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ELECTRON MICROSCOPIC STUDY OF THE BULL SPERMATOZOON VI-VII

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VI STRUCTURE OF THE END PIECE

RESULTS

In suspension materials, the end piece, the terminal structure of a spermatozoon, was shown as a fine slender stick-like structure with comparatively low electron density, being entirely covered by a cell membrane (fig. 49). The fibrillar coil sheath which was observed in the tail piece was not detected in this portion and each of the fibrils consisting the axial fibril bundle ran straight, but sometimes twisting, decreased in the thickness with descent and finally terminated at the end.

The replicas showed no more details, excepting that the end piece was slightly more slender than the tail piece (fig. 50).

By frequent washing with some solutions, the cell membrane was lost and the fibrils of the axial fibril bundle were exposed. By adding more intense physical or chemical treatments, the bundle was separated into several groups of fibrils or isolated fibrils, showing a brush-like form (fig. 52). Sometimes, the fibrils were cut or splitted, thus, it became difficult to calculate the number of the fibrils accurately.

In longitudinal sections, it was noticed that the axial fibril bundle ran straight through this portion, being enveloped by a triple-layered cell membrane (figs. 53 & 54). The end piece was clearly distinguishable from the tail piece in the absence of the fibrillar coil sheath in longitudinal or oblique sections. In transverse sections, the mode of fibril arrangement showed the [9 : 9 : 2] pattern as same as that of the tail piece (figs. 55~57). In the case where some disorder was observed in the pattern, it may be suggested that twisting would occur in this portion.

DISCUSSION

In the age of the optical microscope it has been believed that the end piece in almost of all mammalian sperms is a naked, slender structure below 5 μ long[55,57,59,102,108]. Some authors, however, describe the variety of the length due to species[53,84].

Since BRETSCHNEIDER & ITERSON (1947) reported that the end piece of the

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bull sperm consists of 9 fibrils in electron micrographs from suspended materials, the detailed structure of this portion has been studied in various animals, and it became apparent that the end piece also has the axial fibril bundle. Concerning the morphological appearance of the axial fibril bundle in this portion, however, much discordant opinions have been presented; some authors considered the brush-like structure of the fibrils to be normal[7,37,98,106], while others attributed it a kind of artifacts[26,41,53,92], and also supported the opinion that the cell membrane entirely covers the end piece[3,32,60,62,95].

According to BONADONNA & CURTO, the incidence of "brush formation" varies with species; boar sperms are less frequent in its appearance than bull or stallion sperms. HANCOCK[49] described that the brush formation of the end piece may be a phenomenon caused by death of the sperm, suggesting the possibility of its utilization in differential diagnosis of live and dead sperms in the boar. Although it is not easy to estimate whether the sperm having the brush-like structre was living or not at the time of fixation, the present work did not give any evidence on which the structure should be considered to be original one.

There are many different opinions about the number of fibrils consisting the axial fibril bundle of the end piece. Even among electron microscopists, the number varies from 2 to 20[3,26,29,37,93,95]. On the human sperm, ÅNBERG and SCHULTZ-LARSEN counted the number as 20, respectively, but the pattern of fibril arrangement presented by these authors showed a discordance; the former showing $[0:(2\times 9):2]$, while the latter $[9:9:2]$. The $[9:9:2]$ pattern seems to be essential in the bull sperm, too.

Some disorder in the pattern was sometimes observed, but it may be attributed to an artifact probably caused by a twisted sperm tail (fig. 57). This assumption may be supported by the fact that in cillia of general somatic cells which have not such spiral structure as the fibrillar coil sheath in spermatozoa, the pattern of fibril arrangement never shows out of order[49,89]. Another frequent disorder in cross sections, may be due to some difference either in their original length or degree of contraction by fixation, especially as likely shown in suspended materials (fig. 51).

It is well known that there exists, widely in the biological world, a regularity in the pattern of fibril arrangement; for example, in flagella of Protozoa[22], the germ cell of plants[89] and cillia of general somatic cells[40,89,100] a common pattern, $[0:(n\times 9):2]$, resembling that of the spermatozoon, has been observed. Up to now, however, the origin and significance of the pattern has not yet been well clarified[1,22,40,89]. In this connection, it is interesting that BRADFIELD[22] has suggested the possibility of functional division among the central and peripheral fibrils.

RANDALL & FRIEDLAENDER reported that a coil structure which is composed

of two twisted fine fibrils spirals around the end piece of the sheep sperm, as well as the fibrillar coil sheath in the tail piece. Likewise, FRIEDLAENDER and SCHNALL[92] described a similar structure with counter-clockwise direction of spiral in the human sperm. Other workers, including the present author, who studied with sectioned materials, however, denied the existence of these structures[3,95]

HAMMEN et al. stated that in the human sperm the brush-like structure of the end piece showed "moniliform", but it seems to be a kind of artifacts from the reason already mentioned in chapter V. They described also "sub-fibril". This feature has a close resemblance to those observed in the present work (fig. 52). It seems to be concluded that such features of divergent fibrils should be attributed to some artificial separations caused by chemical or physical treatment rather than to morphological abnormalities. CLELAND & ROTHSCHILD emphasized that in the bandicoot sperm the corresponding fibrils of the inner and outer rings in the end piece were adhered with each other, however, according the present author's observation in the bull sperm, no adhesion occur among these fibrils.

HERRNLEBEN stated that the end piece of the rabbit sperm terminates at a characteristic tubercle-formed end which is not seen in sheep, goat and boar sperms. In the present study of the bull sperm, none of such structure could be seen.

It can be said that the plane of bilateral symmetry in the end piece is decidable as well as other portions of the bull spermatozoon on the basis of the present author's observations. The definition of dorsal or ventral side is also available, although the typical pattern of fibril arrangement is not always easily detectable because of high fragility of this portion lacking in binding structures such as the fibrillar coill sheath.

There are few workers who deny the existence of the cell membrane in this portion[17,37,98]. However, in the electron microscopic study, the structure is clearly demonstrated by many investigators[3,32,33,53,81,95]. The present paper is the first report which deals with the ultramicrotomized end piece of the bull sperm. In addition, no one, except the present author, has so far been demonstrable the longitudinal section of this portion using ejaculated bull spermatozoa.

CONCLUSION

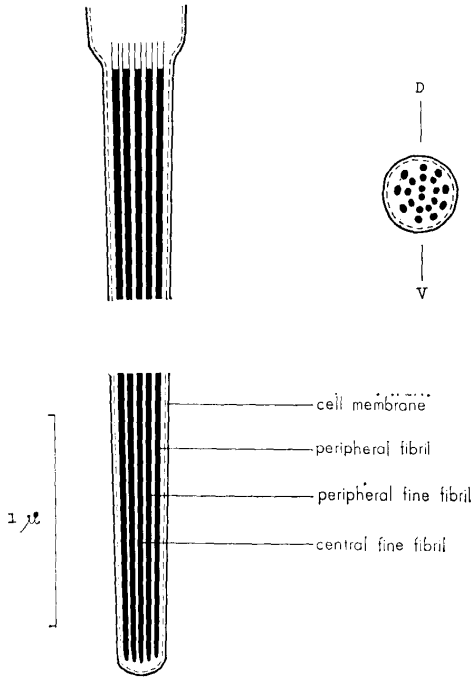
1) The pattern of fibril arrangement in the axial fibril bundle of this portion is completely homologous to that of the tail piece, the [9 : 9 : 2] pattern.

2) Each of the fibrils consisting the axial fibril bundle is approximately equal in thickness.

3) The axial fibril bundle is entirely covered by the triple-layered cell membrane, without any structure between them.

4) The bilateral symmetry is confirmed also in the end piece.

TEXT FIGURE 8 *Schematic diagrams of end piece*



N. B.: Left shows longitudinal section of flattened view. Right indicates cross section at anterior portion of end piece.

VII Summary

The present investigation deals with an electron microscopic study of spermatozoa collected from 4 Holstein bulls with normal fertility by the artificial vagina method. The investigation was carried out by means of three different techniques: suspension method, replica method and sectioning by means of an ultramicrotome. From the results of this investigation, a recommendable schedule for preparing block preparations was discussed by the author.

The findings are as follows:

The head consists mainly of the sperm nucleus of which the anterior half is covered by the head cap, whereas its posterior half is covered by the nuclear sheath. The sperm nucleus which contains homogeneous nuclear substance with high electron density is flat and oval in shape, but it tapers away at the dorsal side of the anterior margin. The head cap consists of a double-layered acrosome membrane, the external and internal acrosome membranes. Both membranes are composed of two layers and are continuous with each other at the posterior margin of the equatorial zone. The interspace between them is filled by the acrosome substance and at the dorsal side of the anterior portion of the interspace the acrosome corpuscle is contained. The equatorial zone is a part of the head cap, forming a circular band around the head. It begins at the portion where the acrosome sack is almost empty of the acrosome substance and terminates at the mid-portion of the head. Its width is smallest at the two lateral sides and enlarges gradually toward the central portion of the dorsal and ventral sides. There are 2 basal granules one at each side of the basal portion of the nucleus, showing small structures; the whole granule shows high electron density. Three fossula-like depressions are observed at the base; the central one is larger than the others, about twice in diameter. Along these depressions, there is a basal plate which connects with the neck.

The neck consists of a mitochondrial sheath, some kinds of radixes and a centriole. The mitochondrial sheath which originates from the basal granules of the head forms 2 spiral structures, the mitochondrial helixes, irregularly surrounding the radixes as a collar. In the anterior portion of the neck, 2 large radixes run along both lateral sides inside of the mitochondrial spirals. Likewise, at the dorsal side 3 small radixes and at the ventral 2 small radixes run, respectively. Thus, in a transverse section, these radixes take elliptic form around the centriole. In the middle portion of the neck, each of the large radixes branches off 2 medium radixes respectively, and at that point each of the pair of medium radixes on each side twists and exchanges its position with the other one. Therefore, in the posterior portion, the neck consists of each pair of medium radixes at both the lateral sides, 3 small radixes at the dorsal side and 2 small radixes at the ventral: these 9 radixes in relation to one anther take a circular form in transverse section. Each radix is composed of about 15 segmental structures with high electron density, the neck platelets. Inside of these radixes there is a centriole which shows a radial structure with high electron density around a central point with low electron density. The centriole extends toward the posterior direction, forming the central radix within a short distance and then branching off 11 fine fibrils, i.e., 9 peripheral fine fibrils and 2 central ones.

The axial fibril bundle forms the central core, running through from middle piece to

the end of the structure. The axial fibril bundle is composed of 20 fibrils: the outer ring, inner ring and central pair. The outer ring consists of 9 peripheral fibrils of which 4 are thicker than the other 5 in the middle piece. The arrangement of these fibrils is characteristic. Two pairs of the thicker peripheral fibrils originating from the 4 medium radixes of the neck are located at the lateral sides. On the other hand, the remaining 5 (3 & 2) peripheral fibrils originating from the small radixes of the neck are located at the dorsal and ventral sides, respectively. The inner ring consists of 9 peripheral fine fibrils with electron density approximately equal to that of the 2 central fine fibrils, and all of them originate from the central radix. The 9 peripheral fibrils, however, become gradually thinner as they approach to the end piece. Thus, in appearance the 20 fibrils resemble each other not only in size but also in electron density at the end piece.

The middle piece consists of a mitochondrial sheath, the axial fibril bundle and a Jensen's ring. The mitochondrial sheath which continues from the neck is composed of 2 mitochondrial helixes twisting regularly about 32 times around the axial fibril bundle from the anterior portion of the middle piece as far as the Jensen's ring. Each helix has a double-layered mitochondrial membrane, mitochondrial cristae and mitochondrial matrix. The Jensen's ring is a circular body with high electron density, located at the junction of the middle piece and the tail piece.

The tail piece consists of a fibrillar coil sheath, 2 longitudinal fibrillar tail strips and the axial fibril bundle. The fibrillar coil sheath consists of 2 fibrillar spirals twisting more than 400 times around the axial fibril bundle from a point just inside of the Jensen's ring to the anterior portion of the end piece. Each spiral shows the same electron density as the fibrils composing the axial fibril bundle. The longitudinal fibrillar tail strips run straight through the tail piece, attached closely inside of the fibrillar coil sheath; one is located at the dorsal side and the other at the ventral.

The end piece consists only of the axial fibril bundle, except for the double-layered cell membrane which covers the entire spermatozoon.

In conclusion, it may be said that the spermatozoon is quite symmetrical on flattened view, but difference between the dorsal and ventral sides can easily be distinguished by investigation ultramicrotomized materials.

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SUPPLEMENTAL REFERENCES

Since the present author wrote this paper in Japanese in March 1962, over three years ago, in the meantime some valuable reports in this field were published. They include critical reviews[125,126] and original papers which are concerned with various animals' sperms[122,131,132] and of the bull sperm[123,124,128,129,134,135], spermatogenesis[130,136,137] technical improvement of sectioning[127] and fundamental study of cell ultrastructures[133].

These papers tell us further important problems about the morphological study of male gametic cells and the present author's mistake, if available.

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EXPLANATION OF PLATES

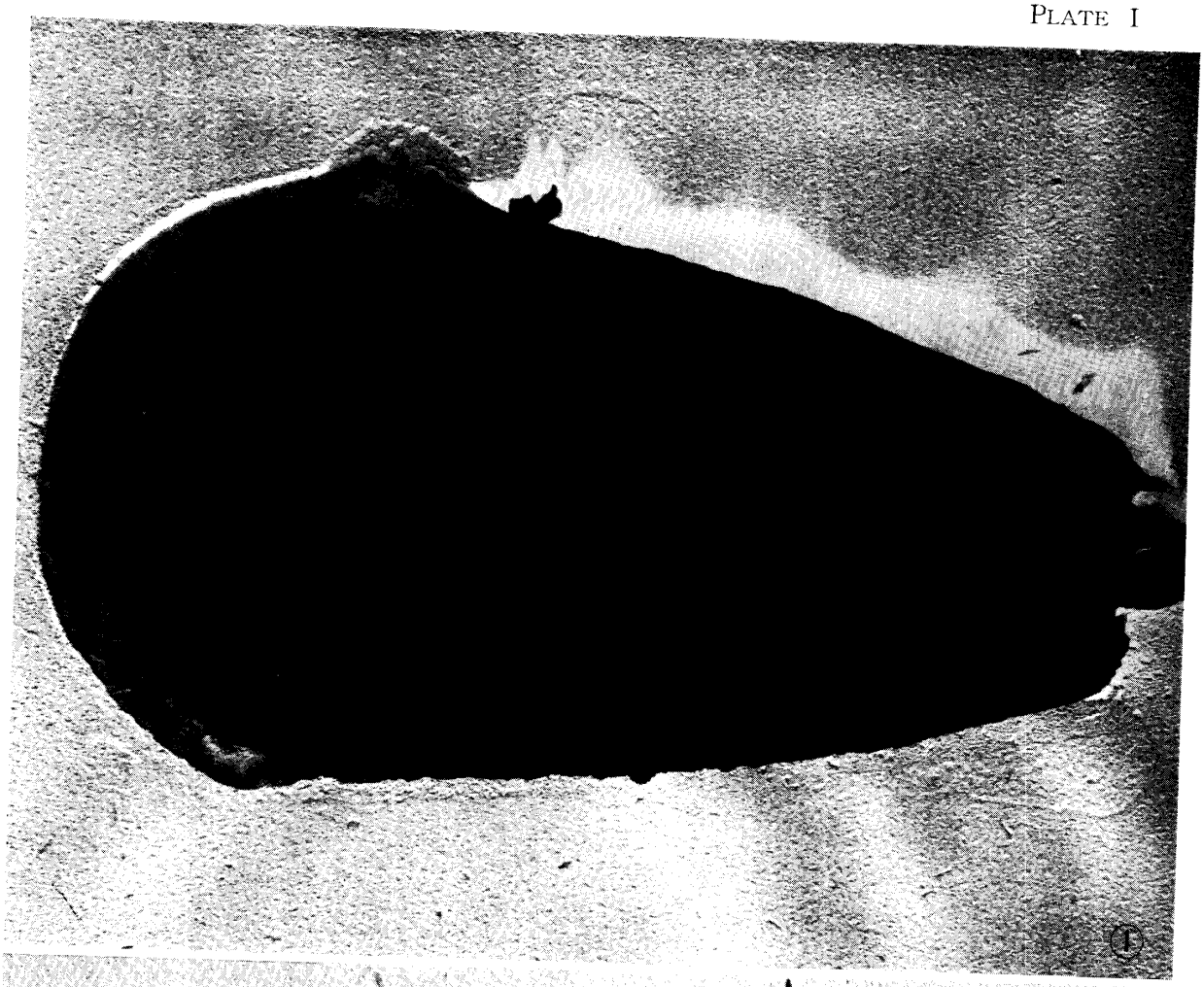
In cases without any indication of magnification in the following explanations, the magnification means 15,000 times.

PLATE I

Each of figures in plates I~IV is based on suspension preparation.

Fig. 1 S-1: A presumably intact head
The head is ovoid in shape, containing nuclear substance with high electron density. At the head top, there is an arc-like structure, the acrosome corpuscle.

Fig. 2 S-2: Swollen head cap after treatment of twice washing with distilled water



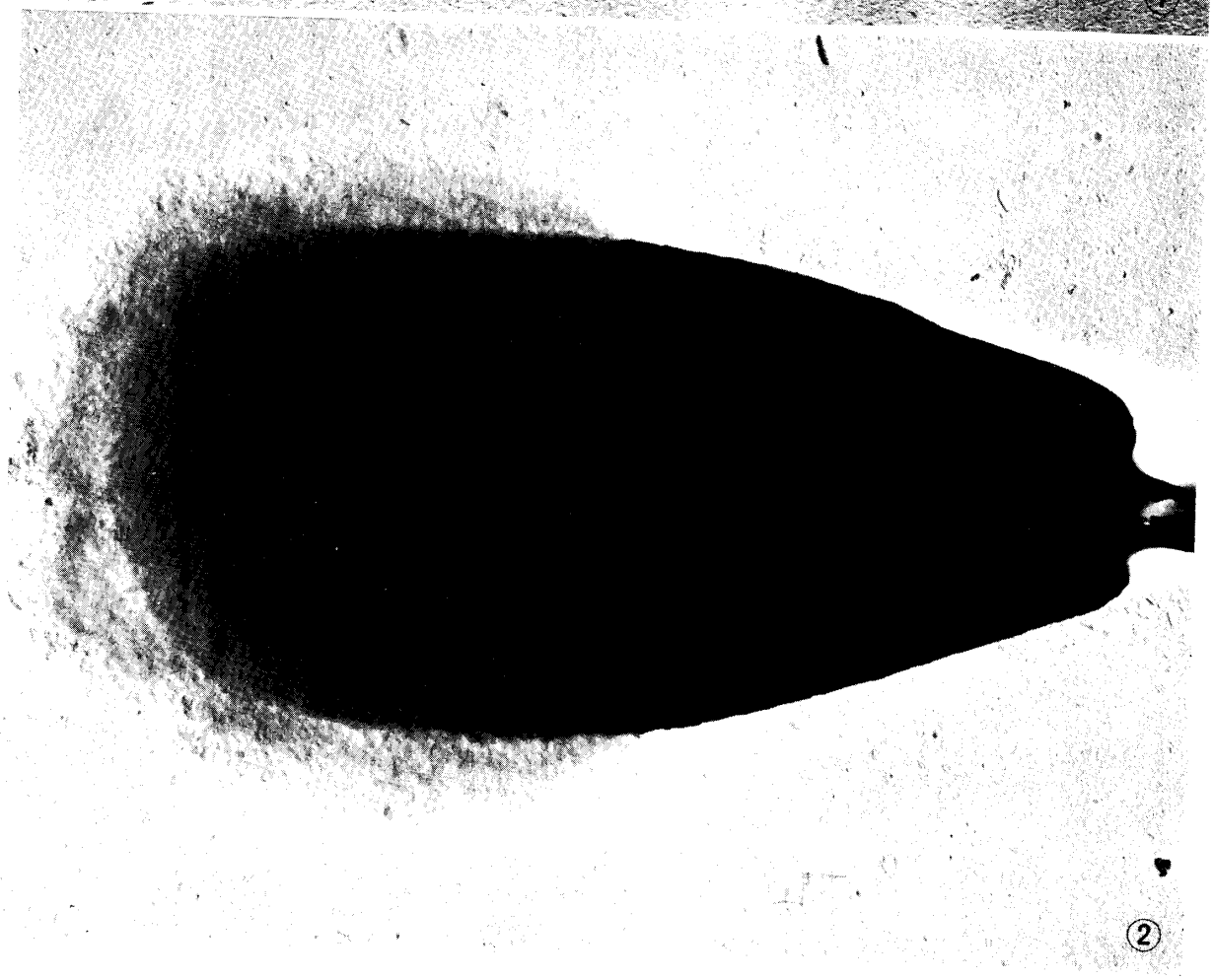
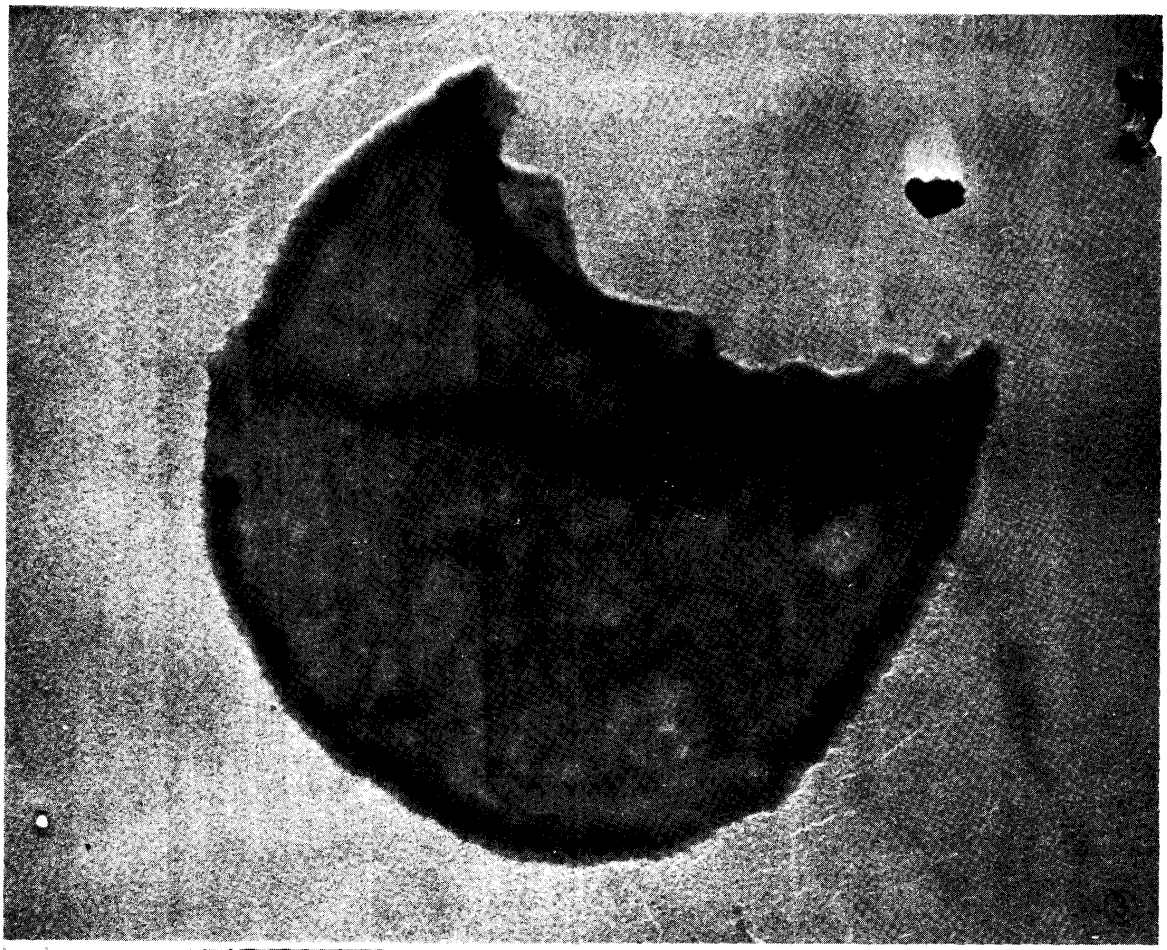


PLATE II

Fig. 3 S-7: A head cap separated from the head proper
It consists of a membraneous substance (acrosome membrane) with low electron density.

Fig. 4 S-2: Acrosome corpuscle and equatorial zone are apparent. External acrosome membrane has lost.
Note the knob-like structures at the posterior margin of the equatorial zone appears.



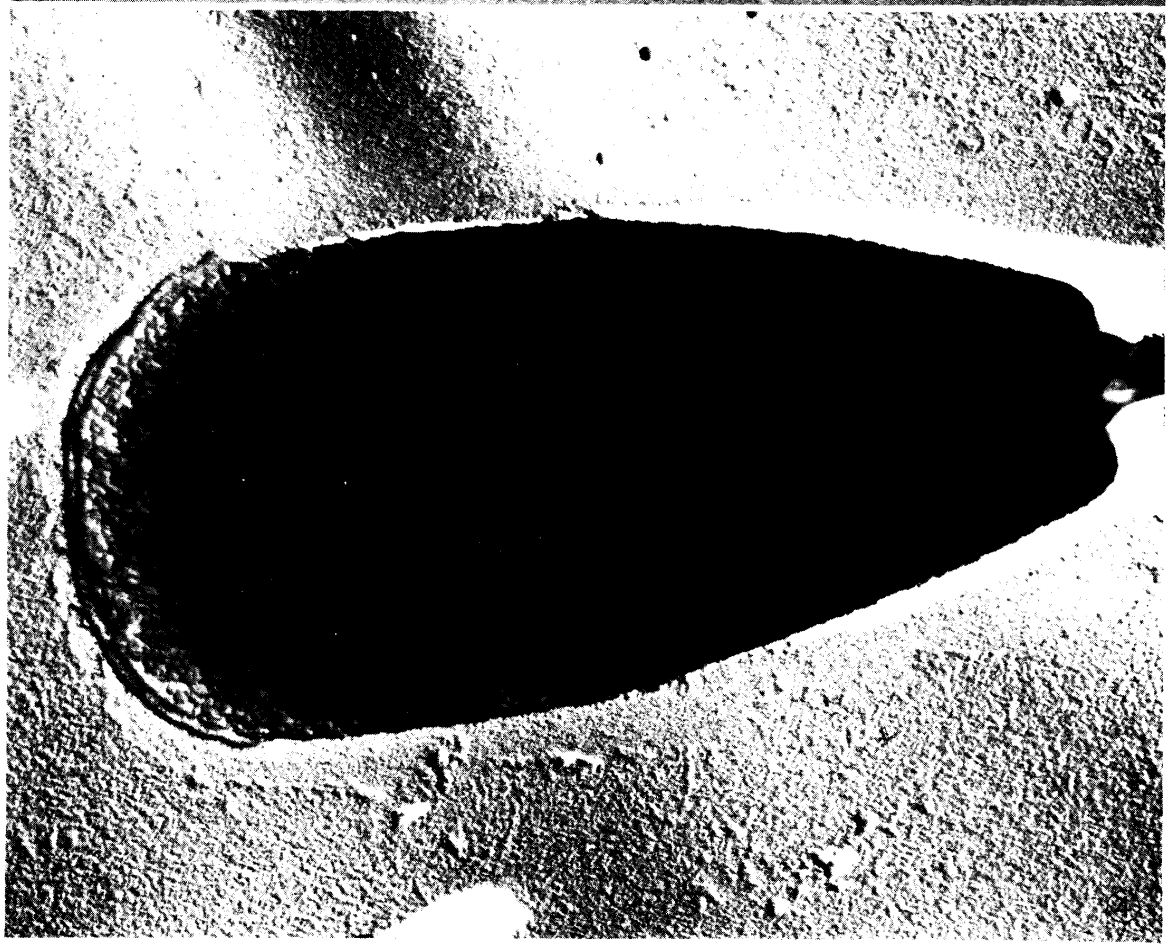
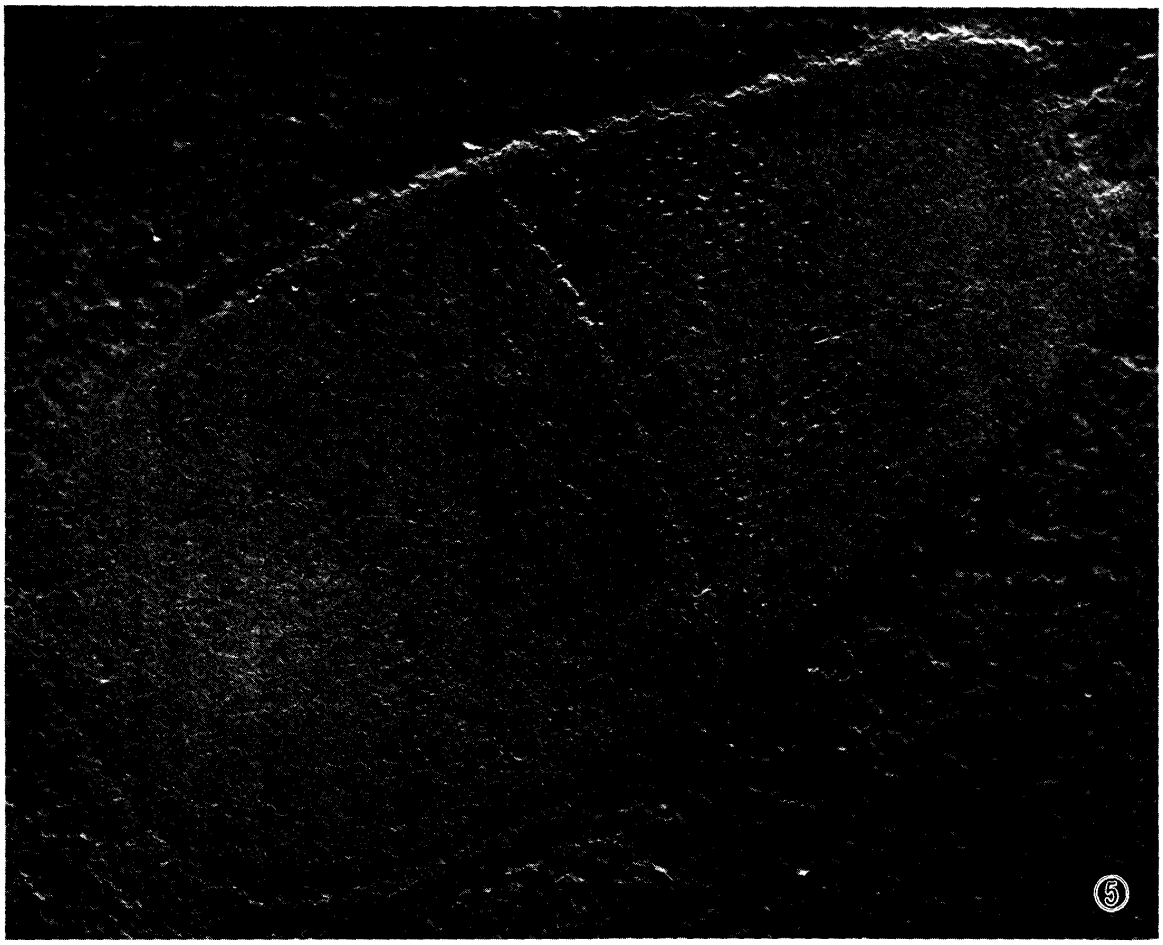


PLATE III

Fig. 5 This figure is based on replica preparation.
Sperm head with head cap
Note equatorial zone and smooth appearance of head cap's replica.

Each of figures in plates III (fig. 6) and IV is based on flattened and longitudinal section.

Fig. 6 E-10: External and internal acrosome membranes are continuous with each other at the middle portion of head.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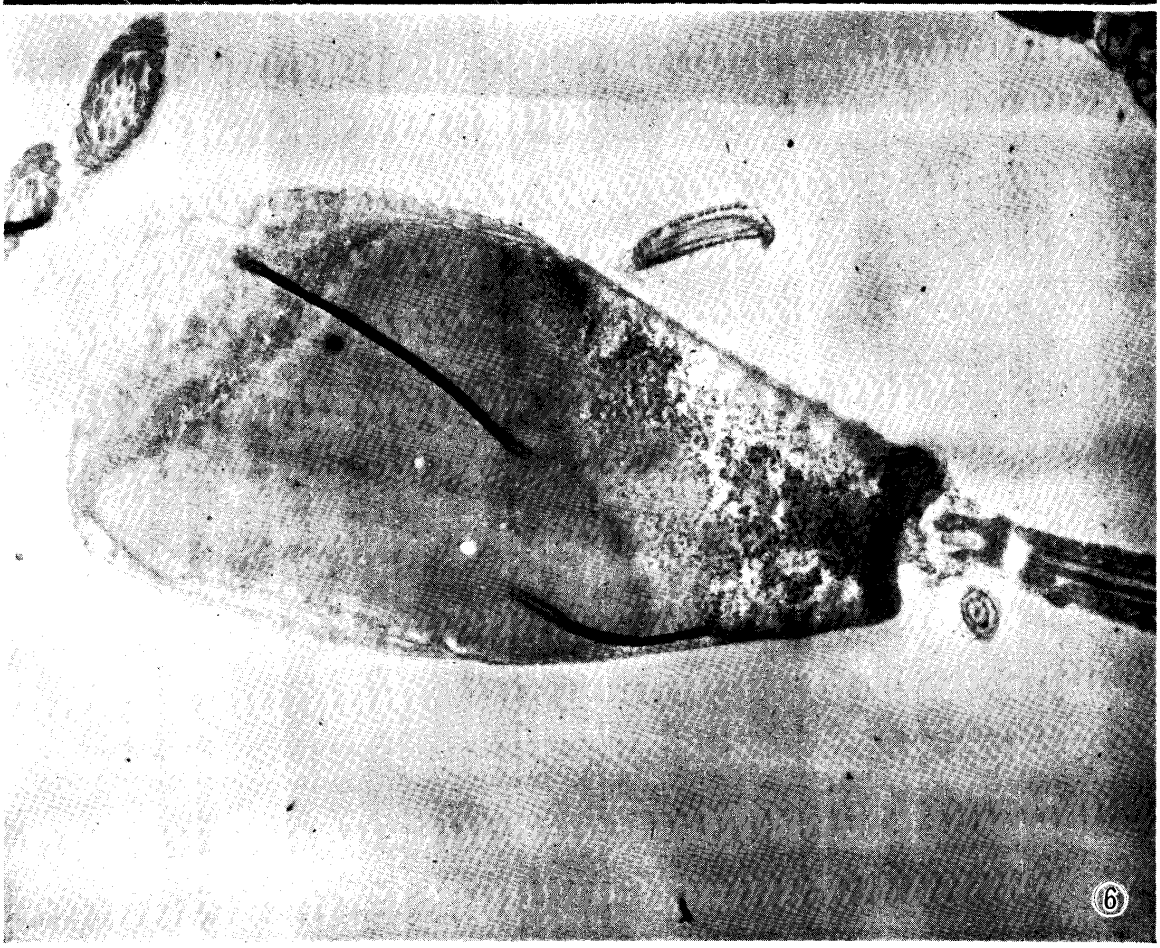
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PLATE IV

Fig. 7 E-10: The cell membrane with a triple-layered structure, unit membrane, envelops the whole of head and neck. Anterior half of head is covered by head cap and also posterior half is inclosed by nuclear sheath. Head cap is composed with a double-layered acrosome membrane (external and internal) and acrosome. Vacuoles of variable sizes and density are present in nuclear substance, predominantly in basal part. Numerous fine vesicles appeared in nuclear proper may suggest holes or pores of nuclear membrane. × 25,000

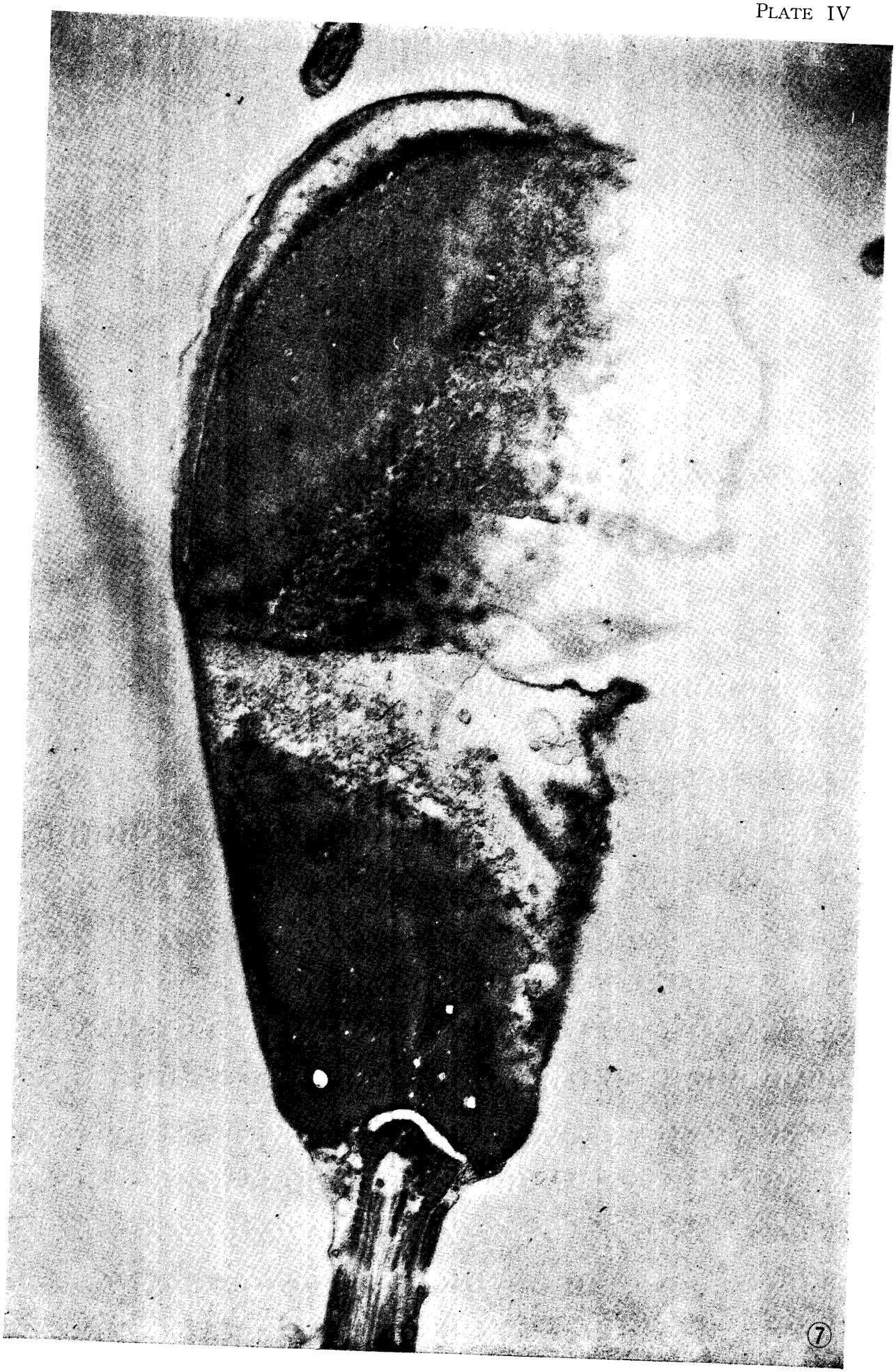
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PLATE V

Each of figures in plate V is based on sagittal and longitudinal section.

Fig. 8 L-1: Profile indicates that head of bull sperm is rather thin and flat. Head contains a nucleus which tapers anteriorly like a sharp sword. At the dorsal side of head top, an acrosome corpuscle is apparent as a dense small mass. The central one of three fossula-like depressions is noticed. Along with the depression, there is a basal plate. × 25,000

Fig. 9 E-10: Triple-layered cell membrane covers entirely the head. Inside of the cell membrane, there is a sac-like structure, head cap, at the anterior half of head, and there is nuclear sheath at the posterior half. The head cap consists of external and internal acrosome membranes, the cavity of which contains acrosome substance and an acrosome corpuscle.

× 25,000

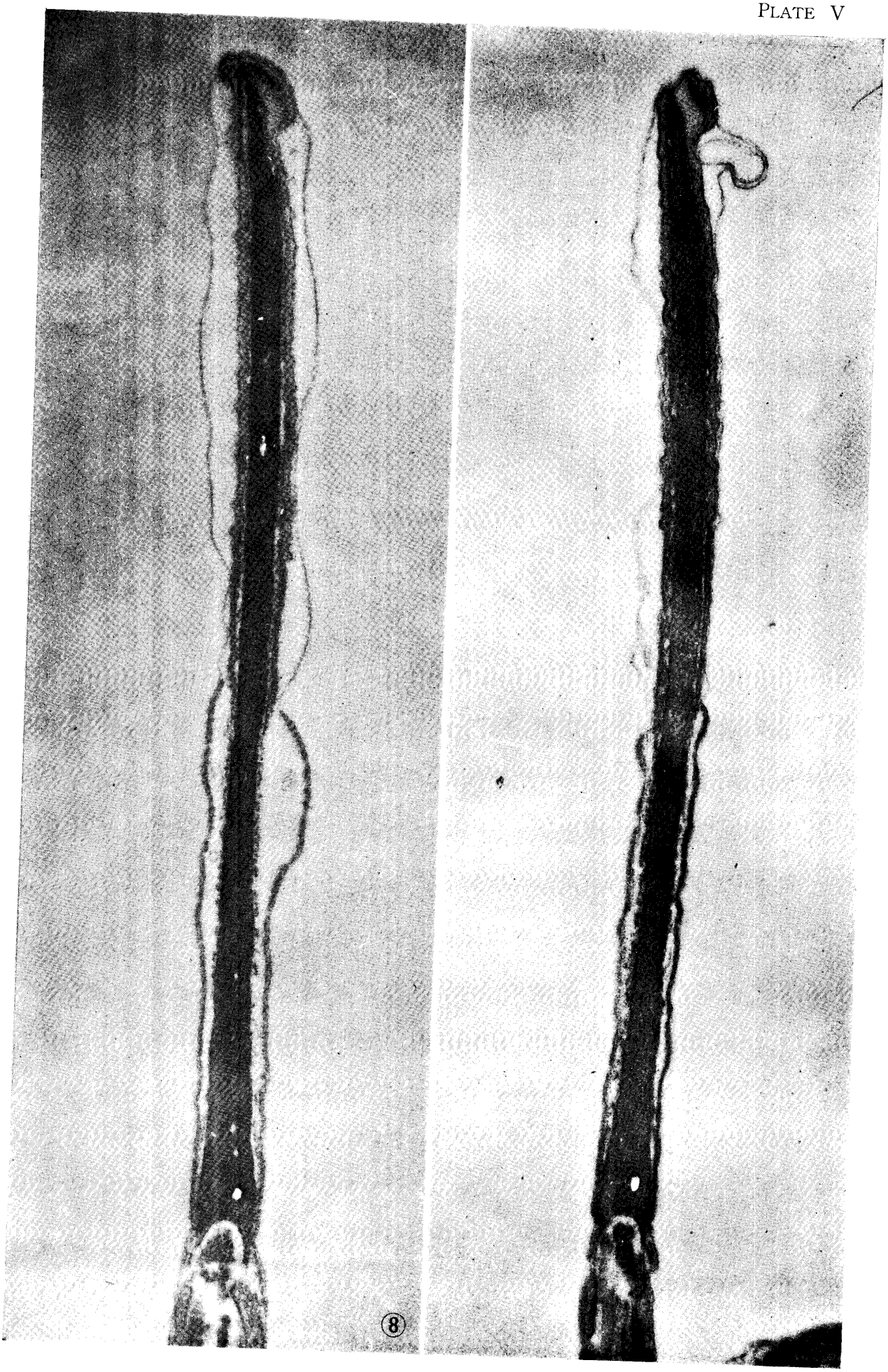
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PLATE VI

Fig. 10 E-10: Cross section of anterior portion of head (left)
Cell membrane attaches to the external acrosome membrane at two points at ventral. Oblique cut of acrosome corpuscle is noted at dorsal.

Fig. 11 L-1: Cross section of anterior portion of head (upper)
Findings resemble as those of figure 10.

Fig. 12 E-4: Flattened and longitudinal section of head
This section is assumedly showing a phase between surface of sperm nucleus and cell membrane. At the ventral side, the cell membrane shows a characteristic attachment pattern. This figure is in extremely accordance with its cross section.

⑩

⑪

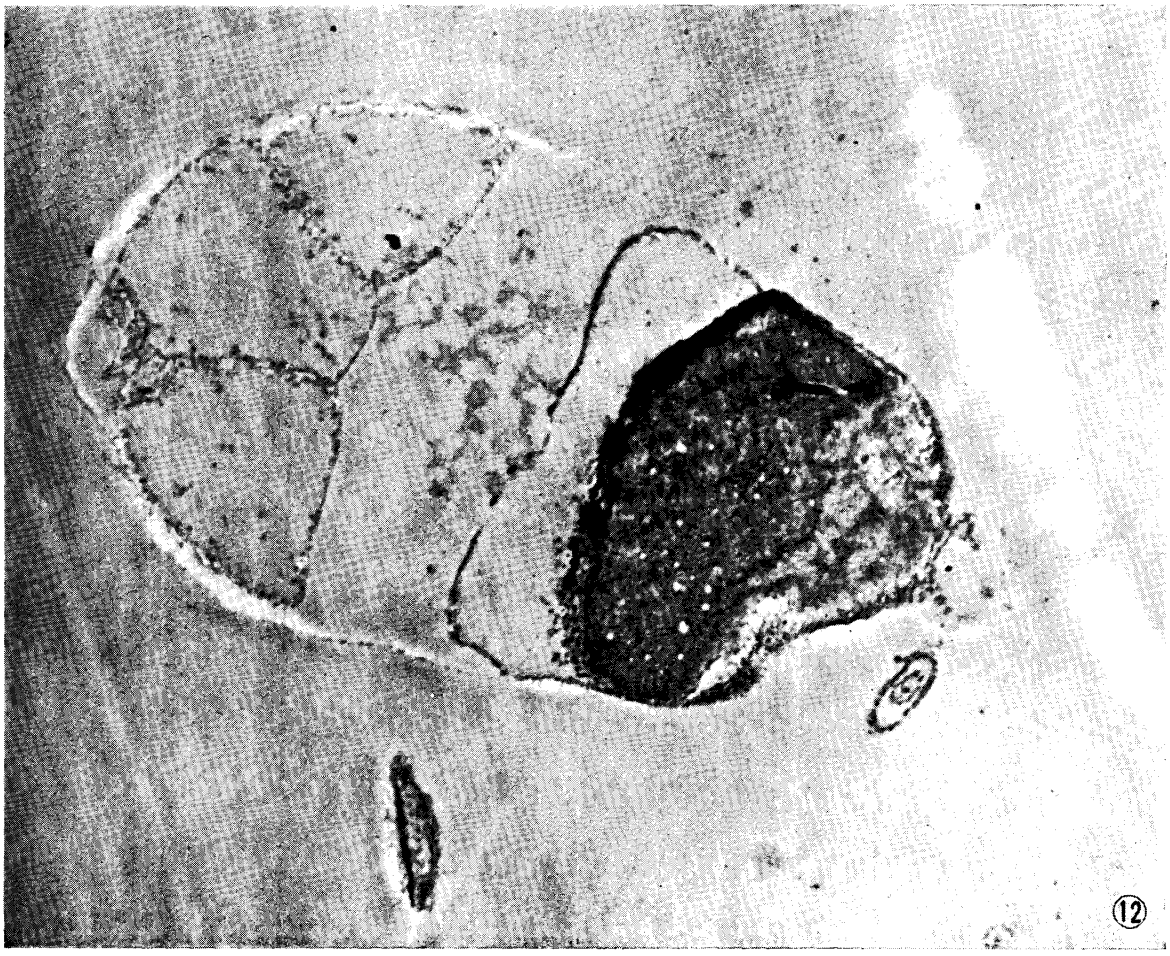
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PLATE VII

Fig. 13 L-1: Flattened and longitudinal section through head, neck and middle piece

Cell membrane entirely covers these regions. At basal portion of head, nuclear sheath covers nuclear substance inside of cell membrane, and a basal granule which consists of several fine granules appears at the left side. At junction part between head and neck there is a basal plate along with three fossula-like depressions. In neck portion, there are two large radixes which consist of several neck platelets. In the central portion of neck, a centriole with easily fragile nature appears. A characteritic mosaic junction is noted between medium radixes of neck and thicker peripheral fibrils of middle piece. Cut surfaces of a mitochondrial sheath appear at both sides of neck and middle piece. × 30,000

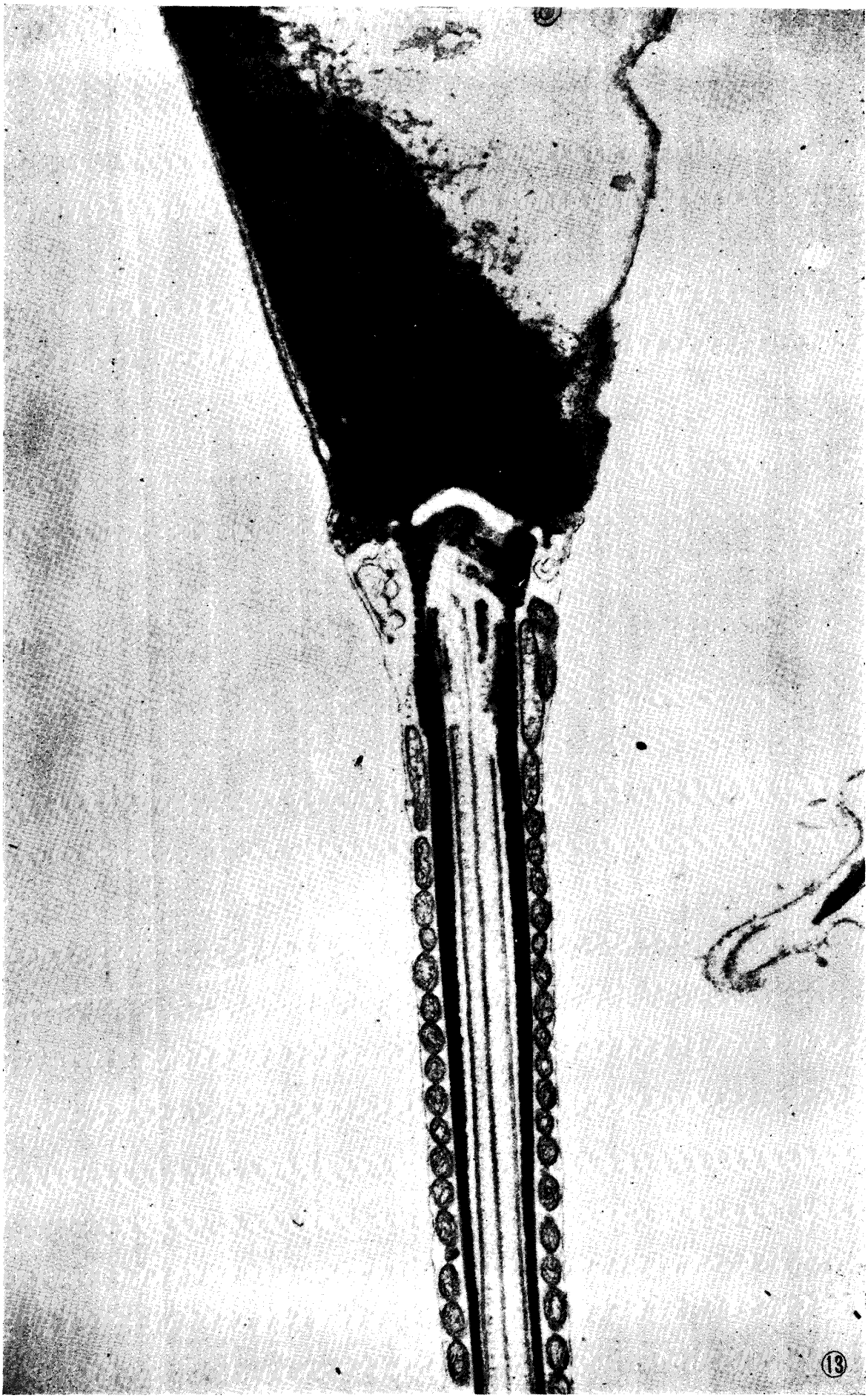
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PLATE VIII

Figures 14 and 15 are based on suspension preparation.

Fig. 14 S-1: A border between neck and middle piece is indistinct.

Fig. 15 S-2: More severely damaged sperm
Neck is completely separated from head. Anterior end of neck shows three protrusions of head. Tail pieces of other spermatozoa are entangled in this photograph.

Fig. 16 Replica of sperm neck × 30,000

14

15

16

PLATE IX

Fig. 17 L-1: Each of two large radixes consists of piled neck platelets forming a triangular shape at anterior end of neck. This is more clear at left side in this figure.

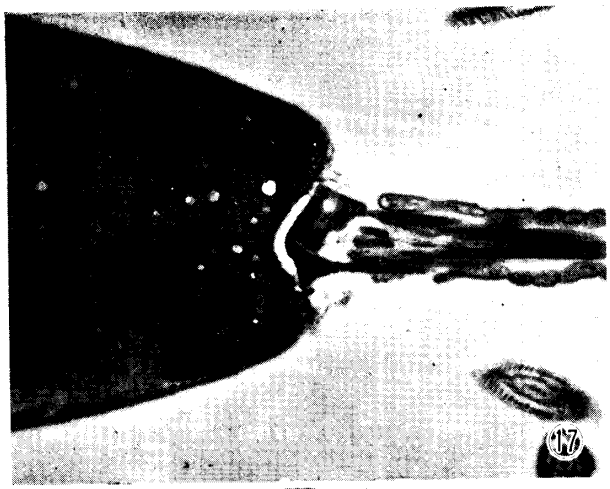
Fig. 18 E-2: Basal granule is clearly visible at each of lateral sides of basal part of head, in which fossula-like depressions and a basal plate are also noted. One of large radixes is more distinct at right side than left.

Fig. 19 E-3: Sagittal and longitudinal section of neck and neighbors Centriole, a ring structure at anterior portion of neck, is distinct.

Fig. 20 E-3: Sagittal and longitudinal sections of neck and neighbors In middle portion of neck, it is clearly shown that a pair of medium radixes originating from a large radix twists and exchanges its position with the other one.

Fig. 21 L-1: Flattened and longitudinal section of neck and neighbors Two small radixes run parallel with central axis in neck. It is assumed that this figure may represent ventral side of sperm.

Fig. 22 E-10: Flattened and longitudinal section of neck and neighbors Three small radixes run through neck and middle piece. This figure may show dorsal side of sperm.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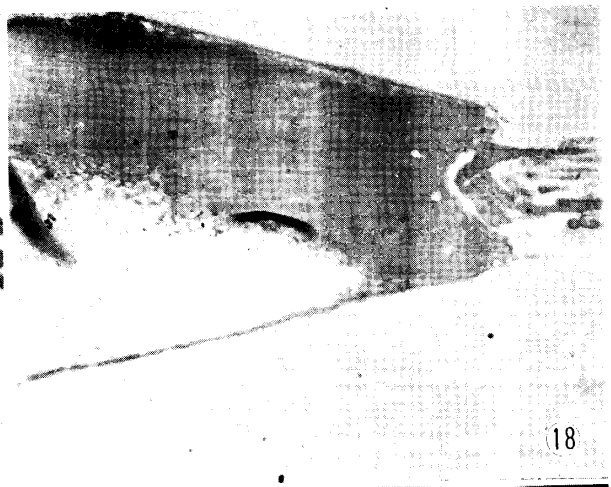
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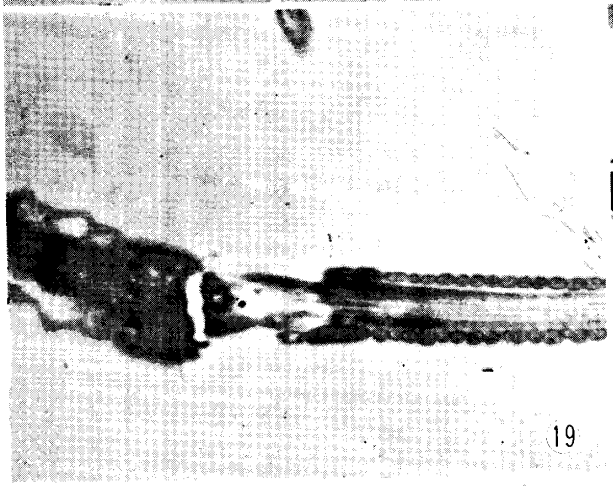
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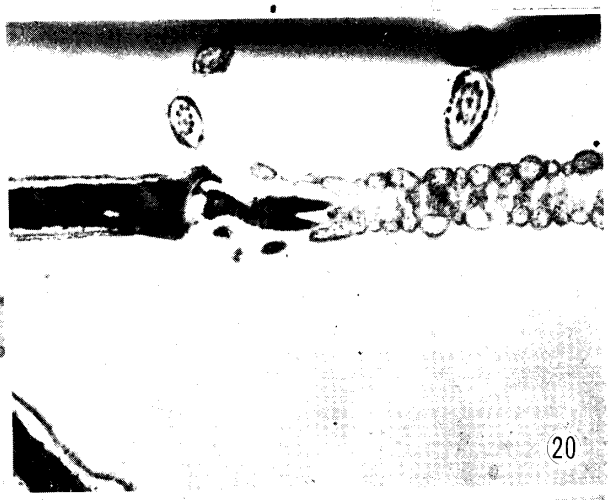
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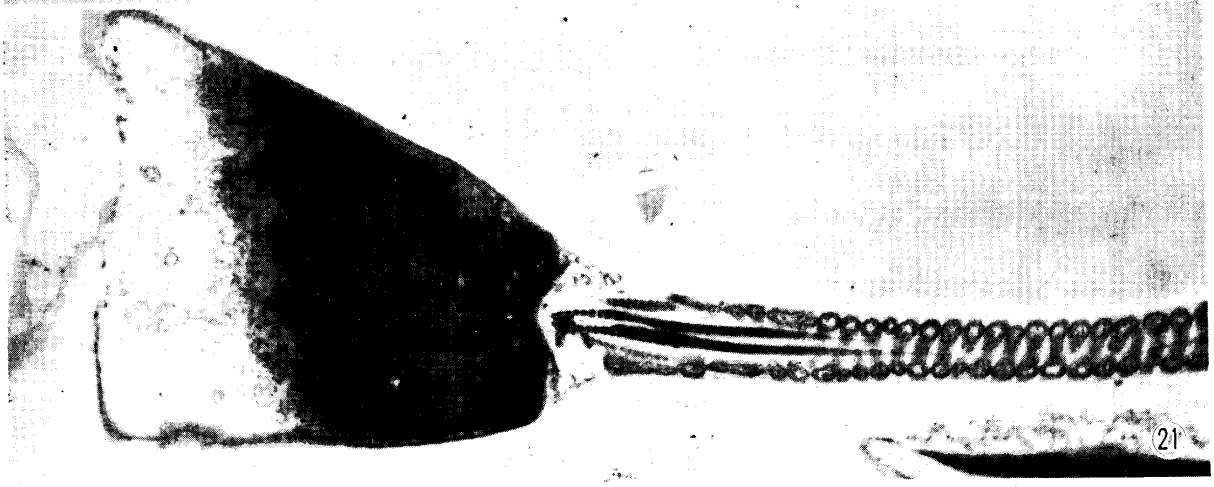
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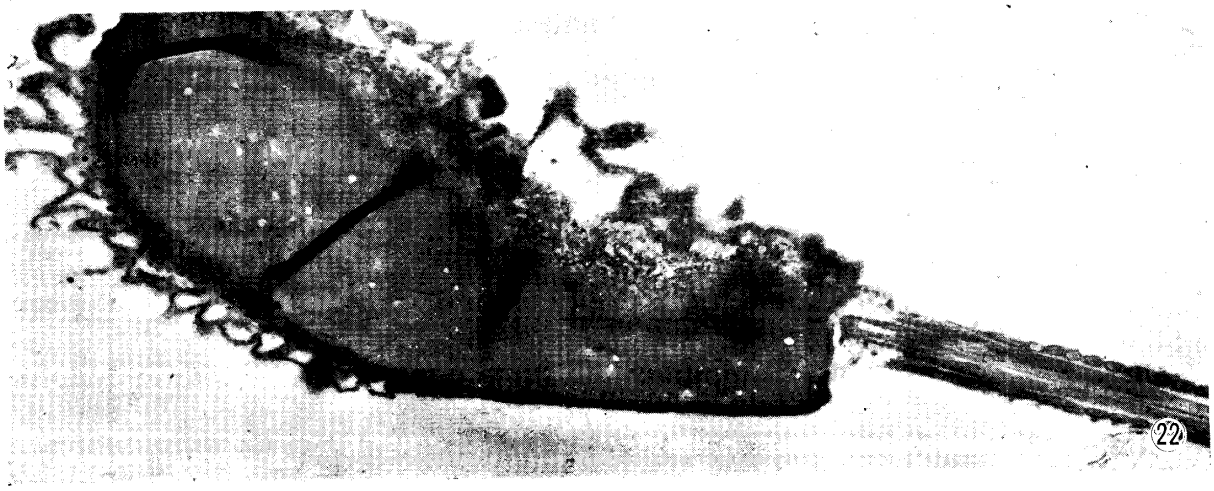
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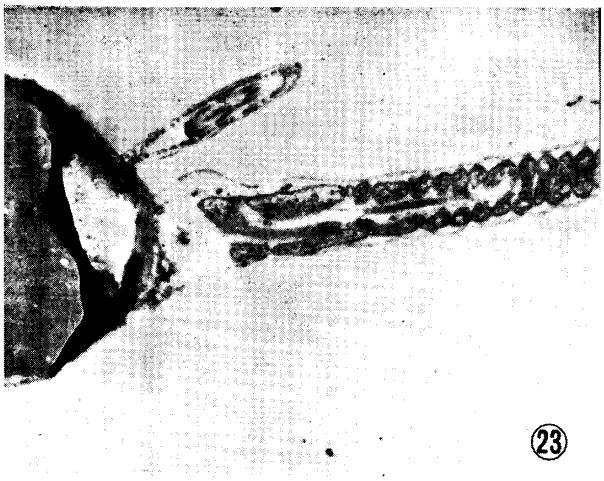
PLATE X

Fig. 23 E-10: Flattened and longitudinal section
Mitochondrial sheath is irregular in neck.

Fig. 24 E-10: Flattened and longitudinal section
This figure may show ventral side, because of presence of two small radixes in neck.

Fig. 25 L-1: Cross section of anterior portion of neck (arrow)
Inside of mitochondrial sheath, each of radixes takes elliptic form around centriole, 2 large radixes locating at both lateral sides, 3 small radixes at dorsal side and remaining 2 small ones at ventral.

Figs. 26, 27 & 28 L-1, and Fig. 29 E-10: Oblique sections of neck with a part of head
Typical arrangement pattern of radixes in neck is noticed in each of these figures, especially in figure 29 (arrow). The pattern seems to be symmetric against an assumptive plane passing through on a pair of central fine fibrils.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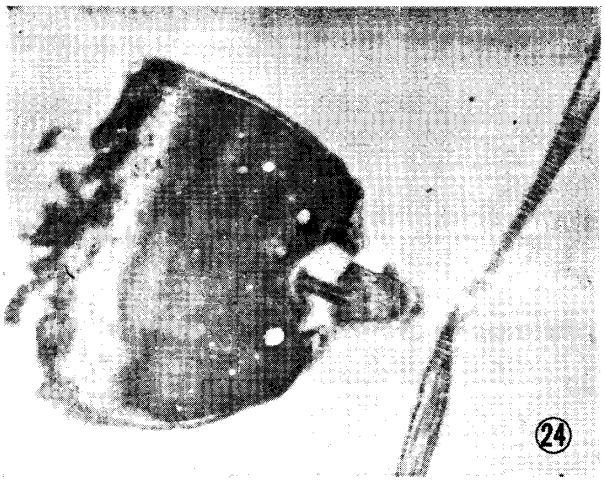
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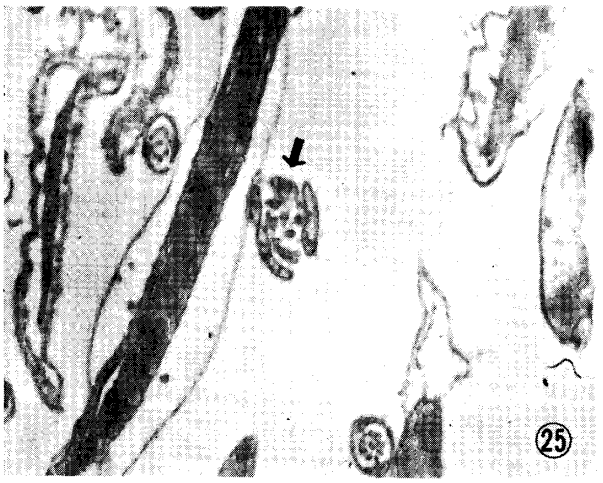
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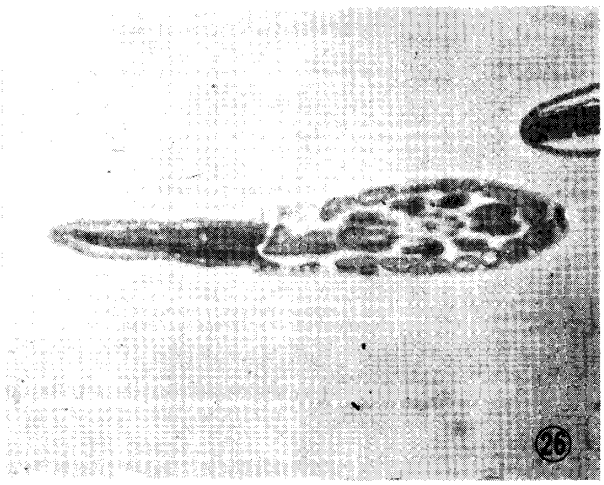
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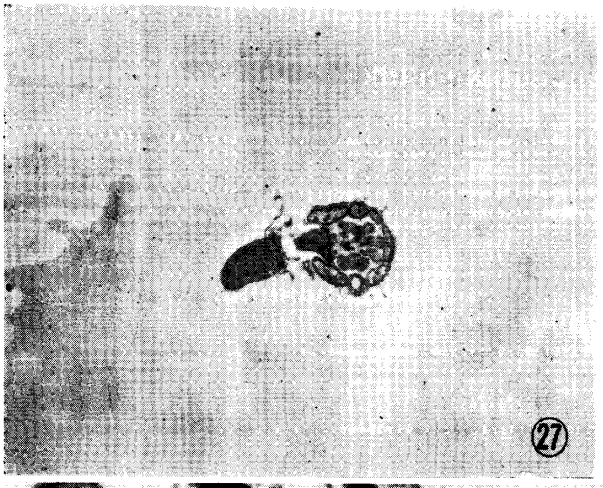
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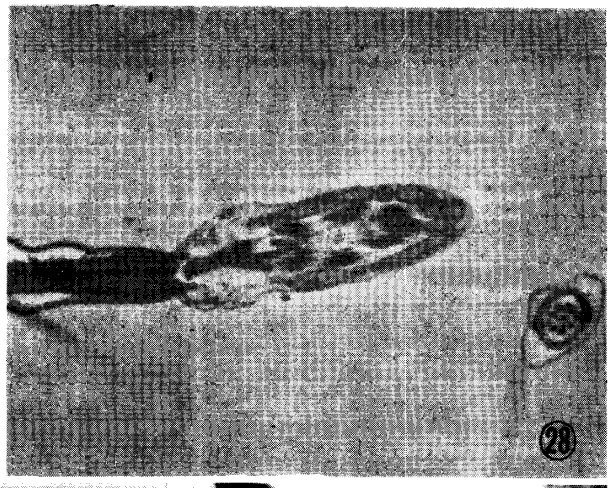
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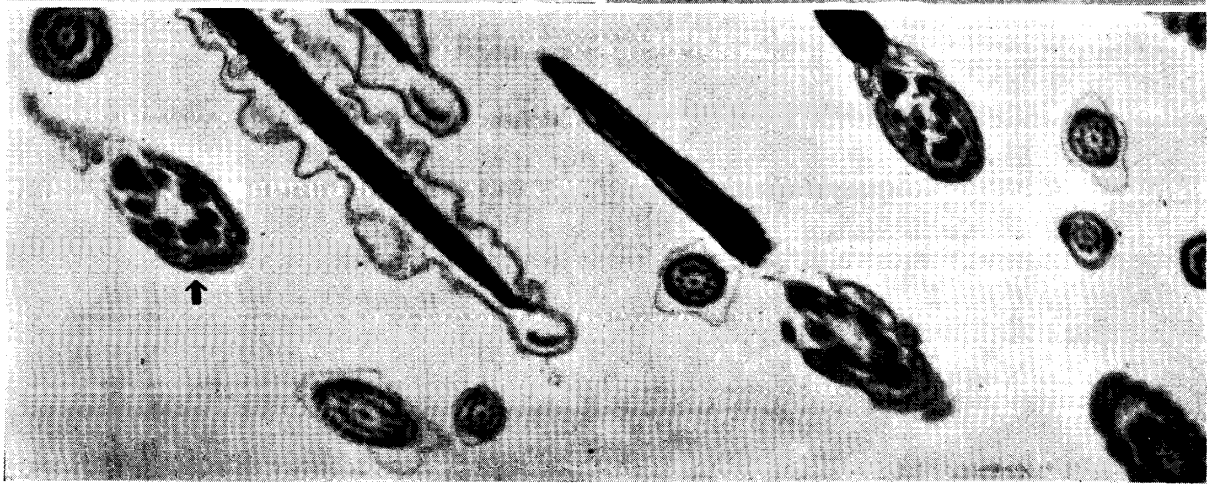


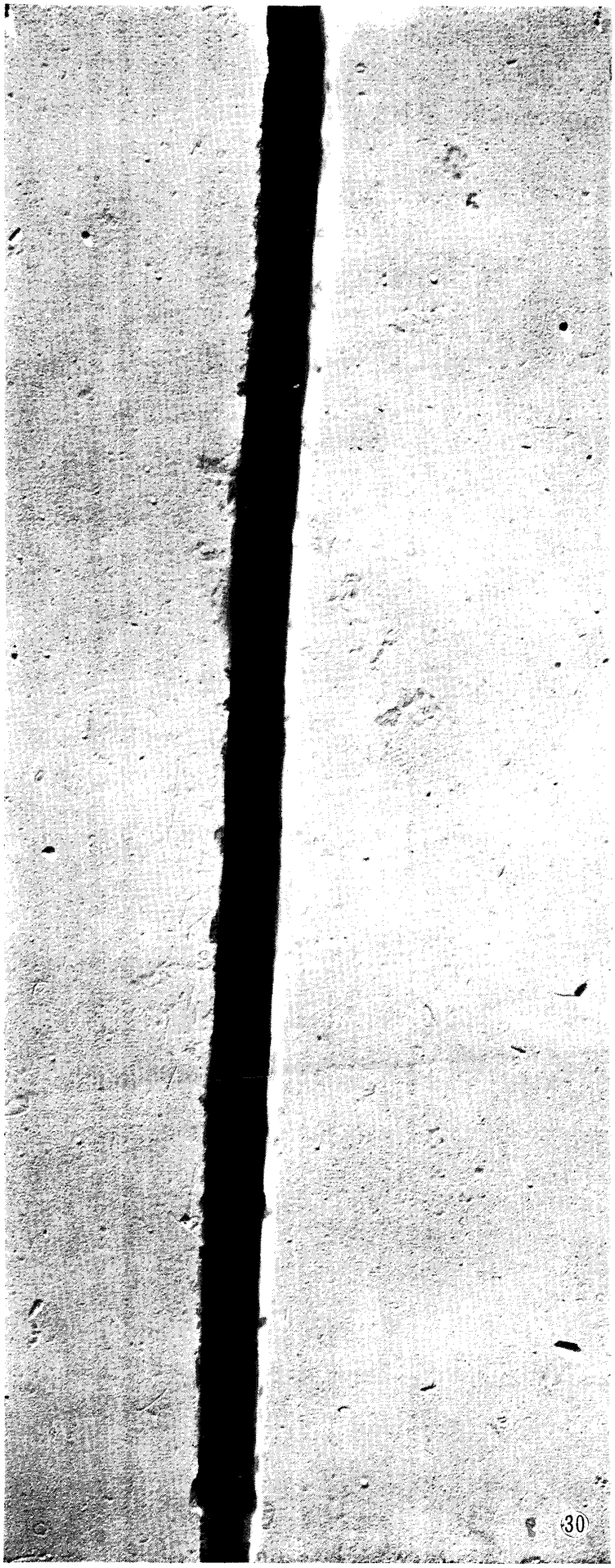
PLATE XI

Fig. 30 S-2: Suspension preparation of middle piece
A boundary neck and middle piece is indistinct, but a boundary middle piece and tail piece is clear. At the transitional part, there is Jensen's ring. Each part of the photographs; figure 2: head, figure 30: middle piece, figure 40: anterior part of tail piece, and figure 41: posterior part of tail piece, is based on a spermatozoon from the suspension material.

Fig. 31 Replica of middle piece
Note distinct replicas of mitochondrial spiral helixes.

Fig. 32 Replica of middle piece and tail piece
Note replicas of helixes of mitochondrial sheath and replica of Jensen's ring.

From the results of figures 31 and 32, the direction of spiral of mitochondrial helixes is constantly observed as counter-clockwise.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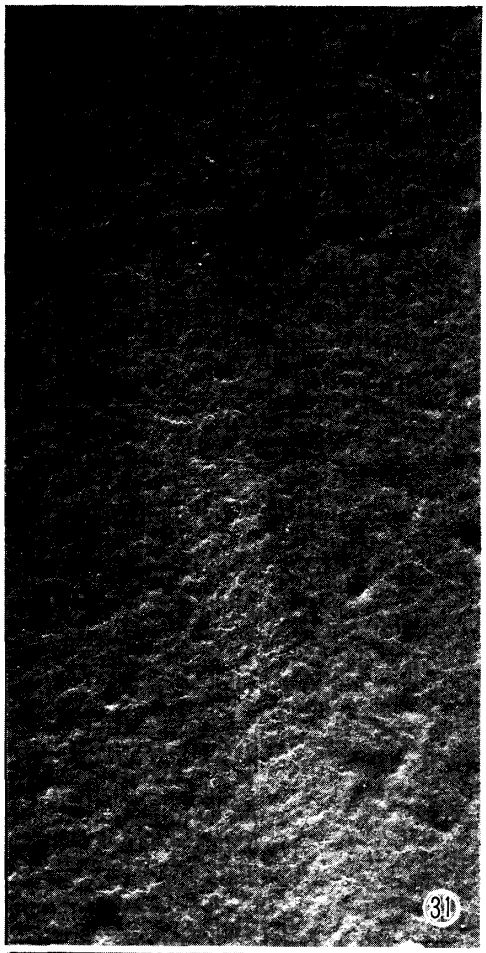
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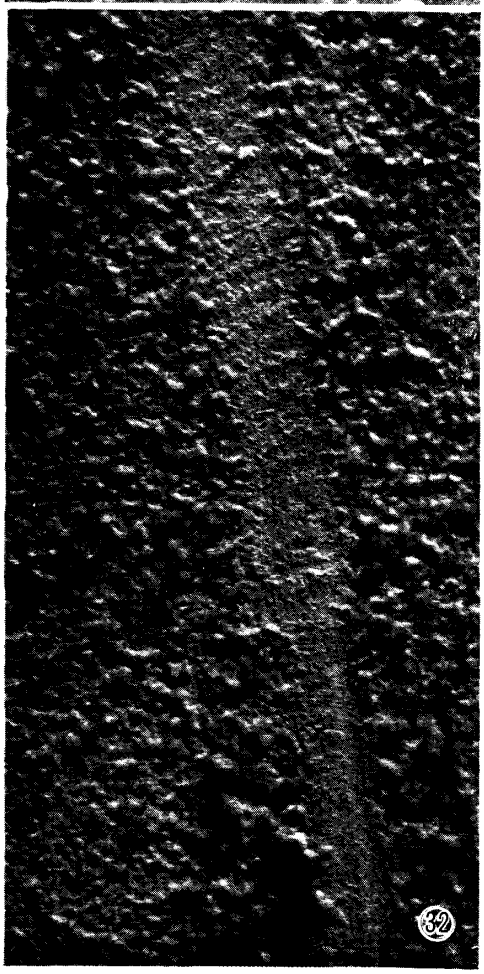
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PLATE XII

Each of figures in plate XII is based on longitudinal section of middle piece.

Fig. 33 L-7: There are many cutting surfaces of mitochondrial helixes with regular arrangement inside of cell membrane. Two thicker peripheral fibrils, two peripheral fine fibrils and one of central fine fibrils are also distinct.

Fig. 34 L-5; Most parts of middle piece are sectioned obliquely. The cutting surfaces of mitochondrial helixes appear as a structure of serial elliptic shape.

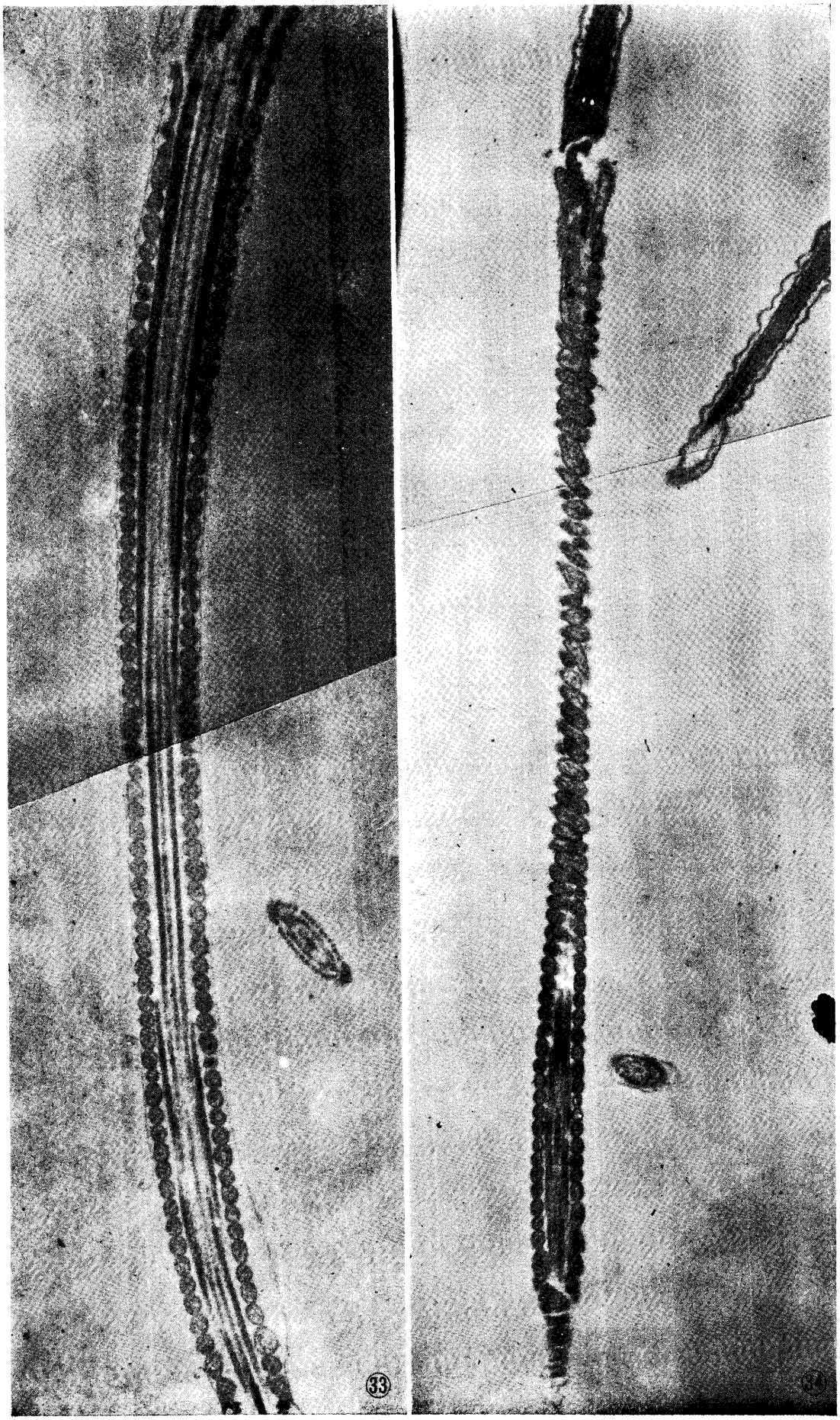
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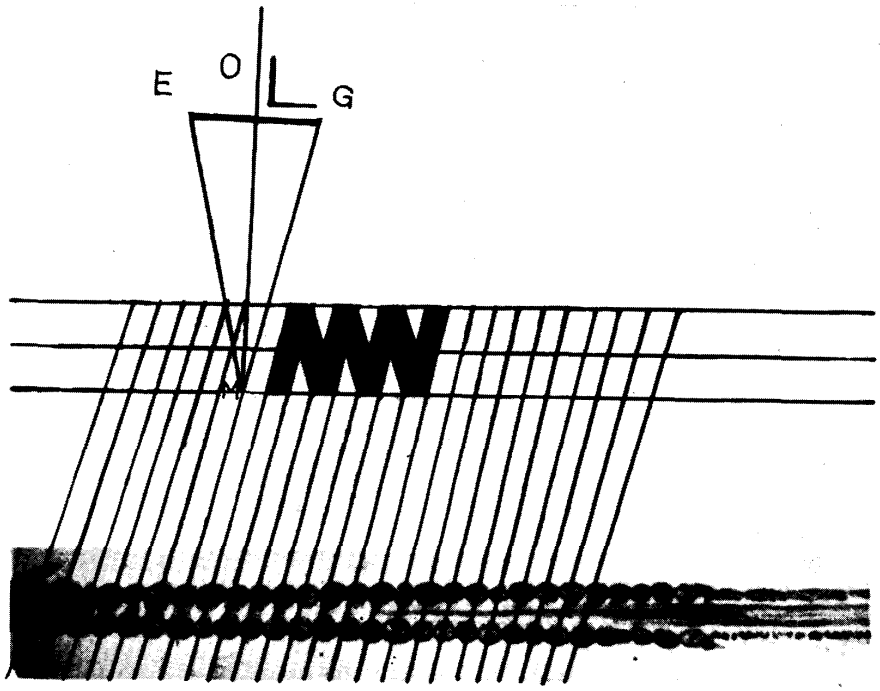
PLATE XIII

Fig. 35 L-1: Longitudinal section of middle piece and its schema
A theoretical explanation of the number of mitochondrial helixes is given by analysis of this schema. It is estimated that mitochondrial sheath is composed of two helixes, since the following two geometrical conditions are satisfied:

$$\angle MOE = \angle R \quad \text{and} \quad \triangle MOE \equiv \triangle MOG$$

Fig. 36 L-1: Successful cross section of middle piece (left)
Note cell membrane, mitochondrial sheath and characteristic arrangement of [9 : 9 : 2] pattern in axial fibril bundle which consists of 9 peripheral fibrils, 9 peripheral fine fibrils and a pair of central fine fibrils. A line passing through the paired central fine fibril may be noticed as a symmetrical one of this part.
× 30,000

Fig. 37 E-3: Cross sections of middle piece
Note the central one, in which two cutting surfaces of mitochondrial helixes are distinct. This figure may strongly support the estimation with figure 35. × 30,000



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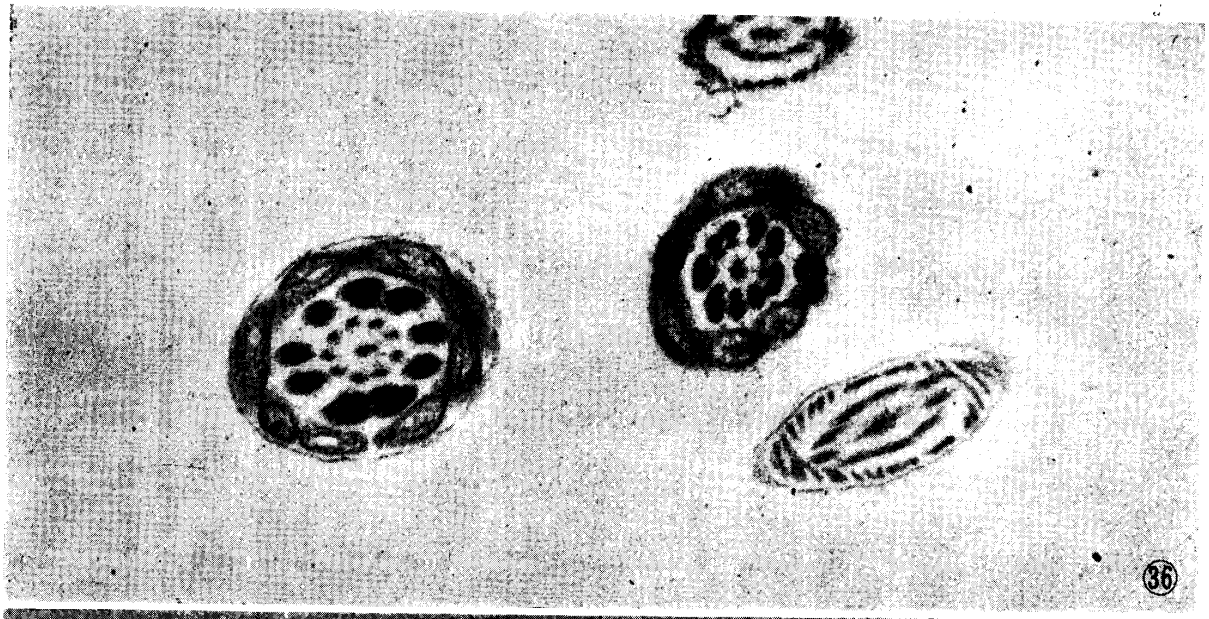

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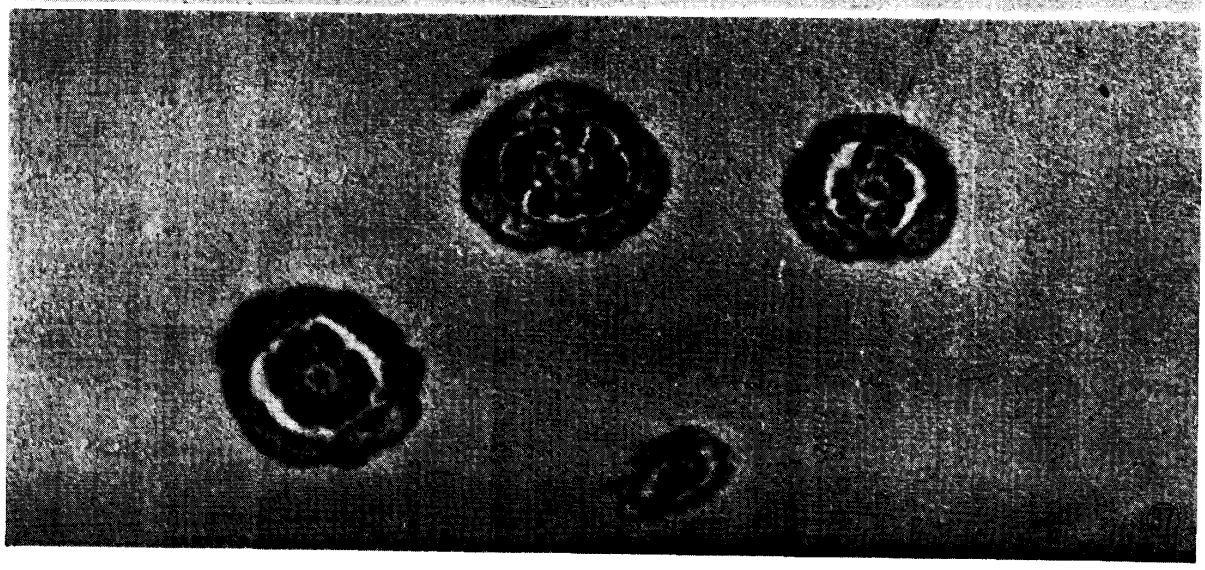

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PLATE XIV

Fig. 38 L-6: Longitudinal section through middle piece to tail piece (center)

Axial fibril bundle runs straight down throughout middle piece and tail piece. Outside of it, there is mitochondrial sheath with regular spirals in middle piece and there is fibrillar coil sheath in tail piece.

Fig. 39 L-1: Oblique sections through head to middle piece

Centriole in neck and a pair of central fine fibrils in middle piece are clearly shown in this figure.

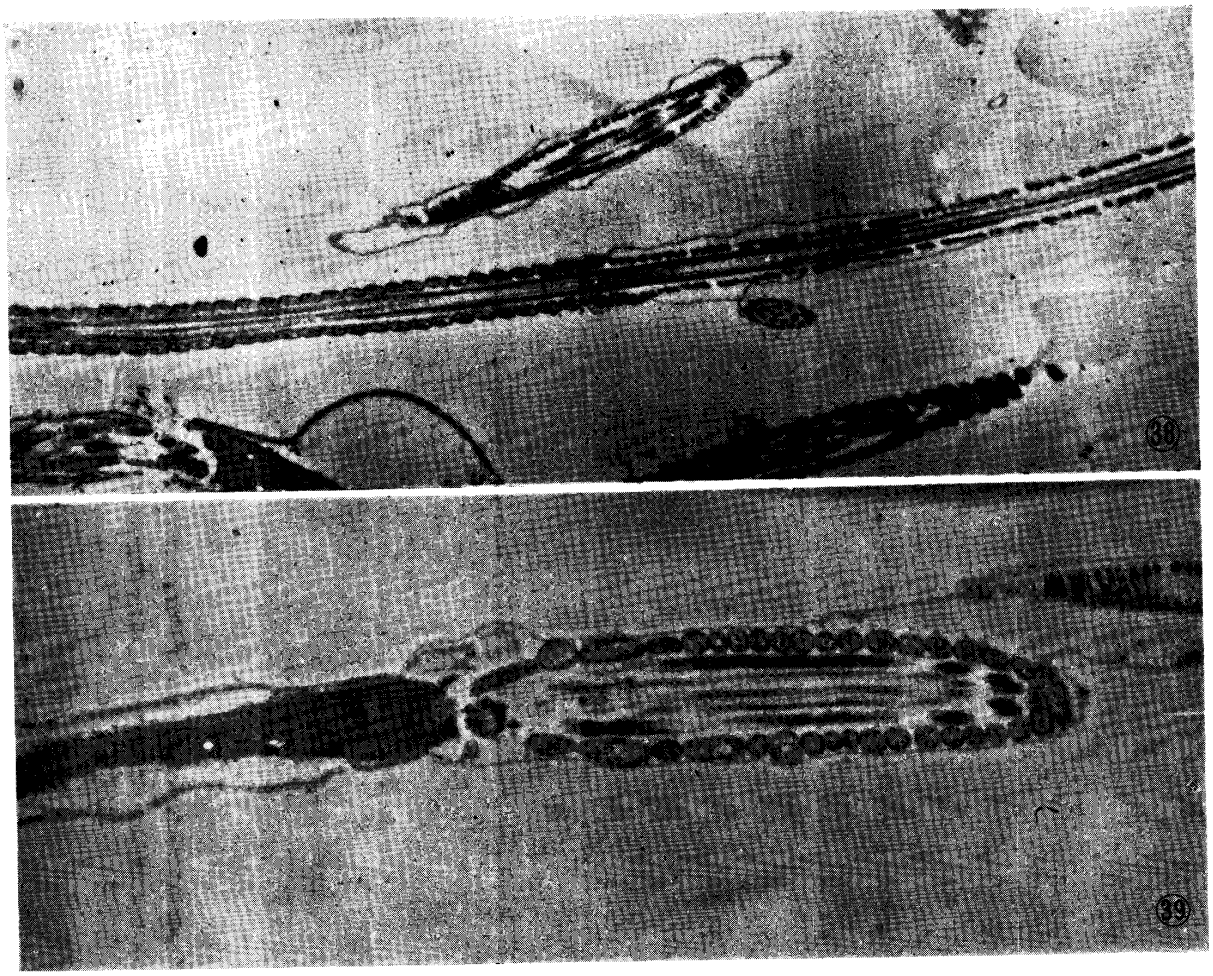
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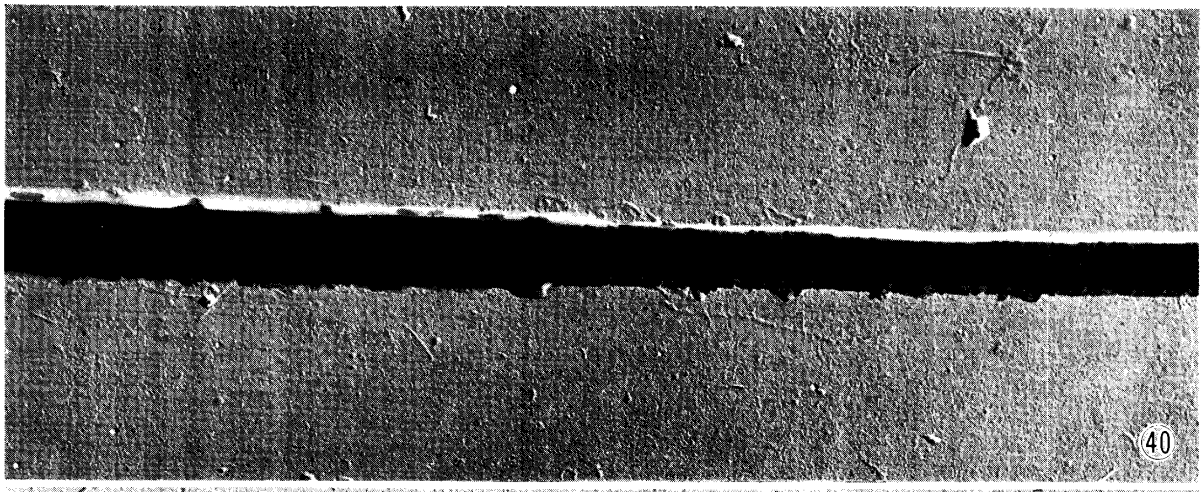
PLATE XV

Each of figures in plate XV is based on suspension preparation.

Fig. 40 S-2: Middle piece and anterior portion of tail piece
Note fibrillar coil sheath in tail piece.

Fig. 41 S-6: Posterior portion of tail piece
Toward posteriorly, tail piece becomes more thinner.
Note axial fibril bundle and fibrillar coil sheath surrounding it.

Fig. 42 S-6: Two typical figures of sperm head with neck and middle piece are shown. Four tail pieces are noted, some of which show fine structures of fibrillar coil sheath. Brush-like appearance is also given in two end pieces (under left and middle right). × 5,000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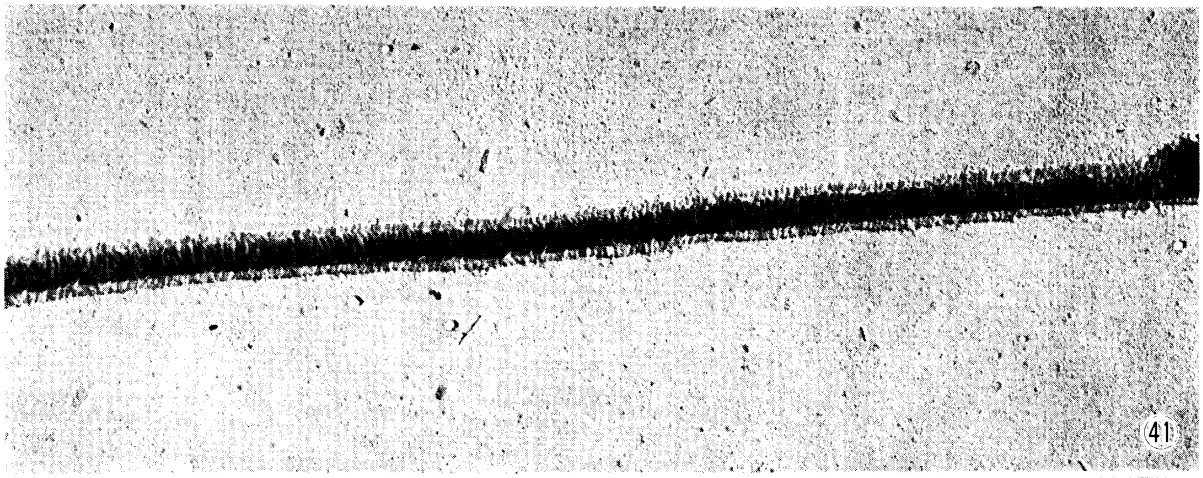
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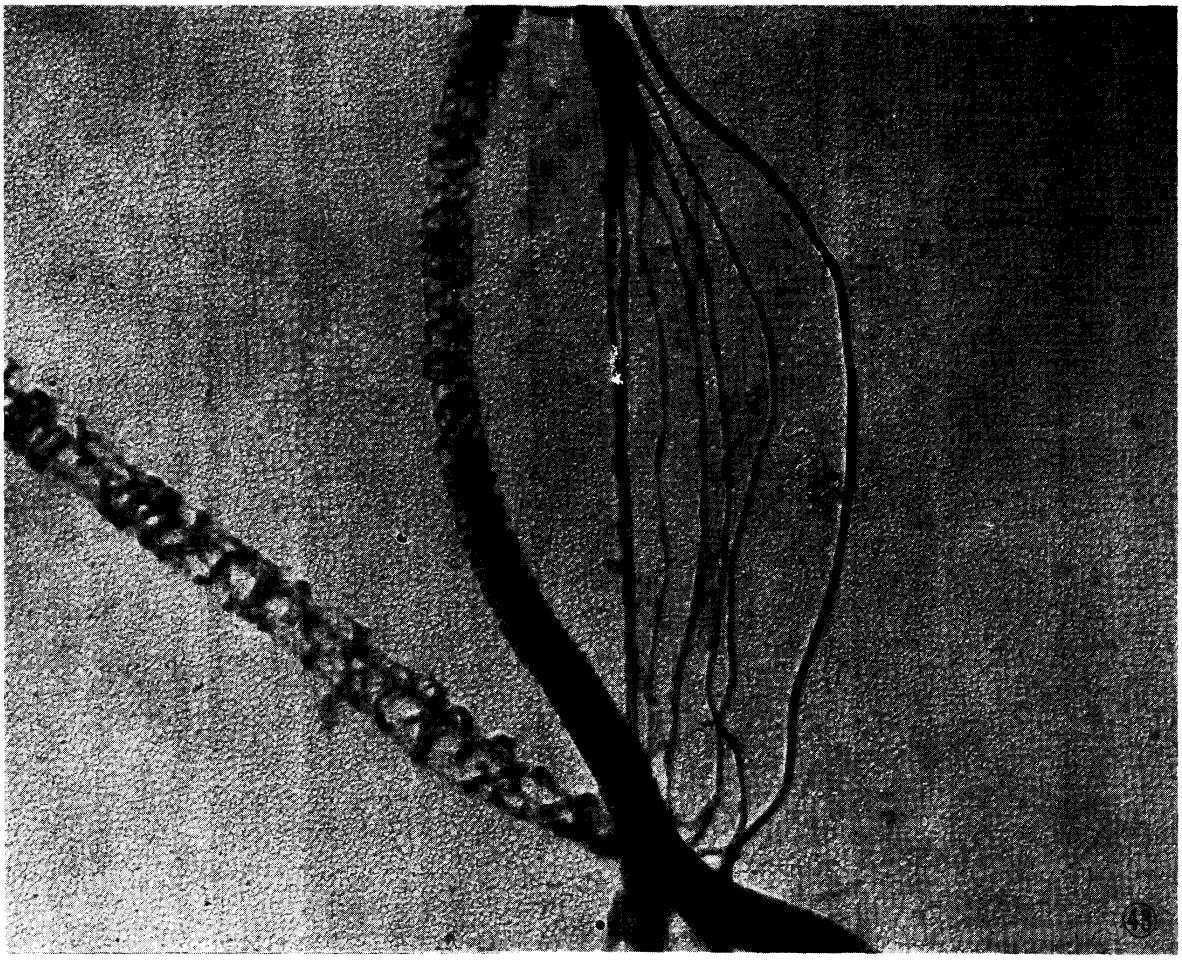
PLATE XVI

Fig. 43 S-3: Damaged tail pieces

Fibrillar coil sheath and longitudinal tail fibrillar strips are highly collapsed, giving a ghost appearance (left and middle). Fibrils of axial fibril bundle are separated.

Fig. 44 S-3: A part of damaged tail piece

Fibrillar coil sheath and fibrils of axial fibril bundle get loose. Nine or 11 fibrils can be counted.



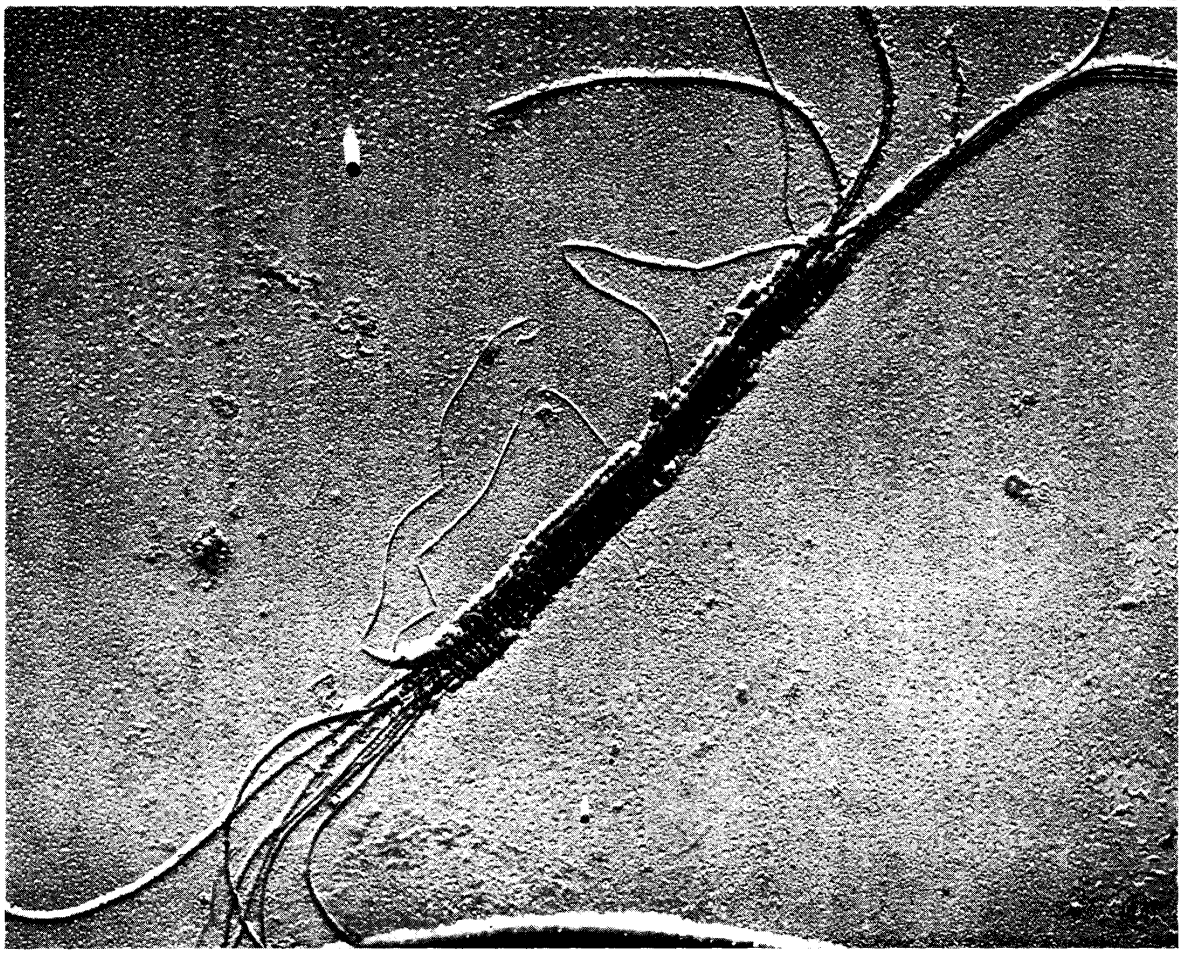


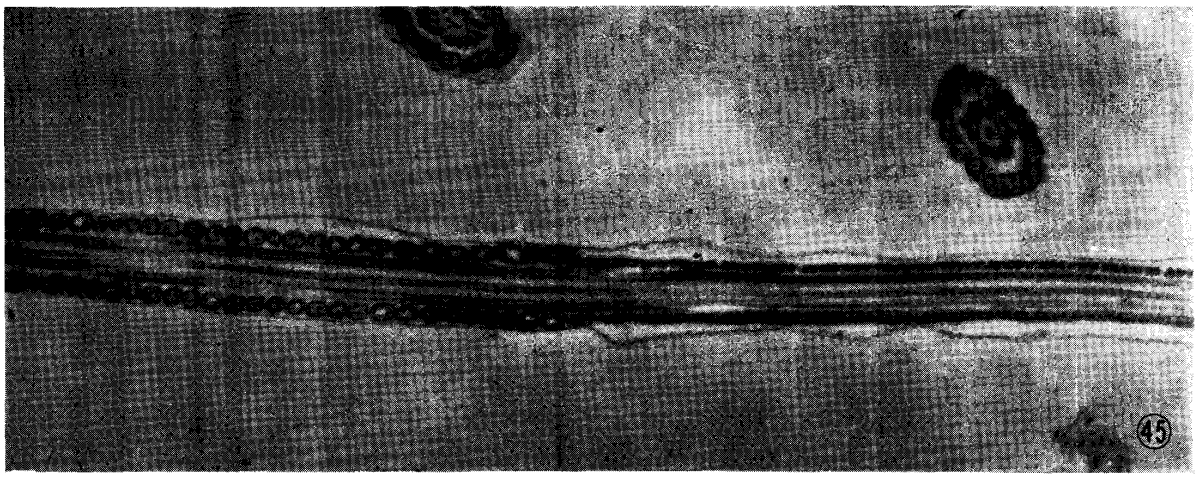
PLATE XVII

Each of figures in plate XVII is based on longitudinal and cross section of tail piece and its neighbors.

Fig. 45 E-10: Longitudinal section of middle piece and tail piece
Inside of cell membrane, fibrils of axial fibril bundle in middle piece run straight into tail piece. Fibrillar coil sheath originates just below Jensen's ring.

Fig. 46 L-1: Anterior portion of tail piece
Cell membrane, fibrillar coil sheath and axial fibril bundle are clearly shown.

Figs. 47 & 48 E-2 & E-1: Cross sections of middle piece and tail piece
Sections of tail piece show cell membrane, fibrillar coil sheath, longitudinal fibrillar tail strips and axial fibril bundle. Althogh differentiation between fine fibrils of inner ring and fibrils of outer ring of axial fibril bundle is difficult in these figures because of their close location, the fundamental arrangement of fibrils of axial fibril bundle in tail piece is [9 : 9 : 2] pattern as same as that of middle piece.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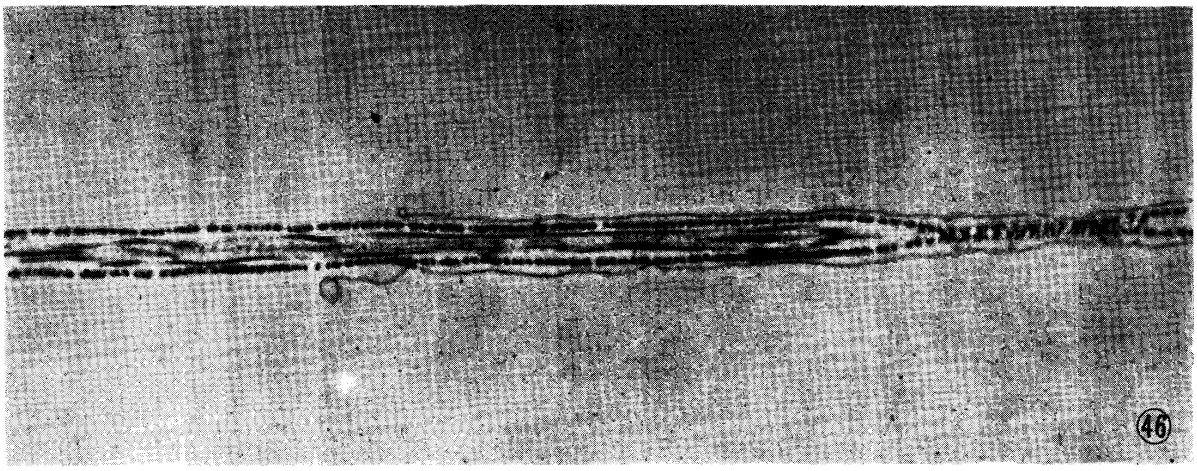
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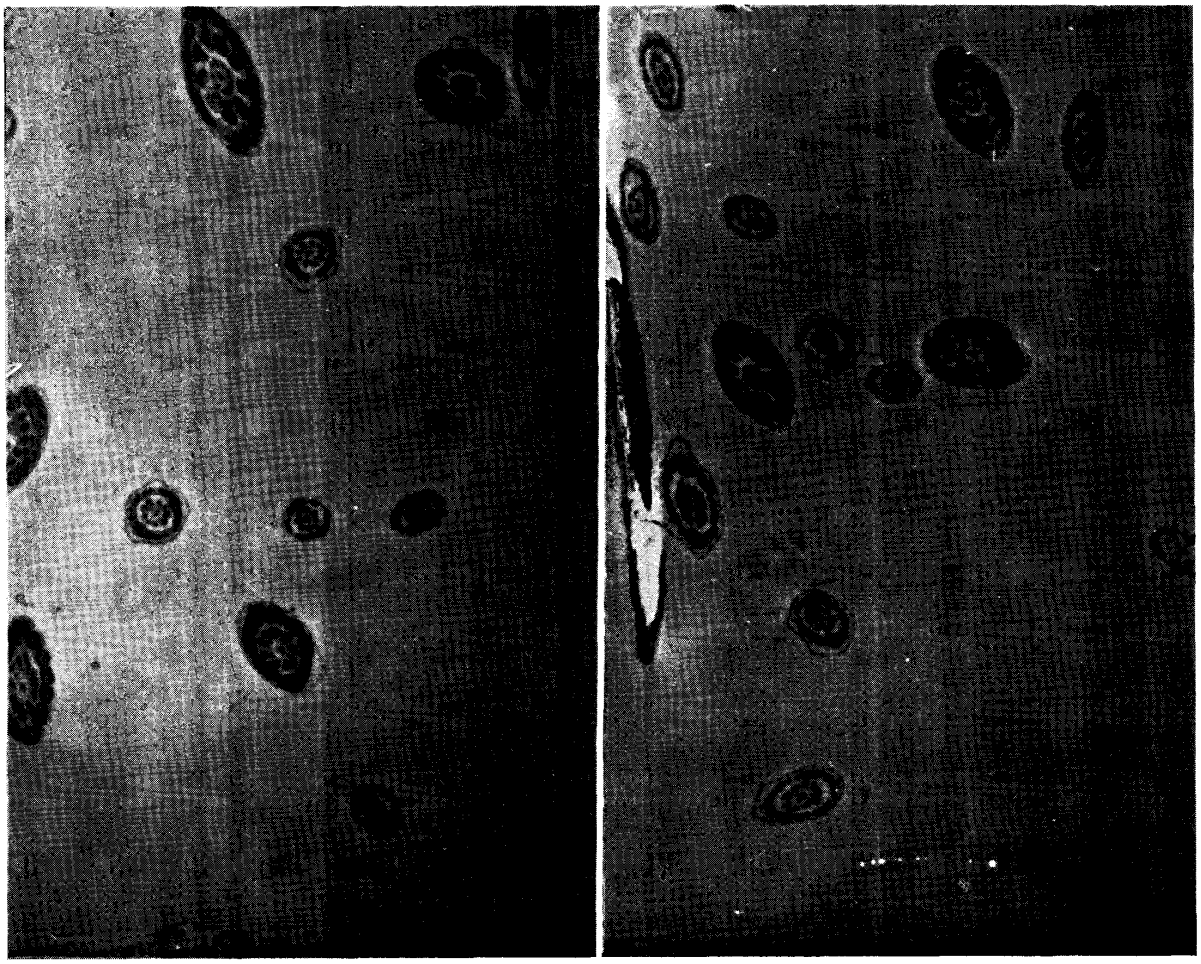


PLATE XVIII

Fig. 49 S-6: Suspension preparation of end piece with posterior portion of tail piece

End piece has cell membrane and axial fibril bundle, lacking in fibrillar coil sheath.

Fig. 50 Replica of end piece with a part of tail piece

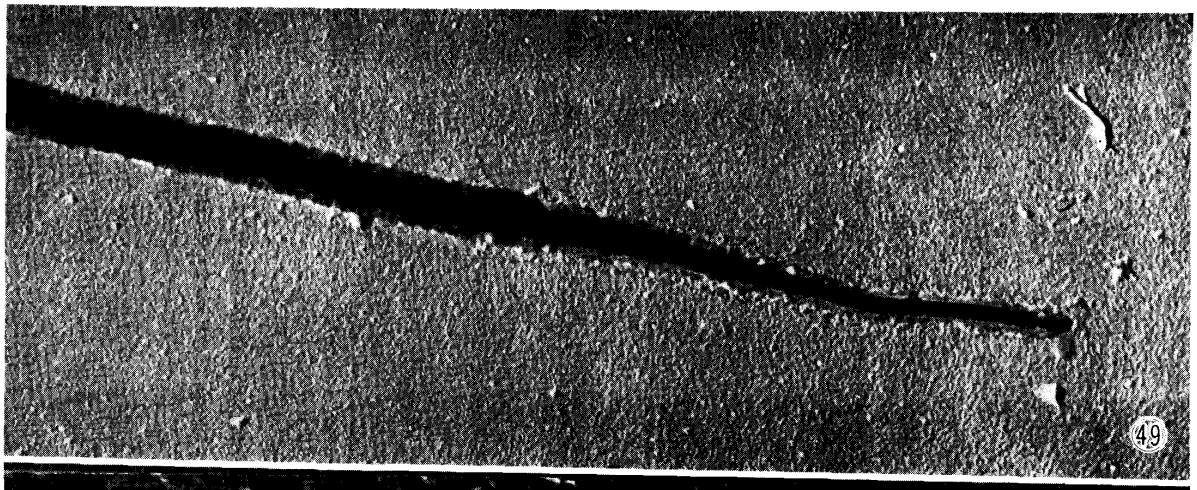
Difference in thickness is distinct between tail piece and end piece.

Figures 51 and 52 are based on suspension preparation.

Fig. 51 S-2: Tail piece and end piece treated by washing

Axial fibril bundle shows brush-like appearance in end piece. This photograph is based on a twice washed spermatozoon, which is also illustrated partially in figures 2, 30, 40 and 41.

Fig. 52 S-3: Some fibrils are separated into original units. This figure actually proves that the axial fibril bundle running through the middle piece, tail piece and end piece, straightly, without changing their number and arrangement pattern.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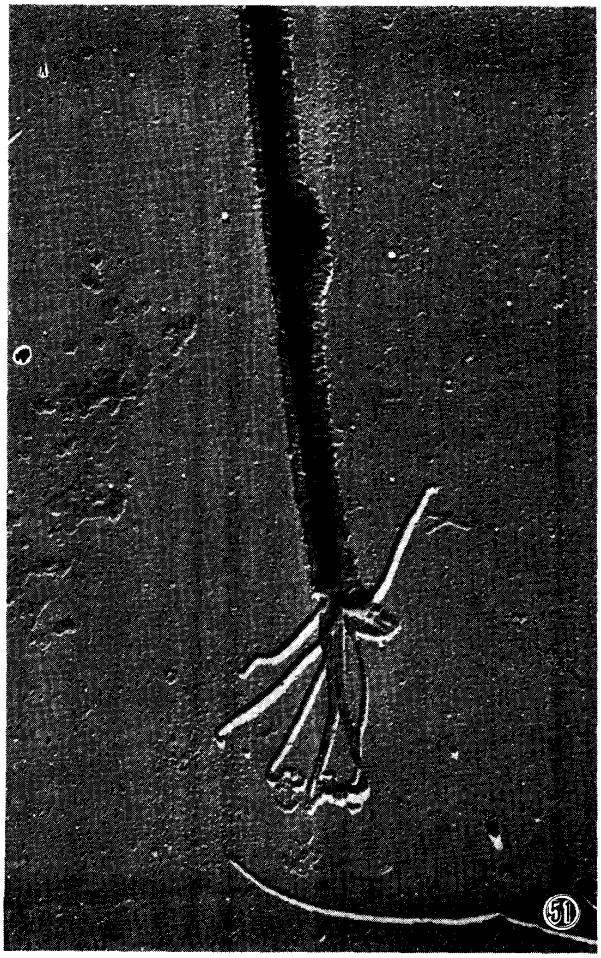
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PLATE XIX

Fig. 53 L-10: Photograph shows a cross section of tail piece (upper), a longitudinal section through tail piece and end piece, and oblique section of middle piece and a longitudinal section of tail piece (right). Note end piece which has neither mitochondrial sheath nor fibrillar coil sheath. × 30,000

Fig. 54 E-10: Longitudinal section through tail piece and end piece End piece consists merely of axial fibril bundle and cell membrane (upper).

Figs. 55 & 56 L-1 & L-10: Cross sections of end piece (arrows) Arrangement pattern of fibrils in end piece is assumed to be invariable as the upper portions.
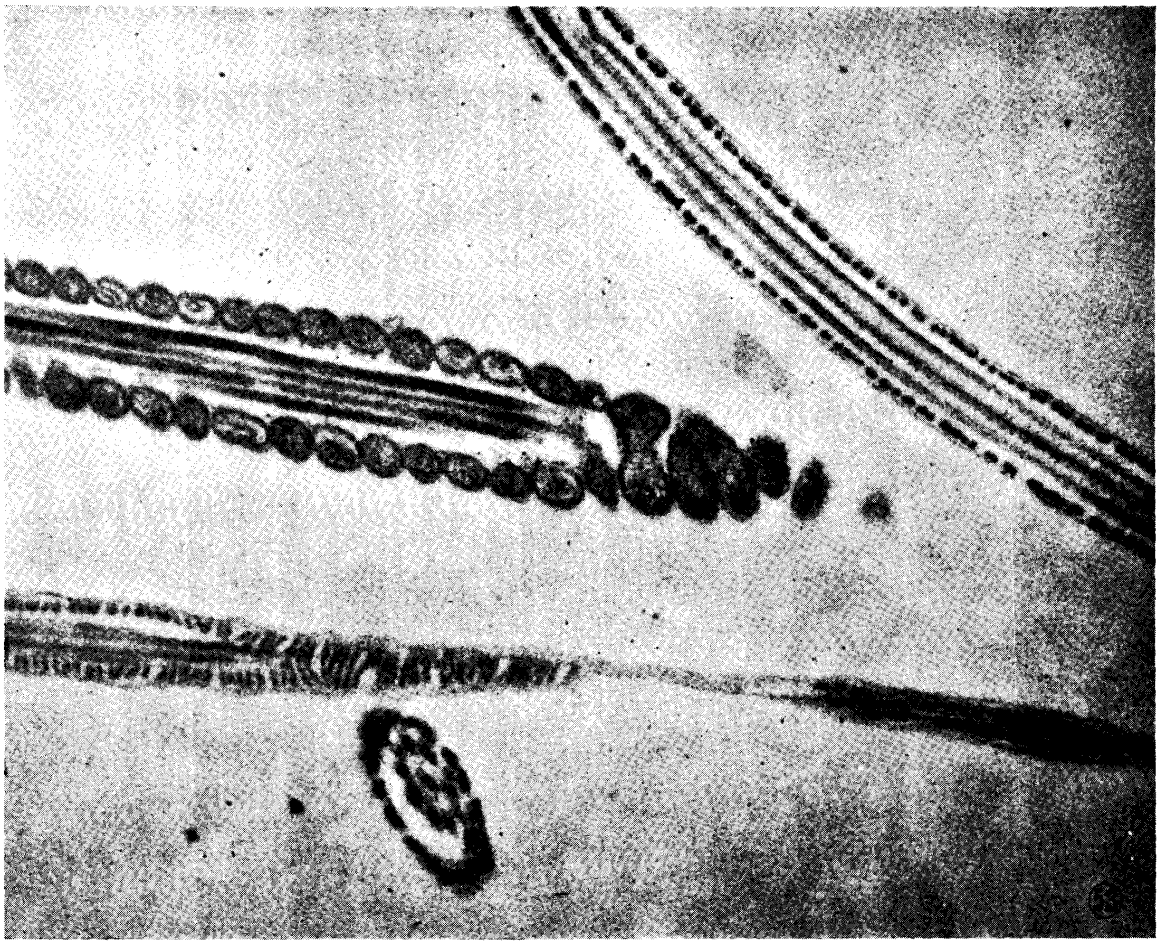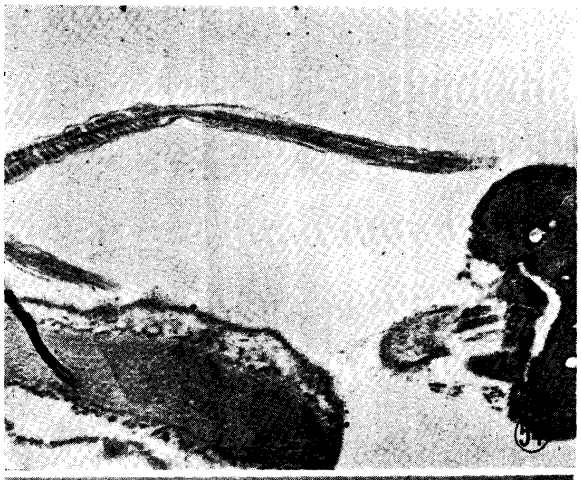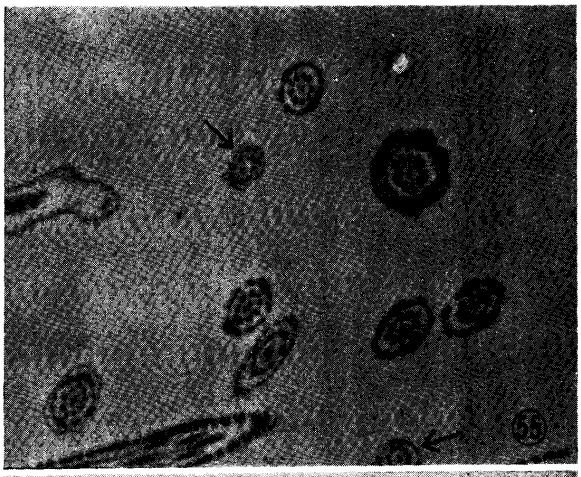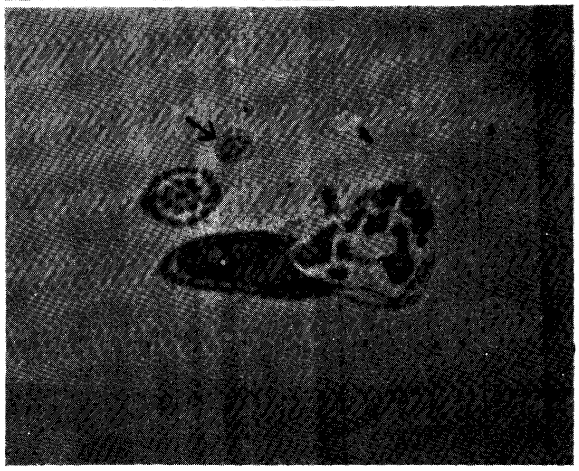
Fig. 57 L-1: Oblique section of transitional part between middle piece and tail piece (left), and cross section of end piece