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ADAPTATION OF A MOTILITY ANALYSIS APPARATUS FOR SPACE SCIENCE & MICROGRAVITY GROUND-BASED EXPERIMENTS

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Introduction

Previous space flight studies have described unfavorable effects of microgravity on testicular morphology and spermatogenesis (Cosmos 1887 Biosputnik flight, 9/29/87 - 10/12/87). The flight animals demonstrated small reductions in testicular and epididymal size, the phenomenon explained as resulting water loss (1). Yet, light microscopic histological preparations revealed few spermatozoa in the rete testis of the flight males compared to control animals. The cause for this finding was subjectively assessed to be due to " the anatomical dislocation of the organs... and a disturbance in testicular blood supply"(1). Unfortunately, the reported effects of microgravity on the reproductive processes (particularly within males) are few and divergent. If habitation in space is a futuristic goal, more objective testing (of male and female gametes) in a microgravity environment will provide insight to the developmental potential of these reproductive cells.

As part of the Marshall Space Flight Centers' Summer Faculty Fellowship Program within the Biophysics Branch, a key component of the research investigation was to develop a test to evaluate individual cell motility and orientation in varying gravitational environments, using computerized assessment of sperm cell concentration, morphology and motility to provide objective, quantitative experimental control. In previous work performed jointly by the author and NASA colleague, it has been shown that macroscopic motile aggregates of spermatozoa were not altered by the absence of microgravity (2). Variations in the number of normal versus abnormal sperm due to microgravity influences have yet to be established. It is therefore of interest to monitor the cytoskeletal matrix (microtubulin) of these organisms as a possible indicator of cell viability and/or function.

Using Image and cell motion analysis we can show in a simple, rapid test that chemical toxicity and other deterrents to motility can be objectively measured and quantified (3). Positive identification of the effects chemical toxicants and other potential environmental contaminants (especially from space) can have on living organisms on earth would be potentially beneficial to space science experimentation and ground - based tests developed for future shuttle and space station missions. For spermatozoa, cell motility responses were unaltered in reduced gravitational vehicles (i.e. - KC-135, RWV Bioreactor). However, developing a simple test of viability using common amino acids found normally in semen (glutamic acid, glycine, arginine, proline) we determined that reduced motility (20-45% of active sperm cells) could be established with all of the selected amino acids except glutamic acid. Glutamic acid, in contrast to the other amino acids enhanced sperm motility and maintained a good rate (i.e. vigorous movement, with about 70-85% active sperm cells) for up to 120 minutes.

For Tetrahymena, test materials consisted of twenty water-soluble chemicals, each dissolved in type II culture grade purified water. Negative controls (no chemical) and two reference standards (acetone and methanol) were used in the testing. Test samples were placed on the stage of an inverted microscope equipped with a CCD-camera coupled to a computer automated cell analysis system which captured a digitized image of the live

sample. Each sample was analysed for 30 seconds in ten frames per second. The observed number of cells and average linear velocity was determined for each chemical application.

Below are the concentrations of amino acids used to test sperm motility:

Amino Acid	Concentration		
	Low	Medium	High
Glutamic Acid	.05	.25	1.00
Glycine	.03	.10	.50
Arginine	.03	.10	.50
Proline	.03	.10	.50

Below are the Rank Order Toxicity effects on Tetrahymena from the Computerized Motility Assay: [3]

Rank order toxicity from computerized assay. Toxicity scores shown as the tolerated dose (dilution factor) with immobilized (a) d_{high}, 90 percent of the swimming cells (high dose); (b) d_{low}, 10 percent of the swimming cells (low dose); (c) the average dose as the reciprocal sum of the high and low dose ($1/d_{avg} = 1/d_{high} + 1/d_{low}$). Rank orders shown for 20 organics (alcohol, ketones, ethers, esters) and salts.

Chemical	10 Percent Motile	90 Percent Motile (HTD)	Computer (ATD)	Rank Order	Silverman
Ethylene glycol	1.000	17.500	18.500	1	11.000
Ethanol	5.310	18.800	24.100	2	13.000
Isopropanol	78.000	22.500	30.300	3	
Methanol	1.000	38.900	39.900	4	
DMSO	1.000	46.500	47.500	5	9.300
3-methyl 2-butanone	18.900	40.000	58.900	6	
Isobutyl acetone	1.000	79.400	71.400	7	19.300
Methyl isobutyl ketone	13.800	58.300	72.100	8	
2-methyl 1-propanol	1.000	78.000	79.000	9	
Methyl ethyl ketone	34.800	55.900	90.700	10	60.000
Acetyl acetone	31.200	122.000	153.000	11	
Butanol	33.000	170.000	203.000	12	
Bleach	44.600	346.000	391.000	13	
Diethylanoamine	120.000	284.000	404.000	14	
2-octanone	250.000	308.000	558.000	15	
Nonanol	178.000	801.000	979.000	16	
1-pentanol	321.000	687.000	1,008.000	17	
Heptanol	694.000	2,250.000	2,944.000	18	
2-methyl 1-butanol	963.000	2,275.000	3,238.000	19	
Hexanol	2,000.000	13,000.000	15,000.000	20	

Conclusions

Simulated Microgravity vehicles such as the KC-135 provides conclusive evidence that the physiological mechanisms governing motility of spermatozoa are not gravity dependent.

Ground-based testing of organisms using cell motion and image analysis further provide support of gravity independent motility of spermatozoa.

Adaptation of the motility analysis apparatus for space science experimentations will yield objective measurements of microscopic, physiological occurrances which could otherwise be misinterpreted, if one merely uses subjective visual observations.

Quantitative analysis identifies a specific range and rate of change in motility upon chemical additions.

Subtle changes in motility characteristics for spermatozoa as well as the protozoan, tetrahymena are noted as apparent changes in linear velocity and direction.

Positive identification of the effects chemical toxicants and other potential environmental contaminants (especially from space) can have on living organisms on earth would be potentially beneficial to space science experimentation and ground - based tests developed for future shuttle and space station missions.

Future Work

Summer efforts have also been focused on proposal development addressing the priority needs for space science experiments in the Microgravity Biotechnology area. Briefly, if the proposal is funded, my NASA Collegue, other University & Industry partners and I, propose to determine if microgravity alters the growth, metabolism and morphological differentiation of reproductive cells (oviductal, endometrial, embryos) cultured in vitro; and determine further, if microgravity alters the biochemical substrate utilization or synthesis of these cells. These initiatives, both ground-based and anticipated shuttle or space station experiments will explore the microgravity environment as a unique and effective 'laboratory' to study living organisms and life on earth.

References

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