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TOXICITY PROBLEMS IN PLASTIC HARDWARE DESIGNED FOR BIOLOGICAL SPACE-FLIGHT EXPERIMENTS

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SUMMARY

In the development of hardware for a biological space flight, various plastic materials were found to be toxic to sea urchin sperm and unfertilized eggs. Glass control chambers were not. Acrylic and polycarbonate plastics were tested as material for the hardware body, while fluorocarbon elastomer rubber, nitrile, three silicone rubbers, butyl rubber and ethylene propylene were tested as "O" ring materials. Fertilized frog eggs were found to be compatible with ethylene propylene and acrylic plastic after careful treatment, principally outgassing by vacuum exposure, but no treatment was discovered which would sufficiently detoxify plastic hardware so that it would maintain sea urchin sperm and unfertilized eggs.

INTRODUCTION

On September 7, 1967, the first successful BIOSATELLITE was launched by NASA from Cape Kennedy. It was recovered two days later with a wealth of biological data from 13 biological experiments designed to study the effects of a space flight on development, growth, and response to radiation.

Preceding the flight, several years of developmental work had gone into the preparation and automation of these experiments; many problems had to be overcome, and new techniques developed. This paper outlines some of the problems encountered with the development of hardware for a sea urchin experiment and a frog egg experiment.

The purpose of these experiments was to study the effects of space conditions, principally weightlessness, on fertilization and early development of eggs. Results from the two experiments were to have been compared with each other as well as with controls. For the sea urchin egg experiment, hardware was designed and constructed that could automatically fertilize the eggs at predetermined times and then later fix them at selected stages in their development.

At the time the hardware was designed, engineering considerations restricted the construction of the containers to plastics rather than glass. Resulting toxicity problems combined with extended hold time ultimately made it necessary to cancel the sea urchin egg experiment for the BIOSATELLITE flight and to modify procedures greatly for the frog egg experiment.

The purpose of this paper is to review the tests and analyses relative to the biological toxicity problems in plastic hardware.

TESTS AND RESULTS

Tests were performed at Ames Research Center to investigate the toxic effects of experimental hardware.

The organisms involved included the sea urchins, Arbacia punctulata and Lytechinus variegatus, first and second choice organisms for flight and readily available in Florida, near the launch site. Strongylocentrotus purpuratus and the sand dollar, Dendraster excentricus are available along the central California coast near Ames Research Center. The complementary breeding seasons and geographic locations of these four echinoderms made it possible to work on the problem at nearly any time of year. The sperm and eggs of all seem to be about equally sensitive to the toxic effects under study.

Background Studies

Fertilized eggs were found to be resistant to these toxic effects, unfertilized eggs less so, and experimentation showed that the storage of sperm prior to fertilization was the critical factor. Consequently, in the tests the sperm and eggs were held separately for varying lengths of time in the material being tested. They were then mixed, and examined for several days thereafter to observe development. As a laboratory baseline for comparison, 90 - 95 percent of the eggs in glass control chambers could be expected to show raised fertilization membranes, followed by first and second cell division. These eggs would then be followed through the motile gastrula stage to early or middle pluteus stage to watch for abnormal development or high mortality rate. For detailed descriptions on obtaining and handling sea urchin eggs, see reference 1.

Toxicity was presumed when few or no fertilization membranes were raised, little or no cell division occurred, the motile gastrula and pluteus either behaved abnormally or appeared abnormal, or the mortality rate was substantially higher than in the controls.

Repeated experiments using glass chambers indicated that separate sperm and eggs could usually be held for periods in excess of 6 hours without unacceptable loss of viability if held in carefully cleaned pyrex glass, in filtered sea water with 1×10^{-3} M EDTA added, and the pH readjusted to 8.2 with NaOH. Temperatures were normally from 13° to 20° C, depending on the species involved. The Florida organisms did well at higher temperatures. Tyler (see ref. 2) was able to get normal development of eggs fertilized with sperm stored up to 24 hours, but that figure was never equaled in the Ames laboratory at normal temperatures. However, 90-percent fertilization was achieved when sperm were held at 6° C for 24 hours and then warmed to 13° C before fertilization. Ground glass stoppered bottles (10 ml volumetric flasks) sealed with high vacuum silicone grease gave satisfactory results and it was also found that sperm and eggs could usually be successfully kept in plastic hardware if the containers were open to the air. The toxic effects were usually only apparent in plastic when the chambers were closed. This discovery necessitated repeating a whole series of tests, and a number of substances previously thought to be safe were found to be unacceptably toxic under sealed conditions. The only two substances found to be entirely safe for sea urchin sperm were pyrex glass and high vacuum silicone grease, which was used to lubricate and seal our systems.

Plastic Hardware Studies

Each unit (fig. 1) of the multiple unit device was designed to hold separately 4.2 ml of egg suspension and 0.45 ml of sperm suspension in sea water. At predetermined times after loading, the contents of the two chambers were mixed. A third chamber contained 5 ml of a fixative (usually formaldehyde) which was added to the suspension of fertilized eggs after various lengths of time had been allowed for development.

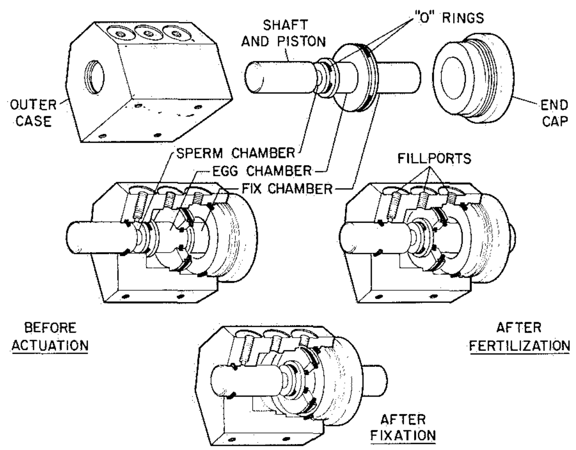

Figure 1.- Sea urchin egg chamber.

When the eggs were fertilized immediately after being placed in the hardware, they developed normally or nearly normally. However, when approximately 1 hour elapsed before fertilization, both the fertilization rate and normal development deteriorated drastically, sometimes below 5 percent. Since the expected hold time had been increased from an originally estimated 3 to 8 or 9 hours (actual hold time at flight was nearly 14 hours), this situation had to be corrected.

At first formaldehyde leakage from the fix chamber to the egg chamber was blamed for the difficulty, and indeed such leakage did occur. The silicone rubber "O" rings used for dynamic seals on sliding pistons were permeable, and over an 8-hour period would allow several parts per million of formaldehyde to diffuse into the chamber.

Several "O" ring materials were tested for both toxicity and permeability. These included fluorocarbon elastomer rubber, nitrile, three different kinds of silicone rubber, butyl rubber, and ethylene propylene. All but ethylene propylene proved either too toxic, too permeable or both for our purposes. Ethylene propylene was found acceptable for frog eggs but was not proven acceptable for sea urchin eggs. It was also found necessary to inspect each "O" ring with a dissecting microscope to ensure against manufacturing blemishes.

Another toxicity problem was due to the plastic material from which the chamber was made. When it was discovered that acrylic plastic was toxic when the hardware was sealed, a polycarbonate was used. In the course of the tests, these plastics were found to contain materials in addition to their basic formulae, probably as impurities and as additions to improve strength and performance, etc. Understandably, the manufacturers declined to divulge proprietary information concerning these additional constituents, but analysis of materials extracted by "hard" outgassing of the plastics showed a variety of potentially toxic substances. Among them were low molecular weight hydrocarbons, and methyl methacrylate monomer in the acrylic and phenol in the polycarbonate. "O" rings contained such volatile materials as low molecular weight hydrocarbons, olefine compounds, and carbon disulfide. All "O" ring materials tested showed at least traces of several metals.

At this point two separate programs were begun to avoid the effects of these materials. One was an outgassing and intensive cleaning procedure and the other was an attempt to coat the inside of the hardware with protective materials. Vacuum plating techniques were used to plate silicone, gold, chromium, aluminum oxide, silicon dioxide, inconel, nickel, and gold, over chromium. These coverings gave very erratic results. Occasionally the results very nearly equaled those in glass, and yet the same hardware seemed highly toxic the next time it was used, while another piece of hardware treated the same way might be toxic on its first usage. This was tentatively attributed to the thinness of the coatings, the possibility of incomplete coverage, and the probability that handling and cleaning, however gentle, would damage the integrity of the layer. The best of these erratic results were obtained with gold.

Outgassing and acid cleaning treatments seemed to hold the most promise for success, and a technique was developed that proved satisfactory for the frog egg experiment and was indeed used for the flight, but was not satisfactory for sea urchin sperm and eggs.

Acrylic hardware and ethylene propylene "O" rings were scrubbed thoroughly in a biological lab detergent, rinsed for a half hour in running deionized water and then placed in an acid-alcohol rinse and vibrated in an ultrasonic cleaner for 15 minutes. The rinse was made as follows:

Concentrated HCl	175 ml
Distilled H_2O	325 ml
95% Ethyl alcohol	500 ml

The hardware was rinsed again for 1 hour in deionized water, placed in a vacuum oven, and heated to 65° C while a vacuum pump was drawing a vacuum of 0.1 micron (0.0001 mm Hg) continuously for 48 hours. The hardware was cooled gradually over about 4 hours to prevent deformation. With hardware that had been used with formaldehyde, this entire procedure was preceded by a 1 hour soak in a 2-percent sodium metabisulfite solution.

This outgassing and acid cleaning technique gave good results for the frog egg experiment in which the eggs were prefertilized, but was unable to rid plastic materials sufficiently of their toxicity to maintain sea urchin eggs and sperm separately.

CONCLUSION

The experiments showed that echinoderm eggs and sperm could be kept safely in hardware made of various plastic materials if open to the air. When the hardware was closed, as would be necessary for space flight, no tested materials other than glass and silicone high vacuum grease allowed normal fertilization and development. Various coatings and treatments, while alleviating this problem somewhat, were unsuccessful in ridding the plastic hardware of harmful effects to sea urchin eggs and sperm.

Ames Research Center
National Aeronautics and Space Administration
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