Electron Microscopy of the Sperm Tail

Results Obtained with a New Fixative

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ABSTRACT

The details of a new fixation procedure using 40 per cent osmium tetroxide in carbon tetrachloride are presented. This fixative is a good general preservative, gives a higher contrast than the ordinary osmium fixatives, and may also preserve structures that are not otherwise readily revealed. Some possible reasons for the increased contrast are discussed.

Micrographs of the sea urchin spermatozoa treated with the new fixative provide more detailed information on the tail structure than has heretofore been obtainable. This information is summarized in the diagrammatic text-figure.

The sperm tail can no longer be regarded as having a bilateral symmetry, and thus, it is possible to assign an index number to each of the nine peripheral filaments.

The nine peripheral filaments have a complex morphology, each one of them seems to be composed of two subunits that have unequal diameters. The slightly larger subunits are all found in the clockwise direction with regard to the other subunit or are all found in the counter-clockwise direction in the sectioned tail. Each of the slightly larger subunits is at intervals provided with two types of projections—referred to as the arms and the spokes—that extend in respective tangential and radial direction. The arms from one filament may be in actual contact with its neighboring filament through a complex bridge-like formation. There is a quantitative difference between the nine filaments with regard to this bridge.

It is assumed that the eleven tail filaments follow straight paths. Some hypotheses on sperm movement are discussed based on this assumption and on the fact that the oscillations of an actively working sperm tail are in one plane. Probably, the nine peripheral filaments have non-equivalent functions in tail movement.

In the centriole the nine peripheral filaments characteristically appear as triplets in a whorl-like arrangement. It is suggested that the inner part of this triplet is a derivation of the arms. A structural abnormality of the tail is described that is characterized by two or three complete sets of tail filaments within one cell membrane.

The most commonly employed fixative for electron microscopy is a water solution of osmium tetroxide. The concentrations used rarely exceed 2 per cent. In fact, it is not possible to obtain a much stronger solution since the solubility of osmium tetroxide in cold water is only about 6 per cent. It is possible, however, to increase the osmium tetroxide concentration of the fixative by omitting the water carrier and using the pure melted osmium tetroxide as a fixation fluid (2, 3) or by using carbon tetrachloride as a carrier for the osmium tetroxide in place of water (3, 4).

These two fixatives were developed in the hope that a more rapid penetration of the osmium tetroxide would be possible, resulting in a better preservation of the sea urchin egg. The appearance of the egg cytoplasm did not differ markedly from the picture obtained after using the more

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traditional osmium fixation methods. It was found, however, that a higher contrast was achieved in the electron micrographs if the new fixatives were used. For detailed analysis of fine structure, when it is necessary to use high electronic magnifications and very thin sections, it has been a problem to obtain enough contrast in the micrographs. Thus, the fact that the new fixatives provide micrographs of higher contrast seems to be a valid reason for their use.

Earlier methods to increase the contrast in the sections include, for instance, the addition of a "contrasting" agent such as phosphotungstic acid to one of the alcohols in the dehydration procedure (15, 25) or the use of potassium permanganate as a fixative (18), which for unknown reasons gives preparations of very high contrast. The disadvantage of the first method is that the tissue blocks may be more difficult to cut, and in the second method the general preservation is not equal to that of an osmium fixation. The two new fixatives—melted osmium tetroxide and osmium tetroxide in carbon tetrachloride—do not have these disadvantages. In fact, the latter fixative has so few disadvantages that it may become a useful contrasting agent in addition to those presently in use.

The aim of this paper is to present the details of a new fixation procedure by osmium tetroxide in carbon tetrachloride and to demonstrate that this fixative, in addition to giving higher than usual contrast, may also preserve structures that would otherwise not be readily revealed. The paper includes a description of the method of fixation; a detailed description of the fine structure of the sperm tail to demonstrate the improved quality of fixation; and a discussion of a probable mode of tail movement based on new information contained in the micrographs.

It may be appropriate here to summarize some data obtained from earlier electron microscopical studies on sperm tails and on cilia. Manton and Clarke examined spermatozoids from *Sphagnum,* first on whole mounts (19) and some years later in sections (20). These authors concluded that the two flagella of the cell contain nine peripheral and two central filaments, all of which were considered to be doublets. Fawcett and Porter (11) and Fawcett (9) examining different kinds of animal spermatozoa and cilia in sections, found the inner two filaments to be single and the peripheral nine to be doublets; another important conclusion was that the direction of the ciliary beat is perpendicular to the plane of the two central filaments. The sea urchin spermatozoon examined by Afzelius (1) showed a similar arrangement: Two single tubules with a size significantly different from that of the elements of the peripheral filaments, which were described as doublets or triplets. A variety of cilia and sperm tails have since been described and only slight modifications have been proposed to the above mentioned schemes. Evidence from most of these papers indicates that sperm tail and ciliary filaments follow a fairly straight course along the tail or cilium.

Methods

Fixation by osmium tetroxide in carbon tetrachloride is performed as follows. An osmium tetroxide ampulla is opened and carbon tetrachloride is added to give a 40 per cent solution. The osmium tetroxide dissolves rapidly. A small droplet of the concentrated cell suspension to be studied or a thin slice of tissue is placed in the ampulla. The watery suspension or tissue slice will not mix with the carbon tetrachloride, but within this two phase system the specimen will blacken rapidly. After fixation for 1 hour at room temperature or at 0°C. the specimen is washed in carbon tetrachloride to remove any excess of osmium tetroxide. Washing for 10 minutes in several changes of carbon tetrachloride seems to be sufficient. The next step is the removal of the water still present in the specimen. This is accomplished by eight changes of absolute alcohol during 1 hour. The alcohol is then exchanged for catalyzed butyl- and methyl-methacrylate in the desired proportions. The concentration of the catalyst, benzoyl peroxide, was 0.2 per cent. Again, eight changes during a total period of 1 hour seem to be sufficient for cell suspensions. From the last change the specimens are transferred to gelatin capsules containing the same unpolymerized methacrylate mixture. Polymerization is then carried out at a temperature of 60°C. Thus, the total fixation and embedding time is just a little more than 3 hours plus the few hours it takes for the methacrylate to polymerize.

In this study, spermatozoa from the sea urchin *Psammechinus miliaris,* were used. It was not necessary to centrifuge down the spermatozoa during dehydration as the spermatozoa kept together in droplets. The sections were cut on a Sjöstrand microtome (22) equipped with a glass knife, but otherwise following the technique of Sjöstrand (23).

An RCA EMU-3C electron microscope provided with the standard equipment was used for this work. The bore diameter of the objective aperture was about 60 μ. The micrographs were taken at an initial magnification of 16,000, and were enlarged photographically to the desired size. The developer, Ko-

dak D 18, was used for plates and paper. The print paper was Kodak F-5. The magnifications at the various settings of the microscope were determined by calibration with a replica of a diffraction grating ruled 28,800 lines to the inch.

RESULTS

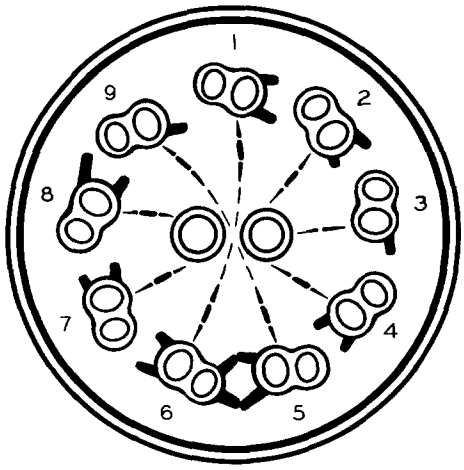
As a fixative, the solution of osmium tetroxide in carbon tetrachloride shows very good preservation properties—at least with regard to the cell suspensions on which it has been tried so far. When applied to sperm the general preservation, as judged by membrane continuity and by the regularity of some structural details, is superior to that following ordinary osmium techniques. The cell membrane follows a fairly irregular course in both types of fixation and this feature is, no doubt, a fixation artifact. Moreover the contrast of the sectioned specimen is higher and the dark lines in the micrographs stand out with greater clarity when the carbon tetrachloride-osmium method is used (*cf.* Fig. 1 here and Fig. 8 in reference 1). In this paper no survey picture of the whole sperm is shown, mainly because such figures look very similar to previously published pictures of spermatozoa fixed with osmium tetroxide in sea water (1). Higher magnifications of the tail filaments, on the other hand, reveal new features that cannot be seen after "osmium in sea water" fixation.

A new concept of the arrangement of the sperm tail filaments and their inter-connections is shown diagrammatically in Text-fig. 1. This scheme is based on micrographs like those in Figs. 1 and 2, and shows two central single filaments surrounded by nine peripheral filament pairs. In the present scheme the two component "strands" of the peripheral filament pairs are shown to be morphologically and topographically distinct from each other. This is evident from a number of different findings:

(*a*) Two short dense protrusions extend from one side of most of the peripheral filaments. These protrusions will be referred to as the "arms." The arms are oriented roughly in the tangential[1] direction and they are connected with only one of the strands in the filament pairs.

(*b*) There are slender threads radiating from about the center axis of the tail to the strands provided with arms. These structures will be referred to as "spokes." Often all nine spokes are clearly visible in one cross-section. The most conspicuous portion is located at a distance somewhat closer to the filament than to the center.

(*c*) The diameter of the strands having arms and spokes is slightly larger than that of the "tubules" without arms and spokes. This feature, exaggerated for demonstration purposes in the diagram, is certainly a distinct and regular phenomenon.



TEXT-FIG. 1. A schematic representation of a typical sperm tail in cross-section. The outlines of the two central filaments are circular, those of the nine peripheral filaments are of a more complex morphology. There are projections between the filaments: the "spokes" in radial direction, here represented by interrupted lines, and the "arms" of the peripheral filaments drawn as solid lines. It is to be noted that the two subunits of one peripheral filament differ. The subunit in the clockwise direction is characterized by its slightly larger radial diameter, the connection with the arms as well as with the spokes, and by the tilting of the peripheral filaments that gives the clockwise side a slightly more central position than the counter-clockwise side. The asymmetry of this filament arrangement has made it possible to assign an index number to each of the peripheral filaments. This was done according to the following system. Filament 1 is the filament that is located at an equal distance from the centers of the two inner filaments, and the increasing numbers are given to the filaments in the direction of the arms. The arms from filament 5 are met by projections in a counter-clockwise direction from filament 6, and the four units seem to form a complex bridge. Further details are presented in the text. (Drawing by John Spurbeck.)

[1] The terms "tangential" and "radial" are used here and below in relation to the circle described by the nine peripheral filaments.

(*d*) The distances from the center of the tail axis to the two respective "tubules" are not the same. This deviation appears in a regular fashion; *i. e.*, the "tubule" with arms and spokes is always closer to the center.

(*e*) In a section through a sperm tail the "tubules" with arms and spokes lie in the clockwise direction with regard to the other "tubule," or they lie in the counter-clockwise direction. It seems highly probable that this direction is actually constant in all sperm tails and only conditioned in the micrographs by the direction of the sperm tail within the section. The implication of this feature is that the sperm tail can no longer be regarded as having a bilateral symmetry; the filaments are asymmetrically arranged.

For descriptive reasons the peripheral filaments have been described above as if they were each made up of two strands or tubules. If this had been the case one would expect to find the thickness of the common wall of the two adjacent "tubules" to be twice that of the free parts of the wall. This is not the case; the radial partition has the same thickness as the wall at other places. It is therefore better to consider the peripheral filament as a complex structure with no simple geometrical cross-section. The fact that the peripheral filaments near the end piece can be split up into two separate units (1) does not seem to invalidate this conclusion.

As mentioned above, the arrangement of the tail filaments is asymmetrical. This information allows one to assign an index number to each peripheral filament. The following system was adopted. The plane that divides the tail into two equally big parts and is perpendicular to the line through the two inner filaments transects one peripheral filament. This filament is called No. 1 and the increasing numbers are given to the filaments in the direction of the arms. This index system is illustrated in Text-fig. 1. One is thus given a means to refer a certain local peculiarity to a specific filament. In some cases it is impossible to determine the position of filament 1, possibly because of distortion during fixation, embedding or sectioning, but as a rule one can identify it satisfactorily and hence ascribe a number to each of the other eight.

It was natural to look for characteristic peculiarities in one or another of the nine indexed filaments. The spokes connected to filaments 3, 4, 7, and 8 are shorter than are those connected to the other five filaments. Otherwise the filaments do not differ with respect to their attached spokes; the presence of an especially prominent spoke or the absence of a spoke within the plane of the section can be seen at all nine filaments and at the same frequency for all nine. The arms may also be missing in a certain cross-section and this is found equally often in all of the filaments.

A notable feature in the present pictures is that the arms from one filament may be in actual contact with its neighboring filament. Two filaments joined by such a bridge superficially resemble a structure made up of five "tubules," but at closer examination the arms forming the bridge can be recognized as such. It can also be seen that the bridge is apparently made up of four structures—two arms from the larger strand and two arm-like protrusions extending backwards from the neighboring smaller strand. These protrusions meet the arms at an angle. The outer one of the two arm-like protrusions is the more prominent of the two. In most cases one of these connections can be seen in a cross-section through the sperm tail (after this fixation), less commonly two connections can be seen, or a connection of three filaments in a row. Rarely are all nine filaments connected to a continuous beaded circle, or are they all separated from each other, as in the middle sperm tail in Fig. 4. It is interesting that these filament connections do not occur equally often between all nine filament combinations; some sites are favored. The connection that occurs most frequently is between filaments 5 and 6. This bridge is represented in Text-fig. 1 and can be seen in all the three cross-sections in Fig. 1 and possibly also in Fig. 2, although the distortion in Fig. 2 makes it difficult to index the filaments. The connections between filaments 9 and 1 and between Nos. 1 and 2 are the two sites that follow in the order of decreasing frequency, while the connections at the other six sites occur less frequently.

So far, we have been concerned with the appearance of the sperm tail in cross-sections. Turning to oblique or longitudinal sections of the tail, it becomes evident that the arms and the spokes represent narrow bridges between the filaments (Figs. 6 and 7). The arms and the spokes are at approximately right angles to the filaments and the shape of the arms seems somewhat irregular. After fixation in a more traditional osmium fixative the arms are, as a rule,

washed away and the longitudinal sections look cleaner and heretofore appear easier to interpret. More longitudinal sections will have to be studied before a more exact description of the arms and the spokes can be made.

High resolution micrographs are also needed to elucidate the finer architecture of the filaments proper. The cross-section of a central filament is circular and the thickness of the dense line is 50 A. In a great many micrographs there is at least an indication that this dense line is double. The same is true for the outline of the peripheral filaments in the cross-section.

The Centriole.—Fig. 3 is a section through the centriole. This is the region where the tail filaments originate. In the figure at least some of the nine filaments can be seen clearly. There is also a dense substance surrounding the filaments. This denser substance would, in a three dimensional picture, be represented as a curved disc situated in the posterior nuclear depression (1).

Two notable features are characteristic of the peripheral filaments at this level: (*a*) The plane through each filament has a greater deviation (about 40 degrees) from the tangential direction than does the plane in a more distal cross-section. (*b*) Each filament shows up as a complex structure of three rather than two subunits embedded in the dark matrix material. It might well be that these three subunits are not equal in composition, and that only the two outer ones are to be compared with the peripheral filament proper, whereas the inner subunit is a derivation of their arms. The structure of the centriole can thus be visualized by letting the nine peripheral filaments be twisted by some 40 degrees and then filling the space between the filaments but not within the arms with a darker substance. This interpretation seems to be simpler than one which assumes a branching of the filament "tubules."

The End Piece.—The end piece is the terminal part of the tail also characterized by a distinctly smaller diameter than the rest of the structure. In the light microscope or in electron micrographs of whole mounts these characteristics make it very easy to recognize the end piece. When examining cross-sections through sperm tails, on the other hand, only a reduced cross-section diameter can be used as a distinguishing mark; the other distinction—namely its terminal position—is, as a rule, not easily revealed. In this paper cross-sections from three different end pieces (as recognized by the reduced diameter and the changed morphology) are shown. The typical appearance of a supposedly more distal part of the end piece is seen in the upper tail cross-section in Fig. 1, which contains several filaments, all of which appear characteristically single. The diameter of these single filaments is smaller than either the diameter of the inner filaments or the "halves" of the peripheral filaments. This is in agreement with earlier observations (1).

The region of the end piece that supposedly is closer to the main part of the tail also has some interesting features. Here the termination of the eleven filaments can be studied to greater advantage. Surprisingly enough there seems to be two different types of end pieces when studied at this level. Both types seem to be equally common, and both types are illustrated in Fig. 4. The lower end piece in the figure represents a type characterized by the presence of two inner filaments surrounded by nine peripheral filaments, all of which (or most of which) are single. In this type some spokes can still be discerned. In the upper end piece in Fig. 4 the other type is shown: the two central filaments have disappeared and the peripheral filaments appear in a reduced number, but otherwise with an unchanged morphology, both the arms and some spokes can be seen. This seems to be true for at least six of the filaments in this particular end piece, whereas the seventh filament seems to be a single filament.

It is difficult to know whether both these morphological types may exist in end pieces from normal spermatozoa, or whether one of the types represents a structural abnormality. This question cannot be settled on the basis of the present evidence.

The Cell Membrane and Some Structural Abnormalities.—The cell membrane of the sea urchin spermatozoon has been described in an earlier paper (1) as a "double membrane" (defined as two dense lines separated by a lighter interspace) about 100 A in thickness. Essentially the same picture is obtained when these cells are fixed in carbon tetrachloride—osmium tetroxide solution. A minor difference can be noted, however; the two dense lines are not of equal thickness, often the inner line seems to be somewhat thicker. The cell membrane is not always well fixed. Sometimes a portion of the cell membrane may be broken open, and the tail contents may or may not be disordered. In a peculiar type of filament disorder, the nine outer filaments are

lined up in a straight row, and seem to be held together by their arms. Usually, however, the cell membrane is continuous around the cell, although its course is quite irregular. As shown in Figs. 1, 2, and 4 it is quite common to find two or three outbulging portions of the cell membrane in a tail cross-section. As the shape and size of these bulges vary and as their respective positions show no relationship to each other or to the filaments, it seems most likely that they represent fixation artifacts.

The cross-sections in Fig. 5 demonstrate a phenomenon of more interest. The upper right cross-section in the figure shows a complex tail in which three complete sets of tail filaments are enclosed by one cell membrane. The cross-section in the lower left corner shows a similar abnormal tail, which is of a type that is fairly often found. In about ten such twin structures the orientation of the two filament sets permitted an analysis of the direction of the arms, and it was found that in all of these cases one set of filaments had the arms in the clockwise direction and the other in the counter-clockwise direction. The arrangement of the eleven filaments within each of the filament sets does not differ from that of a normal tail. It is not easy to know how these double or triple structures originate. It could perhaps be an abnormal sperm tail, that developed two or more filament sets during spermatogenesis. Another possible explanation is that two or more spermatozoa under certain circumstances may fuse after a collision. Perhaps the best evidence for such a view can be obtained from observations on living material like those of Koltzhoff, who reports having seen two clam spermatozoa collide and flow together like two soap bubbles (17). Perhaps this latter concept of a more or less fluid cell membrane could be used to explain some previously reported sperm abnormalities where the tail filaments run alongside the head under the cell membrane (Fig. 3 in reference 1).

DISCUSSION

The Fixative.—Compared to many recently reported osmium fixatives with well balanced ion concentrations and carefully buffered pH-values the present fixative must seem very crude. Nevertheless, it works well with some types of cells and gives a high contrast in the sections. In addition to the increased contrast obtained, this fixation method has other evident advantages: it is a simple and fast technique; it preserves some cytoplasmic details that are not preserved by ordinary osmium techniques and therefore it may be used as a complement to these. There are also some disadvantages: it is an expensive fixative. It is an irrational one, as the fixation fluid and water are immiscible. It is also peculiar in being a mixture of a lipide binder and a lipide solvent.

It is not known what happens during the fixation and to what degree the carbon tetrachloride acts on the cells, but it seems evident that much osmium tetroxide enters the cell, as the contrast is high. It might not be an altogether favorable feature that much osmium gets bound to the cells. In some preliminary experiments, the luminescent organ of the firefly was fixed and embedded, and it was noted that the methacrylate had not penetrated the tissue slice. This might have been due to a massive osmium reaction in the compact tissue that interfered with the subsequent penetration of the methacrylate monomer. This effect has not been observed in the embedded cell suspensions.

It may seem surprising that osmium is bound by more groups in the cell after using the present fixative than after applying other osmium fixatives, that is to say that an increased contrast is noted. Several different mechanisms can be proposed in an attempt to explain this feature. Unmasking of masked groups may occur, exposing more reactive sites, and this unmasking may be due to the increased osmium tetroxide concentration or to the carbon tetrachloride. Osmium tetroxide will probably enter the cells more rapidly from the highly concentrated carbon tetrachloride-osmium solution, and some structures may get fixed that otherwise would be dispersed owing to rapid autolytic changes. The osmium complexes formed in the cell can secondarily be opened by oxidation processes that undo fixation and give osmium tetroxide and organic oxidation products. A higher concentration of the osmium tetroxide may then favor the formation of the bound osmium complexes. It might also be helpful that the dehydration and embedding times are considerably reduced. In the high concentrations of osmium tetroxide in carbon tetrachloride the osmium tetroxide seems to exist in a polymerized state, as has been shown by vapor pressure measurements (6). Thus, there might be a possibility that one reactive site in the cell may bind more than one osmium atom. In this list of suggested explanations, the increased contrast is

explained on the basis of increased amounts of osmium bound to the cells. It is also quite possible that part of the contrast noted in the sections is due to an extraction effect by the carbon tetrachloride on some cell components.

The Filament Pattern.—The main findings in the present study are the following. The peripheral filaments of the tail have a complex morphology; for instance, they appear more complicated than the myofilaments of muscle cells. The eleven filaments are interconnected by cross-bridges and their mutual arrangement gives the tail an asymmetrical cross-section.

These new features are clearly visualized only after fixation in the concentrated carbon tetrachloride-osmium fixative. With a dilute osmium fixative of a more traditional type it is, as a rule, not possible to observe any of these details. It may therefore be appropriate to ask whether the concentrated fixative has introduced more fixation artifacts than are normally encountered. The diagram of the tail cross-section shown in Text-fig. 1 is certainly more complicated than any earlier diagrams based on sectioned spermatozoa (*cf.* references 8, 9, 10), but the arrangement of the filaments and their interlinkages has a highly regular appearance. Since any fixation method is much more liable to cause a destruction of complicated biological structures than to cause the appearance *de novo* of regularly arranged artifactitious formations, it seems highly probable that the present diagram of the tail filaments is a more life-like representation than the earlier schemes.

In a study of the human spermatozoon, Ånberg has described the spokes radiating between the inner and the outer tail filaments (5), and similar structures have occasionally been encountered in other types of spermatozoa and cilia. It is shown in the present paper that the spokes are connected with only one of the two strands in the peripheral filaments, and it is therefore unlikely that they represent a simple precipitation of the sperm tail matrix.

The arms have not been previously described in sectioned spermatozoa or cilia. In whole mounts of *Sphagnum* spermatozoids, on the other hand, Manton and Clarke have demonstrated some structures within the fragmented flagella, that undoubtedly are homologous to the arms described here (19). These structures can be seen in their pictures connecting the filaments like "the rungs of a ladder," or they could be attached to the isolated filaments on one side only, giving the filaments "a curious battlemented appearance." In some of their pictures this battlement material is shown as regularly arranged protrusion pairs very reminiscent of the arms of the present study. Other micrographs demonstrate that the battlement material can disappear completely or liquefy and become spread upon the supporting formvar film. This tendency for water liquefaction of the battlement material is used to explain why cilia fall apart into separate filaments after the death of the cell, and it seems to explain the fact that the battlement material cannot easily be demonstrated in most types of cilia and flagella. Manton and Clarke tried to reconstruct the shape of the battlement material in the intact cilium from their pictures of the fragmented cells. They assumed that the battlement material was situated between the inner and the outer filaments and in the shape of a continuous spiral. Their name for this structure was "the spiral tube lining." This conclusion is not in agreement with the present findings.

The observation that the battlement material may bridge several filaments together in a ladder-like fashion is similar to the present observation that the arms may sometimes bind together most of the peripheral filaments. These considerations raise the question of whether this condition represents the life-like appearance of the tail filaments. From a functional point of view it seems very probable that this is the case and that the filaments need this mutual support. Unsupported rods having a length exceeding the width by a factor of more than 1,000 seem unlikely to be able to act as functional biological units.

If the concept be accepted that the peripheral filaments are provided with arms that can be visualized by proper fixation, it is also possible to explain the triple units seen in the centriole. The inner one of the three "tubules" of each filament in the centriole is likely to be a derivation of the arms. The morphology of the sperm centrioles shown here has a great resemblance to centrioles described in dividing or resting somatic cells, as described by de Harven and Bernhard (13) and by Bessis and Breton-Gorius (7). In the first paper some of the nine filaments contained in the centriole were found to be triple, and in the second paper all were triple. The basal corpuscles of the tracheal ciliary epithelium have a very similar ultrastructure (21).

There have also been some reports on periph-

eral filaments of a triplet type in the more distal parts of some spermatozoa and cilia. This phenomenon is probably better explained if one assumes that the arms here have not been washed away. These triplets were first described in sea urchin spermatozoa (1) and later in cilia from the tracheal epithelium (21) and from human spermatozoa (5). The apparent branching of the two inner filaments, as described in the last two papers and in those of Manton and Clarke (19, 20) may either be due to a difference in material or techniques, or to a confusion produced by the spokes coming in close to the inner filaments.

Functional Aspects.—The information that has been obtained by electron microscopy on spermatozoa and on cilia cannot be said to have provided any direct clue to the mechanism of flagellar or ciliary movements. An attempt will be made on the basis of the present more detailed electron micrographs to see how the new information will fit into some current concepts. The basis for any theory of sperm tail movement must evidently come from studies *in vivo*, and it is fortunate in this connection that there exists a detailed investigation on the movement of the sea urchin spermatozoa. This is the recent study by Gray (12), who used the same sea urchin species, *Psammechinus miliaris*, as that used in the present study. Gray concluded that the oscillations of the tail are restricted to a single morphological plane, and that the sperm rolls about its longitudinal axis, as is evidenced by the rotation of that plane. Furthermore, the wave originating close to the head and propagating towards the end of the tail is asymmetrical; *i.e.*, the degree of bending exhibited by the two sides of the proximal tail is usually different. The difference between the movement of this monoflagellate sperm and ciliary movement is therefore not so fundamental as earlier believed. (Similarly the flagellary movement of the choanocytes in sponges has been given as an example of a three-dimensional conical beat, but recently it has been shown that the flagellary beat is in one plane only, a plane that slowly rotates about its axis (16).)

In an analysis of his data, Gray presents evidence that all the regions along the tail are actively contractile. It is also pointed out that the nature of the internal bending mechanism in the sperm tail requires the presence of both contractile elements and of elements that are capable of resisting compression. Gray indicated that the nine peripheral filaments could be the contractile units, and that the ciliary matrix or the cell membrane might be resistant to compression. The possibility that the two inner filaments have a rigidity function was not considered by Gray. The reason for discarding this possibility is not clear from the paper. It might have been for reasons similar to those expressed by Bradfield (8), who states that "it seems unlikely that they have an important skeletal function since they tend to be more easily dissolved and lost than the other nine." In the opinion of the present author the cell membrane is unlikely to be rigid enough to resist much compression, whereas the position of the two inner filaments makes them a reasonable choice for this function. The two inner filaments were shown to be located in one plane in the spermatozoa of a grasshopper (24), and it is probable that the same is true of sea urchin spermatozoa. This arrangement will probably make the tail resistant to bending in the plane of the inner filaments but free to bend in the perpendicular plane. This is a view similar to that expressed by Fawcett and Porter (11) with regard to the movement of some cilia. The arms interconnecting the nine peripheral filaments are probably also of importance in sustaining the rigidity of the tail structure. An additional possible function of the arms will be discussed below.

The waves exhibited by a normally active tail were analyzed by Gray. The form of the wave may correspond closely to that of a sine curve with an amplitude of 4 μ and a wavelength of 24 μ. From these data it can be calculated that 30 μ of the tail is included in a full wavelength. The total length of the tail averages 41 μ in this species. To explain the development of the waves proximally and their propagation towards the end of the tail, Gray assumes that the peripheral filaments contain contractile elements that may shorten in length when excited by a stimulus.

In the opinion of the present author it seems reasonable to assume that all eleven filaments in the tail follow straight paths. Assuming this, it follows that over a whole wavelength each filament maintains a constant length. Accepting 0.15 μ as the distance between the centers of two opposite filaments, it is possible to calculate the difference in length between the peripheral filaments on the outer and the inner side of half a wavelength. This difference is about 0.23 μ, which is a value smaller than that given in Gray's paper. The filaments must not necessarily be

contractile in the ordinary sense of the word; the work done by them could also be the result of a sliding of the filaments in relation to each other, each filament retaining its original length and thickness. A shift in their relative position of only 0.23 μ could be the sufficient cause to bend the tail to a sine curve of one wavelength. The underlying mechanism for such a filament sliding if it exists, is certainly not understood, but it is probable that the arms would be active in this process. This would thus be a mechanism reminiscent of the contraction model in cross-striated muscles proposed by Huxley (15). From a merely morphological point of view it is interesting to note that both the myofilaments of muscle cells (14, 15, 25), and the filaments of the sperm tail are interconnected by cross-bridges. One notable difference is that the arms of the tail filaments are not preserved by ordinary osmium fixatives.

A model of the sperm tail must take into account not only the formation of waves, but also their propagation. The waves originating proximally must travel along the tail to its tip, in order to give the spermatozoon the forward push that propels it through the water. It is not clearly understood how the mechanism suggested above can be used as an explanation of sperm movement, even if one makes the additional assumption that the position shift of the filament is self-propagating along the length of the tail. Also there remain many other unknown factors that make the speculations somewhat premature. For instance, it would be desirable to know the number of filaments that are actively sliding during tail movement. It might be a minimum of only a few filaments on one side of the central filaments, for example filaments 5 and 6 and to a smaller degree Nos. 4 and 7. This would give the tail beat a high degree of asymmetry. With a high spring-like action of the central filaments the degree of asymmetry can be much reduced. Another possibility is that the filaments on the two sides of the midline are active. The two central filaments and the peripheral filaments 3 and 8, making up the midline, will then retain a fixed position in relation to each other, and constitute the axis against which the other filaments work. A third possibility is that the impulse for filament movement is spread around the nine peripheral filaments, possibly in the order of their numbering. If the two central filaments introduce a resistance to bending in their plane, only the force components in the perpendicular direction will be active in bending the sperm tail, resulting in movement in one plane. More work remains to be done to choose between these possible hypotheses and still others.

It follows from the above that the nine peripheral filaments probably do not have equivalent importance and function in the tail movement. Any difference that can be detected between the nine peripheral filaments in their morphology will be likely to provide information on the mode of the tail movement. So far, only one distinction has been noted. This is the connection between two filaments through an elaborate bridge of four different projections, which is a structure more often found between filaments 5 and 6 than at other sites. The significance of this difference is not clear. It might be due to a relatively firm binding together of these two filaments, and thus lend support to the hypothesis that these two filaments do not move in relation to each other.

In mammalian spermatozoa there is according to several authors an additional circle of nine coarse fibres outside of the nine peripheral filaments (references 8, 9, 10). It is not known what function these additional fibres have; their fine structure differs markedly from that of the active nine filaments in the inner circle. There are interesting structural differences between these additional fibres that might help to understand the mode of tail movement. The coarse fibres outside filaments 1, 5, and 6 are often thicker than are those outside the other filaments (references 8, 10). The coarse fibres outside filaments 3 and 8 are shorter than the others in the human spermatozoon (5), and in the guinea pig spermatozoon these two fibres seem to fuse with a fibrous sheath that surrounds the axial filaments (10). Until more is known about the function of the coarse fibres it is difficult to evaluate these findings, but the differences are in harmony with a concept that some filaments are more active than others in the movement of the spermatozoon.

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EXPLANATION OF PLATES

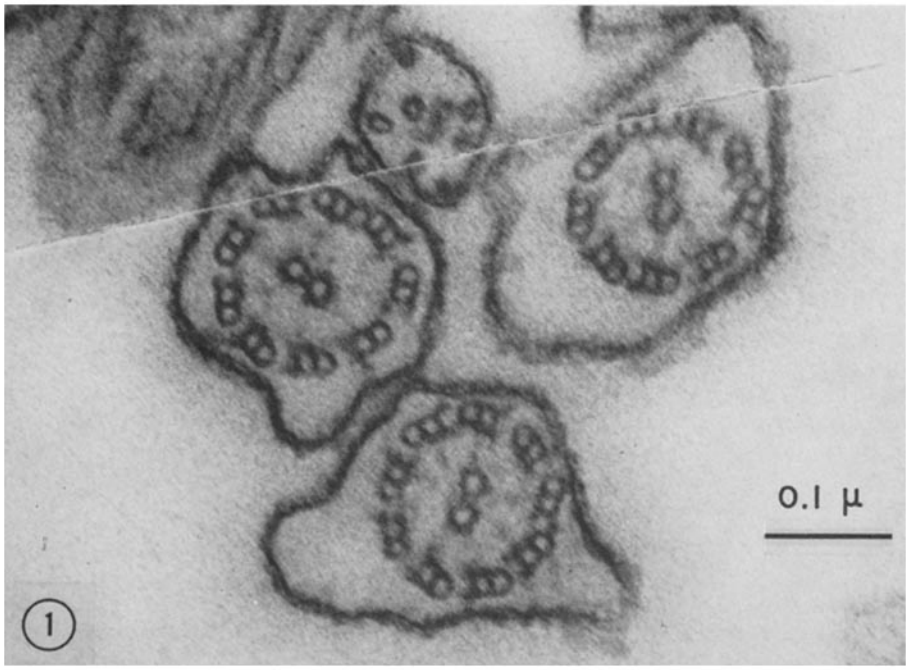
All the electron micrographs shown are from spermatozoa of the sea urchin *Psammechinus miliaris* fixed in 40 per cent osmium tetroxide in carbon tetrachloride. The micrographs were taken at an initial magnification of 16,000 and enlarged photographically to the indicated magnifications.

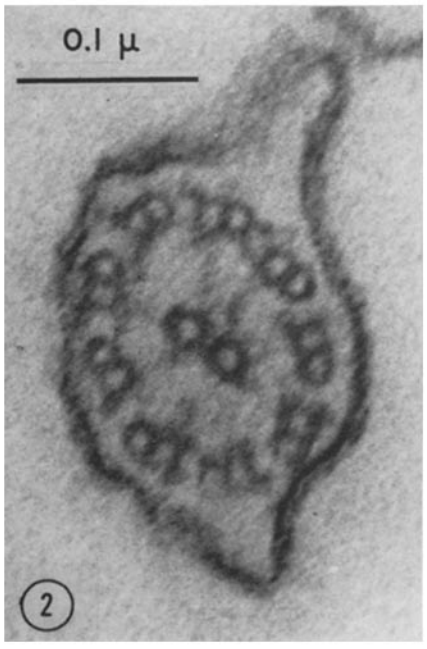
PLATE 119

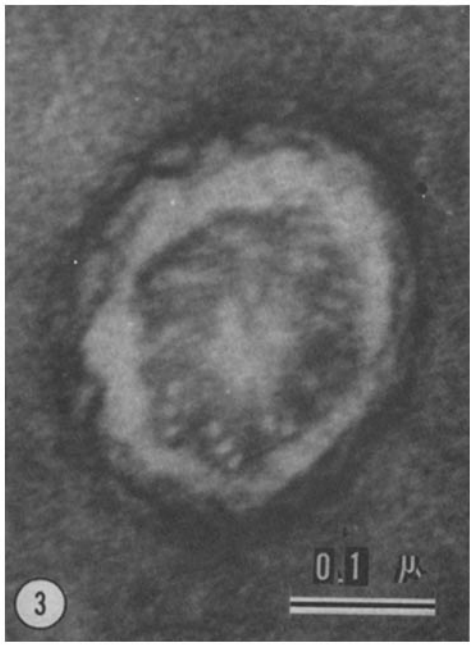
FIG. 1. Cross-section through the main piece of the tail of three spermatozoa and through the end piece of a fourth spermatozoon. In the three sectioned main pieces the arrangement of the filaments with their arms and spokes is similar to that of the drawing in Text-fig. 1. The arms in the clockwise direction in these three tails and the connections between filaments 5 and 6 can be observed. The end piece in the upper part of the figure has a small cross-section diameter and contains several single filaments. × 170,000.

FIG. 2. Cross-section through a sperm tail. The features visible here are the same as those shown in Fig. 1 or in Text-fig. 1. The cell membrane can be seen as a "double membrane" at many places. × 240,000.

FIG. 3. Cross-section through the sperm centriole. At this level the nine peripheral filaments are embedded in a dense matrix that has a ring-shaped cross-section. The centriole is located in a posterior nuclear indentation, and the dense granular substance that surrounds the centriole as a frame in this figure is nuclear material. In the centriole, the filaments have a whorl-like arrangement, and they may have a triplet appearance as is the case with the two lower filaments. × 195,000.







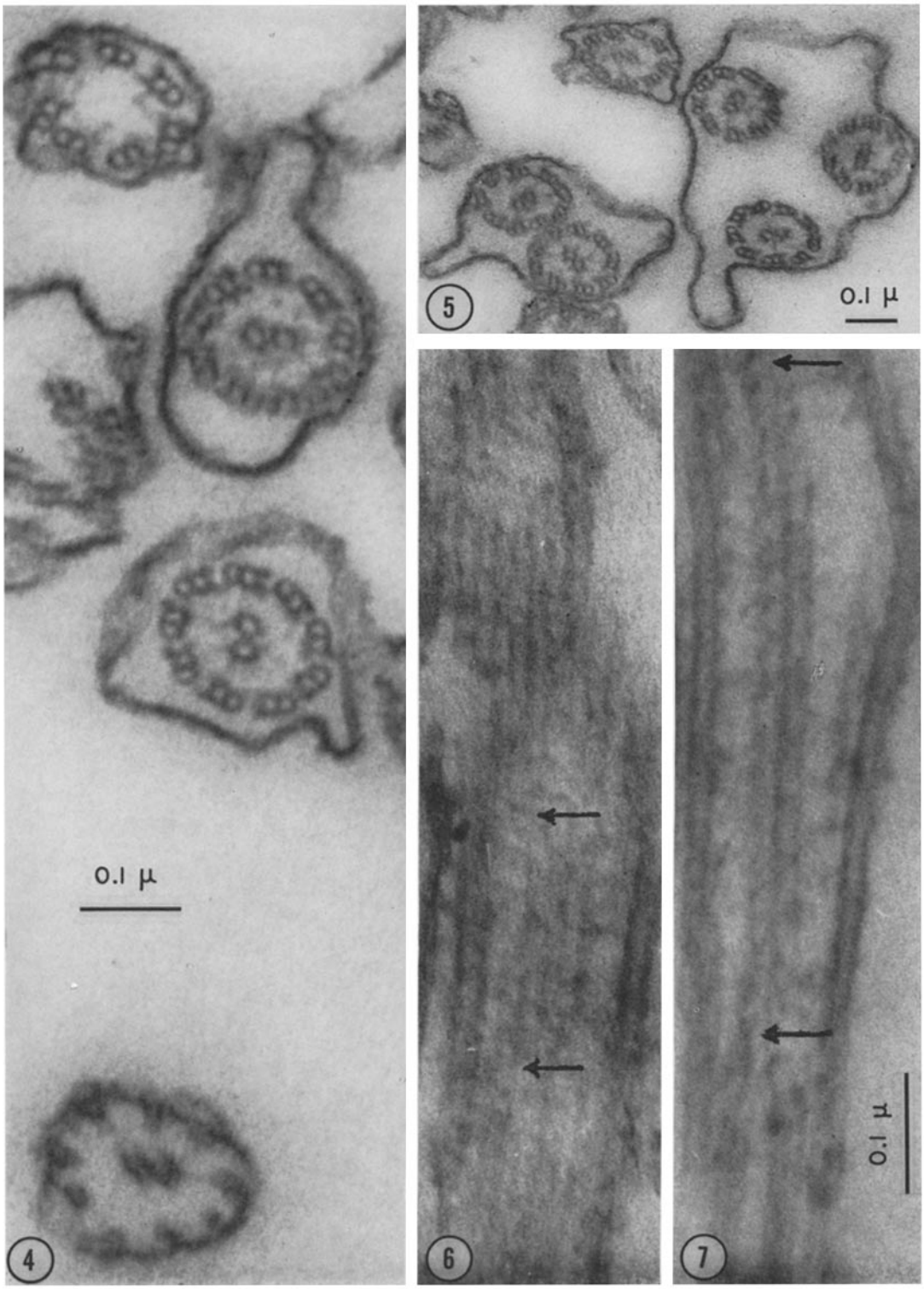
(Afzelius: Electron microscopy of sperm tail)

PLATE 120

FIG. 4. Cross-sections through four sperm tails. The two tails in the middle part of the figure are sectioned through the main piece of the tail, the upper one and the lower one probably represent sections through the end piece. In the tail at the bottom the filament arrangement is that of two single central filaments and nine likewise single peripheral filaments. In the end piece at the top there are seven peripheral filaments, all but one appear double, the seventh in the upper left corner seems to be single. × 155,000.

FIG. 5. Section through two tail abnormalities. The structure occupying the right part of the field apparently represents three complete sets of tail filaments surrounded by one cell membrane. The cross-section in the lower left corner shows a similar abnormality with two sets of tail filaments within one cell membrane. × 80,000.

FIGS. 6 and 7. Sections cut longitudinally along a portion of the tail. The upper half of Fig. 6 contains only peripheral filaments interconnected by arms, whereas in the lower part of the same figure the tail is bent within the section and the obliquely cut central filaments are included. In Fig. 7 the section is oriented to contain a fairly long distance of the two central filaments and the adjacent peripheral filaments on both sides. Some spokes between the central and the peripheral filaments can be seen. The extension of the central filaments within the plane of the section is shown with arrows. × 195,000.



(Afzelius: Electron microscopy of sperm tail)