GOLGI APPARATUS AND LYOSOMAL GRANULES OBSERVED IN SOME CULTURED CELLS

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A bulk of information is available suggesting a universal presence of Golgi apparatus as an organelle, not only in animal cells but also in plant cells (Manton 1960; Parke et al. 1959; Moore and McAlear 1963; Morré et al. 1965; Maruyama 1966; Mollenhauer and Morré 1966). The presence of hydrolytic enzyme or enzymes dephosphorylating several nucleoside diphosphates as well as thiamine pyrophosphate in Golgi apparatus, which was first demonstrated by Allen and Slater (1961) and Novikoff and Goldfischer (1961), offered an intriguing physiological significance to this organelle. Recently, the functional relationship between the Golgi apparatus and the lysosomes, or cytoplasmic granules, has increasingly been evaluated by virtue of electron microscopy (Bertolini and Hassan 1967; Essner and Novikoff 1962; Jamieson and Palade 1964; Sobel and Avrin 1965; Bainton and Farquhar 1966) or by radioautographic techniques (Welling 1963; Caro and Palade 1964; Fedorko and Hirsch 1966; Jamieson and Palade 1966). However, morphological as well as cytochemical knowledge on the Golgi apparatus and the lysosomes has remained meagre in cells cultured in vitro.

The present study deals with a cytochemical investigation of the Golgi apparatus and lysosomal granules in a variety of cells in culture.

MATERIALS AND METHODS

The following types of cells were employed for the present study: 1) fibroblasts isolated from the skin of an adult human female and cultivated for 7 months in our laboratory, 2) strain HeLa cells, 3) mouse whole embryonic cells from the second passage of culture, 4) rat fibroblasts clonally isolated from JTC-4 strain which was established from the heart tissue of suckling rats (Takaki and Sugi 1960), and 5) monkey kidney cells from a primary culture. The culture medium for HeLa and mouse embryonic fibroblasts consisted of Earle's BSS, 0.5% lactalbumin hydrolysate, and 10% bovine serum. Human skin cells were cultured in a medium consisting of 5 parts of Hank's BSS, 3 parts of TC medium 1066, and 2 parts of bovine serum. Rat fibroblasts were cultured with TC medium 199 added with 10% calf serum. Monkey kidney cells were seeded in a medium consisting of Earle's BSS, 0.5% lactalbumin hydrolysate, and 5% bovine serum. The above media contained in each penicillin-G (200 units/ml) and phenol red (0.002%). In all cases, cells were grown on coverslips in Leighton-type tubes for 2 or 3 days after subculture.
The Golgi apparatus was demonstrated as the site of reaction product of thiamine pyrophosphatase (TPPase) activity visualized by the method of Gomori (1939) for acid phosphatase (ACPase) following the incubation in the medium of Allen (1963). The suitable incubation time was 90 minutes at 37°C. ACPase activity for demonstrating lysosomal granules was tested by the technique of Gomori (1941). Prior to these enzymic reaction procedures, cells grown on coverslips were subjected to the freezing substitution technique (Takayama and Ojima 1966). Cells were stained with Giemsa and Heidenhain's iron hematoxylin. Oil droplets were stained with Sudan black B after exposing cells to formalin vapor. Lysosome localization was also confirmed by vital staining with neutral red, and acridine orange. Their specificity has been documented by Koenig (1963), Robbins et al. (1964) and Allison and Paton (1965). Observations of living cells were made with Carl-Zeiss phase contrast optics.

**OBSERVATIONS**

**Human fibroblasts:** Reaction product of TPPase activity occurred in a juxtanuclear position as a bundle of tangled filaments (Fig. 1). This type of Golgi apparatus was found in cells of elongated and spindle shape. The other type of the reaction precipitate image was represented by an irregularly network surrounding the most part of the nucleus (Fig. 2). This was common in flattened cells. In living cells, a bundle consisting of filamentous structure quite similar in appearance to that of the TPPase reaction product as shown in Figure 1, was observed in the vicinity of the nucleus. This seems to appear a living state of the Golgi apparatus. Giemsa staining and hematoxylin staining also revealed a network feature at the Golgi zone (Figs. 3 and 4). Surrounding the nucleus and the Golgi apparatus, there were a number of globules, being $0.2 \mu$ to $10 \mu$ in diameter, mostly $1.5 \mu$; they showed a lower refractability in comparison with oil droplets. Their localization was mostly confined to the central part of cells (Fig. 5). ACPase test showed the occurrence of the reaction product on the globules. The Golgi zone showed a weak, if any, activity for ACPase (Fig. 6). In cells stained with acridine orange globules with flaming red or orange fluorescence were shown under ultraviolet light (Fig. 7). The globules were also easily stained with neutral red. In relatively flattened cells, a small number of stained globules were visible within the Golgi zone (Fig. 8). Evidence presented suggests that the globules are lysosomal in nature.

**HeLa cells:** TPPase activity was shown close to or somewhat apart from one side of the nucleus as a cluster of black filaments or masses, irregular in size and shape (Fig. 9). As shown in Figure 12, ACPase reaction product was observed as fine granules scattered throughout the cytoplasm. In many cells those granules appeared to some extent concentrated in the juxtanuclear position. A similar distribution pattern of granules was observed in fluorescent picture following acridine orange staining (Fig. 10) as well as in neutral red staining (Fig. 11). Oil droplets were much larger than those lysosomal granules and stained with Sudan black B only (Fig. 14). Giemsa staining revealed mitochondria scattering over the cytoplasm and the Golgi apparatus which located surrounding the nucleus. The apparatus could be distinguished from mitochondria.
Figs. 1-8. Human fibroblasts. 1 and 2; TPPase activity. Reaction product occurs in a form of a bundle or network structures on or near the nucleus. ×800. 3; Giemsa staining showing a network structure near the nucleus. ×800. 4; Heidenhain's iron hematoxylin staining showing a network structure near the nucleus. ×800. 5; Phase contrast. A bundle consisting of filamentous structures and the numerous globules are observed surrounding the nucleus. ×800. 6; ACPase activity. Reaction product is confined to the globules. ×320. 7; Globules surrounding the nucleus are stained vitally with diluted neutral red solution. ×800. 8; Acridine orange staining. Globules surrounding the nucleus show prominent fluorescence. ×320.
Figs. 9-14. HeLa cells. 9; TPPase reaction products locate at one side of the nuclei. ×800. 10; Acridine orange staining. Granules show reddish fluorescence. ×410. 11; Neutral red staining. ×800. 12; ACPase activity. Stained granules locate in a juxtanuclear position. ×800. 13; Golgi apparatus and mitochondria stained with Giemsa staining. ×800. 14; Oil droplets stained with Sudan black B. ×800.

Figs. 15-18. Mouse embryonic cells. 15, 16 and 17; TPPase reaction product occurs in various configurations. ×800. 18; ACPase activity. ×800.

Figs. 19-20. Rat fibroblasts. 19; Acridine orange staining. Granules show prominent reddish orange fluorescence. ×410. 20; ACPase reaction product showing filamentous configurations. ×800.

Fig. 21. Monkey kidney cells. TPPase activity is observed in the form of irregular masses or thick filaments. ×800.
by its equivocal image (Fig. 13).

**Mouse whole embryonic cells:** In these cells TPPase reaction product demonstrated various types of Golgi figures; filamentous structure lying on one side of the nucleus (Fig. 15), network or multipolar features (Fig. 16), or irregular masses locating at a part of the nucleus (Fig. 17). Lysosomal granules demonstrated with ACPase reaction was not very prominent (Fig. 18). Such a variety of the Golgi figures seemed to suggest that the cell culture from whole embryos consists of various types of cells.

**Rat fibroblasts:** This type of cells showed no TPPase activity even after the repeated and prolonged incubation. With this respect the rat fibroblast cells appear to be exceptional and quite unique. In contrast, lysosomal granules of the cells were quite prominent, being larger in number and size than those of HeLa cells (Fig. 19). Occasionally ACPase reaction product appeared as filamentous structure occurring adjacent to a part of the nucleus (Fig. 20).

**Monkey kidney cells:** TPPase reaction product was detected in a form of irregular masses or thick filaments. The intensity of the reaction was relatively weak, but as a whole the reaction positive elements were larger in number in comparison with those of the other types of cultured cells. A relatively expanded distribution of the reaction product was also characteristic to this type of cells (Fig. 21).

No test was performed for studying lysosomes, because of an accidental loss of the culture.

**DISCUSSION**

Following Novikoff and Goldfischer (1961) and Allen and Slater (1961), TPPase activity in the Golgi apparatus has been demonstrated by a considerable number of authors. The situation in cultured cells, however, is still left unelucidated. Robbins and Gonatas (1964a, b) reported in HeLa cells that the Golgi apparatus visualized as the precipitation of a TPPase reaction product which occurred in a juxtanuclear region, showed an intricately interwoven complex. Masuda (1966) observed TPPase activity on the Golgi apparatus in cells cultured from meninges encephali of chick embryos. Takayama and Ojima (1966) subjected strain L cells to a test for TPPase activity following a freezing substitution procedure. It was reported that most cells showed the Golgi apparatus in a juxtanuclear position of the cell as a cluster of black filaments or small irregularly shaped bodies. In other cells with larger nuclei the organelles were prominent in nature and appeared covering the nuclear surface, or scattering throughout the cytoplasm.

The present study revealed that four different types of cultured cells, human fibroblasts, HeLa cells, mouse embryonic cells, and monkey kidney cells, showed TPPase reaction products in a configuration characteristic to each cell type. In contrast, rat fibroblasts failed to provide any TPPase reaction product even after repeated and prolonged incubation. The negativity of TPPase activity in this type of cells, however, does not necessarily indicate the absence of the Golgi apparatus. Since the physiological significance for the presence of TPPase activity in the Golgi apparatus is to date still unknown, the problem is left for a subject of future investigation.
Novikoff et al. (1962) recognized the presence of the intimate topographical relation between lysosomes and the Golgi apparatus in the studies of over sixty normal tissues and several experimentally altered tissues. The functional relationship between the Golgi apparatus and lysosomes in hepatomas was also extensively studied by Essner and Novikoff (1962). They suggested that secretory products accumulated within the Golgi cisternae, separating as lysosomes. Robbins and Gonatas (1964b) reported, working on HeLa cells treated with spindle inhibitors, that the Golgi apparatus fragmented and took up a circumferential distribution in a pattern similar to that of the lysosomes. Bainton and Farquhar (1966) showed in normal rabbit polymorphonuclear leukocytes electron micrographs demonstrating the existence of two distinct granule types, lysosomal in nature, and their separate origins from the Golgi complex.

Some autoradiographic studies have been exploring a possible functional significance of the Golgi apparatus in relation to the formation of lysosomes or cytoplasmic granules. Caro and Palade (1964) and Jamieson and Palade (1966) concluded on the bases of their experiments with pancreatic exocrine cells, applying leucine-H\(^4\) to the cultures, that the zymogen granules were formed in the Golgi region. Fedorko and Hirsch (1966) observed a transitional shift of the label of tritiated lysine from the Golgi apparatus to cytoplasmic granules. In addition to the above studies there is a good deal of information which suggests the presence of certain functional relationship between the Golgi apparatus and lysosomes (Dougherty 1964; Jamieson and Palade 1964; Shanthaveerappa and Bourne 1965; Holtzman and Novikoff 1966; Sutton and Weiss 1966).

All cells tested in the present study showed granules or globules positive to ACPase test, neutral red, and acridine orange staining. Evidence presented here seems to be an indication that they are lysosomes, notwithstanding that the definition of the term “lysosome” is to date still uncertain. The fact that rat fibroblasts showed lysosomes but not TPPase activity, may at least imply that TPPase activity does not directly participate in the formation of lysosomes in those cells. Though some information regarding certain topographical relations between the Golgi apparatus and the lysosomes was obtained in the present study, it is premature at the present status to deal with their functional relationship.

**SUMMARY**

The Golgi apparatus and the lysosomes were demonstrated in human fibroblasts, HeLa cells, mouse embryonic cells, rat fibroblasts, and monkey kidney cells, which were all grown in culture. The Golgi elements were generally detected as the precipitation of a reaction product from thiamine pyrophosphatase (TPPase) activity and the lysosomes were revealed as the granules positive to acid phosphatase (ACPase) activity. (The enzymic activity tests were made on cells seeded on coverslips and fixed by means of a freezing substitution technique.) ACPase activity positive granules showed a strong affinity to neutral red, and appeared with reddish orange fluorescence after acridine orange staining. Rat fibroblasts were exceptional by showing the ACPase activity only. It was shown that ACPase and TPPase activities were specific to each cell organelle, and the topographical relationships suggested the occurrence of certain
physiological significance between these organelles.

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