

# COMPARATIVE ANALYSIS OF MAMMALIAN SPERM MOTILITY

DAVID M. PHILLIPS

From the Department of Biology, Washington University, St. Louis, Missouri 63130

## ABSTRACT

Spermatozoa of several mammalian species were studied by means of high-speed cinematography and electron microscopy. Three types of motile patterns were observed in mouse spermatozoa. The first type involved an asymmetrical beat which seemed to propel the sperm in circular paths. The second type involved rotation of the sperm and appeared to allow them to maintain straight paths. In the third type of pattern, the sperm appeared to move by crawling on surfaces in a snakelike manner. Spermatozoa of rabbit and Chinese hamster also had an asymmetrical beat which sometimes caused them to swim in circles. In spite of the asymmetry of the beat, these spermatozoa were also able to swim in straight paths by rotating around a central axis as they swam. Spermatozoa of some species appeared very flexible; their flagella formed arcs with a very small radius of curvature as they beat. Spermatozoa of other species appeared very stiff, and their flagella formed arcs with a very large radius of curvature. The stiffness of the spermatozoan appeared to correlate positively with the cross-sectional area of the dense fibers. This suggests that the dense fibers may be stiff elastic elements. Opossum sperm become paired as they pass through the epididymis. Pairs of opossum spermatozoa beat in a coordinated, alternating manner.

## INTRODUCTION

Gray (1958) was the first to analyze carefully the properties of the beat of mammalian spermatozoa. He used dark-field illumination and a stroboscopic flash to make multiple exposures of swimming bull spermatozoa. Details of the form of the flagellar beat were then extrapolated from these data. More recent studies of bull spermatozoa by Rikmenspoel (1965) and by others (for review see Nelson, 1967) have further detailed the form of the flagellar beat and hydrodynamics of motility of bull sperm. There have also been a number of investigators who have studied mechanisms of motility by using mathematical treatments based on generalized sperm structure (Hancock, 1953); Reynolds, 1965; Taylor, 1952). Branham (1969) has carefully analyzed the paths that rabbit sperm traverse as they swim. He observed several types of patterns which rabbit spermatozoa follow and quantitated

their variation among different bucks, among semen samples from the same buck, and at various pH's. Numerous investigators have studied the effect of different chemical conditions of the environment which affect motility (for review see Salisbury, 1962, and Nelson, 1967). Yanagimachi (1970) recently reported that capacitation affects motile pattern.

In the present study, I have compared motile patterns of several mammalian species and also considered the way in which some aspects of sperm motility relate to ultrastructure. I have not analyzed sperm motility quantitatively in any one species. Rather, I have made some qualitative observations on a number of species which have provided some interesting information on directly observable general features of mammalian sperm motility.

## MATERIALS AND METHODS

### *Electron Microscopy*

Epididymides or semen samples were generally fixed either by interstitial perfusion or by immersion in cold 5% glutaraldehyde buffered with 0.2 M collidine. Tissues were fixed at pH 7.4 for 3–8 hr, rinsed in cold collidine, and postfixed 2–5 hr in collidine-buffered 1% OsO<sub>4</sub>. After dehydration in alcohol, tissues were embedded in Epon, sectioned on an LKB ultramicrotome, stained in 3% aqueous uranyl acetate (12–18 hr) and lead citrate (Venable and Coggeshall, 1965), and were examined on a Siemens Elmiskop IA.

### *Cinematography*

Motile spermatozoa were viewed in a Zeiss Universal microscope with Nomarski, phase, or dark-field optics and photographed with a Locam camera (Red Lake Laboratories, Santa Clara, Calif.) with a 180° shutter at speeds from 24–500 frames/second. A Zeiss 150 watt Xenon light source was employed. Semen samples, uterine, oviducal, or epididymal spermatozoa were diluted with 0.9% NaCl buffered at pH 7.5 with 0.005 M Sorenson's phosphate buffer. Spermatozoa were viewed either in a deep well or in hanging drop. Hanging drops were suspended from a cover slip in a small chamber. Temperature was maintained at 37°C with a Sage Air Cushion Incubator (Sage Instruments, White Plains, N. Y.). Films were analyzed with an L & W photo-optical data analyzer. (The data analyzer is essentially a movie projector equipped with a frame counter. The instrument is capable of running at numerous frame rates and projecting a single frame for as long as desired so that the frames can be studied or traced.)

## RESULTS

### *Mouse Spermatozoa*

I have observed motile spermatozoa from the distal part (tail) of the epididymis of male mice and from the uterus and oviducts of inseminated females. Among spermatozoa taken from the same organ of different animals there is a great deal of variability in the per cent of spermatozoa which are motile and in the type of motile pattern observed. Variability is observed between different samples from the epididymis of different animals, between samples of uterine spermatozoa examined at the same time after insemination, and between samples of oviducal spermatozoa at the same time after insemination. Differences in per cent motile cells and in type of motile pattern

render it difficult to assess differences in motile pattern or per cent motility in different parts of the female tract or among samples of spermatozoa which have been in the female tract for different lengths of time. It is apparent, however, that spermatozoa from either the male or female tract may display more than one type of motile pattern. I have observed three types of motile patterns. These are discussed separately below. One type of pattern has only been observed in spermatozoa from the female tract. The other patterns are observed in sperm samples from both male and female tracts.

**ASYMMETRICAL BEATING (PATTERN 1):** Motile spermatozoa in a deep well or hanging drop tend to move along a surface. This may be either the top surface of the cover slip or the bottom surface of the slide or drop. Mouse spermatozoa which swim on surfaces often swim in circular paths (Fig. 1). When frames of a high-speed motion picture are traced, it can be seen that the beat appears completely two-dimensional. Even with a 1.3 NA objective the entire mouse spermatozoan, 120 μ long, is usually in complete focus. This indicates that the flagella are displaced in one plane as they beat in this motile pattern.

Mouse spermatozoa swimming in circular paths on surfaces have an asymmetrical, two-stroke beat. This is reminiscent of the effective stroke and the recovery stroke observed in cilia (Satir, 1963; Tamm and Horridge, 1970). I shall designate an effective stroke and a recovery stroke since it is easier to explain the beat if I use these terms, although the terms may not be entirely appropriate. The shapes of the effective stroke and recovery stroke in mouse sperm can be visualized in tracings of high-speed films (Fig. 1). In the effective stroke the spermatozoan mid-piece bends very sharply such that at the extreme of the bending phase, the convex side of the sickle-shaped head, forming an arc of about 180° or more, comes very close to the mid-piece. In this stroke the annulus (region between the mid-piece and principal piece) progresses forward. The mid-piece then straightens out in what we consider to be the recovery stroke. The annulus does not progress forward on the recovery stroke. Before beginning the effective stroke again, the mid-piece bends at most a few degrees beyond the fully straightened position. The observation that spermatozoa which progress in this manner tend to travel in circular paths may be a consequence of the asymmetrical form of the beat. This type of

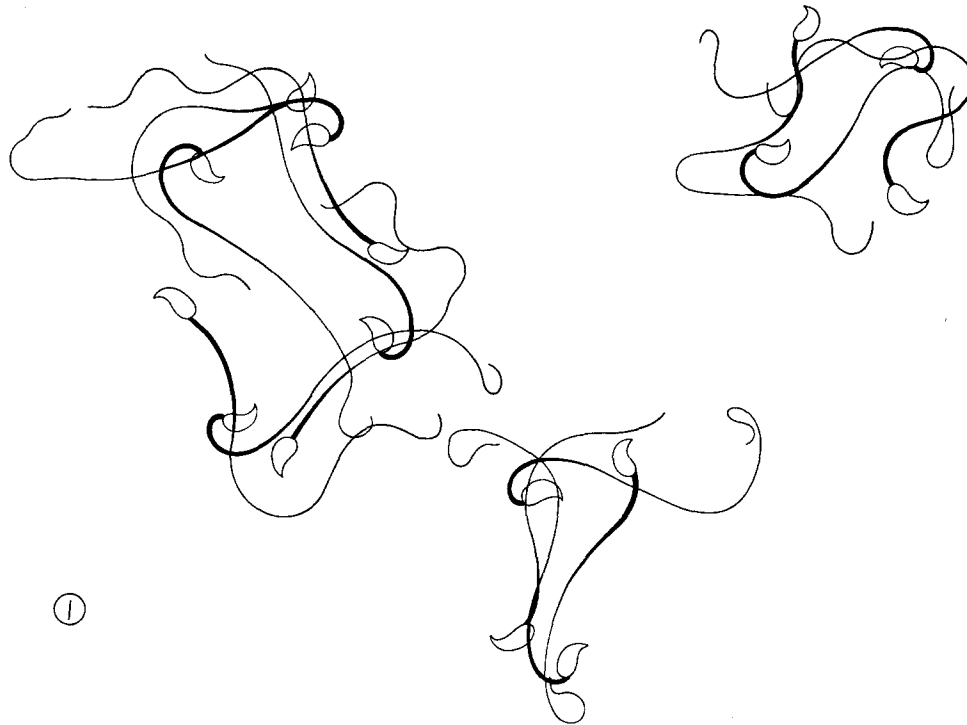


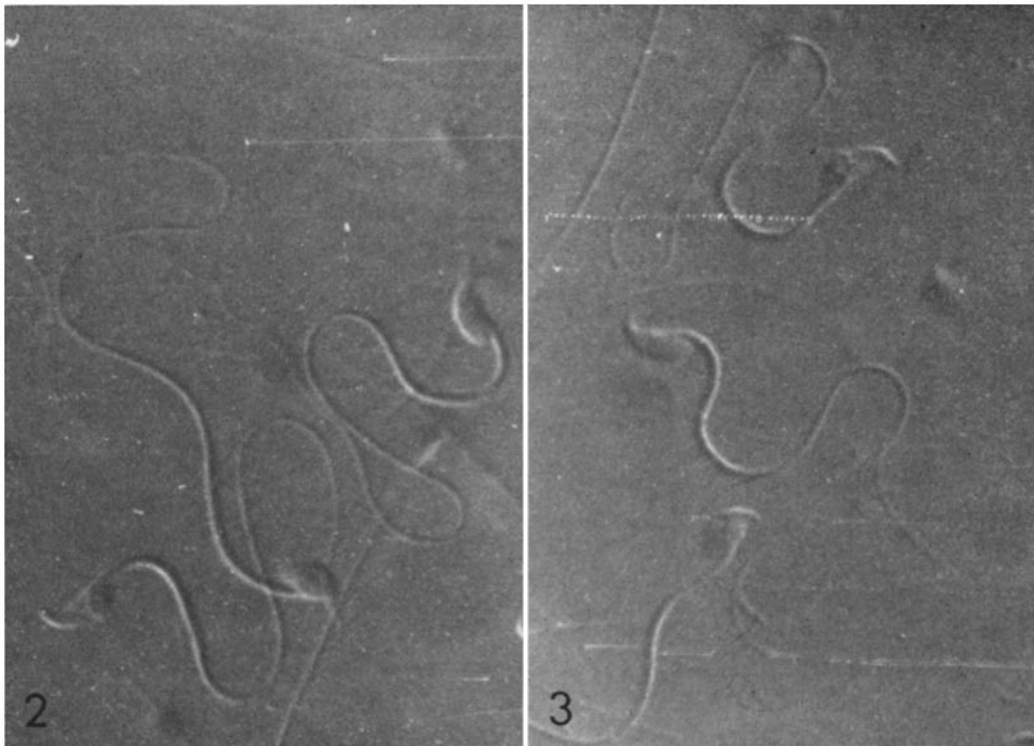
FIGURE 1 Tracings of three mouse spermatozoa from frames of a film taken at 100 frames/second with high-dry Nomarski optics. Each of the three cells was traced at the full extent of alternate effective and recovery strokes. (When the spermatozoa are traced at each rather than alternating effective and recovery strokes, there are so many partially superimposed images that the drawing is confusing.) These three spermatozoa, and many others I have observed, swim in circles. When spermatozoa move in what might be considered the effective stroke (as the annulus moves forward on this stroke), the mid-piece bends very sharply as the convex surface of the head bends back and is disposed near the mid-piece. In what might be considered the recovery stroke, the mid-piece straightens out. It sometimes is slightly bent beyond the fully straight position but never forms the sharp arc which is observed in the effective stroke.  $\times 500$ .

motion is observed in epididymal, uterine, and oviducal spermatozoa.

**CRAWLING (PATTERN 2):** Oviducal and uterine spermatozoa are capable of another type of motile pattern which appears strikingly similar to the motion of a snake as it crawls on the ground. When moving in this way, the spermatozoan does not beat, but the end of the tail appears to follow in the path of more anterior parts; the forward motion appears to come from the pushing of the flagellum against the substrate. Erythrocytes stuck to the slide appear to be deformed by pressure from passing spermatozoa as they push against them. The flagellum is very sharply curved back and forth along its length when spermatozoa move along a substrate in this manner (Figs. 2 and 3). Crawling spermatozoa do not follow circular paths. The characteristics of the crawling

pattern of motility which distinguish it from the asymmetrical beat pattern are strikingly obvious in a film (Phillips, 1970), though perhaps not so clear in prints or tracings of films.

**ROTATING (PATTERN 3):** I have observed that when mouse spermatozoa swim at some distance from surfaces, they generally exhibit a type of motile pattern which is distinctly different from either asymmetrical beating or crawling. This pattern may be a variation of the asymmetrical beating pattern (Pattern 1) and has an effective stroke and a recovery stroke. When such motile spermatozoa are viewed in films taken at 200-500 frames/second, it can be seen that at each effective stroke the spermatozoan rotates approximately  $180^\circ$ . This can be detected because of the asymmetrical, sickle-shaped head which characterizes mouse sperm. The pointed end of the head is



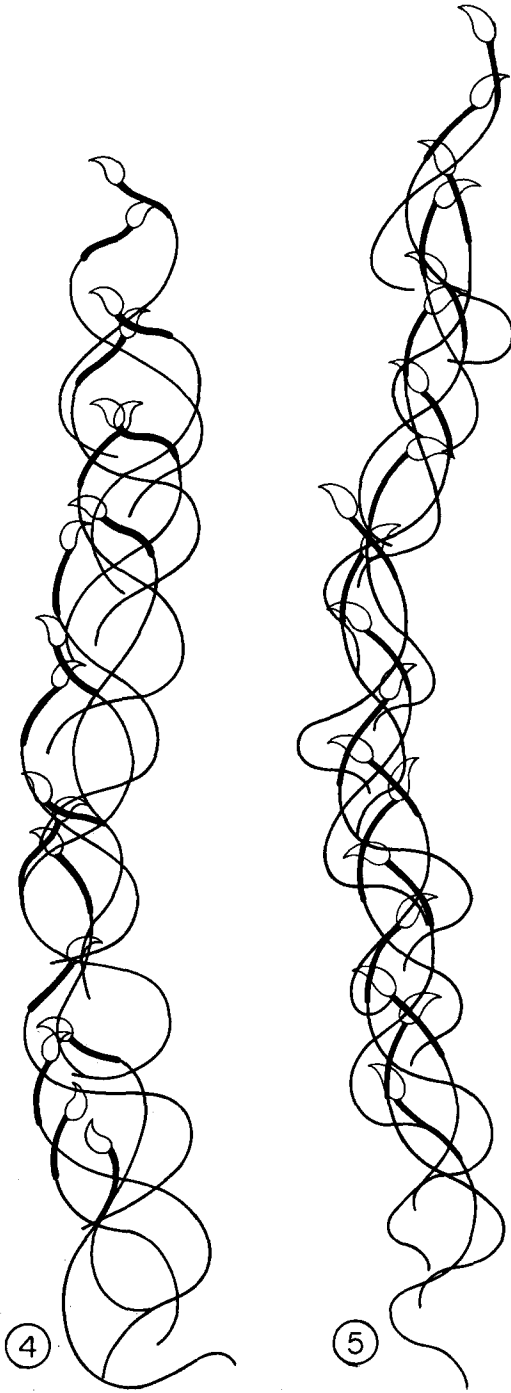
FIGURES 2 and 3 Frames of a film of crawling oviducal mouse spermatozoa 4-5 hr after insemination. Oviducal mouse spermatozoa often crawl in a motion which is reminiscent of the crawling of a snake. When they are progressing in this fashion, the spermatozoa move forward with the end of the tail following in the path of more anterior parts. The sperm appears to progress by pushing against the surface. The flagellum does not move very fast relative to other types of motile pattern.  $\times 800$ .

directed to alternate sides on alternate beats. The head can be seen moving through  $180^\circ$  on nearly every effective stroke (Figs. 4 and 5). Evidence for a similar rotation of spermatozoa in human, rat, and Chinese hamster is presented below and has been described more quantitatively in the bull by several workers (for review see Nelson, 1967). In order to determine whether mouse spermatozoa rotate or flip back and forth, I have studied films in which spermatozoa are swimming in a plane which is slightly above or below the plane of focus of the microscope. I observed in such films that the point of the tip of the head comes into focus on alternate strokes, indicating that spermatozoa rotate rather than flip back and forth.

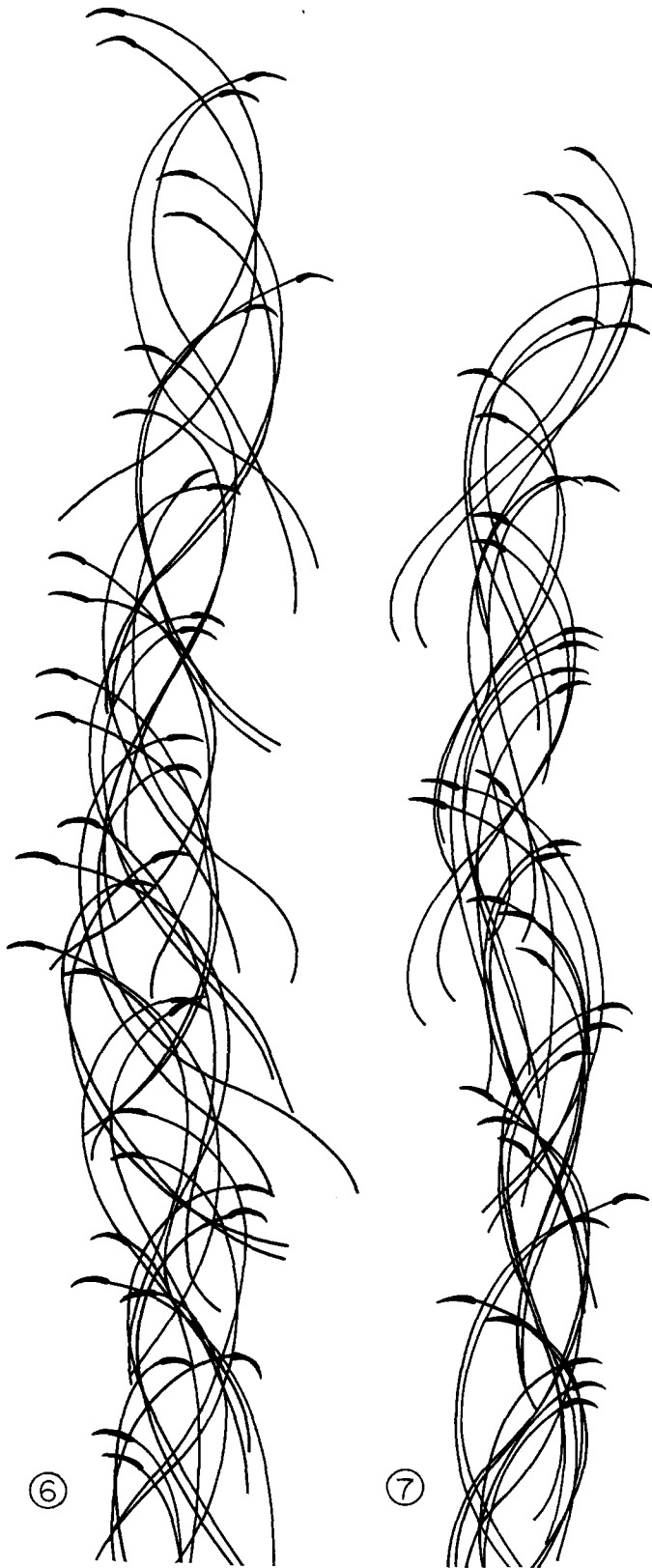
#### *Chinese Hamster Spermatozoa*

Chinese hamster spermatozoa are very long relative to most mammalian spermatozoa; they

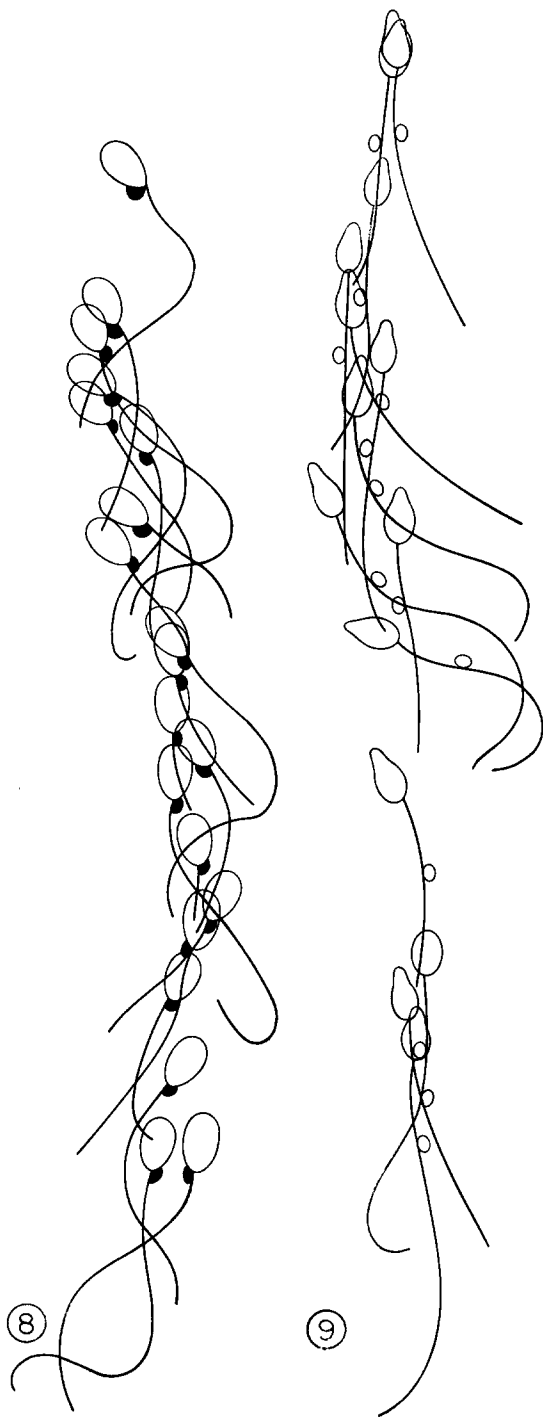
are  $250 \mu$  in length. (The length of rabbit spermatozoa is  $46 \mu$ , that of mouse is  $120 \mu$ , and that of rat is  $190 \mu$ .) I have examined epididymal and uterine spermatozoa of Chinese hamster. Spermatozoa of this species are extremely rigid. When swimming, the sperm of Chinese hamsters always have an effective stroke and a recovery stroke. The entire spermatozoan forms part of one wave and, even at the very start of the recovery stroke, the spermatozoan is bent into only a very gentle arc (Figs. 6 and 7). Even at the full extent of the recovery stroke the spermatozoa do not straighten out fully but remain slightly bowed in the direction of the effective stroke. Chinese hamster spermatozoa have two motile patterns which are similar to the asymmetrical beating and rotation in mouse, which were described above. Spermatozoa of Chinese hamsters do not usually swim on surfaces but undergo both of these motile patterns when away from surfaces. When undergoing the



FIGURES 4 and 5 Tracings of two mouse spermatozoa from frames of a film taken at 300 frames/second with  $25\times$  objective and dark-field optics. Spermatozoa were traced on each beat on the frame in which a broad side of the sperm head was in best focus. In the case of both of these tracings and other similar ones, spermatozoa rotate  $180^\circ$  on each beat without variation. I followed one spermatozoon for 86 beats; the cell rotated through  $86 \times 180^\circ$ . Spermatozoa swimming in this manner travel in more or less straight paths. The motion can be seen to be a rotation rather than a flipping back and forth in films taken with high-dry Nomarski optics where spermatozoa are swimming slightly above or below the plane of focus. The hook of the sperm head is seen to come into focus on alternate frames in these films, indicating that the sperm rotates. In Fig. 4, the spermatozoon progresses only on alternate beats. I have observed this in several cells.  $\times 500$ .



FIGURES 6 and 7 Tracings of two Chinese hamster spermatozoa from frames of a film photographed at 150 frames/second with low-magnification ( $10\times$  objective) dark-field optics. The spermatozoa were traced at the full extent of bending of each beat. A spermatozoan moves by taking two or three beats in one direction, then rotating  $180^\circ$  and taking two or three beats in the opposite direction. Usually, when spermatozoa swim in a deep well, as in this case, either they move out of the plane of focus or the plane of their two-dimensional beat changes. The two shown here were selected because the plane of beat remained parallel to the surface, and they remained in focus for a considerable number of beats. Since Chinese hamster spermatozoa have an effective stroke and a recovery stroke, and since Chinese hamster spermatozoa which do not rotate swim in circular paths, I believe that the rotation results in a straight rather than a circular path. Spermatozoa progress farther on the beat on which they rotate than on other beats.  $\times 325$ .



FIGURES 8 and 9 Tracings of human spermatozoon from frames of film photographed at 500 frames/second with a planapo 100  $\times$ , 1.3 NA, phase objective. Human spermatozoa are difficult to follow since they are small. They are best photographed with a high numerical aperture objective which must necessarily have very little depth of focus. Spermatozoa therefore constantly move in and out of the plane of focus. Since human spermatozoa do not have an asymmetrical hooked head as do spermatozoa of many rodents, I have chosen two spermatozoa which happen to have a protruding protoplasmic droplet in the region of the neck (as in Fig. 8) or the annulus as in (Fig. 9) which imparted visible asymmetry to the spermatozoon. Spermatozoa were traced whenever the droplet came into focus, so the frames are not spaced in any ordered way. It can be seen in both figures that the droplet is sometimes on one side of the cell and sometimes on the other, which indicates that human spermatozoa rotate 180° when beating as do mouse and Chinese hamster spermatozoa. From studying many films of swimming human spermatozoa, I believe that they generally rotate 180° at each beat as do mouse spermatozoa.  $\times$  1300.

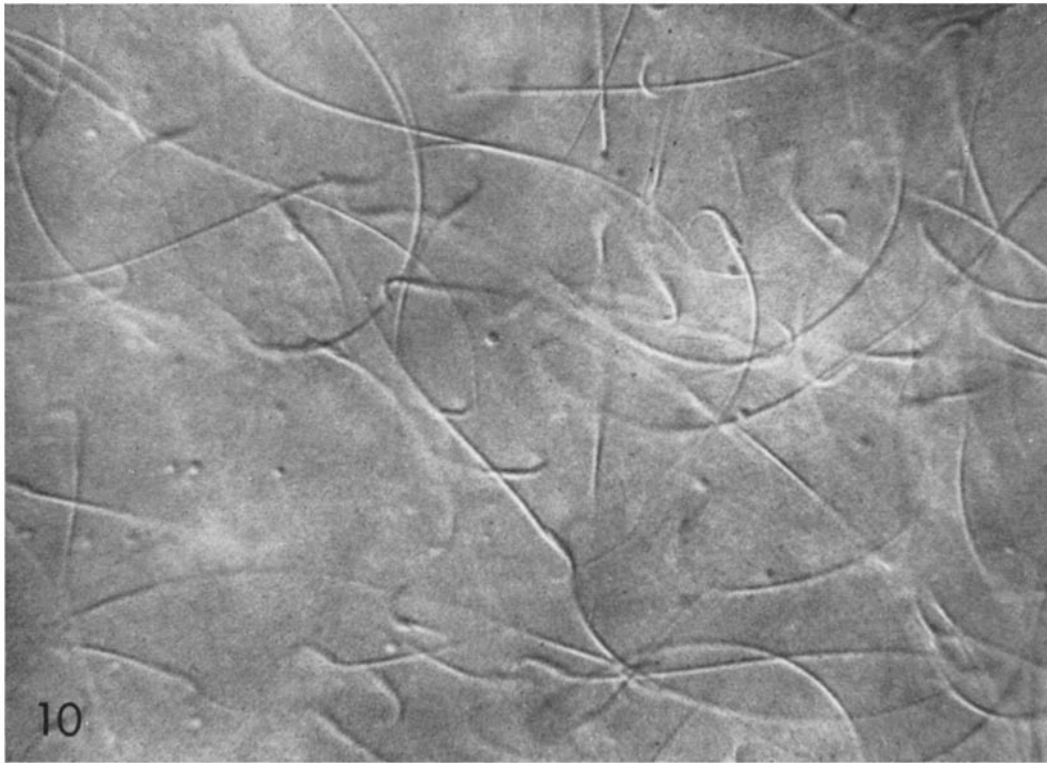


FIGURE 10 Print of swimming rat spermatozoa from film taken at 150 frames/second photographed with high-dry Nomarski optics. Rat spermatozoa are very stiff and bend only in very gentle arcs.  $\times 400$ .

asymmetrical beating pattern, the rigid spermatozoa bend in the effective stroke and straighten out in the recovery stroke and swim in rather tight circular paths. The form of the beat in rotating Chinese hamster sperm is similar to that of the asymmetrical beating pattern except that the spermatozoan rolls through approximately  $180^\circ$  on every second or third recovery stroke (Figs. 6 and 7). It can be seen in Figs. 6 and 7 that the concave surface of the arc-shaped spermatozoan faces the opposite direction after rotation, showing that the entire spermatozoan rolls and not just the head. When moving in this manner, spermatozoa traverse straight paths. Generally, the spermatozoan progresses much farther forward on the effective stroke after the spermatozoan rolls than on other effective strokes.

#### *Rat Spermatozoa*

I have examined epididymal and uterine spermatozoa from rats. Uterine spermatozoa were

observed at various intervals between 10 min and 5 hr after insemination.

Rat spermatozoa progress in a rotating pattern similar to that observed in mouse and Chinese hamster. Rotating rat spermatozoa generally execute two or three beats between rotations, similar to the manner described above for the Chinese hamster. Swimming rat spermatozoa do not form sharp bends such as I have observed in motile mouse spermatozoa, yet the spermatozoa have a smaller radius of curvature than motile Chinese hamster spermatozoa (Fig. 10).

#### *Human Spermatozoa*

Human spermatozoa were studied in films taken at 500 frames/second with 1.3 NA oil immersion phase or Nomarski objectives. These spermatozoa swim in straight paths and presumably do not have a completely planar beat since the entire spermatozoan is never in focus at the same time. It can be seen that the heads of spermatozoa turn, but



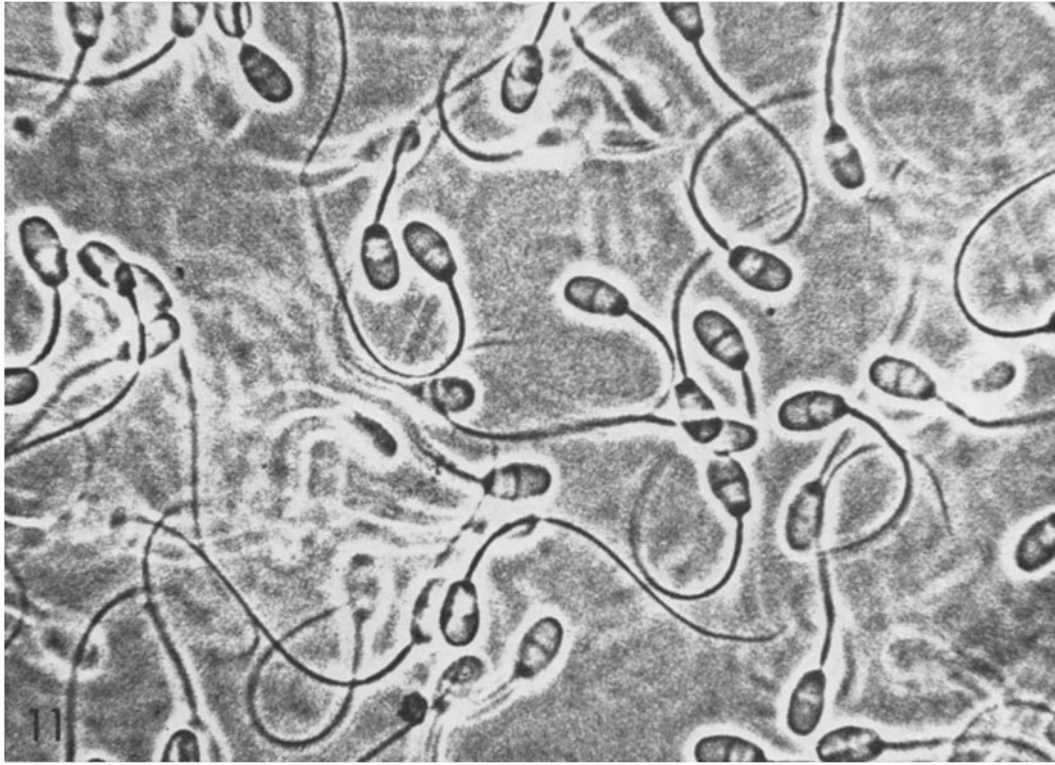


FIGURE 11 Frame of film taken at 300 frames/second with phase optics. Rabbit spermatozoa in a hanging drop preparation frequently come to the surface and swim in counterclockwise circles just below the surface of the cover slip. They have very pronounced effective and recovery strokes in which they bend very sharply in one direction but not the other. Note that all the flagella which are bent are bent to the same side (i.e., to the right in spermatozoa which face toward the bottom of the picture).  $\times 1500$ .

the rotation which we have described in mouse and Chinese hamster cannot be demonstrated in most human spermatozoa since the head is not asymmetrical in shape as is the sperm head of Chinese hamster and mouse. In some human spermatozoa, however, the protoplasmic droplet protrudes from one side of the cell, either in the neck region or the region of the annulus (Figs. 8 and 9). Spermatozoa which have a protruding protoplasmic droplet occasionally remain in focus for a second (500 frames) or more, and, in frames where the protoplasmic droplet is in focus, it can be seen that the droplet is sometimes on one side of the cell and sometimes on the other, indicating that human spermatozoa also rotate. Human spermatozoa appear to have an effective stroke and a recovery stroke as do other spermatozoa.

#### *Rabbit Spermatozoa*

We have studied rabbit spermatozoa obtained by an artificial vagina.<sup>1</sup> The semen was diluted in Hanks' salt solution or saline buffered with very dilute phosphate buffer at pH 7.8.

ROTATING: As is the case with spermatozoa of other species reported here, rabbit spermatozoa often rotate when they swim. Generally, rotating rabbit spermatozoa swim in fairly straight paths as do rotating spermatozoa of other species. Spermatozoa which rotate and swim in fairly tortuous paths, however, were also observed. Various paths

<sup>1</sup>I am grateful to Dr. Richard R. Fox, Jackson Laboratory, Bar Harbor, Maine, for his very helpful advice on collecting semen samples from rabbits.

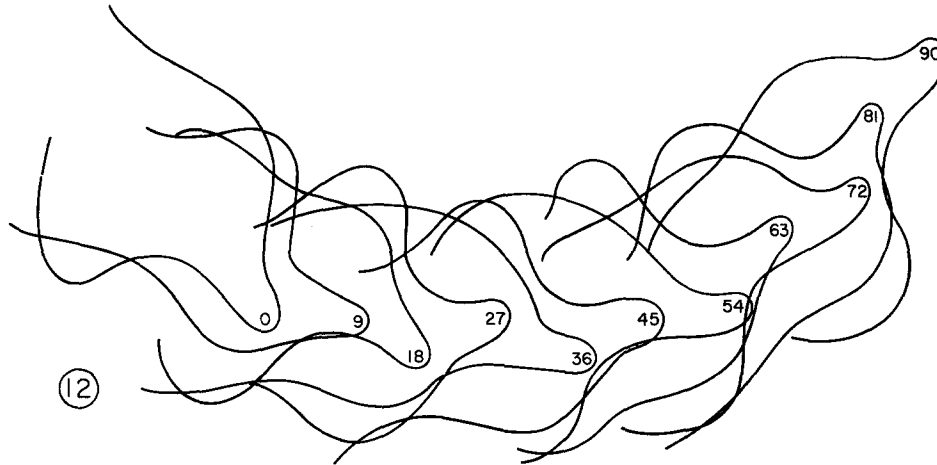


FIGURE 12 Tracing along the leading edge of a pair of spermatozoa of the North American opossum *Didelphis*. I have traced a large number of such pairs. The two spermatozoa of the pair swim in alternating beats. Here I have traced every ninth beat. Note that on each even-numbered beat the upper cell bulges out, whereas on odd-numbered beats the lower spermatozoon bulges, showing alternation of beat.  $\times 600$ .

of swimming rabbit spermatozoa have been described by Branham (1969).

**CIRCULAR PATHS ON SURFACES:** Rabbit spermatozoa which are swimming in a deep well or hanging drop often come to the surface and swim with the broad side of their heads parallel to the coverslip. They swim in a plane parallel to the cover slip just below it. Many of these spermatozoa swim in circles, although some follow fairly straight to very straight paths. The spermatozoa which swim in circles almost invariably swim in counterclockwise direction. When the heads of the spermatozoa are traced, it is found that the diameter of the circular paths is very small, usually less than  $80 \mu$ . Spermatozoa swimming in this manner clearly have an effective stroke and a recovery stroke (Fig. 11). In the recovery stroke the flagellum bends over in a very sharp arc, and it subsequently straightens out rapidly in the recovery stroke.

#### *Opossum Spermatozoa*

I have examined the ultrastructure and motility of epididymal spermatozoa of the North American opossum, *Didelphis marsupiales virginianus*, the woolly opossum, *Caluromys philander*, and the mouse opossum, *Marmosa mitis*. In all three species, single spermatozoa are released from the testis, but spermatozoa in the proximal portion of the epididymis join together in twos. A very close junction forms between the heads where the plasma membranes of the two spermatozoa are

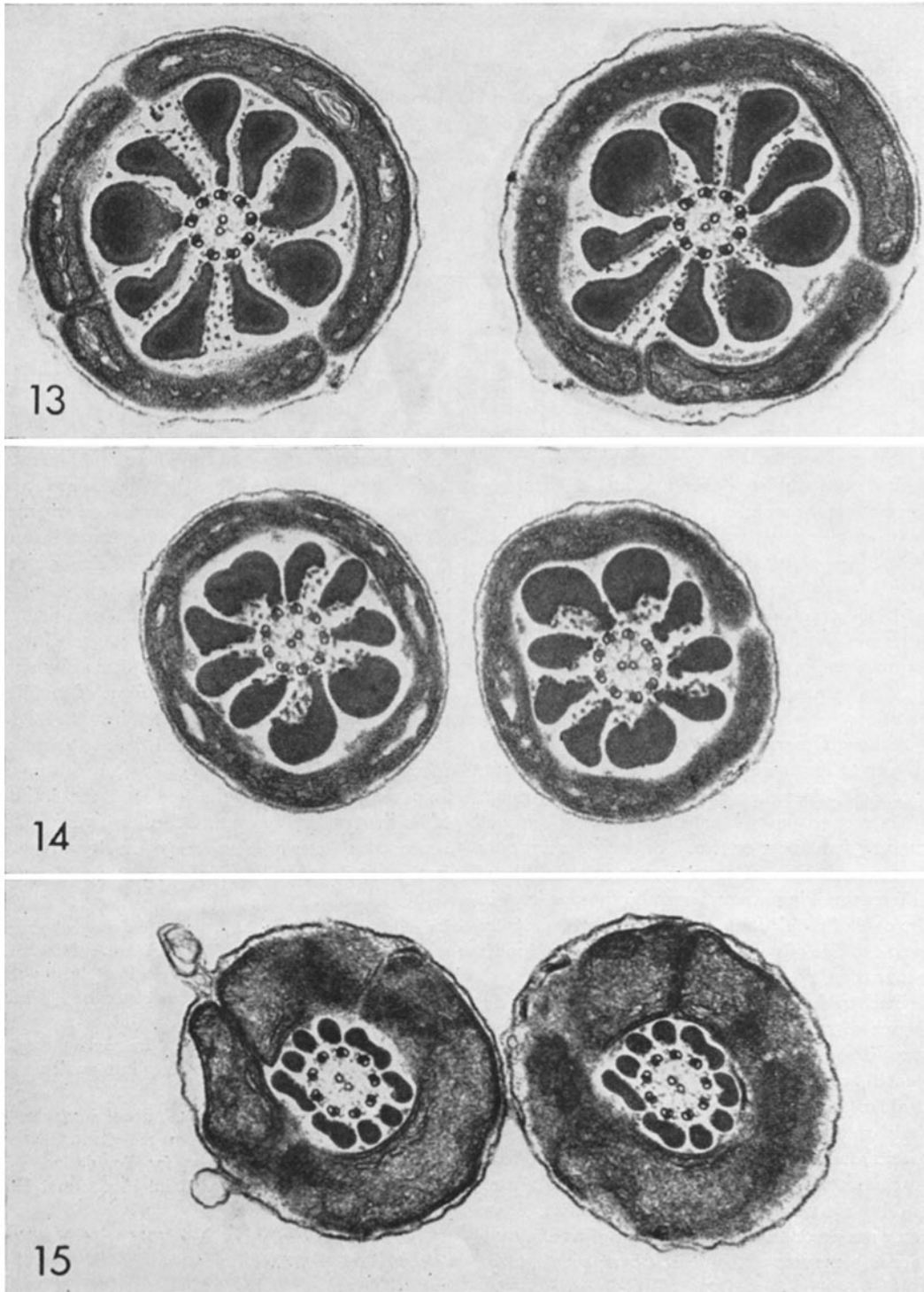
juxtaposed around the rim of the acrosome (Phillips, 1970). Analysis of high-speed films reveals that in all three species the two sperm flagella of a pair beat alternately during swimming (Fig. 12). I followed one pair of spermatozoa (using the data analyzer) for 1000 beats. Each spermatozoon beat 500 times, always alternating beats with the other without missing a beat.

#### *Ultrastructure of the Dense Fibers*

Identifying the structural features of spermatozoa which correlate closely with characteristics of motility may help to elucidate the function of various sperm structures in motility. One structural feature of spermatozoa which shows a high degree of interspecies variability is the size of the dense fibers. Figs. 13–15 illustrate this in some of the species I have examined. The Chinese hamster (Fig. 13) and ground squirrel (Fawcett, 1970) have very large, dense fibers. The rat (Fig. 14) has dense fibers which are somewhat smaller in cross-sectional diameter. Spermatozoa of mouse (Fig. 15), human (De Kretster, 1969; Zamboni et al., 1971), rabbit (Zamboni and Stefanini, 1971), and opossum (Phillips, 1970) all possess very small, dense fibers. Correlation of dense fiber size with motility is discussed below.

#### DISCUSSION

I find that spermatozoa of some mammalian species display different types of motile patterns



FIGURES 13-15 Electron micrographs of transversely sectioned mid-pieces of spermatozoa of Chinese hamster (Fig. 13), rat (Fig. 14), and mouse (Fig. 15) at the same magnification. Although the diameter of the mid-piece of the spermatozoa is about the same in rat and mouse, the cross-sectional area of the dense fibers (coarse fibers) is several times greater in rat than in mouse. Chinese hamster spermatozoa have even larger dense fibers. The size of the dense fibers correlates positively with the stiffness of the spermatozoa. This suggests that dense fibers may be stiff elastic elements.  $\times 49,000$ .

in vitro. In the mouse, I have observed three distinctly different types of motile patterns. I have seen two in the Chinese hamster and rabbit and one in the human spermatozoa. Rikmenspoel (1965) observed two types of motility in bull spermatozoa. He considered the rotating type of motility normal and the motility of a planar type on surfaces abnormal since this type of motility could be produced by cold shock. It is possible that several types of motile patterns can be produced by sperm of many mammalian species, depending upon external conditions. It would be interesting if these different patterns were characteristic of sperm in different parts of the female tract or at different times after insemination. Spermatozoa in many mammalian species must spend a considerable time in the female tract before fertilization. It may be that sperm change their motile pattern, depending on whether they are moving through liquids or on cellular surfaces. It is possible that the type of motility that is observed in semen samples is very different from the type of motility necessary for fertilization and that changes in motile pattern could even be involved in capacitation. Careful studies of oviducal spermatozoa in conditions which approximate the in vivo environment will help to answer these questions.

Although spermatozoa of mouse and human appear to rotate almost constantly as they swim, sperm of Chinese hamster and rat generally rotate on one beat and subsequently progress without rotating for a beat or two. This suggests not only that spermatozoa of some species are capable of different motile patterns, but also that, at least in the case of the Chinese hamster and rat, they are even capable of switching different types of motile patterns on alternating beats.

One striking variable in sperm movement is the degree of stiffness of the spermatozoan. Spermatozoa of some species we have studied (e.g., mouse, human, rabbit, and opossum) appear relatively flexible, and as they beat they form arcs with a small radius of curvature. Spermatozoa of other species, such as the Chinese hamster and rat, have spermatozoa which appear very stiff when beating and have a very large radius of curvature. There is a close correlation between the radius of curvature and the size of the dense fibers; spermatozoa of mouse, human, rabbit, and opossum, which have a small radius of curvature, have small dense fibers. Spermatozoa of Chinese hamster and rat, which have a large radius of curvature, have large

dense fibers. Comparing particularly the spermatozoa of mouse and rat, it is apparent that the cross-sectional diameter of the mid-piece of both species is about the same whereas the cross-sectional area of the dense fibers in the region of the mid-piece of the rat is several times that of the mouse (compare Figs. 14 and 15). These observations suggest that the dense fibers are stiff elements. Thus, the dense fibers may influence the form of the beat by determining the elastic properties of the sperm tail. One might imagine that different-shaped beats in different species might be suited to specific conditions of the female reproductive tract, the cumulus, etc.

My study has provided some understanding of the types of motile patterns which sperm are capable of generating. These various capabilities will have to be taken into consideration in analyzing the mechanisms by which motility is produced. However, it has not yet been determined whether sperm in the female tract actually move in any of the manners described. In the case of the mouse, the lumen of the oviducal tract is very narrow; it would not be surprising if sperm tended to crawl rather than swim in the oviducts. Crawling, though it was the rarest form of motility seen in vitro, might be the principal form of motility in vivo. Other motile patterns seen in vitro might then be the result of undirected flailing about of spermatozoa lacking a physiological substrate. In other words, I have gained some understanding of how sperm can move, but further investigation is needed to determine how they actually do move in the female genital tract.

I would like to thank Dr. Don W. Fawcett who suggested many of the experiments I have done and who has given me valuable suggestions during the course of the work. I would also like to thank Mr. Charles Stolar and Miss Miriam Lifisics for their skillful technical assistance and Mrs. Sylvia Keene for transforming my rough tracings into professional illustrations.

This work was supported by National Institutes of Health Contract 69-2106 from the Contraceptive Development Branch, Center for Population Research, National Institute of Child Health and Human Development.

*Received for publication 10 June 1971, and in revised form 10 January 1972.*

#### REFERENCES

- BRANHAM, J. M. 1969. Movements of free swimming rabbit spermatozoa. *J. Reprod. Fert.* 18:97.

- DE KRETSTER, D. M. 1969. Ultrastructure features of human spermiogenesis. *Z. Zellforsch. Mikrosk. Anat.* **98**:477.
- FAWCETT, D. W. 1968. The topographical relationship between the plane of the central pair of flagellar fibrils and the transverse axis of the head in guinea pig spermatozoa. *J. Cell Sci.* **3**:187.
- FAWCETT, D. W., and D. M. PHILLIPS. 1969. Observations on the release of spermatozoa and on changes in the head during passage through the epididymis. *J. Reprod. Fert.* **6**(Suppl.):405.
- FAWCETT, D. W. 1970. A comparative view of sperm ultrastructure. *Biol. Reprod.* **2**(Suppl.):90.
- GRAY, J. 1958. The movement of spermatozoa of the bull. *J. Exp. Biol.* **35**:96.
- HANCOCK, G. J. 1953. The self-propulsion of microscopic organisms through liquids. *Proc. Roy. Soc. Ser. A.* **217**:96.
- NELSON, L. 1967. Sperm Motility in *Fertilization*. Metz and Monroy, editors. Academic Press, Inc., New York.
- PHILLIPS, D. M. 1970. Cinematographic analysis of mammalian spermatozoa. *J. Cell Biol.* **47** (2, Pt. 2):238 a. (Abstr.)
- REYNOLDS, A. J. 1965. The swimming of minute organisms. *J. Fluid Mech.* **23**:241.
- RIKMENPOEL, R. 1965. The tail movements of bull spermatozoa. Observations and model calculations. *Biophys. J.* **5**:365.
- SALISBURY, G. W. 1962. Ionic and osmotic conditions in relation to metabolic control in *Spermatozoan Motility*. Bishop, editor. (AAAS publication No. 72). 59.
- SATIR, P. 1963. Studies on cilia. The fixation of the metachronal wave. *J. Cell Biol.* **18**:345.
- TAMM, S. L., and G. A. HORRIDGE. 1970. The relation between the orientation of central fibers and the direction of beat in *Opalina*. *Proc. Roy. Soc. Ser. B.* **175**:219.
- TAYLOR, G. 1952. The action of waving tails in propelling microscopic organisms. *Proc. Roy. Soc. Ser. A.* **211**:225.
- VENABLE, J. H., and R. G. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407.
- YANAGIMACHI, R. 1970. The movement of golden hamster spermatozoa before and after capacitation. *J. Reprod. Fert.* **23**:193.
- ZAMBONI, L., and M. STEFANINI. 1971. The fine structure of the neck of mammalian spermatozoa. *Anat. Rec.* **169**:155.
- ZAMBONI, L., R. ZEMJANIS, and M. STEFANINI. 1971. The fine structure of monkey and human spermatozoa. *Anat. Rec.* **169**:129.