FINE STRUCTURAL CHANGES IN THE FLAGELLUM OF THE SPERMATID IN EXPERIMENTAL CRYPTORCHIDISM OF THE RAT

TOSHIO NAGANO, M.D.

From the Department of Anatomy, School of Medicine, Chiba University, Chiba, Japan

ABSTRACT

Rat testes were confined to the abdominal cavity by operation. After 1 to 26 days they were excised, fixed with osmium tetroxide, sectioned, and examined with the electron microscope. Changes in the axial filament complex of the spermatid flagellum appeared 2 days after operation, and the arrangement of filaments in the middle- and main pieces of some spermatid tails was disordered as compared to the 9 + 2 filament arrangement in the tails of the control spermatids and in other flagella and cilia. In cross-sections, the filaments in the experimental material were nine or less in number, and each of them was single and dense. Occasionally some were double, and in those instances one filament was dense and the other was light and tubular. The central filaments were obscure. In longitudinal sections, the filaments were not parallel to the main axis of the flagella or to each other. It was assumed that the central filaments were more sensitive to the experimental conditions than the peripheral pairs of filaments. Furthermore, the light filaments of the peripheral pairs were more sensitive than the dense filaments. Besides the axial filament complex, the fibrous sheath which surrounds it in the main piece was also changed. The plasma membrane of the changed flagella disappeared or became fragmented.

INTRODUCTION

According to Payne (21), in 1891 Piana first reported the structural changes occurring in rat testes removed to the abdominal cavity (22). Griffiths (11) also found these changes in the dog. Later, Moore (15–17) analyzed the factors involved in these changes in some mammalian testes and remarked that "the experimental production of cryptorchid testes was the most beautiful method for studying the biological reactions of the many elements in the gland, . . ." (15). He concluded that the changes were due to the effects of temperature. The degeneration and abnormality of mammalian spermatogenic cells in such experiments have been reported by others using the light microscope (18, 22).

Recently, with the improved methods for electron microscopy, the fine structure of normal flagella and cilia has been revealed in detail (see Fawcett's review, 9). In early studies on the fine structure of the flagella, Bradfield pointed out that the two central filaments were definitely different chemically from the other nine and that they, in particular, were more sensitive to treatment with distilled water and digestive enzymes (8).

Study of the changes in the structure of sperm flagella in cryptorchid testes should reveal interesting phenomena concerning the degenerative process. This paper deals with the fine structural changes in the flagellum of the spermatid in the

rat after experimental cryptorchidism of short duration.

MATERIAL AND METHOD

Young adult white male rats of the Donryu strain with a body weight of 250 to 400 gm were used in this study. These animals were separated from females during the experiment and were fed rat cubes and water *ad lib*.

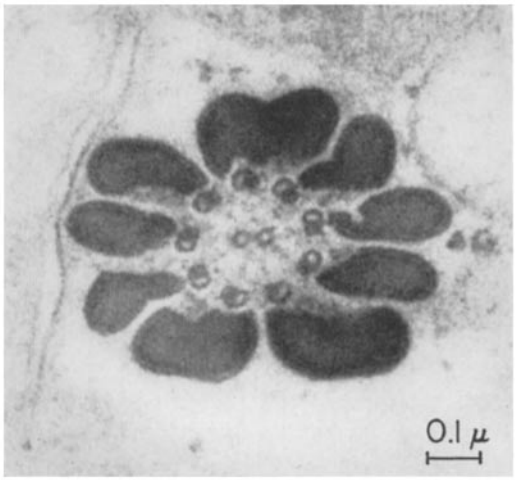


FIGURE 1 Cross-section of the middle-piece of the flagellum of spermatid from the untreated rat. One can see the 9 + 2 pattern of the axial filaments. Two central filaments with tubular appearance and nine pairs of peripheral filaments, each pair consisting of a dense and a light filament, are demonstrated. The dense filaments have processes extending toward the light filament of the next pair. Surrounding the axial filaments, nine coarse fibers are seen. Some dense material is seen between the central and peripheral filaments. × 73,000.

In the operation, which was done under ether anesthesia, the scrotum was opened and the fibrous tissue between the epididymis and the base of the scrotum was cut. Both testes with the epididymides were then displaced into the abdominal cavity through the inguinal canals. The canals and the wound were subsequently closed with stitching. In this operation, the vascular and nerve supplies as well as the vas deferens seemed to be kept intact. The testes were excised at intervals of 1, 2, 3, 5, 10, 12, and 26 days after the operation and were cut into blocks and fixed. Those from untreated animals removed and fixed similarly for use as controls.

Two per cent osmium tetroxide solution buffered with *s*-collidine was used as the fixative (6). After dehydration with ethanol, the tissue blocks were embedded in Epon 812 (13) and cut on a Porter-Blum type microtome with a glass knife.

Sections were stained with 2 per cent uranyl acetate (25) followed by lead solution (14), and examined with a JEM-4C electron microscope. The negatives were taken at a magnification of 7,000 to 13,000.

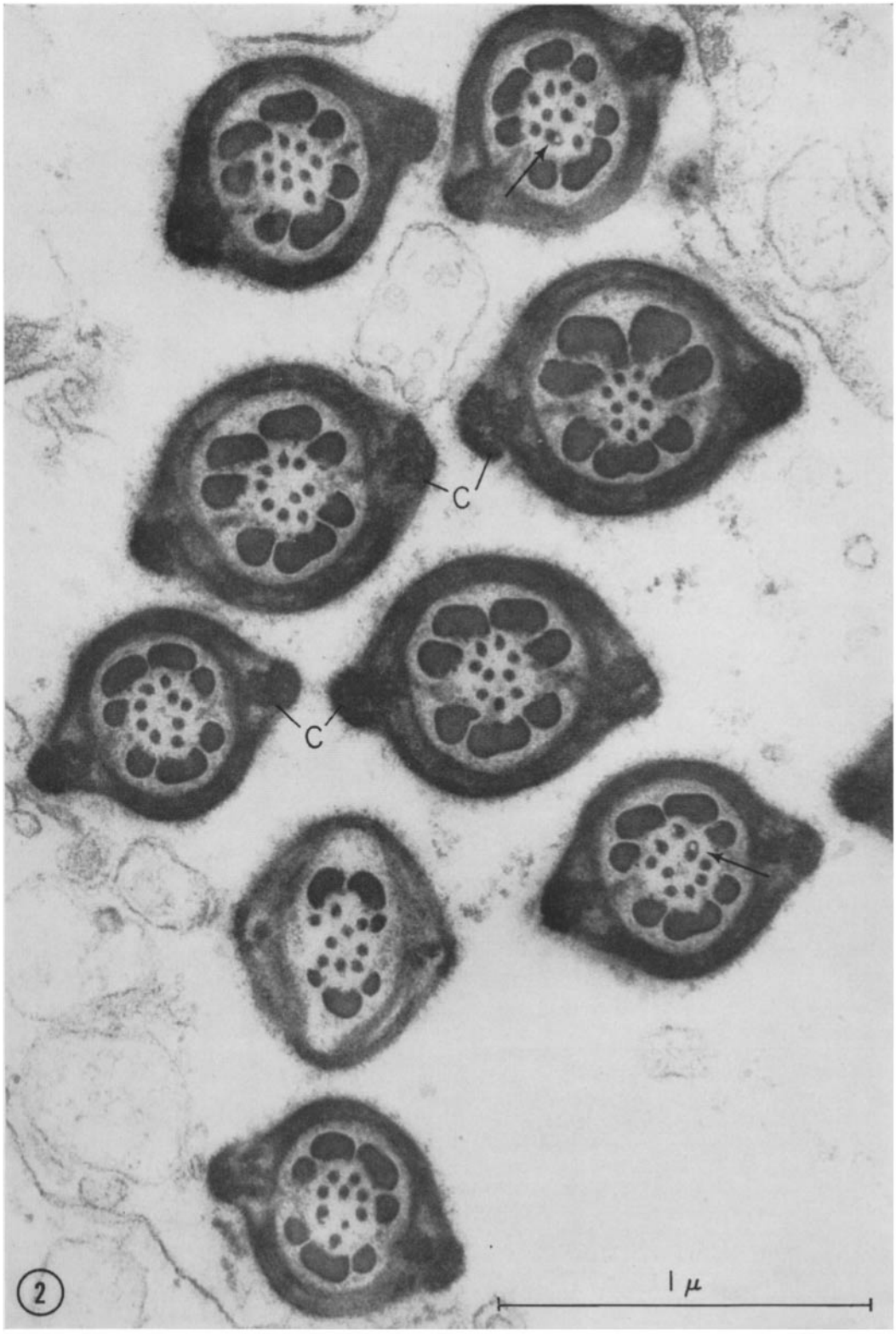
OBSERVATIONS

The Structure of the Sperm Flagellum in the Normal Rat

The present study provides few findings to be added to those already published by other investigators on the flagellum of the normal sperm in the rat (24, 26) and in other mammals (3, 5, 7, 20, 24). The axial filament complex of the rat sperm tail has basically the same pattern as that of other flagella and sperm tails (1, 4, 10, 19). A brief description of the filaments in normal sperm tails, however, will help to clarify the changes that occur in the sperm of the experimental animals.

In cross-sections, the flagellum reveals nine pairs of peripheral filaments arranged circumferentially, at the same angle with respect to the center, and one pair of central filaments which are tubular in appearance. Each peripheral pair consists of a dense and a light filament. The dense filament has arms oriented toward the light filament of the next pair. Some dense material can be observed between the central and the peripheral pairs (Fig. 1). This material may correspond to either the spoke (1) or the secondary fiber system (10), but a clear cut picture of this material is not available. In longitudinal sections, the axial fila-

FIGURE 2 Cross-sections of the main piece of the flagella of rat spermatids 12 days after confinement in the abdominal cavity. The axial filaments of each flagellum are nine in number. They are disarranged in the space surrounded by the coarse fibers. Most of the axial filaments can be seen to be single, while some of the doubles (arrows) consist of a dense and a light filament. The coarse fibers of each flagellum as well as the fibrous sheaths appear normal. The columns at opposite sides of the fibrous sheath are indicated (*C*). Note that each main piece is not covered by a plasma membrane. × 60,000.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ments are found to be parallel to the main axis of the flagellum and to each other.

In certain parts of the flagellum additional structures surround the axial filament complex. In the middle-piece, mitochondria and nine or fewer coarse fibers surround the axial filament bundle. In the main piece, this bundle is enveloped by a dense fibrous sheath. In cross-sections, this sheath is roughly elliptical in shape. On opposite sides of the sheath two "columns" can be observed (24). In the end-piece, the axial filament bundle is enclosed by the plasma membrane alone.

The Structure of the Sperm Flagellum in the Operated Rat

In testes removed on the 1st day after the operation, no structural changes in the sperm flagellum can be observed; 2 days later, however, structural changes begin to appear in some of the flagella.

The pattern of the axial filament complex is found to be disarranged and the number of filaments has decreased. The number of axial filaments is nine or less (Figs. 2, 4 to 6), instead of eleven as found in the controls. They are situated at random in the space surrounded by the outer coarse fibers and the fibrous sheath. Each axial filament appears to be single and dense, but occasionally it seems to be double, with one dense filament and one light, tubular filament in cross-section (Fig. 2). In some instances, the axial filament bundle is only slightly disarranged, and roughly eleven filaments can be identified in the end-piece with the intact plasma membrane. Several of them are dense, as shown in Fig. 7. In this picture, however, one cannot determine whether the disarrangement is due to the cryptorchidism or to the fact that at this level of section the end-piece was unaffected (see Discussion).

Neither spokes, secondary fibers, nor arms were observed in the disorganized flagella. Hardly any flagellar structure could be seen after 26 days of confinement in the abdominal cavity.

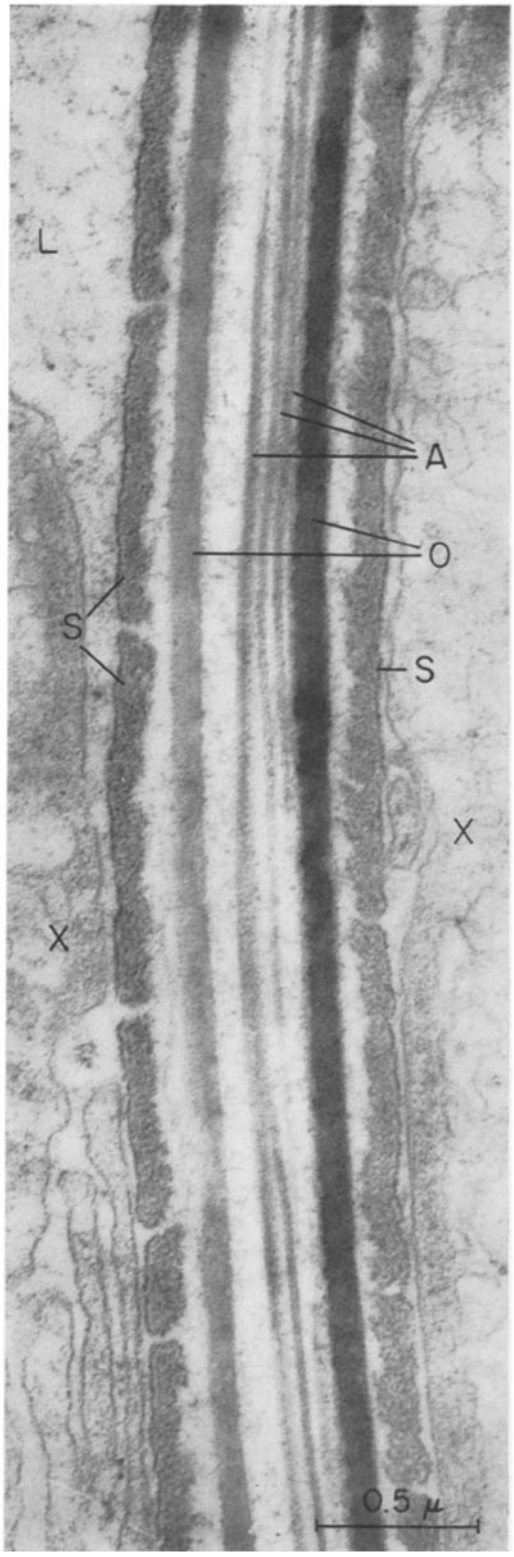

FIGURE 3 A longitudinal section of the flagellum of a rat spermatid 3 days after operation. Three axial filaments (*A*) can be seen. The outer coarse fibers (*O*) and the segmented fibrous sheath (*S*) are sectioned longitudinally. The axial filaments are not parallel to each other, and are rather tortuous in course. The distance between axial filaments is not constant. The segmented fibrous sheath (*S*) directly faces the Sertoli cell surface (*X*), and no plasma membrane of the flagellum is present. The lumen of the seminiferous tubule is indicated (*L*). × 44,000.

In longitudinal sections, some axial filaments are not parallel to the main axis or to each other and, consequently, they seem to be rather tortuous (Fig. 3).

Most of the outer coarse fibers in the middle- and main pieces appear intact during the experiment, but some of the coarse fibers are indistinct in shape and position (Fig. 4). In some cases, the fibrous sheath decreases in density and looks swollen, and the filamentous structure in it becomes apparent (Figs. 4 to 6). The behavior of the mitochondria in the middle-piece is not clear enough to state at present.

No plasma membrane is seen in electron micrographs of such disorganized flagella (Figs. 2 to 6). Furthermore, in the same section, some flagella are disorganized and have fragmented plasma membranes, while others have normal axial filaments with intact plasma membranes (Fig. 6).

DISCUSSION

In his studies on experimental cryptorchidism in the mouse, Payne (21) found with the phase microscope that the abnormally shaped, mature spermatids appeared as early as one day after operation, and he stated that the spermatids changed first, followed by the spermatocytes and spermatogonia. On the other hand, Mori (18) stated that in the rat, studied with the light microscope, the percentage of abnormal spermatozoa from the cryptorchid cauda epididymis was no different from that of the controls until 5 days later. Moore (15) reported that in the adult guinea pig testis confined to the abdomen for 7 days the germinal epithelium lost its typical arrangement; fragmentation of the germinal cells was marked and the lumen of the tubules became filled with debris in which a few ill defined sperm heads were present. Further, he demonstrated that the changes were reversible when the testes were replaced in the scrotum (15, 17). Apparently, in his experiments he did not pay much attention to the early changes. In our studies with the electron microscope, distinct changes in the axial filaments and in the acrosomes (unpublished) appear 2 days after the operation. Moreover, no such changes have been observed in the controls examined so far. Although the fine structural changes could not be demonstrated with the light microscope, the changes observed in the present material are likely to coincide with the observations of Payne (21), but not with the observations of others (11, 15, 18).

In the tails of the spermatozoa of mammals and of lower forms, as well as in protozoan flagella, it has been shown that near the tips of the flagella the axial filaments are distributed at random (1, 3, 10, 20). In such cases, the axial filaments are usually fewer than eleven in number and some are single filaments. Furthermore, some of the other elements of the complex, such as the arms or spokes, also seem to disappear. It may be thought that the changes that occur in the cryptorchid testes, with respect to the disorganization of the axial filament complex of spermatid flagella, appear similar to those taking place near the tips of the flagella, although the changes noted here occur in the middle- and main pieces. Gibbons and Grimstone noted that "the flagellar tips suggest an important role for the arms and secondary fibers in maintaining the spacing and orientation of the central and peripheral filaments" (10). The present study may strongly support their suggestion. The difference in sensitivity between the two filaments of the peripheral pair has now been demonstrated clearly, as well as the difference in density of the two filaments of the peripheral pair. The central filaments seem to disappear first. After these, the light filaments, and then the dense filaments, decrease in number. The arms and spokes or secondary fibers are so fine that it is difficult to determine when they disappear. As previously described, the central filaments are more sensitive to distilled water or enzymes than the peripheral filaments (8). Recently Afzelius found the motile flagella of the normal sperm of the worm *Myzostomum* to be without the two central filaments (2).

It is interesting to note that, in the early development of the fertilized egg of the rat studied by Izquierdo and Vial (12), one cannot see the axial filaments of the spermatozoon in the cytoplasm of the ovum after 58 hours of development, but one can see them after a 24-hour period (see their Figs. 1 to 4). In their figures, the mitochondria and coarse fibers are clearly seen. Szollosi and Ris, in their paper on sperm penetration in the rat ovum (23), observed the axial filaments, but the central filaments are obscure (see their Figs. 1 and 5 to 8). Although neither of these authors mentioned the disappearance of the filaments, a phenomenon similar to the one suggested by our findings may occur.

In addition to the axial filament changes, the fibrous sheath appears swollen. Furthermore, the

disappearance or fragmentation of the plasma membrane of the flagellum apparently plays a very important role in the process of degeneration. However, it is not known whether an indication of death of a spermatid itself or the partial change of the flagella could be classified as the "degenerative" process.

In the present study, it seems that the longer the duration of confinement of the testes in the abdomen, the greater the degenerative changes that take place. Furthermore, the germinal cells, as well as the flagella, are more greatly decreased in number. However, it is difficult at present to make quantitative observations with the electron microscope. Quantitative proof and more precise examination of the changes must be obtained in the future.

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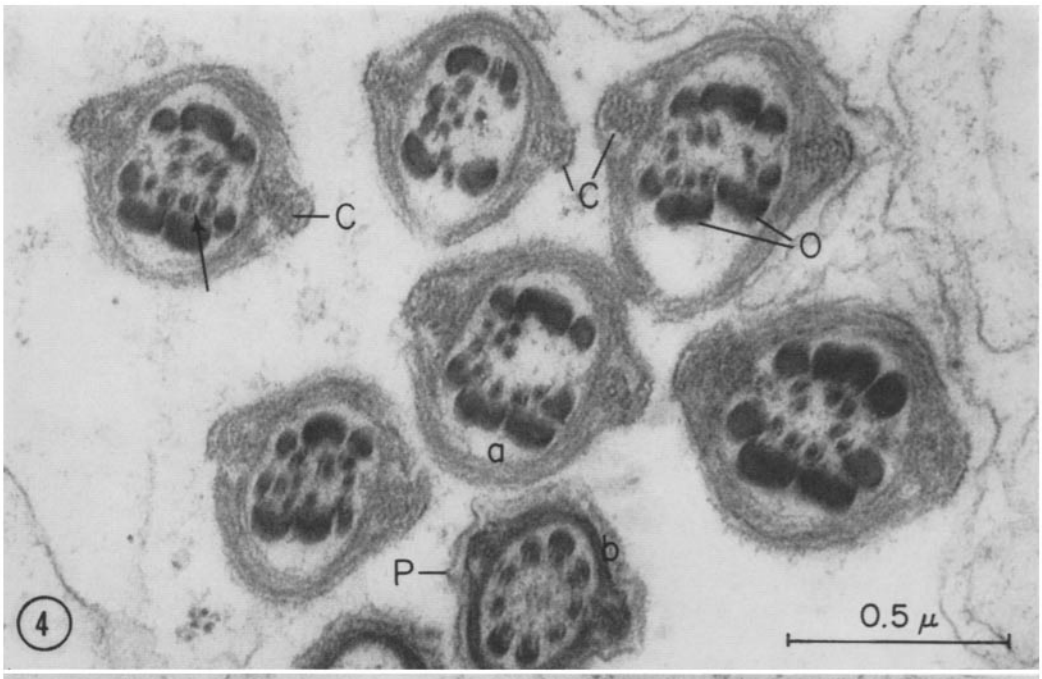
FIGURE 4 Cross-sections of the main piece of the flagella of rat spermatids 2 days after operation. In the flagellum indicated by *a*, some axial filaments are situated circumferentially, while others are displaced. In another flagellum, axial filaments are displaced but can be seen to be double (arrow). Some of the outer coarse fibers are disarranged (*O*). The flagellum indicated by (*b*) seems to be normal and to have a plasma membrane (*P*), but the other six flagella have none. The fibrous sheaths of the six are less dense than that of (*b*), and the filamentous structure and the column (*C*) are apparent. × 60,000.

FIGURE 5 Cross-sections of the main piece of rat spermatids 5 days after operation. The axial filaments are disarranged and the coarse fibers may be normal. The fibrous sheaths are swollen and disintegrated in part. One flagellum (right) is surrounded by the cytoplasm of the Sertoli cell. The plasma membrane is not seen in the other two flagella. × 52,000.



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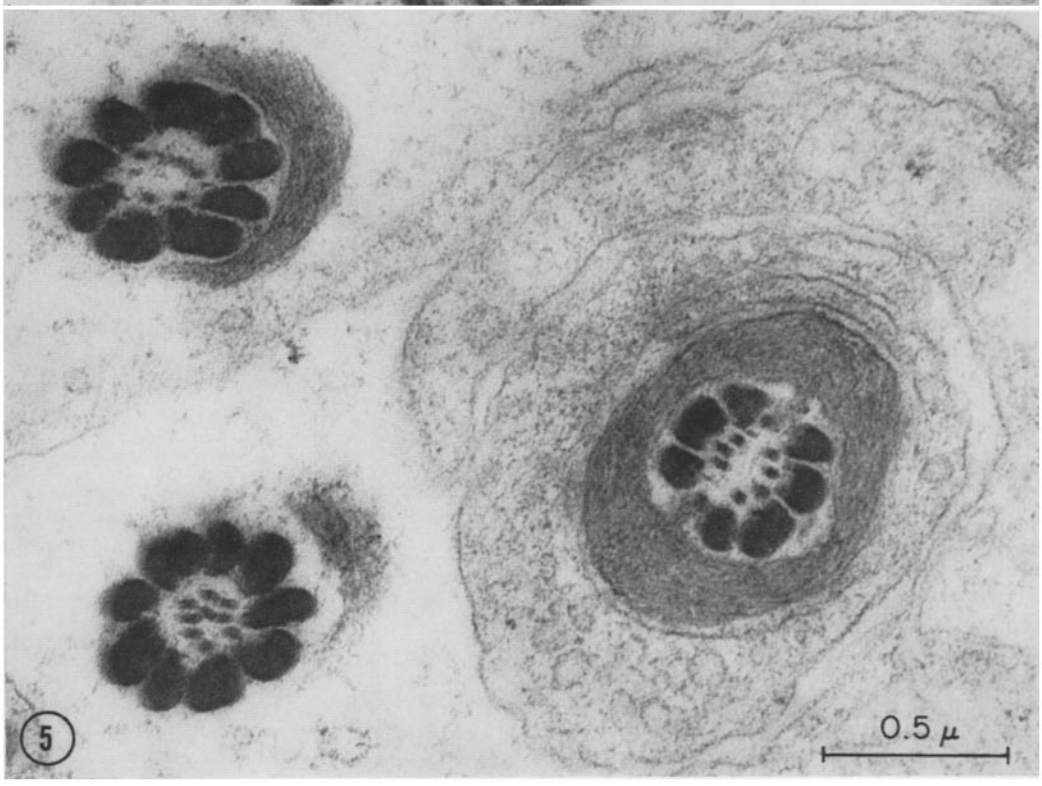

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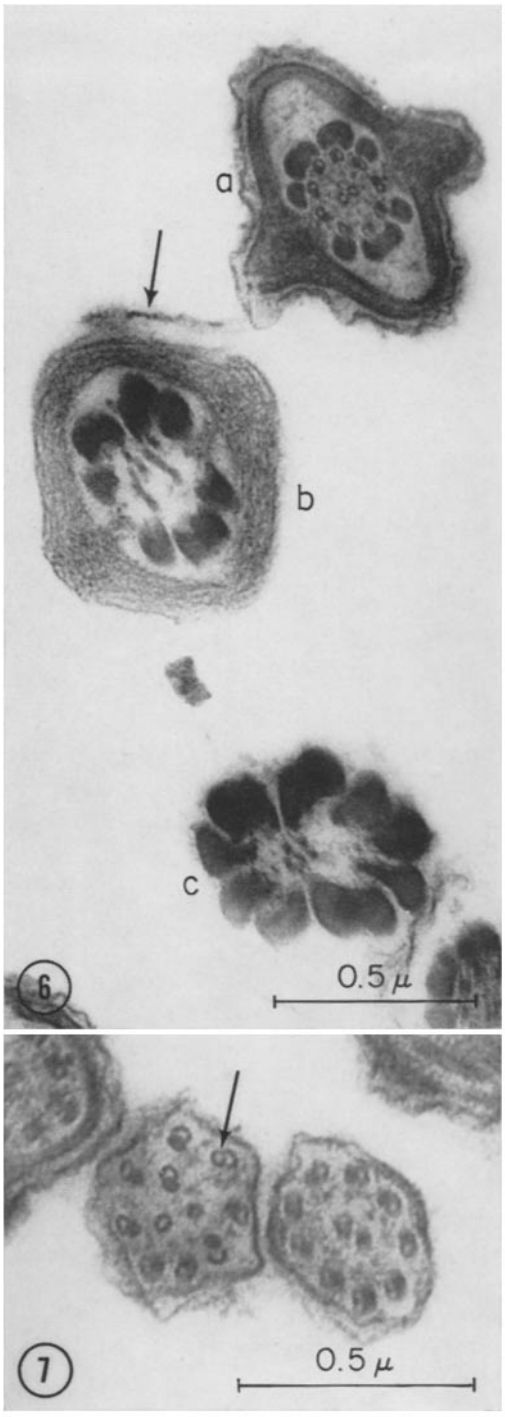

FIGURE 6 Cross-sections of three flagella of rat spermatids 3 days after operation. The flagellum (*a*) with a plasma membrane at the main piece appears to be normal. It has 9 + 2 axial filaments, seven coarse fibers, and a fibrous sheath. Another flagellum (*b*) is sectioned at the main piece. Each axial filament is obscure. The fibrous sheath appears swollen and within it a filamentous texture is observed. A fragment of the plasma membrane is seen outside the fibrous sheath (arrow). The third flagellum (*c*) is probably sectioned at the middle-piece and the axial filaments are displaced. × 55,000.

FIGURE 7 Cross-sections of the end-piece of the flagella of rat spermatids 2 days after operation. The axial filaments of one of the flagella follow a normal pattern, while in the other they are slightly disarranged but are still eleven in number. Some pairs of the peripheral filaments show a different density, but one filament of each pair (arrow) is light and tubular in appearance. Both flagella are enclosed by a plasma membrane. × 64,000.