An Investigation of the Golgi Apparatus by Means of the Phase Contrast Microscope

SAUL WISCHNITZER, University of Notre Dame,[1] Notre Dame, Indiana

In the year 1898 Camillo Golgi (3) the distinguished Italian neurologist, identified an internal recticular apparatus in Purkinje cells of the cat and bar owl. Since that time more than 2,000 papers, including 28 review articles, have been published. Two classical methods, namely impregnation of fixed materials with metal salts and vital staining have been used extensively in studying this organoid.

While the impregnation methods are not specific, the results which they yield justify the conclusion that the blackened networks reveal the presence of some hitherto unrecognized material.

Of the well known vital stains, neutral red was the first to be used. This work led to the vacuolar and canalicular theories of the Golgi apparatus. Subsequent work has cast strong doubt on the validity of both concepts. It should be emphasized, that in view of the fact that slight surface tension changes may convert a canal into a row of droplets or produce the reverse effect, the vacuolar and canalicular theories may be based on a purely physic-chemical transformation of a system of canals.

The Golgi apparatus of fixed preparations is an artifact, in the sense that it conveys an impression which does not fully or accurately represent the state of affairs in the living cell. The main difficulty is that the Golgi apparatus cannot be seen when freshly isolated mammalian somatic cells are examined by means of the light microscope. The phase contrast microscope has been used in the hope of overcoming this difficulty. Investigations of the Golgi apparatus by this means has been very limited and inconclusive.

Materials and Methods

Columnar intestinal epithelial cells, pancreas acini, liver cells, columnar epididymal cells and dorsal root ganglion neurons from albino mice, were examined by means of a Spencer phase contrast microscope, using bright high contrast and medium contrast oil immersion objectives. The slides were prepared from minced tissue fragments and were kept in oxygenated Tyrode's solution.

Results

Examination within 3 to 5 minutes after isolation, failed to reveal the presence of a classical Golgi net in the columnar intestinal epithelial cells. Numerous granular mitochondria are seen in the basal portion of the cell which also contains the oval nucleus. After a short time one can observe spheres in the Golgi zone. After two hours of standing these spheres coalesce into canaliculi. These spheres as can be expected, would

1. The author would like to express his deep appreciation to Prof. Edward O. Dodson for his helpful guidance in this work.

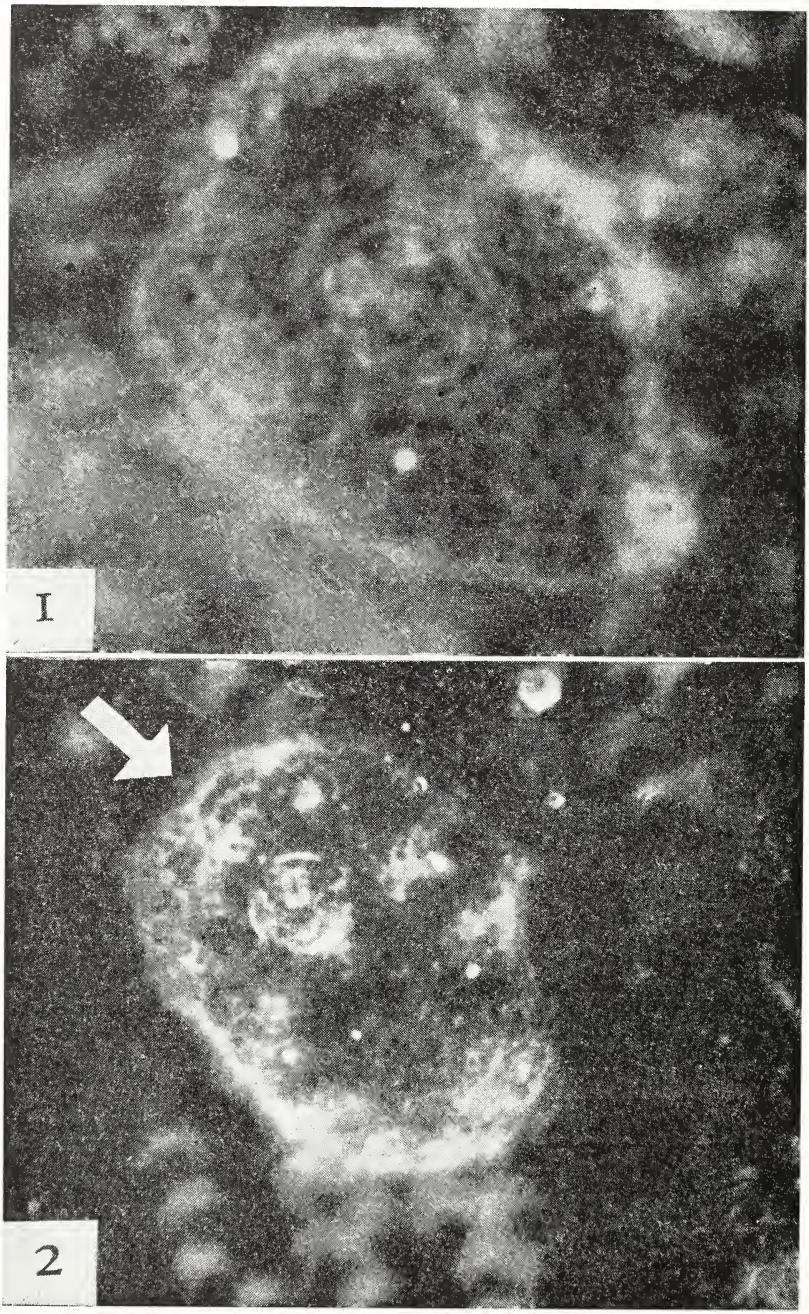

Fig. 1. A phase contrast photomicrograph of a liver cell approximately five minutes after isolation. A classical Golgi image is absent. 1200 X.

Fig. 2. Same cell two hours after isolation. A canaliculus or negative image of the Golgi apparatus (arrow) has developed. 970 X.

offer a surface for the precipitation of metal salts such as Silver or osmic acid used in impregnation. This appears to be exactly what has happened as shown in Dalton's (1) electron micrograph of some columnar intestinal cells.

The cytoplasm of liver cells (Fig. 1) reveals numerous small and large lipid droplets which are highly refractile. These lipid droplets are light or dark depending on their position with reference to the focal plane. A Golgi net is again absent. After two hours of standing (Fig. 2), the presence of a complex juxta-nuclear canalicular appartus is clearly evident. Were it not known that this network had arisen as a direct result of post-mortem intracellular changes, then one would have unhesitatingly called it a negative Golgi image.

Due to the presence of a strong connective tissue reticulum investing pancreas acini, it was extremely difficult to isolate individual pancreas cells. The apical region of these cells contains numerous zymogen granules. It is interesting to note that the nuclei, which are in the basal zone, become visible only after 5 to 10 minutes. A Golgi network is absent from the subnuclear region, where it is normally produced by the classical impregnation methods.

The upper two-thirds of the columnar epididymal cells were found to be filled with numerous large and refractile lipid droplets, pigment granules and thin, dark, filamentous mitochondria. Golgi images were not seen. Dalton and Felix (2) reported the presence of a classical network in fresh material observed by phase microscopy. While one of their photographs, Figure 6, reveals the presence of a Golgi-like net, it is lacking in the filamentous mitochondria which even they admit are present. It is conceivable that the effect of the 4% NaCl solution, which they have subsequently changed, produces aggregation of the mitochondria into such reticular images. That distortion takes place is evident by the appearance of the outer surface of the columnar cell. In any case it should be strongly emphasized, that even if the report of the presence of a Golgi network is verified, this does not necessarily imply its existence in other types of cells.

As for the dorsal root ganglion neurons, clear refractile areas were noted to develop in the otherwise homogenous cytoplasm on standing.

Discussion

An objective examination and analysis of the voluminous literature on this subject will lead one to the conclusion that there exists within a specific region of the cell a substance of lipoidal nature, which exhibits polymorphism when treated with the classical methods. The morphological investigations carried out by means of the phase contrast microscope, as reported in this paper, reveals that the Golgi apparatus as classically considered is probably an artifact. This is to be expected since undoubtedly by means of the classical methods gross distortions are produced due to the forces that are set into action against the lipids of the cell.

Results obtained by the new observational technique are very significant since numerous supporters of the in vivo existence of the Golgi

apparatus explain its invisibility in living untreated cells as being due to the fact that it has the same refractive index as the rest of the cytoplasm. If this were so, observation by means of the phase contrast microscope should make the slight difference between the two substances sufficiently pronounced so that the organoid would become visible. This however is not the case.

Further evidence as to the ease with which the cell is distorted has been brought out as a result of the observation of the effects of the surrounding medium on the tissues studied. These effects consisted of sphere formation and post mortem coalescence of inclusions, formation of clear refractile areas and of canaliculi. Cell shrinkage with the resultant distortion of the intracellular contents contributed to the artifact formation.

A logical explanation of the entire question was originally presented by Walker and Allen as far back as 1927 (7). Based on a series of very convincing experiments they concluded that the Golgi apparatus in impregnated preparations are probably artifacts produced by the methods used to demonstrate them. They also showed the possibility of artificially reproducing the Golgi apparatus, and strongly indicated that probably phospholipids are the materials that form the organoid. Unfortunately this excellent paper received very little attention after its publication. Their conclusions were in the main substantiated by the work of Palade and Claude in 1949 (4).

By realizing that phospholipids are found in varying concentrations in almost all tissues, and in particularly high concentrations in nervous and germinal tissues, the differences in the form and thickness of the network when revealed by the classical methods become readily understandable. The Golgi material has been observed in forms (Fig. 3.) ranging from globular to spherical, rod-like to tubular, from a solid network to a canalicular recticulum, as well as in numerous intermediate forms. The suggestion is therefore made that the Golgi substance is in reality made up of a fine dispersion of phospholipid microscopic or submicroscopic particles located in the juxta-nuclear zone. The morphological variations that are exhibited by this organoid would therefore be determined by the concentration of the "phospholipid dispersion" and the degree to which distortion takes place. Palade and Claude (4) have found that even changes in pH are effective in the production of various different Golgi figures. The phospholipid nature of the dispersion has been confirmed by biochemical analysis as reported by Schneider and Kuff, (5). And recent electron microscopic work by Sjöstrend and Hanzon (6) has shown the presence of submicroscopic lipid particles and lamellae in the Golgi zone.

Conclusions

1. A review of the voluminous literature on this subject indicates that an unknown material is present in a localized area near the nucleus of the cell. This material has a lipoidal nature and when treated with the classical techniques exhibits a polymorphism.

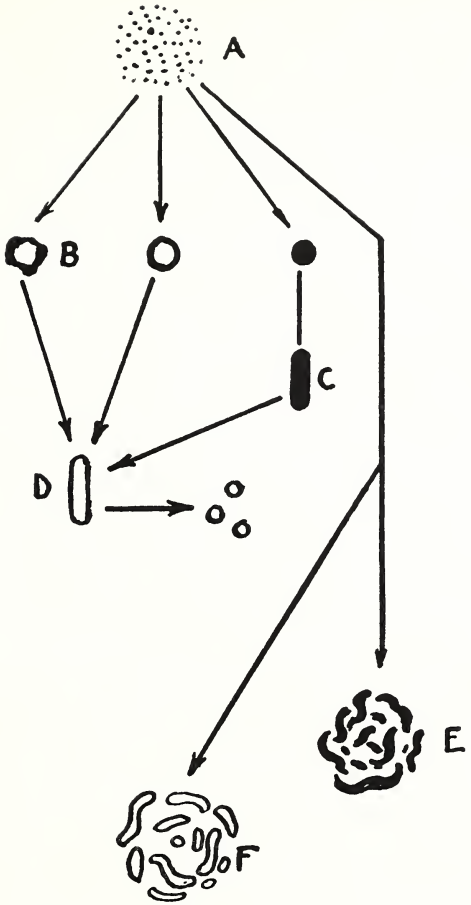

Fig. 3. The various proposed forms for the Golgi apparatus and their interrelationships.

A. Golgi substance made up of submicroscopic and microscopic lipoid particles.
B. Lipochondria of Baker.
C. Golgi material of Gresson.
D. Golgi apparatus of Gatenby.
E. Canaliculus or Trophospongium of Holmgren.
F. Classical Golgi apparatus.

2. Nothing resembling the classical 'Golgi apparatus' or 'Golgi material' is visible on first observation of tissues isolated from living albino mice.

3. With increasing time, post-mortem coalescence of inclusions in the Golgi zone was observed in columnar intestinal cells and distinct refractile areas appear in the otherwise homogeneous cytoplasm of dorsal root ganglion neurons. Canaliculi and network images develop in liver cells on standing.

4. It is suggested that the classical osmiophilic and argentophilic Golgi nets are fixed and stained products of changes associated with the death of the cell.

Literature Cited

1. DALTON, A. J., and MARY D. FELIX. 1953. Studies on the Golgi substance of the epithelial cells of the epididymis and the duodenum of the mouse. Amer. J. Anat. **92**:277-305.
2. DALTON, A. J. and MARY D. FELIX. 1954. Cytologic and cytochemical characteristics of the Golgi substance of the epithelial cells of the epididymis in situ, in homogenates and after isolation. Amer. J. Anat. **94**:171-207.
3. GOLGI, CAMILLO. 1898. Sur la structure des cellules nerveuses. Arch. Ital. Biol. **30**:60-71.
4. PALADE, G. E., and A CLAUDE. 1949. The Nature of the Golgi apparatus. I and II J. Morph. **85**:35-111.
5. SCHNEIDER, W. C., and E. L. KUFF. 1954. On the isolation and some biochemical properties of the Golgi substance. Amer. J. Anat. **94**:209-224.
6. SJOSTRAND, F. S., and V. HANZON. 1954. Ultrastructure of the Golgi apparatus of exocrine cells of mouse pancreas. Exper. Cell Research. **7**:415-425.
7. WALKER, C. E., and M. ALLEN. 1927. On the nature of the 'Golgi' bodies in fixed material. Proc. Roy. Soc. London. **101B**:468-483.