INTERMITTENT SWIMMING IN LIVE SEA URCHIN SPERM

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ABSTRACT

Sperm of the sea urchin *Tripneustes gratilla* repeatedly start and stop swimming when suspended in seawater and observed by dark-field microscopy. While in the quiescent state, which usually lasts about a second, the sperm assume a shape resembling a cane, with a sharp bend of ~3.4 rad in the proximal region of the flagellum and very little curvature in the rest of the flagellum except for a slight curve near the tip. The occurrence of quiescence requires the presence of at least 2 mM Ca^{2+} in the seawater, and the percentage of sperm quiescent at any one time increases substantially when the sperm are illuminated with blue light. With intense illumination, close to 100% of the sperm become quiescent, and this percentage decreases gradually to ~0.3% over a 10^4-fold decrease in light intensity. An increased concentration of K^+ in the seawater also increases the percentage of quiescence, with a majority of the sperm being quiescent in seawater containing 80 mM KCl. The induction of quiescence by light or by increased KCl is completely inhibited by 10 μM chlorpromazine, and ~90% inhibited by 1 mM procaine or sodium barbital. Sperm treated with the divalent-cation ionophore A23187 swim quite normally, although for a relatively short period, in artificial seawater lacking divalent cations, but are abruptly arrested upon addition of 0.04–0.2 mM free Ca^{2+}. The flagellar waveform of these arrested sperm is almost identical to that of light-induced quiescence in the live sperm. The results support the hypothesis that quiescence is induced by a rise in intracellular Ca^{2+}, perhaps as a consequence of a membrane depolarization, and that it is similar to the arrest response in cilia.

KEY WORDS calcium · light-induced quiescence · motility · A23187 · flagella · membrane depolarization

Alterations in the usual pattern of ciliary and flagellar beat have been described in many organisms. Two main types of modified behavior are known: the arrest response of metazoan cilia, and the changes in direction or form of beating, seen in both protozoan and metazoan cilia and flagella. These modifications are transient in nature and of functional importance to the cells, and they can be produced by mechanical, chemical, or electrical stimulation (8, 30, 45). In gill cilia, the arrest response, which is under nervous control, allows the cells to regulate the flow of fluid between the gill filaments (30). In other organisms, alterations in the usual bending pattern are important in tactic behavior (8, 24, 28).

While full details of the molecular mechanisms underlying these responses are not known, there is strong evidence in many cases that they are mediated by a transient increase in the intracellular concentration of free Ca^{2+} upon the opening of voltage-sensitive Ca^{2+} gates (11, 36) that accompanies depolarization of the cell membrane. In live

cells of *Paramecium*, direct electrophysiological study has shown that ciliary reversal is associated with a Ca^{2+} action potential (12, 32), and use of $^{45}Ca^{2+}$ has demonstrated a correlation between Ca^{2+} influx, membrane depolarization, and ciliary reversal (7). Considerable evidence has accumulated in support of the involvement of Ca^{2+} in the arrest response of intact and demembranated gill cilia of *Mytilus* and *Elliptio* (31, 37, 39, 47, 49). In certain other organisms, it has been possible to obtain direct confirmation of the regulatory role of Ca^{2+} by using demembranated cells to demonstrate that an increase in Ca^{2+} concentration to 10 μM causes changes in the direction or symmetry of beating, as in cilia of *Paramecium* (33), and flagella of *Crithidia* (23), and of *Chlamydomonas* (24). A Ca^{2+}-mediated change in the symmetry of flagellar waveforms has been described in several species of sea urchin sperm that have been demembranated with Triton X-100 and reactivated with ATP (3–6, 17). Although this behavior appears closely related to ciliary reversal, the extent to which they share a common mechanism is not yet known.

After dilution into seawater, sea urchin sperm usually swim continuously at physiological temperature for an hour or more. Their motility can be inhibited by gaseous CO_2 (29) or lowered pH (19), but no behavior resembling ciliary arrest has been reported to our knowledge. This paper, however, describes an intermittent swimming behavior, in live sperm from the sea urchin *Tripneustes gratilla*, in which the sperm repeatedly pass in and out of a quiescent phase at irregular intervals, and also reports some physical and chemical factors that influence the occurrence of this behavior. The results show that quiescence in sperm flagella has many characteristics in common with ciliary arrest, and strongly support the hypothesis that it is controlled by a mechanism involving calcium. Preliminary reports of some of this work have appeared previously (15, 17).

The following paper (16) reports a Ca^{2+}-induced quiescent state in demembranated sperm reactivated with ATP. Two other papers present an analysis of the transient waveforms that occur during the stopping and starting phases of flagellar beating in live sperm and an analysis of the patterns of microtubule sliding during the transients (manuscript in preparation and reference 18).

MATERIALS AND METHODS

Sea urchins of the Hawaiian species *Tripneustes gratilla* and *Colobocentrotus atratus* were induced to shed their sperm by injecting them with 0.5 M KCl. The sperm were usually collected in seawater containing 0.2 mM EDTA, adjusted to pH 8.3 and Millipore filtered. This will be referred to as "standard seawater." The sperm were kept as a stock suspension (3–10 mg/ml) at room temperature for up to 4 h, and were diluted further for observation in a Petri dish as needed. A few observations were made with sperm of the Californian sea urchins *Stronglocentrotus purpuratus* and *Lytechinus pictus*.

Artificial seawater was prepared with 465 mM NaCl, 10 mM KCl, 9.8 mM $CaCl_2$, 25 mM $MgCl_2$, 28 mM $MgSO_4$, 2 mM $NaHCO_3$, and 0.2 mM EDTA. In artificial seawater containing altered proportions of KCl and NaCl, the sum of the concentrations of Na^+ and K^+ was kept at 475 mM to maintain the correct osmolarity. For some experiments artificial seawater containing 465 mM choline chloride in place of NaCl was used. Magnesium-free artificial seawater contained 465 mM NaCl, 10 mM KCl, 9.8 mM $CaCl_2$, 2 mM $NaHCO_3$, and 0.2 mM EDTA. For experiments with the divalent-cation ionophore A23187, artificial seawater containing no Ca^{2+} or Mg^{2+} (465 mM NaCl, 10 mM KCl, 2 mM $NaHCO_3$, and 0.2 mM EGTA) was used. All forms of artificial seawater were adjusted to pH 8.3 before use.

Sperm to be treated with the ionophore A23187 were collected in divalent-cation free artificial seawater and diluted into more of the same to a concentration of ~8 mg/ml. Then to 5 ml of the same seawater being vigorously stirred on a magnetic stirrer was added a drop of the diluted sperm suspension followed immediately by 10 μl of a stock solution of A23187 (10 mM in 50% dimethyl sulfoxide-50% ethanol [vol/vol]). Next, 0.25 ml of the suspension of ionophore-treated sperm was added to 2.25 ml of the divalent-cation-free artificial seawater in a Petri dish. Calcium was added in the form of aliquots of a stock solution of $CaCl_2$ in water, and under these conditions the free Ca^{2+} concentration is considered to be the concentration in excess of the 0.2 mM EGTA present in the artificial seawater.

Observation dishes (referred to as Petri dishes) were made by fixing glass rings to 75 × 50-mm microscope slides with epoxy resin, followed by careful washing, and then coating of the inner surface with ~0.03% Formvar in ethylene dichloride to prevent the sperm from sticking.

Sperm swimming in circles at the bottom surface of a Petri dish were observed at 22°–23°C by dark-field microscopy. Routine observations were made with a ×10 0.22 NA objective, a 0.7–0.85 NA dark-field condenser and illumination from a XBO 150W xenon lamp (Osram, Berlin). Photography at low magnification was performed on Polaroid 3000 Type 107C film or on 35-mm Kodak 2475 film. Oscillations of the head from the mean circular trajectory of swimming sperm can be seen easily on time exposures and were used to determine the length of circular arc travelled per beat, called the turning rate (see, for example, Fig. 5). For photography at higher magnification, a ×40 0.75 NA water-immersion objective with a 1.2–1.4 dark-field condenser and 35-mm Kodak 2475 film were used.

The dose response curve with respect to light intensity was measured with a 0.35–0.9 NA dark-field condenser, the same illuminator as above, and a series of calibrated neutral density filters prepared from partially exposed photographic plates. Estimates of the percentage of quiescent sperm were made by rapid visual estimation during the first 1–2 s after movement to a new field. Because the sperm tended to adapt to the light, the percentage of quiescence decreased after a short time to a steady-state value that was usually substantially lower than that during the first few seconds in the illuminating beam. From 11 to 30 such estimates were made for each intensity of light, the experi-

ment was repeated with three different preparations of sperm, and the results were averaged. The estimates must be regarded as approximate, but they were sufficiently accurate to serve our purpose. When it was necessary to avoid the inducing effect of light, a green filter (Zeiss No. VG-9) was used.

The sample of A23187 was kindly donated by Dr. Robert L. Hamill of Lilly Research Laboratories, Indianapolis, Ind. Chlorpromazine hydrochloride and procaine hydrochloride, both from Sigma Chemical Co., St. Louis, Mo., were prepared as 0.1 M stock solutions in water and used the same day. Calcium chloride was reagent grade from J. T. Baker Chemical Co., Phillipsburg, N. J.

RESULTS

Characteristics of Intermittent Swimming

The movement of sea urchin sperm in free fluid is helical, but when their paths impinge on a surface such as the bottom of an observation vessel they become trapped and swim in circles with their plane of beat parallel and close to the surface (20, 22). This circling movement is a consequence of slight asymmetry in the flagellar bending waves, and a quantitative measure of the asymmetry can be obtained by measuring the turning rate of the sperm in rad/beat (3, 5). In most preparations, >90% of the sperm are found to be moving in counter-clockwise circles as seen by an observer looking down toward the sperm moving over a surface (20).

When suspended in seawater and observed in a Petri dish by dark-field light microscopy, sperm of the sea urchin *T. gratilla* repeatedly stop and start swimming. Individual sperm stop at irregular intervals, and then resume swimming after a quiescent period of about a second. However, as the sperm start up from quiescence, their swimming paths are often nearly straight for a short time before they again become circular (Fig. 1).

During an episode of quiescence, the sperm flagellum assumes a characteristic shape that resembles a cane, with a sharp bend of angle of ~3.4 rad in the proximal region of the flagellum near the head, a nearly straight mid-region, and a gentle bend of ~0.4 rad near the tip (Fig. 2*a*); this waveform shows considerable uniformity, both among sperm of a given preparation and from one preparation to another. Analysis of high-speed movie films of sperm undergoing the transition between quiescence and swimming has shown that the bends near the proximal end and the tip of the quiescent flagella are in the same direction relative to the normal circular swim path as the principal bends of beating flagella (13). In 17 quiescent sperm from four preparations, the angles of the principal proximal and distal bends ranged from 3.3 to 3.8 rad and 0.2 to 0.7 rad, respectively. Although the mid-region of the flagellum is usually almost straight, most preparations contain a few sperm in which this region is gently curved. Occasional sperm lacking a distal bend in their flagella are also observed. The waveform of quiescent sperm flagella, like that of beating flagella (22), appears essentially planar. For convenience in this paper, the use of the term "quiescent" shall be limited to immotile sperm whose flagella are bent into this characteristic cane-shaped waveform.

In some cases the flagella of quiescent sperm show occasional irregular flexing and quivering movements. These will be described in detail elsewhere.

In gently homogenized preparations containing some motile sperm with short flagella, it was found that sperm with flagella as short as 10 μm still exhibit intermittent swimming. When stopped, the flagellar waveform of such sperm consists of a proximal bend of ~2.4 rad extending out to ~7 μm from the head, with the rest of the flagellum almost straight (Fig. 2*b*).

Factors that Influence the Occurrence of Intermittent Swimming

Experiments with artificial seawater have shown that the presence of Ca^{2+} is required for the induction of quiescence. In artificial seawater containing <2 mM Ca^{2+}, no quiescent sperm are seen, whereas at concentrations of Ca^{2+} between 5 and 10 mM, the percentage of quiescent sperm appears to be about the same as that in standard seawater.

Apart from this requirement for Ca^{2+}, the factor that influences the occurrence of quiescence most strongly is blue light. The induction of quiescence by light is clearly observed when one moves a Petri dish on the microscope stage to a new field. In the case of strong induction, nearly all the sperm in the newly illuminated field stop swimming within 1–2 s of being moved into the light beam. The effect is seen most dramatically at the bottom surface of the dish, which is where the sperm are usually observed swimming. However, occasional sperm can be seen to stop up in the solution away from the bottom of the dish. The percentage of sperm that stop at the bottom surface is variable from one preparation to another and often seems also to depend on the particular Petri dish being used, although it is not related to the

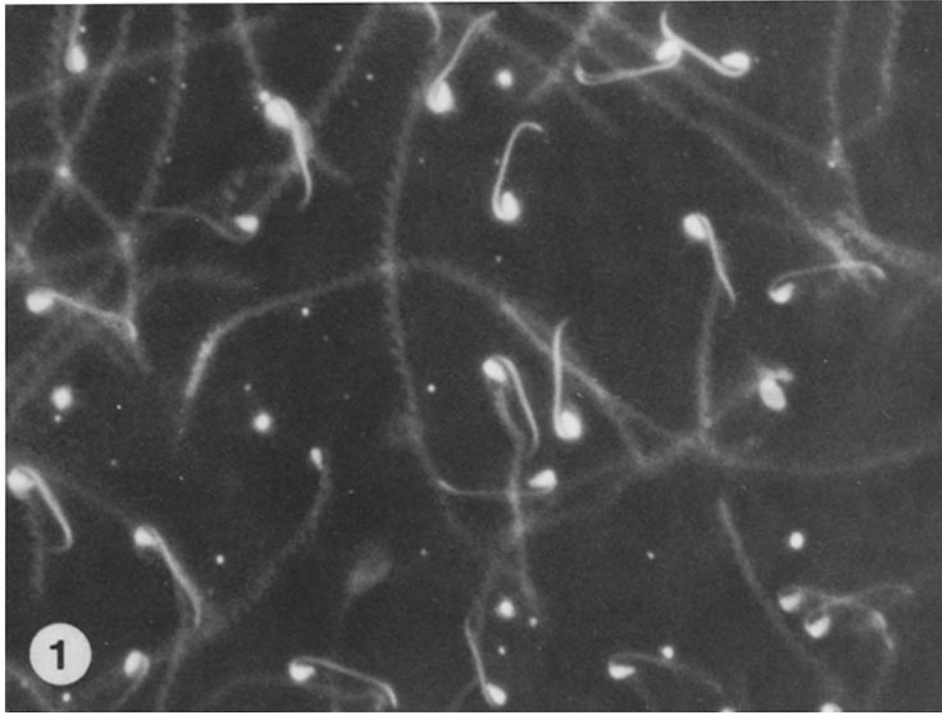

FIGURE 1 Dark-field micrographs of live sperm of *Tripneustes* suspended in natural seawater containing 0.2 mM EDTA and adjusted to pH 8.3 (referred to as standard seawater). The micrograph, which was taken a few seconds after moving this field into the light beam, shows some sperm in light-induced quiescence, and some that are swimming. Among those swimming, most show little asymmetry as indicated by the near straightness of their paths. Exposure: 1 s. × 380.

light absorption properties of the glass. By using a series of filters (Zeiss: UG5, BG3, BG12), the active wavelength in light-induced stopping has been determined to lie in the range 400–500 nm, and the effect can be almost eliminated by use of a green filter (Zeiss, VG9). The dose response curve was measured by estimating the percentage of sperm that stopped upon initial movement into the light beam at different intensities of illumination (Fig. 3). As mentioned, it was found that with the full intensity of our illuminator, close to 100% of the sperm immediately stopped in the quiescent waveform (Fig. 4). A striking aspect of the results shown in Fig. 3 is that the percentage of sperm stopped shows a gradual decrease to ~0.3% as the light intensity decreases over a factor of $\sim 10^4$. The low percentage of sperm that continue to become intermittently quiescent at a relative light intensity of 10^{-4}, or with the green filter inserted, may represent a basal, spontaneous level because under these conditions the sperm usually do not stop immediately upon movement into the light beam. However, we cannot be certain on this point because it is difficult to make an accurate estimate of the percentage of quiescent sperm when this is <1%. Except at the highest intensities, the sperm appear to adapt to the light, so that the percentage of quiescent sperm is highest when they are initially moved into the beam and decreases to a constant lower value after they have been in the beam ~15 s. Exposure of the sperm to the maximum light intensity for several minutes rendered them irreversibly nonmotile, presumably by radiation damage.

The brief period of relatively straight swimming that usually follows quiescence interferes with the measurement of the steady-state asymmetry of the flagellar bending waves of these sperm as indicated by their turning rate. However, with quiescence prevented by suspension of the sperm in artificial seawater containing only 2 mM Ca^{2+}, the average turning rate in a typical preparation was determined to be 0.18 rad/beat (range for 20 sperm was 0.12–0.26 rad/beat). It is believed that this is the

turning rate that would be observed in standard seawater if the sperm did not pass in and out of quiescence.

The presence of a low concentration of EDTA or EGTA in the seawater appears to enhance somewhat the percentage of quiescent sperm, although its presence is not essential. In our laboratory, we usually add 0.2 mM EDTA to seawater because this prolongs the longevity of the sperm (48), presumably by reducing the level of contaminating traces of free heavy metal ions.

Intermittent swimming and the stimulation of quiescence by light do not require the presence of Mg^{2+} in the external medium, and sperm in Mg^{2+}-free artificial seawater show about the same level of quiescence as in normal seawater containing 53 mM Mg^{2+}.

The incidence of quiescence is increased if the sperm are suspended in seawater containing a higher than normal concentration of potassium. Under these conditions, induction by light does not seem to be a factor and the proportion of quiescent sperm is about the same with green light as with normal illumination. A maximal effect appears to be obtained with seawater containing ~80 mM K^+. When sperm are examined immediately after transfer to this solution and mixing, 50–90% are quiescent with their flagella bent in the usual cane-shaped form. Those that remain motile show asymmetric flagellar waves of low amplitude (2) and swim slowly in circles of small radius, some with counterclockwise and some with clockwise motion. Many of the sperm alternate between quiescence and this feeble motility, but the duration of each episode of quiescence is 5 s or more, considerably longer than in standard seawater. This K^+-induced quiescence requires approximately the same Ca^{2+} concentration in the seawater as the quiescence induced by light in seawater containing normal levels of K^+ and Na^+. The effect of elevated potassium is less pronounced at 40 or 60 mM K^+, while at 100 mM K^+ the percentage of quiescence is usually lower than at 80 mM K^+, and the motile sperm are mostly swimming vigorously in circles of small radius. In seawater containing 475 mM K^+ and no Na^+, 80–

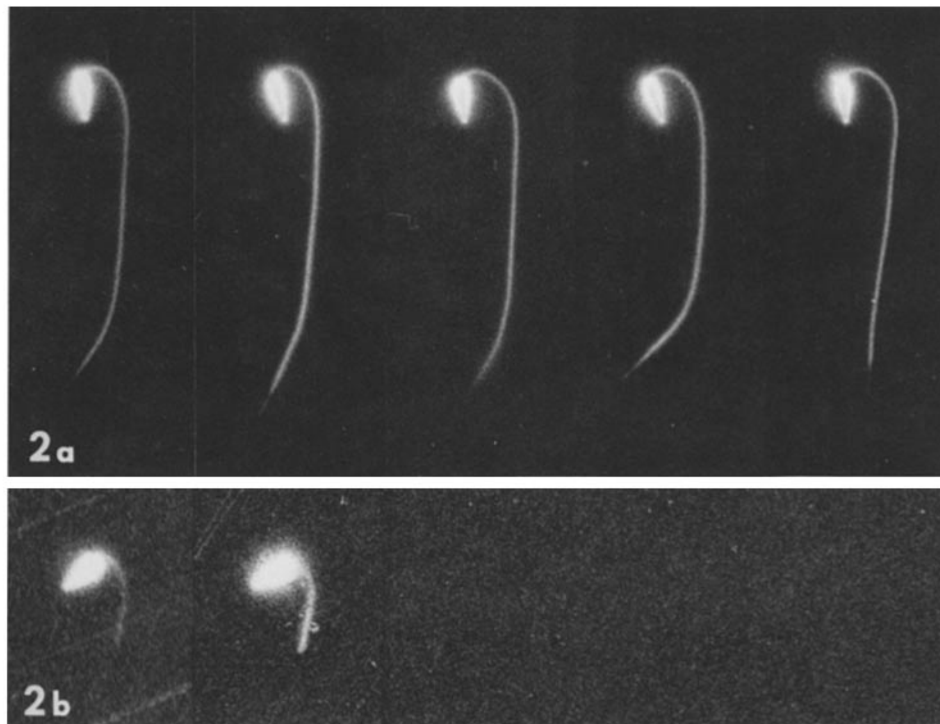

FIGURE 2 Dark-field micrographs showing the flagellar waveforms in typical live sperm during the quiescent phase of light-induced intermittent swimming. (*A*) Intact sperm. (*B*) Sperm with flagella of ~10 μm. Exposure: flash. × 1040.

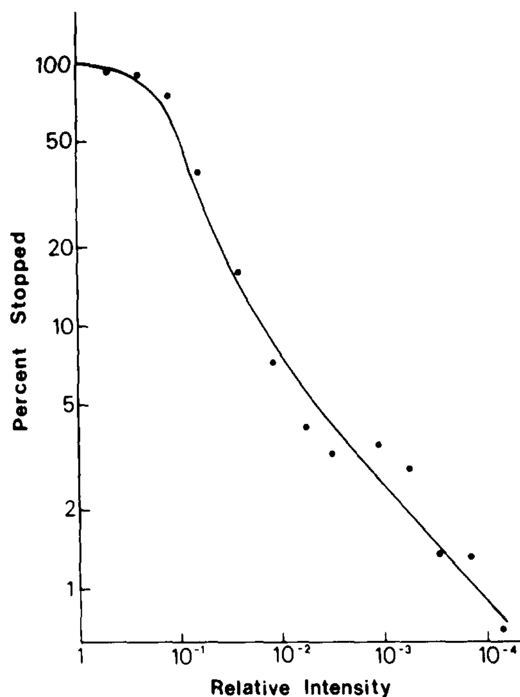

FIGURE 3 Variation of the percentage of quiescent sperm with the intensity of light. Estimates were made during the first 1–2 s after movement of the dish to a new field. A value of 1 was arbitrarily assigned to the intensity of the unfiltered beam of our illuminator.

100% of the sperm are nonmotile initially, while the rest barely twitch. The flagellar waveforms in the majority of the nonmotile sperm resemble the cane-shaped quiescent form, but they are less homogeneous in shape than usual. After a short time, a small percentage of the sperm begin swimming in counterclockwise and clockwise circles of small radius, but no episodes of quiescence are observed in the motile sperm under such conditions. By 5–10 min, all the sperm are nonmotile and do not resume motility if transferred back into standard seawater. On the other hand, in artificial seawater that contains 475 mM Na^+ and no K^+, the light-induced intermittent swimming observed is approximately the same as that in standard seawater.

An attempt has been made to determine whether the intermittent swimming shows a requirement for external Na^+, by replacing it in the seawater with choline (25). In artificial seawater containing 465 mM choline chloride instead of NaCl, the sperm swim in small circles with an average turning rate of ~0.43 rad/beat, indicating that the flagellar bending waves are much more asymmetric than in standard seawater. However, no quiescent sperm are seen even at high light intensities. In this seawater, more than half the sperm swim in clockwise circles.

In addition to discovering the above factors that either are required for, or enhance the intermittent swimming, it has been found that certain drugs eliminate the behavior, overriding even the most strongly inducing conditions. The most effective of these is chlorpromazine hydrochloride, which at 5–10 μM abolishes quiescence even in sperm that are suspended in artificial seawater containing 80 mM KCl, and 0.2 mM EDTA, or are intensely illuminated, and that before the addition of chlorpromazine showed a very high percentage of quiescence. This drug causes that proportion of the sperm that swim with clockwise motion in seawater containing elevated K^+ to convert to counterclockwise swimming. Procaine hydrochloride (35) and sodium barbital also greatly reduce the percentage of light-induced quiescent sperm in standard seawater at concentrations of 0.6 and 1 mM, respectively, although a small percentage of quiescence still persists. In other respects, the sperm appear to be unaffected by the addition of these drugs, except that in the presence of chlorpromazine they are highly sensitized to the destructive effect of light, and become irreversibly nonmotile within 1 min in the light beam used for normal viewing.

Observations with Other Species of Sea Urchin

Neither intermittent swimming nor flagella with the typical quiescent waveform have been seen in sperm from a second species of Hawaiian sea urchin *C. atratus*, even under strongly quiescence-inducing conditions. However, the intermittent swimming and the typical, cane-shaped quiescent form have been seen in sperm of two California species of sea urchin, *S. purpuratus* and *L. pictus*, with ~5% being quiescent at a given moment. In brief experiments with sperm of these latter species, however, no definite inducing effect of light could be demonstrated.

Studies with the Ionophore A23187 and Calcium

The above results, together with the known effects of Ca^{2+} on ciliary arrest and reversal, suggested that one should investigate the effects of introducing Ca^{2+} into live sperm with the divalent-

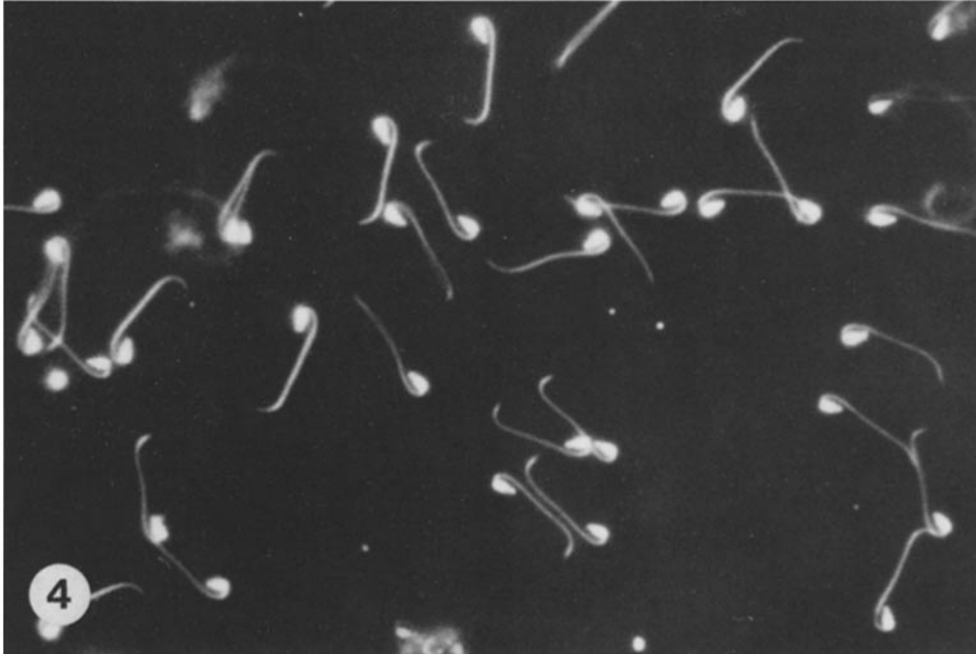

FIGURE 4 Dark-field micrograph showing light induction of quiescence in live sperm in standard seawater. The micrograph was taken immediately after moving this field of sperm into the illuminating beam and shows nearly 100% of the sperm in quiescence. Exposure: ½ s. × 380.

cation ionophore A23187. When freshly shed sperm of *Tripneustes* are diluted into artificial seawater lacking divalent cations, as in the procedure for treating them with A23187, they are initially mostly nonmotile with their flagella in a slightly crescented shape, and then become 95–98% motile within 1–2 min. Under these conditions, the sperm swim continuously in circles at the bottom surface of the dish with a turning rate of ~0.2 rad/beat, about the same as that in seawater containing only 2 mM Ca^{2+} (in which quiescence is prevented). The same pattern of movement is seen with sperm that have been treated with ionophore as described in Materials and Methods (Fig. 5 *A*), or sperm that have been treated with only the ionophore solvent. However, the longevity of sperm in divalent cation-free seawater is relatively short. Whether or not the sperm have been treated with ionophore, the flagellar beat frequency and the percentage of motile sperm gradually decrease, until after 5–15 min the sperm are nonmotile, and their flagella are straight.

A dramatic result is observed if 0.24–0.4 mM Ca^{2+} (corresponding to ~0.04–0.2 mM free Ca^{2+}) is added to the ionophore-treated sperm while they are motile. The sperm immediately become arrested with their flagella uniformly bent into exactly the same cane-shaped waveform as non-ionophore-treated sperm in light-induced quiescence (Fig. 5 *B*). Although the ionophore-treated preparations are very homogeneous initially, they are unstable with time, and after 5–15 min the sperm flagella gradually begin to straighten, sometimes with twitching movements, until they, like the flagella of ionophore-treated sperm that have not been given Ca^{2+}, are straight. With a given preparation of ionophore-treated sperm, the time required for appearance of the straight, nonmotile phase is about the same whether or not Ca^{2+} has been added.

Addition of higher concentrations of free Ca^{2+} (0.6–1.0 mM) to actively swimming, ionophore-treated sperm does not give a uniform quiescent waveform, but instead produces a heterogeneous population in which most of the sperm are stationary with varied flagellar waveforms, while the flagella of a few sperm beat irregularly for ~1 min in a slow, asymmetric fashion. At Ca^{2+} concentrations somewhat lower than those needed to produce quiescence, ionophore-treated sperm are mo-

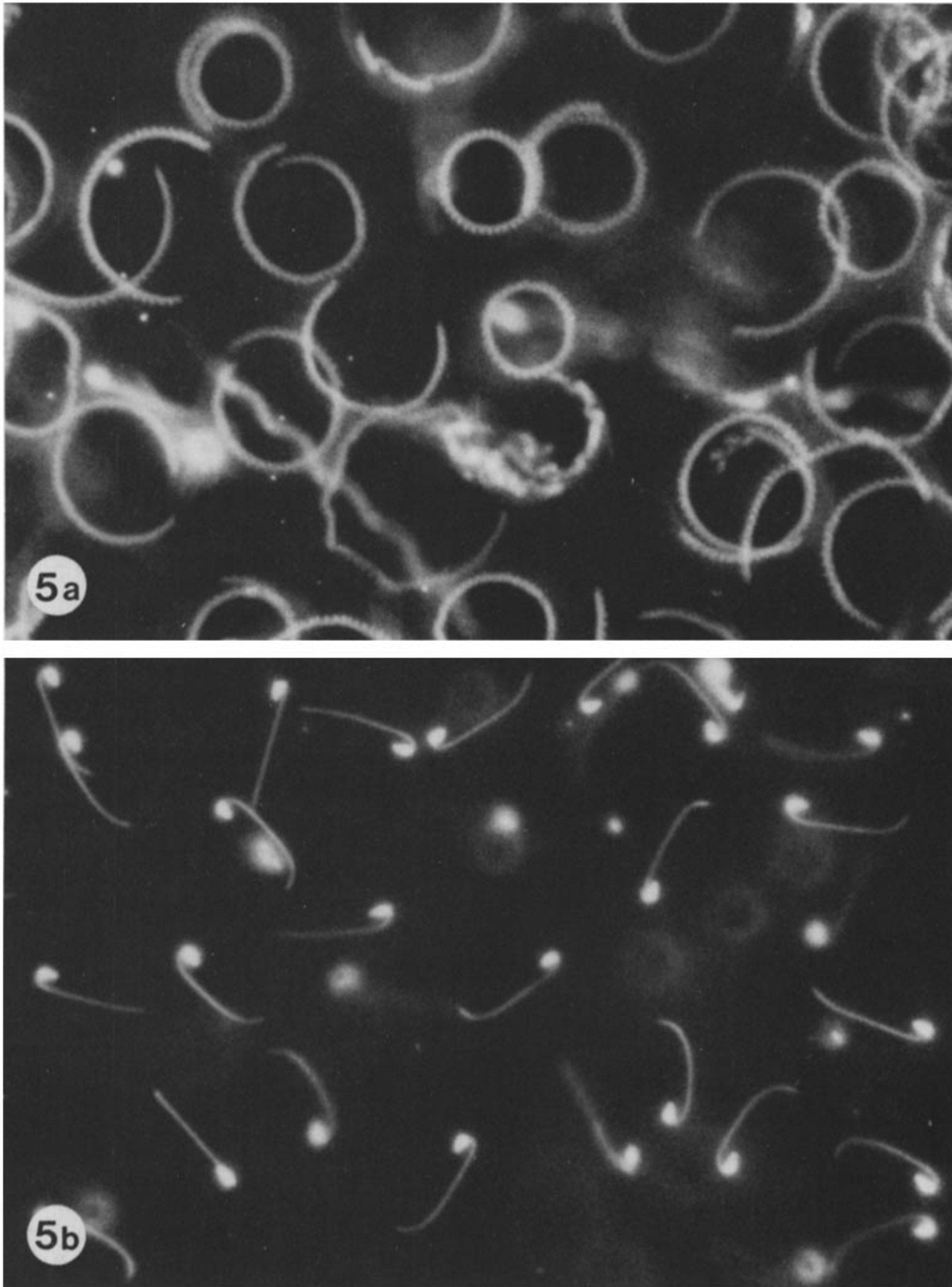

FIGURE 5 Dark-field micrographs of: (*A*) sperm that have been treated with ionophore A23187, then diluted tenfold into artificial seawater containing 0.2 mM EGTA and no divalent cations. The sperm are swimming in circles at the bottom surface of the Petri dish. (*B*) the same preparation of sperm after addition of ~0.1 mM free Ca^{2+}. Nearly all the sperm in the preparation are quiescent. Blurred circles are caused by stationary sperm out of the plane of focus. Exposure: 1 s. × 380.

tile with flagellar waveforms that are more asymmetric than normal, but I have not seen the extreme degree of asymmetry that can be obtained with reactivated sperm of this species (17).

Ionophore-treated sperm cannot be made quiescent by adding Mg^{2+} (0.3 or 5 mM $MgSO_4$) instead of Ca^{2+}. Under such conditions the sperm flagella continue to beat, but become abnormal somewhat more quickly than in the absence of Mg^{2+}. Moreover, subsequent addition of Mg^{2+} to sperm that have been made quiescent with 0.1 mM free Ca^{2+} causes them to resume motility. Addition of 5 mM $MgSO_4$ induces essentially 100% motility, with an initial beat frequency of 10–20 Hz, followed by complete loss of motility after 1–2 min.

Ionophore-treated sperm of *Colobocentrotus* behave quite differently from those of *Tripneustes* in their response to Ca^{2+}. Throughout the range between 0.04 and 1.0 mM free Ca^{2+}, most of the sperm beat with symmetrical waveforms of low amplitude and swim in almost straight paths. Some sperm are nonmotile, but no flagellar waveforms are seen that resemble those of quiescent *Tripneustes* sperm.

DISCUSSION

On the basis of the present results and by analogy with the well substantiated hypothesis explaining ciliary reversal in *Paramecium* (12, 32) and ciliary arrest in *Mytilus* and *Elliptio* (31, 47, 49), we propose that the swimming of sperm of *Tripneustes* can be arrested by an influx of Ca^{2+} into the flagellum, that this influx of Ca^{2+} can be triggered by various stimulae, including blue light, and that it is probably associated with a membrane depolarization. This excess intraflagellar Ca^{2+} is then presumably removed by outward active transport, involving a Ca^{2+}-pump in the flagellar membrane (10, 38), and when the intraflagellar Ca^{2+} concentration has reached a sufficiently low level, the sperm resume swimming in what appears to be a spontaneous manner. Under appropriate conditions, this process leads to an intermittent stop-start swimming of the sperm, in which the flagella alternate at irregular intervals between quiescence and apparently normal motility.

Several lines of evidence support the hypothesis that arrest is induced by a sudden increase in intraflagellar Ca^{2+}. The occurrence of intermittent swimming requires a minimum of ~5 mM Ca^{2+} in the seawater, even under strongly quiescence-inducing conditions. Uniform quiescence is obtained by addition of Ca^{2+} to ionophore-treated sperm. In addition, arrest is eliminated by chlorpromazine, a drug whose action in the pharmacologically active range of 0.1–10 μM is thought to involve competition with Ca^{2+} in its interaction with plasma and nerve membranes (1, 43). This interpretation is confirmed by our finding, reported in the following paper (16), that quiescence can be induced by the addition of Ca^{2+} to reactivated sperm.

In sperm treated with divalent cation-ionophore, ~0.1 mM added free Ca^{2+} is needed to induce quiescence. The concentration of Ca^{2+} that actually reaches the interior of the cell is unknown, for the transport of Ca^{2+} ions through A23187-induced pores may occur with low efficiency and would presumably be opposed by a Ca^{2+} pump in the flagellar membrane (10, 38). However, an upper limit can be placed on the inhibitory concentration by assuming that the intracellular Ca^{2+} concentration is equal to the free Ca^{2+} concentration in the external medium, and this indicates that the intracellular concentration required for arrest does not exceed 0.1 mM. This inhibitory level is lower than that used to induce arrest in ionophore-treated gill cilia (49), although in most studies on cells that have been completely demembranated, ciliary reversal or arrest is induced by a free Ca^{2+} concentration of 1–10 μm (31, 47, 49).

The preliminary finding that the induction and waveform of quiescent reactivated sperm are influenced by the pH (16) suggests the possibility that changes in intracellular pH may also play a role in quiescence in the live sperm. Such changes in pH could arise, for example, through changes in the proton conductance of the membrane during depolarization or as a consequence of a diminished rate of dynein cross-bridge activity and concomitant ATP hydrolysis during the quiescent phase.

The heterogeneous response, including some motility, that was obtained when relatively high levels of Ca^{2+} (1–2 mM) were added to ionophore-treated sperm, is as yet unexplained. A similar response in demembranated sperm of *Tripneustes* is described and discussed in the following paper (16).

The ionophore A23187 is not specific for Ca^{2+}, but promotes transmembrane passage of many types of divalent cation, and, in particular, is at least as effective for Mg^{2+} as for Ca^{2+} (46). For this reason, Mg^{2+} as well as Ca^{2+} was eliminated from the test solutions, or was supplied in a controlled way. The reversal of arrest that was obtained by adding $MgSO_4$ to ionophore-treated

sperm arrested with Ca^{2+} is difficult to interpret because of the general breakdown of the ionic compartmentalization of the cell under these conditions, and awaits further work. The possibility that Mg^{2+} competes with Ca^{2+} for sites in the binding process that leads to quiescence should be considered, although the lack of response of quiescent reactivated sperm to the addition of 10 mM $MgSO_4$ is in apparent contradiction to this (16).

The hypothesis that a membrane depolarization permits the influx of Ca^{2+} in live sperm of *Tripneustes* is based partly on the observation that quiescence is induced by light in live sperm but not in ionophore-treated or demembranated sperm, and partly on the analogy to the extensively studied ciliary response in *Paramecium.* The finding that procaine and barbital greatly reduce quiescence in *Tripneustes* sperm lends support to the hypothesis that a membrane depolarization is involved, since there is evidence that these drugs block membrane excitability and inhibit the influx of Ca^{2+} (9, 43), although their action is complex and not fully understood. Schmidt and Eckert (42) have shown that 10 mM procaine inhibits the photostimulation of flagellar reversal in *Chlamydomonas,* leading them to postulate that Ca^{2+} mediates this phenomenon also.

The cilia of *Paramecium* respond to a sudden increase of K^+ or Na^+ by reversing their direction of beating, thus causing the cells to swim backwards (7, 8). In addition, an increase in the rate of Ca^{2+} influx occurs in cells suspended in increasing concentrations of K^+ up to 10 or 20 mM, and this has been shown to be associated with an increase in the duration of ciliary reversal. At higher concentrations of K^+, however, the rate of Ca^{2+} influx and the duration of reversal decrease. The intermittent swimming of *Tripneustes* sperm responds to elevated K^+ in a way somewhat analogous to that of *Paramecium,* suggesting that a similar ionic gating mechanism may exist in *Tripneustes* sperm. On the other hand, the results with choline artificial seawater and with all Na^+- and K^+-artificial seawater suggest that in *Tripneustes* sperm the initial depolarization step which leads to increased membrane Ca^{2+} conductance may depend on the presence of Na^+, since quiescence was not seen in the absence of Na^+. In the case of the ionic changes that occur upon fertilization of the sea urchin egg (44), the initial event is an influx of Na^+ that depolarizes the membrane and causes opening of the Ca^{2+} gates. However, the increase in asymmetry of the flagellar waveform that was observed in sperm swimming in choline artificial seawater suggests that the ionic interactions are complex, and not fully interpretable at this time. The electrophysiology of *Paramecium* is also far from simple, as the work of Kung and co-workers (26, 40) has shown, and probably involves an array of ionic conductance channels.

The changeover from counterclockwise to clockwise swimming seen in a proportion of sperm swimming in seawater containing elevated K^+ or choline instead of Na^+ is not understood at present. However, the results suggest that the phenomenon, like quiescence, is influenced by the ionic permeability and electrical properties of the membrane.

It is questionable whether the intermittent swimming described in this report is of physiological significance in *Tripneustes* as, for example, in taxis. While the brief period of straight swimming that follows quiescence does remove the sperm from a localized area of intense light, the intermittent swimming appears to occur only infrequently under conditions approximating the natural environment and probably only reflects a susceptibility of the membrane to a low intensity stimulus leading to depolarization. The levels of light at which a significant degree of quiescence was observed are considerably greater than that of natural sunlight. However, the present results suggest that variations in light-induced quiescence may need to be considered as a possible complicating factor when sperm motility is observed by indirect methods that involve illumination of a sperm suspension and measurement of light absorbance or scattering (21, 34).

The light-induced tumbling of bacteria that has been described in *Salmonella* (27) is analogous in several ways to light-induced quiescence in *Tripneustes* in that a maximum effect occurs in the same wavelength range in both cases, a relatively high light intensity is needed, and the response occurs rapidly and is reversible if short pulses of light are used. It differs in that the response in bacteria is graded over a much narrower range of light intensity. These observations have been interpreted to indicate that the bacterial response may be caused by an alteration of electron transport (27).

Two other observations on sea urchin sperm may be relevant to the observations on intermittent swimming. Yanagamachi (50) has described the

occurrence of a modified pattern of flagellar beating called the boring movement in sperm from the sea urchins *Strongylocentrotus nudus* and *S. intermedius* during penetration of the egg jelly just before fertilization. Although this motion has not been described in detail, it appears to be a nonplanar bending of extreme angle and short wavelength, and it requires the presence of Ca^{2+}. More recently, Schackmann et al. (41) have reported that the acrosome reaction triggered by the egg jelly is followed by a large uptake of Ca^{2+} into the sperm, and that the concentration of Ca^{2+} may reach 10 mM in localized regions within the sperm cell. However, since the sperm remain motile during this influx, these observations may be more closely related to the ability of Ca^{2+} to induce different modes of beating, as in *Chlamydomonas* (24), than to quiescence or arrest.

The lack of intermittent swimming behavior in the live sperm and the absence of Ca^{2+}-induced quiescence in ionophore-treated sperm of *Colobocentrotus* imply that whatever Ca^{2+}-sensitive mechanism is present in *Tripneustes* is modified or missing in *Colobocentrotus.* It has been noted that the symmetry of the beating waveforms of demembranated *Colobocentrotus* sperm is significantly less sensitive to Ca^{2+} than is that of demembranated sperm of *Tripneustes* (14, 17). These species differences and the possible relationship between intermittent swimming and Ca^{2+}-induced asymmetric beating are discussed in greater detail in the following paper (16).

Study of modifications in the bending motion of axonemal structures provides an approach to understanding the largely unknown mechanisms that regulate microtubule sliding and convert it to bending. The present work strengthens the already persuasive body of evidence that Ca^{2+} is involved in this regulation, and demonstrates an additional unity in the behavior of flagella and cilia. It may eventually provide a means for determining the localization and nature of the Ca^{2+}-receptors in flagella. Walter and Satir (49) have concluded that these sites must be on the axoneme itself or on the basal apparatus since ciliary arrest can be mimicked in demembranated cells. We have also attempted to approach this problem by investigating Ca^{2+}-induced quiescence in reactivated *Tripneustes* sperm. Although so far it is not possible to conclude with certainty whether or not all the Ca^{2+}-sensitive sites involved in intermittent swimming are localized on the axoneme, further evidence concerning this point is described and discussed in detail in the following paper (16).

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