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Alterations in Primate Sperm Motility With Maturation and During Exposure to Theophylline

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Micropuncture was used to collect pure suspensions of sperm from the caput and cauda regions of chimpanzee epididymides, which were analyzed with a Motion Analysis VP-110. Sperm recovered from the caput region showed no forward motility. Incubation of these sperm with cauda epididymal fluid affected motility in 62%–90% of the sperm. Dilution of cauda sperm into buffer containing >50 mM theophylline resulted in immediate initiation of progressive forward motility. Although this motility was maintained by at least 50% of the sperm for over 5 hr, these "activated" caput sperm did not penetrate zona-free hamster ova. These data show that sperm from the caput epididymis of the chimpanzee have the capacity for normal motility but do not have the capacity to bind to and penetrate an ovum. Cauda epididymal chimpanzee sperm were motile at the time of recovery and this motility was maintained for over 5 hr. These sperm penetrated both hamster zona-free ova and intact chimpanzee ova. These data show that sperm from the cauda epididymis of the chimpanzee have the capacity for normal motility and also have the capacity to bind to and penetrate an ovum. This is the first use of computer assisted analysis to quantify motility in maturing nonhuman primate sperm.

Key words: chimpanzee, computer

INTRODUCTION

During epididymal transit sperm of most mammalian species, including primates, develop the capacity for progressive motility and for fertilization of the oocyte. Caput epididymal sperm are immotile and infertile, whereas cauda epididymal sperm are motile and have potential fertilizing capacity [Orgebin-Crist et al., 1975; Mann, 1964]. These two changes are not synonymous in that even when the motility pattern of caput epididymal sperm is indistinguishable from that of ejaculated sperm the lack of fertility persists [Cornwall et al., 1986].

The specific components of epididymal fluid that stimulate sperm motility are unknown. There is evidence that a component, forward-motility protein (FMP), present both in cauda epididymal fluid and in seminal plasma, stimulates motility of epididymal sperm [Acott & Hoskins, 1981; Hoskins et al., 1978; Serres & Kann,

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1984]. Moreover, Cornwall et al. [1986] have demonstrated that sulfhydryl oxidizing agents are needed to prevent flagellar angulation in newly motile sperm and that fatty acid contamination of albumin can prevent the initiation of normal motility patterns. The lumenal fluid concentrations of carnitine, glycerylphosphorylcholine, sodium, potassium, and calcium [Turner & Giles, 1981; Hinton & Setchell, 1980] also may be involved in the initiation of motility in maturing sperm. The intracellular concentration of cyclic AMP is important in sperm motility. In mammals, the mechanism of cAMP action has been explored in sperm from the bull [Vijayaraghavan & Hoskin, 1986; Lindemann, 1978], boar [Dacheux & Paguignon, 1980; Garbers et al., 1975], hamster [Morton et al., 1973; Morton et al., 1974], guinea pig [Hyne & Garbers, 1979; Yanagamachi & Usi, 1974], and human [DeTurner et al., 1978; Schoenfeld et al., 1975]. Phosphodiesterase inhibitors, including theophylline, and dibutyl cAMP increase motility of caput epididymal sperm [Garbers et al., 1975; Garbers & Kopf, 1980], whereas imidazole and its analogs depress motility of cauda epididymal sperm [Appleman et al., 1973]. It is probable that elevation of intracellular cAMP, either by stimulation of synthesis or inhibition of breakdown, results in phosphorylation of a protein(s) essential for motility. This could be a Ca^{2+} dependent protein as Ca^{2+} alone increases motile activity in hamster [Morton et al., 1974], guinea pig [Hyne & Garbers, 1979], ram [Breitbart et al., 1985], and human [Yanagimachi & Usui, 1974; Fakih et al., 1986] sperm, and the calcium ionophore A23187 activates bovine sperm motility [Babcock et al., 1976].

Quantitating changes in motility in the pattern and speed of the sperm have presented difficult technical problems [Stephens et al., 1981]. Recognition of the limitations of subjective methods for evaluation of sperm motility have led to the development of objective methods for semen analysis. Initial methods monitored the "integrated" sperm motility using measurement of changes in intensity of diffused light, diffusion of light, uv absorption, optical density, or microphotometry [Boselaar & Spronk, 1952; Dubois et al., 1975; Timourian & Watchmaker, 1970]. More recent methods have utilized cinephotography or videomicrography, with various levels of computer aided analysis of the video data [Schoevaert-Brossault, 1984; Katz et al., 1985; Burke & Kapinos, 1985; Samuels & Van Der Horst, 1986]. In this study changes in chimpanzee sperm-motility patterns during sperm maturation both before and after exposure to theophylline have been quantitated with digitized video techniques.

MATERIAL AND METHODS

Six adult male chimpanzees (*Pan troglodytes*) of proven spermatogenic status, and weighing between 42 and 48 kg, were used in this study. Ejaculates were provided from each animal by masturbation or by the use of an artificial vagina. The coagulum was maintained at 37°C for 45 min, and the volume of liquefied semen was measured. Sperm were sedimented by centrifugation at 800*g* for 5 min at room temperature (RT), resuspended in Ham's F-10 (Sigma Chemical, St. Louis, MO) pH 7.4, 280 mOsm/liter (Ham's F-10) to a volume 5 times that of the liquefied semen, and centrifuged at 800*g* for 5 min at RT. A population of motile sperm was obtained with the swim-up method of Courtot et al. [1981]. The sperm pellet first was disturbed gently with one drop of Ham's F-10 made 10% with human cord serum (Ham's F-10/HCS) and then one ml of Ham's F-10/HCS was layered carefully over the pellet and the suspension was maintained at 30°C for 30 min. The layer containing the motile sperm was removed and diluted to a final concentration of 5×10^6 sperm/ml in Ham's F-10/HCS.

Epididymal samples were recovered by micropuncture of the convoluted ducts of the caput and cauda regions of one epididymis of each chimpanzee [Hinton & Setchell, 1980; Young et al., 1985]. Samples were diluted into 100 μl of PBS, and

sperm were pelleted by centrifugation at 800*g* for 5 min at RT, washed in Ham's F-10 by centrifugation, and resuspended to a final concentration of 5×10^6 sperm/ml in Ham's F-10/HCS.

The samples of washed caput epididymal, cauda epididymal, and ejaculated sperm each were divided into aliquots. Four aliquots were made 0, 5, 10, or 30 μg with theophylline (Th). A fifth aliquot was incubated in 30 μg of cauda epididymal fluid (CdF) for 30 min, washed in 10 volumes of Ham's F-10 by centrifugation at 800*g* and resuspended to 5×10^6 sperm/ml in Ham's F-10/HCS.

Sperm motility was observed in a Makler (Sefi Products, Haifa, Israel) counting chamber on a Leitz light microscope, at × 250 magnification, and a videotape record was made with a JVC HD150C VHS videotape recorder. Approximately 30 sec of tape, recorded from each of at least three fields in the counting chamber, were analyzed with a Motion Analysis System Motion Analyzer with a VP110 digital processor and a dedicated IBM PC-AT computer (Motion Analysis Corp., Santa Rosa, CA).

Motion analysis involves the generation of a digitized image of the swimming sperm. For each frame of data, a centroid is calculated that represents the position of each sperm head visible in the frame. The centroids are then connected to identify the path taken by each sperm. Calculation of linearity and speed depends only on mathematical relationships within the video field and will be accurate provided the video screen is calibrated correctly by recording of a micrometer grid prior to recording of sperm data. These analyses are subject to arbitrary parameters which govern such factors as the maximum and minimum object size that will be identified as a "sperm," the extent to which the computer will project a path when connecting centroids, and the degree of movement that represents an immotile sperm. These avoid the program counting nonsperm objects as sperm, ensure that complete paths are calculated even though vertical movement of a sperm may move it out of focus for one or two frames, and account for particle movement in the recorded field resulting from vibration and Brownian motion. To ensure accurate recording, computer-generated data were compared with observed data. Program parameters were selected to provide comparable results for swimming pattern, total number of sperm, and percentage of motile sperm. It is impractical to list all these parameters. However, a copy of the programs used is available from the authors, and the basic parameters are provided in Table I. Criteria used for analyses are listed in Table II.

Quantitative analyses of records obtained with each sample were conducted at the times shown in Table III. The time selected demonstrates the maximal effect observed with each treatment.

RESULTS

Data derived from computer printouts of analyzed video data from the recorded motion of chimpanzee sperm are provided in Table IV. Examples of raw data are shown in Fig. 1.

Caput epididymal chimpanzee sperm were immotile (velocity less than 10 μm/sec), and showed a predominant (>50%) type 1 motility pattern. However, after 30 min in in vitro culture, twitching of the tail was observed in approximately 10% of these sperm. Incubation of caput epididymal sperm in 5 mM Th for 30 min (CpS-5-30) had a small but significant effect on motility. The percentage of motile sperm increased to 47% and mean velocity to 22.8 μm/sec ($P < .05$). As the recorded velocity resulted from sporadic twitching of the sperm tails in a type 2 sperm motility pattern, the progressive motility remained below 3 μm/sec. Linearity was not significantly different after 30 min in the presence or absence of 5 mM Th ($P > .05$). Incubation in 30 mM Th for 30 min (CpS-30–30) resulted in a significant increase in velocity to 43.4 μm/sec and in progressive motility to 23.8 μm/sec. Approximately

TABLE I. Basic Parameters for Semen Analysis

Frame rate	30/sec
Duration of data capture	1 sec
Frames analyzed	30
Minimum number of sperm	100
Threshold velocity	5 μm/sec
Micron:pixel ratio	1.546
Sperm dilution	$6 - 15 \times 10^6$

TABLE II. Semen Evaluation Parameters

Count	Number of sperm/ml
Percentage motile	Percentage of sperm showing motility
Percentage live	Percentage of sperm with intact membranes [Martin & Davidson, 1976]
Linear speed	Speed of sperm along the recorded path
Progressive speed	Progressive forward motility of sperm
Net/gross ratio or linearity	Progressive speed/linear speed
Predominant pattern	Path patterns most frequently observed 1. Immotile 2. Irregular 3. Straight 4. Irregular straight 5. Hyperactivated

10% of Cp-S-30–30 sperm showed evidence of hyperactivity (pattern 5) (Fig. 1A), ($P < .001$), 45% showed either a type 2 or a type 3 pattern, and 45% remained immotile. Linearity of the motile sperm increased significantly from 0.041 to 0.41 ($P < .05$), and the CpS-30-30 sperm showed active forward progressive motility (mean 19.2 μm/sec).

After 30 min exposure of caput epididymal sperm to chimpanzee cauda epididymal fluid (30 μg) followed by washing and resuspension in Ham's F-10/HCS, 62% of the sperm were motile at 0 min (CpS/CdF-0-0), and 90% were motile at 30 min postincubation (CpS/CdF0–30). The predominant pattern was either type 2 or type 4. There was no change in velocity or in progressive motility of the sperm during the 30-min period. Progressive motility was less than 8 μm/sec, compared with approximately 20 μm/sec after exposure of caput sperm to only 30 mM Th for 30 min (CpS-30-30). Linearity for CpS/CdF-0-30 sperm was 0.15, compared with 0.41 for CpS-30-30 sperm ($P < .05$). Caput sperm, incubated in cauda fluid, washed, and resuspended in Ham's F10/HCS containing 30 mM Th (CpS/CdF-30-0 and CpS/CdF-30-30), showed a progressive motility of approximately 20 μm/sec. Although this activity was maintained for up to 5 hr in in vitro culture, such sperm did not penetrate hamster zona-free oocytes or intact chimpanzee ova and are therefore considered infertile.

Cauda epididymal chimpanzee sperm were motile at recovery (CdS-0-0) and showed a linear velocity of 112 μm/sec and a progressive motility of approximately 33 μm/sec. The motility pattern was usually type 3 and was maintained for more than 5 hr in in vitro culture. When incubated in the presence of 30 mM Th for 30 min (CdS-30-30), approximately 20% of CdS showed a type 5 pattern of motility, and the remaining 67% showed a type 4 motility pattern.

TABLE III. Samples Analyzed

	Theophylline (mM)	Cauda fluid	Minutes of incubation	Abbreviation
Caput epididymal	0	–	0	CpS-0-0
Chimpanzee sperm	0	–	30	CpS-0-30
	5	–	30	CpS-5-30
	30	–	30	CpS-30-30
	0	+	0	CpS/CdF-0-0
	0	+	30	CpS/CdF-0-30
	30	+	0	CpS/CdF-30-0
	30	+	30	CpS/CdF-30-30
Cauda epididymal	0	–	0	CdS-0-0
Chimpanzee sperm	0	–	30	CdS-0-30
	0	–	30	CdS-30-30
Ejaculated	0	–	0 preswimup	ES-0-0
	0	–	0 postswimup	ES/S-0-0

TABLE IV. Semen Motility Parameters[a]

Source pattern	Predominant pattern	Percent motile	Percent live	Velocity of motile sperm[b,c]	Linearity of motile sperm[b,c]	Progressive motility[b,c]
CpS/0-0	1	5	89	8.9 ± 0.4	0.068 ± 0.02	1.2 ± 0.2
CpS/0-30	1	12	89	9.3 ± 0.6	0.092 ± 0.03	1.1 ± 0.31
CpS/5-30	1/2	47	89	22.8 ± 1.59	0.041 ± 0.01	2.9 ± 0.72
CpS/30-30	3/4	55	89	43.4 ± 4.07	0.41 ± 0.07	23.8 ± 5.1
CpS/CdF-0-0	2/4	62	89	32.2 ± 1.8	0.157 ± 0.015	7.6 ± 1.47
CpS/CdF-0-30	2/4	90	89	34.6 ± 1.26	0.126 ± 0.01	6.57 ± 1.1
CpS/CdF-30-0	4	84	89	55.8 ± 3.64	0.251 ± 0.026	24.2 ± 2.31
CpS/CdF-30-30	2/4	97	89	56.6 ± 5.4	0.17 ± 0.031	19.8 ± 1.2
CdS-0-0	3	100	94	112.0 ± 2.12	0.288 ± 0.013	33.15 ± 5.12
CdS-0-30	3	95	94	101.0 ± 1.79	0.252 ± 0.022	29.21 ± 1.45
CdS-30-30	4/5	87	94	107.0 ± 2.11	0.421 ± 0.029	23.12 ± 2.61
ES	4	87	90	42.0 ± 0.97	0.241 ± 0.021	16.21 ± 0.81
ES/S-0-0	3/4	97	99	54.0 ± 0.72	0.397 ± 0.015	21.68 ± 2.25

[a]See Table II.
[b]Mean of six samples ± S.E.
[c]μm/sec.

Washed, motile, ejaculated chimpanzee sperm before swimup had a mean velocity of 42 μm/sec and a progressive motility of 16.21μm/sec. After swimup, these values increased but not significantly, to 54 ± .72 and 22 ± 1.2 μ/sec, respectively. The predominant pattern was type 4. These values are not significantly different from those observed with CpS exposed both to CdF and to 30 mM Th (CpS/CdF-30-30). However, motility of the ES/S, both linear and progressive, was significantly less than that of CdS ($P < .05$). There was no significant difference in mean linearity between ES and CdS; and therefore, the observed change in motility probably reflects a less uniform distribution of motility in the ejaculated sperm.

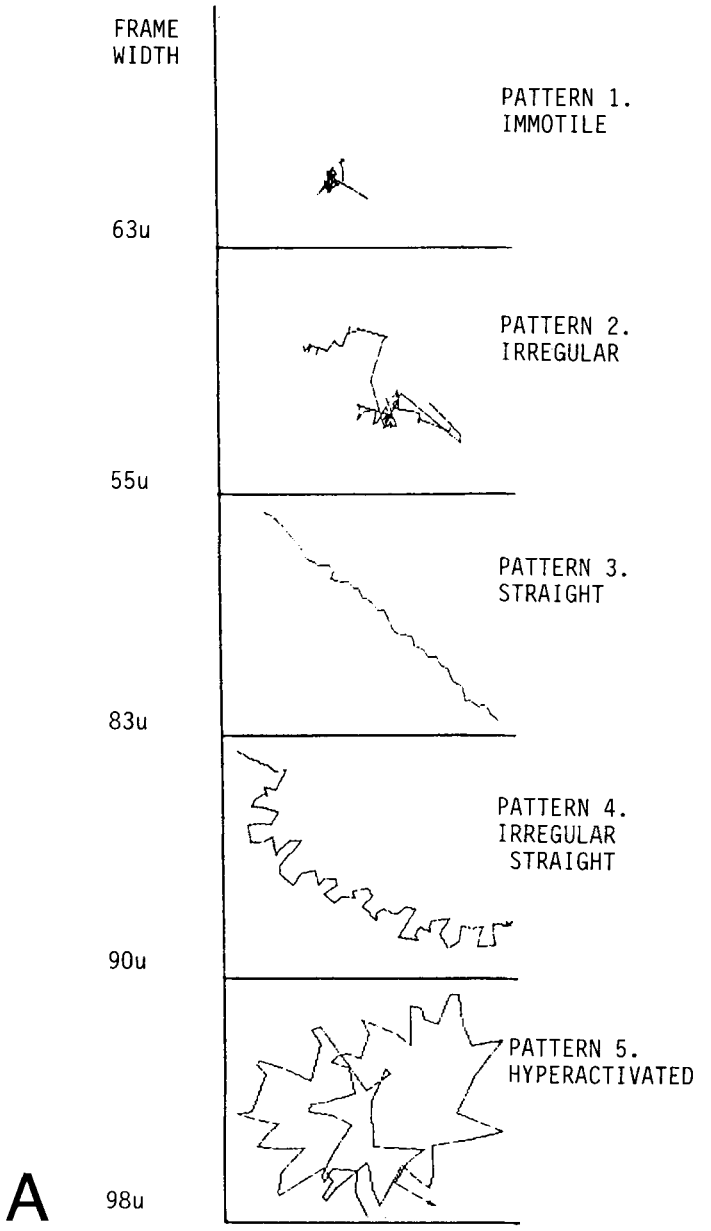
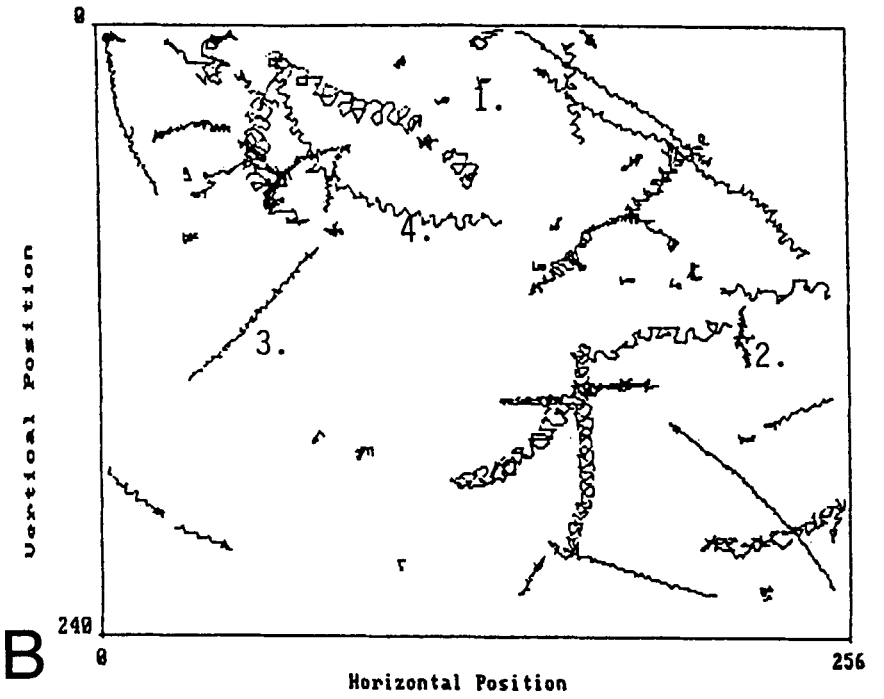


Fig. 1. A: Swimming paths of spermatozoa used for classification of sperm-motility; path shows the relative progressive motility of each pattern. B: Representative plot of sperm paths: patterns 1–4 are identified paths plotted for 3 sec. Original sample: ejaculated sperm.



DISCUSSION

Computer based analysis of videotaped records of sperm motility, although still requiring some degree of operator intervention to establish acceptable parameters, does provide quantitative data on alterations in sperm motility. Using this methodology to study maturational changes in chimpanzee sperm motility, we have demonstrated: 1) caput epididymal chimpanzee sperm are immotile; 2) motility can be initiated in these sperm by exposure to fluid from the chimpanzee cauda epididymis or to theophylline; 3) cauda epididymal chimpanzee sperm are motile; 4) the motility pattern of these sperm can be modified by exposure to theophylline; and 5) quantitative changes in the motility pattern of chimpanzee ejaculated sperm occur subsequent to capacitation.

Sperm recovered from the caput region of the epididymis of all mammalian species are immotile, although "twitching" of the tails sometimes may be observed [Bedford, 1975]. The capacity for forward progressive motility develops as these sperm mature during epididymal transit [Bedford, 1975]. Whereas sperm recovered from the cauda region of the epididymis of various mammalian species demonstrate different motile activity, cauda epididymal sperm of the rabbit [Turner & Reich, 1985], chimpanzee [Young et al., 1985], and macaque (Gould, unpublished observations) are highly motile; it has been suggested that this motility results from the effect of a factor in cauda epididymal fluid that specifically stimulates sperm motility [Hoskins et al., 1978]. However, cauda epididymal sperm of the human, hamster, guinea pig, and rat are immotile [Turner & Reich, 1985]. This immotility has been attributed to the presence in cauda epididymal fluid of a motility-inhibiting factor [Turner & Reich, 1985; Usselman & Cone, 1983]. Nevertheless, capacity for motility

in these cauda epididymal sperm is demonstrated by the acquisition of forward progressive motility on dilution into appropriate buffered salt solutions [Acott & Carr, 1983; Turner & Howard, 1978].

Because of difficulties involved in obtaining epididymal samples from primates uncontaminated by extraneous fluid or cells, the effects of epididymal fluids on initiation and characteristics of primate sperm motility have been difficult to study. Sperm recovered by dissection of the caput epididymis of a chimpanzee 6 hr post mortem showed significant motility (Gould, unpublished data), which was attributed to their exposure to tissue proteins. Therefore, in this study, we used in vivo micropuncture to recover pure samples of lumenal contents from the caput and cauda regions of the chimpanzee epididymis [Young et al., 1985], a procedure that enabled quantitative analysis of epididymal sperm motility and of the effects of cauda epididymal fluid on caput epididymal sperm.

Sperm recovered from the chimpanzee proximal caput epididymis by micropuncture are immotile. Quantitative analyses of the motility patterns induced in these sperm after incubation in chimpanzee cauda epididymal fluid, in theophylline, and sequentially in cauda epididymal fluid and then theophylline showed significant differences. Exposure of caput epididymal sperm to cauda fluid did stimulate a percentage of sperm to show forward, progressive motility. Approximately 50% of chimpanzee sperm were stimulated to move, equivalent to the stimulation reported for caput epididymal hamster sperm incubated in hamster cauda epididymal fluid [Cornwall et al., 1986]. This percentage is significantly greater than the stimulation reported for caput epididymal rat sperm [Turner Giles, 1982] and for caput epididymal hamster sperm [Cornwall et al., 1986] incubated in seminal fluid, but similar to that reported for caput epididymal bull sperm incubated in seminal fluid [Acott et al., 1983].

Motility inducing components in reproductive fluids may be nonspecies specific as both human seminal plasma and bull seminal plasma did induce some motility in caput epididymal hamster sperm [Cornwall et al., 1986]. This induced motility was described as "inadequate" as compared to that of ejaculated hamster sperm in three specific areas: 1) the presence of a "flagellar angularity" in motile caput sperm; 2) a low percentage of motile sperm; and 3) a longevity of motile sperm less than 2 hr. With chimpanzee sperm, approximately 30% of caput epididymal sperm, stimulated by exposure to cauda epididymal fluid, showed "flagellar angularity." It has been suggested that this flagellar angularity results from a deficiency in disulfide bonding in immature caput sperm [Calvin & Bedford, 1971] and can be corrected by addition of sulfhydryl oxidizing agents to the incubation medium [Cornwall et al., 1986].

Increasing levels of cAMP in mammalian sperm during epididymal maturation have been shown to correlate with increasing motility [Hoskins et al., 1975; Amann et al., 1982]. Elevation of cyclic AMP levels effected by the in vitro addition of cyclic AMP phosphodiesterase inhibitors induced motility in immotile and immature caput epididymal sperm [Garbers et al., 1973; Hoskins et al., 1975]. It has been suggested that this response to cAMP results from alterations in calcium levels within the cell [Hong et al., 1985].

Immotile caput epididymal chimpanzee sperm exposed to >5 mM theophylline show motile activity There is a significant increase both in their velocity and in their linearity, compared with those exposed to cauda fluid alone. In fact, the motility pattern of caput sperm exposed to both cauda fluid and theophylline closely resembles that of cauda sperm exposed to theophylline alone. Although these caput epididymal sperm remained motile for more than 5 hr in vitro, they were shown to be infertile, both in heterologous sperm penetration assay and in homologous in vitro fertilization assay [Gould, 1983; Young et al., 1987].

In the chimpanzee, almost 100% of cauda epididymal sperm are highly motile. Their velocity is greater than that of sperm from any other portion of the male tract, including ejaculated sperm. This finding is in contrast to the 65% motility reported for cauda epididymal hamster sperm [Cornwall et al., 1986] and the 50% reported for cauda epididymal bull sperm [Hoskins et al., 1978]. However, the effect of fatty acids, reported to impair sperm survival, has been invoked by Cornwall et al. [1986] and by Hoskins et al. [1978] as a reason for poor survival of cauda epididymal hamster sperm and cauda epididymal bull sperm in vitro. Fatty acids are associated with the BSA used in many in vitro culture media; the absence of BSA from the media we used may account for the enhanced survival rates noted in our studies.

Phosphodiesterase inhibitors also have been shown to increase the motility of cauda epididymal and ejaculated sperm [Amelar et al. 1980]. Using a subjective assay system, Turner and Giles [1981] demonstrated that the addition of 10 mM caffeine to rat sperm recovered from the distal region of the cauda epididymis stimulated "full" motility. Exposure of cauda epididymal chimpanzee sperm to 30 mM theophylline resulted in a significant increase in linearity, with no significant alteration in velocity. The linearity of cauda sperm stimulated with theophylline was approximately equal to that of caput sperm stimulated with theophylline, but remained greater than that of caput sperm stimulated with cauda fluid or with cauda fluid and theophylline. This could reflect the addition of suppressive components in the corpus and cauda epididymis that prevent the exhibition of hyperactivity before incubation in utero. The presence of such a repressive mechanism would then be similar to, but quantitatively less than, the total suppression of motility observed in cauda epididymal sperm of other mammalian species [Turner & Reich, 1985].

Ejaculated chimpanzee sperm show a motility pattern with a velocity lower than that of cauda epididymal sperm and a linearity that is lower, i.e., the swimming pattern is more convoluted. Incubation of ejaculated chimpanzee sperm in vitro under conditions compatible with capacitation, as demonstrated by the subsequent in vitro fertilization of chimpanzee oocytes [Gould, 1983], resulted in an increase in velocity and a drop in linearity, with 25–40% of the sperm demonstrating highly irregular patterns of motion resembling hyperactivation. Incubation of ejaculated chimpanzee sperm in utero for 60 min before recovery and washing also resulted in a decreased linearity and an incidence of hyperactivation not significantly different from that observed after incubation in vitro. Hyperactivation has been clearly demonstrated in hamster, guinea pig, mouse, and dog [Yanagimachi & Usui, 1974]. The occurrence of hyperactivation is less clearly established in primate sperm, although there is some evidence indicating that it does occur in the human [Burke & Kapinos, 1985]. The time relation of the initiation of hyperactivity, which takes approximately 1 hr in vivo, but is almost instantaneous for caput sperm incubated in cauda fluid and theophylline in vitro, when taken together with the fact that caput epididymal sperm are infertile, shows that the motility pattern is not a direct indicator of capacitation. This supports the contention of Barros [1974] that activated sperm are not always capacitated.

Acquisition of motility by maturing sperm is not a simple progression, as is shown by the fact that cauda epididymal sperm exposed to theophylline show an increase in linearity but do not show the hyperactivated pattern of motility exhibited by caput epididymal sperm exposed to theophylline. This may reflect the presence of components in the corpus and cauda epididymis that suppress the initiation of hyperactivity before incubation in utero. Glyceryl phosphorylcholine and carnitine cause quiescence of cauda epididymal rat sperm but do not prevent induction of motility [Turner & Giles, 1981]. In the chimpanzee, we have shown a temporal relationship between addition, in the cauda epididymis, of a specific 27 Kd compo-

nent with inhibition of the potential for hyperactivity [Young et al., 1987]. Removal of this 27 Kd component during incubation in utero correlates with recovery of the potential for hyperactivation. However, any direct relationship of this 27 Kd component to the changing motility patterns observed in this study remains to be determined.

CONCLUSIONS

1. Sperm from the caput epididymis of the chimpanzee have the capacity for normal motility but do not have the capacity to bind to and penetrate an ovum.

2. Sperm from the cauda epididymis of the chimpanzee exhibit normal motility and have the capacity to bind to and penetrate an ovum.

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