THE FINE STRUCTURE OF CHROMOSOMES IN THE MEIOTIC PROPHASE OF VERTEBRATE SPERMATOCYTES*

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Several studies have been made with the electron microscope on the structure of chromosomes (7, 9, 8, 1, 5). The results have generally been disappointing in that they have failed to confirm the presence of structural components reported earlier by workers using the light microscope and they have failed to reveal the ordered internal structure expected from the indirect evidence of cytogenetics. In electron micrographs of ultrathin sections, the giant chromosomes of Diptera show variations in density along their length that correspond to the cross-banding visible with the light microscope (1), but in general, the chromosomes of dividing somatic cells in most animal species are surprisingly homogeneous. They lack a limiting membrane and seem to be composed of closely aggregated fine granules of uniform size and moderate density. Ordinarily no chromonemata or other oriented, filamentous subunits are visible. In dividing germ cells of crayfish and grasshopper, however, Moses (6) has recently observed in the chromosomes a central core having a characteristic fine structure. The observations presented here confirm and extend his findings by demonstrating a central dense structure in the interior of the chromosomes of dividing spermatocytes in pigeon, cat, and man.

Material and Methods

The observations recorded here were assembled from numerous electron micrographs of testicular tissue made in the course of earlier studies on spermatogenesis in pigeons, cats, and humans. In brief, the technical procedures were as follows: Small pieces of tissue approximately 2 mm. on a side were fixed by immersion for 2½ hours in 1 per cent osmium tetroxide adjusted with veronal-acetate buffer to pH 7.6–7.8. The tissue was washed briefly in distilled water, dehydrated in increasing concentrations of ethyl alcohol, infiltrated and embedded in *n*-butyl methacrylate. Sections 25 to 30 mμ in thickness were cut on a Porter-Blum microtome and examined with RCA electron microscopes models EMU 2E and EMU 3B. For a more detailed account of the methods, the reader is referred to the previous papers on the fine structure of the testis (2–4).

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OBSERVATIONS

In electron micrographs of the prophase nucleus of pigeon spermatocytes (Figs. 1 and 7) the chromosomes are recognizable as homogeneous masses of fine granules (*circa* 100 A). The chromosomes are elongated in form, and about 750 mμ in thickness with a sharply defined but irregular outline and they stand out clearly against a background of karyoplasm of very low density. Adhering to their surface are numerous small aggregations (75 to 100 mμ) of granules that have a greater density than those comprising the chromosomes. Smaller clusters of these dark granules are also distributed through the karyoplasm. Running longitudinally through the axis of each chromosome is a core consisting of three linear densities that have the appearance of parallel fibrils spaced about 300 A apart and lying in the same plane (Figs. 5 and 6). The two outer elements are of the same thickness (450 A), while the middle one is much more slender (120 A) and in some specimens is barely discernible. These structures are seen to best advantage when cut longitudinally, or nearly so, but in most sections of prophase nuclei the majority of chromosomes are cut at too great an angle. In these cases the chromosomal core is seen end-on and is easily overlooked, but on careful inspection, it can usually be made out as three dots in a row, a small central one, flanked by two somewhat larger (see at arrows in Figs. 1 and 7).

In cat and in human spermatocytes the filamentous core seems to be present very early in prophase before chromosomes, as such, are visible in the electron micrographs. Short segments of parallel fibrils cut at various angles are often seen in spermatocyte nuclei which appear quite homogeneous except for a prominent nucleolus (Figs. 2 and 3). As it winds through the karyoplasm, the ribbon-like structure formed by the parallel fibrils may be twisted on its long axis so that the outer fibrils pursue a loose spiral course around the smaller central element. Evidence for this is presented in Fig. 4. There, it is possible to follow one of the outer fibrils from the upper part of the figure, where it lies on the right side of the central dense line, to the lower part of the figure, where the same fibril appears to lie on the left of the midline. Not infrequently the filaments seem to end upon, or attach to, the nuclear membrane (Fig. 8). Later on in meiotic prophase, nebulous masses of delicate granules accumulate around the parallel fibrils to form recognizable chromosomes. To date, such fibrillar cores have not been identified in interkinesis nor in chromosomes of metaphase or anaphase. They seem therefore to arise as one of the earliest events of prophase of the first meiotic division and apparently they are transient structures which function only during this one phase of karyokinesis.

In the course of these observations on human spermatocytes a peculiar behavior of the nucleolus was noted in early prophase. The nucleolus is compact in its organization and inhomogeneous in density. A spherical central

mass is present which is more dense than the rest of the nucleolus and, in addition, there is one or more sharply demarcated vacuolar areas containing granular material of relatively low density (Fig. 8). At the stage when chromosomal cores are present in the nucleus but distinct chromosomes have not yet formed, the nucleolus is found at the periphery of the nucleus and the nucleolar vacuoles appear to be discharging their contents into the karyoplasm along the nuclear membrane (Fig. 8). This phenomenon has been observed repeatedly in human spermatocytes but has not been seen in other species. Its significance in relation to cell division is entirely unknown.

DISCUSSION

The foregoing description of the central structure of the chromosomes in pigeon, cat, and human spermatocytes differs somewhat from Moses' interpretation (6) of this structure in the meiotic chromosomes of crayfish and grasshopper. According to his description, the chromosomal core when cut longitudinally, had the appearance of a series of alternate, parallel light and dark lines. The three-dimensional form postulated to account for this appearance was that of a slender dense rod embedded in a less dense material and surrounded by varying numbers of thin concentric shells. No such concentric organization of the central structure of the chromosome was observed in the species examined in the present study. Instead of a cylindrical form, the core appears to be a ribbon-like structure with its margins formed by two parallel dense fibrils evenly spaced on either side of a third slender linear density. It is difficult to determine from our micrographs whether the narrow line in the middle should be regarded as a third fibril of smaller size, or whether it is merely a linear condensation of material at the interface between two cylinders of karyoplasm organized around the two larger lateral fibrils. The latter interpretation seems somewhat more acceptable. One can speculate that the two dense fibrils and their surrounding envelope of less dense karyoplasm correspond in some way to the chromonemata of light microscopy. On the other hand, if one is obliged to regard the midline density as an odd, third fibril it is difficult indeed to equate the components observed in electron micrographs to structures either seen with the light microscope or predicted by currently accepted cytogenetic theory. Any interpretation of the findings at the present time is probably premature but it is encouraging that current methods of specimen preparation for electron microscopy are able to preserve these delicate structures in the interior of chromosomes and it is certain that their significance will not long remain obscure.

SUMMARY

The prophase chromosomes of the first meiotic division in pigeon, cat, and man contain a central structure or core consisting of a pair of dense fibrils

(450 A) that are parallel to one another and equidistant from a delicate linear region of increased density midway between them. These parallel strands are present early in prophase and the chromosomes seem to arise by congregation and organization of the chromatin granules around them. They have not been observed in mitosis or in other stages of meiosis.

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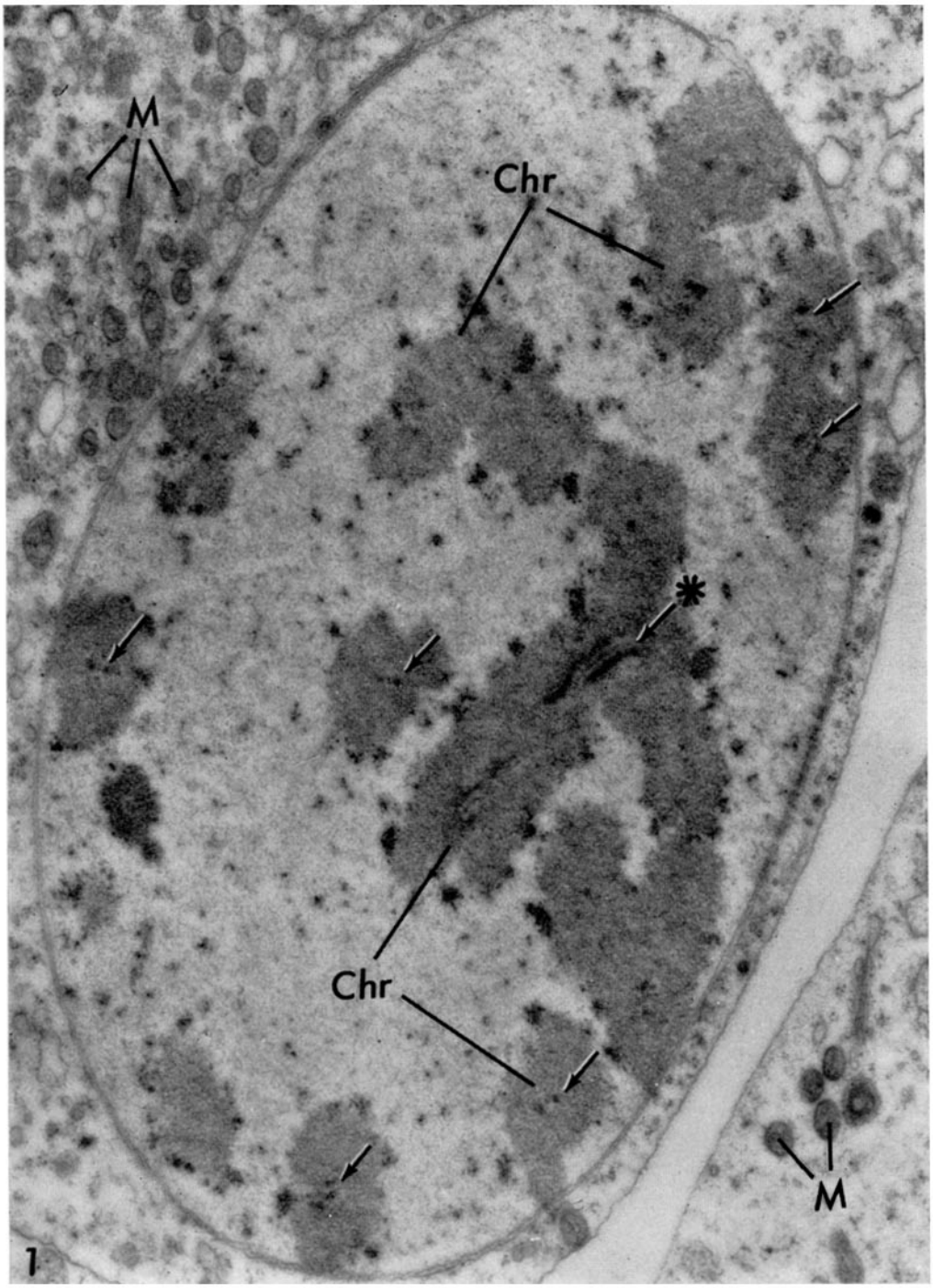
EXPLANATION OF PLATES

PLATE 99

FIG. 1. Spermatocyte from pigeon testis in prophase of the first meiotic division. The chromosomes (*Chr*) stand out clearly against a background of low density. Numerous aggregations of small dense granules are present. Some of these adhere to the surface of the chromosomes, others are randomly distributed through the karyoplasm. In the interior of the chromsome marked with an asterisk, three, parallel linear densities are visible. Two of these are interpreted as fibrils that form a core running longitudinally through the axis of the chromosome. At the several other arrows these structures are seen end-on in chromosomes cut nearly transversely. The cytoplasm adjacent to the nucleus is crowded with very small mitochondria (*M*). × 26,000.



(Fawcett: Chromosomes in meiotic prophase of spermatocytes)

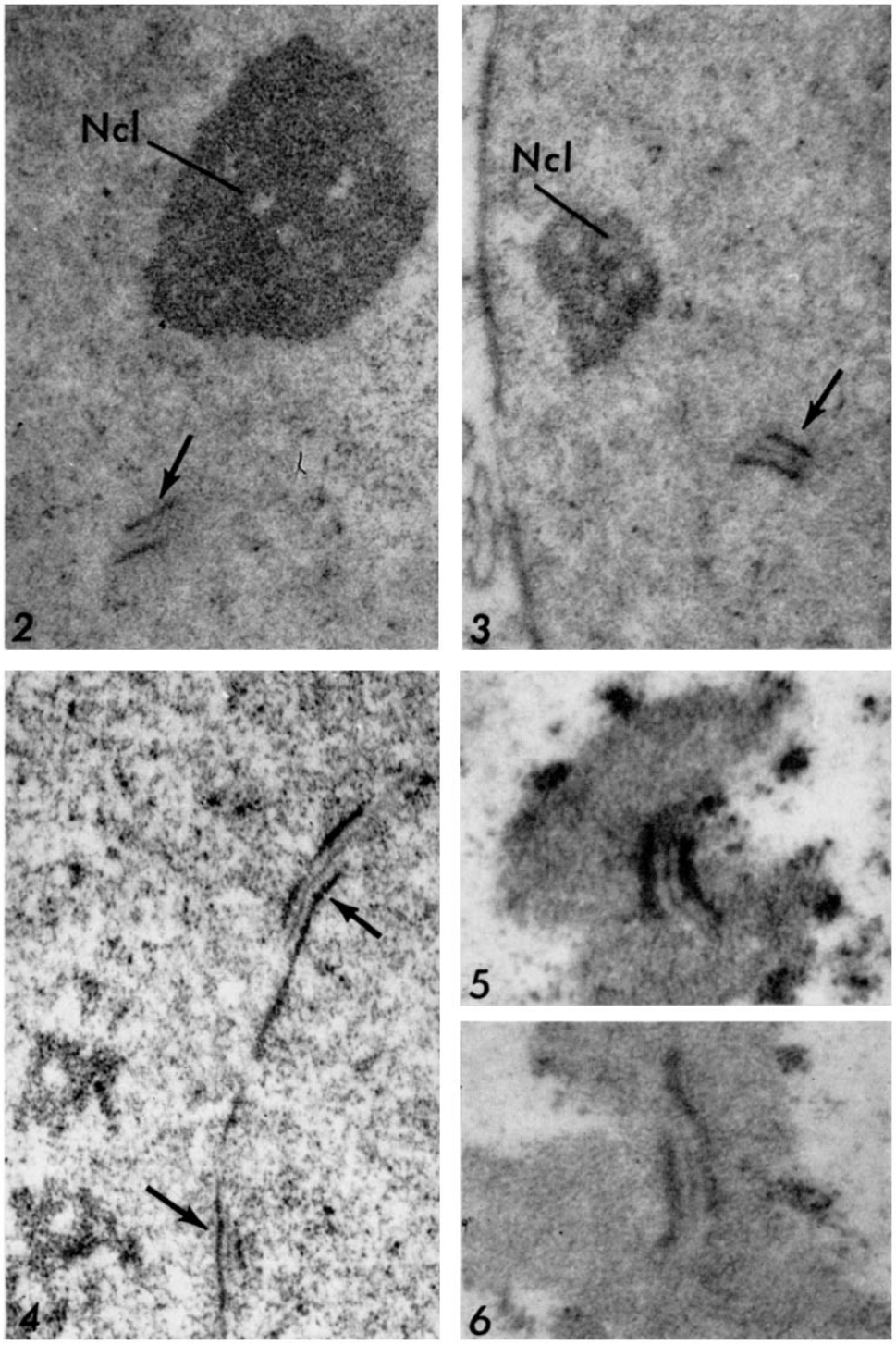
PLATE 100

FIG. 2. A small area of the nucleus of a spermatocyte from human testis. Near the nucleolus (*Ncl*) are short segments of parallel fibrils (at arrow) that form ribbon-like structures running a serpentine course through the karyoplasm. This nucleus is believed to be in very early prophase. No chromosomes are yet recognizable but it is thought that they would soon have arisen by condensation of chromatin around the filamentous structures illustrated here. × 29,000.

FIG. 3. Another example of parallel linear structures of greater density in the nucleus of a human spermatocyte. When cut transversely, these appear as two or three dots in a row and hence are generally overlooked. It is only when their course coincides with the plane of the section for a short distance that they are easily recognizable as fibrils. × 30,000.

FIG. 4. An area of karyoplasm from a cat spermatocyte. In this micrograph a twisting of the ribbon-like structure is suggested by the fact that the fibril situated on the right at the upper arrow can be followed downward toward the lower arrow where it is clearly on the left. × 28,000.

FIGS. 5 and 6. Chromosomes of pigeon spermatocytes at higher magnification. A short segment of the fibrous core has been cut longitudinally. It is seen to consist of two parallel dense structures about 450 A thick interpreted as fibrils. These run along either side of a less dense zone about 700 A wide. In the middle of this zone is a linear density about 150 A in thickness. It is not clear whether this is a third fibril or simply an interface between two cylindrical zones of karyoplasm organized around the lateral fibrils as centers. × 48,000.

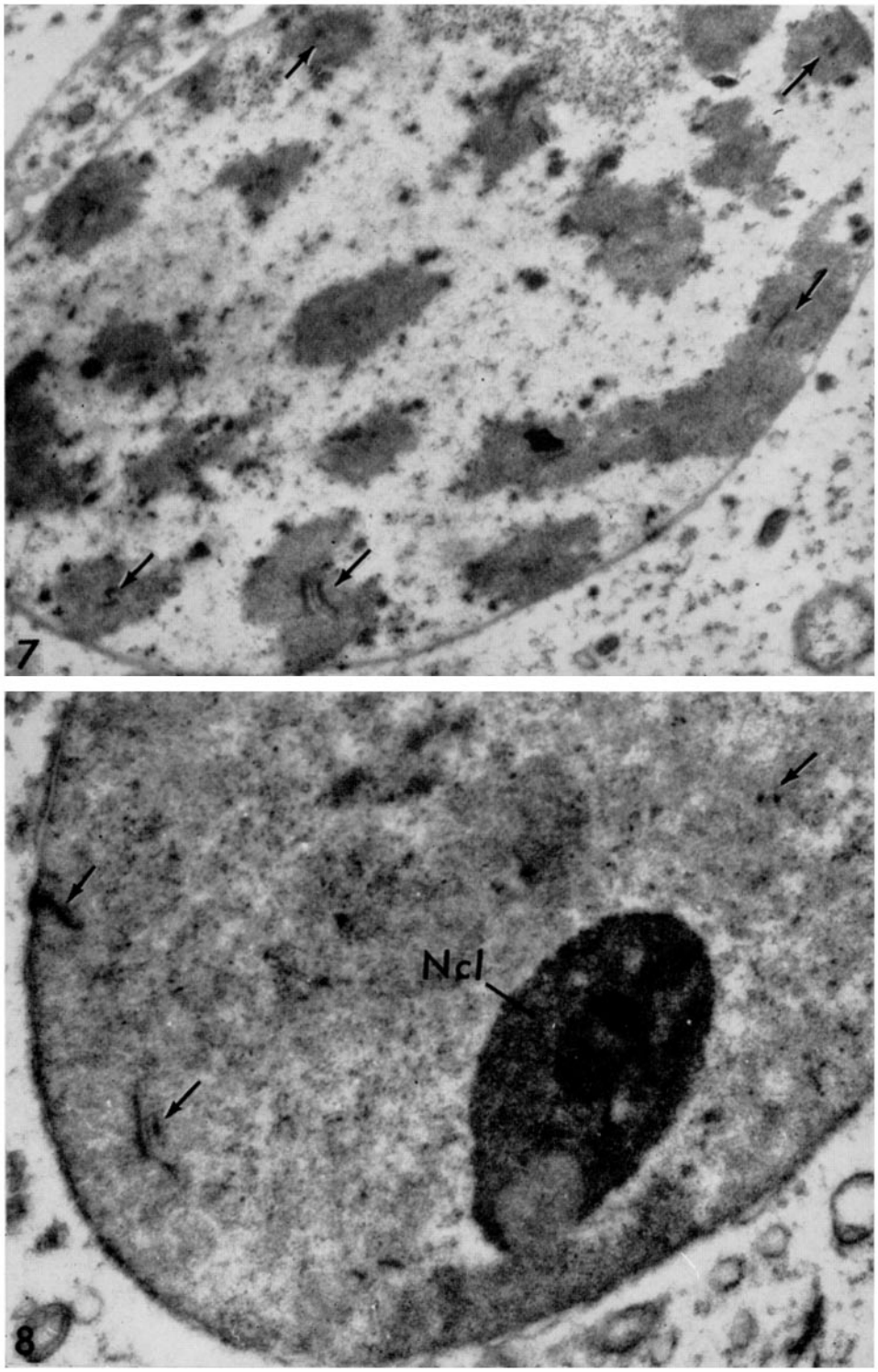


(Fawcett: Chromosomes in meiotic prophase of spermatocytes)

PLATE 101

FIG. 7. Low power electron micrograph of the nucleus of a pigeon spermatocyte in prophase. The chromosomes are, for the most part, cut transversely. The several arrows point to the places where the chromosomal cores are visible. × 20,000.

FIG. 8. A portion of the nucleus of a human spermatocyte believed to be in very early prophase. The fibrous cores of future chromosomes are seen at the arrows. One at the upper right is cut transversely and hence appears as a pair of dots. At the left, one of the dense fibrils ends in close relation to the nuclear membrane. The nucleolus (*Ncl*) has an oval central mass of greater density and a vacuole containing fine granular material of relatively low density. The vacuole appears to be discharging its contents into the karyoplasm adjacent to the nuclear membrane. × 25,000.



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