

STUDIES ON THE GOLGI APPARATUS BY ELECTRON
MICROSCOPY WITH PARTICULAR REFERENCE
TO AOYAMA'S TECHNIQUE

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PLATES 97 AND 98

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Observations made with the aid of the light microscope have led some workers to believe that the classical osmium and silver impregnation techniques for the Golgi apparatus do not reveal a single homologous inclusion but a variety of dissimilar objects. It has been variously suggested that the osmium or silver deposits on neutral-red or lipoidal bodies (41-44, 31, 26, 2-7, 53), mitochondria (51), myelin figures (37, 38), or chemically modified cytoplasm lying between crowded secretion granules (4, 5, 7). As a result of this Baker (4, 7) has urged that the term "Golgi apparatus" be completely abandoned. However, other workers who have used only the light microscope have concluded that the classical methods do reveal an homologous inclusion and that there is every justification for referring to it by the name Golgi apparatus (12, 8, 9, 18-24, 32, 27-30). According to several of these workers the Golgi apparatus in vertebrate somatic cells is a canal-like or vacuolar structure with chromophilic walls and a chromophobic lumen (22, 23, 33, 28).

During recent years an inclusion has been observed in cells studied by electron microscopy, which consists of parallel lamellae, vacuoles, and small granules (16, 17, 48, 50, 40, 13). Some workers have identified this inclusion as the Golgi apparatus (16, 17, 48, 50, 13) while others have not felt justified in coming to this conclusion (40). Bearing this in mind, and the fact that one of the classical osmium impregnation techniques has already been studied in vertebrate cells by electron microscopy (15), it has been thought worth while to see exactly where the silver is deposited when one of the classical silver techniques is used.

Method

The pancreatic tissue was obtained from five female mice, of average weight 24 gm. and aged 2 to 3 months. Each mouse was killed by a sharp blow on the head and small pieces of tissues were (a) fixed in 1 per cent buffered osmium tetroxide solution (34) for 4 hours, rinsed in distilled water, dehydrated, and embedded in a 1:3 mixture of *n*-methyl and *n*-butyl

methacrylate and (b) treated according to Aoyama's technique for the Golgi apparatus. In the latter case the tissue was placed rapidly into Aoyama's fixative (1) and left for 5 hours, rinsed quickly with two changes of distilled water, and then left in 1.5 per cent silver nitrate solution for 18.5 hours in the dark. Next the silver nitrate was decanted, the tissue rinsed quickly with distilled water as before, and then placed into Aoyama's reducer for 6 hours. The tissue was then washed for 1 hour, dehydrated, and subsequently embedded in a 1:3 mixture of the plastic monomers.

The material was sectioned on a modified Cambridge rocking microtome (14). The sections were mounted on copper grids coated with formvar films and examined by means of a Philips E.M. 100 type electron microscope and a Siemens type electron microscope.

OBSERVATIONS AND INTERPRETATIONS

Aoyama's Method

General Cell Structure.—Fixation appeared generally good although in some cells there were signs of shrinkage. The cytoplasm consisted of alternate arrays of material of high and low scattering density with the zymogen granules and mitochondria confined to the former region. In view of this it appears that the region of low scattering density corresponds to the endoplasmic reticulum or ergastoplasm described by several workers in material fixed usually in buffered osmium tetroxide solution (45-47, 34, 39, 33, 11, 52). The region of high scattering density is interpreted as hyaloplasm or cytoplasmic matrix. Neither cytoplasmic double membranes (49, 46, 11) nor particles (36, 46, 11, 49) were observed in these preparations.

Various gradations were observed between nuclei that were well fixed and those that were badly fixed. Typically the well fixed nuclei showed little evidence of shrinkage and retained their oval or spherical form whereas the badly fixed nuclei were shrunken and distorted.

The zymogen granules were well fixed and consisted of a dense outer pellicle enclosing a less dense homogeneous substance. Near the zymogen granules were other inclusions consisting of a thin rim of a dense material surrounding a denser core. These inclusions are interpreted as lipoidal bodies (prozymogen bodies of Bensley (10)) which, in light microscopical preparations, consist of a lipoidal sheath enveloping a developing zymogen granule (3, 27, 30). The mitochondria were seen as filamentous or elliptical bodies displaying some scattering density. They lacked the fine membrane structure described by Sjöstrand (49) and Palade (35) in material fixed in buffered osmium tetroxide solution.

Distribution of the Silver and the Location of the Golgi Apparatus

The silver within the cells was confined almost entirely to two main regions: (1) within the dense cytoplasmic material (hyaloplasm or matrix) and (2) along one or both sides of a series of closely apposed vacuoles lying within the Golgi zone; *i.e.*, lying distal to the nucleus and in close proximity to the zymogen granules (Fig. 1). The silver within the dense cytoplasmic material was in the

form of many fine submicroscopic grains. The silver outlining the vacuoles was in the form of an opaque mass lying well within the limits of resolution of the light microscope. Bearing this in mind, and the position of the silver deposits within the cell, it seemed reasonable to identify the large deposit of silver with the chromophilic region of the Golgi apparatus and the vacuoles with the chromophobic part. Silver was rarely observed elsewhere in the cell except in the form of occasional submicroscopic grains on the nucleus and mitochondria.

The Golgi Apparatus of Cells Fixed in Buffered Osmium Tetroxide Solution

A structure similar to that revealed in the present work by Aoyama's method has been described recently by Sjöstrand and Hanzon (50) in exocrine cells of the mouse pancreas fixed in buffered osmium tetroxide solution. They identified this structure as the Golgi apparatus without the use of classical techniques but on the basis of its characteristic position within the Golgi zone of the cells. The present work supports their identification.

According to Sjöstrand and Hanzon the Golgi apparatus consists of pairs of Golgi membranes, which often contain a dense substance, intercalated Golgi vacuoles, Golgi granules, and a Golgi ground substance. In the present study similar results were obtained although a distinct ground substance was not observed. It was also observed that: (1) Some of the pairs of Golgi membranes gradually diverge from one another as they approach the vacuolar substance while the dense material they enclose becomes correspondingly less dense (Fig. 2). (2) The Golgi vacuoles correspond to terminal dilations of the paired Golgi membranes (Fig. 2). (3) Often more pairs of membranes are found on one side of the dilations than on the other (Fig. 2). (4) Osmiophilic strands, possibly representing membranes, are sometimes seen and link each member of a pair of Golgi membranes (Fig. 2).

DISCUSSION

In the present work similar results have been obtained by the use of Aoyama's silver method to those obtained by Dalton (15) by the use of classical osmium techniques. The results are similar also to those obtained by several cytologists who have studied vertebrate somatic cells, treated by classical methods, and have examined them only with the light microscope (22, 23, 33, 28).

It would appear that when Aoyama's method is used the silver impregnates mainly the borders of a series of closely apposed vacuoles and reveals a distinct cytoplasmic inclusion, corresponding to the Golgi apparatus. Moreover, except in rare cases, the only deposit of silver that lies within the limits of resolution of the light microscope is that found on the Golgi apparatus. The fine granules of silver which occur within the cytoplasm are of submicroscopic dimensions although as a whole they probably account for the yellow background seen in light microscopic preparations. Silver-impregnated myelin figures are not ob-

served to be formed with Aoyama's technique. Finally, the mitochondria and lipoidal bodies (prozymogen bodies of Bensley (10)) are either not impregnated or at most contain only a few submicroscopic grains of silver. It may be emphasised that these results were obtained by a study of Aoyama's method without recourse to the technical improvements of postchroming, gold toning, or differentiation.

Comparison between the appearance of the Golgi apparatus in exocrine cells as revealed by Aoyama's technique and after fixation in buffered osmium tetroxide solution leaves little doubt that the silver impregnates the paired Golgi membranes outlining the vacuoles (*i.e.* dilations of the paired membranes). The fact that sometimes more silver is deposited on one side of the vacuoles than on the other corresponds with the observation that the paired membranes are sometimes unequally distributed on either side of the vacuoles (compare, Fig. 1 with Fig. 2). Neither the Golgi membranes nor small Golgi vesicles are retained in Aoyama preparations (Fig. 1). It must be pointed out that the Golgi apparatus was observed more consistently in tissue fixed in osmium tetroxide solution than in tissue treated by Aoyama's method. This may be due to the general accepted difficulties in the application of these silver impregnation techniques.

An inclusion similar to that revealed by classical osmium or silver impregnation techniques has been observed in the Golgi zone of many types of cells fixed in buffered osmium tetroxide solution (16, 17, 48, 50, 13). In all cases this inclusion has been identified as the Golgi apparatus and been found to consist of paired membranes, usually in association with vacuoles and numerous small vesicles. Recently Palay and Palade (40) have observed an inclusion with a similar fine structure in neurones, the type of cell in which the Golgi apparatus was first described nearly sixty years ago (25). Palay and Palade state that the pairs of "agranular" membranes they observed probably corresponded to the membranes (Golgi membranes) described by Dalton and Felix (16) but are cautious about identifying the inclusion as the Golgi apparatus. There can, however, be little doubt that with its characteristic fine structure this inclusion is the Golgi apparatus.

It is now apparent, from the pioneer studies of Dalton and from more recent work that the evidence from electron microscopy supports the view (see introduction) that in a wide variety of cells the classical methods reveal an homologous inclusion. It seems unreasonable, therefore, to contend that the name of Golgi should not be associated with this inclusion.

SUMMARY

1. Aoyama's silver impregnation method for the Golgi apparatus has been used on exocrine cells of the pancreas of the mouse and studied by electron microscopy in order to determine as precisely as possible where the silver is

deposited. Similar cells have also been fixed in buffered osmium tetroxide solution and compared with cells treated by the silver technique.

2. Examination of the Aoyama preparations usually revealed a light deposition of silver in the cytoplasm (hyaloplasm or matrix) and a heavy deposition of silver around a series of closely apposed vacuoles. The heavy deposition of silver was regarded as revealing the chromophilic region of the Golgi apparatus while the vacuoles were identified as the chromophobic component.

3. Comparison of the silver preparations with those fixed in buffered osmium tetroxide solution showed that the silver was primarily deposited in the region of the Golgi membranes.

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EXPLANATION OF PLATES

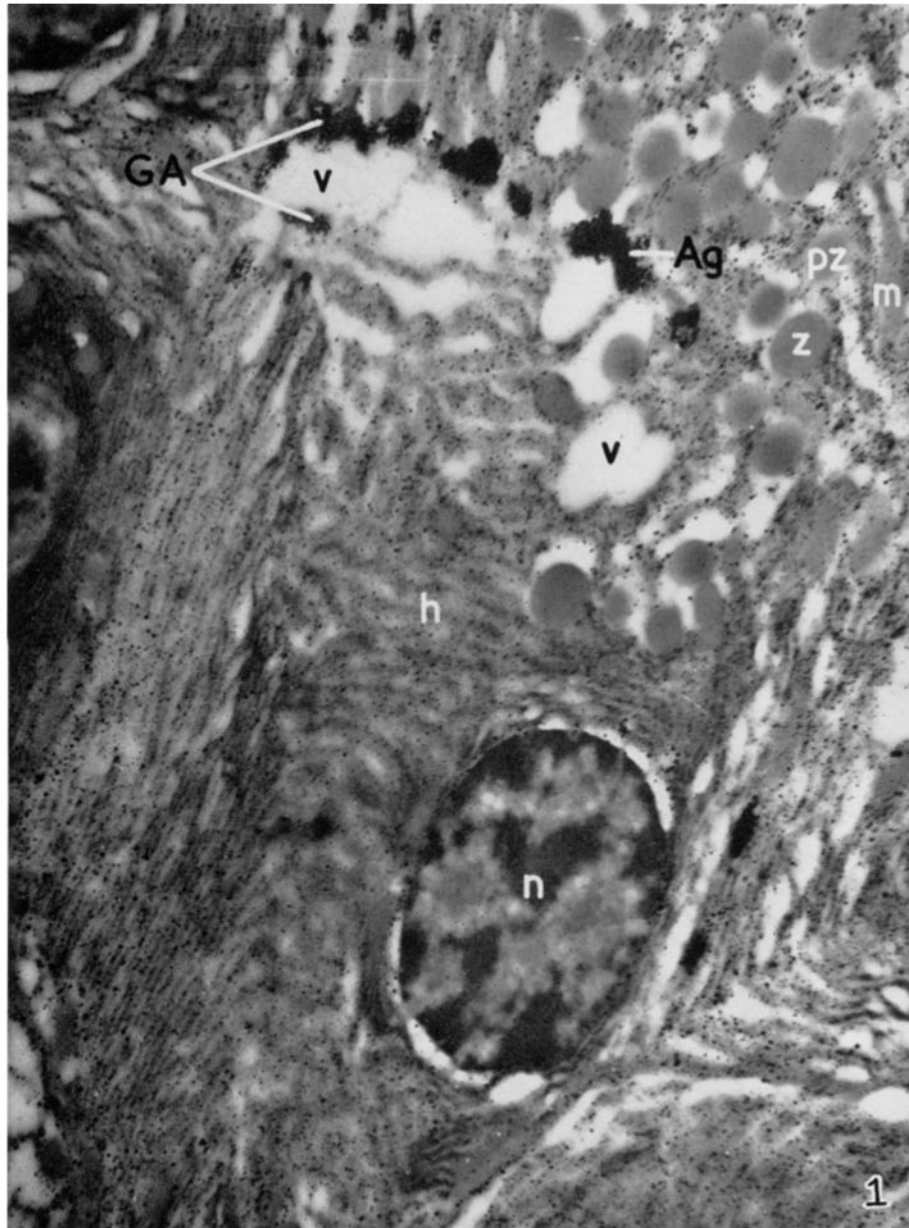
PLATE 97

FIG. 1. A micrograph of an Aoyama preparation.

In the Golgi zone of this cell is a large deposit of silver (*Ag*) lying within the limits of resolution of the light microscope, which is regarded as revealing the chromophilic region of the Golgi apparatus (*GA*). This region outlines a series of closely apposed vacuoles (*v*) which represent the chromophobic component of the Golgi apparatus.

A fine deposit of silver, lying well below the limits of resolution of the light microscope, can be seen in the main body of the cell. This fine deposit actually occurs on alternate strands of a dense material (*h*) in which various cell inclusions are embedded. These strands represent, therefore, the hyaloplasm or cytoplasmic matrix which lies between the lamellae of the ergastoplasm or endoplasmic reticulum.

Near the Golgi apparatus are spheroidal inclusions of two kinds: (1) those which are almost homogeneous in appearance but with a slightly dense pellicle and (2) those with a dense core surrounded by a much less dense cortex. The former are identified as zymogen granules (*z*) while the latter appear to correspond with the prozymogen bodies (*pz*) of Bensley. *n*, nucleus; *m*, mitochondrion. $\times 15,000$.



(Lacy and Challice: Aoyama's technique in studies on Golgi apparatus)

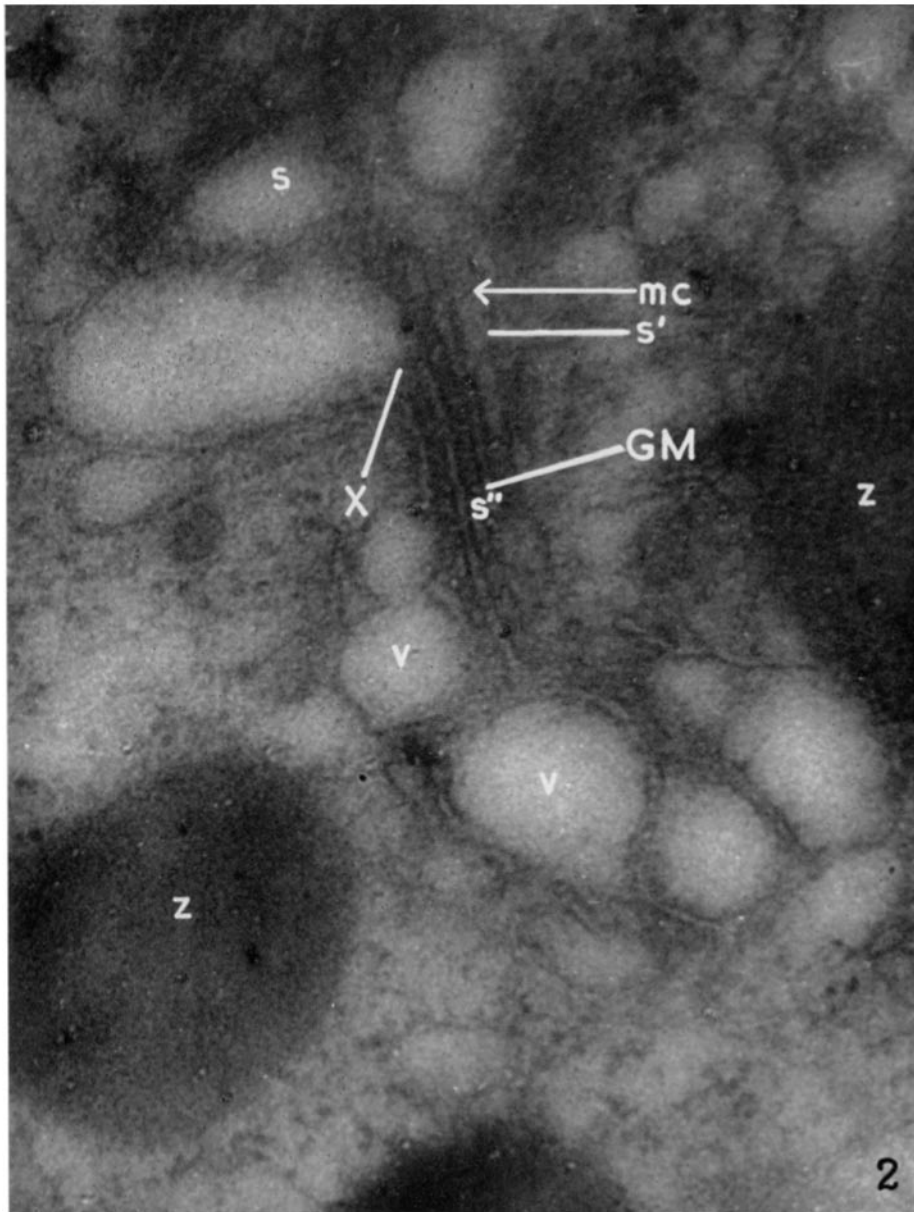
PLATE 98

FIG. 2. A micrograph showing the Golgi apparatus as seen after fixation in buffered osmium tetroxide solution.

The micrograph shows the paired Golgi membranes (*GM*) and vacuoles (*v*) as reported by several previous workers. It also shows that in pancreas cells the vacuoles correspond to dilations of pairs of membranes (as at *X*) within which are large amounts of a substance (*s*) which does not react with the fixative. A further substance (*s''*), which does react with the fixative, may be seen and this lies within the narrow spaces that separate the paired membranes. Finally one pair of membranes is more widely spaced than the others and encloses a substance (*s'*) which has a density intermediate to that shown by the other two substances (*s*, *s''*). It is possible, therefore, that the more dense substance (*s''*) may gradually be converted into a less dense material (*s'*) and finally into the vacuolar substance (*s*) seen within the dilations.

Several fine dense strands (*mc*) may be seen connecting each member of a pair of Golgi membranes. These may represent additional membranes.

It is apparent, from the comparison between this micrograph and the preceding one (Fig. 1), that when Aoyama's method is used the silver deposits in the Golgi membrane region. *z*, zymogen granule. $\times 150,000$.



(Lacy and Challice: Aoyama's technique in studies on Golgi apparatus)