

# THE MOVEMENT OF SPERMATOZOA

by Charles J. Brokaw

A spermatozoon is a device for the transport of a package of DNA containing a male complement of the genetic information of a species. In most animals the delivery of this package to the egg depends in part on the active propulsion of the spermatozoon by undulations of its tail. The sperm tail is an example of the class of cell structures known as flagella or cilia, which are widely distributed among organisms wherever movements are required at the microscopic level. As far as we know, all cilia and flagella share basic mechanisms of motility. But as yet we know very little about these mechanisms.

The sea urchin spermatozoon is a favorite material for studying flagellar motion. Sea urchins are abundant near Caltech's Kerckhoff Marine Laboratory. They are "ripe" throughout most of the year and produce generous amounts of nearly pure spermatozoa. Their spermatozoa are simpler in structure than those of humans and other mammals, and their motion is somewhat simpler to study since the sperm flagellum bends almost entirely in one plane. The external and internal morphology of these spermatozoa—and other cells with flagella and cilia—has been intensively studied since the early 1950's, when the electron microscope became a widely available research tool. However, the detailed morphology of their movements has seldom been examined because very specialized techniques are required to record their movements.

The force causing forward propulsion of a spermatozoon is generated by the propagation of bending waves along the sperm flagellum, in much the

same way that an eel or a snake propels itself by the passage of bends along its body. The bending waves originate near the head of the spermatozoon and are repeated regularly at frequencies of 30-40 beats per second. The waves pass backwards along the tail at speeds of the order of 1,000 microns per second and propel the spermatozoon forward at speeds of 150-200 microns per second. Since the sperm flagellum itself moves laterally through the water at speeds of about 400 microns per second (2,000 times its width per second) exposure times of 100 microseconds or less are required to obtain accurate photographs showing the form of the bending waves during movement. Because of its small size, very intense illumination is also required to photograph it. To study how the shape of the flagellum changes with time as it generates its bending waves, photographs taken at intervals of a few milliseconds are desirable. Only a few electronic flash illumination systems have been built that meet these particular requirements.

In 1955 Sir James Gray, professor of zoology at Cambridge University, published a description of the movements of sea urchin spermatozoa, based on photographs taken with exposures of 2 milliseconds duration. He concluded that the shapes of the bent sperm tails shown on his photographs could be closely matched by sine waves. This conclusion formed the basis for most theorizing about flagellar movement for the next ten years. The relationship between the bending waves and the forward swimming of the spermatozoon was developed mathematically, and mechanisms for generating bending waves were proposed on the assumption that the waves were similar to the familiar sinusoidal vibrations of an elastic rod, modified to include internal amplification to prevent a decrease in amplitude as the sinusoidal waves passed along the flagellum.

My own interest in flagellar movement dates back to the period from 1955 to 1958, which I spent as a graduate student in Sir James Gray's depart-

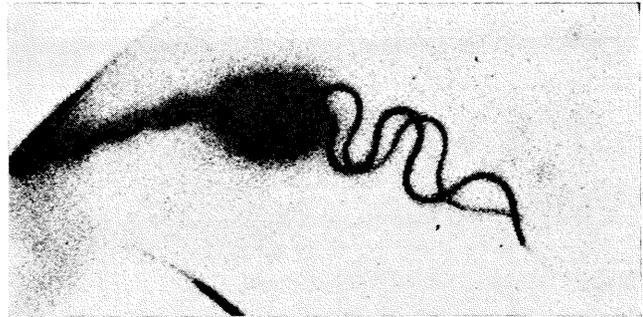
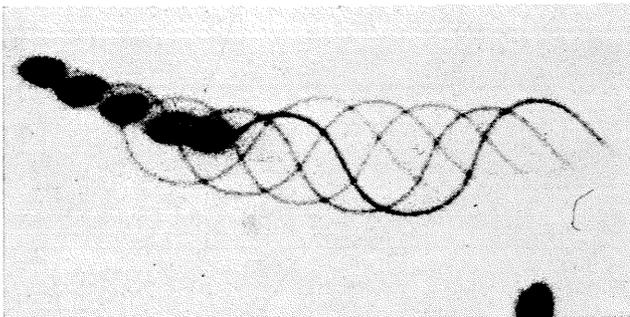
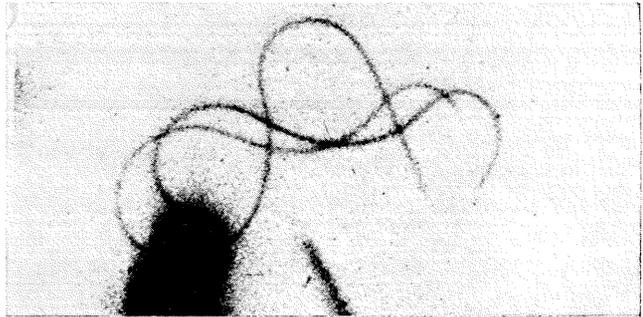
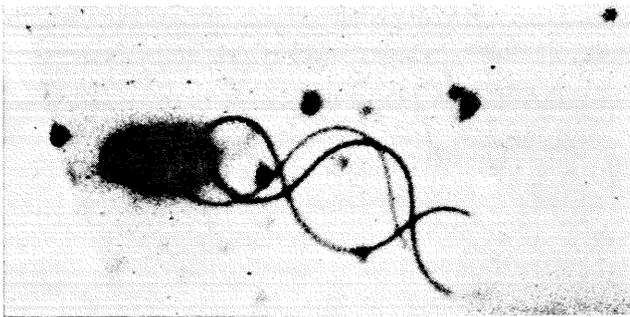
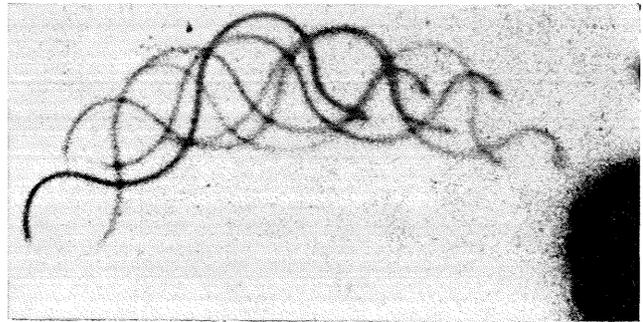
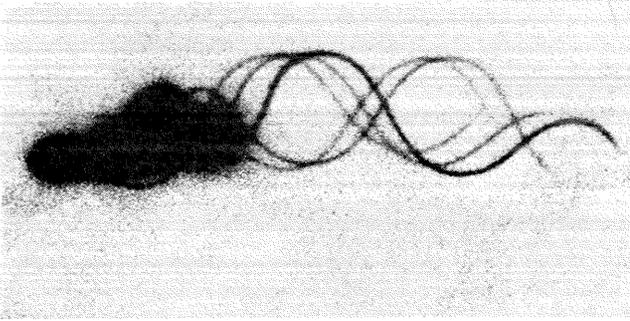
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CHARLES J. BROKAW, '55, *associate professor of biology at Caltech, is studying how spermatozoa make the movements which enable them to swim. Much of this work is carried on at Caltech's Kerckhoff Marine Laboratory at Corona del Mar, where the marine animals used for these studies are abundant. Dr. Brokaw has just been awarded a three-year grant from the United States Public Health Service for the continuation of this work.*

## PHOTOMICROGRAPHS OF SWIMMING SPERMATOZOA

This series of pictures (reproduced here as negative prints at 1600-fold magnification) was taken to obtain information about the changes in the shape of the tail of a swimming spermatozoon. The photographs are made using dark-field illumination and a sequence of flashes, so that each photograph is a multiple exposure showing one spermatozoon in several positions. The first flash in the sequence can usually be identified because it is more intense than the subsequent flashes.



1. A swimming spermatozoon of a sea urchin photographed at 50 flashes per second.

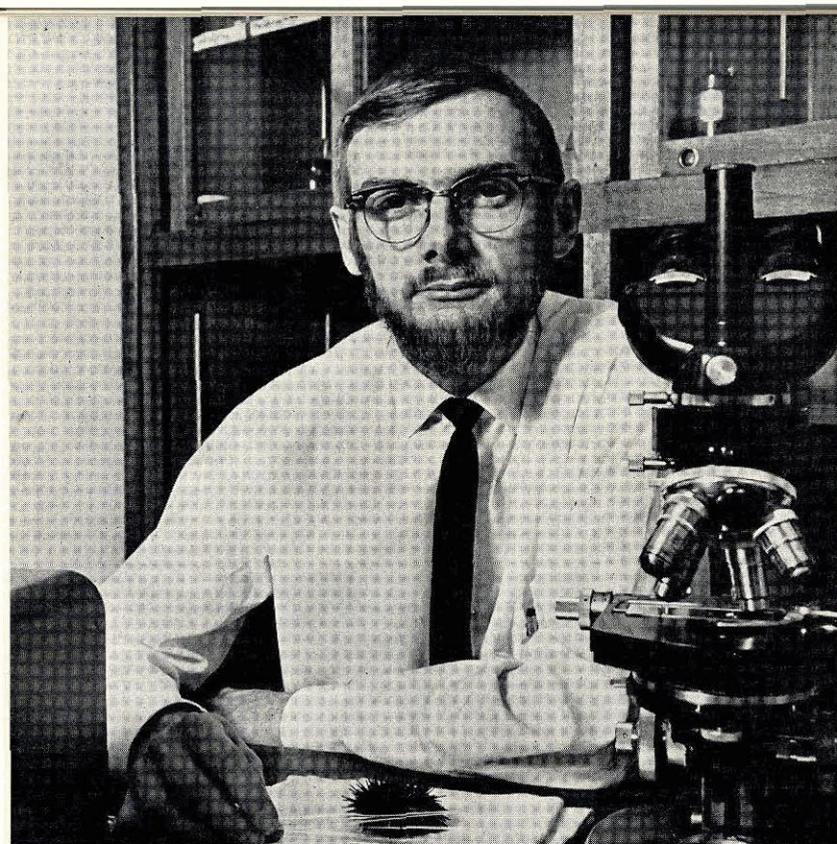
2. A swimming spermatozoon of an annelid worm photographed at 50 flashes per second.

3. Spermatozoon of a tunicate—at 40 flashes per second, only slightly faster than the beat frequency of the spermatozoon, showing the approximate forward progress made during each beat cycle.

4. The movements of a sea urchin spermatozoon after the head and midpiece have been removed by shearing a sperm suspension. The movements last for only a few seconds but prove that all the machinery needed to generate bending is located in the tail.

5. A spermatozoon which has been inactivated with 50 percent glycerol and then reactivated with ATP.

6. One type of movement obtained when spermatozoa are swimming in a solution containing methyl cellulose to increase the viscosity. Under these conditions the flagellum can form much sharper bends than in normal swimming.



Charles Brokaw, associate professor of biology.

ment at Cambridge. After some attempts to study the biochemistry of isolated flagella and to correlate this with movement, I realized that the available information on the movement of flagella was inadequate. In 1963, using a new flash illuminator built at Caltech, I obtained photographs of the morphology of moving spermatozoa which suggested that Gray's earlier conclusion was incorrect.

These photographs, having considerably greater resolution, showed that the shape of the bent flagellum did not match a sine wave. Instead, it contained regions that appeared to be bent into circular arcs. These bent regions, alternating in direction along the flagellum, appeared to be separated by shorter regions where the flagellum remained straight. Although the difference between such a wave and a sine wave is small—easily hidden by the limited resolution of Gray's original photographs—it is not completely trivial. It makes possible a simpler and more complete analysis of the relationship between the bending waves and the forward swimming of the spermatozoon. More significantly, it suggests a simple model for the generation of bending waves in all types of cilia and flagella. This model could never have been seriously considered as long as it was believed that the waves had to be sine waves.

According to this new model, each bend on the flagellum forms an arc of a circle within which the curvature of the flagellum remains constant. At any moment, bending is occurring only at the leading edge of the bend, where the shape of the flagellum changes rather abruptly from straight to curved.

Unbending is occurring only at the trailing edge of each bend. These transition regions, which mark the ends of each bend at any given moment, are the only points where the flagellum is actively doing anything. As the flagellum bends at one end and unbends at the other end of the bend, the bend itself moves along the flagellum. Each pair of transition regions passing along the flagellum causes a propagated bend, and two opposed pairs of transition regions form a complete bending wave.

This new way of thinking about the bending waves of flagella allows us to see more clearly the questions which must be answered before we can understand how the internal mechanisms of the flagellum generate its movements:

1. What are the internal structural correlates of the bent and straight states of the flagellum? How is the transition between these two states brought about?

2. What sort of information transmission is responsible for the point-to-point propagation of transitions along the flagellum?

3. How is the properly timed sequence of transitions initiated at the head end of the flagellum? These are the questions which our research is now trying to answer.

The smallness in the size of the flagellum has so far prevented us from knowing whether bending is causally correlated with the contraction of subunits, analogous to muscles, on each side of the flagellum. We do know, however, that if the flagellar membrane and the energy-converting systems of the sperm midpiece are destroyed by treatment with 50 percent glycerol, the movements of the

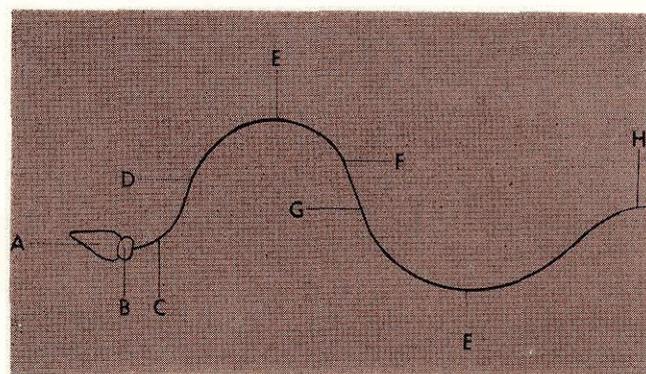


Diagram of a sea urchin spermatozoon. A. Head, containing genetic information and devices for entering the egg. B. Midpiece, containing a store of fuel and the enzymatic machinery needed to convert it to a usable form—ATP—supplied to the tail. C. Tail, or flagellum—its bending movements propel the spermatozoon. D. Unbending point. E. Bent region. F. Bending point. G. Straight region. H. Terminal piece; significance unknown.

spermatozoa can be reactivated by adenosine triphosphate (ATP). ATP is the biochemical fuel used by muscles and by most other cellular systems that produce work.

I am now trying to measure how much ATP is used at each transition region as it moves along the flagellum. Some results suggest that each bending transition may involve the use of just one molecule of ATP by each of the enzyme molecules involved. The next step will be to try to measure the number of molecules used under conditions where more extreme bending occurs, such as may happen when spermatozoa are swimming in solutions with much greater than normal viscosity.

Because flagella are so small, most speculations made about their internal mechanisms involve very simple solutions. The simplest way to transmit information from point to point along the flagellum, to turn on a transition at the next point, might be to use as a signal the structural strains which will inevitably arise near the transition between a bent region and a straight region. The details of this idea have been worked out, and we are now trying to test it experimentally.

One experimental approach to this question, which is being pursued by Stuart Goldstein, a graduate student in biology working at the Kerckhoff marine lab, is the use of a laser microbeam to damage small portions of the spermatozoon. For these experiments a redesigned flash illuminator for photographing the spermatozoa is being built, which can be synchronized with the laser discharge and the movement of the target spermatozoon.

These experiments should allow us to discriminate between the characteristics of the bending, unbending, and information transmission processes and to distinguish the properties of a special region near the base of the flagellum that may be responsible for the initiation of bending and unbending transitions.

The flagellum is an example of what may be termed an "oligomacromolecular" system. The answers to our questions about its mechanisms may lie tantalizingly between the cellular level, at which techniques such as optical and electron microscopy can give clear results, and the molecular level, at which highly developed techniques exist for the biochemical study of macromolecules and their interactions with other molecules in solution. The unanswered questions pose challenging problems, which become even more absorbing when one watches spermatozoa swimming under the microscope and sees the beauty of the regular and seemingly effortless rhythm of their movements which photographs cannot capture.



**Cora Black willed  
her estate to  
"The University of  
Southern California,  
commonly known  
as UCLA."**

The legal name of **Caltech** is California Institute of Technology.

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