ISOLATION OF GERM CELL GOLGI APPARATUS FROM SEMINIFEROUS TUBULES OF RAT TESTES

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ABSTRACT

Intact Golgi apparatus have been isolated with good purity from rat testis by a simplified sucrose gradient technique. The procedure is inherently selective in that most of the Golgi apparatus in the isolate are from germ cells in late spermatocyte or early spermatid development. No Sertoli cell Golgi apparatus are present in the fraction. Biochemical analyses showed that the enzyme N-acetylglucosamine galactosyltransferase is enhanced in the band containing Golgi apparatus. Because they are easy to isolate, and because they are involved with the formation of a specific internal cellular component (the acrosome), these Golgi apparatus will be useful objects for comparison with other kinds of Golgi apparatus and for other studies leading to a better understanding of the basic functioning of Golgi apparatus.

INTRODUCTION

Fractionation and direct biochemical characterization of cellular organelles are powerful tools in elucidating the functional mechanisms and molecular organization of cells. Beginning with the work of Kuff et al (13, 34), progress in the isolation and purification of Golgi apparatus has made possible significant studies of the chemistry and enzymology of this secretory organelle (4, 10, 11, 12, 15, 21, 23, 24, 27, 29, 32, 33, 36). In many tissues the Golgi apparatus is relatively unstable and, therefore, is often one of the most difficult cellular organelles to extract in reasonable quantity, purity, and state of preservation. Techniques for the isolation of significant quantities of purified Golgi apparatus have been reported for only a few tissues, particularly rat liver from which most of the chemical data has been obtained. Other tissues have sometimes required treatments such as chemical fixation (6, 26) and modification of the tissue by diet or storage conditions (18, 25), in order to obtain adequate yields of Golgi apparatus. These unusual procedures are undesirable since they may introduce alterations in the morphology and metabolism of the isolated organelles.

In this report we present a simplified fractionation procedure applicable to rat testis. This procedure yields a highly purified, enzymatically active, Golgi apparatus fraction in adequate quantity for biochemical analyses. The morphology of the isolated Golgi apparatus suggests that most of them are derived from germ cells in late spermatocyte or early spermatid stages of development. These Golgi apparatus are of particular interest because of their physiological role as the source of the sperm acrosome (1, 2, 3, 9, 14). The detailed steps in acrosome formation have not been elucidated, however, and it is hoped that this technique for Golgi apparatus isolation may facilitate biochemical analysis of this process.
MATERIALS AND METHODS

Isolation Procedure

Adult male albino rats (Holtzman, 250-400 g) were killed by cervical dislocation, the testes excised, the tunica albuginea and testicular artery removed, and the soft mass of seminiferous tubules was dropped into ice-cold medium (referred to hereafter as 0.5 M) containing 0.5 M sucrose, 0.05 M Tris-maleate buffer pH 6.5, 1% dextran (Type 200C, Sigma Chemical Co., St. Louis, Mo.), 0.005 M MgCl₂, and 0.005 M mercaptoethanol. In a typical experiment, three rats weighing 300 g each were used, yielding 9-10 g wet weight of testicular material. This material was homogenized in 20 ml of 0.5 M medium for 20 sec at the lowest setting on a Polytron 10ST (Kinematica, Lucerne, Switzerland; now sold by Brinkmann Instruments Inc., Westbury, N.Y.). The homogenate was diluted with additional 0.5 M medium to a total of 50 ml and centrifuged for 10 min at 5000 rpm (2800 g average) in an IEC No. 283 swinging bucket rotor (International Equipment Co., Needham Heights, Mass.). The pellet formed during this centrifugation contained two easily recognizable layers. The supernatant solution was discarded and a few milliliters of a 1 M solution were added to the tube. This solution contained the same ingredients as the 0.5 M homogenizing medium above, except that sucrose concentration was increased to 1.0 M. The tan-colored top layer was separated from the pink-colored bottom layer with a glass stirring rod and poured into a glass Potter-Elvehjem homogenizer. It was resuspended in the 1.0 M medium by using a loose-fitting Teflon pestle (about 35 ml of medium for the tan layer from six testes), and transferred to four centrifuge tubes. 2 ml of the 0.5 M medium were layered on top of each tube, and the tubes were spun at 35,000 rpm (140,000 g average) for 30 min. The Golgi apparatus float upwards in the 1.0 M medium and form a thick white band at the interface with the 0.5 M medium. A small amount of lipid accumulates at the top of the tube which can be removed by touching the surface with a piece of tissue paper. The 0.5 M layer was removed with a Pasteur pipette and also discarded. The Golgi apparatus layer was removed with as little 1.0 M medium as possible, diluted with the 0.5 M medium (about 20 ml for the combined Golgi layers from four tubes), and centrifuged at 15,000 rpm (22,000 g average) for 10 min. The pellet was homogenous, white, and firmly packed. 9 g of fresh testes yielded about 5-6 mg of protein. The preparation begins to deteriorate in a few hours both morphologically and enzymatically. The purity of the Golgi apparatus can be improved by further resuspension and centrifugation, but this procedure increases the rate of deterioration. Freezing and thawing causes a complete disappearance of morphologically recognizable Golgi apparatus.

It is possible to prepare Golgi apparatus by an even simpler procedure which eliminates the initial centrifugation and resuspension. The seminiferous tubules are dropped directly into the 1.0 M medium and homogenized in the centrifuge tube. The tube is then filled with 1.0 M medium and centrifuged in an IEC SB-110 rotor at 20,000 rpm for 1 hr. The Golgi apparatus float up as before and are removed along with the lipid layer. The Golgi apparatus can be separated from the lipid contaminant by a low-speed centrifugation in the 0.5 M isolation medium in which the Golgi apparatus sediment and lipid floats. This technique allows more tissue to be handled in a shorter time, but results in slightly less purification. The ratio of testis material to 1.0 M medium is not critical if the cellular contents do not dilute the 1.0 M sucrose too much and thereby alter the density of the final solution. We have found that a wt/vol of no more than 20% is acceptable.

Enzyme Assays

UDP-galactose: N-acetylglucosamine galactosyltransferase activities were determined as described previously (24). The reaction mixture contained 2 μmoles Tris-HCl pH 7.5, 1 μmole MgCl₂, 0.5 μmole MnCl₂, 5 μmoles mercaptoethanol, 61 μmamoles uridine diphosphate galactose (UDP Gal), containing 9 picomoles UDP Gal-C₁₄ from New England Nuclear Corp., Boston, Mass., specific activity: 252 picocuries/mole), 90 μmamoles N-acetylglucosamine (NAG), and 0.01-0.2 mg protein in a total volume of 0.3 ml. Incubations were carried out at 37°C for 10-30 min. The reaction was stopped by placing the reaction mixture on a 1 ml BioRad AG₁-X₂ column (BioRad Laboratories, Richmond, Calif.). N-acetylasaminogalactose and galactose were eluted from the column with three 0.4 ml washes with distilled water which were collected in scintillation vials and counted in Aqualux (New England Nuclear Corp.) in a Nuclear-Chicago Unilux II scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.) with an efficiency of 64%. To correct for nonspecific hydrolysis of UDP-Gal, a control without NAG was run for each determination. The unit of specific activity is μmamoles galactose released per hour per milligram protein.

Electron Microscopy

For electron microscopy, the Golgi apparatus fraction was pelleted by spinning in 0.5 M medium

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1 Abbreviations used in this paper: ER, endoplasmic reticulum; NAG, N-acetylglucosamine; PTA, phosphotungstic acid; UDP-Gal, uridine diphosphate galactose.
at 15,000 rpm in the IEC No. 283 swinging bucket rotor. Pellets were cut into sectors, which preserved the layering and orientation of sedimentation, and fixed for 3 hr in cold 2% glutaraldehyde in 0.05 M collidine buffer pH 7.3–7.4. They were washed 5 × 10 min in 0.1 M collidine + 0.12 M sucrose and post-fixed 5 hr in cold 1% OsO4 in 0.1 M collidine. They were dehydrated in a graded acetone series and embedded in the Spurr, low-viscosity epoxy resin (35). Sections were double stained with saturated uranyl acetate and alkaline lead citrate, and observed and photographed with a Philips EM 200 electron microscope.

For negative staining, portions of the same pellets were resuspended before fixation in distilled water and diluted to a concentration of about 1 mg protein/ml. A drop of this suspension was pipetted onto a carbon-coated grid, a drop of 2% phosphotungstate neutralized to pH 6.8 with KOH (K-PTA) was added, and the grid was blotted with filter paper and examined immediately in the electron microscope.

RESULTS

Sedimentation Characteristics of Testis Golgi Apparatus

Centrifugation of the simplified gradient used in this study results in the formation of two bands and a pellet as illustrated in Fig. 1. The upper band at the top of the 0.5 M layer is a thin, milky-white film consisting mainly of lipid. The pellet at the bottom of the tube is tan in color and contains mainly mitochondria with a few sperm, nuclei, blood cells, and vesicles intermixed.

The band at the interface between the 1.0 and 0.5 M layers is creamy-white in color and consists predominantly of large, hemispherical Golgi apparatus. A low-magnification view of a negatively stained preparation and a section through a pellet made from this band are shown in Figs. 2 and 3, respectively. These figures represent "average" fraction purity of the entire band. A few mitochondria, dense granules, and cisternae of rough endoplasmic reticulum (ER) are also present as minor contaminants in this fraction. The density of the 1.0 M medium is about 1.135 at 20°C. By comparison, rat liver Golgi apparatus are buoyant at a density of about 1.160.

Morphology of Isolated Golgi Apparatus

All of the Golgi apparatus in these fractions are similar in form and consist of large aggregates of dictyosomes (stacks of cisternae about 1 μ in width) interconnected by tubules. Their size and characteristic organization suggest that all of them are derived from germ cells, most probably in late spermatocyte or early spermatid stages of development. The widely separated dictyosomes characteristic of Sertoli cell Golgi apparatus (20) are entirely absent from these fractions.

At least three different developmental stages of germ cell Golgi apparatus are recognizable in these fractions (see Fig. 4). One is small and consists of only a few cisternae which appear in cross-section in a nearly continuous circle with a relatively empty center region (shown in lower left of Fig. 4). In several places the cisternae of this type seem atrophied and shrunken. These Golgi apparatus resemble those of stages 4–7 of spermatid development (see Leblond and Clermont, reference 14 for definition of stages). The most conspicuous type of Golgi apparatus (center of Fig. 4) is large and has five or six dictyosomes (stacks of cisternae) discernible in cross-section, and clusters of tubules which interconnect and surround each dictyosome. The concave, interior portion contains swollen cisternae, vesicles, and more tubules, and the convex surface is associated with pieces of ER. Although not shown here, Golgi apparatus of this type sometimes have centrioles adjacent to the concave face. These Golgi apparatus are thought to be from stages 1–3 of spermatid development (14) or late stages of spermatocyte development. These Golgi apparatus are the predominant form present in the Golgi apparatus fraction. The third Golgi apparatus form is depicted in the upper left portion of the micrograph; it is smaller but otherwise similar to the type just described and probably represents only...
FIGURE 2 Very low magnification picture of an entire specimen grid opening showing the typical distribution of purified Golgi apparatus derived by these procedures. Negatively stained preparations such as this are particularly useful in assaying fraction purity and showing that the Golgi apparatus remain intact. These Golgi apparatus are equivalent to those shown in Figs. 3 and 6. X 4000.

FIGURE 3 Section through a pellet of Golgi apparatus illustrating "average" fraction purity. All of the Golgi apparatus are large and most of them seem to be derived from spermatocytes or spermatids (see Figs. 4 and 5). The smaller, more widely distributed Golgi apparatus of Sertoli cells are totally absent, suggesting that they fragment during isolation or have significantly different sedimentation properties than germ cell Golgi apparatus. The principal contaminants in the Golgi apparatus fraction are lysosomes, mitochondria, and segments of rough ER. An occasional residual droplet (not illustrated in this field) may also be present. X 12,000.
a slightly different stage of spermatocyte or spermatid development.

The network of anastomosing tubules which surrounds and interconnects the dictyosomes in the Golgi apparatus are most vividly observed in negative contrast. Fig. 6 shows a single isolated Golgi apparatus stained with K-PTA.

Morphology of Golgi Apparatus

In Vivo

A number of reports are available (1, 2, 3, 9, 14, 28, 31) which describe spermatogenesis, the formation of the acrosome, and other forms of the Golgi apparatus in mammalian testis. A portion of a spermatid cell is shown in Fig. 5 for comparison with the isolated Golgi apparatus. Note the cisternae of ER on the convex, or forming, face and the thickened cisternae and dark staining vesicles at the concave or maturing face. Similar thickened cisternae were seen in guinea pig spermatids (20) and are probably associated with the last stages of secretory vesicle formation.

Enzyme Analysis

Table I shows some representative UDP-Gal: NAG galactosyltransferase activities. The activity in the absence of an acceptor is a measure of nonspecific hydrolysis of UDP-Gal. The specific activity reported in the presence of the acceptor has been corrected for hydrolysis and is thought to represent true transfer activity. This reaction was linear with both time and protein concentration. No determinations were made in this study of the affinities in this reaction for substrates other than UDP-Gal, or acceptors other than NAG. In these experiments, the purification of this enzyme activity in the Golgi apparatus fraction compared to the whole homogenate was approximately 13-fold. There is also a 13-fold higher rate of transfer activity in the Golgi apparatus fraction than hydrolysis activity. The recovery of UDP-Gal transferase activity in the Golgi apparatus fraction is only about 4% of the total activity in the whole homogenate, and the fraction itself represents only about 0.5% of the total testicular protein. Though this figure appears relatively low it represents, in fact, at least 25–50% of the total testis Golgi apparatus of the kind and stage of development that is present in the isolates (i.e., the Golgi apparatus characteristic of a late spermatocyte or early spermatid stage of development). This is calculated from the data of Albert (see Ewing and Schanbacker, reference 7) who showed that spermatocytes occupy only 14.7% and spermatids only 41% of testicular volume. Consider further that the Golgi apparatus is only a small fraction of the germ cell (in the neighborhood of 5%) and that the procedures outlined here select toward the midstage of germ cell development. This selection causes a sizeable portion (calculated about 60%) of early- and late-developmental stages of Golgi apparatus to be lost from the preparation. Therefore, because of this selectivity, the per cent yield of specific Golgi apparatus is very high in spite of the fact that the total yield is relatively low.

Discussion

In many cells, the Golgi apparatus is one of the most sensitive intracellular membrane systems and reacts rapidly when the cell is damaged or disrupted (19). This is demonstrated also by the fact that Golgi apparatus have been isolated in
Figure 5  Section through a spermatid Golgi apparatus *in situ* showing its general form and its relationship to the acrosome. Endoplasmic reticulum is always associated with its outer (or convex) surface, and secretory vesicles and tubules are present in the inner (or concave) portion of the Golgi apparatus. × 35,000.
Entire germ cell Golgi apparatus can be visualized by negative contrast as illustrated in this figure. Tubules interconnect and overlay the flattened portions of the cisternae and, therefore, appear to be the most prominent feature of the Golgi apparatus. \( \times 22,000. \)

good quantity from only a few types of tissues. Little is known about the forces which maintain the close association of the stacks of cisternae or about the factors which tend to disrupt the apparatus. It is believed that digestive enzymes play a significant role in degrading the Golgi apparatus, but this cannot be the only factor involved. It is easy to demonstrate complete disruption of the...
Hydrolase activity represents a nonspecific hydrolysis of UDP-Gal measured without NAG present in the incubation medium as described in Materials and Methods.

Golgi apparatus in some tissues in times as short as 30 secs, and it is difficult to relate a digestive process to this kind of time scale. Thus, it is, that much of the success of Golgi apparatus isolation (as is also the case with mitochondria, endoplasmic reticulum, nuclear envelope, and plasma membrane) depends upon a fortuitous choice of operating parameters, including here choice of tissues as well as the technical procedures used. Some of the factors which we believe contribute to the success of the isolation procedure presented in this report are discussed below. These factors can be divided, more or less, into choice and/or availability of tissues, choice of homogenizer, choice of isolation media, and choice of gradient.

**Tissue Choice**

Rat testes are readily obtainable in the laboratory in a fresh state under controllable conditions. The soft seminiferous tubules are easily broken, allowing cell disruption by mild treatments that neither disrupt the Golgi apparatus mechanically nor release excessive hydrolytic enzymes which are able to digest the structures before they can be purified. The high ratio of Golgi apparatus membranes to other cytoplasmic organelles in these tissues also aids in subsequent fractionation. It seems now, however, that all testes may not be equally useful. Thus, preliminary attempts to scale-up this procedure by using testes from larger animals have not been successful. This is due possibly to the fact that these testes were septate and far more difficult to homogenize than rat testes.

In some procedures previously reported for the isolation of rat liver Golgi apparatus (18), the age, diet, and source of the rat was important to the success of the isolation. We have used both Sprague-Dawley and Holzman rats, fed or starved, with equal success in this technique. Large rats (400 g) yield more fresh weight of testis material but not proportionately more Golgi apparatus than do small (250 g) rats. The testes do not deteriorate rapidly after death, and excising the tissues rapidly is not critical to this procedure.

**Homogenization**

The type of homogenizer used to break the cells is extremely important in Golgi apparatus isolation. Early studies (25) showed that chopping of tissues with razor blades provides gentle cell breakage and is very effective (and far superior to most other methods of homogenization) in releasing intact Golgi apparatus. The method is equally applicable to plant or animal tissues, but it is somewhat tedious even with a motorized (5) chopper.

Some time ago it became apparent (17) that the Polytron had some characteristics which were well suited for making isolates of Golgi apparatus and that it was, in fact, almost as good as hand chopping in maintaining dictyosome form, and significantly faster in operation. Strictly speaking, the Polytron does not “cut” each cell since its blades are spaced nearly 0.008 inch apart, and this spacing is significantly greater than the cell diameter. It appears to work primarily by cutting large segments of tissues which then undergo such shear stresses that the cells are pulled apart. The production of sonic energy (for which the Polytron was designed) is not a factor at the slow speeds used in these applications.

A number of Polytron generators are now avail-

| Fraction          | Specific activity | % of recovery |
|-------------------|-------------------|---------------|
|                   | Total protein     | Hydrolyase activity | Transferase activity | Protein | Hydrolyase activity | Transferase activity |
|                   | mg                | umoles/mg protein per hr |                        |         | umoles/mg protein per hr |                        |
| Total homogenate  | 1040              | 24             | 12             | 100.0   | 100.0              | 100.0              |
| Golgi apparatus   | 4                 | 12             | 160            | 0.4     | 0.2                | 4.4                |

*Hydrolase activity represents a nonspecific hydrolysis of UDP-Gal measured without NAG present in the incubation medium as described in Materials and Methods.*
able and each is likely to give slightly different results. Therefore it is expedient to carefully establish and monitor the homogenization technique so that optimum conditions are obtained. This is accomplished by selecting conditions which ensure complete cell breakage, yet produce a minimum of damage to the Golgi apparatus. When cell breakage is not complete there will be an excessive number of cytoplasmic inclusions in the Golgi apparatus fraction. The inclusions appear to be portions of intact spermatids (without nuclei) or some form of residual body. They are probably derived from the inner cells of the tubules as a result of the very gentle homogenization stresses. They can be disrupted by increasing Polytron speed slightly and/or by decreasing molarity of the homogenization medium. Until the procedure is optimized, each preparation should be assayed visually by fixation and sectioning of the Golgi apparatus fraction. We have found that negative staining may give erroneous results and should not be used for these assays.

**Medium**

The medium in which the cells were disrupted was adapted from previous experiments with rat liver (22). This medium seems effective and no attempts were made to determine the efficacy or optimum concentration of its various components with the exception of mercaptoethanol. As shown previously (22), eliminating (or adding) this sulfhydryl reagent had no observable effect on Golgi apparatus morphology but it was definitely required for preservation of the galactosyltransferase activity.

**Gradient**

The first attempts to purify rat testis Golgi apparatus in this study employed discontinuous step sucrose gradients previously reported effective for rat liver (22). The size and complex reticular organization of the Golgi apparatus reduces the effectiveness of step gradients, however, since these organelles tend to accumulate at interfacial boundaries and then trap smaller organelles as they sediment past. The unusually high buoyant density of the germ cell Golgi apparatus makes it possible to separate them from mitochondria and other membrane fragments by flotation. This technique has several advantages. First, the organelles separate and move in opposite directions while in fairly dilute suspension rather than filtering together through a dense layer trapped at an interface. This means that more material can be handled per centrifuge tube since loading is less of a problem. Second, it is much easier to make the gradients since they consist of only two layers which have widely differing densities. Mixing of the layers poses little problem.

Sections of pellets, and negatively-stained preparations (see Figs. 2, 3, and 4), show that preservation of Golgi apparatus morphology by this technique is excellent. The entire secretory complex is present including vesicles, tubules, and even the cisternae of ER characteristically associated with the convex forming face of the Golgi apparatus. Intactness is an important consideration in any isolation procedure since disruption of the Golgi apparatus means a loss of intercisternal constituents as well as portions of forming and maturing cisternae. Collectively these losses could represent some 20–50% of the Golgi apparatus fraction. By morphological criteria, the purity of the Golgi apparatus is relatively high. Small numbers of other cellular components (particularly germ cell mitochondria) are present but they usually represent only a few per cent of the total material in the fraction. In one sense, the cisternae of ER which are present in this preparation are not contaminants since their close association with the forming face of the Golgi apparatus suggests that they are a part of the secretory complex.

Though the over-all size of testis Golgi apparatus differs significantly from that of liver and most plants, the subunits of all of these Golgi apparatus (i.e., the dictyosomes) are remarkably similar. Each consists of a stack of cisternae, most of which are composed of a central plate-like region and a peripheral region of attached tubules (1, 5, 8, 16). The differences in appearance between Golgi apparatus depend, then, on how many dictyosomes are contained and how closely they are inter-associated.

A complex Golgi apparatus, structurally similar to that of rat testis, has also been isolated with good purity from a mucopolysaccharide-secreting gland of snail (30). It is also remarkably similar in respect to the recovery of galactosyltransferase, the association with centrioles and ER, and its general appearance in negative stain.

The over-all isolation procedure is selective toward germ cell Golgi apparatus and, therefore,
the fractions consist almost entirely of one class of Golgi apparatus. Without this selection the procedure would not be particularly useful since the diverse number of cell types and stages of development in testes would yield a very heterogeneous mixture of Golgi apparatus, each with distinct and markedly different functions.

The enzymatic transfer of galactose from UDP-Gal to NAG with the formation of the corresponding disaccharide (N-acetylaminolactose) has been shown to be localized in Golgi apparatus membranes from rat liver (24, 32) and to be highly active in "Golgi-rich" fractions isolated from bovine liver and epididymis (10). The data obtained in this study suggest that this enzyme is also present in testis Golgi apparatus.

Spermatocyte and spermatid Golgi apparatus differ significantly from liver Golgi apparatus in that they are involved in a form of internal secretion; i.e., they participate in the formation of the acrosomal particle which is retained within the cell. Liver Golgi apparatus, in contrast, give rise to vesicles which migrate to the cell surface and fuse with the plasma membrane. In addition to differing with respect to the composition and fate of their products, these two types of Golgi apparatus also differ in structural organization. The germ cell Golgi apparatus is a large, highly ordered structure, whereas the liver Golgi apparatus is smaller and more widely distributed. The technique presented in this report makes possible a comparison of the biochemical properties of these two types of Golgi apparatus which it is hoped may reveal some of the details of the synthesis of secretory products and the mechanisms involved in membrane transformations and membrane flow mediated by the Golgi apparatus. It is also hoped that these preparations of Golgi apparatus may help solve some of the specific problems in acrosome formation, and may contribute to the understanding of sperm competence.

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BIBLIOGRAPHY

1. BEAMS, H. W., and R. G. KESSEL. 1968. The Golgi apparatus: Structure and function. Int. Rev. Cytol. 23:209.

2. BRÖKELMANN, J. 1963. Fine structure of germ cells and Sertoli cells during the cycle of the seminiferous epithelium of the rat. Z. Zellforsh. Mikrosk. Anat. 39:820.

3. BURGOS, M. H., and D. W. FAWCETT. 1955. Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids in the cat (Felis domestica). J. Biophys. Biochem. Cytol. 1:287.

4. CHEETHAM, R. D., D. J. MORRÉ, and W. N. YUNGHANS. 1970. Isolation of a Golgi apparatus-rich fraction from rat liver. II. Enzymatic characterization and comparison with other cell fractions. J. Cell Biol. 44:492.

5. CUNNINGHAM, W. P., and H. H. MOLLENHAUER. 1970. Isolation and purification of Golgi apparatus from rat testis. J. Cell Biol. 47:44 a.

6. CUNNINGHAM, W. P., D. J. MORRÉ, and H. H. MOLLENHAUER. 1966. Structure of isolated plant Golgi apparatus revealed by negative staining. J. Cell Biol. 33:105.

7. EWING, L. L., and L. M. SCHANBACKER. 1970. Early effects of experimental cryptorchidism on the activity of selected enzymes of rat testes. Endocrinology. 87:129.

8. FAVARD, P. 1970. The Golgi apparatus. In Handbook of Molecular Cytology. A. Lima-de-Faria, editor. North Holland Publishing Co., Amsterdam.

9. FAWCETT, D. W. 1958. The structure of the mammalian spermatozoon. Int. Rev. Cytol. 7:195.

10. FLEISCHER, B., S. FLEISCHER, and H. OZAWA. 1969. Isolation and characterization of Golgi membranes from bovine liver. J. Cell Biol. 43:59.

11. KEENAN, T. W., and D. J. MORRÉ. 1969. Phospholipid class and fatty acid composition of Golgi apparatus isolated from rat liver and comparison with other cell fractions. Biochemistry. 9:19.

12. KEENAN, T. W., D. J. MORRÉ, and R. D. CHEETHAM. 1970. Lactose synthesis by a Golgi apparatus fraction from rat mammary gland. Nature (London). 228:1105.

13. KUFF, E. L., and A. J. DALTON. 1959. Biochemical studies of isolated Golgi membranes. In Subcellular Particles. T. Hayashi, editor. The Ronald Press Company, New York. 114.

14. LEELOND, C. P., and Y. CLERMONT. 1952. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann. N. Y. Acad. Sci. 55:540.

15. MAHLEY, R. W., R. L. HAMILTON, and V. S. LEQUIRE. 1969. Characterization of lipoprotein particles isolated from the Golgi apparatus of rat liver. J. Lipid Res. 10:433.

16. MOLLENHAUER, H. H., and D. J. MORRÉ. 1966.
Golgi apparatus and plant secretion. Annu. Rev. Plant Physiol. 17:27.

17. Mollenhauer, H. H., D. J. Mørkæ, and L. Broman. 1967. Homology of form in plant and animal Golgi apparatus. Anat. Rec. 158:313.

18. Mollenhauer, H. H., D. J. Mørkæ, and C. Kogut. 1969. Dietary modification of the stability of rat liver Golgi apparatus. Exp. Mol. Pathol. 11:113.

19. Mollenhauer, H. H., W. G. Whaley, and J. H. Leech. 1960. Cell ultrastructure responses to mechanical injury. J. Ultrastruct. Res. 4:473.

20. Mollenhauer, H. H., and W. Zebrun. 1960. Permanganate fixation of the Golgi complex and other cytoplasmic structures of mammalian testes. J. Biophys. Biochem. Cytol. 8:761.

21. Mørkæ, D. J. 1969. In vivo incorporation of radioactive metabolites by Golgi apparatus and other cell fractions of onion stem. Plant Physiol. 45:791.

22. Mørkæ, D. J., R. L. Hamilton, H. H. Mollenhauer, W. R. Mailey, W. P. Cunningham, R. D. Cheetham, and V. S. LeQuere. 1970. Isolation of a Golgi apparatus-rich fraction from rat liver. I. Method and morphology. J. Cell Biol. 44:884.

23. Mørkæ, D. J., T. W. Keenan, and H. H. Mollenhauer. 1971. Golgi apparatus function in membrane transformations and product compartmentalization: Studies with cell fractions isolated from rat liver. Proceedings 1st International Symposium Cell Biology and Cytopharmacology, Venice, 1969. In press.

24. Mørkæ, D. J., L. M. Merlin, and T. W. Keenan. 1969. Localization of glycosyl transferase activities in a Golgi-rich fraction isolated from rat liver. Biochem. Biophys. Res. Commun. 37:813.

25. Mørkæ, D. J., and H. H. Mollenhauer. 1964. Isolation of the Golgi apparatus from plant cells. J. Cell Biol. 23:295.

26. Mørkæ, D. J., H. H. Mollenhauer, and J. E. Chambers. 1965. Glutaraldehyde stabilization as an aid to Golgi apparatus isolation. Exp. Cell Res. 38:572.

27. Mørkæ, D. J., S. Nystedt, and E. Rivera. 1970. Lecithin biosynthetic enzymes of onion stem and the distribution of phosphorylcholine-cytidyl transferase among cell fractions. Plant Physiol. 45:800.

28. Nath, V. 1956. Cytology of spermatogenesis. Int. Rev. Cytol. 5:395.

29. Nystedt, S. E., R. Barr, and D. J. Mørkæ. 1970. Ubiquinone from rat liver Golgi apparatus fractions. Biochim. Biophys. Acta. 208:532.

30. Outracht, L., D. J. Mørkæ, and L. M. Merlin. 1969. Isolation de l’appareil de Golgi d’une gland sécrétrice de mucopolysaccharides chez l’escargot (Helix pomatia). J. Microsc. 2:899.

31. Roosen-Runge, E. C. 1962. The process of spermatogenesis in mammals. Biol. Rev. (Cambridge). 37:343.

32. Schachter, H., I. Jabbal, R. L. Hudgin, L. Pinteric, E. J. McGuire, and S. Roseman. 1970. Intracellular localization of liver sugar nucleotide glycoprotein glycosyltransferases in a Golgi-rich fraction. J. Biol. Chem. 245:1090.

33. Schachter, H., I. Jabbal, and S. Roseman. 1969. Subcellular localization of sialyl and N-acetylglucosaminyl transferases in a smooth-surfaced membrane fraction of rat liver. Proc. Can. Fed. Biol. Sci. 12:77.

34. Schneider, W., and E. L. Kuff. 1954. On the isolation and some biochemical properties of the Golgi substance. Amer. J. Anat. 94:209.

35. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastuct. Res. 26:31.

36. Wagner, R. R., and M. A. Cynkin. 1969. Enzymatic transfer of 14C-glucosamine from UDP-N-acetyl-14C-glucosamine to endogenous acceptors in a Golgi apparatus-rich fraction from rat liver. Biochem. Biophys. Res. Commun. 35:139.