OBSERVATIONS OF CENTRIOLE FORMATION
IN MALE MEIOSIS

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ABSTRACT

Centriole formation in male meiosis of the hyrax, *Heterohyrax syriacus*, and the Berdmore palm squirrel, *Memetes berdmorei*, was investigated by serial section analysis of selected regions of the seminiferous epithelium and isolated meiotic cells. Two periods of centriole formation were observed, a first in cells in transition between zygotene and pachytene and a second in the secondary spermatocytes. The duplication events before each meiotic division insured the presence of a centriolar duplex at each division pole. The parental member of each duplex of the second division was closely associated, at its distal end, with the plasma membrane. This orientation was established in the secondary spermatocyte and persisted until the completion of telophase II. Subsequently in early spermatids, each duplex assumed a characteristic orientation adjacent to the nucleus.

INTRODUCTION

Throughout spermiogenesis of most vertebrate species, two centrioles, which are oriented at right angles to each other, are present juxtaposed to the nuclear membrane. The distal centriole functions as a basal body for the flagellum. However, in some cases it undergoes a gradual dissolution at the completion of spermiogenesis and may be absent from the mature spermatozoa (Fawcett and Phillips, 1969). The role of the proximal centriole in spermiogenesis is not apparent. The spermatid duplex, consisting of two mature centrioles, has been observed at the initiation of the spermiogenic process. However, the distribution and duplication pattern of the centriole during meiosis from which this duplex is derived has not been carefully investigated.

Several studies of invertebrate meiosis suggest that the centriolar complement is established before the first meiotic division. In the silkworm, Lanaka (1955) has observed four duplexes before metaphase I, each possibly representing a duplex to be found in the early spermatid. Further, in neuropteran insects (Friedlander and Wahrman, 1966) centriolar duplication in prophase is sug-

gested by the observation of two centriolar duplexes, one at each pole of metaphase I. After the first meiotic division, parent and daughter centrioles separate and a single centriole occurs at each pole of the second division. This centriole, partitioned to each spermatid, represents the single elongate basal body characteristic of the spermatozoa of this species.

Ultrastructural investigations of cells of several organisms have allowed the correlation of centriolar behavior with the somatic cell cycle (Brinkley and Stubblefield, 1969; Robbins et al., 1968). These studies suggest that centriolar formation in mitosis is achieved by the generation of a daughter structure at right angles to the proximal tip of the parent. This assembly process is initiated during the period of nuclear DNA synthesis and results in the regular partitioning of two mature centrioles to each daughter cell.

In order to investigate the distribution and duplication pattern of the centriole in meiosis, testis cells of the hyrax, *Heterohyrax syriacus*, and the Berdmore palm squirrel, *Memetes berdmorei*, were examined by serial section analysis. Evidence

for centriole formation in cells in the zygotene-pachytene transition and secondary spermatocytes as well as a description of the behavior of the centriole duplex during division events will be presented

MATERIALS AND METHODS

Testes of the hyrax, *Heterohyrax syriacus*, and 'the Berdmore-palm squirrel, *Memetes berdmorei*, were dissected into a Petri dish containing Millonig's phosphate buffer or collidine buffer at pH 7.4. A portion of each testis was cut into 1 mm pieces and samples were transferred to bottles containing either 3% glutaraldehyde in collidine buffer or 3% glutaraldehyde in Millonig's phosphate buffer. After 1 hr the tissue was postfixed in a 1% osmium tetroxide solution buffered as described above, and dehydrated in a graded ethanol series and embedded in Epon 812 The remainder of the testis was touched to a Falcon plastic Petri dish (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.), allowing free cells to adhere to the dish surface. These preparations were fixed and embedded by the method of Brinkley et al. (1967). Such preparations provided a cell monolayer containing a variety of spermatogenic stages.

Several criteria were employed to stage meiotic cells present in Epon monolayer and thick section preparations. Prophase cells were identified by the morphology of the synaptonemal complexes Cells with nuclei containing synaptonemic cores were considered to be in the zygotene stage. Cells in which initiation of chromosomal pairing as evidenced by short segments of synaptonemal complex were considered to represent cells in transition between zygotene and pachytene, the latter stage identifiable by the presence of completely formed synaptonemal complexes Cells in which fragments of the synaptonemic cores were present and chromatin condensation appeared pronounced were considered to represent diplotene Cells in which the chromosomes had condensed and shortened were identified as diakinesis.

Several cytological features allowed the differentiation of cells of the first and second meiotic divisions. Cells of the first meiotic division were large and ovoid in shape (in hyrax = 26 5–28.0 μ). The meiotic apparatus displayed a central position in the cell cytoplasm, and a large chromatin mass was evident. In contrast, the cells of the second meiotic division were considerably smaller (in hyrax = 11.0–13.0 μ) and more spherical in shape, the chromosomes appeared thinner in diameter, and the spindle extended the complete length of the cell. Secondary spermatocytes were found in areas containing both first and second meiotic divisions. The central spherical nuclei contained chromatin in several stages of condensation. The more dense areas appeared to have

the shape of "x's" and "y's". A larger sample of meiotic cells was examined in the hyrax, and stages showing centriolar replication events were selectively examined in the Berdmore palm squirrel. The number of meiotic hyrax cells from the monolayer preparations and from the seminiferous epithelium from which serial section analysis of the complete centriolar complements was obtained were: leptotene (5), zygotene (3), late zygotene–early pachytene (8), pachytene (5), diplotene (2), diakinesis (2), metaphase I (7), anaphase I (5), telophase I (5), secondary spermatocytes (7), metaphase II (4), anaphase II (3), telophase II (3)

Serial section analysis was done on both intact tissue and isolated cells Regions of the seminiferous tubules were preselected by analysis of Epon-thick sections. Contiguous sections in the silver-to-gold range, cut with a diamond knife on the LKB ultratome III, were collected on copper slotted grids coated with a 1% collodion solution in amyl acetate. Cells from the Petri dish monolayer were preselected by phase-contrast microscopy, using a 100 × phase oil immersion objective lens, and scored with a needle. The area containing the selected cell was cut from this Epon disc with a cork borer and cemented on an Epon blank. Subsequently, the blank was trimmed to approximately 0.5 × 0.5 mm. Sections were cut and collected by the method described above and examined in a Siemens Elmiskop I microscope operated at 60 kv.

RESULTS

Meiotic Prophase

Serial sections of eight cells identified in the leptotene-zygotene stages revealed a single pair of centrioles 0.5–0.6 μ in length. During zygotene the paired centrioles lost their right-angled orientation and separated to a distance of 0.2–0.3 μ. During this period each centriole was embedded in an electron-opaque matrix Protions of this. matrix frequently appeared structured, perhaps indicating initial procentriolar organization (Figs. 1 and 1 A). In eight cells containing nuclei in which short synaptonemal complexes could be identified (zygotene-pachytene), procentrioles were present at right angles to each parent structure (Fig. 2). In the hyrax the procentriole was generated at right angles to the proximal-most tip of the parent. However, in the Berdmore palm squirrel the procentriole appeared more central to the body of the parent structure (Figs 2 A and 2 B). Procentriolar elongation was apparent in serial sections of four diplotene-diakinesis cells
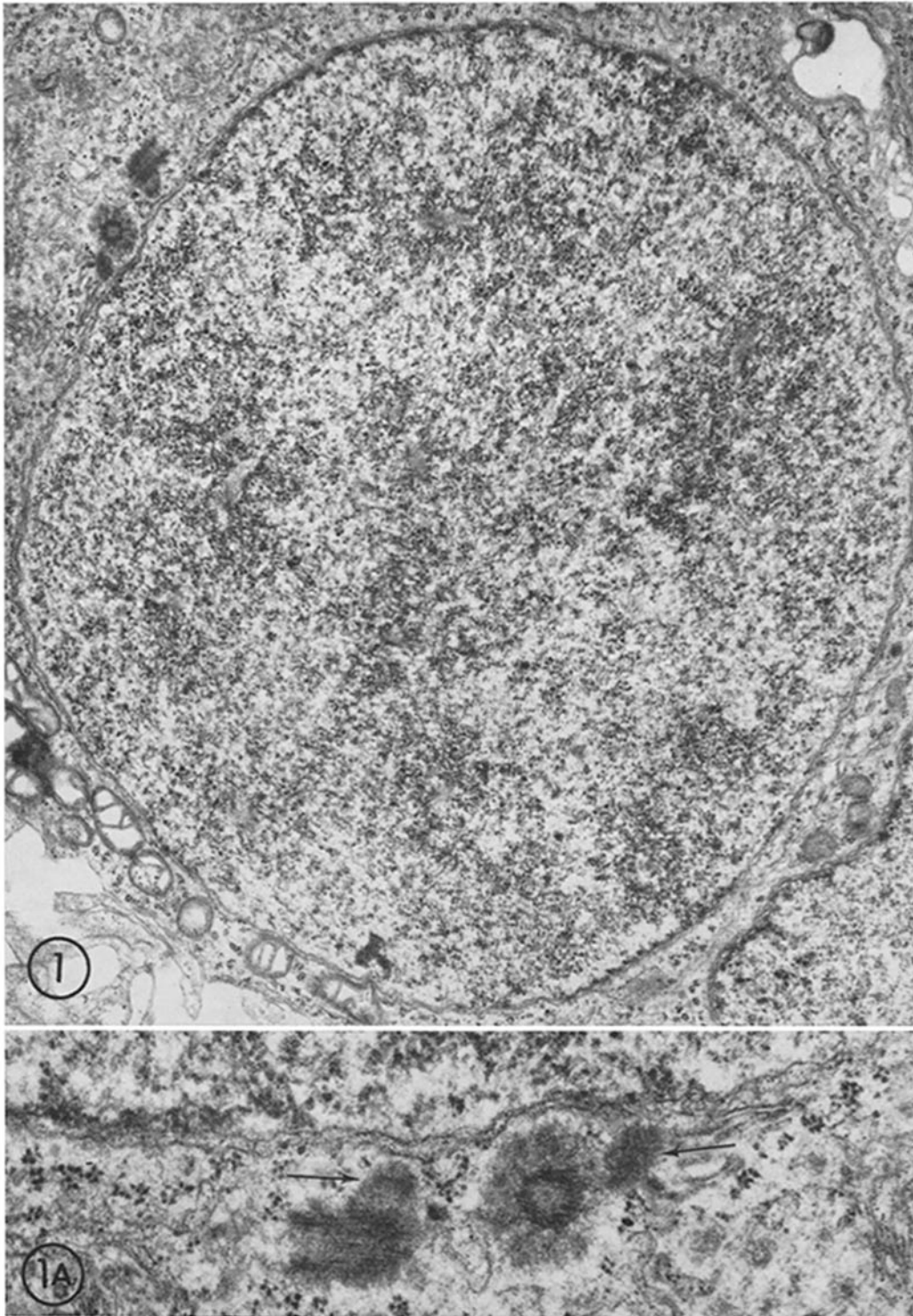
FIGURE 1   Zygotene cells from the seminiferous tubule in which the two members of the centriolar du-
plex have separated and are encased in an electron-opaque matrix. *Heterohyrax syriacus.* × 11,800.

FIGURE 1 A   High magnification of the centriolar duplex from Fig. 1. Localized densities (arrows) may
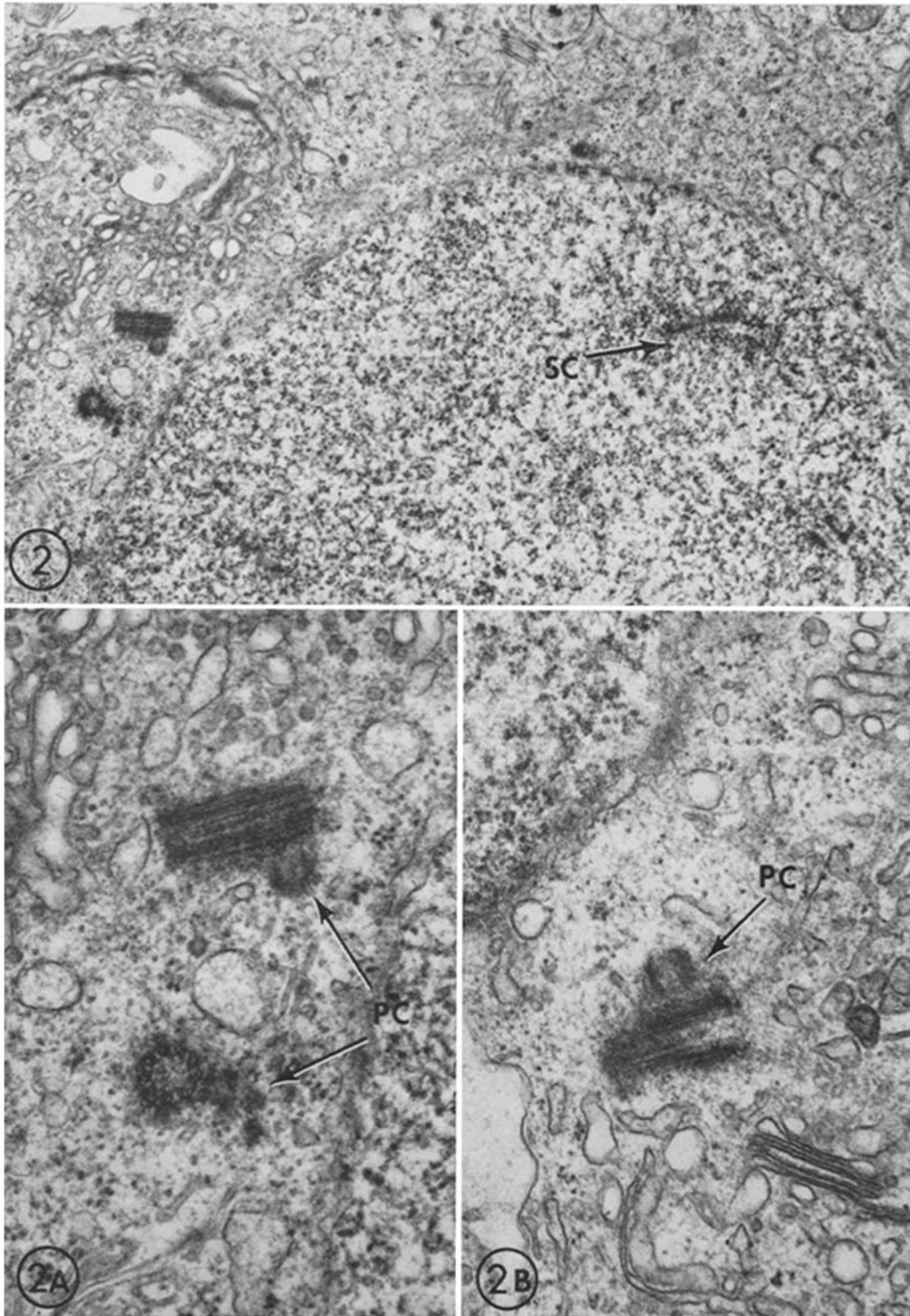indicate the initial organization of the procentrioles. *Heterohyrax syriacus.* × 36,200.

FIGURE 2   Zygotene-pachytene cell from the seminiferous epithelium Short segments of synaptonemal complexes (SC) are present in the nucleus. *Heterohyrax syriacus.* × 13,000.

FIGURE 2 A   High magnification of the two centriolar duplexes shown in Fig. 2. Procentrioles (PC) are present at the proximal tips of each mature centriole. *Heterohyrax syriacus.* × 36,000.

FIGURE 2 B   Centriolar duplex from early pachytene cell of the Berdmore squirrel. Procentriole formation (PC) is initiated central to the body of the mature parent. *Memetes berdmorei.* × 34,000.

(Figs. 3 and 4) at which time the daughter centriole attained its final length

First Meiotic Division

At the onset of the first meiotic division (prometaphase) two centriolar duplexes were present: peripheral and at either side of the central chromosomal mass. The parent centrioles displayed a right-angled orientation with respect to one another. This orientation was maintained throughout the remaining division events, and each duplex maintained a position several micra from the plasma membrane (Fig. 5). Microtubular elements projected from the pericentriolar cytoplasm in all directions. As metaphase was established, two classes of microtubular elements, chromosomal and continuous, could be identified and astral elements extended from the centriole towards the plasma membrane. At late telophase the chromatin mass had a "u"-shaped contour (Fig. 6) and the duplex was positioned at the edge of the lumen of the "u". Microtubules persisted between the chromatin and centriole pair until the completion of nuclear membrane formation. As the nuclei became spherical in shape and assumed a central position in the cell, the centriolar duplex migrated towards the plasma membrane.

Secondary Spermatocyte

Early secondary spermatocytes were characterized by a single centriolar duplex, the parent member closely applied at its distal end to the plasma membrane. Subsequently, the daughter detached from the parent and moved laterally 0.3–0.5 μ. Once this conformation was obtained, a second duplication event occurred as evidenced by the appearance of procentriolar structures (Fig. 7). Procentriole formation occurred at right angles to the parent along the surface proximal to the nucleus. As elongation proceeded, the two duplexes moved apart, the duplex derived from

the parent centriole of the first division maintaining its orientation along the plasma membrane.

Second Meiotic Division

We have observed 10 cells of the second meiotic division by serial section analysis. During prometaphase the duplex derived from the daughter centriole of the secondary spermatocyte division completed its migration to the opposite pole and became associated with the plasma membrane in a manner analogous to that of the duplex derived from the parent centriole of the first division The parental member of each duplex then lay along a plane parallel to the longitudinal axis of the spindle. The parent centrioles maintained their close orientation with the plasma membrane but often lost the relationship juxtaposed to the membrane surface (Fig. 8). The astral elements of the spindle were absent from the meiotic apparatus of this division. At the completion of telophase events the duplex contains two mature centrioles. Subsequently, the duplex assumed a perinuclear orientation characteristic of early spermatids of these species.

DISCUSSION

Examination of meiosis of the hyrax and Berdmore squirrel revealed two periods of centriolar formation, the first initiated during the zygotene-pachytene transition and a second in the secondary spermatocyte Studies of centriolar replication in somatic cells have suggested a temporal relationship between nuclear DNA synthesis and centriolar replication (Stubblefield and Brinkley, 1967; Robbins et al., 1968) This temporal relationship does not appear to be prerequisite for both centriolar replication periods in meiosis. The bulk of nuclear DNA synthesis occurs before meiotic prophase with only a minor synthetic period during zygotene-pachytene (Hotta et al., 1966, Lima-de-Faria, 1968). Although this minor synthetic period coincides with the first

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Figure 3 Diplotene cell preselected from an Epon monolayer. The maturing daughter centriole has a length of 0.40 μ (arrow). The parent has been transected obliquely in this section and shows a mature length of 0.6 μ. *Heterohyrax syriacus.* $\times$ 12,000.

Figure 4 Early diakinesis cells from an Epon monolayer. The daughter centriole has completed elongation. Microtubules now extend from the duplex to the adjacent chromatin. *Memetes berdmorei.* $\times$ 20,000.
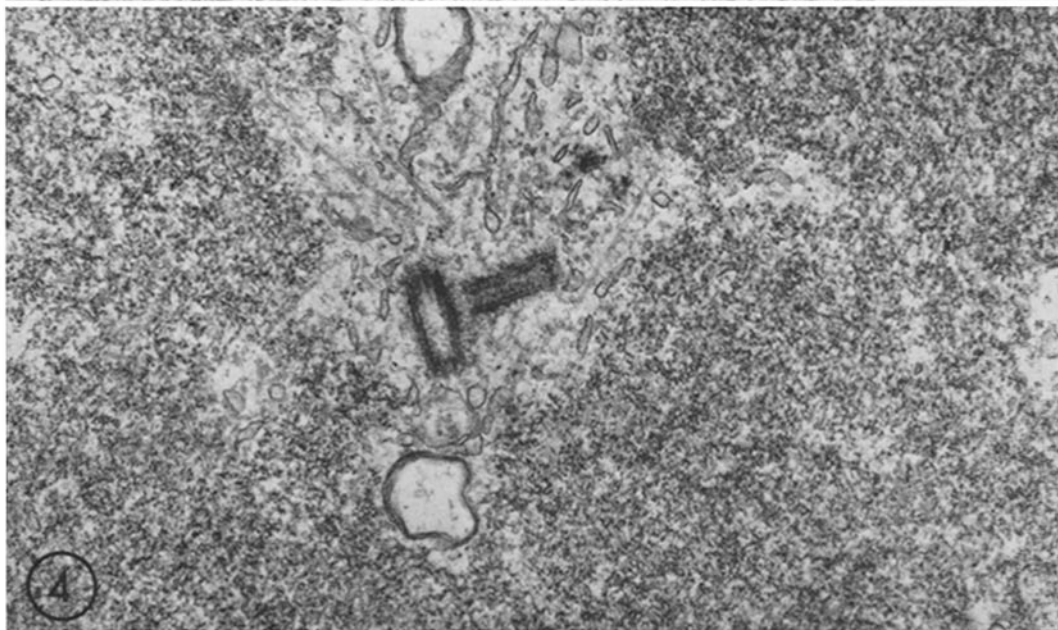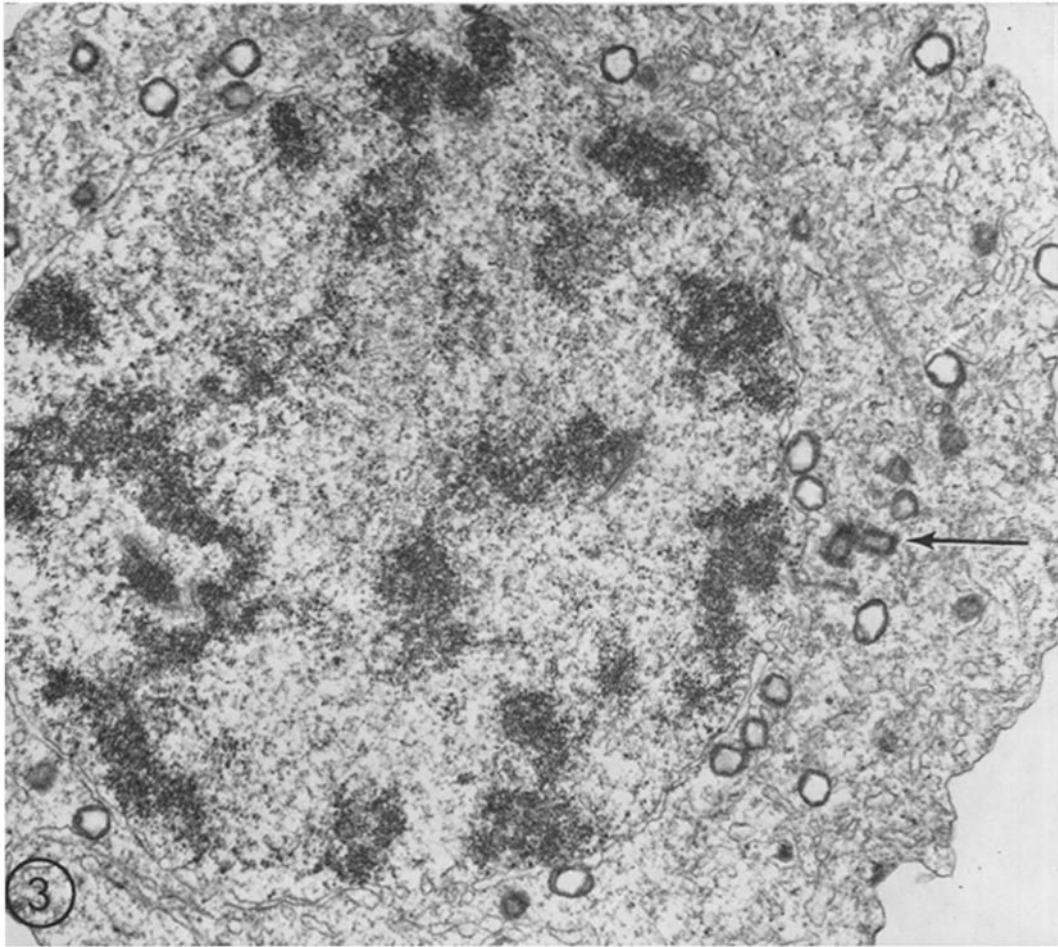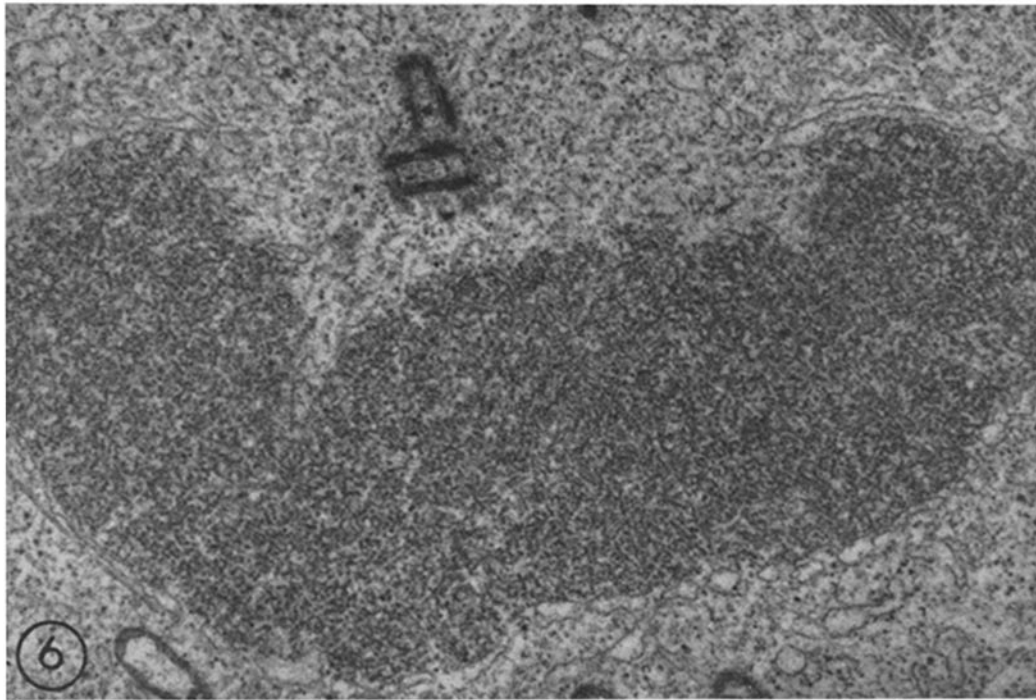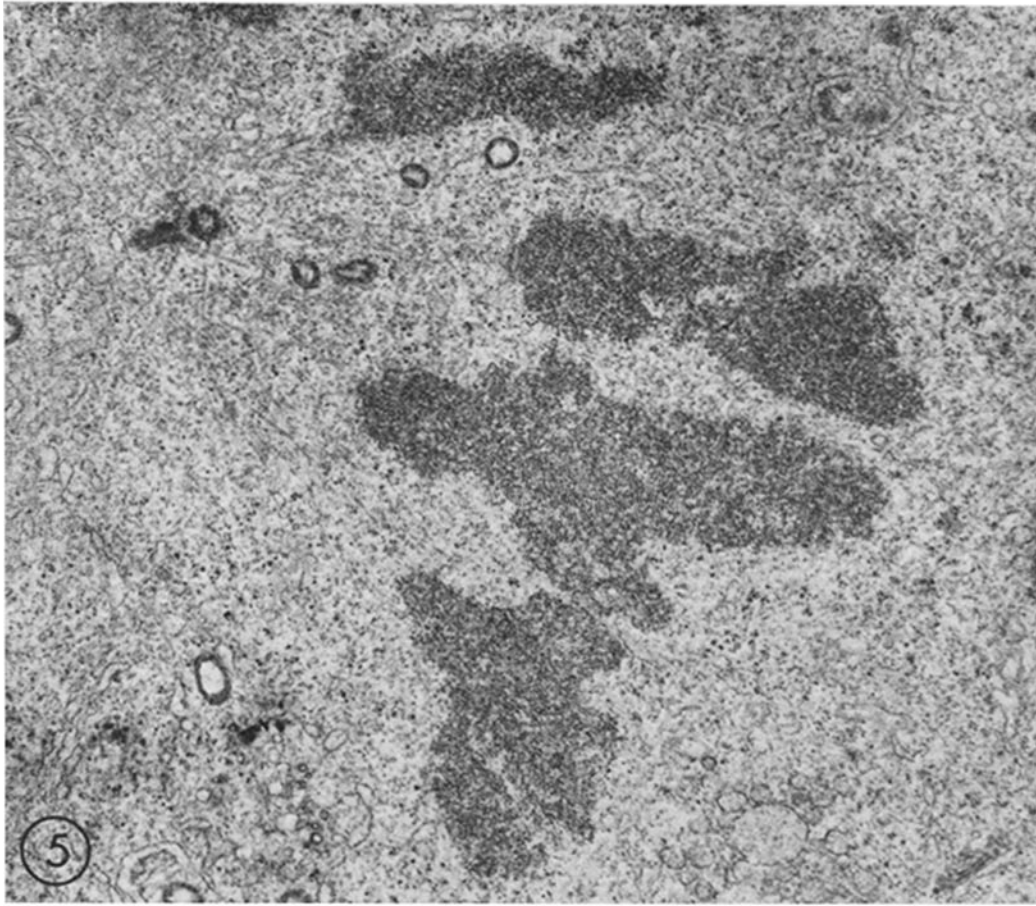
FIGURE 5   Anaphase I displaying a centriolar duplex at one pole. *Heterohyrax syriacus.* × 15,000.

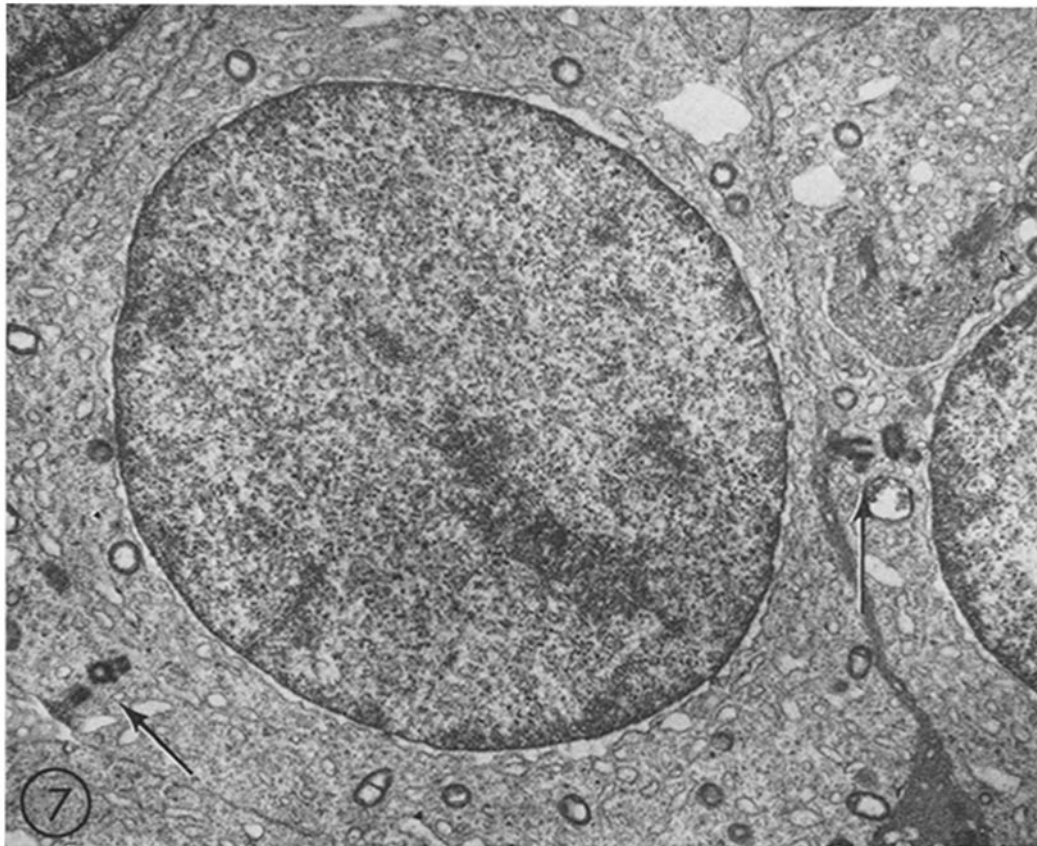FIGURE 6   Telophase I from the seminiferous epithelium. *Heterohyrax syriacus.* × 28,000.

FIGURE 7 Two adjacent secondary spermatocytes (arrows). The tip of each mature member is associated with a procentriole. The original parent from the first division shows a close association with the plasma membrane. *Heterohyrax syriacus.* × 10,000.

period of centriolar replication, there is no detectable synthesis during the second duplication period within the secondary spermatocyte.

The architecture of the centriole and procentriole formation and maturation during meiosis was not observed to deviate in general from descriptions of mammalian somatic cells (see Brinkley et al., 1969; and Pitelka, 1969, for review). However, generation of procentriolar structures during meiosis of the Berdmore squirrel appears to occur at a region central to the body of the parent rather than the proximal tip. Further, the parent centriole of the second division displayed a close association with the plasma membrane. This association, established within the secondary spermatocyte, may function in assuring the partitioning of only one duplex to

each spermatid or controlling the polarity of the events of the second meiotic division. It seems unlikely, however, that this phenomenon is associated with the initial organization of the spermatid since the centriole loses its attachment to the membrane at the completion of the meiotic events and undergoes a migratory phase in the early spermatid (Fawcett and Phillips, 1969).
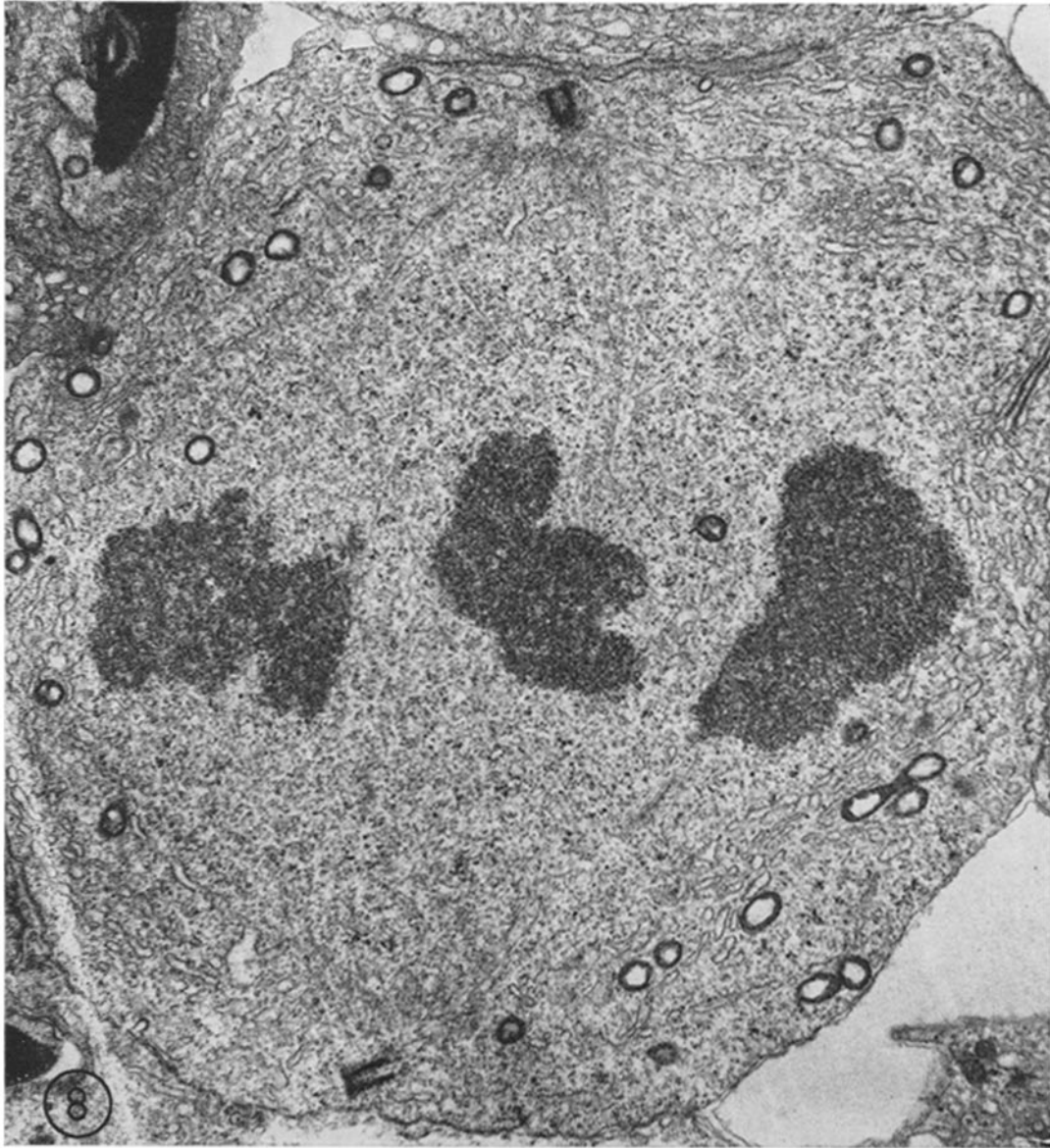
FIGURE 8 Metaphase II cell from the seminiferous epithelium. The parental member of each duplux is adjacent to the plasma membrane. Serial sections revealed the presence of a daughter at right angles to each parent. *Heterohyrax syriacus.* × 16,000.

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