Association of Golgi vesicles containing acid phosphatase with the chromatoid body of rat spermatids[1]

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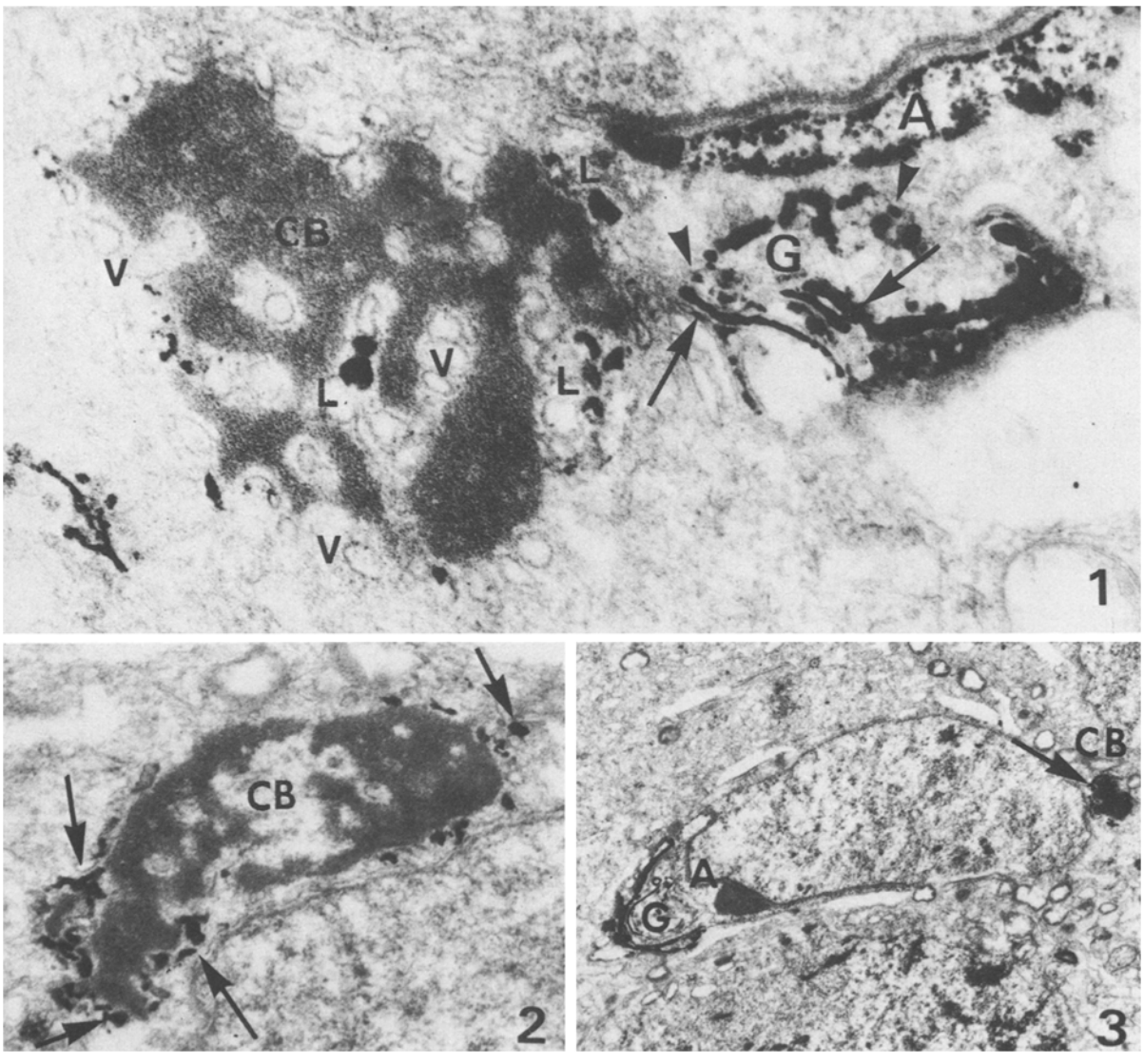
Summary. The relationship between the Golgi apparatus and the small vesicles associated with the chromatoid body was investigated using a cytochemical technique. It was observed that in early spermatids, when the chromatoid body appears in close contact with the Golgi complex, and all through its migration to the caudal pole of the nucleus, some of the vesicles that accompany the organelle display acid phosphatase activity. It is concluded that these smooth vesicles originate in the Golgi apparatus.

The chromatoid body is a dense irregular cytoplasmic organelle which is first visible in the pachytene stage of male germ cells; it persists during the second meiotic division, and becomes very conspicuous in early spermatids[2,3].

Morphological similarities between the chromatoid body and the nucleolus have led some investigators to consider that the former has a nucleolar origin; however, no cytochemical technique has succeeded in revealing RNA in the chromatoid body[4] although recent publications reported



Figures 1–3. Cytochemical preparations of sections of rat spermatids stained for acid phosphatase. Fig. 1. Association of the Golgi apparatus (G) with the chromatoid body (CB). Intense acid phosphatase reaction is present in lamellae (arrows) as well as in primary lysosomes (arrowheads). Numerous vesicles (v) are seen around and between the lobules of the chromatoid body, and some of them have lead deposits, indicating acid phosphatase activity (L); also the acrosome (A) appears with an intense reaction. × 40,000. Fig. 2. Migration of the chromatoid body (CB). All along its migration around the nucleus the chromatoid body is accompanied by small vesicles displaying acid phosphatase reaction (arrows). × 31,000. Fig. 3. Electron-micrograph of a more differentiated spermatid. The Golgi complex (G) is near the anterior pole of the elongated nucleus and shows a strong acid phosphatase reaction whereas the acrosome (A), with the acrosome granule, displays almost no reaction; on the opposite side of the nucleus appears a chromatoid body (CB) with a few small acid phosphatase positive vesicles (arrows). × 8300.

the detection of RNA by autoradiography of spermatids[5] and pachytene spermatocytes[6] labeled with ^3H uridine.

The migration of the chromatoid body from the acrosomal to the caudal pole of the nucleus during spermiogenesis and its association with the Golgi apparatus in the early stages of the process have been observed by phase contrast and electron microscopy[7]. Furthermore, it has been demonstrated that multiple small vesicles accompany the body during that movement.

In the present study a cytochemical staining for acid phosphatase activity has been used in order to investigate the relationship between the Golgi apparatus and the small vesicles associated with the chromatoid body.

Materials and methods. Adult Wistar rats were anesthetized by an i.p. injection of urethane (120 mg/100 g b.wt) and the testes were perfused through the internal spermatic artery with 5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 as previously described[8]. Sections 50–70 µm thick were obtained with a Smith Farquhar tissue sectioner or with a Lipshaw cryotome, they were then incubated for 60 min at 37 °C in Gomori's lead salt mixture[9] for acid phosphatase containing β-glycerophosphate, grade I (Sigma Chemical Co.) as substrate; postfixed in buffered OsO_4, stained with 0.5% uranyl acetate, dehydrated, and embedded in Maraglas. Sections incubated in the same medium which also contained 0.01 M sodium fluoride were used as controls, as well as mixtures lacking the substrate. Ultrathin sections were obtained with a Porter Blum microtome and the grids were observed unstained or slightly stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Zeiss EM-9 A electron microscope.

Results and discussion. The presence of acid phosphatase in some lamellae, Golgi vesicles, and lysosomes resembling dense bodies in early spermatids has been reported before[10,11] and was confirmed in the present study. Furthermore, a strong positive reaction was occasionally observed in the acrosome (fig. 1).

It is a well known fact that the chromatoid body migrates during spermiogenesis from the region where the acrosome is being formed by the Golgi apparatus towards the caudal pole where the flagellum is growing out[3,7]. At the beginning of that journey, when the chromatoid body is in close association with the cisternae of the Golgi complex, many small vesicles that look like Golgi primary lysosomes and show a positive acid phosphatase reaction appear around the body; in the spaces that separate the negatively stained multiloculated structure of the chromatoid body only a few of those vesicles are seen (fig. 1).

Neither the fine structure of the chromatoid body nor its association with the small vesicles are altered during the migration to the other side of the nucleus (fig. 2). When the caudal pole of the nucleus (opposite to the acrosome) is reached, the chromatoid body is still accompanied by vesicles, some of which show lead deposits caused by acid phosphatase activity (fig. 3).

The fact that only some of the vesicles associated with the chromatoid body show the acid phosphatase reaction could be due either to functional differences[12] or to differences in enzymatic content that could, perhaps, be disclosed by another cytochemical technique.

The association of the chromatoid body with multiple small vesicles in stage 1 of spermiogenesis, as well as the contacts with the Golgi apparatus during stages 2 and 3, were also observed by Parvinen and Jokelainen[7], who proposed the possible participation of the chromatoid body in the formation of the acrosomal system; however, the present observations suggest that the transient interaction with the Golgi complex could be directed to the collection of vesicles whose hydrolytic enzymes were to be used in a later step of spermiogenesis.

It has been suggested by Clermont[13] that the smooth-surfaced vesicles frequently associated with the chromatoid body proceed from the endoplasmic reticulum; however, the presence of acid phosphatase activity in some of those vesicles indicates that some of them, at least, are provided by the Golgi apparatus.

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Structural alterations in rat kidney proximal tubules perfused with fresh autologous serum[1]

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Summary. Two min of intraluminal perfusion of the rat proximal tubules with autologous serum induced marked ultrastructural alterations including extensive cytoplasmic vesiculation due to swelling of rough endoplasmic reticulum cisternae and occasional extrusion of nuclei and cytoplasm into the lumen. Within 4 min pronounced vesiculation of mitochondria was observed. These findings are consistent with the notion that serum-induced inhibition of proximal tubular fluid absorption is due to cell lysis, presumably mediated by complement activation.

Fresh autologous serum perfused directly into the lumen of rat proximal tubule induces depolarization of intracellular potential difference, abolishes electrical resistance of the luminal cell membrane and virtually abolishes fluid transport. This effect occurs within 2 min and it is not reversible[3–5]. Although the precise mechanism for such a dramat-