(2)

CHEMICAL RESEARCH, DEVELOPMENT & ENGINEERING CENTER

CRDEC-TR-295

CHANGES IN RABBIT SPERM MOTION CHARACTERISTICS DURING INCUBATION AND ITS USE IN THE ASSESSMENT OF CHEMICAL CYTOTOXICITY

R. J. Young
W. C. Starke
RESEARCH DIRECTORATE

Barry A. Bodt
Elizabeth A. Laurie
U.S. ARMY BALLISTIC RESEARCH LABORATORY

August 1991

U.S. ARMY ARMAMENT MUNITIONS CHEMICAL COMMAND

Aberdeen Proving Ground, Maryland 21010-5423

Disclaimer

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1991 August	Final, 89 Jun - 90 Aug

4. TITLE AND SUBTITLE
Changes in Rabbit Sperm Motion Characteristics During Incubation and Its Use in the Assessment of Chemical Cytotoxicity

5. FUNDING NUMBERS
PR-F8J2-10-005

6. AUTHOR(S)
Young, R.J., and Starke, W.C. (CRDEC),
Bodt, Barry A., and Laurie, Elizabeth A. (BRL)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
CDR, CRDEC, ATTN: SMCCR-RST, APG, MD 21010-5423
DIR, BRL, ATTN: SLCBR-SE-P, APG, MD 21005-5066

8. PERFORMING ORGANIZATION REPORT NUMBER
CRDEC-TR-295

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The CellSoft Motion Analysis System was used to study the changes in motion characteristics of rabbit sperm during incubation in a medium in which capacitation occurs. Sperm velocity reached a maximum after 1 hr of incubation and then declined linearly. The mean velocity at 5 hr was significantly higher than the mean at 0 hr. A steady increase in average amplitude of lateral head (alh) was observed, the values at 0 and 5 hr being significantly different. The motility characteristics of individual sperm cells also changed during incubation; however, this change was not reflected in the motion parameters. Positive correlations were observed only between curvilinear and straight line velocities and between maximum and average alh. A new toxicological endpoint, motility concentration index$_{50}$, MCI$_{50}$, was defined.

14. SUBJECT TERMS		15. NUMBER OF PAGES
Rabbit	Motility	17
Sperm cells	Motion parameters	16. PRICE CODE
Toxicology	Velocity	

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	SAR

Blank

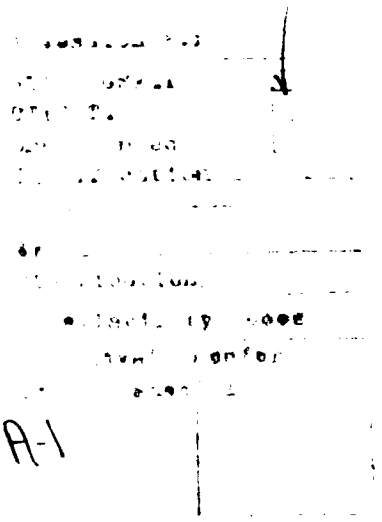
PREFACE

The work described in this report was authorized under Project No. F8J2-10-005. This work was started in June 1989 and completed in August 1990.

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.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for release to the public.



Blank

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHANGES IN RABBIT SPERM MOTION CHARACTERISTICS DURING INCUBATION AND ITS USE IN THE ASSESSMENT OF CHEMICAL CYTOTOXICITY

1. INTRODUCTION

The swimming pattern of rabbit sperm cells undergoes continuous change during their ascent of the female reproductive tract. Although several motility characteristics of rabbit sperm cells in vitro have been described,[1] no information is available on either the effect of prolonged incubation in vitro on these characteristics or on the motility parameters such as curvilinear velocity, linearity, and amplitude of lateral head displacement. The CellSoft Motion Analysis System has been used to measure these parameters as a function of time of incubation. The results, as well as a reanalysis of the effects of several chemicals on the parameters,[2] are described in this report.

2. MATERIALS AND METHODS

2.1 Animals.

New Zealand white rabbits were individually housed in standard rabbit cages. The room was maintained at 25 ± 2 °C, and laboratory rabbit chow and water were available ad libitum.

2.2 Collection and Purification of Sperm Cells.

Rabbit semen was collected and purified by centrifugation through a percoll gradient previously described.[3]

2.3 Incubation, Videotaping, and Motion Analysis of Sperm Cells.

A suspension of sperm cells (1-2 by 10^7 mL) in defined medium (DM)[4] was incubated at 37 °C under an atmosphere of 5% CO_2 and 95% air. At 0, 1, 3, and 5 hr, 5 µL of sperm suspension was placed in a warm, 20 µm deep chamber, and the chamber was placed on a microscope stage heated to 37 °C. A stream of air heated to 37 °C was directed onto the chamber to help maintain the temperature at 37 °C. Videotaping was carried out as previously described;[2] two drops of each suspension and 5 microscope fields of each drop were videotaped. The videotapes were analyzed with the CellSoft Motion Analysis System with the previously determined software settings and procedure.[5] Each segment of tape corresponding to a field was tracked for 30 video frames.

3. RESULTS

At 0 hr, the predominant type of sperm cell motion was linearly progressive with head rotation. A variable number (1-20%) of the cells moved in anticlockwise circles of different radii without head rotation. The latter type of motion was observed almost exclusively in a 10-μm chamber and probably represents movement along the surface of the chamber. After 1 hr of incubation, linearly progressive sperm exhibited a much higher frequency of head rotation and appeared to move with more vigor and a higher velocity. Over the next 2 hr, an increasing number of sperm developed a "jerky" motion characterized by a side-to-side head movement about the direction of travel superimposed upon a high rate, and frequently high radius, of head gyration. The frequency and degree of yawing varied considerably among sperm samples. During this period, a small number of sperm were observed with different movement patterns. Individual sperm changed the direction of progression with or without a pause in movement; progressed in spurts; changed back and forth from circular movement without head rotation to linear progression with head rotation; progressed with a trajectory resembling a sine curve with a long wavelength; rapidly spun or whirled over a confined space; or traced a circle with a short radius. The heads of the spinning, whirling, or turning sperm also rotated rapidly. There was a great deal of variation in the motion patterns seen between sperm preparations from different rabbits and even in different preparations from the same rabbit; however, no attempt was made to determine the proportion of each type of motion pattern. Sperm moving with either a "jerky" or one of the other types of motion patterns were often present after 1 hr of incubation. Incubation beyond 3 hr resulted in a marked decrease in the number of sperm exhibiting the "jerky" as well as the other types of motion. After 5 to 6 hr of incubation, the percentage of motile sperm was reduced by 50-90%, and much fewer progressive sperm exhibited yawing or rapid head rotation.

The changes that occurred over time in the motion parameters of sperm obtained from four rabbits are shown in Table 1. Table 2 shows the changes in time-motion parameters in samples obtained 6 days later from three of the rabbits. The motion parameters are sample averages determined by pooling the means over five fields from each of two drops taken from sperm suspensions at 0, 1, 3, and 5 hr of incubation in DM. To determine if the motility parameters (varied between different rabbits) either changed with incubation time or were related to one another, a two-fold nested factorial design, consisting of the fixed effect, time, random effects, sperm sample, drop within a sample, and field within a drop, was analyzed for each of the motility parameters: curvilinear velocity (Vc), straight line velocity (Vst), linearity (lin), average amplitude of lateral head (aalh) displacement, maximum amplitude of lateral head (malh) displacement, and beat cross frequency (bcf). The factor,

Table 1. Changes in Rabbit Sperm Motility with Time of Incubation

Rabbit	Time	Vc μm/sec	Lin	AALH μm	BCF hz	Vc μm/sec	Lin	AALH μm	BCF hz
510	0H	77.4±5.1 (20-175)	8.05±0.29 (0.13-9.97)	1.79±0.31 (0.09-9.77)	14.8±0.9 (2.4-25.7)	98.8±5.4 (20-158)	8.44±0.56 (0.45-9.95)	2.11±0.41 (0.42-7.96)	14.2±0.7 (5.6-24.8)
173	0H	65.6±5.7 (20-146)	8.29±0.26 (0.17-10)	1.43±0.24 (0.01-11.1)	14.6±0.6 (2.7-28.3)	102.0±7.5 (20-187)	8.64±0.39 (0.15-10)	2.14±0.48 (0.18-7.37)	14.5±0.6 (2.4-24.6)
564	0H	72.6±4.6 (20-192)	8.03±0.37 (0.06-10)	1.98±0.24 (0.11-9.36)	13.7±0.6 (3.5-27.8)	73.8±9.2 (20-203)	7.61±0.70 (0.05-10)	1.96±0.29 (0.11-9.3)	14.5±0.7 (3.2-28.6)
971	0H	64.5±6.5 (20-168)	8.29±0.34 (0.02-10)	2.15±0.2 (0.15-10.1)	14.1±0.6 (3.3-27.8)	94.5±6.2 (20-199)	8.24±0.38 (0.15-10)	2.37±0.34 (0.4-17.5)	13.6±0.5 (2.1-27)
510	3H	82,4±6.6 (33-148)	8.17±0.95 (0.14-10)	1.99±0.42 (0.32-7.06)	14.1±1.2 (2.5-28.3)	86.9±14.4 (33-149)	8.31±1.38 (0.13-9.94)	2.16±0.52 (0.54-9.35)	13.8±1.7 (5.6-17.9)
173	3H	86.7±3.9 (20-154)	8.45±0.36 (0.14-10)	2.17±0.35 (0.34-5.82)	13.8±0.6 (3.6-25.1)	87.6±6.9 (21-131)	8.67±0.32 (0.39-10)	2.20±0.29 (0.310.57)	13.8±0.7 (2.1-23.3)
564	3H	78.5±7.0 (31-162)	9.0±0.94 (0.11-10)	1.76±0.31 (0.16-6.92)	14.1±0.8 (4.5-24.6)	65.8±8.9 (30-145)	7.51±0.76 (0.18-10)	2.19±0.47 (0.13-8.39)	14.4±1.7 (3.6-27.8)
971	3H	88.5±4.7 (30-165)	0.73±0.42 (1.16-10)	2.26±0.41 (0.29-8.63)	12.6±2.03 (4.1-27)	84.9±6.4 (30-159)	8.67±0.21 (0.94-10)	2.09±0.4 (0.27-10)	13.6±1.1 (1.9-28.9)

Average ± S.D. (range).

Spermatozoa were incubated in UM. At 0, 1, 3, and 5 hours five fields from each of two drops of the sperm suspensions were tracked for 30 frames.

Table 2. Changes in Rabbit Sperm Motility with Time of Incubation

Rabbit	Time: 0H Vc μm/sec	0H Lin	0H AALH μm	0H BCF hz	1H Vc μm/sec	1H Lin	1H AALH μm	1H BCF hz
510	81.8±8.7 (20-190)	7.72±1.01 (0.16-10)	1.86±0.47 (0.11-7.15)	13..0±1.4 (3.2-27.8)	86.6±16.9 (30-187)	8.12±0.78 (0.18-10)	1.63±0.53 (0.17-7.91)	15.1±1.8 (2.4-25.7)
173	64.7±4.3 (20-227)	8.29±0.89 (0.16-10)	1.61±0.38 (0.15-8.42)	13.5±1.1 (1.19-28.3)	87.6±8.1 (21-180)	7.83±0.97 (0.15-10)	1.90±0.35 (0.24-9.14)	12.6±1.8 (4.1-27)
564	78.8±10.3 (20-193)	8.57±0.82 (0.13-10)	1 67±0.23 (0.07-7.08)	13.8±1.0 (1.9-27)	94.6±7.1 (20-195)	8.37±0.56 (0.06-10)	1.91±0.36 (0.22-7.89)	13.5±1.5 (2.4-23.6)
	3H Vc μm/sec	3H Lin	3H AALH μm	3H BCF hz	5H Vc μm/sec	5H Lin	5H AALH μm	5H BCF hz
510	85.8±23.1 (30-150)	7.38±1.42 (0.07-10)	2.10±0.39 (0.19-10.22)	13.8±3.2 (3.6-23.3)	87.8±21.4 (30-175)	7.78±2.66 (0.09-10)	2.24±1.29 (0.36-5.14)	12.8±2.9 (3.5-27.9)
173	83.8±16.2 (30-170)	8.60±3.6 (0.1-10)	2.23±0.63 (0.28-10.27)	12.9±3.0 (3.6-28.6)	72.4±13.2 (31-173)	7.54±1.49 (0.12-10)	2.29±0.97 (0.25-5.83)	12.2±2.9 (4.5-27)
564	91.7±9.5 (20-182)	8.35±0.38 (0.05-10)	1.94±0.31 (0.29-7.79)	14.1±1.3 (3.3-25.7)	79.5±7.4 (23-135)	8.50±0.81 (0.28-9.97)	1.79±0.25 (0.33-4.47)	14..2±1.2 (3.6-23.9)

Average ± S.D. (range)

Spermatozoa were incubated in DM. At 0, 1, 3, and 5 hours five fields from each of two drops of the sperm suspensions were tracked for 30 frames. Sperm samples used in the experiments of Tables 1 and 2 were collected 7 days apart.

time, was examined at 0, 1, 3, and 5 hr. Two sperm samples, obtained 6 days apart from each of three rabbits and one sample from a fourth rabbit, were independently considered.

Analysis of variance indicated that incubation resulted in a significant change in Vc and Vst at the $\alpha = 0.01$ level and aalh at the $\alpha = 0.05$ level. The Newman-Keuls Test for pair wise comparisons indicates that the means for Vc and Vst reached a maximum after 1 hr of incubation and then decreased over the next 4 hr of incubation. The mean for Vc at 5 hr was significantly higher than the mean for Vc at 0 hr. However, deviations from this behavior were observed with rabbit 564 (Table 1). Although the mean for Vst was also higher at 5 hr than at 0 hr, the difference was not significant. Partitioning of the variability among 1, 3, and 5 hr into linear and quadratic effects suggested that the decline was linear. A steady increase in the response means for aalh was observed over time; however, the Newman-Keuls Test indicates that the difference was significant only between 0 and 5 hr. Differences ($\alpha = 0.05$ level) were found in the velocity means of sperm samples between and within (rabbit 564) the four rabbits and between drops taken from the same sample ($\alpha = 0.025$ level). Partitioning out the variability induced by rabbits and drops increased the sensitivity of the test on incubation time.

To determine if a consistent pattern is present in correlations existing between motility characteristics, the correlation matrix was examined for each sperm and time combination. Among the seven samples, significant ($\alpha \leq 0.10$) positive correlations were frequently observed only between the Vc and Vst and between aalh and malh. Examining the correlation matrix over time did not reveal consistent associations between changes in one motility characteristic and changes in another for any of the other possible pairs of motility parameters.

In a previous study, the CellSoft Motion Analysis System was used to analyze the effect of several chemicals on the motility characteristics of rabbit sperm cells.[2] This study used manufacturer suggested and other software settings that were determined to be inappropriate by a later study.[5] The videotapes recorded during that study were reanalyzed with new software settings, and the effect of the chemicals on the percentage of motile sperm cells, Vc, lin, and aalh was assessed. The results are shown in Table 3. Each of the five compounds studied inhibited motility of the sperm cells; but only triethyl phosphite (TEP) also reduced Vc, lin, and aalh to values that were 50% or less of the control value. Figures 1 and 2 show that the decrease in the motility parameters is time and concentration dependent. Two of the compounds (ammonium oxalate and hydroquinone) inhibited motility; but, even at the concentration at which motility was inhibited, the motility parameters Vc, lin, and aalh did not decrease to values that were 50% of the control values.

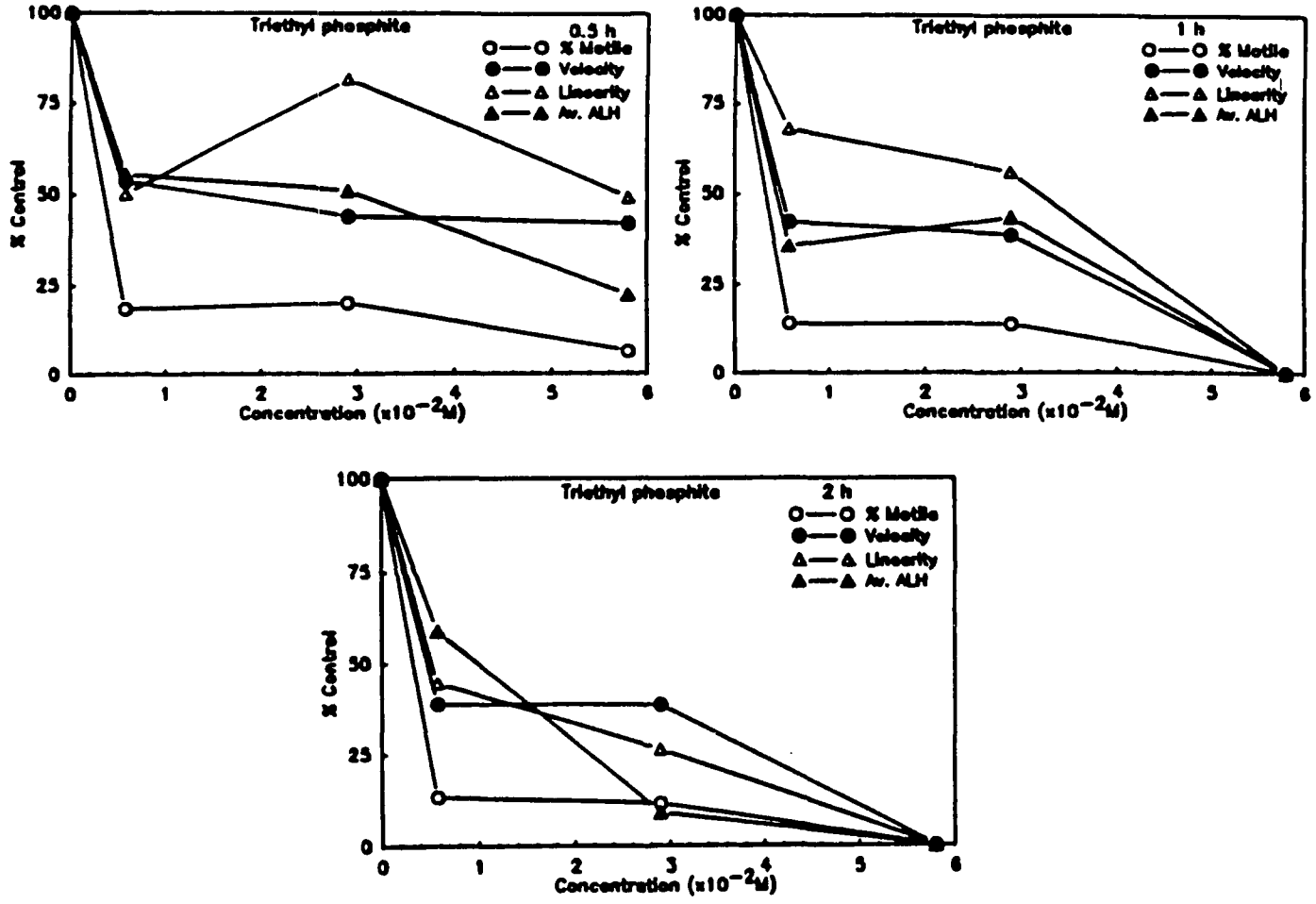


Figure 1. Effect of TEP on Rabbit Sperm Cell Motion Parameters. Rabbit sperm cells 2.1 x 10^7/mL and different concentrations of TEP were incubated in DM at 37 °C under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2

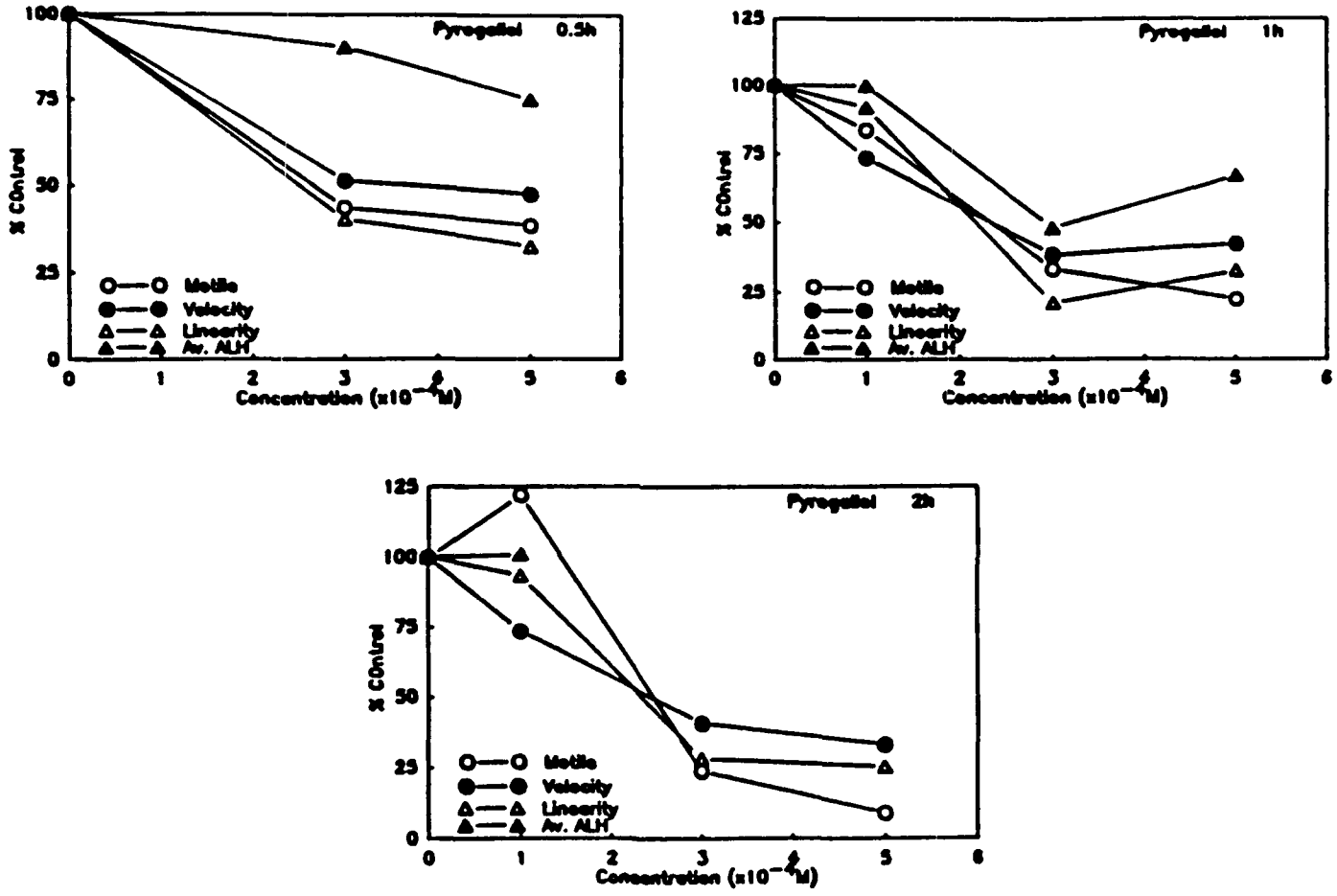


Figure 2. Effect of Pyrogallol on Rabbit Sperm Cell Motion Parameters. Rabbit sperm cells 1.41 x 10^7/mL and different concentrations of Pyrogallol were incubated in DM at 37 °C under an atmosphere of 5% CO_2, 8% O_2, and 87% N_2.

Table 3. Effect of Chemicals on Motion Parameters of Rabbit Sperm*

Compound	% Motile	Vc (μm/s)	Lin	aalh (hz)
Pyrogallol	+	+	+	-
Pinacolyl Alcohol	+	+	-	-
Ammonium Oxalate	+	-	-	-
Triethyl Phosphite	+	+	+	+
Hydroquinone	+	-	-	-

*Decrease in sample mean to a value ≤50% of the control mean.

To normalize the toxicological endpoint for the compounds, a motility concentration index$_{50}$ (MCI_{50} = C_{50}/SC, where C_{50} is the concentration of the compound that reduces the percentage of motile cells by 50%, and SC is the sperm concentration in millions per milliliter) was calculated. The C_{50} for pyrogallol is shown in Figure 3, and the MCI_{50} is 0.00038M/14.1 = 2.7 x 10^{-5} M/10^6 sperm at 1 hr and 0.00044M/14.1 = 3.1 x 10^{-5} M/10^6 sperm at 0.5 hr. Table 4 shows the MCI_{50} and the oral lethal dose expected to kill 50% of the exposed population (LD_{50}) values for the five compounds and the amount of the five compounds that reduced the uptake of neutral red dye by 50% (NR_{50}) compared to control in mouse 3T3 cells [personal communication between D.M. Starke (Rockefeller University, New York, NY) and Dr. O.J. Olajos (U.S. Army Chemical Research, Development and Engineering Center)]. There is good agreement among the three, suggesting that they all point to the same or similar toxicological endpoints.

Table 4. Comparison of Toxicological Endpoints

Compound	MCI_{50} (M/10^6 Sperm)	LD_{50} (Oral) (mg/Kg) Rat	Rabbit	NA_{50} (μg/mL)
Hydroquinone	<2.5x10^{-6}	320	200	16
Pyrogallol	2.9x10^{-5}	-	-	38
Ammonium Oxalate	5.29x10^{-4}	-	-	>200
Triethyl Phosphite	7.6x10^{-4}	3200	-	3000*
Pinacolyl Alcohol	2.39x10^{-3}	-	-	10000

*Triisopropyl phosphite

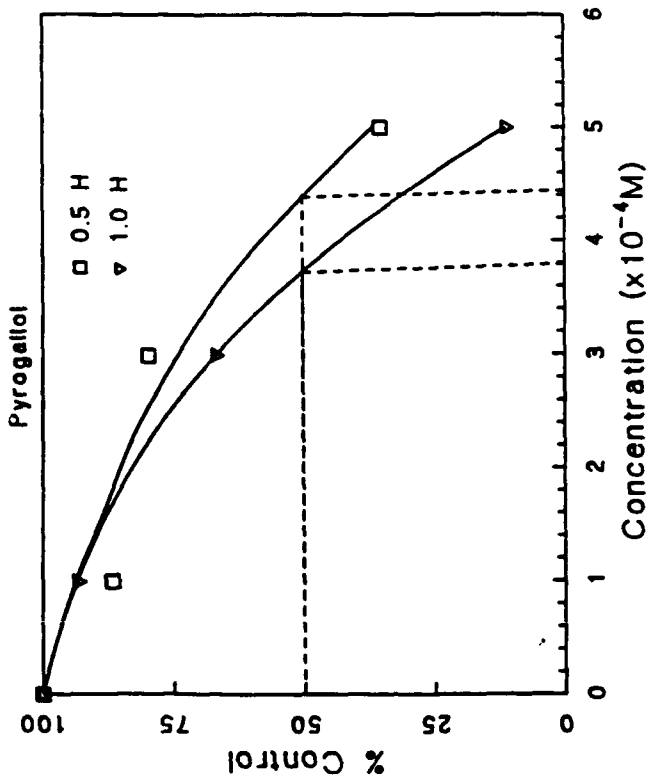


Figure 3. Decrease with Respect to the Control in the % Motile Rabbit Sperm Cells upon Exposure to Different Concentrations of Pyrogallol for 1 hr

4. DISCUSSION AND CONCLUSION

The initial low values for curvilinear velocity (Vc) and average amplitude of lateral head (aalh) most probably reflect the suboptimal conditions (18-23 °C, air) and mechanical stress to which the sperm cells were exposed prior to incubation at 37 °C under an atmosphere of 5% CO_2, 8% O_2 and 87% N_2. The decline in the percentage of motile sperm cells during incubation without a concomitant decrease in the sample means for Vc, beat cross frequenccy (bcf), and aalh suggests that the medium is deficient in one or more of the components necessary for the maintenance of motility but not as a source of energy. Although the difference in sample means for aalh at 0, 1, and 3 hr is not significant, a trend towards higher mean aalh is apparent, and this parallels the appearance of sperm cells whose heads either yawed about the path of progression or progressed with a large radius of gyration. Individual sperm cells that exhibited either a high radius of gyration or degree of yawing had high aalh values, and high aalh values may be characteristic of these types of swimming patterns. The mean and individual bcf values did not

alter over the 5 to 6 hr of incubation and were also unaffected by exposure of sperm cells to chemicals; the relationship of this motion parameter to the swimming motion of sperm is unclear. Similarly, the high frequency of head rotation observed after 1 hr of incubation and the wavy, spinning, and whirling trajectories present in some sperm cells after 3 hr of incubation are not reflected in the changes in population means for the motion parameters. Nevertheless, the time- and concentration-dependent decreases in these means and in the numbers of motile sperm that occur in the presence of added chemicals show that the changes are indicators of the inhibition of sperm cell motility by the added chemicals, and thus, are measures of cell viability. The correspondence between the motility concentration index$_{50}$ and lethal dose$_{50}$ values and another in vitro measure of cell viability, the decrease in uptake of the supravital dye neutral red by mouse 3T3 cells, indicates that analysis of the effect of chemicals on sperm motion may serve as a means for the in vitro assessment of chemical toxicity. Validation of this hypothesis will require studying the effects of many more compounds on sperm cell motion. This procedure will reduce, if not entirely eliminate, the use of animals in toxicity assessment, is cheaper, faster, and environmentally benign because the procedure does not require the use of radioisotopes.

LITERATURE CITED

1. Branham, J.M., "Movements of Free-Swimming Rabbit Spermatozoa," J. Reprod. Fertil. Volume 18, p 970105 (1969).

2. Young, R.J., and Starke, W.C., Computer-Assisted Analysis of Several Chemicals on Rabbit Sperm Cell Motion, CRDEC-TR-142, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD, January 1990, UNCLASSIFIED Report.

3. Young, R.J., and Starke, W.C., A Procedure for the Removal of Vesicles and Prostate Secretions from Motile Rabbit Sperm Cells, CRDEC-TR-88129, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD, August 1988, UNCLASSIFIED Report.

4. Brackett, B.J., and Oliphant, G., "Capacitation of Rabbit Spermatozoa In Vitro," Biol. Reprod. Volume 12, pp 260-274 (1975).

5. Young, R.J., Starke, W.C., Bodt, B.A., and Laurie, E.A., Statistical Validation of the CellSoft Motion Analysis System for the Study of the Motility Characteristics of Rabbit Sperm Cells, CRDEC-TR-214, U.S. Army Chemical Research, Development and Engineering Center, Aberdeen Proving Ground, MD, January 1991, UNCLASSIFIED Report.