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Some Observations on the Internal Structure of the Sperm Head of the Grasshopper, *Oxya yezoensis*, after Dehydration and Hydration Treatments[1)]

By

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(*With 4 Text-figures*)

Recently, the internal structure of the sperm head ot animals, particularly of insects, has attracted the attention of cytologists and geneticists (Nakamura 1951, Cooper 1952, Shimakura 1954, Shigenaga and Yoshida 1954, Herskowitz and Muller 1954). They have presented evidence of a chromonema-like structure within the sperm head. The present author undertook this study in a hope to inquire into the internal fine structure of the sperm head of the grasshopper making use of dehydration and hydration treatments.

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Method

The adults of the grasshopper, *Oxya yezoensis*, were adopted as material for this study. Hanging-drop preparations were made as follows : spermatozoa were quickly removed from the testis-follicles with the aid of a sharp knife and placed on a clean coverslip in a small pool of body fluid derived from the operated animal. The coverslip was then inverted over a depression slide. Just before inverting the coverslip, a small amount of human saliva was put on the bottom of the depression of the slide to make a moist chamber. The edge of the slip was sealed with liquid paraffin. Observations were made with a phase microscope in the optical combination of a positive medium contrast 100 × objective, or a bright medium contrast 90 × objective, and a Leitz Periplan 10 × ocular.

Observations

1) Living spermatozoa : The heads of spermatozoa which have reached maturity are lance-shaped and exhibit no observable structure, being uniformly dense in appearance. The general feature of the sperm-head after non-treatment is as shown in Figure 1.

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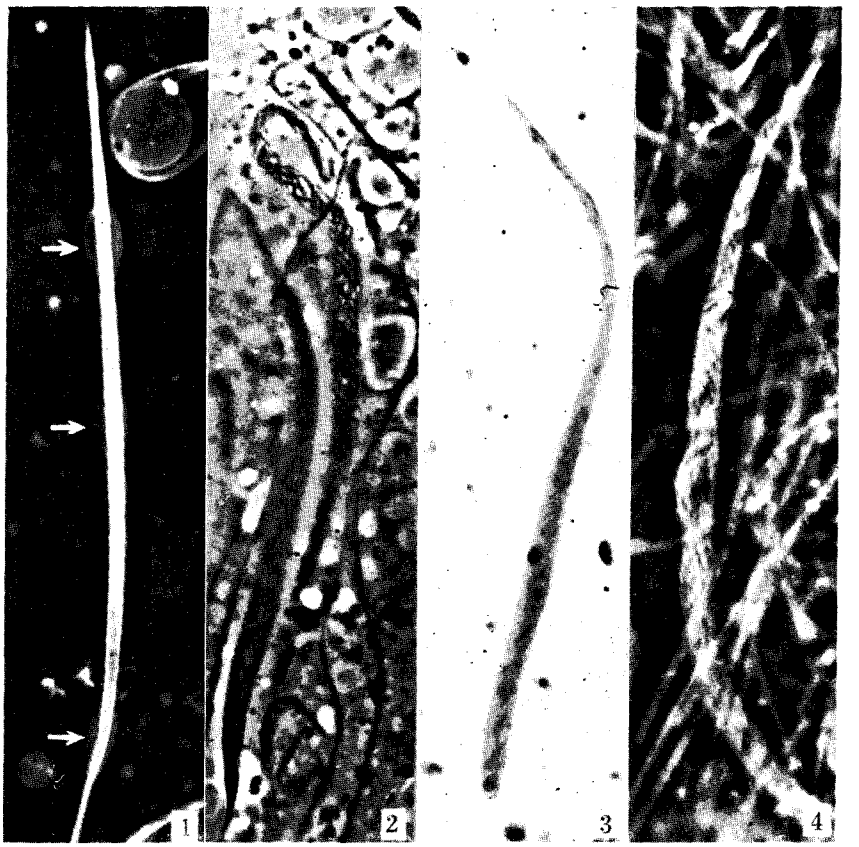
2) The structure of the sperm-head after dehydration and hydration treatment : The hanging-drop preparation of spermatozoa can be dehydrated through exposure to heat applied on the surface of the coverslip and the underside of the slide.[1] With these treatments the spermatozoa are gradually hydrated by the



Figs. 1–4. Photomicrographs of sperm-heads of the grasshopper, *Oxya yezoensis*, in living and treated material. × 1300. 1, living, nearly mature sperm-head, uniformly dense in appearance. Arrows indicate cytoplasmic masses. Bright phase-contrast. 2, the sperm-head after dehydration and hydration with heat, showing the threads in the head. Dark phase-contrast. 3–4, sperm-heads after treatment with glycerin and water. 3, acetic dahlia preparation, showing the deeply stained threads in the sperm-head. 4, non-stained hanging-drop preparation, showing the threads in the sperm-head. Bright phase-contrast.

1) The heat source here used is an electric heater (40 W) set up at a distance of about 10 cm from the coverslip or slide glass.

vapor from the saliva placed on the bottom of the depression. After this treatment, fine and distinct threads have begun to appear within the sperm-head (Fig. 2). Each thread coils into a spiral, and the sperm-head shows a network, as a whole. The long, coiled threads, being about ten in number, are seen running along the long axis of the sperm-head. It is, however, difficult to count exactly the number of the threads, because they are lost from view with time after hydration. Meanwhile, the sperm-head swells and becomes homologous in appearance.

3) The structure of the sperm head after glycerin and water treatments: The fresh testis was placed in a glass tube containing glycerin. After 5 to 15 minutes the testis-follicles were strongly dehydrated showing shrinking. Then, the glycerin was gradually diluted with distilled water. The testis-follicles showed swelling after about 20 minutes of this treatment. After having been gently washed with distilled water, the testis-follicles were squashed on a slide to smear the spermatozoa, and the excess of water was removed with a blotting paper. Then the smears were covered with acetic dahlia. In this preparation, the deeply stained threads were observable within the sperm-head by the use of the ordinary microscope (Fig. 3). The threads, however, are very hazy in outline, it being rather difficult to count their number. Further, the hanging-drop preparation after glycerin and water treatments showed a similar feature which was observable by phase microscopy (Fig. 4). However no threads were observed when the material was treated with water only. From this evidence, it is probable that the spermatozoa are dehydrated and then hydrated.

Similar experiments were undertaken in two species of grasshoppers, *Podisma sapporense* and *Acrydium japonicum*, with nearly similar results.

Discussion

The formation of the sperm-head in insect spermatogenesis has attracted much attention from both cytologists and geneticists. The nuclear origin of the sperm-head has been well established by cytologists, but its internal structure has remained open to question. In living spermatozoa, as well as in those fixed with ordinary fixatives, the sperm-head generally shows no visible internal structure. Shimakura (1954) with the aid of a polarization microscope observed coiled threads which showed strong birefringence and seemed to be chromonemata in the nearly mature sperm-head of the grasshopper, *Chloealtis genicularibus*.

The results of the present observations indicated the presence of the threads within the sperm-head after dehydration and hydration. In the early prophase nucleus of grasshopper spermatocytes, the existence of the chromonemata could not be demonstrated in living state. The chromonemata became visible when the spermatocytes were dehydrated (Nakanishi, unpublished). Ris aud Mirsky (1949) reported that in the interphase nucleus of the injured cell of the grasshopper (*Melanoplus femurrbrum*), the chromatin structure became apparent. Shinke

(1941) also observed the chromonemata in the resting nuclei of *Tradescantia* after treatment with hypertonic or hypotonic solution. According to him, the invisibility of chromonemata in the resting nuclei is due to the difference in the configuration of the chromonemata which depends mainly on the grade of hydration or dehydration of the nuclei or of the chromosomes. The sperm-head is without question the transformed interphase-nucleus which surely re-enters division after fertilization. Therefore, evidence presented in this study indicates that the threads observable in the sperm-head may be no other than chromonemata. Probably, the chromonemata which are invisible in the usual condition become visible following dehydration and hydration. The Feulgen positive threads within the sperm-head of the grasshopper, *Acrida lata*, demonstrated by Nakamura (1951) after treatment with 1 M NaCl are similar ones to those observed in this study.

Shigenaga and Yoshida (1954) using the spermatozoa of the grasshopper, *Oxya vicina*, after treatment with 3-5 M NaOH solution showed by electron microscopy that the sperm-head consists of two chromonemata. After treatment with 10 M NaOH solution, some constrictions took place in the sperm head, which are nearly identical with the haploid number of chromosomes. Further, they indicated that the chromosomes are arranged in linear series like a railway line. Gowern and Gay (1953), working with *Drosophila*, measured the sizes both of the sperm-head and of the individual chromosomes at oogonial metaphase. The close correspondence between the length of the sperm-head and the total length of a haploid set of metaphase chromosomes, together with the close similarity in chromosome and sperm-chromatin widths, led them to conclude that the chromosomes were arranged end to end (presumably in a straight line). Cooper (1952) reported that in the sperm-head of *Drosophila melanogaster* all the chromosomes were similar in size as well as in proportions to those of the chromosomes at an ordinary mitotic metaphase, and that they were arranged in single file along the long axis of the sperm-head in certain fixed preparations. On the other hand, Herskowitz and Muller (1954) stated that, according to figures and information furnished by Cooper, the chromatin of the nearly mature spermatozoa had the appearance not of a straight line but rather of a coarse helix with more or less side-by-side arrangement of its several coils, and when squeezed apart these were seen to be made up of chromosomes of metaphase appearance. Further, in the measurement of the lengths of the chromatin masses in spermatozoa of *Drosophila melanogaster*, Herskowitz and Muller (1954) have reached the conclusion that the sperm-head chromosomes do not have a straight end-to-end arrangement but as a rule overlap each other. The chromonemata visible in the sperm-head of *Oxya yezoensis* seem to show the appearance of a side-by-side arrangement of several coils along the long axis of the sperm-head; this seems to support the possibility of the view of Herskowitz and Muller (1954).

Summary

This study deals with the internal structure of the sperm head of the grasshopper, *Oxya yezoensis*, observed by phase microscopy after dehydration and hydration treatments.

The nearly mature sperm-head in living condition is lance-shaped, and shows no observable structure. After dehydration and hydration with heat, or with

glycerin and water, fine threads become visible within the sperm-head. Each thread coils into a spiral, and the sperm-head shows a network as a whole. The threads seem to be chromonemata which show a side-by-side arrangement of several coils parallel to the long axis of the sperm head.

It is the author's great honor to dedicate this piece of work to Professor Tohru Uchida in celebration of his 60th birthday.

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