Submicroscopic Structure of the Sperm-Head as Revealed by Electron Microscopy

By

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With the help of an electron microscope, Yasuzumi and coworkers (1948, 1949) succeeded in demonstrating a helical or annular arrangement of chromomere-like particles in the sperms of *Drosophila virilis* and *melanogaster*. The number of arrangement was also ascertained to be four in the *D. melanogaster* and six in the *D. virilis*. With regard to the identification of chromatin granules in the sperm-head, Bretschneider’s interpretation (1949) based on his silver technique attracts our attention. Pollister and Mirsky (1946) suggested the presence of chromosomes in the trout sperm-head, which had been treated with 1/M NaCl. But, Fischer, Hug and Lippert (1952) were positive in denying this finding. In the present study, the submicroscopic structure of bull sperm-heads treated with 5% *Saccharas ferricus medicus* has been clarified with the help of shadow-casting.

Recent advance of the technique for making ultrathin sections for electron microscopy have made it possible to study the submicroscopic structure of sperm-head under condition that proved to be more favourable than those previously encountered in smear preparations. Unfortunately no obvious submicroscopic structure concerning the sperm-head structure was observed in the works of electron microscopy of ultrathin sections of mammalian testes (Yasuzumi and Minamino 1954, and Burgos and Fawcett 1955). We present here a study with the electron microscope of the developing sparrow sperm-head, in which we have been able to distinguish its submicroscopic structure in longitudinal and transverse sections.
Material and Method

Bull semen was obtained from a butchery in Osaka. The purification of the material was required to eliminate mucus and other substances attached to the sperms, thus complicating interpretation by obscuring the structure. The purification consists in removal of the semen plasma by washing with Ringer solution in pH 7.3. Routine washing was done by repeated centrifugation for five minutes at 3,000 RPM. The pure sperm solution was spread over a collodion-covered glass slide in a manner similar to that employed in making blood smears for light microscopic study. The film of sperm layers was immersed in 5% Saccharas ferricus medicus solution for about 48 hours at 37°C. Throughout the experiments wet and dry samples were examined under a light microscope. After the materials was fixed with 2% osmium tetroxide vapour and allowed to dry in air, the contrast of the object was increased with the chromium shadow technique. The Siemens electron microscope model ÜM-100 with a maximal operating voltage of 100 kV was employed in the present study.

Small blocks of adult sparrow (Passer montanus saturatus Stejneger) testis were fixed in 1% osmium tetroxide buffered to pH 7.4 with Michaelis acetate-veronal (Palade 1952), dehydrated and embedded in a mixture of 80% butyl methacrylate and 20% methyl methacrylate (Newman, Borysko and Swerdlow 1949). Sections 20 to 50 mp in thickness were cut on a Shimadzu thin sectioning microtome and examined in a Hidachi electron microscope model HU-10 with a maximal operating voltage of 100 kV, without removing the plastic.

Results

Fig. 1 shows a fairly typical smear preparation of a mature bull sperm-head treated with 5% Saccharas ferricus medicus solution for about 48 hours. The head is composed of complex structure which gives an appearance of fine granules ca 50 mμ in diameter in the distal third portion and of granules ca 0.1–0.2 μ in diameter in the proximal two-third part. When fine granules are visualized, they appear always in a group in the appointed area. The large granules are not separated one another, but they are connected so as to make entangled threads. The middle-piece is easily isolated from the neck portion and is composed of longitudinally running fibrils which have
been enclosed by the helical sheath.

Fig. 2 illustrates the appearance of the same preparation of two bull sperm-heads. This treatment in general has markedly deleterious effects on the structure of the head cap, but usually results in a very satisfactory improvement in contrast, showing a high degree of preservation of structural order in the karyoplasm.

Fig. 3 demonstrates an enlargement of a portion of Fig. 1. The helical thread structure is obviously visible at the points marked by the arrows. The structure which looks like granules 0.1–0.2 μ in diameter corresponds to gyres in the helical threads. In Fig. 4 are conspicuous a number of fine helical structure at the edge of dense bodies. They demonstrate the double helical filaments about 15 mμ in diameter. Each one is only 6 mμ in diameter.

Further information concerning the fine structure of the sperm-head can be derived from thin sections of the sparrow testis. When spermatid nucleus has been projected outside the cell body, the nucleus (primitive head) is of a conical shape with a well defined head cap. The size of the primitive sperm-head in this stage is 1.9–2.05 μ in length and 0.85–1.15 μ at the base (Fig. 5). The fibrillar structure in the head can be seen along its longitudinal axis. The fibrils are so closely packed in the center of the head as well as at the peripheral portion. The oblique section of the nucleus is occupied by the fibrils 20–45 mμ in diameter which are immersed in the matrix, the whole being enclosed by a thin head cap. The cross sections of fibrils have appeared as bodies of an irregular round shape (Fig. 6). The fibrils are less clearly defined in the longitudinal section than cross or oblique section: this seems to be due to a twisted array of fibrils along the longitudinal axis of the head.

In a sufficiently thin oblique section of primitive sperm-head, longitudinal and cross sections of fibrils are visualized, suggesting a helical or banded structure 6 mμ in diameter in certain fibril in the present magnification (Fig. 7).

Discussion

It is very difficult to observe constituent elements of mammalian sperm-head in situ. The present authors succeeded in demonstrating the submicroscopic structure in the bull sperm-head treated with 5% Saccharas ferricus medicus which has dissolved the head cap and has a chemical affinity with nucleoproteins. After being treated with 5%
Saccharas ferricus medicus for 48 hours the sperm-head is positive in the Feulgen’s reaction. The helical threads appeared in the bull sperm-head must be chromosomes or their derivatives. If it would be possible to calculate the number of the helical threads, the genesis of the helical threads could be ascertained.

A group of a fine granules appears in a round shape in the distal third portion of the head. The present authors have been inclined to interpret that this structure is of nucleolus, because Yasuzumi (1950) has already observed a nucleolus-like body in the half posterior region of the bull sperm-head, which has been treated with desoxyribonuclease.

In the course of a study on the spermatogenesis of various vertebrates (Yasuzumi 1956), characteristic fibrillar inclusions have been observed in the developing sperm-head in the sparrow testis. Thus, visualization of the chromosomes or their derivatives as individual entities in the sperm-head has been possible in the smear preparation and the thin section. The helices 6 mµ in dicmeter running along the longitudinal axis of the fibril have been recognized in the smear preparation. But it is not sure whether such helices are the constituent components of fibrils or artefacts produced by shadow-casting. However, this novel feature of the chromosome or its derivative structure seems to be in harmony with that revealed on the thin section of sparrow testis, which first has been demonstrated in the vertebrate sperm-head.

Summary

The submicroscopic structure of bull sperm-head, which has been treated with 5% Saccharas ferricus medicus, has been clarified with the help of the Siemens electron microscope. The sperm-head consists of a multitude of helical threads 0.1–0.2 µ in diameter as well as nucleolus-like body, which is composed of a considerable number of fine granules ca 50 mµ in diameter. The helical thread is composed of a large number of helical filaments 6 mµ in diameter: this finding has been also obtained on the thin section of sparrow sperm-head with the help of the Hidachi electron microscope.

Acknowledgements

The authors are gratefully indebted to Prof. E. Ruska, Dr. Wiesenberger and Dr. C. Weichan at Abteilung für Elektronenoptik der
Siemens & Halske Aktiengesellschaft in Berlin, and Dr. I. Makino and Mr. C. Ito in the electron microscope laboratory of the Hidachi company at Hidachi for help with the electron microscope. The authors would like to express their appreciation to Prof. T. Kasai and Dr. T. Yamanaka for their suggestion.

Literature Cited


Explanation of Plate figures

Fig. 1. Smeared mature bull sperm treated with 5% Saccharas ferricus medicus for 48 hours and shadowed with chromium. The fine granules (FG) ca 50 µm in diameter are visible in the distal third area of the head, suggesting the nucleolus. Note the granules that seem connected one another in the proximal two-third portion of the head. The middle-piece (MP) is composed of longitudinally running fibrils which are enclosed by the helical mitochondrial sheath. Micrograph was taken with ÜM-100 at 3,500 diameters and enlarged optically to x16,000.

Fig. 2. Smeared mature bull sperm-heads treated with 5% Saccharas ferricus medicus for 48 hours and shadowed with chromium. They show a structure similar to that demonstrated in the previous figure. Micrograph was taken with ÜM-100 at 3,500 diameters and enlarged optically to x16,000.

Fig. 3. A part of the same field as Fig. 1, illustrating more clearly helical threads at the points marked by the arrows. Micrograph was taken with ÜM-100 at 15,000 diameters and enlarged optically to 34,500.

Fig. 4. Electron micrograph showing at a higher magnification the double helical filaments at the point marked by the arrow. Micrograph was taken with ÜM-100 at 30,000 diameters and enlarged optically to 138,000.

Fig. 5. A vertical section through the primitive head in the sparrow testis, showing a more or less regular array of fibrillar derivatives of the chromosomes. The conical shaped primitive head is enclosed by a thin head cap (HC) composed of apparently many layers. Micrograph was taken with HU-10 at 10,000 diameters.
and enlarged optically to $\times 100,000$.

**Fig. 6.** An oblique section through the primitive sperm-head, showing cross and oblique sections of fibrils more prominently than in the previous figure. Micrograph was taken with HU-10 at 10,000 diameters and enlarged optically to $\times 65,000$.

**Fig. 7.** Micrograph represents at a high magnification the helical filaments in the fibrils indicated by the arrows. Micrograph was taken with HU-10 at 40,000 diameters and enlarged optically to $\times 170,000$. 
Plate III

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HC

0.5 μ
Plate IV

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