Occurrence of "Nuages" and "Lamellae Anulata" during Spermatogenesis in *Dermatobia hominis* (Diptera: Cuterebridae)

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Various types of "nuages" and "lamellae anulata" can be found during Dermatobia hominis *spermatogenesis. In spermatogonia, the "nuages" occur as granules juxtaposed to the cytoplasmic face of the nuclear envelope or as cytoplasmic granules similar to glycogen granules. In spermatocytes, in addition to the "nuages", dense spherical bodies of approximately 1.0 µm in diameter are also observed. In the spermatids the "nuages" can be of the following types: perinuclear granules, spherical granules with diameters varying in length from 0.5 to 1.0 µm, granules similar to glycogen granules, granules with variable diameters which accumulate at the flagellum base forming the centriole adjunct, or remain in the cytoplasm. "Nuages" can also be observed in these cellular types as dense masses, without a definite outline and are common to animal germinal cells in general. The "lamellae anulata" on the other hand, are observed only in spermatocytes I and in early spermatids, being always immersed in electron-dense material of indefinite outline. In spermatids, the "lamellae anulata" are close to the nuclear envelope suggesting, in spite of opposing opinions, that these cells are envolved in the synthesis and transport of material from the nucleus to the cytoplasm.*

Key words: *Dermatobia hominis* - spermatogenesis - "nuage" - "lamellae anulata"

Cytoplasmic electron-dense materials not surrounded by membranes are a characteristic of animal germinal cells, generically known as "nuages". They occur in different sizes and shapes, receive different names and are present during the whole life cycle of these cells. They are abundant during oogenesis and spermatogenesis, contain RNA, appear isolated in the cytoplasm close to the nuclear envelope and are associated with mitochondria (Eddy 1975) or with "lamellae anulata" (Kessel 1983). "Lamellae anulata" are transient organelles, composed of stacks of cisterns having pores, similar to nuclear pores. They are found in several cells, especially in animal germinal cells, being well developed in oocytes (Kessel 1983, 1992). Despite the innumerable efforts their function so far is unknown (Merisko 1989, Kessel 1992).

We studied the different types of "nuages" observed during the spermatogenesis of *Dermatobia hominis*, their morphology, their relationship with cellular organelles and their period of occurrence, and we compared them to the ones described in the male germinal cells of other animals. Our aim is to contribute to a better understanding of them.

MATERIALS AND METHODS

For this study, pupae were developed in the laboratory from larvae supplied by highly infected bovines. The animals used in this experiment were confined overnight in stables with concrete floors covered by a wooden grid, with 7 cm spacing. Mature larvae abandoned the hosts and fell to the ground during the early morning hours; they were protected from being stamped upon by the spaces in the wooden grid. Each morning larvae were collected, taken to the laboratory and separated by weight; those weighing between 500 to 600 mg developed male imagos (Lello 1979). They were then placed in plastic boxes containing a 6 to 8 cm layer of humid soil where they penetrated to pupate. Boxes were kept at 25°C, with 70-80% relative humidity in a BOD incubator (Fanem, Brasil) during the entire pupal stage which lasted from 30 to 34 days.

From the very beginning of the pupal stage, pupae were removed daily from the soil and dissected in a saline solution for the removal of the insects' gonads; these were preserved in 2.5% glutaraldehyde solution with phosphate buffer, at a 0.1 M, pH of 7.3, for 2.5 hr, and then placed in 1% osmium tetroxide with the same buffer, for 2 hr, in a darkened area, and embedded in araldite. The ultrathin sections were highlighted using a saturated solution of uranyl acetate in 50% alcohol and with lead citrate (Reynolds 1963); observa-

This research was supported by FUNDUNESP, grant no. 573/90
Received 17 October 1994
Accepted 6 April 1995

tions and photo micrographs were made using a Phillips EM 301 transmission electron microscope.

RESULTS

At the time of cyst formation the definitive spermatogonia (Hannah-Alava terminology 1965) in *D. hominis*, mitochondria and lipid droplets were present in the cytoplasm that were poor in other organelles; nuclei were circular, nucleoli had a well-developed granulated region, almost reaching the nuclear envelope (Fig. 1). Various peri-

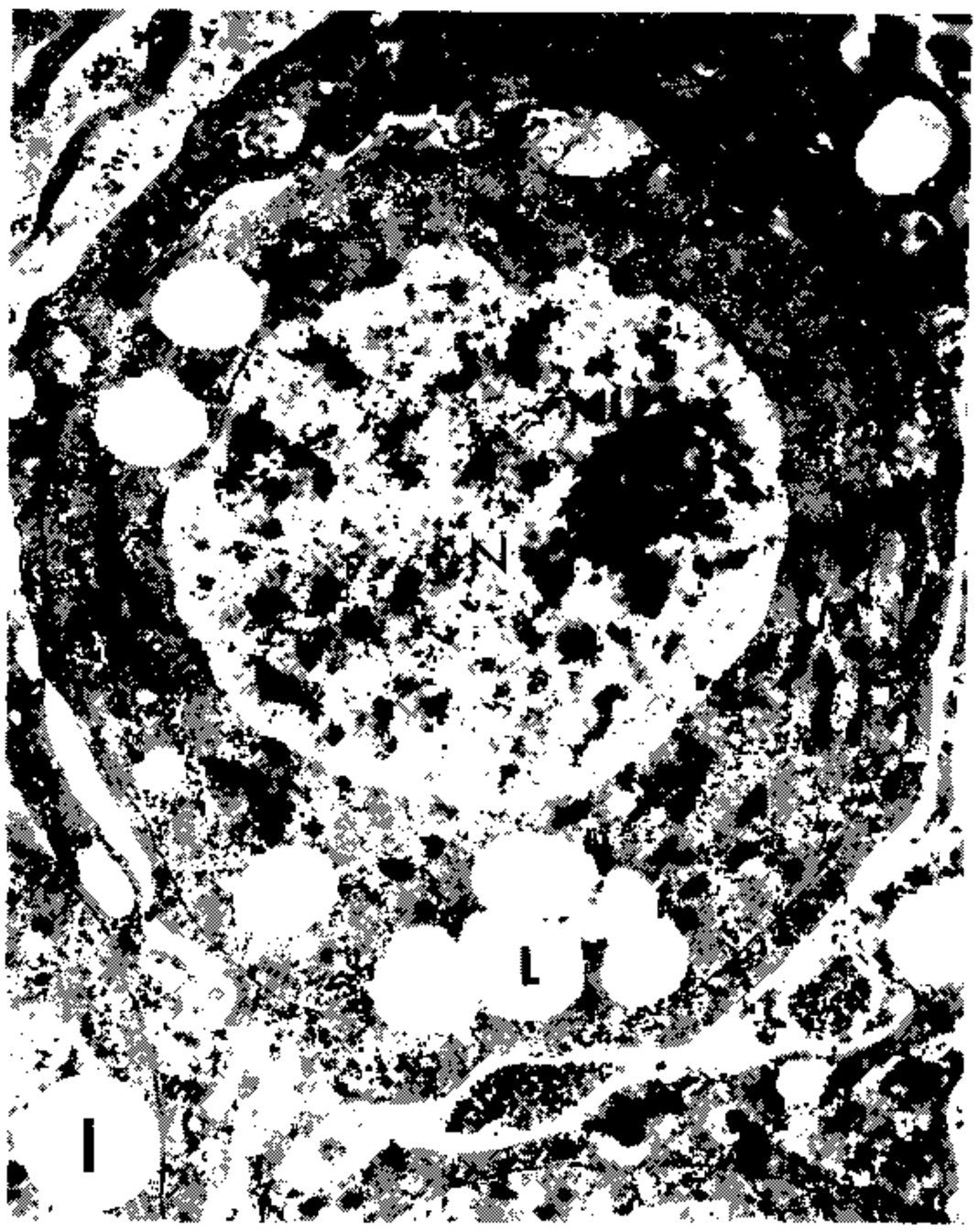
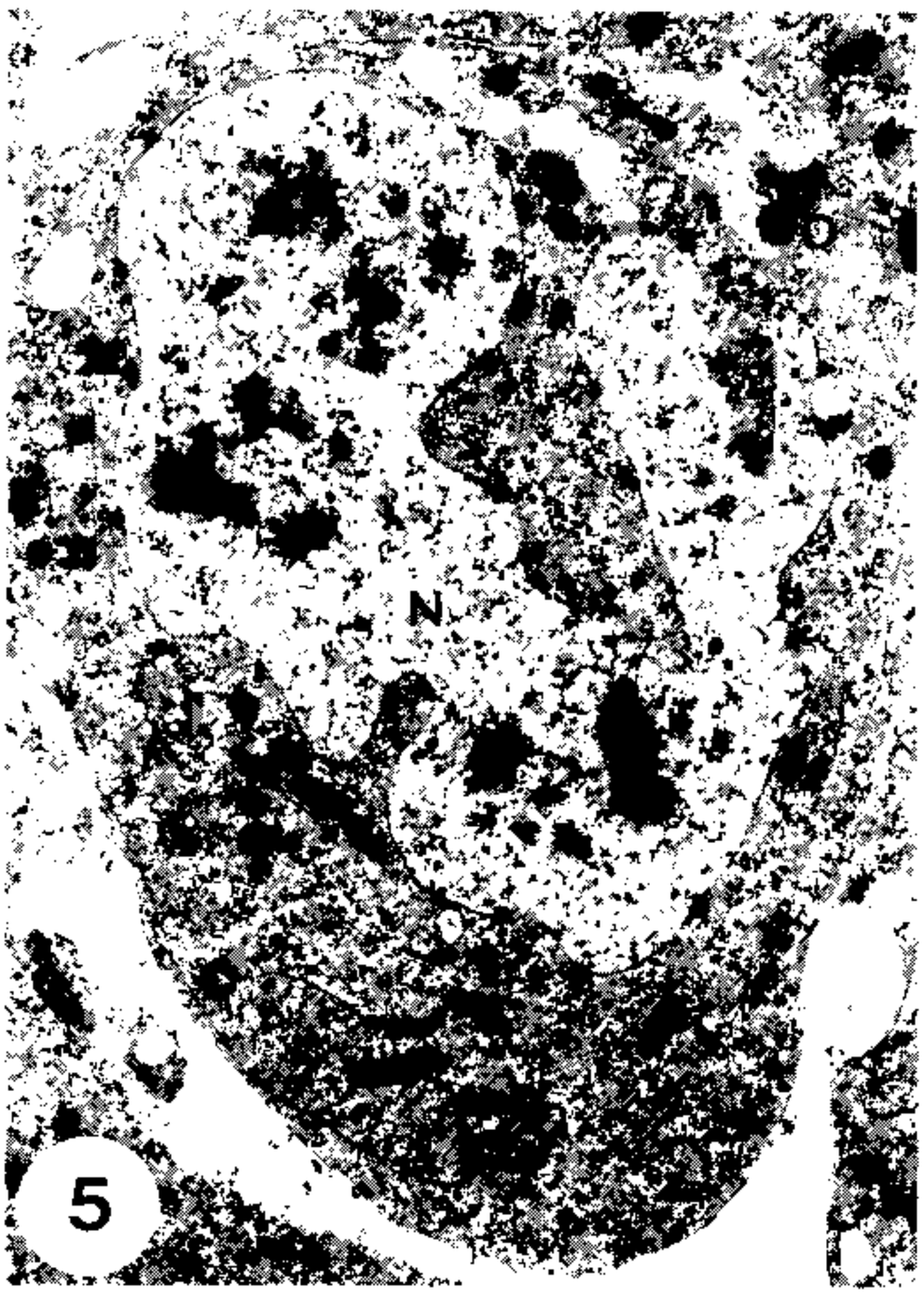
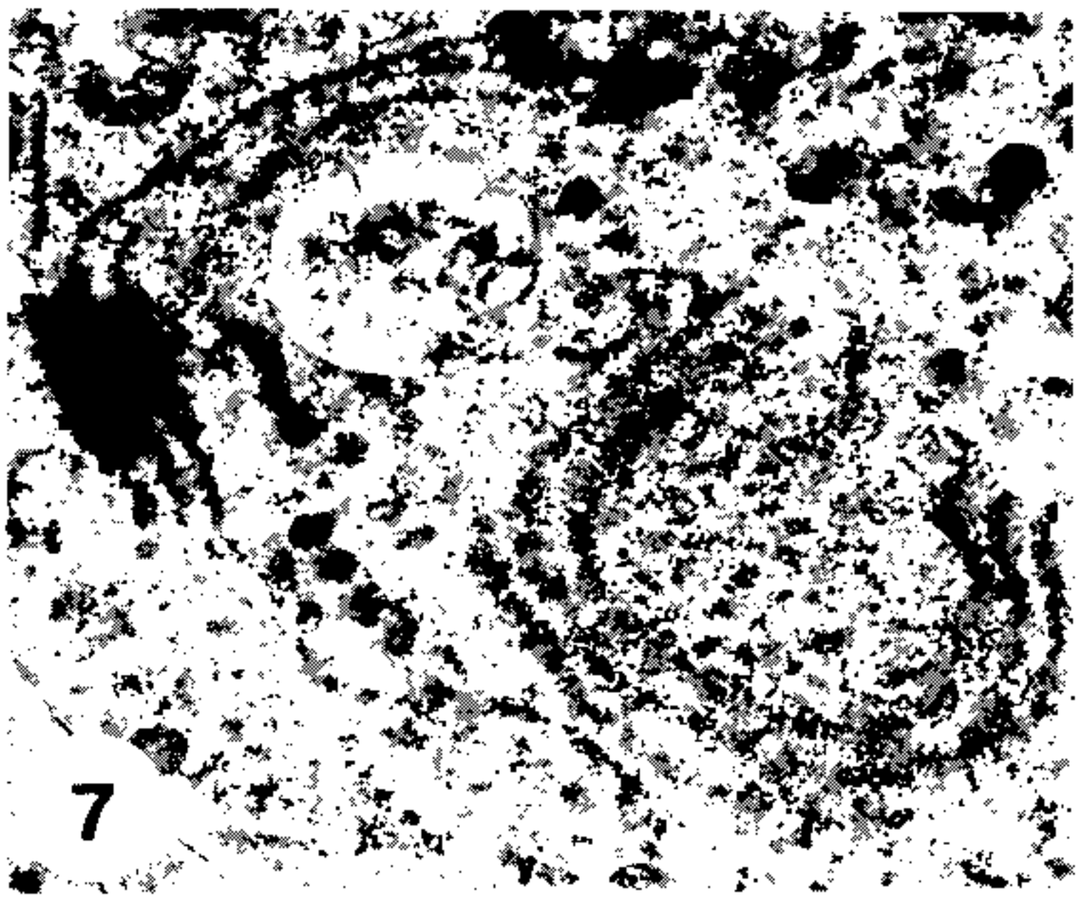
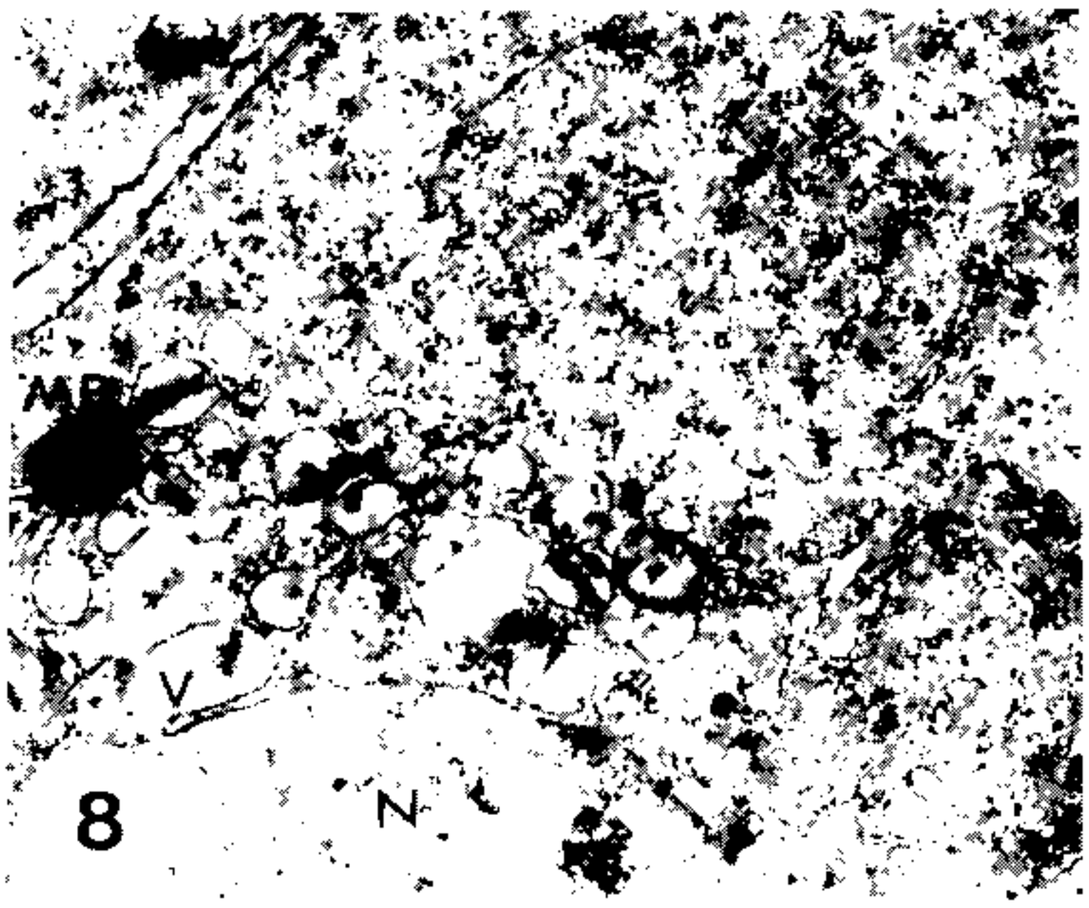


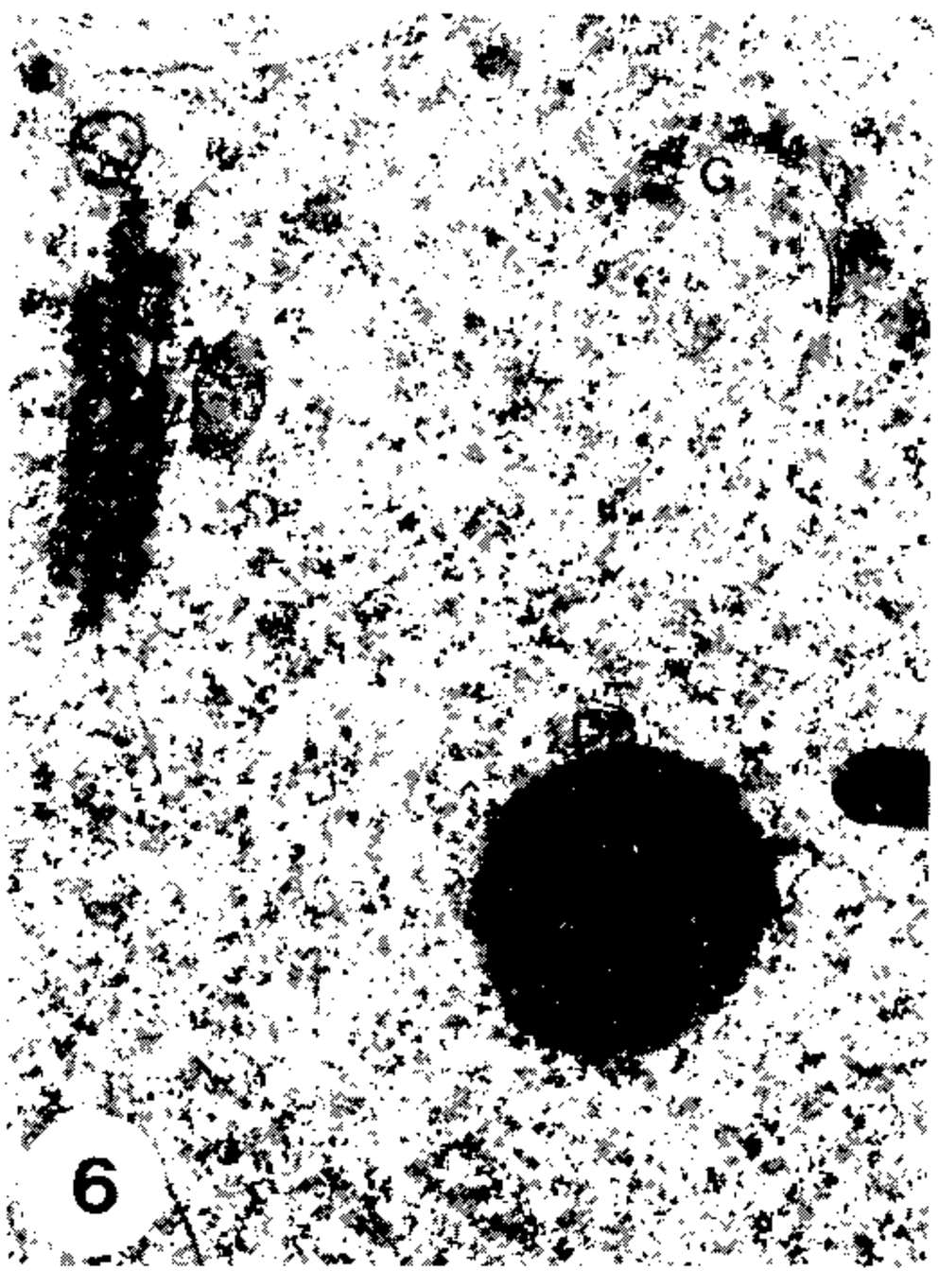
Fig. 1: early cyst with one spermatogonium. L = lipid drop; MI = mitochondria; N = nucleus; NU = nucleolus. X 11000. Figs 2, 3, 4: pre-mitotic spermatogonia. PG = perichromatic granules; L = lipid drop; MI = mitochondria; N = nucleus; B = cytoplasmic bridge; arrow = granule with 90 to 120 nm diameter; double arrow = granules with 60 to 90 nm diameter. Fig. 2 X 15500; Figs 3 and 4 X 16500.

chromatic granules from 90 to 120 nm in diameter were observed in the nuclei during interphasic periods. Similar granules were also observed in the cytoplasm, next to a deep penetration of the nuclear envelope (Fig. 2). Mitochondria were either isolated or organized in clusters having an electron-dense material in the center (Fig. 3), or associated with lipid droplets (Fig. 4). Other stron-









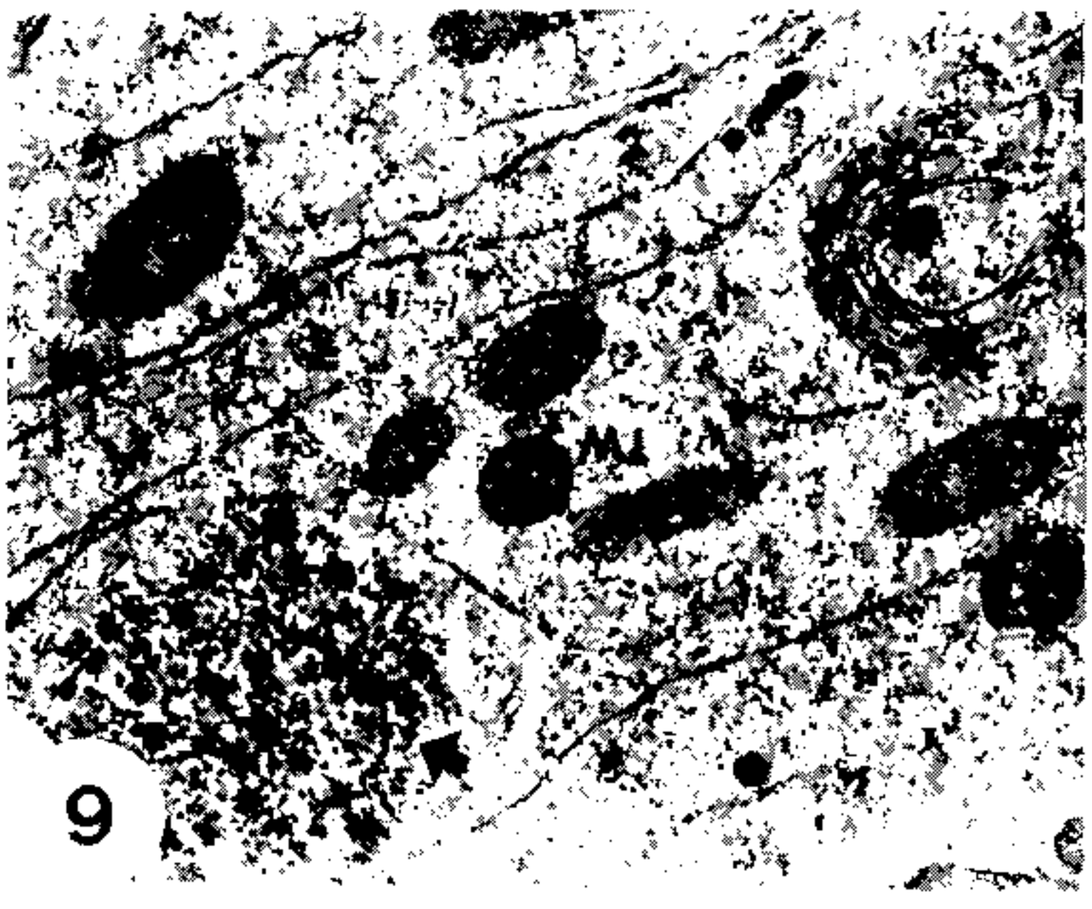


Fig. 5: spermatocyte in prophase I. D = diplosome with a short microtubular organella developed from one of the centrioles; G = Golgi; MI = mitochondria; N = nucleus; Arrow = granules with 90 to 120 nm diameter. X 13000. Figs 6, 7, 8, 9: different views of spermatocytes in prophase I. DB = dense body around 1 μm diameter; MB = multivesicular body; G = Golgi; LA = "lamellae anulata"; M = membranes; MI = mitochondria; V = heterogeneous vesicles; Arrow = granules around 30 nm diameter. Figs 6 and 7 X 21000; Fig. 8 X 26600; Fig. 9 X 28800.

gly electron-dense granules with a 60 to 90 nm diameter, were randomly distributed in the cytoplasm or associated with lipid droplets (Fig. 4).

Like spermatogonia, spermatocytes at the beginning of the meiotic prophase I, had granules varying from 90 to 120 nm in diameter close to the cytoplasmic face of the nuclear envelope. Short microtubular organelles developed from the centrioles (Fig. 5). Besides this there was an extensive system of membranes to which the "lamellae anulata" were associated (Fig. 7). The "lamellae anulata" were formed by at most five cisterns, with

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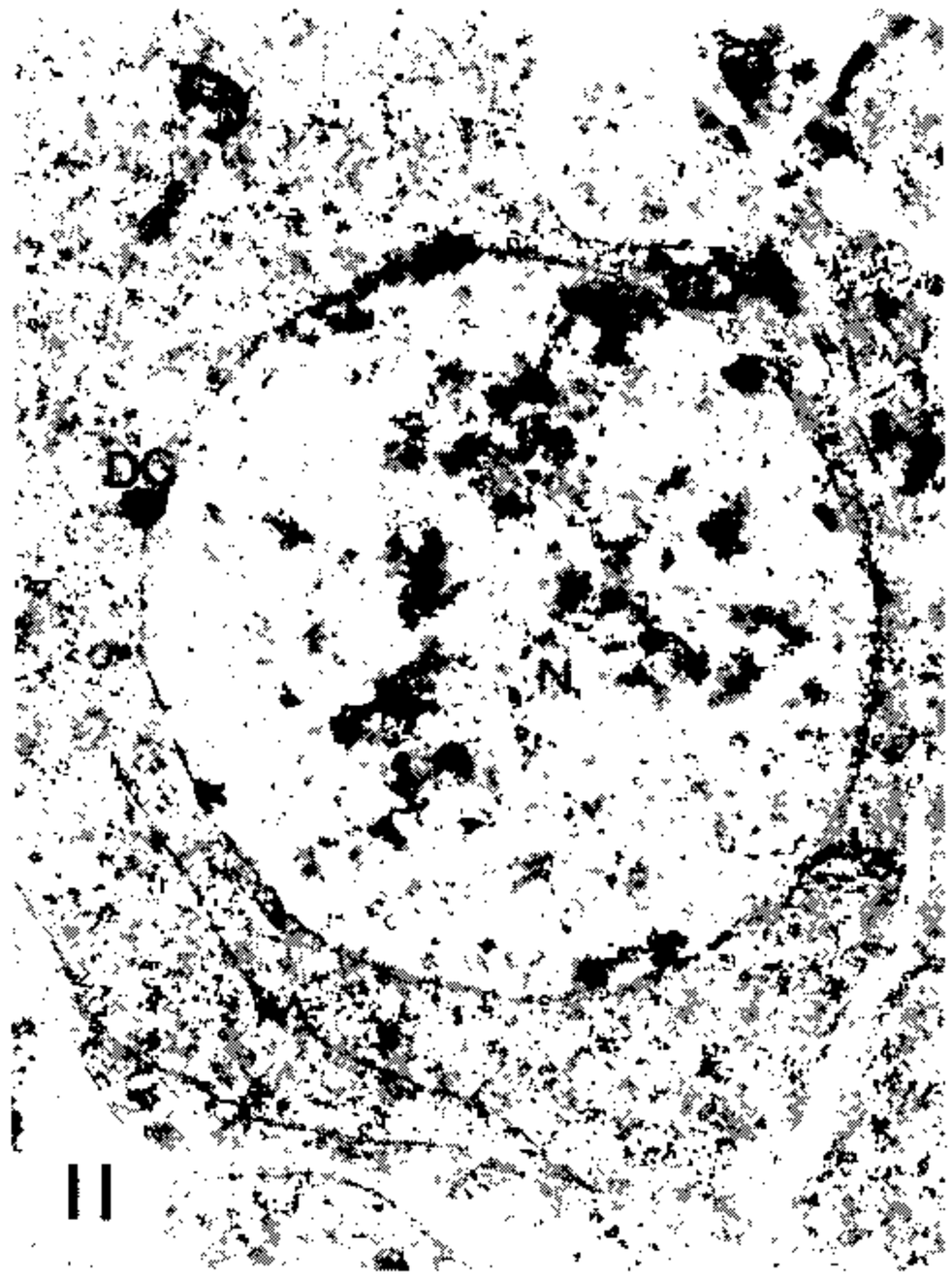

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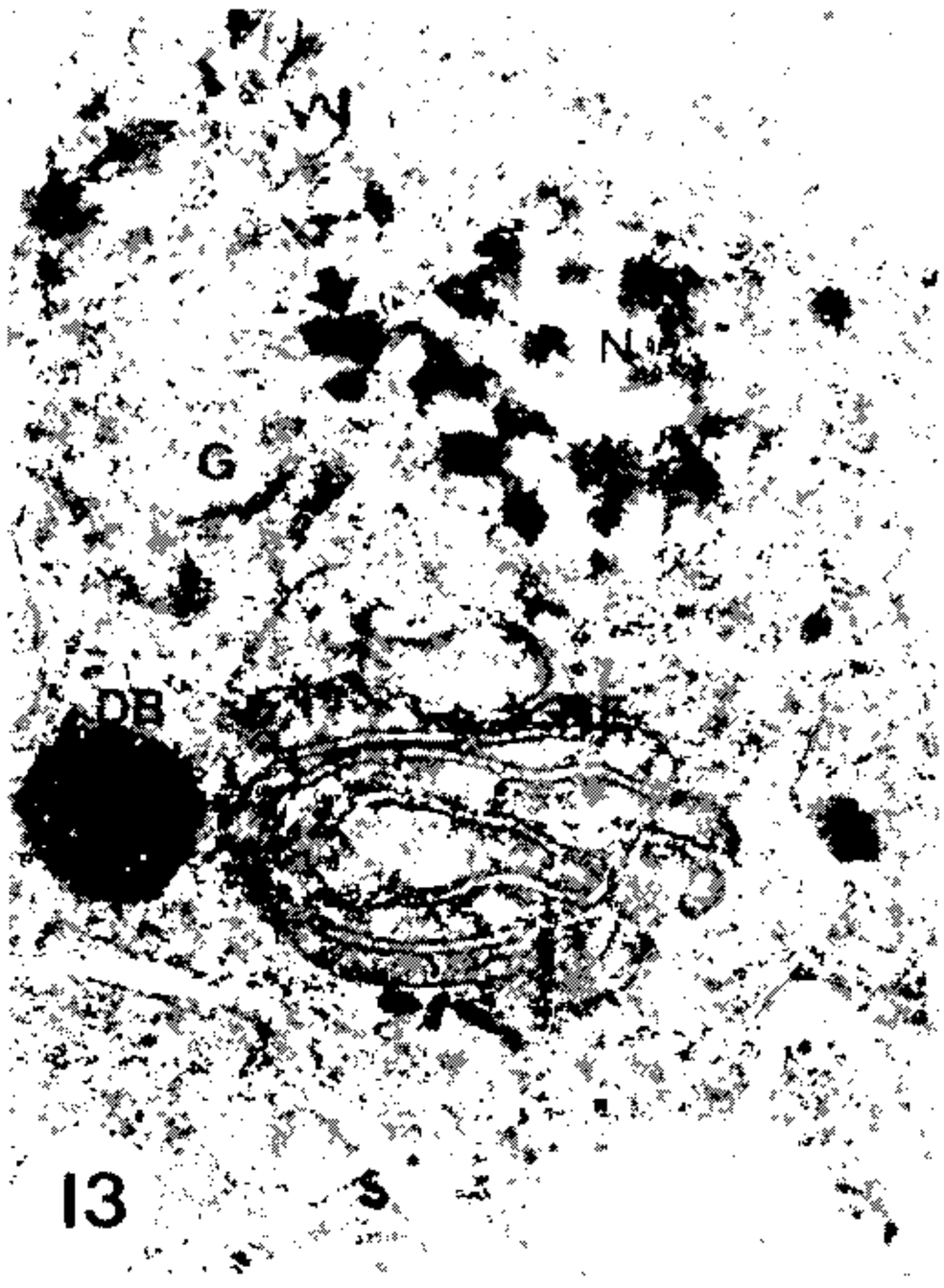

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Fig. 10: spermatocyte in telophase. CH - condensed chromatin; PG - peri chromatic granules; MI - mitochondria; N - nucleus. X 20100. Figs 11, 12, 13: early spermatids. DB - dense body around 1 µm diameter; FL - flagellum; G - Golgi; DG - dense granule around 120 nm diameter; LA - "lamellae anulata"; M - membranes; N - nucleus; NE - "nebenkern"; arrow - dense granules of variable diameter; Fig. 11 X 21000; Fig. 12 X 16500; Fig. 13 X 21000.

pores similar to nuclear pores. They were linear, organizing themselves in stacks, and appearing immersed in an electron-dense material without a definite outline (Figs 6, 7). In addition to the "lamellae anulata" other spherically dense bodies, about 1.0 µm in diameter and clusters of elec-

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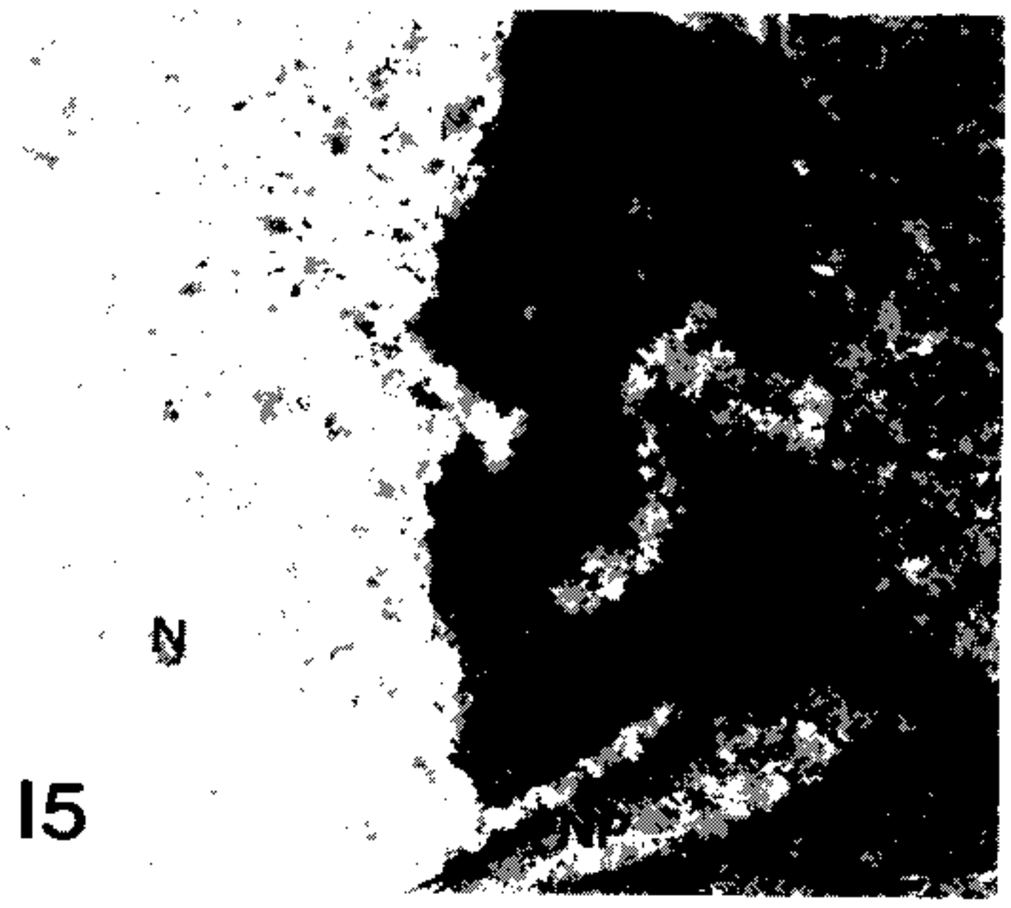

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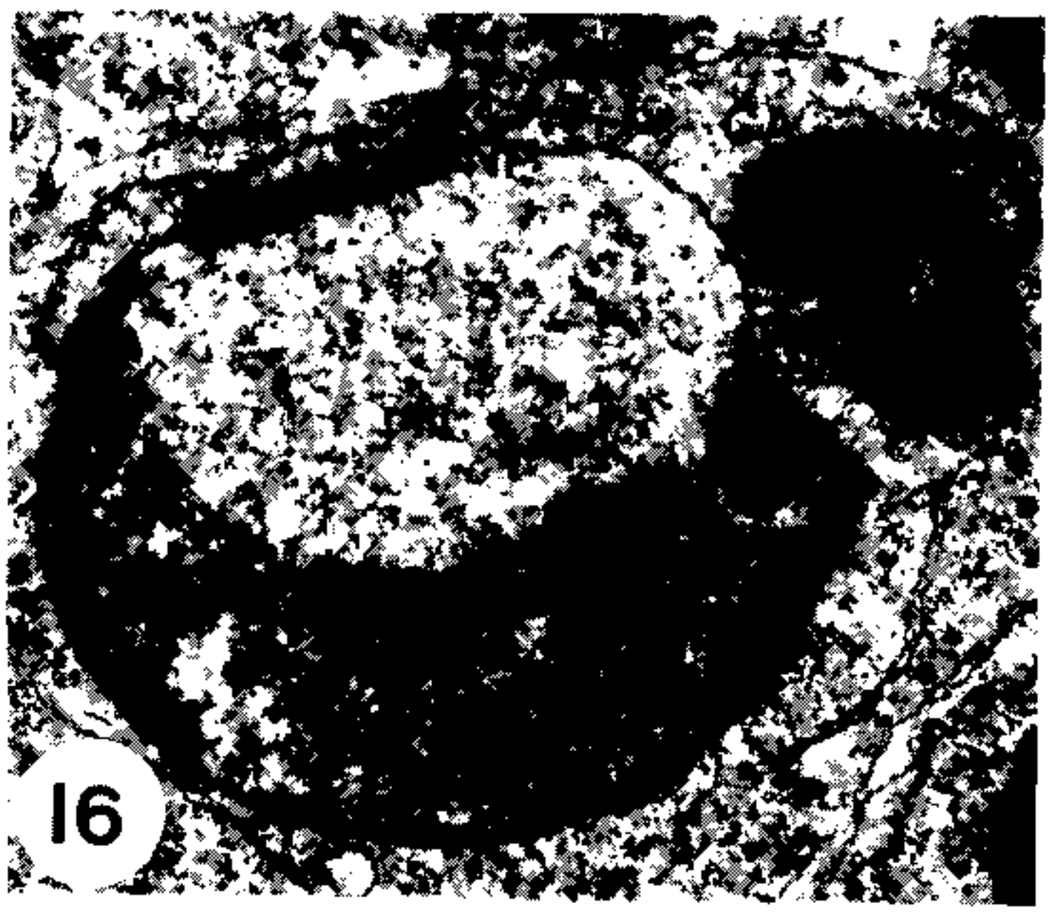

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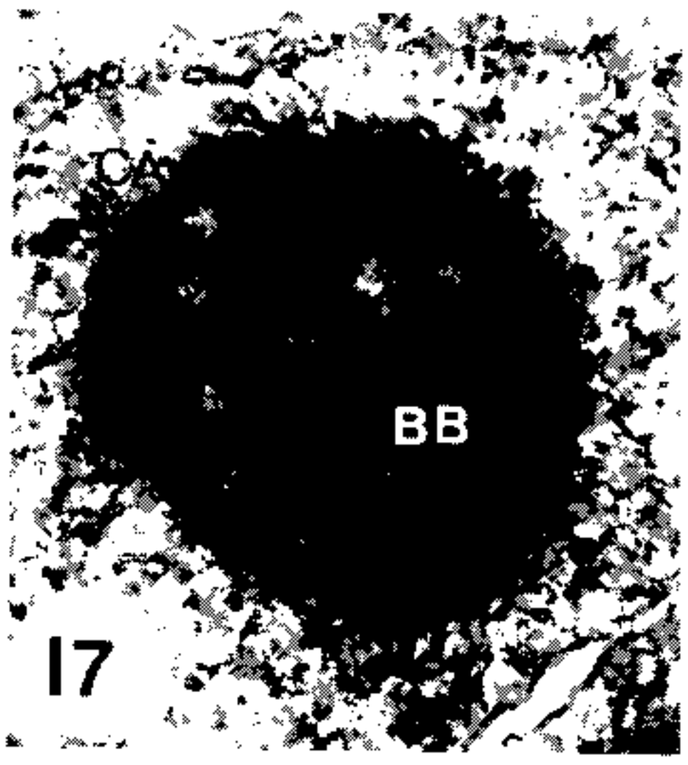

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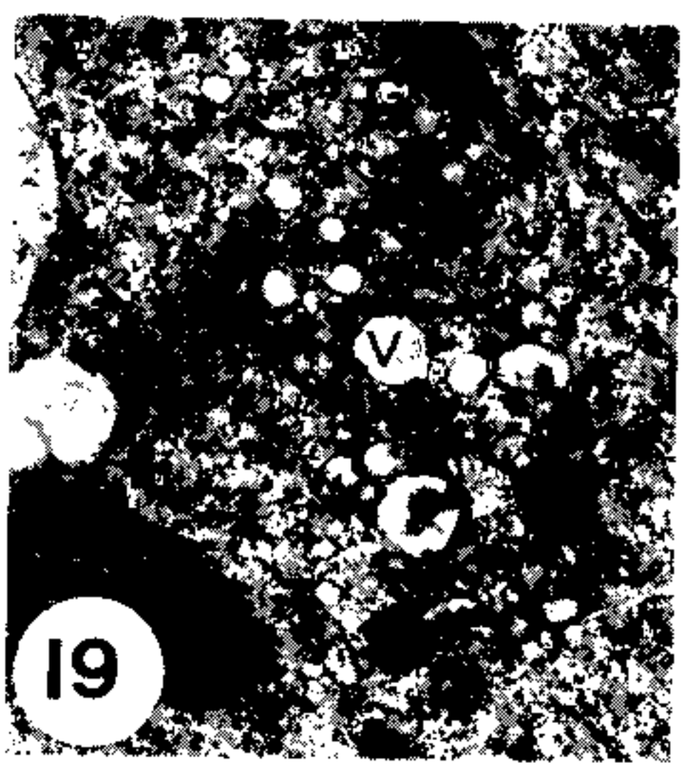

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Fig. 14: elongating spermatid. N - nucleus; NE - "nebenkern"; NP - nuclear pores; Arrow - dense granules around 30 nm diameter. X 20800. Figs 15, 16, 17: elongating spermatids - base of flagellun region. CA = centriole adjunct; BB - basal body; DG - dense granule; N - nucleus; NP - nuclear pore. Fig. 15 X 20300; Fig. 16 X 26400; Fig. 17 X 37400; Fig. 18: elongating spermatids - caudal end. MB - multivesicular body. X 52200; Fig. 19: elongating spermatids - views of cytoplasm. MB = multivesicular body; G - Golgi; V - heterogeneous vesicles. X 23400.

tron-dense particles, about 30 nm in diameter, appeared in the cytoplasm (Fig. 9). A well-developed Golgi apparatus, randomly dispersed mitochondria, multivesicular bodies and vesicles with a heterogeneous content (Figs 6, 8, 9) were also observed.

The only type of "nuage" observed during the entire meiosis, was a cluster of particles about 30 nm in diameter.

By the end of telophase II, the nuclei of the newly formed cells assumed a spherical shape, and their still condensed chromatin appeared surrounded by perichromatic granules varying in length from 90 to 120 nm (Fig. 10). At the beginning of their differentiation the spermatids presented spherical nuclei and diffused chromatin. Electron-dense granules, of about 120 nm in diameter, could be occasionally observed opposed to the cytoplasmic face of the nuclear envelope (Fig. 11). Like the spermatocytes, the spermatids presented "lamellae anulata". They were linear, continuous with the endomembrane system, being formed at most by three stacks of cisterns close to the nuclear envelope; they were immersed in an electron-dense material with an indefinite outline, reaching from the nuclear pores to the lamellar pores (Fig. 12). The presence of a well-developed Golgi apparatus, a cluster of mitochondria composing the "nebenkern", early flagellum, elongated membranes, and randomly dispersed spherically dense bodies with diameters varying in lenght from 0.5 to 1.0 μm and others varying in length from 100 to 300 nm were also characteristic of these cells (Figs 11, 12, 13).

During the initial period of spermatid elongation the cytoplasm contained clusters of particles, about 40 nm in diameter, a deposit of electron-dense material around the base of the flagellum (the centriole adjunct) and occasional electron-dense granules, approximately 0.2 by 0.4 μm in length attached to the nuclear envelope (Figs 14, 15). The material around the flagellum base increased, forming a dense mass intermingled with irregular spaces filled with cytoplasm (Figs 16, 17). Multivesicular bodies and vesicles of heterogeneous content were found in the cytoplasm (Fig. 19).

Multivesicular bodies were also at the caudal end of the elongated spermatids (Fig. 18).

DISCUSSION

The electron-dense granules, varying from 90 to 120 nm in diameter observed in the spermatogonia, spermatocytes I and initial spermatids of *D. hominis* associated to the pore region of the nuclear envelope, had dimensions and electron density similar to the perichromatic granules found in the nuclei. The position occupied by such granules and their similarity to the perichromatic granules suggested the occurence of an intense process of synthesis and transport of material from the nucleus to the cytoplasm.

Russel and Frank (1978) described rat spermatogonia and spermatocytes granules of the same dimension and electron density, located next to the Golgi or between this and the nuclear envelope. Electron-dense granules associated to the nuclear envelope of the spermatocytes I were also observed by Fuge (1976) in *Pales ferruginea* (Diptera: Tipulidae) and by Kessel in *Drosophila melanogaster* (Diptera: Drosophilidae); the granules were composed of RNA and proteins (Fuge 1976, Kessel 1981) and therefore, this is in accordance with the supposition that they must be the nuclear material being transferred to the cytoplasm.

The electron-dense material, observed among clusters of mitochondria in the cytoplasm of *D. hominis* spermatogonia, is a common feature in animal germinal cells (Eddy 1975). However, its occurrence during spermatogenesis in insects was only reported for *D. melanogaster* spermatocytes (Kessel 1981). This material, known as intermitochondrial cement, presents various types of RNAs, proteins, cytochromes and lipids (Toury et al. 1977). The fact that most of these proteins present the same electrophoretic mobility in polyacrylamide gel as mitochondrial proteins, led Toury et al. (1977) to the conclusion that their presence was associated to the origin, development and biogenesis of these organelles. The present knowledge of the interaction between the mitochondrial and nuclear genoma and the processes of synthesis and transference of the mitochondrial proteins to its interior (Darnell et al. 1990, Alberts et al. 1994), brought a new and strong support to their supositions.

Cruz-Landim (1979) studying *Myogryllys* sp. (Orthoptera) spermiogenesis observed that mitochondria appeared associated to the nuclear envelope, before the development of "nebenkern". This fact also led her to suggest the transference of material from the nucleus, which would participate in mitochondrial differentiation during spermiogenesis.

Another kind of electron-dense granule observed during *D. hominis* spermatogenesis was approximately 30 nm in diameter and occured in the form of clusters in the cytoplasm of the spermatocytes and early spermatids. Similar granules were previously reported by Russel and Frank (1978) in rat spermatocytes; according to these authors they were not ribosomes because unlike to the ribosomes, they reacted intensely to ferrocyanide.

Russel and Frank (1978) also eliminated the possibility that these granules be glycogen gran-

ules because they differed in morphology from the glycogen granules described by Russel and Burguet (1977) in rat Leydig cells. Our experience in *D. hominis* led us to believe that they really were glycogen granules. We share this supposition with Wolf et al. (1987), who described similar granules in the spermatocytes of *Orgya thyellina*. However, proper cytochemical tests for the validation of this supposition must be done.

The electron-dense spherical bodies, approximately 1.0 µm diameter, observed in the spermatocytes I and in the early spermatids of *D. hominis* are not similar to the "nuages" described by Russel and Frank (1978). However, such bodies could correspond to the "spongious bodies" observed by them in the spermatocytes I of rats, or to the "chromatoid body satellite" described by Fawcett et al. (1970) in hamster spermatocytes, or to the "dense cytoplasmic masses" observed by Kessel (1981) in *D. melanogaster* early spermatids, or even to the "lamellar bodies" reported by Henning and Kremer (1990) in *D. hydei* spermatocytes and early spermatids. They certainly correspond to the electron-dense material, described in early spermatids of *Melipona quadrifasciata anthidioidis*, as "chromatoid body" by Cruz-Landin et al. (1981). In our opinion, the electron-dense spherical bodies would be composed of RNA and proteins, just like the dense cytoplasmic masses (Kessel 1981) and the "lamellar bodies" (Henning & Kremer 1990).

The electron-dense material that accumulates around the base of the flagellum in *D. hominis* early spermatids and in most of the early spermatids of insects is known as "centriole adjunct" (Gatenby & Tahmisian 1959, Phillips 1970, Baccetti 1972, Yasuzumi 1974). The development of centriole adjunct in *Acrida lata* spermatids described by Yasuzumi et al. (1970) suggests that the dense bodies that at first appear in the cytoplasmic face of the nuclear envelope contribute to the centriole formation. According to Cruz-Landim (1979) these dense bodies were the result of an aggregation of ribosomes. The aspect and position of the centriole adjunct in the early spermatids in insects are similar to those of the chromatoid body in early mammaliam spermatids (Sud 1961, Eddy 1970, 1974, Fawcett et al. 1970, Comings & Okada 1972, Yasuzumi, 1974, Russel & Frank, 1978). The chromatoid body in mammals is an electron-dense structure, with an irregular outline, a fibro-granular aspect, in the cytoplasm, appearing during the meiotic prophase and which, in the early spermatids, migrates to the base of the flagellum. Chemical analysis of the centriole adjunct showed that it contains RNA and protein (Yasuzumi et al. 1970, Taffarel & Esponda 1980), detected previously in chromatoid bodies in mammals (Sud 1961, Eddy 1970, Soderstrom & Parvinen 1976). For this reason, we believe that the centriole adjunct of insects and the chromatoid bodies in mammals are equivalent cellular structures, as already suspected, but refuted by Yasuzumi et al. (1970).

We also observed in *D. hominis* spermatocytes during prophase I and in early spermatids, another kind of "nuage", that occured as a cloud of electron-dense material associated with "lamellae anulata". In his various studies about "lamellae anulata", Kessel (1981, 1985) reported the same type of association in *D. melanogaster*.

Kessel (1981) observed that in early spermatids "lamellae anulata" differentiate in the interior of what he described as "dense cytoplasmic masses". He also demonstrated that these dense masses correspond to the perinuclear material found in spermatocytes I, which, in the spermatids, change their position and relocate in the cytoplasm. Furthermore, Kessel (1981) demonstrated that the masses are made up of granular and fibrillar subunits comparable to the nucleolar subunits, to the material associated to the nuclear pores and to the poliribosomes, possibly being formed by RNA and proteins. Kessel (1981) based on the rhythm of the RNA and protein synthesis during spermatogenesis in *Drosophila* sp. (Olivieri & Olivieri 1965, Henning 1967, Gould-Somero & Holland 1974), and his own observations, suggested that the dense cytoplasmic masses of early spermatids contain genic products of long duration such as rRNAs and mRNAs. He also proposed the hypothesis that the activation of these products in the spermatids should be associated with the appearance of "lamellae anulata" or specifically to the pores of these lamellae. In a further study, Kessel (1985), demonstrated that in *D. melanogaster* spermatocytes I, the "lamellae anulata" which had a perinuclear location differentiate in the interior of a fibrogranular material, of possible nucleolar origin, aligned continuosly from the nuclear to the lamellar pores.

In *D. hominis*, the electron-dense material associated with the "lamellae anulata" did not have a definite outline and was observed in the cytoplasm of spermatocytes I, without a fixed localization. In early spermatids it was arranged in a continuous manner between the nuclear envelope and the "lamellae anulata". This arrangement led us to assume that there was a recent synthesis of this material, perhaps by the spermatid.

This contradicts previous data regarding the post meiotic synthesis of RNA during spermatogenesis in insects (Olivieri & Olivieri 1965, Henning 1967, Brink 1968, Gould-Somero & Holland 1974). However, it finds support in data about other animal species (Monesi 1965, Moore 1975, Kierszenbaum & Tres 1975, Iatrou et al. 1978,

Monesi et al. 1978, Hecht et al. 1986, Oliva et al. 1988, Klemm et al. 1989).

Our proposition is corroborated by Muckenthaler (1964) in *Melanoplus differentialis* and more recently by Huyser et al. (1990) in *D. hydei*.

ACKNOWLEDGEMENTS

To EM Laboratory of IB, Botucatu, for the facilities; to Mr Antonio Vicente Salvador for technical help and Mrs Sandra Aparecida Andrades da Silva for the typewritting.

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