

STRIATIONS IN THE PERIPHERAL FIBERS OF RAT AND MOUSE SPERMATOCYTES

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INTRODUCTION

In mammalian spermatozoa the peripheral fibers of the flagellum surround the proximal part of the axial filament complex, extending distally beyond the end of the mid-piece. The nine fibers are not all the same length and in most species they are also dissimilar in their cross-sectional outline. From the study of thin sections

it is known that each fiber has a narrow cortical zone which appears more or less dense (depending on the methods of preparation) than its center or medulla (Telkka, Fawcett, and Christensen, 1961). Phosphotungstic acid appears to stain the cortex selectively, leaving the medulla unstained, and the demarcation between cortex and medulla is shown to be clearest in the abaxial segment of the fiber (Gordon and Bensch, 1968). The

peripheral fibers seem to arise as outgrowths, one from each of the doublets of the axial filament complex (Fawcett and Phillips, 1969). The analogous peripheral fibers of insect spermatozoa also develop in this way (Cameron, 1965).

This paper is a preliminary report of further structural detail in the peripheral fibers. Fragmented spermatozoa of rats and mice have been studied by the technique of surface replication.

MATERIALS AND METHODS

The rats used were of the Sprague-Dawley strain and the mice were from the outbred Edinburgh "Q" stock. After killing the animals and waiting 30 min for them to cool to room temperature, I removed spermatozoa from the cauda epididymidis and suspended them in 0.85% saline. The suspension was sonicated sufficiently to detach the sperm heads and to break most of the flagella and then neutralized formalin was added to give a final concentration of 2% formaldehyde. The suspension was centrifuged at 2000 *g* for 20 min, the supernatant was removed, and the pellet was partially resuspended in a few drops of distilled water and spread onto a microscope slide. After drying at 10^{-4} mm Hg for 3 hr, the slide was rotated through 360° while a layer of carbon, such as to appear light grey, was evaporated onto it in a vacuum evaporator. The carbon film was floated onto 4 *N* NaOH solution and then transferred to more of this solution at 70°C, where it remained for 2½ hr. The replicas were washed with distilled water and shadowed with either 40% palladium gold or platinum, from an angle of about 25°. They were examined in a Philips EM75 electron microscope.

In subsidiary experiments, sonicated rat spermatozoa were examined as whole-mount preparations in an A.E.I. 6B electron microscope. Some of these preparations were negatively stained with 2% phosphotungstic acid according to the method of Horne (1965).

OBSERVATIONS

In carbon replicas, the peripheral fibers show regular striations on their abaxial surface, the surface which, in the mid-piece, is subjacent to the mitochondria. These striations very rarely appeared to be transverse but were usually oblique, and, since the obliquity in different specimens was always of the same direction, it is interpreted as genuine. The direction of the obliquity is demonstrated in Figs. 1–3. It is noted that the two mitochondrial helices, being sinistral, lie across the peripheral fibers with this same

obliquity. The period of the striations, measured perpendicularly to them, was estimated to be 398 Å. In the above respects, there was no obvious variation either between the nine fibers or between individual spermatozoa. Furthermore, the peripheral fibers in rat and mouse spermatozoa were essentially similar. The periodic appearance of these fibers was most often revealed in the mid-piece owing to the dislodgement of mitochondria, but there is little doubt that the striations are characteristic of the entire length of the fibers. Peripheral fibers, with striations, were often seen protruding from broken sections of the main piece of the flagellum.

Striations could not be detected in the fragments examined *in toto*, even after negative staining.

DISCUSSION

The striations described are not similar to those known to exist in the connecting piece of the spermatozoan flagellum. The latter are transverse, with a major period of 665 Å (Fawcett and Phillips, 1969). This contrasts with the situation in a mollusk, *Helix aspersa*, in which the peripheral fibers have the same transverse periodicity as the connecting piece (Anderson and Personne, 1967). In mammals, however, striations have not been reported in the peripheral fibers of spermatozoa fixed and sectioned by conventional methods nor have they been seen after negative staining. It is thought, in view of this, that the appearance of striations in carbon replicas may be due to the desiccation which must precede the deposition of carbon on the specimen. (Tests have shown that formalin fixation is not the responsible factor.) It is possible, then, that the periodicity reflects a differential hydration of the protein in the fiber.

Further questions of morphology are currently being investigated—for example, whether the striations are a feature of the cortex of the fiber or of the medulla too; whether they extend around each fiber or are restricted to its abaxial surface. If the striations do encircle each fiber, their arrangement could resemble either an irregular helix or, alternatively, a stack of oblique plates or annuli. In addition, it is not certain whether the striations of any individual fiber are in register with those of adjacent fibers. Even when cells least disrupted by sonication are observed, this question is complicated by two

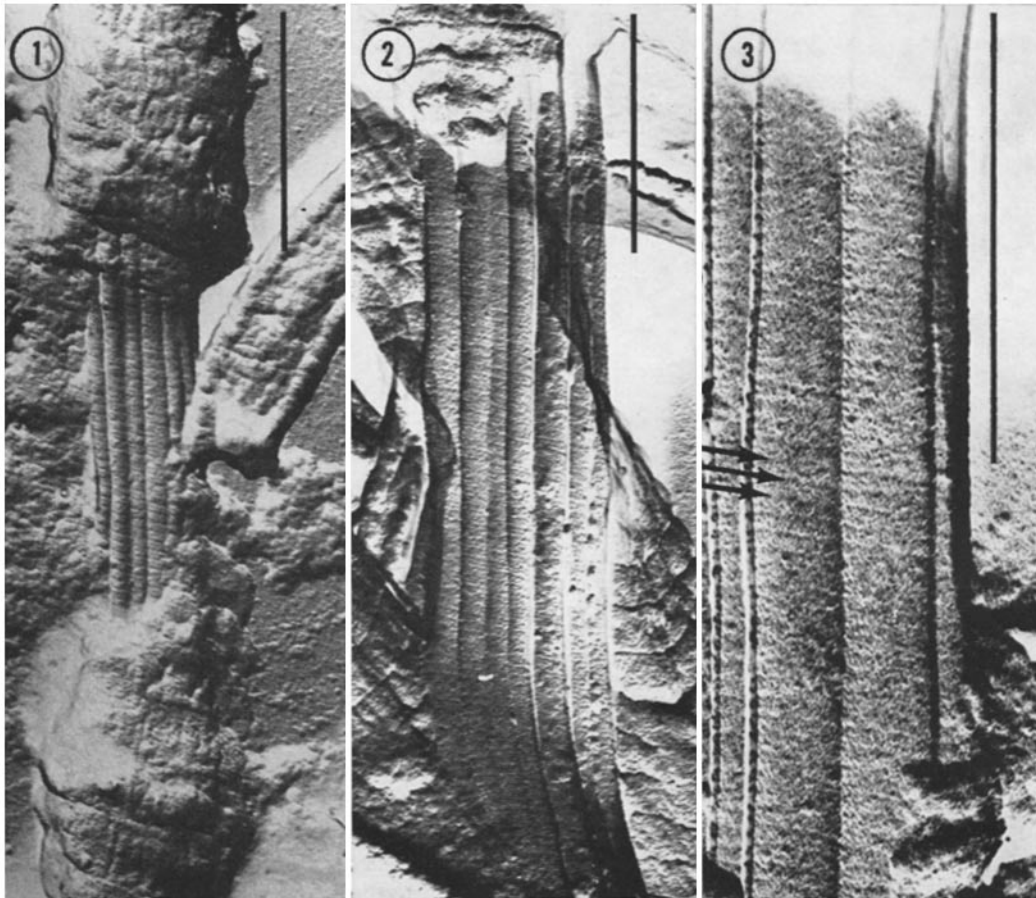


FIGURE 1 Peripheral fibers in the mid-piece region of a mouse spermatozoon. Mitochondria detached by sonication. Carbon replica shadowed with platinum. $\times 32,000$.

FIGURE 2 Comparable region of a rat spermatozoon after the same treatment. Carbon replica shadowed with palladium gold. $\times 32,000$.

FIGURE 3 Peripheral fibers of a rat spermatozoon at higher magnification. The arrows locate three consecutive oblique striations. Carbon replica shadowed with palladium gold. $\times 60,000$.

factors. Firstly, because of the curvature of the bundle, a "parallax effect" would be expected, giving the illusion that in-register fibers are out of register (or vice versa), an effect which would vary with the degree of flattening of the specimen. Secondly, the largest of the peripheral fibers have a shallow groove running longitudinally in their abaxial surfaces (seen in cross-sections, e.g., Elfvin, 1968) and therefore contribute "double columns" to the surface of the bundle-of-nine fibers. In some specimens the double columns are well seen, with their striations running in register across the whole fiber. However, the distinction

between double and single columns is not always clear and an illusion that separate fibers are in register may be created.

What function have the peripheral fibers? There is some evidence, of two sorts, to suggest that they are contractile. Fawcett (1962) noted that the thickest of the fibers, which are also the longest, are situated in the presumed plane of bending of the flagellum, where they would be most effective as contractile elements. Also, the cytochemical and immunocytochemical studies of Nelson and coworkers (reviewed by Nelson, 1967) have indicated some resemblance between the

proteins of the peripheral fibers and the contractile proteins of skeletal muscle. It is not yet proven, however, that the fibers are contractile; it is still possible that their role is simply to strengthen the flagellum. The oblique striations themselves do not demonstrate contractility, but the possibility that they may underlie the generation or propagation of contractions is seen as a stimulus to further investigation.

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