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# ELECTRON MICROSCOPIC STUDY OF THE BULL SPERMATOOZON V

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## V STRUCTURE OF THE TAIL PIECE

### RESULTS

In suspension materials, the tail piece was shown as a slender stick-like structure with comparatively low electron beam permeability (figs. 40~42). It began at the Jensen's ring of the middle piece, where the middle piece showed a sudden decrease in thickness, and it terminated just above the end piece. The tail piece was covered by a cell membrane which continued from Jensen's ring. Inside of the cell membrane there was a fibrillar coil sheath spiraling around the axial fibril bundle like a mantle. This interrelationship has a strong resemblance to that between the mitochondrial sheath and the axial fibril bundle in the mitochondrial sheath of the middle piece. By repeated washing with distilled water or some other solutions, the cell membrane was first removed, then the fibrillar coil sheath was collapsed and the inside structures became to appear. Sometimes, a successful preparation with the washing treatment clearly demonstrated about 11 fibrils consisting the axial fibril bundle (figs. 43 & 44). Between these fibrils and the spiral cords consisting the fibrillar coil sheath, there is a comparatively close resemblance in thickness and electron density.

The replicas could not reveal the further details in the surface structure (fig. 32).

In longitudinal sections, it was apparent that the fibrillar coil sheath begins from just inside of the Jensen's ring and covers entirely the tail piece (fig. 45). The axial fibril bundle which continued from the middle piece ran straight down in the tail piece (figs. 45 & 46). Each of the fibrils forming the axial fibril bundle decreased in thickness with descent, the difference between the thicker and the thinner became small and the interspace among them became narrow, thus they became to approach with each other, but without adhesion.

In oblique sections of the transitional part from the middle piece to the tail piece, the arrangement pattern of the fibrils was common to the middle piece [9 : 9 : 2].

The findings of the tail piece in the cross section (figs. 47 & 48), well explained those of the longitudinal section mentioned above; each cut surface of the fibrils became smaller than those of the middle piece, the tubular structure of the fine fibrils became more obscure, the fibrils of the outer ring approached to the fine fibrils of the inner ring, and the central ones also approached with each other. The spiral cords of the fibrillar coil sheath was more obscure compared with the mitochondrial helix of the middle piece, because of its indistinct

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outline. In addition, there appeared in the cross sectioned tail piece a pair of characteristic structures with crescent-like shape. These corresponded with cutting surfaces of the longitudinal fibrillar tail strips which are located inside of the fibrillar coil sheath and outside of the axial fibril bundle, near the assumptive line through the 2 central fine fibrils. This structures gave an almost nearer circular form to the cross section of the tail piece (figs. 47 & 48).

Each of the fibrils forming the following three kinds of structures in the tail piece, the fibrillar coil sheath, the longitudinal fibrillar tail strip and the axial fibril bundle, has an approximately equal high electron density, making a clear contrast with matrix of low electron density which occupied the interspace between these structures.

The tail piece was also covered by a common triple-layered cell membrane which continued from the above structures.

#### DISCUSSION

From the age of the optical microscope, it has been recognized that the portion between the Jensen's ring and the end piece is longest in all portions of the sperm<sup>17,26,102,108</sup> (about  $30\mu$  in the bull sperm), and this portion has been called the tail piece.

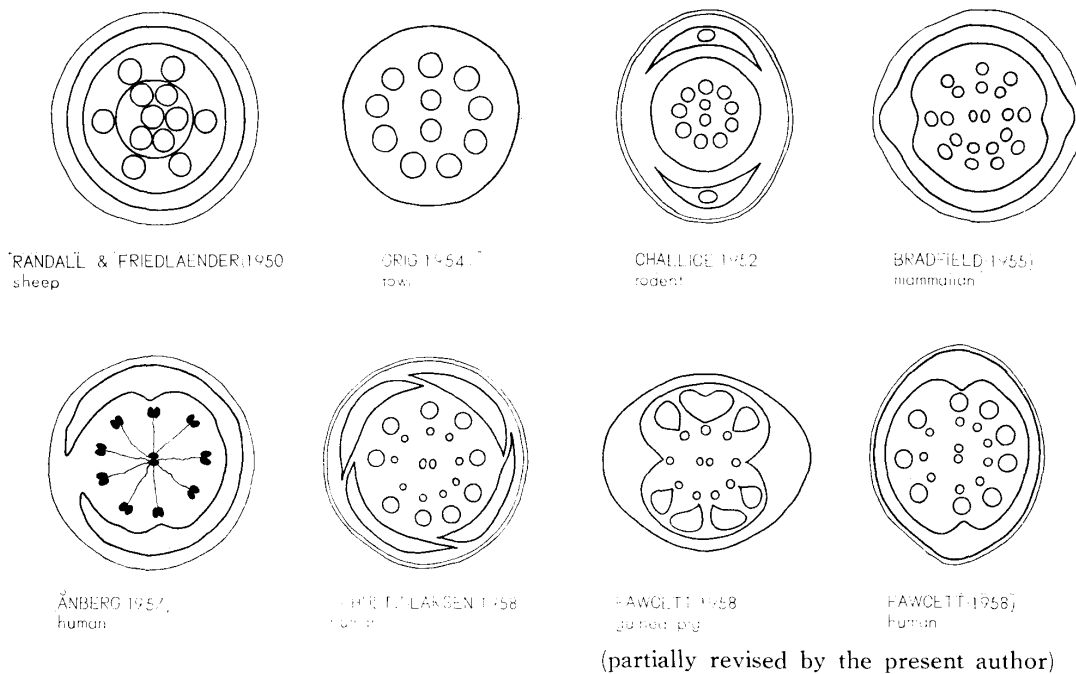
In that age, the axial fibril bundle was regarded as a single fibril<sup>85,86</sup> or as a complex of several fibrils<sup>4,108</sup>. The fibrillar coil sheath was not recognized, and it was thought as a kind of cytoplasmic component<sup>85,102</sup>. By applying the electron microscopy, SEYMOUR & BENMOSCHE first studied fine structures of the tail piece of the human sperm with suspension method. Afterward, many authors followed them, and reported that the axial fibril bundle of the tail piece of the sperm in the higher developed animals is composed of 9~12 fibrils<sup>6,7,26,46,47,62,81,110</sup>. It has gone about 60 years, since BALLOWITZ first reported that the axial fibril bundle of the tail piece of avian sperms consists several fibrils. On the other hand, the fibrillar coil sheath was discovered too, and its morphological characteristics, especially the number of spirals surrounding the axial fibril bundle, has attracted many authors' interests.

In suspension materials treated by frequent washing, it is revealed that the fibrillar coil sheath surrounds a bundle which is composed of several fibrils (figs. 43 & 44). In order to observe the tail piece from various directions, some experimental trial such as breakage of the supporting collodion film by electron beam was carried out. The thickness of the tail piece, however, was almost constant. This may indicate that the tail piece has a cylindrical form. Even when further physical or chemical treatments were added in order to collapse or break down the component elements of the tail piece, the suspension method failed to show accurately the number of the fibrils composing the axial fibril bundle.

With the advance of the ultra-sectioning technic, the fine structures of the

sperm in the testis in many animals, including the tail portion, has been widely investigated<sup>30,32,38,113~120</sup>. However, the ultra-sectioning study of the tail piece about ejaculated semen was carried out by only two workers, ÅNBERG and SCHULTZ-LARSEN, using human materials. The results of these two authors accord with each other in the fact that the number of the fibrils composing the axial fibril bundle is 20, but did not in the morphologic feature of the fibrils and the pattern of their arrangement (text fig. 6). The former author considered that the

TEXT FIGURE 6 *Schematic diagrams of tail piece in cross section postulated by various authors with several different species*



fibrils composing the axial fibril bundle of the tail piece were derived from only the peripheral fine fibrils of the middle piece, and he illustrated an arrangement pattern of the axial fibril bundle as  $[0 : (2 \times 9) : 2]$ , while the latter gave the  $[9 : 9 : 2]$  pattern as same as that of the middle piece. The latter author's findings are strongly supported by the results obtained from sectioned sperms in the testis, which present that the axial fibril bundle of the mammalian sperms consists of a pair of central fine fibrils and 9 pairs of thicker and thinner ones<sup>22,30,33,38,113,120</sup>.

The present author's results are, as a whole, quite agreeable with SCHULTZ-LARSEN's pattern,  $[9 : 9 : 2]$ . There are some cases where it seems as if a  $[9 : 0 : 1]$  pattern would be reasonable. In these cases, however, the  $[9 : 0 : 1]$  pattern is undoubtedly due to faulty observations because of the positional close relation between each of the 9 outer fibrils and of the 9 inner fine fibrils, or each

of a pair of central fine fibrils with each other. This may be aided in understanding by the findings obtained from longitudinal sections of the tail piece (figs. 67 & 68). Likewise, it can be easily understood that the [9 : 0 : 2] pattern offered by many other authors, the [8 : 0 : 1] pattern by BRETSCHNEIDER<sup>27)</sup> (bull) or the [6 : 0 : 6] pattern by RANDALL & FRIEDLAENDER (sheep) may be also probably due to similar faults.

YASUZUMI<sup>116)</sup> stated that in some species of which sperms carry out external fertilization and are unnecessary on vigorous mobility, such as Amphibia and Pisces, have not the outer ring fibrils in the tail piece. This hypothesis is worthy to attention in connection with BRADFIELD's assumption that concerning the function of the axial fibril bundle, the central fine fibrils may play a role as an impulse conducting system and the fibrils of the outer and inner rings may participate in contraction of the tail. From the review of literature CLELAND & ROTHSCCHILD concluded that the fibrils composing the flagella of Protozoa or the cilia of the somatic cells in various animals are greatly different in size among species, as compared with the sizes of these donor cells, whereas, the fibrils of the tail piece of the sperm in many species are almost constant in size, as compared with the sizes of these animals' tail piece. As seen in text figure 6, however, not a few authors stressed a considerable large degree of species difference in size and shape of this structure.

There are some workers describing that each of the fibrils in the tail piece has a tubular structure as similar as that of the middle piece<sup>1,33,38)</sup>. In the present study, the present author can not say exactly whether such structure may exist, because of lacking any sufficient evidence to show clearly such structure.

ÅNBERG described that a pair of central fine fibrils were connected with 9 pairs of peripheral fine fibrils of the inner ring by fine thread-like structures, "interconnections", although the structures were denied by the later authors<sup>38,87,95)</sup>. Furthermore, CLELAND & ROTHSCCHILD (bandicoot sperm in testis) and AFZELIUS (sperm of sea-urchin) actually demonstrated similar structures called "lamellae" and "arm" in the tail piece, respectively. In the present work, however, none of them was detected. Further comparative studies will be required to clarify whether these structures are specific in some species.

The fibrillar coil sheath has been noticed by many authors in suspended materials<sup>26,49,81,92)</sup>, sections of the testis<sup>32,33,38)</sup> and of free sperms<sup>3,95)</sup> in various animals, but uniformity in the form of this structure is not yet gained. There are major two opinions; one is that the coil elements of the sheath would spiral around the axial fibril bundle without changing their thickness<sup>3,22)</sup>, while the other is that with descent toward the end piece the thickness would decrease. Besides, as it was so in the middle piece, the number of coil elements also varies

with authors, such as single<sup>3,91)</sup>, double<sup>38,41,81,92)</sup>, triple<sup>26,32)</sup> and forth<sup>95)</sup>, in mammalian sperms.

According to SCHULTZ-LARSEN, the fibrillar coil sheath consists of two pairs, four in total, of coils which twist with each other. But, the "twisting" feature seems to be an artifact, because his photographs with suspension materials have a close resemblance to the knob-like structure or moniliform which are observed by the present author in the bull sperm added with physical or chemical treatments (figs. 43 & 44). The present author's finding of the fibrillar coil sheath is generally in accord with that of FAWCETT in the human sperm; a pair of spirals (not two pairs and without "twisting") coil regularly around the axial fibril bundle.

There are few workers who paid their attention to the spiral direction of the fibrillar coil sheath. Most of them illustrated it as counter-clockwise without any evidence, while FAWCETT claimed the clockwise direction in the human sperm. In the present work, the direction could not be decided, but it is suggested that the possibility of counter-clockwise direction seems to be most likely on the basis of the findings of suspension materials (figs. 41 & 44) and also in the relationship to the spiral direction of the mitochondrial helix.

In order to clarify the spiral number of the coil sheath, the cutting surfaces of the coils which appeared in 34 micrographs obtained from longitudinal section preparations at the portion near the middle piece about 4.5 cm long in 15,000 magnification (corresponding to  $3\mu$  in the actual length calculated theoretically) were counted. The figure obtained averages 80.6, (rang 66~102). As the actual whole length of the tail piece in the bull sperm is about  $30\mu$ , the total spiral number will be about 800. If the sheath consists of a pair of coils, the spiral number per one coil should be about 400. Moreover, as the tail piece gradually decreases in thickness toward the end piece, the spiral number may theoretically have to more increase. As compared with SCHULTZ-LARSEN's figure in the human sperm (2 coils, about 100 times, respectively) and BRETSCHNEIDER's<sup>26)</sup> in the bull sperm (3 coils, about 150), the spiral number estimated by the present author (2 coils, about 400) is considerably large. But, the present author does not consider that these figures are likely to have an important meaning, because of the reasons already mentioned in the end of the chapter I.

Concerning the origin of these coils, BRETSCHNEIDER<sup>27)</sup> attributes it to the Jensen's ring, while other workers who deny the existence of the Jensen's ring<sup>3,95)</sup> think that the coils are likely to have a relation to the mitochondrial sheath. It is undoubtedly obvious that the coils of the tail piece have their origin in the inside surface of the Jensen's ring (fig. 45). In addition, that the fibrillar coil sheath consists of 2 spiral coils is homologous to the fact that the mitochondrial sheath is composed of 2 mitochondrial helices. So, it is suggested that the

possibility of some connection through the Jensen's ring may exist between the above mentioned structures. This subject, however, will be unable to be explained until more detailed information will be obtained.

In the cross section, there appears a pair of crescent-shape structures with high electron density inside of the fibrillar coil sheath. These are cutting surfaces of the longitudinal fibrillar tail strips. The structure was first found by CHALLICE in the mouse sperm in the testis. He thought that there are 2 ribs between the doubled fibrillar tail sheath (text fig. 6). Thereafter, this structure has been reported in various species. BRADFIELD<sup>22)</sup> (mammalian sperms) and ÅNBERG (human sperm) considered it as a thickened portion of the fibrillar coil sheath. On the other hand, FAWCETT (human sperm) regarded it as a pair of band-like structures which run through the tail piece just inside of the fibrillar coil sheath, with parallel to the axial fibril bundle. In his diagram, this structures are illustrated just on a presumptive straight line passing through the two central fine fibrils, moreover, the two pairs of the peripheral fibrils of the inner and outer rings are also drawn on this line, therefore, the remaining fibrils of the axial fibril bundle are separated into 8 and 6 or 6 and 8 at each side of this line (text fig. 6). In the same species, SCHULTZ-LARSEN could not find out this structure.

The outline of the present author's results on the longitudinal fibrillar tail strips is generally in accord with the diagram given by FAWCETT. That is, two longitudinal fibrillar tail strips run straight down through the tail piece, near a presumptive line passing on the two central fine fibrils, just along inside of the fibrillar coil sheath (figs. 43, 45 & 47). In the cross section of the portion near the middle piece, each of the structures represents a considerably wide, crescent-shaped cut surface, but with descent toward the end piece it becomes smaller and finally at the transitional portion from the tail piece to the end piece it becomes undetected.

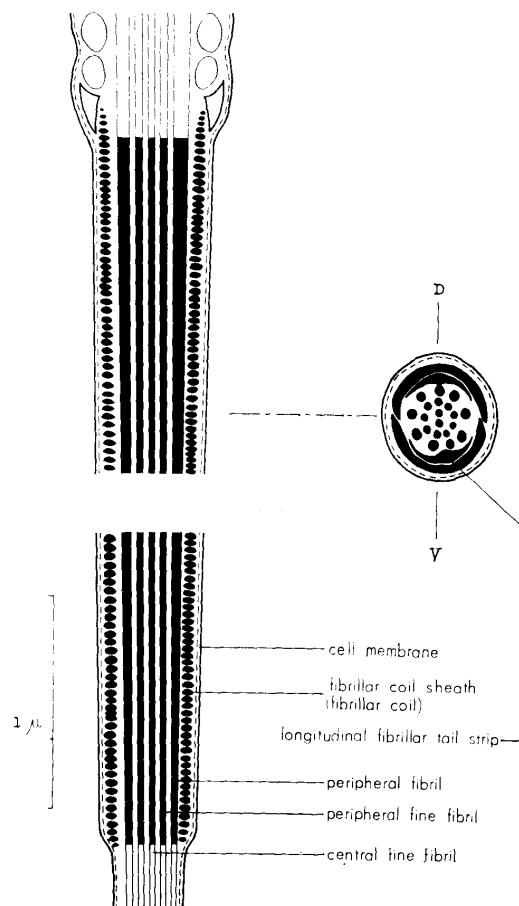
The problem of symmetry is to be a subject to discuss in the tail piece, too. If it is true that the fibrils of the axial fibril bundle run straight through the middle piece and the tail piece, it cannot be said that the tail piece has a complete bilateral symmetry, because one of the longitudinal fibrillar tail strips was located at a slightly sided portion ( $\pm 20$  degrees) against a presumptive line passing through the central pair of fibril, while the other strip did just on the line (figs. 47 & 48).

Does such an asymmetric pattern represent the actual character of the tail piece? The answer is greatly questionable. It should be pointed out that there exists a possibility of the false feature in which the longitudinal fibrillar tail strips may tend to remove toward either side against the plane of bilateral symmetry because of vigorous motion of the sperm tail, even if the tail piece, in nature, be

quite symmetric.

The plane of bilateral symmetry in the pattern of fibril arrangement which is presented by ÅNBERG and FAWCETT, respectively in the human sperm, passes through between the central pair, while in the case of the present author in the bull sperm, the plane passes through on the central pair, thus, the former is corresponding to be perpendicular to the latter. Even if the schemata presented by ÅNBERG and FAWCETT might be correct, the plane of bilateral symmetry is suitable only to the tail piece, but not to the upper portions. This may contradict against the findings obtained from longitudinal sections of the axial fibril bundle continuing from the middle piece to the tail piece without changing its fundamental pattern.

TEXT FIGURE 7 Schematic diagrams of tail piece



N. B. : Left indicates longitudinal section of flattened view and right shows cross section at anterior portion of tail piece.

In view of these considerations, it should be reasonable to assume that the tail piece also is bilaterally symmetric, as was in the upper portions, and that the asymmetric pattern observed may be due to an artifact.

RANDALL & FRIEDLAENDER (sheep) and CLELAND & ROTHSCHILD (bandicoot) reported that the tail piece of the sperm is enveloped by three membrane-like structures. No one, except SCHULTZ-LARSEN in the human sperm, illustrates that the double-layered cell membrane covers the whole sperm including the tail piece. A system with a triple-layered membrane is entirely accord with the unit-membrane structure in general somatic cells reported by ROBERTSON. In the bull sperm, the present work supports completely a triple-layered cell membrane.

#### CONCLUSION

The present author could add newly the following results in respect of the fine structure of the tail piece (text fig. 7).

- 1) The fibrillar coil sheath consists of a pair of spiral coils completely surrounding the axial fibril bundle. The number of spiraling in each of the coils is estimated at more than 400 times.
- 2) There are two longitudinal fibrillar tail strips running through the tail piece inside of the fibrillar coil sheath at the dorsal and ventral sides.
- 3) The mode of fibril arrangement in the axial fibril bundle is common between the middle piece and the tail piece, showing the [9 : 9 : 2] pattern.
- 4) Each of the fibrils gradually decreases in thickness with the descent, becoming to approach to a nearly equal thickness.
- 5) The problem of symmetry in this portion is also discussed.