

THE MOVEMENT OF SEA-URCHIN SPERMATOZOA

By J. GRAY

Department of Zoology, University of Cambridge

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(With Plates 3 and 4)

Although the mechanical activity of spermatozoa plays an essential role in the process of fertilization there is little precise information concerning the type and speed of movement of individual cells in their normal state of activity. This paper represents an attempt to fill this gap with respect to a sea-urchin whose spermatozoa, being of the typical monoflagellate type, provide valuable material for the study of flagellar propulsion in general.

Accurate observation of the movements of fully active spermatozoa encounters three main difficulties: first, the diameter of the tail—on whose activity the propulsion of the head depends—is near the limit of resolution by transmitted light; secondly, the normal frequency of the tail's movements is far above that which can be followed by the human eye; thirdly, direct observation is almost entirely restricted to cells constrained to move in the plane of the microscope's field by the presence of a glass/water or water/air interface. The motion of cells moving freely in a bulk of fluid is extremely difficult to observe. Proximity to a surface not only affects the freedom of the cell to roll or pitch about its own axes, but also exposes the cells to physical conditions different from those in open water.

The difficulty arising from the very small radius of the tail has long been recognized. Fifty years ago Buller (1903) noted that, under normal transmitted light, only the head of an echinoid spermatozoon was visible; he recorded a translatory speed of $120\ \mu/\text{sec.}$ for the spermatozoa of *Echinus microtuberculatus* by observations on the rate of displacement of the head. More recently the movements of the heads of the spermatozoa of *E. esculentus* and of *Psammechinus miliaris* have been recorded photographically by Rothschild & Swann (1949) using darkground illumination; these records indicate translatory speeds of the order of $190\ \mu$ per sec. at 18°C. Apart from the observations of these authors there appear to be few recent observations relevant to the present inquiry.

Some, but not all, of the difficulties of observing the movements of a highly active spermatozoon can be overcome by the use of stroboscopic darkground illumination or by appropriate photographic technique. The results recorded in this paper were obtained by means of a low-power parabolic darkground condenser using a $\times 20$ objective and $\times 10$ ocular. Unless otherwise stated, the observations apply to fresh suspensions of the sperm of *P. miliaris* (P. L. S. Müller; Gmelin) in sea water at about 18°C. when observed on glass slides without cover-slips. The best results

were obtained when the slide was previously rinsed in sea water containing a small amount of a wetting agent such as saponin; although, in this way, it is possible to obtain a very thin uniform film yielding good optical contrast, it intensifies such complications as may ensue from closer proximity of the moving sperm to the surface of the slide or water/air interface. As observed in this way, the head of a motionless sperm appears as a triangular* or conical structure about 3μ long and 1μ in diameter of base; the outline of the head is brilliantly illuminated. The ovoid or spherical middle piece is also brilliantly illuminated, whilst the tail—less bright than the head and middle-piece—is seen as a straight or sickle-shaped filament $40-45\mu$ in length (Pl. 1, figs. 1-3); only on very few occasions has a stationary tail exhibited a wave-like form; the characteristic shape of a motionless tail suggests that it possesses some degree of natural elasticity (see p. 794 seq.). The diameter of the tail cannot be much more than 0.3μ in diameter (Rothschild, 1951); it may end in an extremely fine terminal filament about 5μ in length.

OBSERVATIONS

(a) *Movements of fresh active sperm on the surface of a slide*

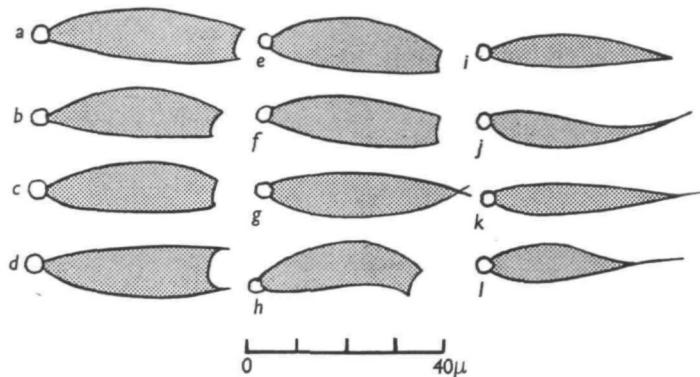
Under darkground illumination an active spermatozoon provides a striking contrast to a sperm at rest. As observed by eye, the moving cell is illustrated diagrammatically by Text-fig. 1. The image of the head is considerably broader than that of a stationary sperm and gives the impression of two images one on each side of the main axis of progression. Behind the head is a less brilliantly illuminated area, variable in shape but, in general, approximating in outline to that of a bullet or of an ellipse. This area is $30-35\mu$ in length; its breadth varies according to the state of activity of the sperm (see Text-fig. 1 and Pl. 1, figs. 7-14), but in fully active preparations it is of the order of $6-10\mu$; it represents the 'optical envelope' of the tail's movements.†

The relationship between the form of the envelope and the activity of the sperm tail is clearly revealed by examination with intermittent light of appropriate intensity and frequency. At $15-18^\circ\text{C}$. the motion of the tail is 'frozen' at a frequency of illumination of $30-40$ per sec.; using flashes of approximately $\frac{1}{500}$ sec. duration (Pl. 1, figs. 4-6), the tail is seen as a brightly illuminated undulatory line, rather more than one wave-length long, and the whole sperm progresses over the field of the microscope without apparent change of form and with only one image of the

* A few observations suggest that the head may be somewhat flattened.

† When viewed by a carbon arc fed by alternating current (or inadequately smoothed direct current) the envelope displays an alternation of light and dark bands similar to those described by Uhlela (1911) for the envelopes of the flagella of some Protozoa. When viewed by an arc fed by direct current of steady voltage the envelope is uniformly illuminated; the banding is therefore an artefact due to pulsations in the intensity of the light source and not, as suggested by Uhlela, to the intrinsic properties of the vibrating flagella. These visual observations have been confirmed photographically. Pl. 1, figs. 10-13, show the bright lines characteristic of illumination from an inadequately smoothed direct current arc; Pl. 1, figs. 7-9, show photographs taken by means of an arc fed by batteries; the broad bands in the latter case are due to the fact that the duration of the exposure was not an exact multiple of the period required for the passage of one complete wave over the tail.

head visible. If the frequency of illumination is then slightly decreased, waves of curvature pass antero-posteriorly along the tail sufficiently slowly to be followed by the eye. A return to continuous illumination at once yields a return of the characteristic envelopes. The envelope seen under continuous illumination is the result of persistence of vision by the eye of the observer.*



Text-fig. 1. Optical appearance of envelopes of spermatozoa moving over the surface of a glass slide; the head is indicated diagrammatically by a circle. Figs. a-h from fresh suspensions; Figs. i-l from older suspensions.

(b) *The form of the waves generated by the tail*

The visual impression of a wave of changing curvature passing along the tail is an expression of two facts:

- (1) That each element of the tail is undergoing a cycle of change in its radius of external curvature each time it sweeps transversely across (or round) the axis of propulsion of the waves (Gray, 1953).
- (2) That each element exhibits a phase of bending slightly ahead of that reached by its more posteriorly situated neighbour.

If all the elements constituting the tail bend and execute the whole cycle of their transverse motions in one and the same plane, the envelope of the tail constitutes a plane lamina; whereas, if each element undergoes bending in two planes simultaneously, and these two cycles of deformation are equal in amplitude and always

* Persistence of vision occurs whenever the image of an object moves across the retina; it does not occur if the axis of the eye follows the moving object and thereby yields an image stationary with respect to the retina. The visual impression of the tail as an illuminated envelope and the double image of the head are due to the fact that the axis of the eye cannot follow either a point on the tail or the head along an axis normal to the path of the sperm's progression, nor can it follow successive wave crests as they move backwards along this path. On the other hand, the axis of the eye readily follows the head along the axis of progression (since this motion is always in the same direction and is relatively slow), and therefore no persistence of vision of the head occurs along this axis. An interesting demonstration of this principle occurs when the stage of the microscope is abruptly moved; the head then yields a brightly illuminated undulatory track superimposed on the less brilliantly illuminated envelope of the tail, because the image of the head is travelling across the stationary retina; the resultant visual impression is of the same fundamental nature as the tracks recorded on a stationary photographic plate by Rothschild & Swann (1949). (See also Plate II figs. 2-6 of this paper.)

one-quarter of a complete cycle out of phase with each other, the envelope of the tail's motion constitutes a cylinder or cone. Under visual observation, the outline of the envelope might be the same in both cases, but their dynamic properties would differ (Gray, 1953). It is therefore important to know at the outset whether each individual element of the tail executes its movements in a plane, in an ellipse or in a circle; in other words, whether the waves passing along the tail are two- or three-dimensional.

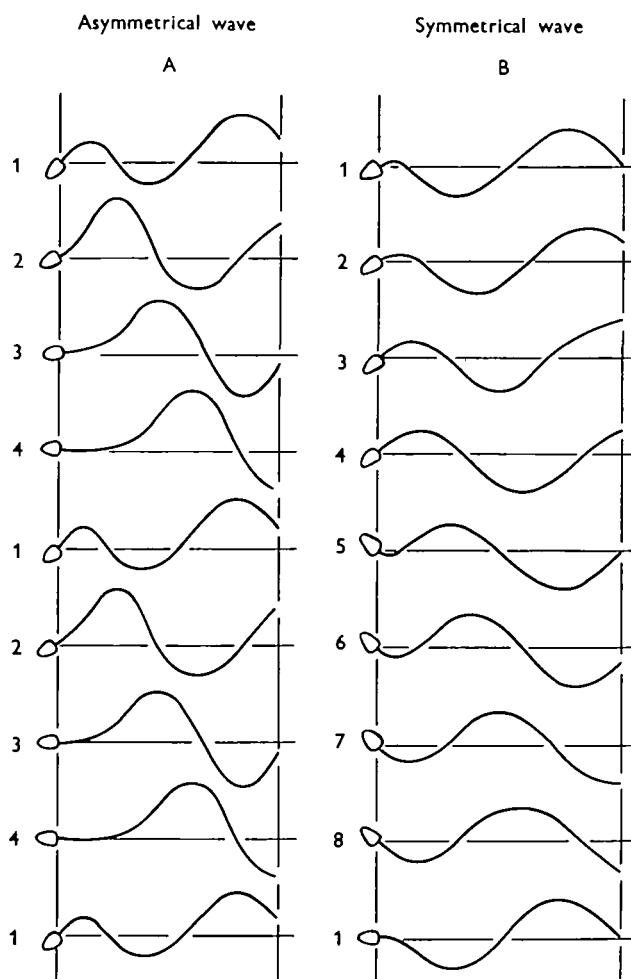
If the plane of observation of a very fresh active suspension of spermatozoa is slightly raised above the level of the slide, individual cells can be observed swimming more or less straight across the field of the microscope. Such cells exhibit one characteristic feature. The visual image of the envelope of the tail alternates rhythmically between the elliptical outline already described and a relatively straight illuminated line (see Pl. 2, fig. 1); the frequency of this change is always very much less than that at which the waves are passing down the tail. Of fourteen cells recorded photographically, the average frequency of this change in form of the envelope was 3 per sec.; the maximum was 6 and the minimum 1·5. The frequency of beat of the tail could not have been less than 24 per sec. It is very difficult to explain these facts on any basis other than that the oscillations of the tail are effectively restricted to a single morphological plane, and that cells, not in contact with an external surface, roll about their longitudinal axes. It does not follow that the contractile fibres responsible for bending are all located in the plane in which the bending occurs.

(c) *Formation and propagation of individual waves*

The development of individual waves at the proximal end of the tail and their subsequent propagation towards the end of the tail can be followed visually by illuminating individual cells at a frequency slightly less than that at which the waves are being generated. Observations of this type indicate that the degree of bending exhibited by an element situated near to the front end of the tail is seldom symmetrical on its two sides. As shown diagrammatically in Text-fig. 2A each wave develops as a transverse displacement of the proximal elements of the tail towards one side of the axis of propulsion. In very few of the cases examined was a transverse displacement of these elements towards one side of the axis followed by one of equal amplitude towards the other (Text-fig. 2B); in other words, the degree of bending exhibited by the two sides of the proximal end of the tail is usually different. These observations are confirmed by the photographic data obtained from spermatozoa where the frequency of the waves was sufficiently low to permit of cinematographic records and where the asymmetry of bending is very marked (see Text-fig. 3).

For quantitative analysis the characteristics of the waves exhibited by a normally active spermatozoon are best determined photographically. Pl. 1, figs. 4–6, are typical of a series of photographs of $\frac{1}{500}$ sec. exposure; these photographs yield images of the tail identical with those recorded by the eye when an active suspension

is observed under intermittent light of appropriate frequency with flashes of $\frac{1}{600}$ sec. duration.* As a wave passes away from its point of origin behind the head, its amplitude and wave-length increase whilst its asymmetry usually decreases until

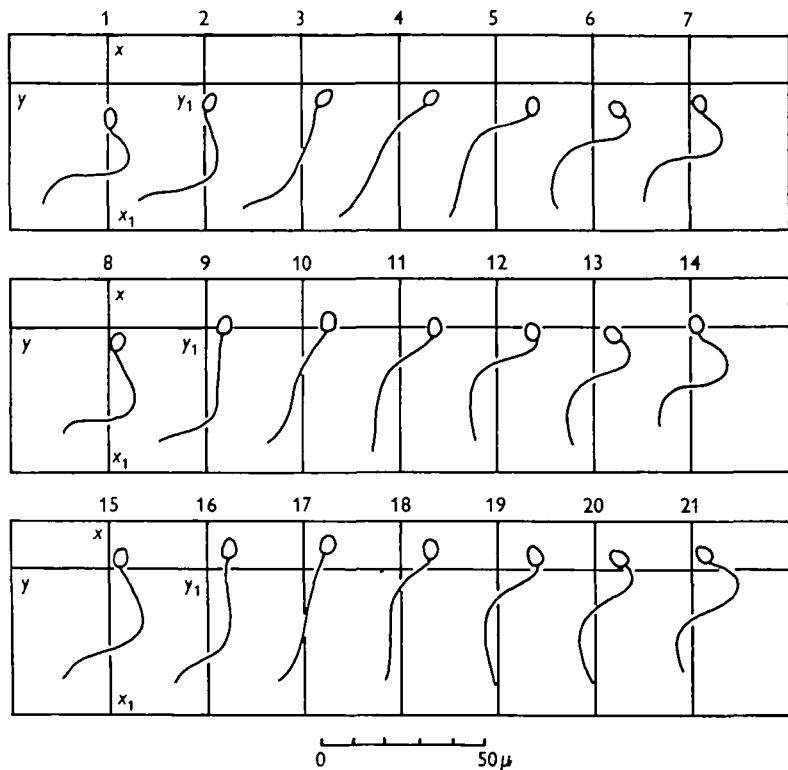


Text-fig. 2. A. Diagram showing asymmetry in the bending on the two sides of the proximal region of the tail. Four phases of bending are shown for each of two waves. Note that the anterior region of the tail only becomes concave on its left side. B. Diagram showing symmetrical bending on the two sides. Eight phases of a single wave are shown.

the wave crest has reached a point about half-way along the tail. At this phase the form of the waves varies in different samples of spermatozoa, but is relatively constant during the early life of any given suspension. Table I refers to a

* The intensity of light required to yield an adequate photographic image of the tail causes extensive scatter from the surface of the head; the latter appears in these photographs as a relatively large circle of light. This difficulty does not arise in visual observations with lower intensity of illumination.

suspension in which the average wave-length (λ) was 24μ and the average amplitude (b) was 4μ , giving an average value of $b/\lambda = 0.17$. In some instances the form of the wave conforms very closely to that of a sine curve (see Text-fig. 4); in others, the asymmetry characteristic of the proximal region is observable over the whole length of the tail.



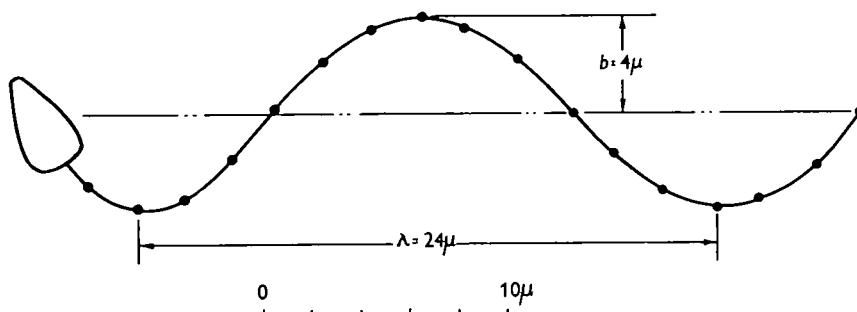
Text-fig. 3. Tracings from a cinematograph record (twelve frames a second) of a spermatozoon propagating three waves in 1.75 sec. Note the asymmetry of bending: the transverse grid-line yy_1 and the vertical grid-line xx_1 is the same in all the figures; the head advances when the tail straightens out (e.g. phases 1-4) and moves backward during the bending phases (phases 4-7).

From a dynamical standpoint it is useful to have a quantitative estimate of the amount of deformation undergone by each element of the tail during a complete cycle of its movements. An element is bent to its maximum extent when it is situated at the crest of a wave; but, as the amount of bending changes relatively rapidly along the curve, only very rough values are available by direct measurements of curvature; useful estimates can be derived from waves of regular form. The radius of curvature at the crest of a sine wave approximates to $\lambda^2/4\pi^2 b$, and therefore the radius of maximum curvature of each side of each element of the tail in Text-fig. 4 changes from +4 to -4 μ during each complete contractile cycle. As the radius of the tail is less than 0.2 μ the difference in length between the two sides of a fully bent element is not more than 10% of the length of a central neutral

axis. A laterally situated contractile fibre 1μ in length (when neither contracted nor stretched) would shorten to 0.95μ when fully shortened and extend to 1.05μ when fully stretched. The process of shortening occupies one-quarter of the whole cycle, and consequently for a wave frequency of 33 per sec. the rate of shortening or elongation would be of the order of $6-7\mu$ per sec. Both the total degree of shortening of each tensile element and the rate at which this occurs are thus quite small.

Table I

Sperm no.	Length of envelope in μ	Wave-length (λ) in μ	Amplitude (b) in μ	Total length of tail in μ	Ratio b/λ
1	35	23	4	45	0.174
2	33	24	4	38	0.167
3	34	27	3.5	42	0.130
4	35	27	4	42	0.148
5	35	23	4	40	0.174
6	34	21	4	42	0.190
7	32	24	3.5	42	0.146
8	34	24	4	42	0.167
9	32	22	3.8	40	0.170
10	33	24	3.8	40	0.156
11	32	23	4	38	0.174
12	35	29	5	40	0.172
13	29	24	5	40	0.209
14	30	21	3.2	40	0.160
Average	33	24	4	41	0.167



Text-fig. 4. The outline of the tail was derived from a photograph $\frac{1}{500}$ sec.) of an active spermatozoon; the points are derived from a sine curve of amplitude 4μ and wave-length 24μ .

The duration of full activity of spermatozoa in a dilute suspension is limited to a few minutes. Towards the end of this period a marked change frequently occurs in the form of the tail's envelope (see Text-fig. 1, i-1 and Pl. 1, figs. 14-17); its width decreases progressively from the anterior to the posterior end until only a short region behind the middle piece and head remains active; finally, the whole tail becomes motionless. This progressive reduction in the amplitude of the waves as they pass along the tail is closely comparable to the damping effect of external viscosity on waves passively generated in an elastic rod (see p. 794 seq.), and strongly

suggests that the normal movements of distal elements of the tail can only be sustained by energy generated by the elements themselves and are not the mechanical result of movement executed by more proximally situated elements (see p. 794 seq.).

(d) *Frequency and rate of propagation of waves*

The average frequency of the waves as determined stroboscopically for the cells concerned in Table 1 was 33 per sec., and since the wave-length was 24μ the average speed of conduction of the wave (as measured along their axis of propagation) was 800μ per sec. The average speed of forward movement of the whole sperm was, for these particular cells, 180μ per sec., giving a ratio of propulsive speed to wave speed of 0.23. An alternative method of estimating the frequency of the waves is available by counting the number of undulations exhibited by photographs of the track traced out by the head in a known time, since each undulation represents the passage of one wave along the tail (see p. 783). A series of such observations, the average frequency for which was 36.9 per sec., is shewn in Table 2; the photographs published by Rothschild & Swann (1949) indicate frequencies of 30–40 per sec. Over a considerable range of frequencies the head travels forward between 5 and 6μ during each wave, so that a propulsive speed of 200μ per sec. indicates wave frequencies between 33 and 40 per sec.; this figure agrees with the stroboscopic determinations.

Table 2

Frequency of waves per sec.	20-24	25-29	30-34	35-39	40-44	45-49	50-54	36.9
No. of cells	0	2	7	11	6	3	0	Arith. mean

For waves 24μ in length, frequencies varying from 33 to 40 per sec. would be equivalent to velocities of propagation of the waves relative to the head of $800-1000\mu$ per sec., according to the frequency of propagation. The ratio of the speed of wave propagation to the speed at which the spermatozoon propels itself through the water is of fundamental importance in an analysis of the dynamical principles involved during the propulsion of the cell; this aspect of the problem will be considered in a separate paper (Gray & Hancock, 1955).

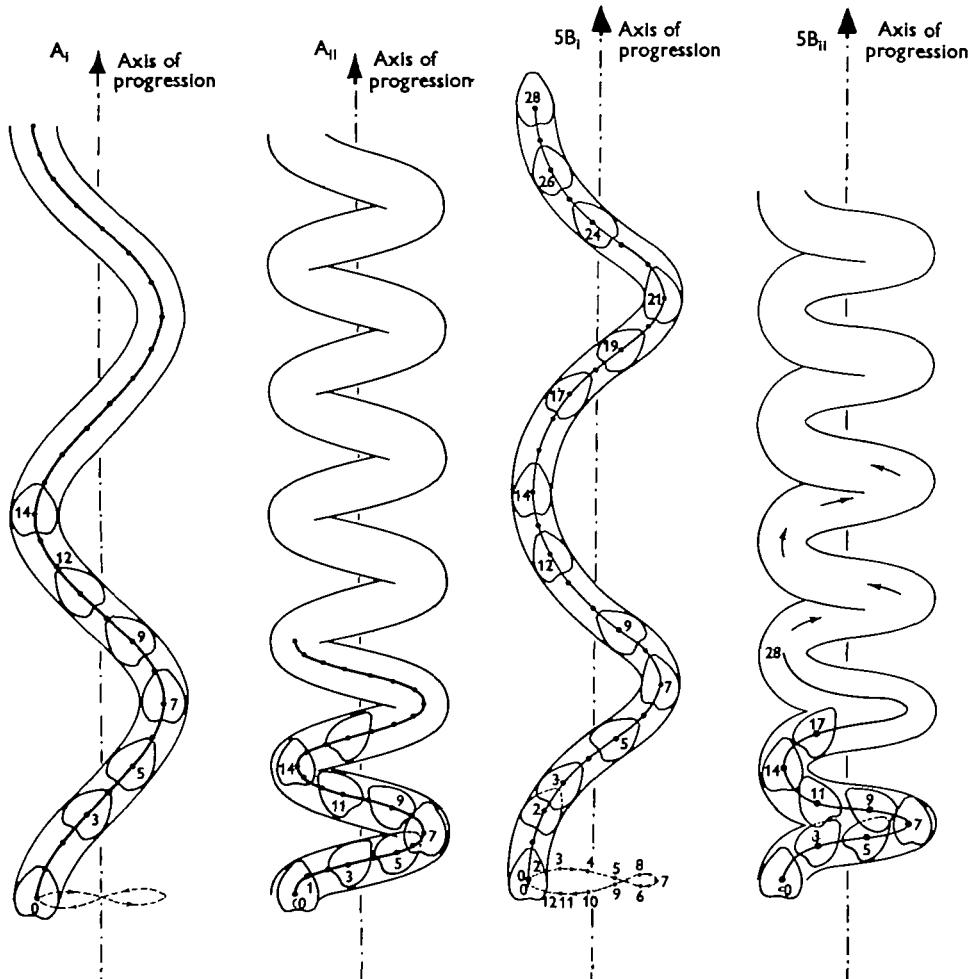
At present, little information is available concerning the frequency with which waves are generated by spermatozoa approaching the end of their active life. Two types of change in wave frequency have been observed:

(i) Cells which stop abruptly after a phase of full activity; in some cases the cessation of movement appears to be permanent, in others active movement is suddenly resumed.

(ii) Cells in which the frequency of the tail's movement gradually decreases without any very drastic change in the form of the waves. The characteristic features of one of these slowly moving cells are illustrated by Fig. 3, the amplitude of the waves being somewhat larger than that of fully active cells.

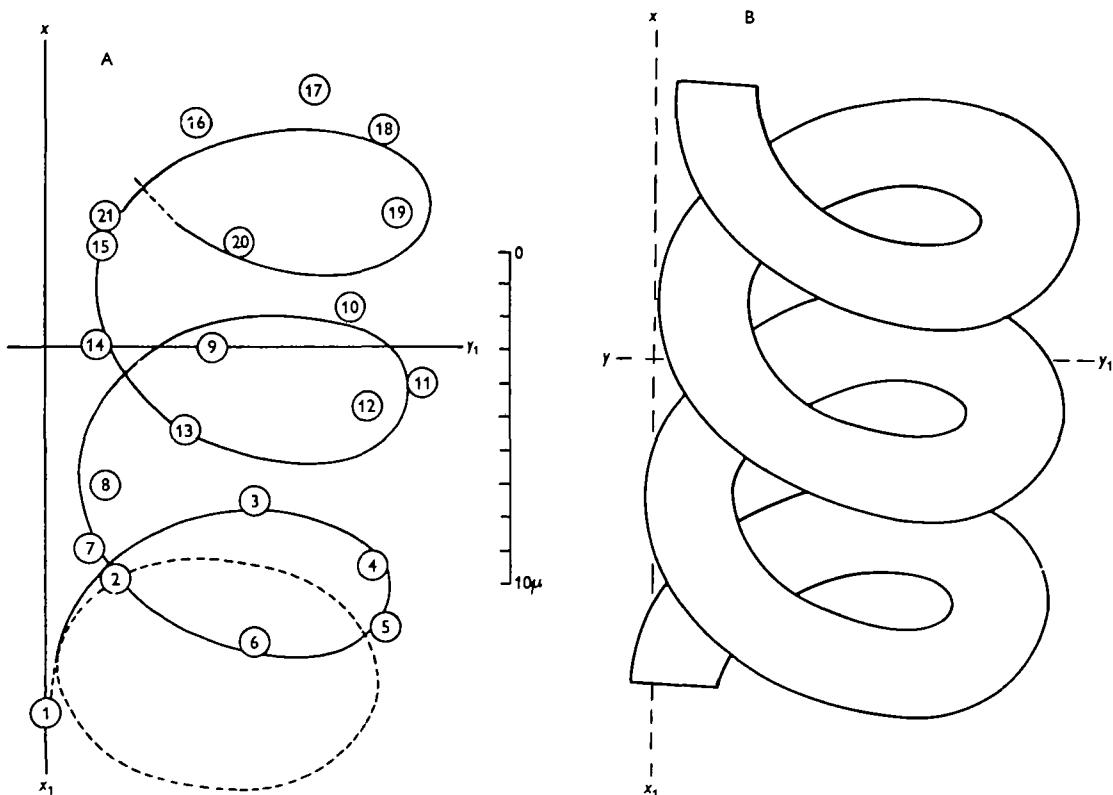
(e) The path of movement of the head

As noted by Rothschild & Swann (1949), and seen in Pl. 2 of the present paper, the track of the head of a spermatozoon is by no means always symmetrical about the main axis of progression; the rate of forward movement of the head is usually greater when the head is displaced to one side of the axis of progression than to the



Text-fig. 5. In Text-fig. 5 A, the bending wave is symmetrical on the two sides of the tail; if the spermatozoon were imagined to slide forward within a closely fitting rigid tube the head would move along a symmetrically undulating track (shown by the dark line running through the axis of the head) at a speed equal to that at which the waves were propagated backward over the tail. When a spermatozoon is moving in a fluid medium the forward speed is very much less than the speed of propagation of the waves, and the head follows the dark line shown in Text-fig. 5 A_{II}—this line being symmetrical about the axis of propulsion. Text-fig. 5 B is similar to Text-fig. 5 A except for an asymmetry of curvature on the two sides of the body—note the asymmetry of the head track in Text-fig. 5 B_{II}. In Text-figs. 5 A_I and 5 B_I, the dotted line at the point o shows the approximate movement of the head if the forward speed of propulsion were zero.

other.* If the waves developed at the front end of the tail were symmetrical about their axis of propagation, the head of the moving sperm would exhibit a symmetrical figure of eight displacement relative to axes passing through the centre of gravity of the sperm, and a symmetrical sinuous track relative to fixed axes (Text-fig. 5 A). If, on the other hand, the waves are asymmetrical, the track of the head must also be



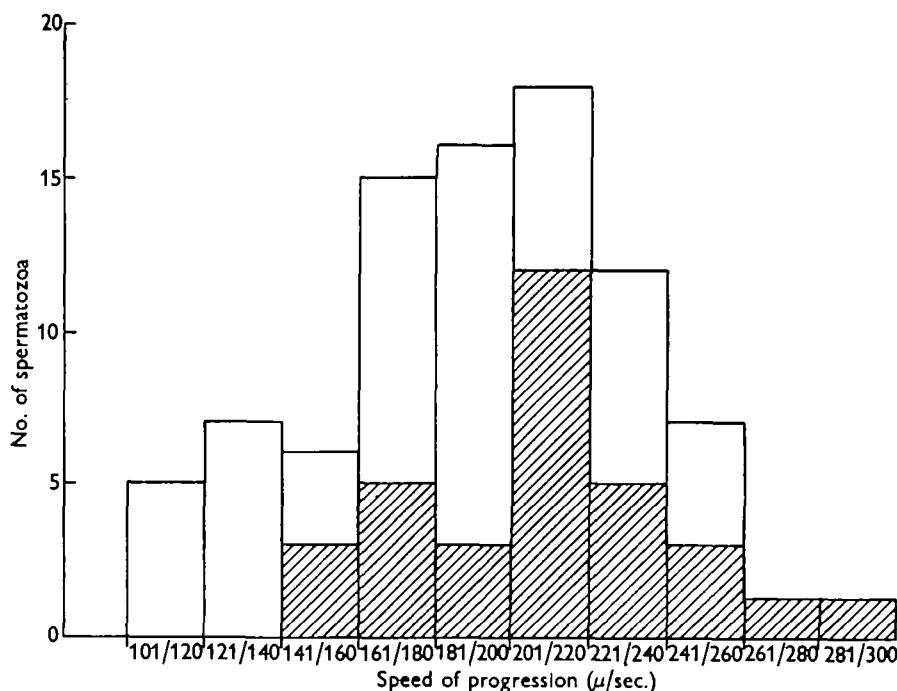
Text-fig. 6. A. Track of the central point of the head of the spermatozoon shown in Text-fig. 3; the grid lines xx_1 and yy_1 are the same in both figures. Successive positions of the head in Text-fig. 6 are shown by numbered circles and correspond to the numbers in Text-fig. 3. The thick continuous line shows the track which would have been followed had the head moved along the surface of the dotted ellipse and also moved forward at a constant speed of about 6μ during the passage of each wave (i.e. in about $\frac{1}{2}$ sec.). B. Diagrammatic illustration of the illuminated head track of Text-fig. 6A. Compare with Pl. 2, figs. 2-6.

asymmetrical (see Text-fig. 5 B). Text-fig. 6 shows the relationship between the track of the head and the passage of an individual wave derived from the cinematograph record of the slowly moving sperm shown in Text-fig. 3. As a wave forms at the proximal end of the tail, the head may even move backwards relative to the ground; as the wave subsides the head moves forward at its maximum speed.

* The asymmetry of the head tracks was interpreted by Rothschild (1951) as an oblique projection of a regular helix resulting from three-dimensional waves passing along the tail. The axis of microscopic vision is, however, so nearly normal to the plane of observation as to render the microscopic projection of a regular helix indistinguishable from a two-dimensional sine wave.

(f) Speed of translatory movement

The average speed of translation of the sperm of *P. miliaris* as calculated by Rothschild & Swann (1949) from the length of the head track during $\frac{1}{4}$ or $\frac{1}{2}$ sec. was 190μ per sec. at about $18^\circ C$. Data obtained in this way from thirty-three cells during the present investigation yielded an average speed of 208.5μ per sec. for an average wave frequency of 36.9. In another suspension the average translatory speed for fifty-six cells (as determined cinematographically) was 181μ per sec., for



Text-fig. 7. Histogram of variation in speed of progression. The hatched areas indicate observations derived from the length of head tracks traversed in $\frac{1}{4}$ or $\frac{1}{2}$ sec.; the clear areas are from cinematograph records.

an average wave frequency (as determined stroboscopically) of 33 per sec. The average speed of the eighty-nine cells was 191.4μ for an average wave frequency of 34.5 per sec., or 5.5μ per wave.

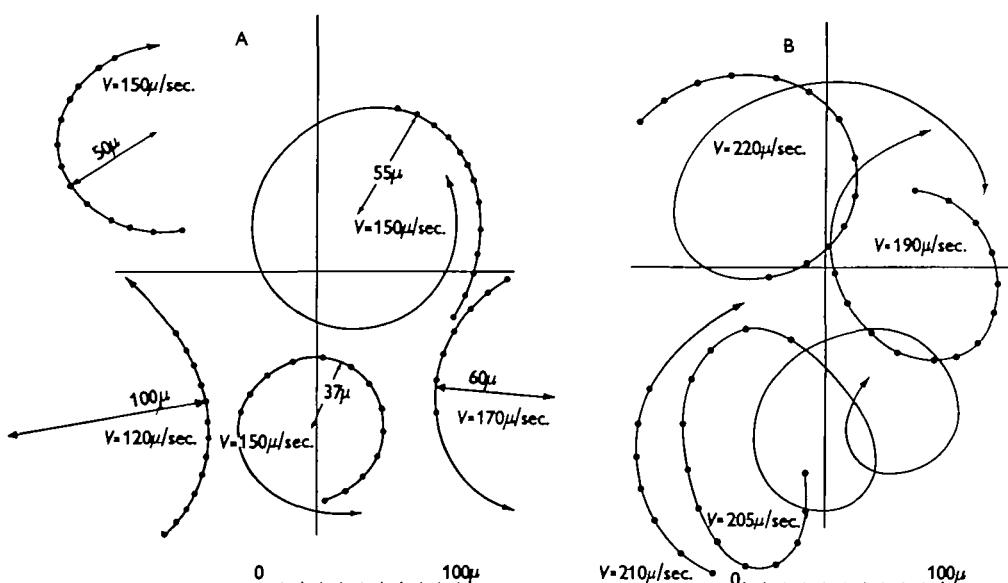
The scatter of translatory speeds observed in the eighty-nine cells referred to above (see Text-fig. 7) is presumably due to two main factors—variation in wave form and variation in wave frequency. As the width of the tail's envelope decreases with increasing age of the suspension the translatory speed falls, the average translatory speed of cells with envelopes of the form shown in Text-fig. 1, *i-l*, and Pl. 1, figs. 15-17, was of the order of 50μ per sec. Unfortunately, no data are available concerning the frequency of the waves generated by these cells, but the drop

in speed appears to be greater than that which can be accounted for by change of wave form alone.

All the above records apply to spermatozoa moving over the surface of a glass slide. Reliable records of cells moving freely in a bulk of liquid are very difficult to obtain, but among fourteen cells free to roll about their longitudinal axes (see p. 778) the maximum propulsive speed was 204μ per sec., and the minimum 130μ per sec.; these speeds do not differ greatly from those of cells moving closer to a surface.

(g) Direction of translatory movement

Very few of the cells moving over the surface of a slide swim along a straight line; the great majority travel along a curved track whose radius of curvature varies considerably from one spermatozoon to another (Buller, 1903); in *P. miliaris* the radius usually varies from 30 to 100μ (Text-fig. 8). In some cases, the track of the

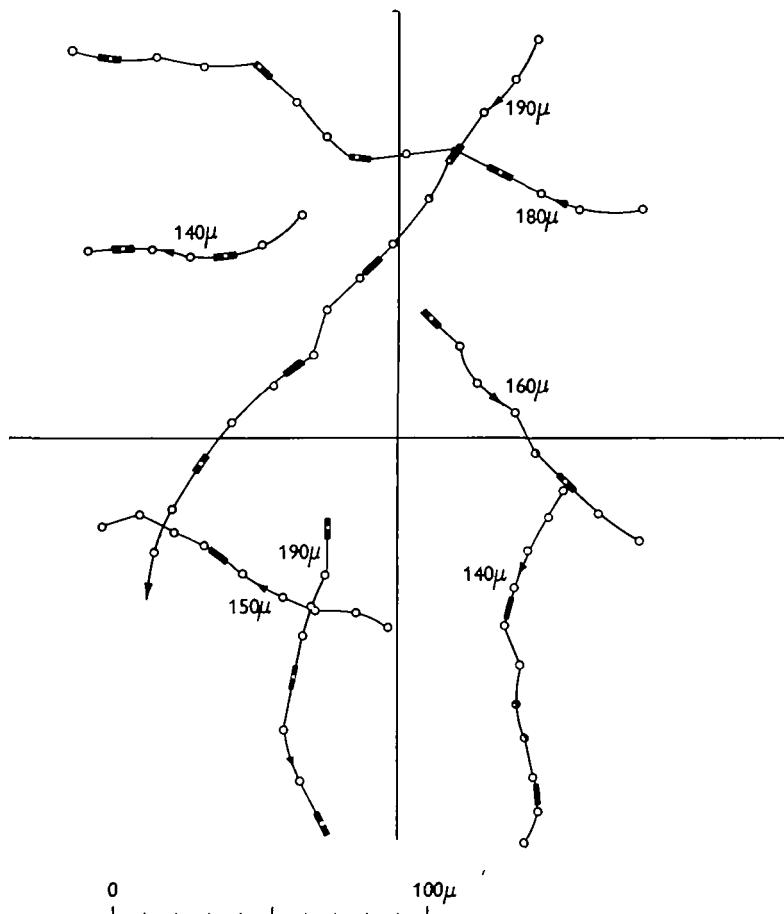


Text-fig. 8. A. Head tracks (from cinematograph records) of five cells moving over the upper surface of a slide and viewed from above, the radius of yaw varying from 37 to 100μ . Four out of the five cells are rotating counter-clockwise. The dots indicate the position of the head at $\frac{1}{10}$ sec. intervals. B. Head tracks of four cells moving over the top surface of a relatively deep drop—also viewed from above: all four cells are moving clockwise.

sperm is almost circular, a complete circle being effected in 1.0 - 3.0 sec.; in other cases, the radius of curvature varies and the resultant track forms a flat spiral; in other instances the radius of curvature varies more irregularly and may occasionally change in sign. No obvious relationship appears to exist between degree of curvature of the track and speed of translation along the track. In contradistinction to a cell moving over the surface of the slide (with its tail beating in a plane parallel to this surface) a cell which rolls about the median longitudinal axis of its envelope

has a relatively straight track (Text-fig. 9). The effect of a roll on the path of progression of a yawing cell is to transform this path from the arc of a circle to a helix.

For many purposes it is necessary to describe the movements of a spermatozoon relative to three internal morphological axes. The first of these (xx_1) is provided by the



Text-fig. 9. Track of head (from cinematograph records) of spermatozoa rolling about their longitudinal axes. The lines are thickened at positions where the envelope of the tail appears edge-on in the record. The dots indicate positions at $\frac{1}{16}$ sec. intervals. The speed of progression is shown in μ per second.

median longitudinal axis of the head; the second (yy_1) can be arbitrarily fixed by a line normal to xx_1 and lying in the plane of the tail's envelope. If yy_1 is drawn through the head of the sperm at any point O on the xx_1 axis, the third axis (zz_1) is provided by a line normal to xx_1 and yy_1 ; in this way four points ($y, y_1; z, z_1$) are fixed on a cross-section of the head through O .

Strictly speaking the movements of the sperm should be defined in terms of the three axes xx_1 , yy_1 and zz_1 . Rotation of the whole sperm about xx_1 constitutes a *roll*, rotation about yy_1 a *pitch*, and rotation about zz_1 denotes *yaw*. For most purposes, however,

reference to xx_1 , yy_1 or zz_1 proves cumbersome, and it is convenient to replace it by an arbitrary, but precise, use of the terms 'dorsal', 'ventral', 'right' and 'left'. If the point z on the surface of the head be denoted as 'dorsal', then z_1 is 'ventral', y is 'left' and y_1 is 'right'.

A *roll* about the axis xx_1 can therefore be either towards the 'right' (y_1) side or towards the 'left' (y) side; similarly, a yaw about zz_1 can either be towards the 'right' (y_1) or towards the 'left' (y); if this nomenclature is adopted it is essential to bear in mind that the terms dorsal, ventral, right and left refer to the internal morphological axes of the spermatozoon and not to axes which refer to an external environment or observer.

If the forces propelling a spermatozoon drive the cell forward along the longitudinal axis of the tail's envelope and, at the same time, cause this axis to yaw steadily to one side, the head of the sperm travels along the arc of a circle whose radius (ρ) depends on the velocity of the sperm's progression (V) and on the frequency per second (ϕ) with which the cell yaws through 360° :

$$\rho = \frac{V}{2\pi\phi}$$

If, in addition to a steady yaw, the spermatozoon also rolls about its longitudinal axis n times per sec., the length of track (V/n) traversed during each complete cycle of roll is transformed from a plane arc into one complete turn of a regular helix. The radius (r) and the pitch (p) of the helix depend on the values of V , n and ϕ :

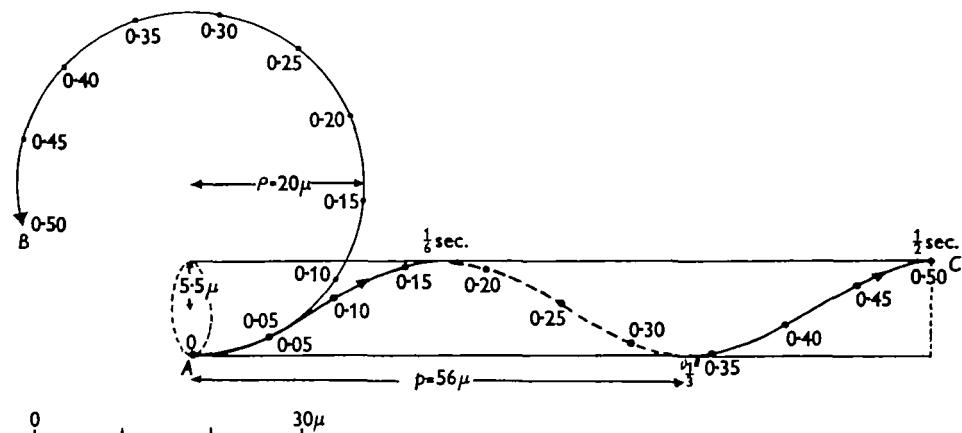
$$r = \frac{V^2}{4\pi^2 n^2 \rho} = \frac{V\phi}{2\pi n^2},$$

$$p = \left[\left(\frac{V}{n} \right)^2 - 4\pi^2 r^2 \right]^{\frac{1}{2}}.$$

If a spermatozoon travelling in a plane along an arc of a circle 75μ in radius at a translatory speed of 200μ per sec. begins to roll twice a second, the resultant helical path of progression has a radius of about 3.3μ and a pitch of nearly 100μ . So long as the cell rolls about its longitudinal axis, the axis of progression is a straight line, and the more frequently it rolls the less is the cell's displacement (r) from this line. For observed values of V , n and ϕ the pitch of the helix is large compared with its radius. The greater the rate of yaw the greater is the frequency with which the cell must roll in order to maintain the same radius of helical progression. From a functional standpoint the ability to roll provides the spermatozoon with a mechanism for maintaining a rectilinear axis of progression even when the forces operating in a 'lateral' plane tend to induce a very pronounced and persistent yaw.

When a spermatozoon is moving forward with both yaw and roll its motion (relative to the surrounding medium) is essentially that of a lamina moving over a 'screw' surface, each element of the tail sweeping 'transversely' across this surface much more frequently than the cell, as a whole, moves along one complete turn of the screw. A rolling cell which progresses without yaw follows a straight line, but its envelope continues to lie on the surface of a screw whose width is the

width of the envelope and whose pitch depends on the forward speed and frequency of roll. The envelope can be regarded as a strip slightly twisted about its long axis. The effect of yaw and roll on the path of the sperm is illustrated by Text-fig. 10.



Text-fig. 10. Diagram showing the effect of rolling on the path of progression of a spermatozoon whose path of motion is otherwise the arc of a circle of radius 20μ and whose forward speed is 200μ per sec.; the spermatozoon traverses the arc AB in $\frac{1}{6}$ sec., the points on the arc indicate the position of the head at 0.05μ sec. intervals. If the spermatozoon rolls 'anti-clockwise' through 360° three times a second, the path of progression becomes a helix AC of approximate radius 5.5μ and pitch 56μ ; the path of the spermatozoon describes 1.5 turns of the helix in 0.5 sec. The diagram does not show that the plane of the arc AB is inclined at an angle to the axis of the helix. (See Text-fig. 11.)

(h) Apparent predominance of anticlockwise yaw

As recorded many years ago (Dewitz, 1886), the spermatozoa of insects show a marked tendency to move in an anticlockwise direction when viewed towards the surface over which they move. This remarkable phenomenon, also observed in *Sphaerechinus granularis* sperm by Buller (1903), has been noted during the present work on *P. miliaris*. In a relatively deep suspension, the spermatozoa show a marked tendency to concentrate at, and swim over, either the surface of the slide or the upper (water/air) surface of the drop. Table 3 shows the direction of curvature of their movement as derived from cinematograph records.

Although an asymmetry of propulsive forces on the two sides of the tail may well be the result of an asymmetry in the form of the waves, this does not explain why a large proportion of the cells move in an anticlockwise direction when viewed against the surface of the slide. Buller suggested that the spermatozoa of sea urchins should be regarded as essentially bi-laterally symmetrical systems, possessing morphological definable right, left, dorsal and ventral surfaces, and that they applied the same morphological surface to any external surface with which they came into contact. A simpler picture can, however, be derived from the fact that the motion of a majority of spermatozoa involves yawing and rolling components.

Relative to its own internal morphological axes (see p. 787), a yawing cell has two

degrees of freedom; it can yaw either towards its right side or towards its left. Similarly, the cell can roll either towards its right side or its left; in the first case the direction of roll is clockwise relative to the cell's own internal axes and to an observer viewing the cell from its posterior end; in the second case the roll appears anticlockwise relative to the same observer. The problem is to define the circumstances under which 80% of the cells will appear, under the microscope, to yaw over the upper surface of a slide in an anticlockwise direction relative to the observer. As already explained the path of progression followed by the head of a rolling and yawing spermatozoon is a helix. This helix can be either right-handed or left-handed; it is right-handed if the cell is rolling to the right, i.e. in a clockwise

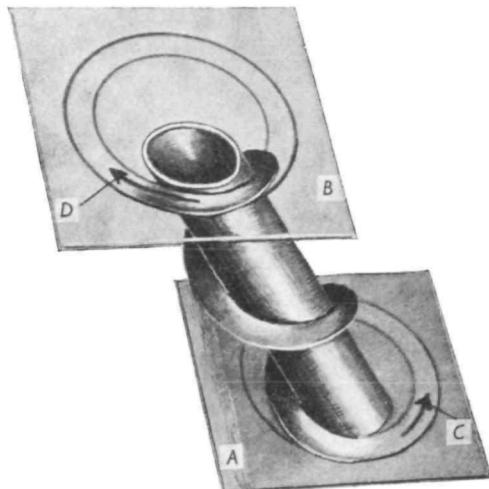
Table 3

Sperm on upper surface of slide (viewed from above)				Sperm at upper surface of drop (water/ air interface) (viewed from above)			
Suspen- sion	Clock- wise	Anti- clock	Doubt- ful	Suspen- sion	Clock- wise	Anti- clock	Doubt- ful
A	7	56	7	A	51	6	3
A	2	33	4	A	35	3	9
A	3	26	6	A	26	3	6
A	1	6	0	A	54	7	14
B	0	8	0	B	7	0	0
C	6	29	9	C	43	4	8
Total	19	158	26	Total	216	23	40
%	9.3	77.8	12.8	%	77.4	8.2	14.3

direction relative to an observer viewing the cell from its posterior end; the helix is left-handed if the direction of roll, relative to such an observer, is anticlockwise; the right-handedness or left-handedness of the helix does not depend on whether the cell is yawing towards or away from its own right surface. These principles can be visualized from Text-fig. 11 in which the helix of progression is traced on the surface of a cylinder. If the orientation of the sperm relative to the helix is such that its right side lies on the helix whilst its left side is away from the surface of the circumscribed cylinder, (i.e. the envelope is lying on the screw surface seen in Fig. 11 with its right side in contact with the surface of the cylinder) the sperm must be yawing towards its right side; if the left side is toward the cylinder the sperm is yawing to its left side. Relative to an observer viewing the cell from its posterior end, however, the direction of roll is always anticlockwise for a left-handed helix and clockwise for a right-handed helix; relative to an observer viewing the cell from its anterior end the apparent direction of roll is, in both cases, reversed, e.g. a cell advancing toward the observer along a left-handed helix appears to be rolling clockwise. If for any reason a cell is prevented from rolling its path becomes restricted to the surface of a plane and the cell will, relative to the observer, yaw over this surface in the same direction as that in which it was originally rolling; if the original roll was anticlockwise relative to the observer, the direction of yaw on the plane surface will also be anticlockwise

relative to the observer. The fact that 80% of the cells conform to this rule is thus explicable on three relatively simple assumptions:

- (i) That the forces exerted by the medium on the large majority of cells causes them to yaw towards the right or left side of their own median longitudinal axis, the relative numbers yawing in the two directions being immaterial.
- (ii) That the reaction from the water, in at least four cells out of five, forces the cell to roll in an anticlockwise direction relative to an observer looking along the axis of progression in the direction of movement; in other words, in open water the motion of the cell is equivalent to that of a left-handed screw.



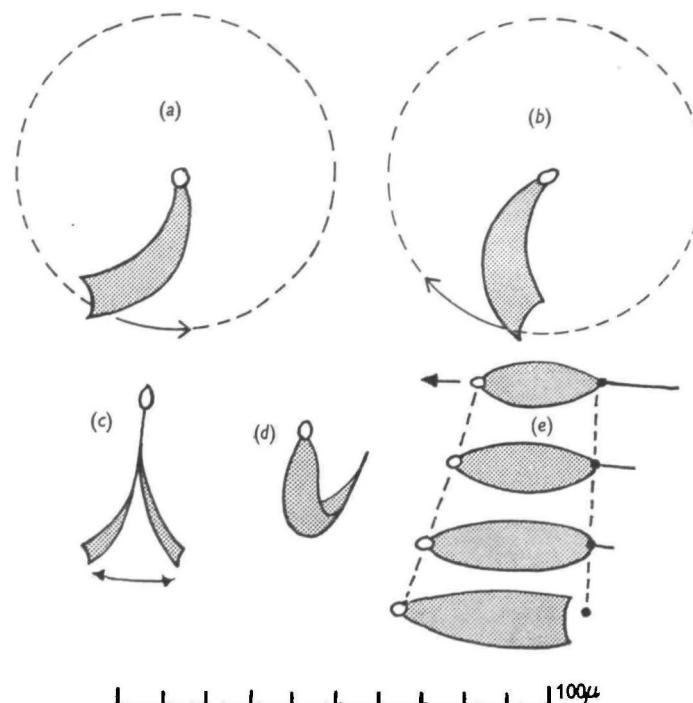
Text-fig. 11. The diagram shows a left-handed screw surface projecting from the surface of a circular cylinder, and terminating smoothly on two plane surfaces *A* (slide) and *B* ('cover-slip'). Spermatozoa may be visualized with the plane of their envelopes lying on the screw surface, and moving up or down this surface. All spermatozoa moving down the surface must be rolling anticlockwise relative to an observer viewing them from *B* to *A*; if, on reaching the surface *A*, the cells cease to roll, they will proceed to yaw over this surface in the direction of the arrow *C*, i.e. in an anticlockwise direction. All spermatozoa moving up the screw surface must roll clockwise relative to the observer, and will yaw clockwise over the surface of *B* in the direction of the arrow *D*.

- (iii) That close proximity to a glass or other plane surface prevents the cell from rolling. Four cells out of five will, relative to the observer, move anticlockwise over the surface of the slide, irrespective of whether they are yawing towards their own right or left sides. The apparent direction is reversed relative to an observer looking at a coverslip towards whose under surface a cell is approaching or has come into contact (see Text-fig. 11).

Until data are available concerning the relationship of yawing and rolling frequencies to the frequency of the propulsive waves passing down the tail, it is difficult to say how far the marked tendency to behave as a left-handed screw is the result of static structural asymmetry of the head, and how far to the dynamic effect of a slight left-handed twist in the envelope of the tail's movements.

(i) *Behaviour of sperm when attached to a glass surface*

Within a few minutes of placing a drop of a suspension on a slide, an increasing number of sperm become attached to the glass surface by their heads although their tails continue to vibrate actively. In nearly every case, the envelope of the tail yaws round the point of attachment of the head with a frequency of about two or three times per second. The tail envelopes of these sperm differ from those



Text-fig. 12. *a.* Spermatozoon on glass surface rotating anticlockwise about the head. *b.* Similar cell rotating clockwise. Note the convex 'leading' surface. Plane of envelope is parallel to slide. *c.* Spermatozoon attached by head, with plane of envelope vertical at proximal end and twisted at distal end. The whole cell oscillated in the direction of the arrows—the distal end untwisting at the reversal of the direction of movement. *d.* Twisted envelope. *e.* Inhibition of bending waves by restraint from a foreign particle (shown by the dot) attached to the slide.

swimming freely over the surface of the slide in that their 'leading' surfaces are more markedly convex and their 'trailing' surfaces more concave (see Text-fig. 12); if, as occasionally happens, the direction of yaw is reversed, the curvature of the surface is also reversed. There can be little doubt that the yawing movement about a fixed head is an extreme case of the yawing phenomenon already described for the majority of cells moving over the surface of the slide.

In the large majority of cases the plane of the envelope of a fixed sperm is parallel to that of the surface to which the head is attached, but a few cases have been observed when the plane of vibration of the tail is oblique or normal to the

slide. Attachments of this kind are not only comparatively rare, but tend to be transitory; they are therefore difficult to record photographically. On four occasions visual observations were made of cases in which the plane of vibration of the tail was effectively normal to the plane of the slide; the form and motion of the envelope were then comparable to those of a thin elastic lamina (viewed edgewise) vibrating from side to side through an arc of about 30° . The envelope resembled a brightly illuminated bent and twisted blade with its leading surface convex and its trailing surface concave. The posterior region of the envelope from time to time widened considerably, giving very clearly the impression of a twisted blade. Whilst undue weight should not be placed on phenomena which are relatively seldom observed, it will be noted that the behaviour of the sperm described above conforms with the conclusion that the envelope of the tail's movements is essentially a lamina whose surface may be bent or twisted under appropriate circumstances.

Occasionally a sperm may be attached to the slide by the tip of its tail, leaving the head free to move over the surface; in such cases the envelope tapers posteriorly towards the point of attachment round which the whole sperm rotates. An interesting modification of this phenomenon was seen on one occasion when the sperm tail was attached at a point about one-half of its length behind the head; when first observed the region of the tail proximal to the point of restraint was actively beating and giving a well-defined envelope; the region distal to the point of attachment remained stationary. The whole sperm moved slowly forwards—the region proximal to the restraint gradually increasing in length and the envelope increasing accordingly. It would appear that waves cannot pass a region of mechanical restraint (see p. 797).

The general impression derived from the observation of an ageing suspension is that the versatility of the tail's movements should not be underestimated, particularly when any part of the cell is subjected to any form of external mechanical restraint.

DISCUSSION

The rhythmical changes in form executed by the tail of an echinoid spermatozoon are fundamentally the same as those of most, if not of all, other undulatory organisms. Some or all of the successive regions of the body can, by active internal effort, induce a change in the radius of their external curvature and, during this process of bending, apply equal but opposite bending couples to the elements lying distally and proximally to itself; provided the phase of bending reached by an element differs by an appropriate amount from that of its neighbour, waves of changing curvature pass along the body and propulsion ensues (Gray, 1953). Two main problems require investigation—the nature of the internal bending mechanism and the nature of the system which maintains the difference in phase between adjacent segments.

Bradfield (1953) and more recently Afzelius (1955) have shown that the tail of a *Psammechinus* spermatozoon contains nine fibrils radially arranged within the perimeter of a cylindrical membrane and two finer fibrils lying along the axis

of this cylinder. Whilst any attempt to assess the functional significance of these structures must be regarded as highly speculative, it is perhaps permissible to consider certain general principles which may eventually have to be taken into consideration. In order that any short element of the tail should be capable of active bending it must contain contractile elements and at the same time be capable of resisting compression. It is impossible to say whether the radial fibrils are contractile and the matrix or membrane of the tail able to resist compression or vice versa. For the time being it seems not unreasonable to regard the nine radially arranged fibrils as contractile, in the sense that when excited by a stimulus they shorten in length and develop tension against the resistance of the compression elements present in the tail and against external resistances. From this point of view it is useful to note that a radially arranged series of fibrils can yield rhythmical changes in external curvature restricted to a single plane provided the pattern of excitation reaching the fibrils is bilaterally symmetrical about the desired plane of bending (see Appendix). If the fibrils pass straight down the tail, each fibril must be visualized as comparable to an earthworm, in that one region is relaxed when another region is contracted. The speed of conduction of a contractile wave would then be of the same order as the speed of conduction of the bending waves travelling along the tail, and the length of the bending wave determined by the length of fibril exhibiting one complete cycle of shortening. On the other hand, if the fibrils are spirally disposed along the length of the tail, the same mechanical result could be obtained from fibrils which shorten or lengthen simultaneously along their whole length; in this case the length of the bending wave would be determined by the length of tail covered by one complete turn of the spirally twisted fibrils.

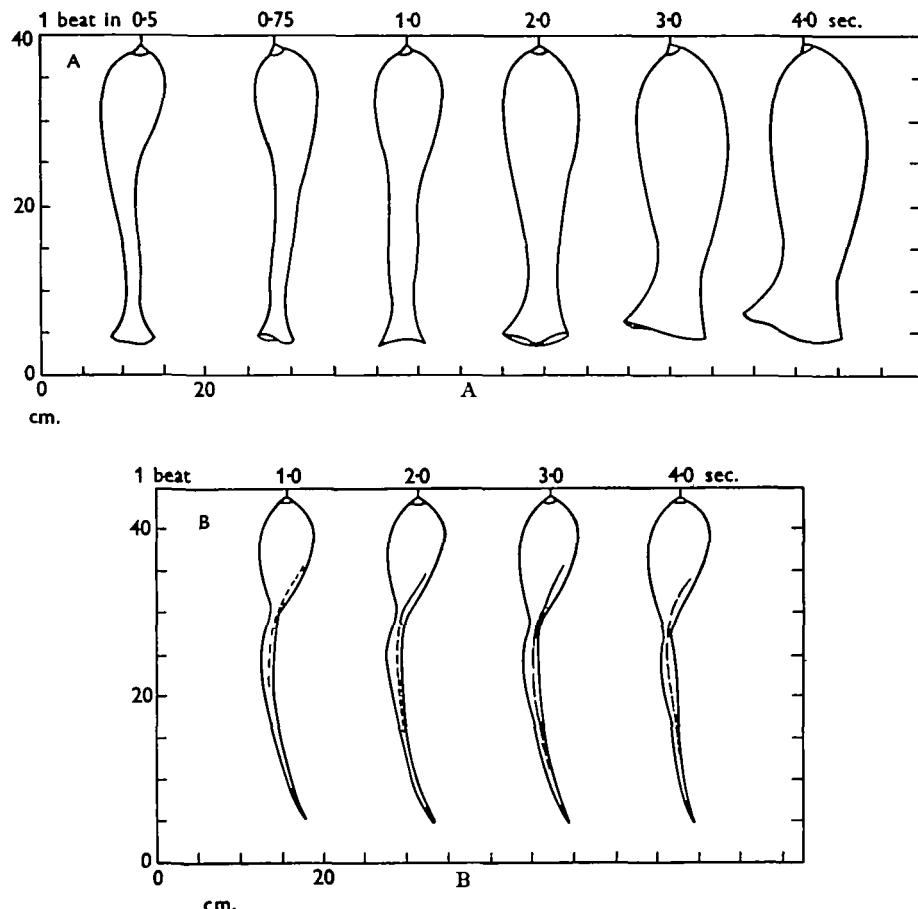
For many years two possibilities have been advocated concerning the mechanism whereby successive elements along a flagellum maintain a characteristic difference in phase of bending from that of their neighbours:

- (i) That actively bending elements are restricted to the proximal region of the flagellum—the remainder of the flagellum representing an inert filament driven by the proximal active element, conduction along the flagellum depending on the latter's natural elasticity.
- (ii) That all elements of the flagellum can actively change their shape and so contribute to the supply of energy necessary for propulsion.

The first hypothesis is based on the principle that bending waves can be passively propagated along an elastic filament immersed in water by oscillating one end by an external source of power; as already indicated, the form of a motionless sperm suggests that the tail possesses some degree of natural elasticity.

The similarity between the envelope of the waves passing down the tail with those passing passively along an elastic filament is illustrated by Text-fig. 13A. At first sight, therefore, the possibility of describing the movement of the sperm tail in terms of passive deformation of an elastic filament operated from a source of power localized at its proximal end seems worth consideration. There are, however, serious objections against this view. In the first place, the passive propagation of waves comparable in form to those exhibited by the active tail only occurs in an

inert elastic filament if one end is constrained to move about a fixed hinge; in the spermatozoon the only source of restraint is the head. An actively bending element situated at the proximal end of the tail could not apply turning or bending forces to the rest of the tail greater than those which it applies to the head, and since the



Text-fig. 13. A. Envelope of waves propagated along a tapering strip of celluloid (37 cm. long, 0.025 cm. thick, and 2 cm. wide at proximal end). The strip was oscillated transversely in water through 90° at various frequencies about a fixed terminal hinge. Note the increase in width of the envelope with decreasing frequency of oscillation. Compare with Text-fig. 1 a-h. B. Envelope of waves propagated by the same strip in a more viscous medium. Note the damping effect on the distal end of the strip. Compare with Text-fig. 1 i-l.

resistance of the water to movements of the head is probably relatively small compared with the resistance operating against the tail (see Gray & Hancock, 1955) an active element located close to the head could only oscillate the head from side to side without effecting any significant displacement of the rest of the tail. Two further differences exist between the movements of a passively oscillated filament and those of the sperm tail. In the case of the filament the form of the waves

(induced by a given type of terminal oscillation) depends on the frequency of the latter; the amplitude decreases markedly with increase of frequency; there is only slight evidence that this is so in the case of the sperm. Again, the form of the waves, and their ability to propagate themselves, is, in the case of an inert filament, markedly affected by the viscosity of the external medium (Fig. 13B). In a highly viscous medium, the envelope of the passive waves approximates in form to that of an 'ageing' spermatozoon—and the change is due to the damping effect of the medium. In the case of the sperm tail, envelopes of this type develop without any change in external viscosity. Finally, in drawing a comparison between the behaviour of a sperm tail and that of a passively oscillated rod, it is necessary to bear in mind that the behaviour of the latter is usually largely determined by its inertia. In the case of the sperm tail, the inertia of the system is negligible compared with the viscous resistance of the medium; the amount of kinetic energy present in an actively beating tail could not sustain its motion for more than a very small fraction of a second. All the evidence strongly suggests that the tail of a spermatozoon cannot be regarded as a passive rod oscillated by a localized source of power. The concept of sources of energy distributed along the whole tail—or at any rate the greater part of it—seems inescapable. At the same time, it is probably going too far to maintain that the passive elastic properties of the tail play no part in the mechanism of movement.

As the tail possesses natural elasticity, mechanical energy must be stored whenever an element is bent, and be released when it straightens out; it may well be that this energy is utilized in the useful work of propulsion and plays some part in the liberation of further energy for use by the contractile elements. It would be interesting to know whether the elastic energy stored in an element when it is bent is large or small in comparison with the total energy necessary to propel the element against the resistance of the medium. If a straight rod is bent into the arc of a circle the amount (U) of elastic energy stored is

$$U = \frac{EIl}{2r^3},$$

where E = Young's modulus, I = 'moment of inertia', l = length of rod, r = radius of curvature.

Applying this equation to a rod of the dimension of a sperm tail, each element of which bends into an arc of 4μ eighty times a second, and using a value of E of the same order as that determined by Mitchison & Swann (1954) for the cortex of sea-urchin eggs, suggests that the total rate of liberation of elastic energy by the tail is of the same order as that calculated by Rothschild (1953) for the propulsion of a mammalian spermatozoon at a comparable speed. The validity of this comparison is, of course, open to doubt, but the argument suggests that it may be unwise to assume that the passive storage of elastic energy by the tail is a minor consideration in the energetics of propulsion.

Reverting to the conclusion that a transformation of chemical into tensile energy occurs along the whole length of the tail, it is relevant to recall that regions of the

tail mechanically isolated from the middle piece are invariably motionless. The middle piece thus appears to function as a 'kinetic centre' the activity of which is essential for the maintenance of the tail's movements. The instance quoted on p. 793 suggests, however, that the excitatory effect exerted by this centre on the tensile units of the tail cannot, in a sea-urchin spermatozoon, pass a region of mechanical restraint. It may be that the excitatory effect of the middle piece only leads to a liberation of tensile energy if the element concerned is mechanically stretched; under such conditions an active release of tensile energy would occur at the stage at which the amount of passively stored energy was at a maximum, and the rate of transmission of the wave would depend on the rate at which the total available energy could drive the element against the resistance of the water, and the form of the wave would depend at least in part on the natural rigidity of the tail. As an element moved from a position of maximum displacement towards the axis of propagation of the waves, the release of energy would be partly from an active source and partly from the energy already passively stored during the preceding process of bending; as the element moved away from the axis the energy available for propulsion would be the active energy minus the energy required for passive storage during the process of bending.

SUMMARY

1. The spermatozoa of *Psammechinus miliaris* (P. L. S. Müller; Gmelin) propel themselves by projecting transverse bending waves along their tails. All points on the tail normally execute their movements in approximately the same plane, their envelope forming a plane (or slightly twisted) lamina. The radius of maximum curvature is of the order of 4μ .
2. In fresh suspensions at about 18° C. the waves are generated at a frequency of 30–40 per sec. and travel along the tail at a velocity of 800 – 1000μ per sec. The average amplitude of the waves is 4μ and the average wave-length 24μ .
3. Elements of the tail situated near the head seldom bend to the same extent on their two sides. The symmetrical bending cycle of the central elements, on the other hand, sometimes imposes on the tail the form of a sine curve.
4. When moving over the surface of a glass slide the passage of each wave along the tail propels the head of the spermatozoon through a distance of 5 – 6μ , and at the same time causes it to oscillate laterally through a distance of about 4μ . The rate of forward propulsion of the head is seldom constant during all phases of the bending cycle; in extreme cases the head may move backwards during certain phases of the cycle. This asymmetry is probably due to the asymmetry of bending on the two sides of the tail.
5. Spermatozoa swimming over the surface of a slide travel at an average speed of about 190μ per sec., but their path of progression is seldom straight; most cells travel along a curved track whose radius is usually 30 – 100μ . Cells swimming farther away from the surface of a slide roll about their longitudinal axes with a frequency of 0.5 – 3.0 per sec., and their axis of progression is straight. The path of

a cell which yaws and rolls as it progresses forms a helix whose properties depend on the rate of yaw, frequency of roll and the rate of the cell's forward progression.

6. Eighty per cent of the cells moving over the surface of a slide yaw in a counter-clockwise direction relative to the observer. This phenomenon can be explained on the assumption that most of the cells when moving freely in a bulk of fluid behave as 'left-handed' screws rolling counter-clockwise as they advance; proximity to a surface prevents the cells from rolling and impresses on them an appearance of yawing in a counter-clockwise direction irrespective of whether they are yawing towards their right or left sides.

7. Evidence is presented which supports the view that all regions of the tail are actively contractile although mechanical forces may affect the propagation of bending waves along the filament.

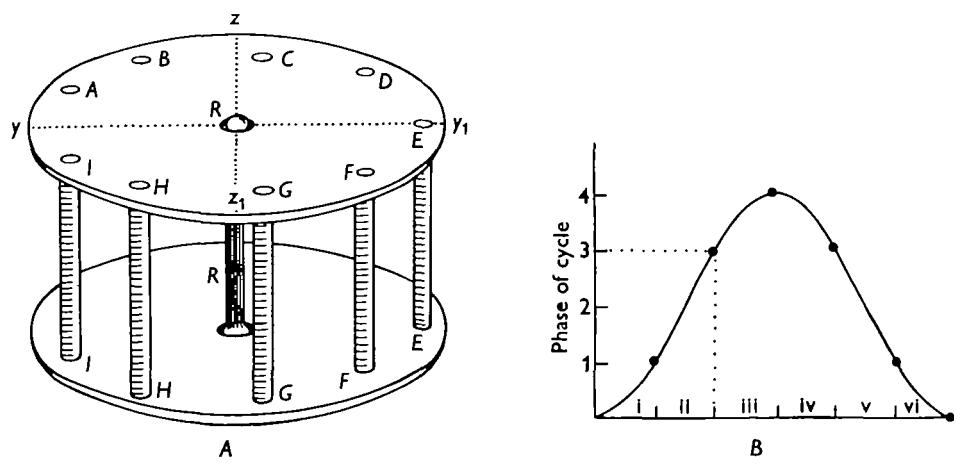
My very sincere thanks are due to a number of colleagues, particularly to Prof. M. M. Swann for his help with some of the earlier photographs; to Prof. T. Fox and Dr K. E. Machin for help with the physical aspects of the problem; and to Lord Rothschild for many fruitful discussions. Finally, I owe the whole of the cinematograph recording to the patience and skill of Mr K. Williamson.

APPENDIX

Text-fig. 14*A* illustrates a mechanical system analogous to a short segment of the tail of a spermatozoon. Two circular plates are hinged concentrically by universal joints to the ends of a rigid rod (*R*), the plates and the rod representing the compression elements of the tail. The plates are also connected by nine radially arranged contractile fibres (*A-I*). If the top plate is to oscillate about one axis (ZZ_1) only, the distribution of tension between the individual fibres must conform with two conditions; (i) the resultant of all the tensile forces exerted by the fibres shall (together with force exerted by the rod *R*) yield a couple, about the axis of oscillation, which varies rhythmically in magnitude and direction during each cycle of oscillation; (ii) at no phase in the oscillation shall there be any resultant couple about an axis (yy_1) normal to the axis of oscillation (zz_1). The first of these conditions is satisfied if each fibre undergoes a contractile cycle identical with that of all the others but maintains a constant phase difference from its immediate neighbours. The second condition is satisfied if the distribution of tension between the different fibres is such that it is bilaterally symmetrical about an axis (yy_1) normal to the axis of oscillation (zz_1). These principles are illustrated by the following example.

Let the form of the contractile cycle of each element approximate to that of a sine wave and produce a maximum tension of four arbitrary units and let the phase difference between adjacent fibres be one-sixth of a cycle. Each cycle can then be divided into six equal intervals of time and the relative levels of tension at the beginning of each of the six consecutive intervals will be approximately 0, 1, 3, 4, 3, 1 (Text-fig. 14*b*). If the conditions prescribed above are to be fulfilled, the

distribution of tension throughout the whole contractile cycle must be that shown in Table 4, which shows that each fibre is always one-sixth of a cycle out of phase with its immediate neighbour and that the distribution of tension is bilaterally symmetrical about the radial axis (yy') passing through fibre E ; fibre A is always in phase with fibre I , fibre B with fibre H , fibre C with fibre G , and fibre D with fibre F .



Text-fig. 14.

Table 4

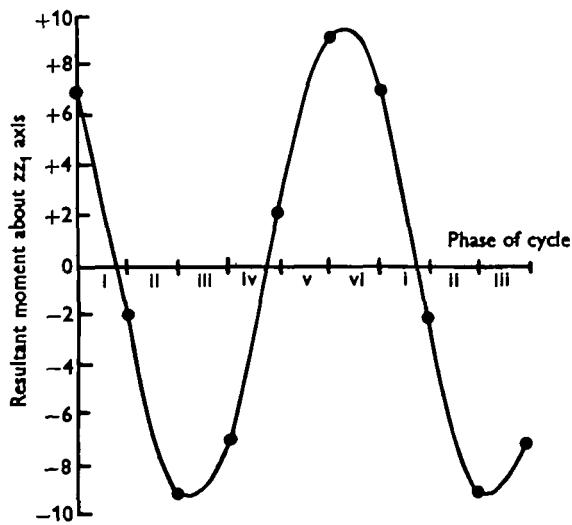
Fibril	Tension at beginning of successive phases					
	i	ii	iii	iv	v	vi
<i>A</i>	3	1	0	1	3	4
<i>B</i>	4	3	1	0	1	3
<i>C</i>	3	4	3	1	0	1
<i>D</i>	1	3	4	3	1	0
<i>E</i>	0	1	3	4	3	1
<i>F</i>	1	3	4	3	1	0
<i>G</i>	3	4	3	1	0	1
<i>H</i>	4	3	1	0	1	3
<i>I</i>	3	1	0	1	3	4

For the sake of convenience the cycle is assumed to start when the tension of fibre E is zero.

That this distribution of tension maintains a regular oscillation about zz_1 is shown by taking moments of all the tensions about zz_1 ; tensions exerted by fibres A , B , H , I are denoted as 'positive', and those by C , D , E , F and G as 'negative'. These moments together with their resultant value at the beginning of each phase of the cycle are shown in Table 5 and Text-fig. 15; one oscillation occurs during each cycle of fibrillar contraction.

Table 5

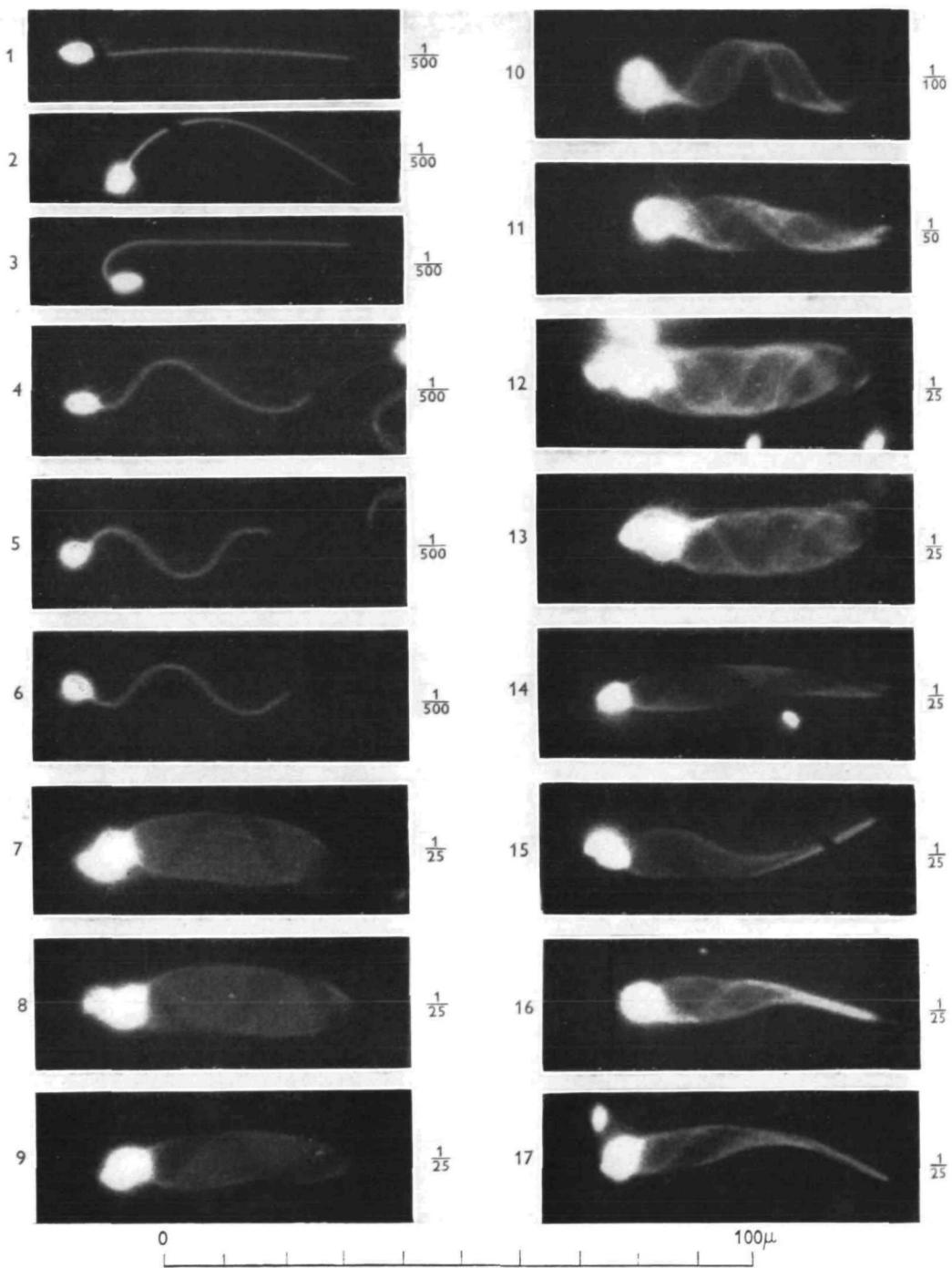
Fibril	Sign of moment	Moments at beginning of phase					
		i	ii	iii	iv	v	vi
A	Positive	2.82	0.94	0	0.94	2.82	3.76
B	Positive	2.00	1.50	0.50	0	0.50	1.50
C	Negative	0.522	0.696	0.522	0.174	0	0.174
D	Negative	0.766	2.298	3.064	2.298	0.766	0
E	Negative	0	1.000	3.000	4.000	3.000	1.000
F	Negative	0.766	2.298	3.064	2.298	0.766	0
G	Negative	0.522	0.696	0.522	0.174	0	0.174
H	Positive	2.00	1.50	0.50	0	0.50	1.50
I	Positive	2.82	0.94	0	0.94	2.82	3.76
Total		+7.064	-2.108	-9.172	-7.064	+2.108	+9.172



Text-fig. 15.

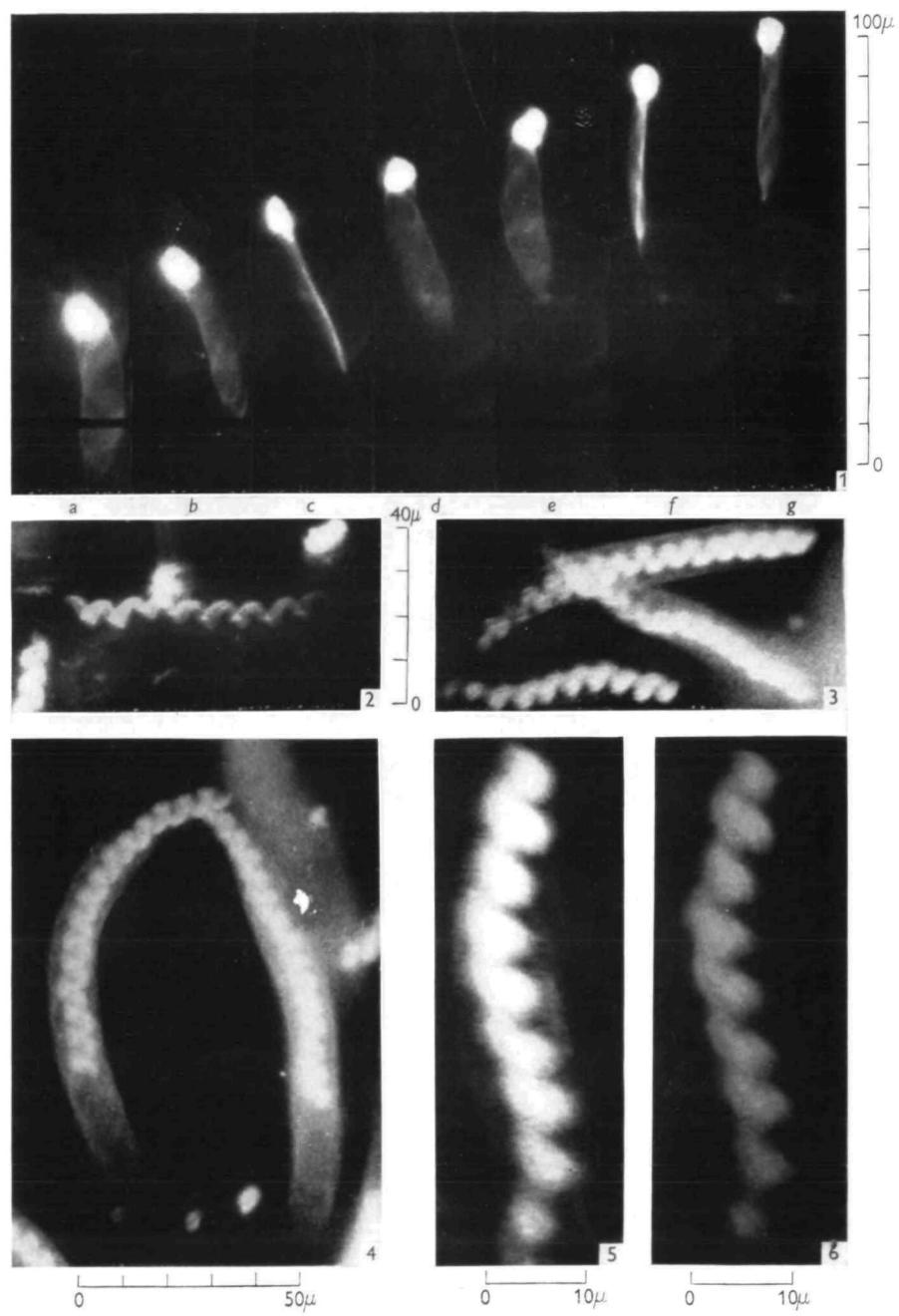
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GRAY—THE MOVEMENT OF SEA-URCHIN SPERMATOZOA

(Facing p. 800)



GRAY—THE MOVEMENT OF SEA-URCHIN SPERMATOZOA

EXPLANATION OF PLATES

PLATE 3

Figs. 1-3. Dead or motionless spermatozoa. Figs. 4-13. Active spermatozoa from fresh suspensions.
Figs. 14-17. From older suspensions.

The approximate exposure of each photograph is shown in fractions of a second.

Figs. 7, 8, 9, 14, 15 are photographs taken with an arc of steady voltage. Figs. 10-13; 16, 17 were taken with a variable arc.

PLATE 4

Fig. 1. Seven successive photographs (*a-g*) from a cinematograph record (12 frames per sec.) of a spermatozoon rolling about its longitudinal axis 2·0 times per sec.

Figs. 2-6. Tracks of heads.

Figs. 3, 4, 5. Showing envelopes of tails.