The Fine Structure of the Meiotic Spindle of the Crayfish.*

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Although the existence of a fibrous orientation within the living spindle can no longer be doubted (3, 6, 7), spindle fibers have been demonstrated with the electron microscope only in a small number of vertebrate tissues (1, 4). Centrioles have previously been investigated in interphasic and dividing cells of vertebrates (1, 4) and as basal granules in sperm middle pieces (2, 11). Centromeric regions, identified as the points of termination of spindle fibers at the chromosomes, have been demonstrated as granular condensations in a micrograph published by de Harven and Bernhard (4). Species of crayfish seemed suitable objects for a continuation of this work. The high chromosome number of about 100 bivalents offers the possibility of frequent encounter and identifications of centromeres and spindle fibers in the thin sections.

Methods

Thin sections from the testis of *Cambarus virilis* were used for this study. The testis was rapidly removed from the decapitated animal and fixed in 1 per cent osmium tetroxide which was buffered to a pH of 7.4 with veronal acetate. The tissue was embedded in *n*-butyl methacrylate and the plastic containing 1 per cent 2,4 dichlorobenzoylperoxide was polymerized by ultraviolet light. The thin sections were cut with a Porter-Blum microtome equipped with a glass knife. The electron microscope used was an RCA EMU-3b, operated at 50 kV.

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Results and Discussion

Axially sectioned meiotic spindles show the centrioles as cylinders of about 0.15 to 0.2 μ diameter and 0.3 μ length. In favorably oriented longitudinal sections of spindles two such cylinders were seen side by side during the first maturation division. In such cases, one of these cylinders always appeared cut across while its sister structure is sectioned through the axis or parallel to it through the cylinder wall (Fig. 2). Both cylinders must therefore be oriented at right angles to each other and to the axis of the spindle. Micrographs from the second division (metaphase, or telophase, as in Fig. 3) showed only one centriolar cylinder. Mitotic metaphases are reported to have one centriole at each spindle pole (1, 4). The existence of a double structure at each pole of the first meiotic metaphase may well be peculiar to meiosis and suggests that all four prospective spermatid centrioles are formed during the first meiotic prophase. However, further observations on both meiotic and mitotic material are necessary before a definite conclusion about the number and orientation of the centrioles can be drawn.

The immediate vicinity of the centriole is generally free of the vesicles of endoplasmic reticulum. Each cylinder is surrounded by material of moderate density which appears amorphous or else shows vague indications of a fibrillar organization (Figs. 2 and 3). Proceeding from this shell toward the interior, the density changes are sufficiently abrupt to define a cylinder wall and a light interior. Comparison of a longitudinally sectioned cylinder with its transversely cut sister structure shows the wall material to be organized into a number of fibrils or tubules. The latter are parallel to each other and the axis of the cylinder. The outlines of the in-

dividual units are but poorly defined. A determination of their number was, therefore, not possible. As the fibrils seem to occupy the whole width of the cylinder wall (400 to 600 A), only 12 to 15 such units could find space if the greatest density of packing is assumed.

Whenever fibrillae were demonstrated in the vicinity of the centriole, they seemed to enter the cylinder wall (Fig. 2). Because of the thinness of the sections, these fibrils can be followed for only short distances away from the centriole. Their possible connection with the spindle fibers remains thus uncertain. Other fibrils are extensions of the wall structure of the cylinder. Fig. 2 shows such tufts of extension fibrils on either side of the longitudinally sectioned centriole.

The appearance of spindle fibers depends very much upon the thickness of the sections. Very thin sections show only short wisps of filamentous material. Relatively thick sections show the fibers for considerable portions of their lengths if cut parallel to the spindle axis (Fig. 1). Each chromosome receives a bundle of such fibrils which terminates in an extremely dense structure. The latter is located poleward at the apex of the chromosome and is clearly differentiated from the remaining chromosomal matter. The spindle fibers are seen to be of much lower density than the membranes of the endoplasmic reticulum. Each fiber consists, in sections parallel to the spindle axis, of two delicate parallel lines which enclose a light central strip. Although problems of contrast make the visualization of these structures difficult, it appears that the two parallel lines unite wherever the section passes obliquely through a fiber bundle. It is, therefore, concluded that the fiber resembles structurally a canaliculus bordered by an extremely fine wall. The maximum width of the spindle fibers is 200 A.

The endoplasmic reticulum was found to contribute to a great extent to the spindle material. Its component vesicles are frequently elongated and preferentially oriented toward the spindle poles. In longitudinally sectioned spindles, the vesicles of the endoplasmic reticulum appear between the chromosomes of the metaphase plate (Fig. 1). These vesicles are elongated in the direction of the spindle. Cross-sectioned metaphase plates show a prevalence of small nearly circular profiles between the chromosomes. This indicates that the reticulum pervades the whole spindle body and may perhaps provide a continuous internal framework in which the chromosomes and the spindle fibers are embedded. Porter (9), in contrast this result, finds no endoplasmic reticulum in the spindle of rat sarcoma cells. However, he described elongated double structures which sometimes exceed 100 mμ in diameter and consist of a pair of tubules in close apposition. As these profiles were coated on the outside with the small granules described by Palade (8), it seems possible that one is dealing with a structure related to the endoplasmic reticulum. The reticular vesicles in the crayfish spindle were always found to be free of such small granules. Besides the endoplasmic reticulum, there is also a great amount of finely granular and seemingly amorphous material of low density within the spindle apparatus.

The results on centriole structure are in general agreement with those of de Harven and Bernhard (4) on different cells from mitotic and interkinetic vertebrate tissues and of Amano (1) on mouse lymphoblasts. The centriole appeared in all instances as a hollow cylinder of almost the same dimensions as those found in crayfish meiocytes. Identical results were obtained by Burgos and Fawcett (2) for the proximal centriole of the cat spermatid. All the authors mentioned find the wall of the centriolar cylinder composed of about nine parallel fibrils. In the present case, the fibrils were not distinct enough to be counted.

Crustacea are known to lack ciliated epithelia and are further characterized by the possession of non-flagellar sperms. The centriole can in this case be active as a spindle component only. It does not give rise to basal granules during any part of its life cycle. Yet its structural plan is basically the same as that of basal granules of cilia (5) and tail fiber granules of spermatozoa.

All available micrographs indicate a transverse orientation of the centrioles with respect to the spindle axis. This agrees with accounts in the literature, including the direction of the cylindrical centriole relative to the tail fiber in sperm middle pieces (2, 11). It is, therefore, difficult to envisage a spindle construction of nine continuous elementary fibers on the basis of simple extension of the centriolar fibrils as postulated by Amano (1). Only the nine fibrils of ciliary basal granules seem to be directly continued into the nine marginal fibrils of the cilium (5).

The spindle fibers demonstrated by de Harven and Bernhard (4) have the same dimensions as those of crayfish meiocytes (200 A). Their identity

with the chromosomal fibers of light microscopy is evident from the termination of these 200 A elements at the centromeric condensations, as shown in the present paper, and the destructive effect of colchicine treatment (4). The continuous fibers of light microscopy (see Schrader (10)) may well be identical with oriented vesicles of endoplasmic reticulum.

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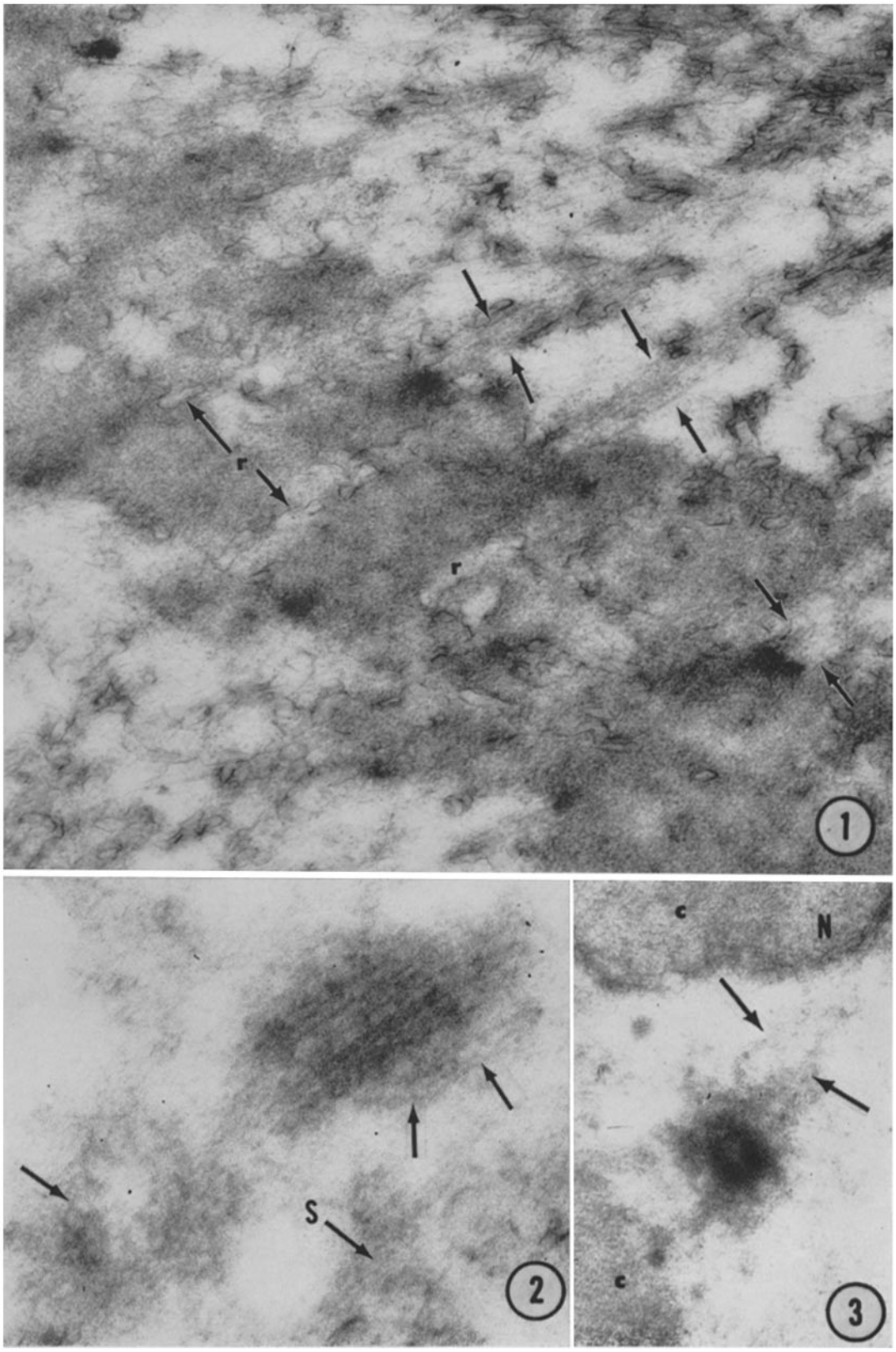
EXPLANATION OF PLATE 85

FIG. 1. Paraxially sectioned metaphase of the second meiotic division. This micrograph of a relatively thick section shows the spindle fibers for considerable portions of their lengths. Bundles of fibers (indicated by the pairs of arrows) terminate in dense granules associated with the chromosomes. Note the rows of vesicles of endoplasmic reticulum (*r*) between the chromosomes and in the half spindle region. × 31,000.

FIG. 2. Two centrioles at the pole of a metaphase spindle (first maturation division). One of the two cylindrical centrioles is sectioned at right angles to its axis (left), the other centriole is cut longitudinally, the section passing through the cylinder wall. Note also the fibrils which appear as extension tufts of the wall of the longitudinally sectioned cylinder, and the fibrous matter entering the cylinder wall (the two arrows at the right). *S*: Direction of spindle axis. × 120,000.

FIG. 3. Centriole at telophase II (early spermatid), sectioned at an acute angle through the cylinder axis. Note the dense fibrils of the cylinder wall and the relatively dense matter surrounding the centriole. Some of this material appears to be fibrous (arrows) and might actually represent contracted spindle fibers. Chromosomes at *c*; the nuclear membrane has been reformed at *N*. × 45,000.



(Ruthmann: Meiotic spindle of crayfish)