

## A QUANTITATIVE DESCRIPTION OF FLAGELLAR MOVEMENT IN GOLDEN HAMSTER SPERMATOZOA

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*Accepted 31 July 1984*

### SUMMARY

Flagellar movement of golden hamster spermatozoa obtained from the testis and the caput and cauda epididymides was observed by a light microscope while holding them at their heads with a micropipette. Flagellar movement of capacitated spermatozoa and of reactivated spermatozoa demembranated with Triton X-100 was also observed. Testicular and caput epididymal spermatozoa showed weak movement in Tyrode's solution, whereas cauda epididymal spermatozoa showed vigorous movement. The flagellar bends of the cauda epididymal spermatozoa were almost planar. Capacitated spermatozoa moved with waves of a large amplitude. Demembranated spermatozoa reactivated with ATP only had a latent period before the initiation of flagellar movement, and beat at low frequency, whereas demembranated spermatozoa reactivated with both ATP and cAMP began to move immediately at high frequency. Thrust and hydrodynamic power output were calculated using the parameters for the typical waveforms of cauda epididymal spermatozoa before and after capacitation. The possible role of the large amplitude beat in capacitated spermatozoa is discussed. A comparison of the 'principal' and 'reverse' bends in golden hamster sperm flagella as defined by Woolley (1977) with those in sea urchin sperm flagella suggests that the so-called 'principal' bend in golden hamster sperm flagella corresponds to the reverse bend in sea urchin sperm flagella and *vice versa*.

### INTRODUCTION

It is well known that mammalian spermatozoa exhibit sequential changes in their motility characteristics during their passage through the male reproductive tract (Bedford, 1975) and during capacitation in the female reproductive tract (Yanagimachi, 1981). Hamster spermatozoa taken from the testis and the caput epididymides are virtually immotile in Tyrode's solution, whereas cauda epididymal and ejaculated spermatozoa are capable of active progressive movement in it. Capacitated spermatozoa show very vigorous motility characterized by whiplash-like beating of their flagella. When the spermatozoa of these types are demembranated

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Key words: Capacitation, flagellar movement, golden hamster, spermatozoa, epididymides.

with Triton X-100 and exposed to ATP (and cAMP), all begin active movement (Mohri & Yanagimachi, 1980).

These changes in motility characteristics of the sperm flagella are reflected by changes in their beating pattern. Therefore, it is important to describe the flagellar movement of the spermatozoa as exactly as possible to investigate the mechanism underlying the change in the beating pattern.

In the present study, golden hamster spermatozoa were used as the material and the flagellar movement of living spermatozoa obtained from the testis, the caput and cauda epididymides as well as that of capacitated sperm and demembrated, reactivated spermatozoa were recorded for analysis while holding them at their heads with a micropipette. The effects of cAMP on the movement characteristics of demembrated spermatozoa were also examined. A preliminary account of a part of this work has been reported (Mohri, Ishijima & Hiramoto, 1983).

#### MATERIALS AND METHODS

The testis and the caput and cauda epididymides of the golden hamster, *Mesocricetus auratus*, were placed separately in 10 ml of albumin-saline (0.9% NaCl and 1 mg ml<sup>-1</sup> bovine serum albumin, Reheis Chemical Co., Chicago, Illinois, pH 7.0) and minced with fine scissors. The extruded sperm were filtered through a double layer of tissue paper (Kimwipes, Kimberly-Clark Corp.) and centrifuged at 3000 rev./min for 5 min. The sediment of about 1 µl was resuspended in 10 ml of Tyrode's solution.

Capacitation of the cauda epididymal spermatozoa was induced according to the method of Yanagimachi (1978).

In order to prepare demembrated spermatozoa, 5 µl of the sedimented spermatozoa obtained from the testis and the caput and cauda epididymides were added separately to 0.2 ml of an extracting solution (0.1% v/v Triton X-100, 0.2 mol l<sup>-1</sup> sucrose, 25 mmol l<sup>-1</sup> potassium glutamate, 1 mmol l<sup>-1</sup> dithiothreitol and 20 mmol l<sup>-1</sup> Tris-HCl buffer, pH 7.9) in a Petri dish. After the spermatozoa had been treated with the extracting medium for 30 s at room temperature, 1 mmol l<sup>-1</sup> (final concentration) of ATP and various concentrations of cAMP were added to the medium to reactivate their flagella.

The prepared sperm suspensions were put into a trough (T in Fig. 1) on the stage of a microscope for observation. One of the spermatozoa was captured by its head with a braking micropipette (P) (cf. Hiramoto, 1974a) attached to an instrument collar (I) and held with a micromanipulator (M) (Ernst Leitz GmbH., Wetzlar, W. Germany) as shown in Figs 1 and 2. The orientation of the spermatozoon was adjusted by tilting and rotating the micropipette so as to bring the entire length of the flagellum into the focal plane of the microscope. Because the micropipette was fixed to the instrument collar after passing through a hole set on the axis of the instrument collar, the spermatozoon at the tip of the micropipette was always in the microscopic field when the micropipette was rotated. Observations were made at 37°C.

A Nikon differential interference microscope or a Nikon phase contrast microscope was used for observation. The images of flagellar movement were

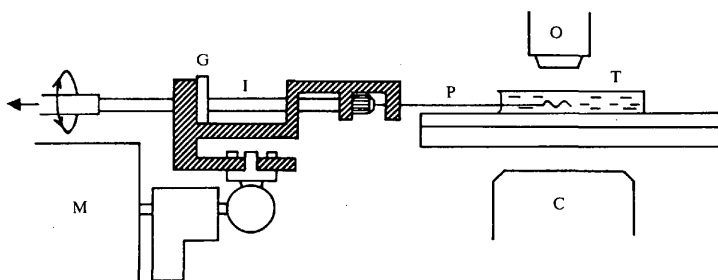


Fig. 1. Experimental setup. C, long-focal-length condenser; G, graduator measuring rotation angle of micropipette; I, instrument collar holding micropipette; M, micromanipulator; O, objective lens; P, micropipette; T, horizontal trough.

recorded on video tape at 60 frames per second using a video camera (AVC-1550, Sony Corp., Tokyo) synchronized with a Chadwick-Helmuth Strobex power supply and lamp (Model 100, Chadwick-Helmuth Co., Inc., Monrovia, California). The images of flagellar movement were also recorded on 35 mm Kodak Tri-X film moving at  $1 \text{ m s}^{-1}$  with stroboscopic flashes at 200 Hz.

For a detailed frame-by-frame analysis, the images of the flagella were traced on paper. The wave axis, which was drawn through the median position of several superimposed tracings, was taken as the centre line of the flagellar waveform. The wavelength was determined by reference to the wave axis. The amplitude was defined as one-half the maximum transverse displacement from the wave axis.

Golden hamster spermatozoa have a hook-shaped head. From an analysis of waveforms of hamster spermatozoa fixed by rapid freezing, Woolley (1977) concluded that the 'principal' bend occurs in the direction in which the head projection points as shown in Fig. 2. This terminology was used in the present study and is discussed below.

In preliminary experiments, the flagellar movement of spermatozoa held at their heads with a micropipette was compared with that of freely swimming spermatozoa. Capturing the sperm head had little or no effect on their beat frequency.

## RESULTS

### *Flagellar movement of intact spermatozoa*

When the intact testicular spermatozoa were suspended in Tyrode's solution, about 20% of the spermatozoa began to move immediately, showing weak movement with waves of very small amplitude (about  $7 \mu\text{m}$ ) and low beat frequency (about 0.3 Hz); most of the remaining spermatozoa showed similar movement only after a 5 min lag period. Propulsive movement was not observed. Movement of the intact spermatozoa from the caput epididymides was similar to that of the testicular ones, except that they beat with a slightly higher frequency (about 0.7 Hz) and

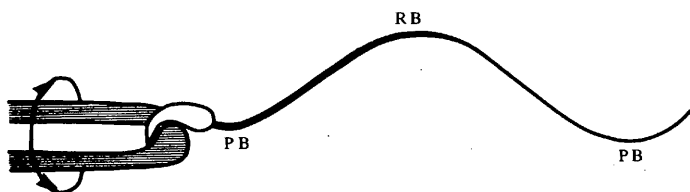


Fig. 2. Technique for holding a golden hamster spermatozoon at its head to the tip of a micropipette. The 'principal' bend (PB) or 'reverse' bend (RB) of the flagellum is shown as defined by Woolley (1977).

larger amplitude (about  $16\text{ }\mu\text{m}$ ). In contrast with the testicular spermatozoa, some of them showed very slow progression.

When the spermatozoa from the cauda epididymides were suspended in Tyrode's solution, most of these spermatozoa began to move within about 30 s. Cauda epididymal spermatozoa held by their heads with the micropipette showed vigorous movement as shown in Fig. 3. Bending waves were initiated at the base of the flagellum and propagated toward the tip. The proximal part of the flagellum underwent little bending, whereas the distal part moved with a large transverse amplitude. Therefore, the amplitude of the bending wave progressively increased towards the distal end. The mean values of beat frequency, amplitude and wavelength for the beating wave of cauda epididymal spermatozoa were 11 Hz,  $18\text{ }\mu\text{m}$  and  $90\text{ }\mu\text{m}$ , respectively, as shown in Table 1.

The flagella of cauda epididymal spermatozoa held by their heads with the micropipette could be observed over almost their entire length when their orientations were properly adjusted under the microscope. When the micropipette holding the spermatozoa was rotated by  $90^\circ$  about its axis so as to bring the beating plane of the flagellum perpendicular to the focal plane of the microscope, the parts of the flagellum in focus were seen as a straight train of dashes as shown in Figs 3D, E, F and 4B. As shown in Fig. 4A, the bending wave of the cauda spermatozoa was asymmetrical.

#### *Flagellar movement of capacitated spermatozoa*

After the spermatozoa from the cauda epididymides had been incubated at  $37^\circ\text{C}$  in the capacitating medium for 3–4 h, their movement was observed as described above. They beat actively for a few minutes and then their movement rapidly ceased. The mean value of beat frequency (10 Hz) was similar to that of the intact cauda epididymal spermatozoa. Some of the capacitated spermatozoa moved with waves of a much greater amplitude (cf. Fig. 5B), although such a pattern could also be observed in a few of the intact ones.

#### *Flagellar movement of demembranated spermatozoa*

When testicular spermatozoa were demembranated in the extracting medium, they became motionless almost instantly. Most of these spermatozoa began to move

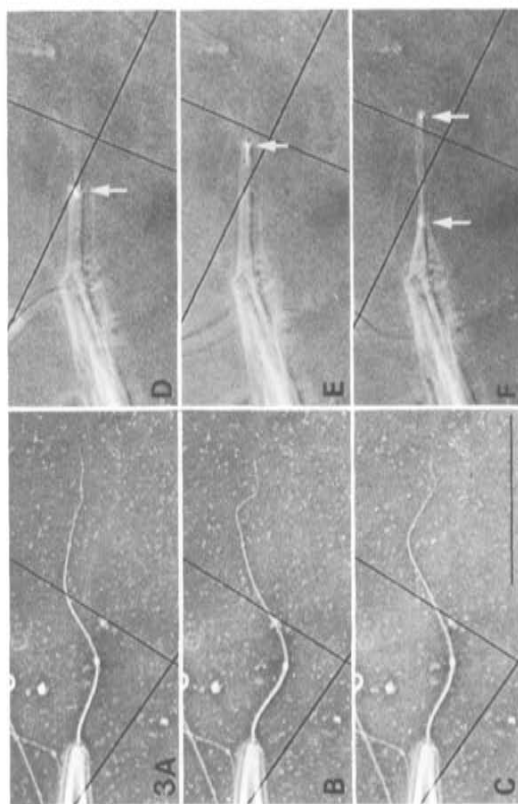


Fig. 3. Successive photomicrographic records of flagellar movement of intact spermatozoa from the cauda epididymides at 1/200 s intervals. Phase-contrast optics. A, B, C; the beating flagellum observed from a direction parallel to the beating plane. Arrows indicate the parts of the flagellum in focus observed as spots moving from the base to the tip of the flagellum. This is not the same cell as in Fig. 3A, B, C. Scale bar = 100  $\mu$ m.

vigorously in a manner similar to that of intact caudal spermatozoa about 5 min after the addition of ATP to the medium to reactivate their flagella. This vigorous movement lasted for about 10 min and then the waveform became rather asymmetrical with a low beat-frequency as shown in Fig. 6A. The mean values of the beating demembrated flagella of the testicular spermatozoa were 5.4 Hz for beat frequency, 23  $\mu\text{m}$  for amplitude and 90  $\mu\text{m}$  for wavelength (Table 1).

Most of the demembrated spermatozoa from the caput epididymides began active movement 3–5 min after the addition of ATP. The movement of the proximal part of the flagellum stopped within 10 min, whereas the distal part of the flagellum continued to beat as shown in Fig. 6B. As summarized in Table 1, the movement characteristics of these spermatozoa were similar to those of demembrated testicular spermatozoa reactivated with 1  $\text{mmol l}^{-1}$  ATP, except that the former had a slightly higher beat frequency.

Almost 100% of the demembrated cauda epididymal spermatozoa moved actively within 1 min after addition of ATP to the extracting medium (Fig. 7), and

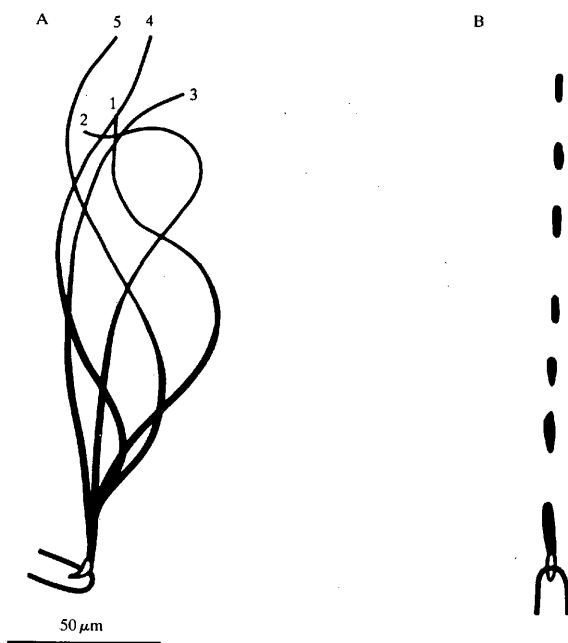


Fig. 4. Traces of a flagellum during a single beat cycle of a typical intact spermatozoon from the cauda epididymides. (A) The beating flagellum as observed from a direction perpendicular to the beating plane. The numbers indicate the positions of the flagellum at 1/60s intervals. (B) The beating flagellum as observed from a direction parallel to the beating plane (see Figs 3D,E,F).

Table 1. *Movement characteristics of golden hamster spermatozoa held with a micropipette\**

		Medium	Beat frequency (Hz)	Amplitude ( $\mu\text{m}$ )	Wavelength ( $\mu\text{m}$ )
Intact spermatozoa	Testicular	Tyrode's solution	$0.3 \pm 0.1$	$7 \pm 1$	$183 \pm 17$
	Caput epididymal		$0.7 \pm 0.2$	$16 \pm 2$	$117 \pm 10$
	Cauda epididymal		$11.0 \pm 1.8$	$18 \pm 7$	$90 \pm 12$
Demembranated spermatozoa	Testicular	$1 \text{ mmol l}^{-1}$ ATP	$5.4 \pm 0.8$	$23 \pm 5$	$90 \pm 17$
	Caput epididymal		$7.2 \pm 1.0$	$22 \pm 3$	$87 \pm 10$
	Cauda epididymal		$6.5 \pm 1.4$	$24 \pm 7$	$86 \pm 8$
	Testicular	$1 \text{ mmol l}^{-1}$ ATP $50 \mu\text{mol l}^{-1}$ cAMP	$7.8 \pm 0.5$	$21 \pm 1$	$91 \pm 11$
	Caput epididymal		$8.2 \pm 1.7$	$35 \pm 1$	$87 \pm 2$
	Cauda epididymal		$8.0 \pm 1.4$	$25 \pm 6$	$92 \pm 6$
Capacitated spermatozoa	Cauda spermatozoa		$10.0 \pm 2.8$	$38 \pm 6$	$82 \pm 9$

\* Each value represents average of 5–13 sperm.

this movement continued for 20–30 min. The amplitude of the bend in the proximal part (the mid-piece) of the flagella of the demembranated cauda spermatozoa was  $24 \mu\text{m}$ , larger than in the intact cauda spermatozoa. The beat frequency ( $6.5 \text{ Hz}$ ) and the wavelength ( $86 \mu\text{m}$ ) were similar to those of the testicular and caput epididymal spermatozoa and were somewhat lower than those of the intact cauda spermatozoa. The bending wave of the demembranated cauda epididymal spermatozoa was asymmetrical and the curvature of the 'reverse' bend was much greater than that of the 'principal' bend. When the bending wave was observed from a direction parallel to the beating plane, the displacement from the plane at the distal region of the demembranated cauda sperm flagellum was larger than in the intact cauda sperm flagellum (cf. Fig. 7B). The displacement from the plane in the distal region tended to be smaller in flagella showing relatively symmetrical bending waves than in those exhibiting asymmetrical and irregular bending waves.

When cAMP was added to the reactivating medium containing  $1 \text{ mmol l}^{-1}$  ATP, initiation of flagellar movement in demembranated spermatozoa occurred faster than in reactivating medium containing ATP alone. The effect was proportional to cAMP concentration up to  $10 \mu\text{mol l}^{-1}$ . No significant differences in the effects on the initiation and the movement characteristics of flagellar movement were found with cAMP at concentrations above  $10 \mu\text{mol l}^{-1}$ . When demembranated testicular spermatozoa were suspended in reactivating medium containing  $50 \mu\text{mol l}^{-1}$  cAMP and  $1 \text{ mmol l}^{-1}$  ATP, most spermatozoa moved within 30 s with waves of  $21 \mu\text{m}$

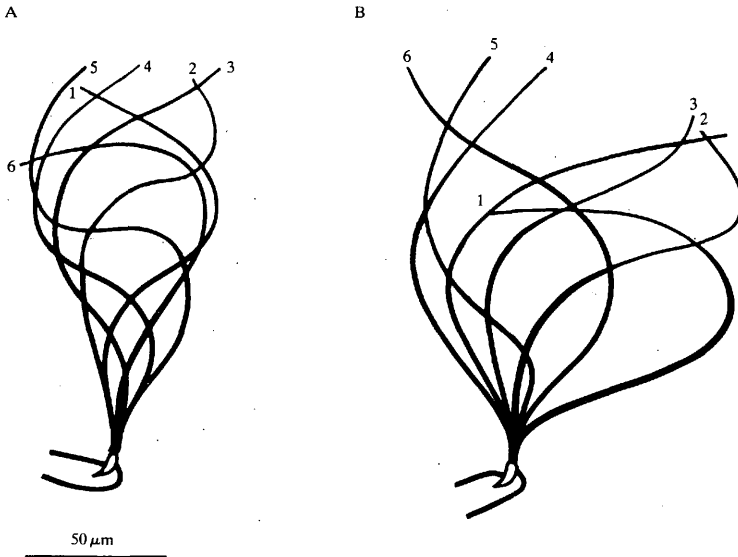


Fig. 5. Traces of a flagellum during a single beat cycle of typical capacitated spermatozoa. The numbers indicate the positions of the flagellum at 1/60 s intervals. (A) Usual beating pattern. (B) Beating pattern with a large amplitude.

amplitude and  $24\text{ }\mu\text{m}$  wavelength at a beat frequency of 7.8 Hz. The beat frequency became larger than that of spermatozoa reactivated with  $1\text{ mmol l}^{-1}$  ATP only and approached the value observed for intact cauda spermatozoa (Table 1). When demembranated spermatozoa from the caput and cauda epididymides were suspended in the medium containing  $50\text{ }\mu\text{mol l}^{-1}$  cAMP and  $1\text{ mmol l}^{-1}$  ATP, most of these spermatozoa began to move actively after about 30 s and beat in a manner similar to that of demembranated testicular spermatozoa reactivated with cAMP and ATP.

Table 1 summarizes the values of beat frequency, amplitude and wavelength obtained with intact, capacitated and demembranated spermatozoa.

#### DISCUSSION

From the geometry of the sperm flagella fixed by rapid freezing, Woolley (1977) reported that the bending plane twisted clockwise (viewed proximally). In the present study, however, when the cauda epididymal spermatozoon was held by its head with the micropipette so that it was beating in a plane parallel to the focal plane

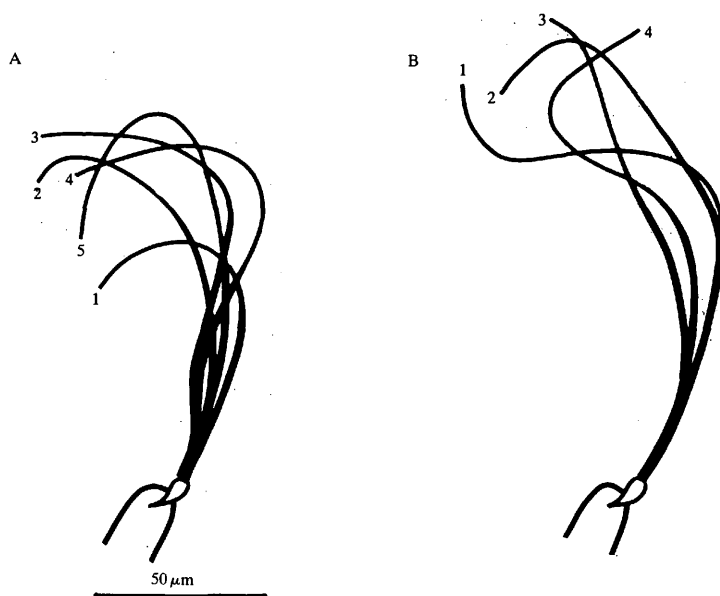


Fig. 6. Traces of a flagellum during a single beat cycle of typical demembrated spermatozoa reactivated with  $1 \text{ mmol l}^{-1}$  ATP. The numbers indicate the positions of the flagellum at  $1/30 \text{ s}$  intervals. (A) Testicular spermatozoon. (B) Caput-epididymal spermatozoon.

of the microscope, almost the entire length of the flagellum could be observed in sharp focus. Moreover, when flagellar movement was observed from a direction parallel to the beating plane, the image of the beating flagellum was seen as a straight train of dashes. These observations indicate that the flagellar movement of the cauda epididymal spermatozoa is almost planar. Close to the coverslip, golden hamster spermatozoa swam along circular paths without rotation about the axis of propulsion, whereas at some distance from the coverslip rotation of the sperm head was observed as reported by Yanagimachi (1970). This rotation is probably caused by a three-dimensional component of the flagellar waveform (cf. Woolley, 1979). As reported by Hiramoto & Baba (1978), the existence of a minor three-dimensional component below the limit of resolution of the light microscope cannot be excluded by the present observations.

When the bending wave of demembrated cauda epididymal spermatozoa reactivated with ATP was observed from a direction parallel to the beating plane,

the deviation from planarity of the distal region was larger than in intact ones. Considering that the beat frequency of the intact spermatozoa was about two times larger than that of the demembrated and reactivated spermatozoa, the activity of the flagellar movement may be closely related to the mechanism of the generation of planar bending waves in the flagella. Miki-Noumura & Kamiya (1979) reported that doublet microtubules isolated from sea urchin spermatozoa formed left-handed helices. Paddock & Woolley (1980) also reported that dense fibres released from golden hamster spermatozoa showed a helical form. Because the flagellar movement of demembrated and reactivated spermatozoa is less active than in intact spermatozoa, the helical nature of the doublet microtubules and/or dense fibres may be reflected in their movement.

The beat frequencies of intact and demembrated golden hamster spermatozoa under various conditions were reported by Mohri & Yanagimachi (1980). Their

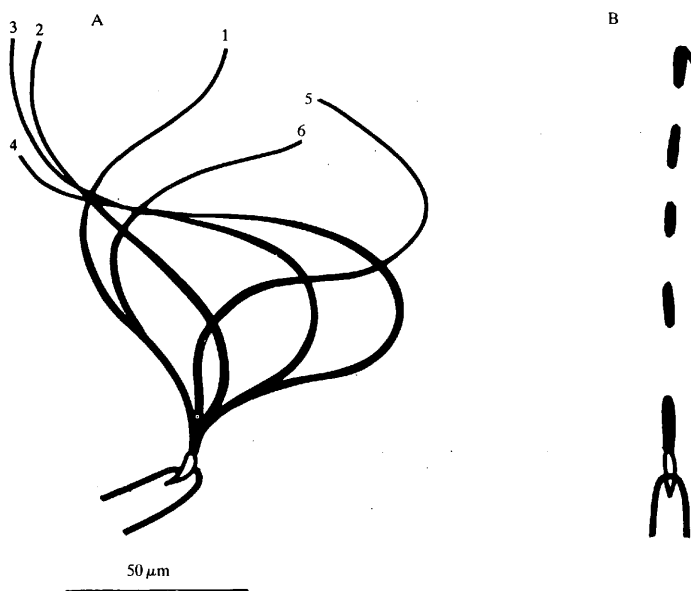


Fig. 7. Traces of a flagellum during a single beat cycle of a typical demembrated cauda epididymal spermatozoon reactivated with  $1 \text{ mmol l}^{-1}$  ATP. (A) The beating flagellum as observed from a direction perpendicular to the beating plane. The numbers indicate the tracings of the flagellum at  $1/30 \text{ s}$  intervals. (B) The beating flagellum as observed from a direction parallel to the beating plane.

values for intact and demembrated spermatozoa were generally smaller than those obtained in the present study. The beat frequencies of the sperm flagella measured stroboscopically in the present study may be more correct than those visually estimated by them.

The testicular and the caput epididymal spermatozoa, which were both virtually immotile when they had an intact plasma membrane, became motile after demembration and reactivation with ATP. This was interpreted by Mohri & Yanagimachi (1980) as indicating that the motor apparatus (the tubulin-dynein system) in these spermatozoa is already functionally assembled in the testis. The present study confirms this conclusion. In these experiments, the initial movements of all the demembrated spermatozoa reactivated with ATP were nearly equivalent to those of intact cauda epididymal spermatozoa. This result suggests that the motor apparatus of the spermatozoa is essentially unchanged during the epididymal transit, as reported by Chulavatnatol & Treetipsatit (1983) for rat spermatozoa.

The average amplitude of the capacitated sperm flagella was about twice that of the intact cauda spermatozoa (cf. Table 1). These data support quantitatively the conclusions of Yanagimachi (1970) that the pattern of movement of capacitated spermatozoa is quite different from that of uncapacitated spermatozoa, and that the amplitude of the capacitated sperm flagella is greater than that of the intact cauda spermatozoa. These phenomena were first called 'activation' of spermatozoa by Yanagimachi (1970) and have more recently been termed 'hyperactivation' (Yanagimachi, 1981).

In rodents, the waveform of the beating flagellum is changed by capacitation as mentioned above. The biological significance of this change is not yet clear (Yanagimachi, 1981). The thrust and hydrodynamic power output developed by the flagellum were numerically computed by the method of Gray & Hancock (1955) with modifications by Lighthill (1976), using parameters for the typical waveform as obtained in this study [(cf. Figs 4A and 5B),  $0.4\text{ }\mu\text{m}$  for the radius of the flagellum,  $180\text{ }\mu\text{m}$  for the total length of the flagellum and  $7 \times 10^{-4}\text{ Pa}\cdot\text{s}$  for the viscosity of the medium (cf. Katz, Yanagimachi & Dresdner, 1978)]. The average thrust exerted by a typical capacitated spermatozoon (cf. Fig. 5B) was  $252\text{ pN}$ , about twice that by a typical intact cauda epididymal spermatozoon (cf. Fig. 4A),  $120\text{ pN}$ . The average hydrodynamic power output developed by a typical capacitated spermatozoon was  $7.9 \times 10^{-13}\text{ W}$ , 2.5 times larger than that by a typical intact cauda epididymal spermatozoon ( $3.2 \times 10^{-13}\text{ W}$ ). In contrast, Katz *et al.* (1978) calculated that the hydrodynamic power output of hamster spermatozoon was about one-tenth that obtained in this study, and they reported that the power output did not change appreciably due to hyperactivation. In the data of Katz *et al.* (1978), the amplitude of the capacitated spermatozoa was about twice that of the intact cauda epididymal spermatozoa, while the beat frequency was about one-half that of the intact ones. In our experiments, as shown in Table 1, the amplitude of the capacitated spermatozoa was also about twice that of the intact caudal epididymal spermatozoa, but the beat frequency was almost unchanged. In rough approximation, the hydrodynamic power output is proportional to the square of both the beat frequency and the amplitude (cf. Taylor, 1952). Therefore the difference in the

power output as determined by Katz *et al.* (1978) and ourselves may be due to the difference in the beat frequency of the flagellum. The difference in the power output is probably due to the difference in the amplitude of the flagellum used for calculation. Katz & Yanagimachi (1981) qualitatively discussed that the change in waveform after capacitation would maximize forcing of the sperm head against the zona material. The two-fold increase in the thrust developed by the flagellum of the capacitated spermatozoa may play an important role in penetrating into the egg.

Cyclic AMP seems to play a significant role in the initiation of sperm motility (Morisawa & Okuno, 1982). In the present study, cAMP accelerated the initiation of flagellar movement in demembranated golden hamster spermatozoa. This fact suggests that cAMP is responsible for the initiation of sperm motility. Moreover, the effects of cAMP on the initiation of flagellar beating was larger in demembranated spermatozoa taken from the testis than in demembranated spermatozoa taken from the cauda epididymides. This result suggests that cAMP modulates the changes which occur during maturation.

There are several reports that cAMP enhances the flagellar beating of demembranated spermatozoa (cf. Tash & Means, 1983). In the present study, the flagellar movement of demembranated golden hamster spermatozoa taken from the testis and the caput and cauda epididymides was enhanced with cAMP. The characteristics of flagellar movement enhanced with cAMP were similar to those of the intact cauda spermatozoa. These results suggest that cAMP may be essential to development of sperm motility during maturation. The mechanism by which cAMP brings about this effect is still uncertain.

As shown in Figs 4A, 5 and especially 7A, the waveform of beating flagella of golden hamster spermatozoa held with a micropipette was asymmetrical and seems to resemble that of beating cilia (cf. Hiramoto, 1974b). The 'reverse' bend of the flagella in the golden hamster is equivalent to the bend propagating from the base to the tip of a cilium during the recovery stroke, whereas the 'principal' bend in the golden hamster is equivalent to the bend during the effective stroke. Mohri & Yano (1981, 1982) drew the same conclusion from comparison of  $\text{Ca}^{2+}$ - and vanadate-induced arrest in mussel gill cilia and in demembranated and reactivated hamster sperm flagella. However, from observations of the final shapes of the sperm flagella arrested with  $\text{Ca}^{2+}$  and vanadate, they surmized that the principal bend in hamster spermatozoa corresponds to the bend propagating during the recovery stroke of cilia (Mohri & Yano, 1982). Based on the observations reported here, this should be revised as described above. Gibbons & Gibbons (1980) discussed that the principal bend of sea urchin sperm flagella is equivalent to the basal bend of the cilium arrested by  $\text{Ca}^{2+}$  and that doublet microtubules nos 5 and 6 would lie on the outer side of the axoneme in the principal bend of the sperm flagellum. Considering these results and the fact that the convex edge of the 'reverse' bend of the flagellum contains doublets nos 5 and 6 in golden hamster spermatozoa (Woolley, 1977), the 'principal' bend defined by Woolley (1977) in golden hamster spermatozoa seems to correspond to the reverse bend in sea urchin sperm flagella, and the 'reverse' bend in golden hamster spermatozoa seems to correspond to the principal bend in sea urchin sperm flagella.

The authors are indebted to Professor Y. Hiramoto of the Tokyo Institute of Technology and Dr M. Okuno of the University of Tokyo for their valuable discussion. The authors also thank Professor Y. Ishijima of the Tokyo University of Agriculture for supplying male golden hamsters. They thank Dr G. B. Witman for critical reading of the manuscript. This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 56480015 and 58340041) from the Ministry of Education, Science and Culture, Japan.

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