

# THE GOLGI ZONE OF THE RAT SPERMATID AND ITS ROLE IN THE FORMATION OF CYTOPLASMIC VESICLES

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PLATES 43 AND 44

The advent of the electron microscope has raised hopes for a settlement of the controversies centering on the structure of the Golgi zone. Dalton and Felix (6) described in the Golgi substance of epididymal cells a system of vacuoles, granules, or microvesicles associated with lamellae composed of flattened sacs. The same elements were observed in the Golgi zone of many other cell types (7, 8, 10, 11, 15, 16, 17, 19).

In the course of a study on the histology of the rat testis, the Golgi zone proved to be conspicuous in spermatids (12). It is associated with the growing acrosomic system during the early phases of spermiogenesis, but later separates from the acrosome and head cap to become a "residual Golgi zone" in the cytoplasm of the maturing spermatid and finally disintegrates as the cell transforms into a spermatozoon.

It was therefore decided to examine spermatids with the electron microscope in the hope that structural details would stand out clearly in this prominent Golgi zone (5). The results presented hereafter confirmed works carried on independently (2, 15) and added some new information. It was thus possible to show the relationships existing between the vesicular elements seen in the Golgi zone and those present elsewhere in the cytoplasm of the cell.

## *Materials and Methods*

Small pieces of rat testis were fixed for one hour in 1 per cent buffered osmic acid (13), washed in distilled water, dehydrated through increasing concentrations of alcohol, and embedded in *n*-butyl methacrylate. Sections 20 to 40  $\mu$  thick, cut with the Porter-Blum microtome, were examined with an RCA EMU-2E electron microscope.

## OBSERVATIONS

*The Golgi Zone of Young Spermatids*<sup>1</sup>.—The Golgi zone of young spermatids formed a dense area, not sharply separated from the surrounding cytoplasm, and containing a complex system of vesicles. These vesicular structures were of two main types: flat vesicles and spheroidal vesicles of various sizes (Figs. 1 and 2, *FV*, *SV*).

In the sections the flat vesicles appeared as delicate non-anastomosing closed

<sup>1</sup> The acrosomic elements in relation to the Golgi zone will not be described here since they have been extensively discussed elsewhere (4).

tubules. These vesicles often formed stacks or piles of lamellar appearance (Figs. 1 and 2, *FV*). The distance between the two membranes of each flat vesicle varied considerably and was approximately 100 to 150 A at the narrowest points. The distance between two adjacent flat vesicles was occasionally very small (approximately 50 to 60 A), in some of the stacks described above (Fig. 2, *FV*).

In addition to the flat vesicles the Golgi zone displayed numerous spheroidal vesicles of various sizes, which appeared circular or oval in section. The smallest were slightly wider than the flat vesicles and had a diameter of 200 to 300 A. They were often seen at the edges of the stacks of flat vesicles from which they seem to arise by budding or fragmentation (Fig. 2, *X*). The largest spherical vesicles reached a diameter of 200 m $\mu$  and were generally seen at the periphery of the Golgi zone (Figs. 1 and 2).

Although some dense granules similar in size to those described by Palade (14) were seen in the cytoplasm of the spermatid in the vicinity of the Golgi zone (Fig. 2, *P*) none were observed within the zone itself.

The stacks of flat vesicles were often distributed toward the periphery of the Golgi zone, thus outlining a central area which contained only small and medium size spherical vesicles (Figs. 1 and 2). At the stage of spermiogenesis when the growing acrosomic system was applied to the surface of the nuclear membrane (Fig. 2, *A*), an area containing mostly spherical vesicles was seen close to the acrosomic structure while the flat vesicles were disposed in the form of a crescent toward the periphery of the Golgi zone.

Outside the Golgi zone, no flat vesicles were observed but spherical vesicles of various sizes were present (Figs. 1 and 2, *V*).

*The Residual Golgi Zone.*—As spermatids matured, the Golgi zone became separated from the acrosomic system and moved toward the portion of the cytoplasm lying along the tail, where it could be identified by the presence of both flat and spherical vesicles (Figs. 3 and 4, *FV*, *SV*). The residual Golgi zone differed from that of the young spermatid by having a smaller number of flat and small spherical vesicles and by a greater number of large vesicles, which gave to the whole structure a vacuolated appearance. Here again, the large spherical vesicles, although numerous in the Golgi area proper, were not restricted to the Golgi zone, since many of them were seen in the surrounding cytoplasm (Figs. 3 and 4, *V*).

#### DISCUSSION

When the light microscope was used to examine the Golgi zone of the rat spermatid stained with osmic acid or impregnated with silver, the main components were dense chromophilic granules, rodlets, or plates (called Golgi elements or dictyosomes) arranged concentrically about a lightly stained core, the idiosome (1, 9). The chromophobic vacuoles or canaliculi visible in the Golgi

areas of other cells were not seen. However, under the electron microscope, the submicroscopic components appeared to be the same in the Golgi zone of spermatids as in that of other cell types, that is, flat and dilated vesicles of various sizes. Granular components were absent. The parallel arrays of flat vesicles, the membranes of which are readily impregnated with osmic acid, evidently correspond to the so-called Golgi elements or dictyosomes seen with the light microscope. Furthermore, the peripheral disposition of the flat vesicles around a central core was evidently responsible for the lamellated pattern described in this zone by the first electron microscopic investigations of the rat spermatid (3, 18).

Haguenau and Bernhard (10), studying the Golgi zones of several types of cells, and Burgos and Fawcett (2) describing the Golgi zone of the cat spermatid, suggested that the small vesicles formed either by budding or by fragmentation of the flat vesicles. The present series of observations support this view. In addition it appeared that the large vesicles seen at the periphery of the Golgi zone were formed by direct swelling of the flat vesicles. Similarly, medium size and large vesicles probably arose from the progressive swelling of the small vesicles. That the flat vesicles transform into spherical vesicles rather than the reverse is suggested by the fact that the Golgi zone of the spermatid becomes progressively vacuolated as spermiogenesis progresses.

The presence of large and small vesicles close to, but outside the Golgi zone of the spermatids at various phases of its development, was also suggestive of a passage of vesicles from the Golgi zone to the surrounding cytoplasm. The movement of the cytoplasm caused by an active undulation of the cell membrane as observed in living spermatids with the phase contrast microscope (Clermont, unpublished) makes the migration of cytoplasmic elements quite plausible. According to this view, the numerous vesicles seen in the cytoplasm of the spermatid (considered by others to be cisternae of the endoplasmic reticulum (14, 15), or ergastoplasmic sacs (2)), would be formed in the Golgi zone.

#### SUMMARY

Electron microscopic study of the Golgi zone of the rat spermatids revealed two main types of vesicular structures: flat vesicles generally accumulated in stacks and spheroidal vesicles of various sizes. The Golgi zone of young spermatids showed a predominance of flat vesicles and small spherical vesicles, while the "residual" Golgi zone of the maturing spermatids displayed an increased number of large spherical vesicles and thus became increasingly vacuolated in appearance.

The images presented were suggestive of a transformation of the flat vesicles into spherical vesicles by fragmentation and dilatation and also of a passage of the spherical vesicles from the Golgi zone to the surrounding cytoplasm.

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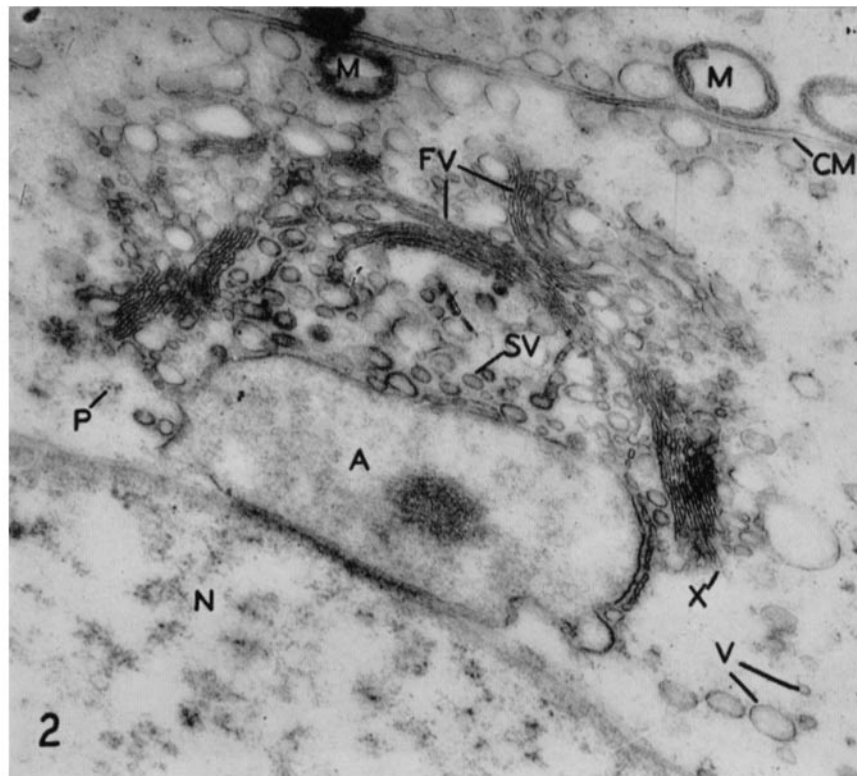
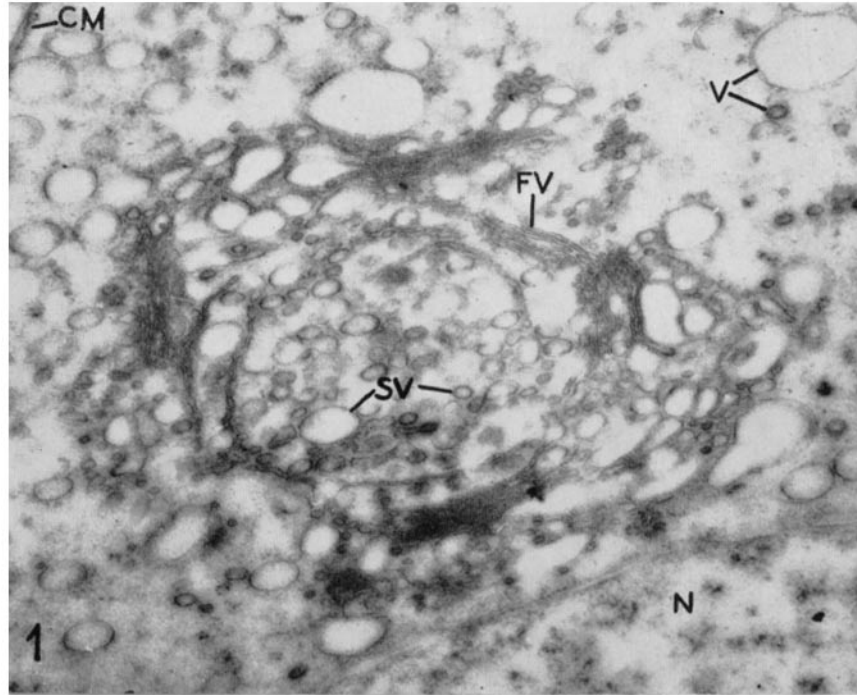
## EXPLANATION OF PLATES

## PLATE 43

FIG. 1. Electron microphotograph of the Golgi zone of a newly formed spermatid. Flat vesicles (*FV*) and spheroidal vesicles (*SV*) of various sizes are seen in the Golgi zone. The flat vesicles occur singly or in stacks and are concentrically arranged around a central core containing mostly spherical vesicles. Vesicles of all sizes (*V*) are also seen in the surrounding cytoplasm. The photograph also shows part of the nucleus (*N*) and the cell membrane (*CM*).  $\times 28,500$ .

FIG. 2. Electron microphotograph of the Golgi zone of a young spermatid with a developing acrosomic system (*A*) at the surface of the nucleus (*N*).

Stacks of flat vesicles (*FV*) are seen arranged in the form of a crescent around an area containing mostly spheroidal vesicles (*SV*). Small vesicles are numerous throughout the Golgi zone and seem to be formed by the fragmentation of the extremities of the flat vesicles (*X*). Large and small vesicles (*V*) are also present in the surrounding cytoplasm, as well as small granular particulates (*P*). Mitochondria (*M*) are visible along the cell membrane (*CM*).  $\times 28,500$ .



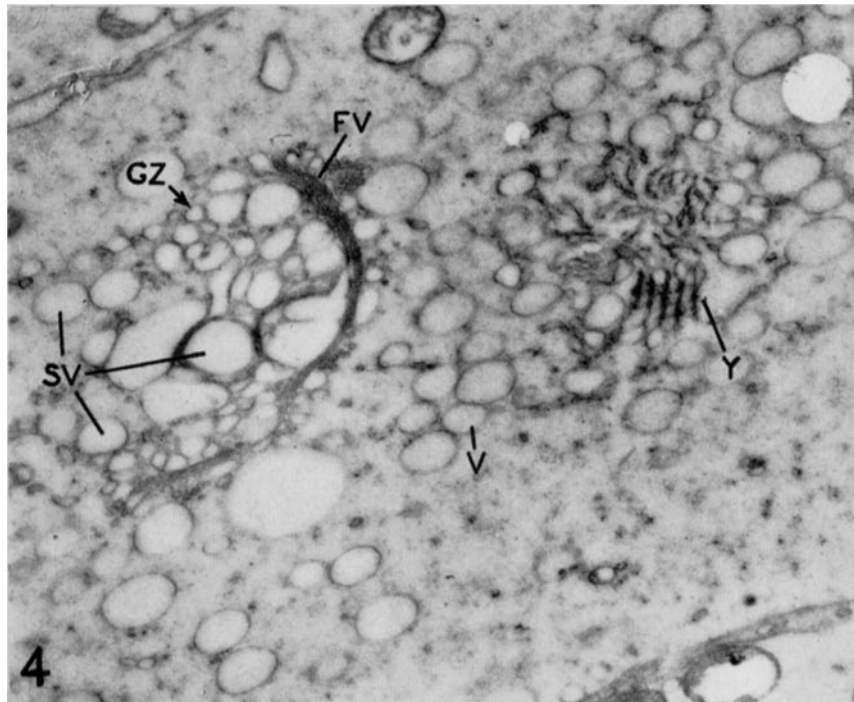
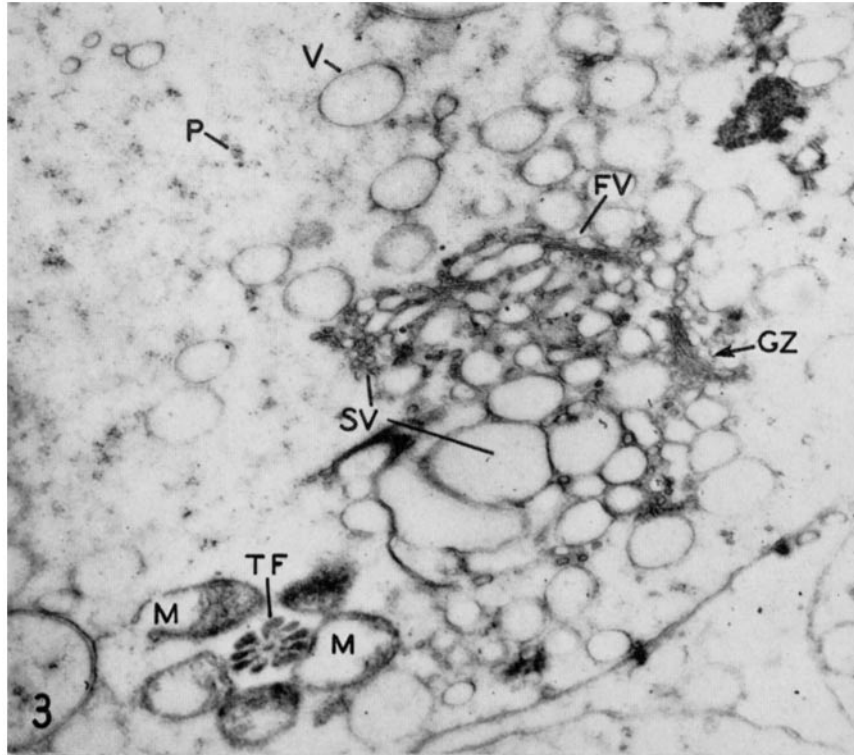
(Clermont: Golgi zone of rat spermatid)

PLATE 44

FIG. 3. Electron microphotograph of a section through the caudal cytoplasmic body of a maturing spermatid. The residual Golgi zone (*GZ*) can be recognized, close to a cross-section of the tail of the spermatid showing the tail filaments (*TF*) and mitochondria (*M*). The Golgi zone contains some flat vesicles (*FV*) and small and large spheroidal vesicles (*SV*); the large vesicles are numerous and give to this structure a vacuolated appearance. Vesicles (*V*) and granular particulates (*P*) are also present in the adjacent cytoplasm.  $\times 10,500$ .

FIG. 4. Electron microphotograph of the residual Golgi zone (*GZ*) of a maturing spermatid showing flat vesicles (*FV*) and numerous spheroidal vesicles of large size (*SV*).

In the adjacent cytoplasm, in addition to the numerous spheroidal vesicles that can be seen (*V*), there is a group of vesicles with irregular outlines (*Y*). These vesicular structures, which are only present in the cytoplasm of the maturing spermatid, are always seen outside the Golgi zone proper.  $\times 10,500$ .



(Clermont: Golgi zone of rat spermatid)