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HYPERACTIVATED MOTION PATTERNS OF RABBIT SPERM CELLS

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RESEARCH AND TECHNOLOGY DIRECTORATE

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Sperm cells from some rabbits develop a high speed random non-progressive motion characterized by wide bending and whip lashing of the tail, known as hyperactivated motility, when incubated for longer than 12 hr in a defined medium (DM). Three types of hyperactivated motions with different motility parameters were identified. As hyperactivated motility is a requirement for fertilization, the ability to recognize and measure the types of hyperactivated cells before and after exposure to chemicals may be a means to screen and assess chemicals for their reproductive effects.

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PREFACE

The work described in this report was authorized under Project Nos. F8J2-10-005 and 1N6A. This work was started in June 1989 and completed in October 1991.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institute of Health Publication No. 85-23, 1985, as promulgated by the committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council (Washington, DC). These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the laboratory Animal Use and Review Committee (LAURC), U.S. Army Chemical Research, Development and Engineering Center (CRDEC),* which oversees the use of laboratory animals.

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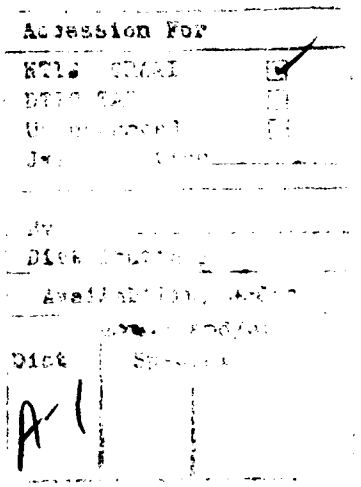

*CRDEC is now known as the U.S. Army Edgewood Research, Development and Engineering Center.

QUALITY ASSURANCE

This study, governed by protocol number 210910430000, was examined for compliance with Good Laboratory Practices as published by the U. S. Environmental Protection Agency in 40 CFR Part 792 (effective 18 September 1989). The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase Inspected	Date Inspected	Date Reported to Study Director/Manangement
Video-taping	05 Nov 1991	05 Nov 1991
Final Report	28 Jun 1993	30 Jun 1993

To the best of my knowledge, the methods described in this report were the methods followed during the study as indicated by the raw data found in the laboratory notebook. The report was determined to be an accurate reflection of the raw data recorded.

Kenneth P. Cameron

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Quality Specialist
Life Sciences Department

30 June 1993
Date

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HYPERACTIVATED MOTION PATTERNS OF RABBIT SPERM CELLS

1. INTRODUCTION

The quality of sperm cell motion in semen is routinely used as an indicator of fertility even though seminal sperm cells are unable to fertilize an egg. Eutherian sperm cells undergo a final maturation process, known as capacitation, in the female reproductive tract environment that permits fertilization. During capacitation, the character of sperm motility changes so that at the time and site of fertilization, sperm possess an energetic and random swimming pattern characterized by wide bending and whip lashing of the tail.[1-4] As development of hyperactivated motility is a necessary preliminary to fertilization, any substance that inhibits hyperactivation could be conceived as a potential reproductive toxicant. Based on changes in sperm cell swimming profiles, an in vitro system design for assessing and screening of potential reproductive toxicants requires some knowledge of the swimming patterns of sperm cells throughout the period of capacitation. This report describes the motility patterns of rabbit sperm cells incubated in a capacitating medium.[5]

2. MATERIALS AND METHODS

2.1 Animals.

New Zealand white rabbits were individually housed in standard rabbit cages in a room maintained at 20-23 °C and 50 ± 10% relative humidity (RH) with a 12 hr light/dark cycle. Certified approved laboratory rabbit chow and water were available ad libitum.

2.2 Collection and Purification of Sperm Cells.

Semen was collected and sperm cells purified by centrifugation through a discontinuous Percoll gradient as previously described.[6]

2.3 Incubation and Videotaping of Sperm Cells.

Incubation of the purified sperm cells in Defined Medium (DM)[5] and videotaping of sperm motion was carried out as previously described.[7]

2.4 Analysis of Sperm Motion Characteristics.

Analysis of sperm cell motion trajectories recorded on videotape was carried out with the CellSoft motion analyzer, series 3000 with ALH, Beat frequency, Circular Motion and

Research Modules. Software settings used for analysis of the trajectories of cells incubated for up to 6 hr were those determined previously.[7] Minimum number of frames set for sperm cells, percentage of motile cells, curvilinear velocity (Vc), linearity (Lin), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), and circular motion were 1, 2, 7, 3, 7, 7, and 10, respectively. Analysis was carried out at 30 frames/s and each microscope field was tracked for 30 frames. Other settings were color, white; dilution factor 0.5 (20 μm deep chamber used for videotaping); ALH parameters, Vc > 20 μm/s and Lin 0; and circular parameters, Vc > 20 μm/s and maximum radius 100 μm; size range 5-70 pixels; and minimum and maximum velocities 20 and 200 μm/s. The pixel scale for the digital image system described[7] was 0.97 μm/pixel. The gray level settings were adjusted for analysis of each field. Because of the rapid and random motion of hyperactive sperm cells resulting in movement in and out of focus of the video camera, the maximum velocity setting was increased to 500-1200 μm/s and the pixel size range was changed to 1-120 pixels.

3. RESULTS

3.1 Linear Motion.

Rabbit sperm cells suspended in a chamber 20 μm deep displayed two types of cellular motions in which the tail beat was symmetrical, namely, linear progression with rotation of the sperm head about the axis of progression and circular motion in an anti-clockwise direction without head rotation. When suspended in a chamber whose depth was less than the length of the cell, only circular motion was observed. At the start of incubation, the velocity of movement and rate-of-head rotation was low. After 1 hr incubation, sperm cell motion appeared more vigorous with cells exhibiting a higher rate of head rotation and velocity of progression, in agreement with the increase in Vc previously observed.[7] Over the next 2 hr, subpopulations of linear progressive sperm cells developed different patterns of movement. This is shown in Figure 1 where Figure 1a-1c are trajectories at the beginning of incubation and Figure 1d-1h are trajectories 2 to 3 hr later. A previous study showed that the average amplitude of lateral head displacement (AALH) of the sperm population increased during 2-6 hr incubation.[7] The AALH of the subpopulations of sperm cells possessing a sinusoidal trajectory (Figure 1c-1h) was higher than the AALH at the commencement of incubation. The increases in head rotation rate and AALH were not reflected in any noticeable change in the overall trajectories of the population of sperm cells. Sperm cells traced circles of different radii, but here also trajectories of cells with high AALH were not different from those with low AALH (Figure 2). These types of motion patterns were found during 6 hr of incubation.

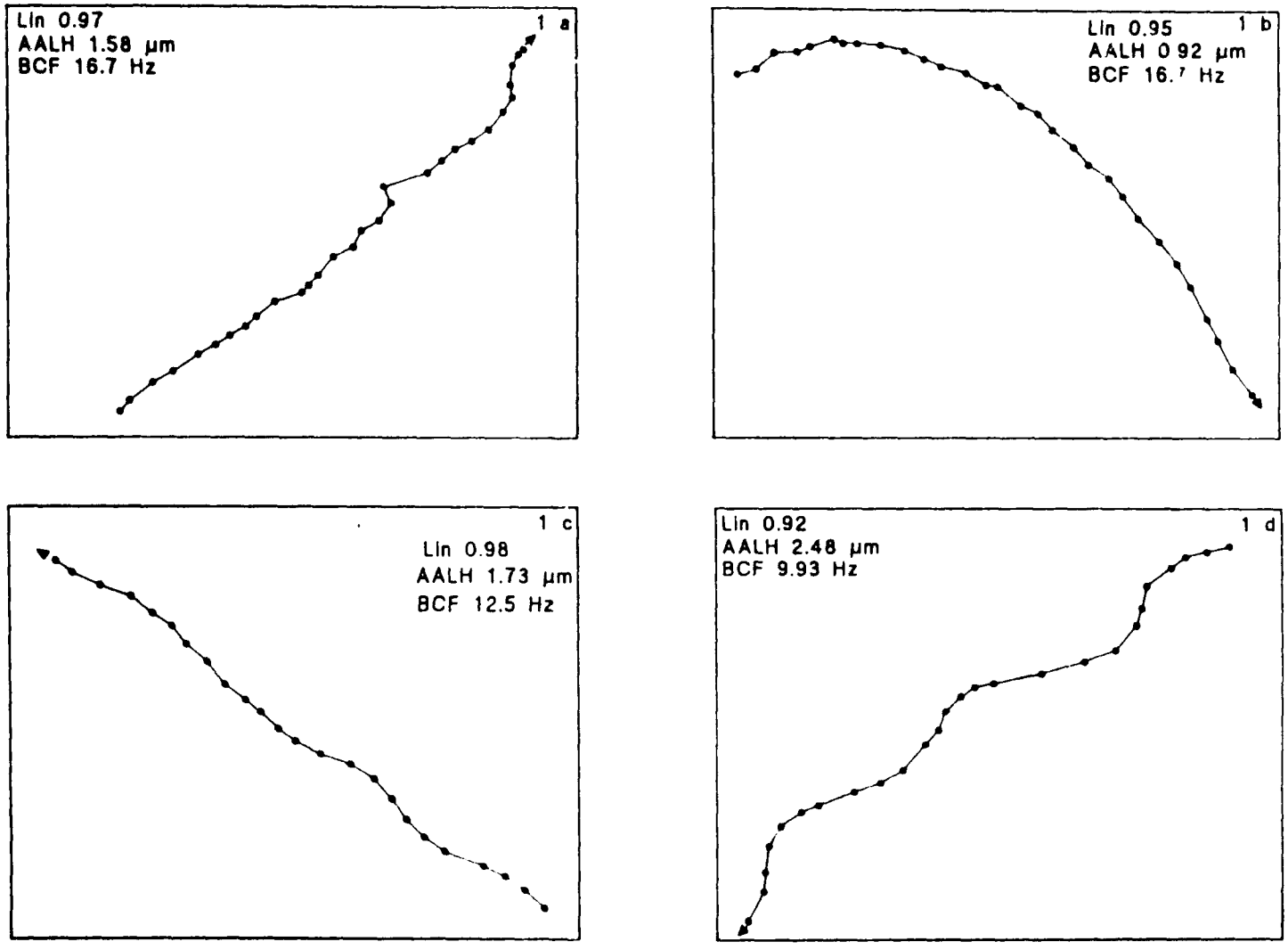

Figure 1. Trajectories of Rabbit Sperm Cells During Incubation in DM.
1a-1c: Progressive trajectories at commencement of incubation.
1d-1h: Progressive trajectories after 2-3 hr incubation.

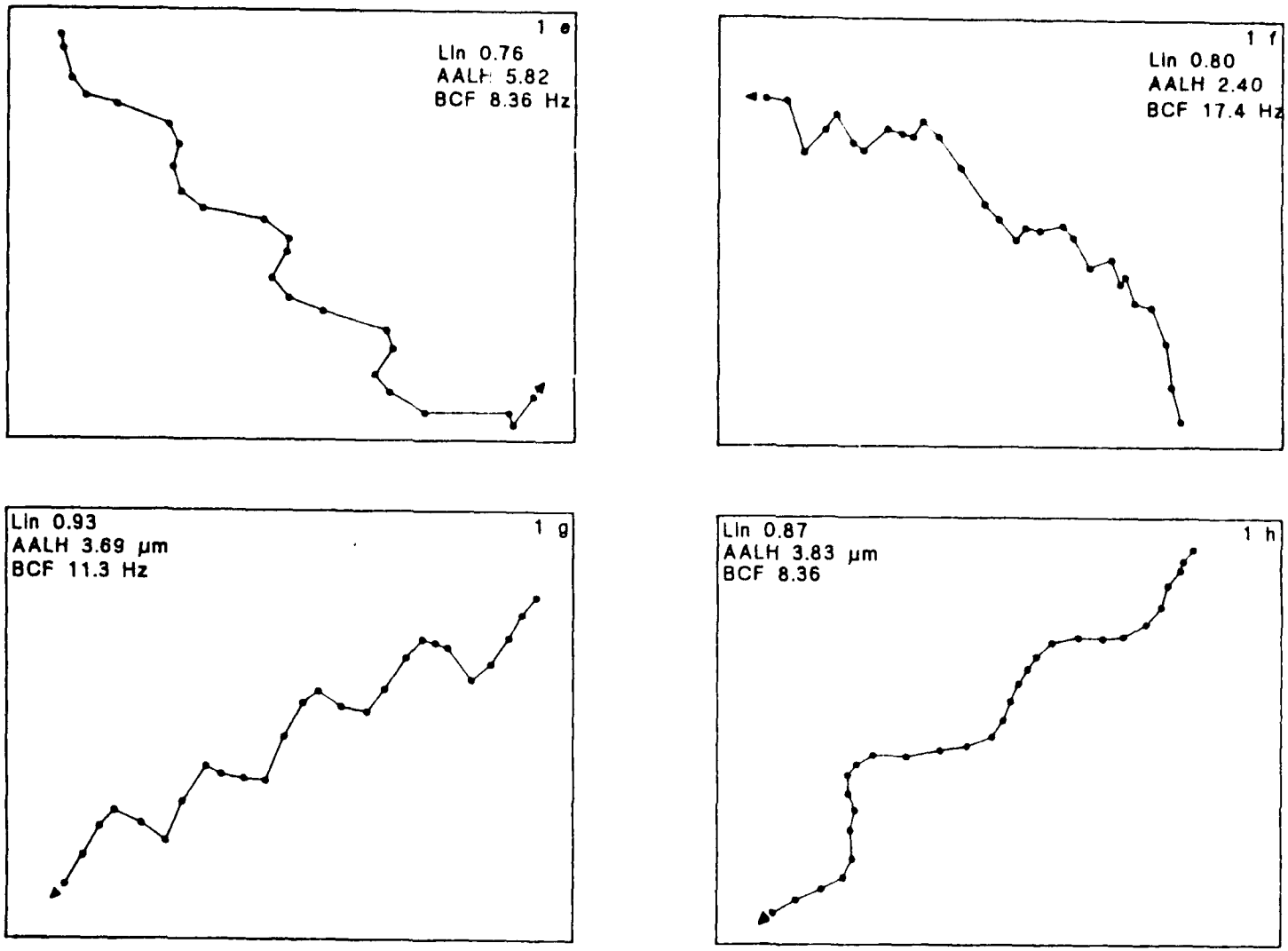

Figure 1. Trajectories of Rabbit Sperm Cells During Incubation in DM.
1a-1c: Progressive trajectories at commencement of incubation.
1d-1h: Progressive trajectories after 2-3 hr incubation. (Continued)

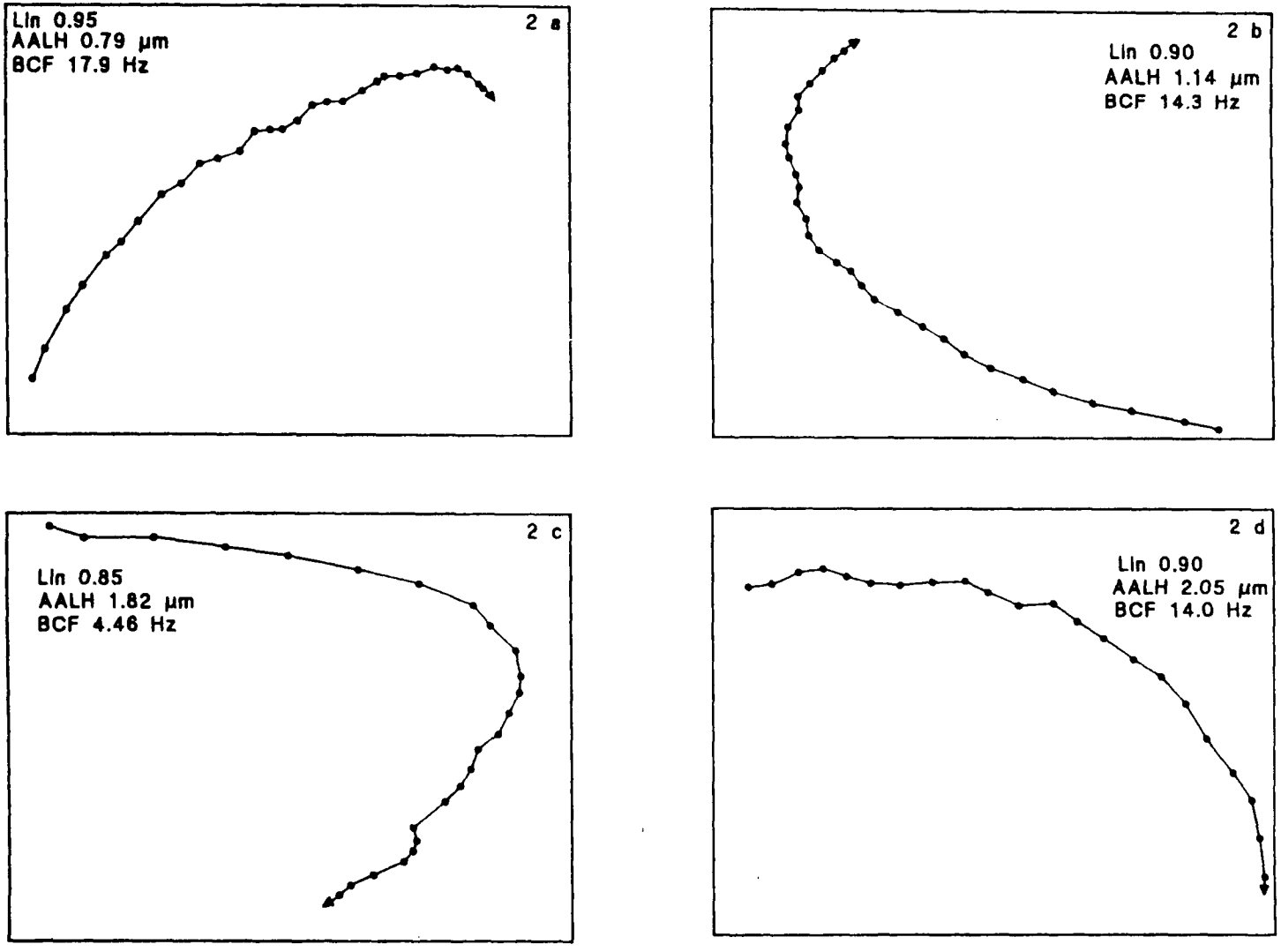

Figure 2. Trajectories of Rabbit Sperm Cells Over 6 hr Incubation in DM, Circular Motion with Different Radius and AALH

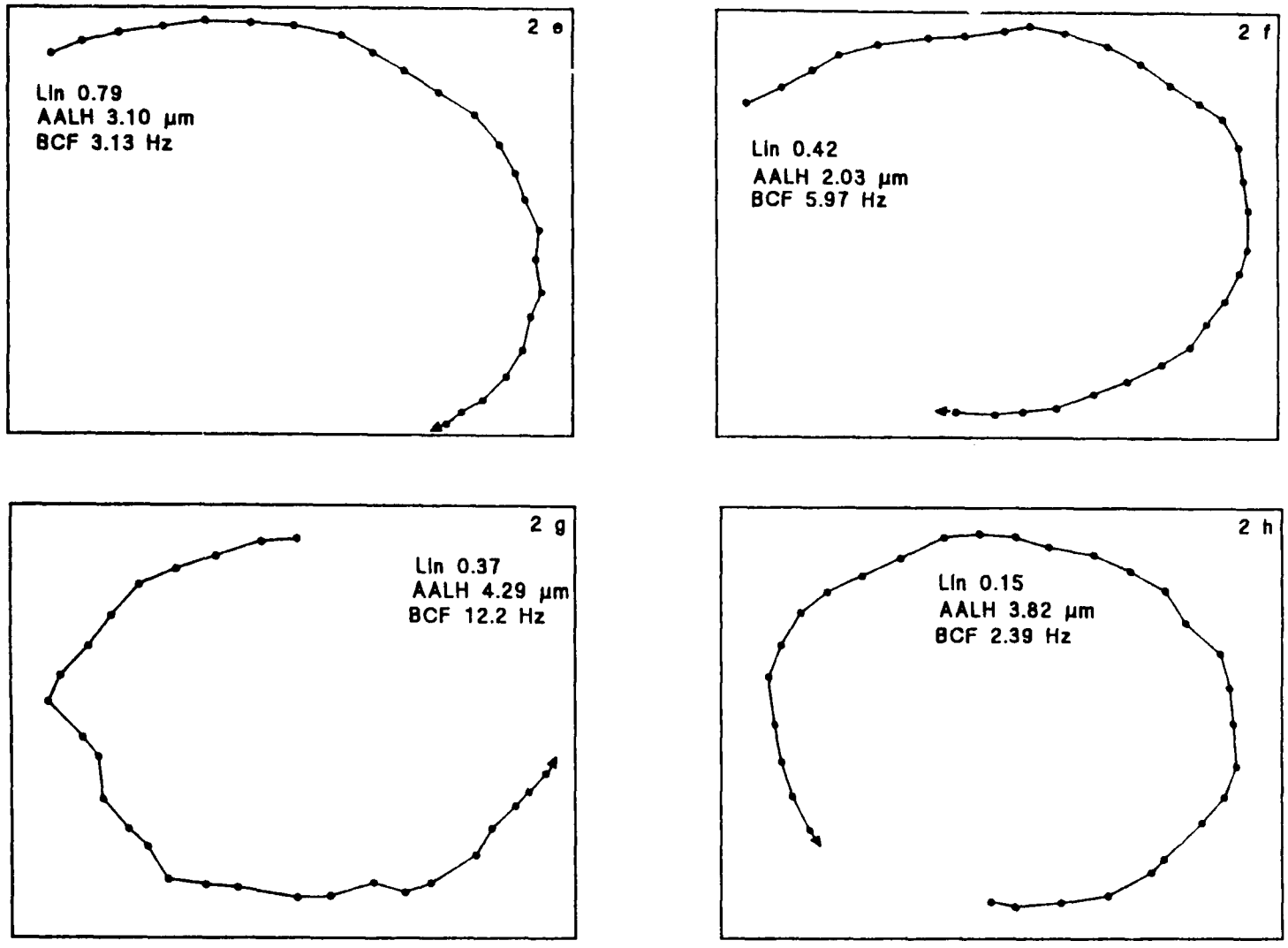

Figure 2. Trajectories of Rabbit Sperm Cells Over 6 hr Incubation in DM, Circular Motion with Different Radius and AALH (Continued)

3.2 Hyperactivated Motion.

When incubation was continued for 16-18 hr further, sperm cells developed entirely new patterns of movement. Some cells still progressed either linearly or circularly but other cells moved with high speed in a random nonprogressive manner characterized by a wide bending and whip lashing of the tail, a movement defined as hyperactivated motility.[8] Sperm cells from only 60-70% of the rabbits in the colony were still motile after overnight incubation, and sperm from only 50-60% of these rabbits developed hyperactive motility. Thus, sperm cells from <50% of the rabbits developed hyperactivated motility in vitro. The percentage of cells that developed hyperactive motility after 22-24 hr incubation varied from rabbit to rabbit and was in the range 10-90%. This response of sperm cells to incubation was quite constant from one specimen to another obtained from the same rabbit. Tracking the trajectories of hyperactivated sperm cells initially proved to be rather slow and difficult because of the rapid and unpredictable three directional movement. It was necessary to constantly adjust software settings for analysis. The maximum velocity setting required repeated changes to successfully track the cells. A low or high setting resulted in failure to track the hyperactive cell. A value of 750 μm/s was generally successful, but the value ranged from 500-1200 μm/s. The pixel size was also varied. A range of 5-90 pixels was usually successful, but a value as low as 1 pixel and as high as 120 pixels was sometimes required to capture cells as they moved in and out of focus. On rare occasions, cells could not be tracked for the minimum 15 frames[8] necessary for accurate and stable measurement of motion parameters. This usually occurred when the cell moved rapidly in and out of focus. The motility patterns of 563 cells that were tracked for a minimum of 15 frames could be classified into 3 types. Type I motions were highly random but movement occurred over a confined space producing a compact and multidirectional trajectory that encompassed a sphere (Figure 3). A second group of cells displayed a highly erratic pattern of movement in which the frequent directional changes were essentially over 180° (Figure 4) in contrast to Type I motion that occurred over 360°. Cells with Type III motion (Figure 5) moved essentially progressively but with a "jerky" motion in which the head yawed. Frame by frame observation revealed that these cells were orientated in planes that were not normal to the video camera. Movements in these planes were random and characterized by whip lashing and bending of the tail into the shape of C, U, or S. Smaller number of cells displayed a biphasic or multiphasic motility in which cellular motion alternated between movement patterns of the three groups.

The motility parameters of the three types of hyperactive cells differ from each other. Type III hyperactivated cells possess higher Lin and straightness (Str) values than the

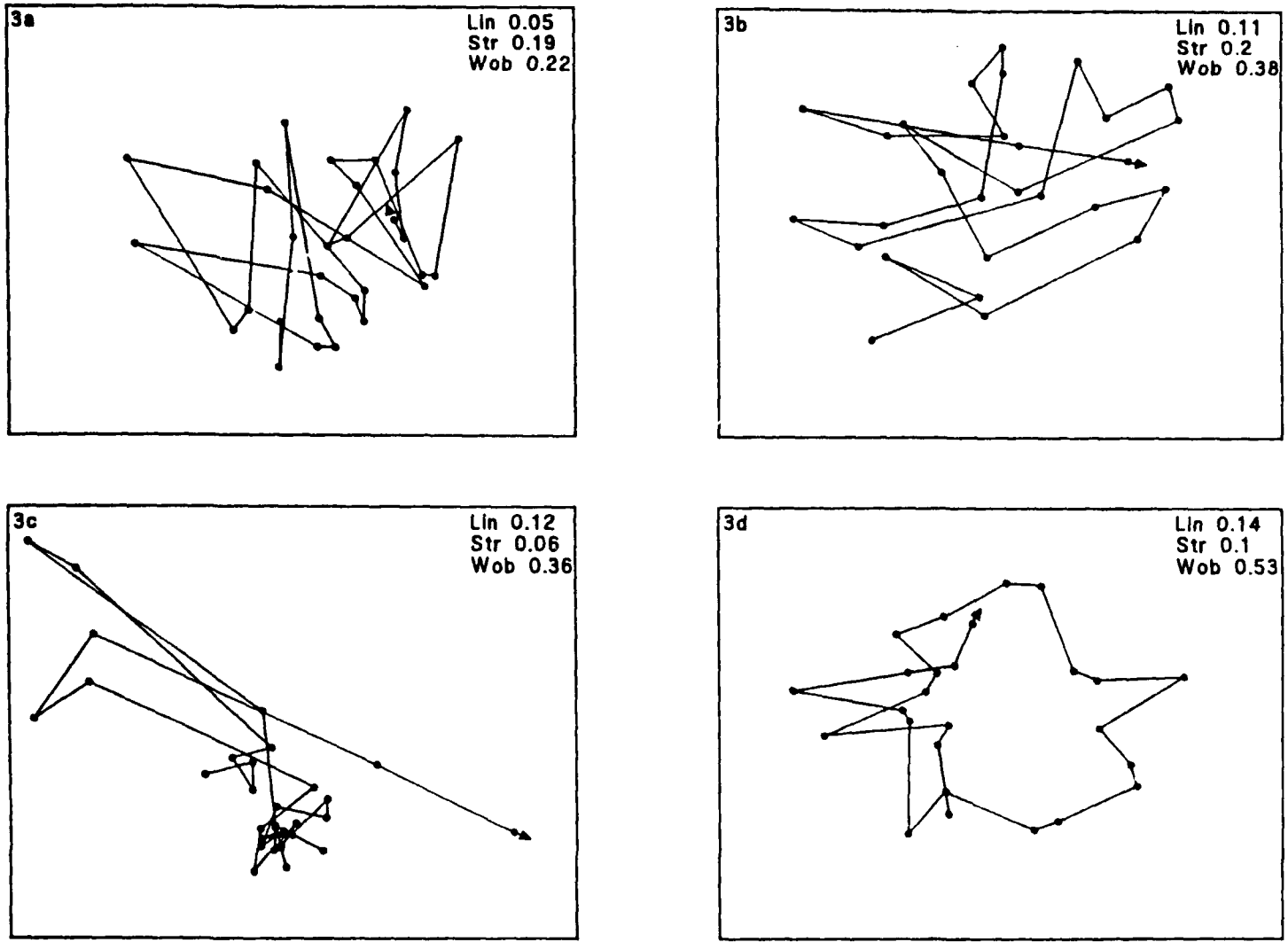

Figure 3. Type I Trajectories of Hyperactivated Rabbit Sperm Cells, Movement Over 360°

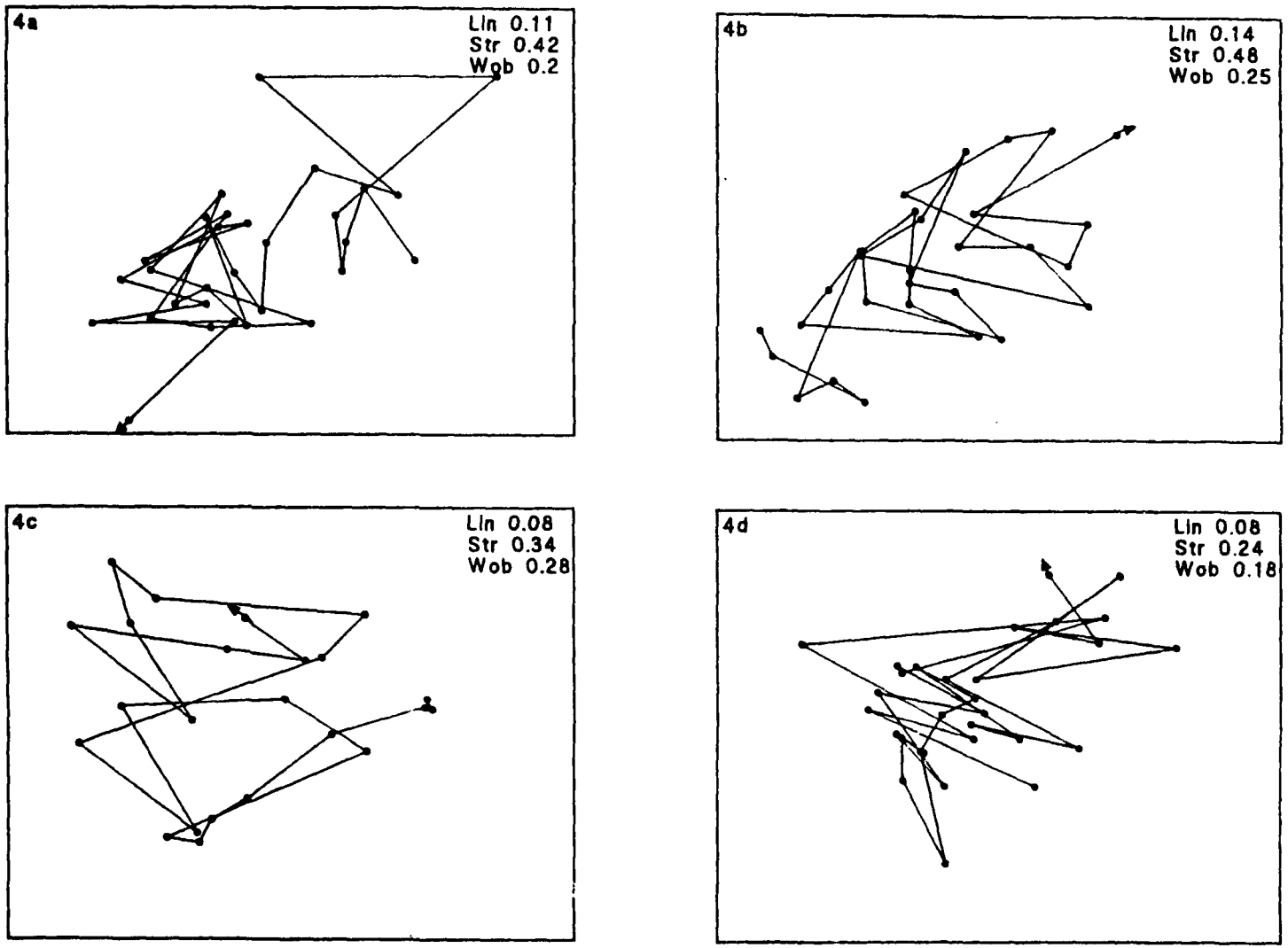

Figure 4. Type II Trajectories of Hyperactivated Rabbit Sperm Cells, Movement Over 180°

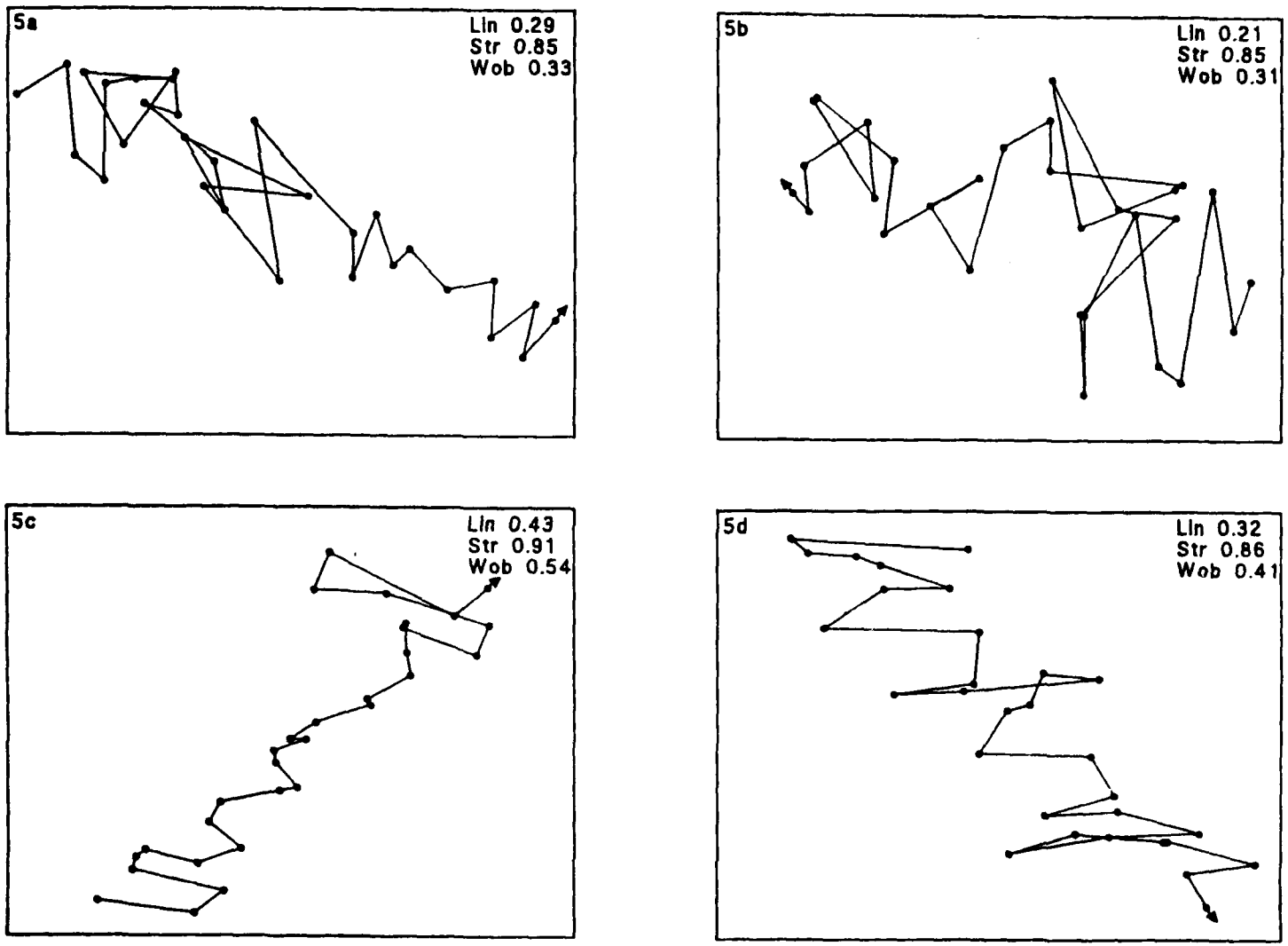

Figure 5. Type III Trajectories of Hyperactivated Rabbit Sperm Cells, "Jerky" Progressive Motility

other two types. Type I cells are differentiated from Type II by lower Str and higher wobble (Wob) values.

4. DISCUSSION AND CONCLUSION

The ability to induce hyperactive motility in vitro provides a methodology for the rapid and facile screening of the potential antifertility effects of chemicals. Underlying the application of this procedure is that hyperactive motion of sperm can be readily identified by visual inspection. However, quantitative assessment requires computer-assisted digital image analysis of sperm cell motion recorded on videotape. The high velocity and erratic movement of hyperactive sperm cells initially made analysis difficult, but appropriate adjustment of software settings and careful choice of tape segments to avoid areas of soft focus and high cell density permit tracking for a minimum of 15 frames of virtually all hyperactive cells. Modification of the optical system to increase the depth of focus, and/or use of a video camera with a higher shutter speed (60-200 frames/s) to enable analysis at frame rates of 60-200 frames/s, should overcome any difficulties in motion analysis. Moreover, video recording and analysis at higher speeds will result in more accurate determination of motility parameters. The potential application of hyperactivated motility measurements for clinical diagnosis of infertility in men has resulted in CellSoft developing a module for automated identification and quantification of hyperactivated human sperm. Automated analysis of hyperactivated rabbit sperm is under current study. Acquisition of hyperactive motility is a necessary preliminary for fertilization, but the physiological significance of the different types of hyperactivated and multiphasic motions is unknown. The motion parameters Lin, Str, and Wob can be used to distinguish between the motility types. The ability to recognize and measure the percentage of each type of cellular motion before and after exposure to chemicals may bring an added dimension to the screening and assessment of the potential adverse reproductive effects of chemicals of military interest.

By judicious use of software settings, the Cellsoft motion analysis system can be used to track the motion patterns of sperm cell induced to hyperactive by long incubation in Medium DM. This system provides a rapid means to screen chemicals for their potential adverse reproductive effects.

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