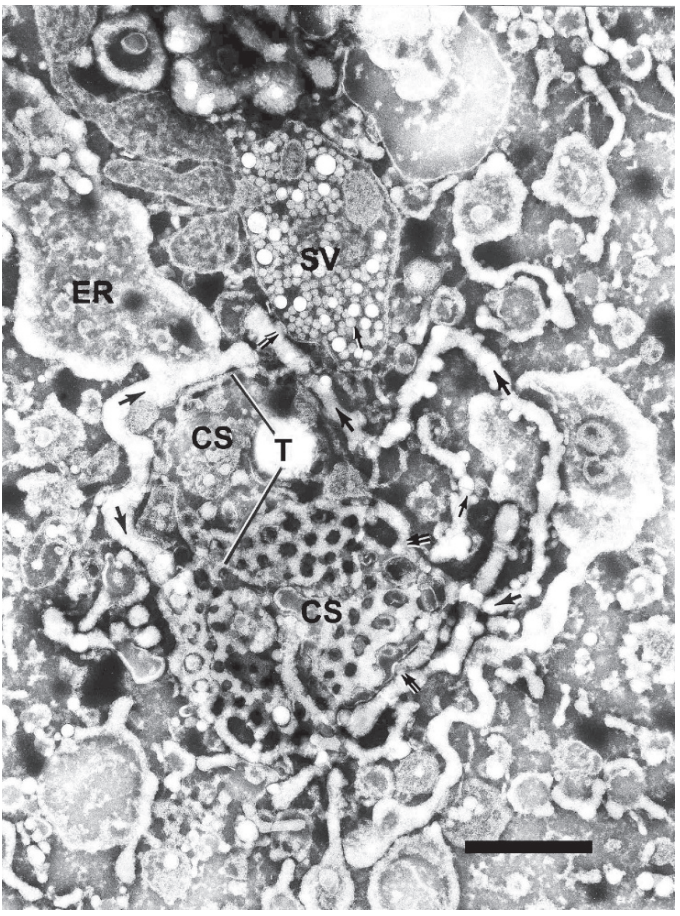


**Fig. 2.10.** Tubules (T) of the Golgi apparatus (boulevard périphérique) connecting (large arrows) endoplasmic reticulum (ER) and lipoprotein particles (small arrows) containing secretory vesicles (SV) of a Golgi apparatus preparation isolated from rat liver. CS = central saccule. Reprinted from Morré and Ovtracht, 1981, Ultrastruct. Res., 74, 284-295, Copyright 1981 with permission from Elsevier. Scale bar = 0.5 μm.

is referred to as the pole proximal to endoplasmic reticulum or proximal pole, or as the "cis" face. For biogenetic considerations, it has also been referred to as well as the "forming" face. The opposite pole or face of the dictyosome is the distal pole, also known as the mature(ing), secreting, or trans face. Membranes of the proximal pole or cis face cisternae are morphologically and cytochemically similar to the membranes of endoplasmic reticulum (Chapter 6). Toward the opposite pole or trans face, the morphology and staining characteristics of the cisternae become progressively more like those of plasma membrane.

Associations between endoplasmic reticulum (or nuclear envelope) and Golgi apparatus take many forms. One of the first to be noted was that of an endoplasmic (nuclear envelope) cisterna lying parallel to the cis face of the Golgi apparatus (Fig. 2.16). Attached ribosomes were present on the cytoplasmic