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J. Vellinger and M. Deuser
SHOT, Inc.
Floyd Knobs, IN

R. Hullinger
Purdue University
West Lafayette, IN

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ENGINEERING SUPPORT OF MICROGRAVITY LIFE SCIENCE RESEARCH: DEVELOPMENT OF AN AVIAN DEVELOPMENT FACILITY

J. C. Vellinger [1] , M. S. Deuser [1] and R. L. Hullinger [2]
[1]Space Hardware Optimization Technology (SHOT), Inc.
Floyd Knobs, Indiana 47119 USA
[2] Purdue University, School of Veterinary Medicine,
West Lafayette, Indiana 47907 USA

ABSTRACT

The Avian Development Facility (ADF) is designed to provide a "window" for study of embryogenesis in space. It allows researchers to determine and then to mitigate or nullify the forces of altered gravity upon embryos when leaving and reentering earth's gravity. The ADF design will allow investigations to begin their incubation after their experiments have achieved orbit, monitor embryogenesis during orbit, and shut down the experiment and fix specimens before leaving orbit. In effect, the ADF makes every attempt to minimize launch and reentry effects in order to isolate and preserve the effects of the experimental variable(s) of the space environment.

INTRODUCTION

Experimental biologists are increasingly recognizing the advantages and conveniences the avian egg provides, as a model for examining embryogenesis and developmental dysfunctions in microgravity conditions.[1] The avian egg is a self-contained, individual development package with its own life-support system, requiring no additional food, water, or waste-removal systems. Since there is no dependence on a maternal host, embryos are unaffected by maternal responses to the microgravity environment. Furthermore, selected embryos can be experimentally manipulated without jeopardizing other members of the clutch and making selective collection of avian specimens in microgravity far easier than with most species. Thus, the avian model offers many research opportunities that can be structured for exploring human development and diseases of immobility and chronic bed rest, as well as those problems associated with adaptation to the weightless environment.[2]

This growing interest in the avian model has propagated a demand for innovative space flight hardware that can provide unique capabilities and flexibility in order to maximize these experimental opportunities. The ADF is an unprecedented flight hardware instrument, which provides complete control of all experimental variables during avian embryogenesis experiments. The first U.S. Avian embryogenesis experiment was conducted during Space Shuttle Discovery's five-day mission in March, 1989.

CHICKEN EMBRYOGENESIS IN MICROGRAVITY ABOARD SHUTTLE STS-29

This multidisciplinary experiment was to determine if exposure to 5-days of weightlessness during embryonic development would effect:

- Embryogenesis
- Bone Formation
- Vestibular Apparatus Development
- Utilization of Minerals from the Shell
- Post-Hatch Chick Performance

Flight and control groups each included sixteen 2-day old chicken embryos and sixteen 9-day old embryos. The investigators chose to use 2-day and 9-day embryos for the 5 day mission. 2-day embryos were chosen for these reasons: 1) the viability of the embryo at this age can be detected by candling, and 2) the otolith formation begins. The 9-day embryo was selected since ossification of the long bones occurs in the time frame of 9-14 days when the embryos would be on orbit. All sixty-four eggs were of Ross-Ross strain, chosen for shell strength and high hatchability.

Eggs were selected from a flock experiencing its peak hatch rate and were candled to insure fertility, proper air cell position, and shell integrity. Flight and control eggs were housed in two identical incubators, engineered to handle the vibration and acceleration of launch. Ground-based ascent acceleration testing had carefully monitored and revealed that an "upward" air cell position improved hatchability. Centrifugation tests indicated embryonic survivability was unaffected by acceleration.[3]

Eighteen hours before launch, the flight incubator was loaded onto the shuttle, while the control was maintained at the launch site. Both incubators were rotated twice before launch to counter the effects of gravity. Immediately after launch, shuttle vibration simulation was conducted on the control incubator. Once in microgravity, flight eggs were no longer rotated, while earth-control eggs were rotated 90° once every hour. Post-flight analysis revealed that the incubators had successfully prevented shell cracking and provided ideal temperature and humidity for embryo development.

In a double-blind manner, half of the embryos were sacrificed for analysis 2 hours after landing while the remaining eggs were allowed to incubate to anticipated hatch date. The high hatch rate of both control (100% for both 2- and 9-day) and experimental groups (0% for 2-day and 100% for 9-day) certified the life sustaining performance of the hardware.

Results

At the end of the flight, half of the eggs were randomly dissected while the rest of the eggs continued to be incubated. Of the eggs allowed to hatch, none of the two day flight embryos hatched, while 100% of the embryos hatched from both the 9-day group and the controls. These results show that turning of the eggs in microgravity is no longer necessary beyond 7-9 days. Turning may be a major factor in early development during spaceflight or, as seems more likely, the microgravity environment causes the death of the early embryos. Of the 2 day flight embryos that died, 1 survived to 6.5 days, 2 died at 5 days, 4 died at 4 days, 2 died at 3.5 days, 1 died at 3 days, and 6 had no embryo, but there was evidence of early embryonic development.[4,5] The sex ratios of the living embryos were as follows:[6]

6 male/2 female for the 2 day control
6 male/2 female for the 9 day space flight
4 male/4 female for the 9 day control

With respect to shell mineral content, there was no change in the amount of shell calcium, phosphorous, the magnesium in of the shell in either the control or both of the 9-day groups. Time of hatching was compared between the flight eggs and the controls. A 24-hour vigil on the 20th day of incubation revealed that there was no change in hatching time. Behavioral signs 30 minutes post-hatch were investigated and determined that there was no difference.

Vestibular nerve conduction velocity response of the flight chicks was investigated and indicated that 3 of the 8 space flight chicks had abnormal vestibular responses 28 days after the flight and 21 days after hatching.[7]

Perching ability of the hatched flight chicks was subjectively tested and there was no difference between the controls and the flight chicks up to 249 days.[6]

The only deaths seen in the 9-day embryos were in 4 of the controls, which upon neuropsy, revealed leg abnormalities as the cause which is a common problem in earth-bound chicks.

Egg production was studied through adulthood. Upon sexual maturity, there appeared to be no change in the age the females produced their first egg, the weight of the first egg, the hatchability of the egg, or the body weight of the hatched offspring. There was also no change in the concentration or quality of the sperm of the males. Second generation studies were also done with the following pairings:

Space flight male X Space flight female
Control male X Control female

In these studies, there was no difference in fertility, hatchability, body weight or feed consumption. In addition, mortality rate was normal at 2%.

In conclusion, 5 days of embryonic development in a microgravity environment (embryonic age 9d-14d) did not affect the egg shell mineral content of those chicks hatched or their post-hatch performance.[8]

AVIAN BIOTECHNOLOGY INSTRUMENTATION

The first U.S. avian microgravity hardware was designed by Space Hardware Optimization Technology (SHOT), Inc., for the Chicken Embryogenesis in Microgravity (CEM) experiment, which was a payload aboard the ill-fated Space Shuttle Challenger (STS-51L) in January, 1986. The hardware for this experiment was designed to house and protect the eggs throughout the duration of the 5 day space shuttle flight.

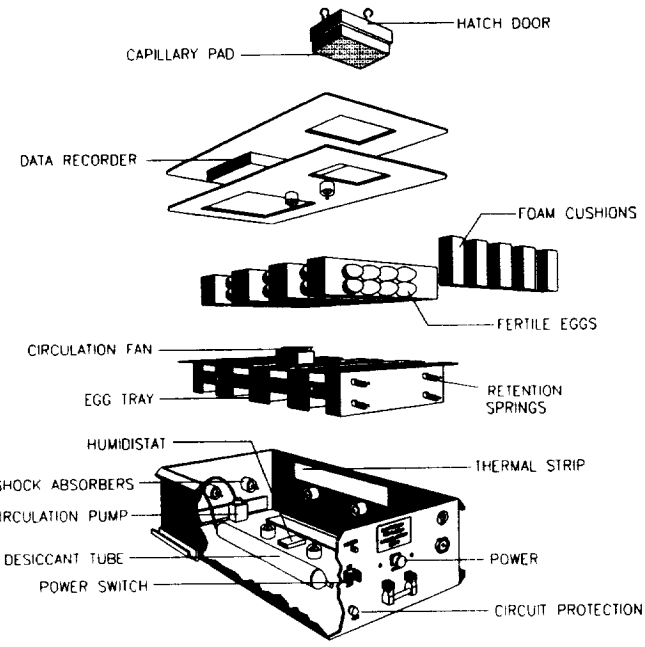

Figure 1. *The incubator developed for the STS-29 mission isolated the eggs from the vibration and acceleration of launch and landing and created optimum environmental conditions.*

The incubator (illustrated in Figure 1) was developed to carefully control temperature, humidity, and airflow. Special precautions were taken to isolate the eggs from the shock and vibration associated with the launch and landing and to insure the experiment did not pose any crew hazards.

Following the Challenger tragedy, the experiment hardware was re-flown on Space Shuttle Discovery (STS-29) in March, 1989. This three-year delay allowed several critical design improvements to be made to the hardware, improvements which significantly enhanced the quality and performance of the incubator. Hardware improvements included better shock/vibration isolation, better environmental control, and easier assembly of the payload prior to launch. Since the CEM experiment, SHOT has been actively designing and developing new and improved systems for avian microgravity hardware. SHOT is currently developing the ADF which has been identified by many gravitational biologists as having many and significant research applications for microgravity life science. This paper explains the primary functions of the ADF and its subsystems and how this technology can also be applied to understanding human dysfunction in space and chronic bedrest.

AVIAN DEVELOPMENT FACILITY (ADF)

Interest in the avian model has propagated a demand for innovative space flight hardware that can provide unique technical capabilities and flexibility in order to maximize experimental opportunities. The ADF is an unprecedented flight hardware instrument, which provides complete control of all experimental variables for avian embryogenesis experiments. Feasibility of this novel instrument has been established under a NASA Small Business Innovative Research Phase II contract, scheduled to deliver a high fidelity, fully-operational prototype in June 1996. This instrumentation offers unique engineering solutions to factors associated with concomitant controls, launch and landing forces, delayed initiation of incubation, embryonic viability, and embryonic fixation and preservation prior to reentry into earth's gravitational field. The ADF controls these variables, by combining a centrifuge, refrigeration capabilities, viability detection, and automated injection systems, along with flight-proven technologies already demonstrated in previous shuttle flight experiments. The flight hardware provides pioneering capabilities that will revolutionize avian gravitational biology and contribute to a better understand-

ing of human dysfunctions and problems with adapting to the space environment.

The ADF provides a test apparatus for supporting microgravity experiments involving either chicken or Japanese quail embryos. The ADF accommodates 24 chicken eggs or 48 quail eggs. It has the capability of providing delayed initiation of embryo development by virtue of its programmable temperature controlled environment. It also offers the investigator the opportunity for conducting concomitant controls by way of the centrifuge which subjects varying numbers of the embryos to unit gravity. The ADF is equipped with an automatic, programmable device for turning the eggs under both centrifuged and non-centrifuged conditions. Also, this facility can monitor embryo viability with the potential to down link data for real time experimental decisions. Finally, the ADF has the capability to chemically preserve any embryo during spaceflight.

The following sections briefly describe each system within the ADF and its capability for researching human dysfunctions in space. The hardware has been designed with the input of the scientific community to expand on existing avian hardware design capabilities.

Centrifuge System

The centrifuge provides the investigator the opportunity to expose egg specimens to artificial gravity and thus research opportunities to investigate the effects of varying-G on embryonic development. Carousels within the ADF can incorporate either chicken or quail eggs within the same volumetric configuration. Furthermore, by stacking carousels and designating only one or two of the carousels to function as a variable-G centrifuge, the control group of eggs can experience all the other environmental factors, the same as the experimental group.

The force of gravity created by the centrifuge acts perpendicular to the longitudinal axis of the egg. This horizontal orientation of the egg creates the least change in the gradient, since the variation in field intensity decreases as the radius increases and the gradient becomes smaller.[9] Maximizing the radius in this orientation also decreases the resulting Coriolis acceleration. Since this orientation is coincident with the natural position of most eggs in a nest this orientation for the centrifuge was adopted. Because the centrifuge provides artificial gravity for the eggs while in microgravity, these eggs must be turned. An internal gear-driven mechanism turns (or rotates) the eggs just as the hen typically moves the eggs in the nest on Earth, i.e., around their longitudinal axis.

The availability of the centrifugation in the ADF creates the opportunity to run variable gravity experiments with simultaneous controls within the same incubator. This makes possible studies of variable "dose" from zero to 1-G conditions and variable duration of exposure. Space adaptation initially hinges upon physiologic adaptation, e.g., lower body to upper body fluid shifts and altered integration of visual and proprioceptive neural signals, often producing space adaptation syndrome. The mobilization of bone mineral and degradation of skeletal muscle protein soon follow. These adaptations, especially the cardiovascular and lymph vascular changes and musculoskeletal changes, parallel those of chronic bed rest as experienced in 1-G. Experiments with the avian, complex vertebrate model during and after development will contribute to this basic research. The goal is to develop an understanding of the degree and duration of artificial gravity necessary to maintain normal embryonic development and prevent the physiologic changes, acute and chronic, which contribute to human dysfunction in space.

Automated Injection System

The automated injection system is designed to preserve embryos in microgravity prior to readaptation to earth's gravitational field during reentry and landing. The injection system automatically dispenses fixatives and/or drugs to either chicken or quail embryos in their shells. The injection system provides a sealed environment around the egg which creates a container for fixative storage. The injection system first anesthetizes the embryos and then delivers the fixative.

The ADF's injection system creates the possibility of preserving the effects of the microgravity environment before reentry to the 1-G (or fraction of 1-G) environment. Some microgravity effects can be reversed within a very short time of reentry, making them unobservable or unmeasureable. By delivering a chemical fixative, however, structural and functional indicators of these effects (enzymatic, secretions, and changes in position or distribution) can be fixed, preserving them for study following reentry. The injection system also provides a means of introducing drugs, hormones, viruses, microbes, micronutrients, growth factors, or whole-cell inoculates

into the embryo. This capability opens experimental opportunities for studying basic metabolism, system distribution, elimination of drugs, and responses of the immune and general defense systems to foreign bodies. Results of such experiments can be extrapolated to human responses in the closed environment of space.

By reversing the injection system, the ADF can extract from the egg, directly or indirectly, embryonic cells or cell-free samples. Such samples can be analyzed for embryo or shell electrolyte uptake and release. They also lend themselves to analysis of cell physiology and molecular biology. Such analyses are vital to understanding human responses to microgravity.

Viability Detection System

The viability detection system is designed to monitor embryo viability without invading the outer shell membrane. SHOT's innovative-design and method attaches electrodes to the outside of the egg shell and implants the sensor between the shell and outer membrane. These electrodes are connected to slip rings to prevent the wires from twisting due to the rotation of the centrifuge. The signal (see Figure 2) from the electrode is transmitted to the microprocessor to be processed and recorded. This system provides the investigator with the opportunity to make real-time experiment decisions based on the viability and health of the embryos, e.g. fixation.

The availability of a viability detection system is critical to determination of embryonic life and its continuation. By this "motion/flow" detection system normal heart function, fluid movement in major blood vessels, and passive/active movements of limbs can also be measured. With this capability it will be possible to determine dysfunction and more specific timing of any embryonic death. The latter is necessary in order to preserve those embryos for post flight analysis without lysis of the embryo.

Finally, the ADF's viability detection system, which allows investigators to determine dysfunction or embryonic death in real-time (and immediately preserve the embryo for post-flight analysis), promises to be an indispensable tool for understanding the requisite conditions of fertilization and early embryonic development. From such studies may come our understanding of the possibility of human reproduction during space flight or in space station colonies.

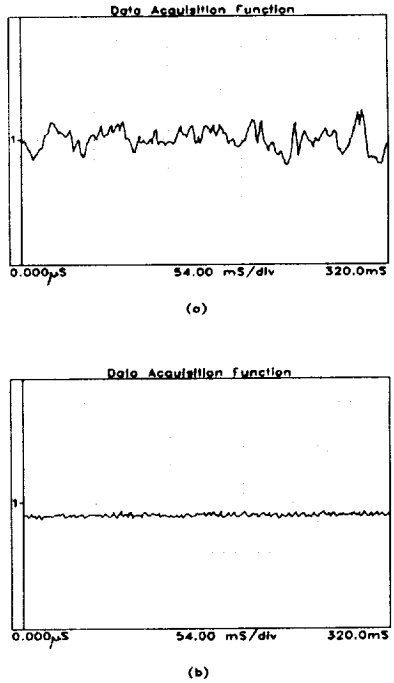

Figure 2. *Viability signal produced surface recordings of embryo activity for a) viable embryo b) embryo after death.*

Environmental Control Systems

Refrigeration Subsystem- Chilling eggs to suspend embryo development is commonly employed in the hatchery industry to equalize and manage day-to-day poultry production. The living embryo has an optimum environmental temperature at which it completes its growth. When temperatures are below this optimum value, development is drastically reduced. The desired chilling temperature for chicken embryos is 16°C, whereas quail embryos require 13°C to suspend initiation of embryo development for periods less than 1 week.[10,11] Possible applications to space biology include, e.g., the opportunity to amplify effects of space radiation at selected stages, etc.

The ADF employs a refrigeration system to take advantage of this industrial procedure during flight experiments. The refrigeration subsystem is composed of both active and passive components. The active part of the cooling system employs thermoelectric devices, which use Peltier effects for removal of heat. The passive system employs Phase Change Material since the desired chilling temperatures are well suited for their application.

Thermal Subsystem- The ADF heating system is an active system that can be energized once the orbiter has reached microgravity or whenever the investigator wants to initiate the experiment. When the thermal subsystem raises the temperature to 37.8°C, embryonic development is activated and the experiment commences.

The heating system that maintains uniform temperatures throughout the ADF must maintain a temperature at 37.8°C[12] for the first nineteen days for chickens and 37.6°C the first fourteen days for quail.[10,11,13] The temperature in the ADF is lowered during the last few days of incubation by 1.1°C for chickens and 0.3°C for quail.[10,11,13]

Oxygen & Carbon Dioxide Subsystems- The free movement of oxygen, nitrogen, carbon dioxide, and water vapor through the pores of the egg shell are important to the developing embryo. The embryo requires a constant supply of oxygen and an exhaust of carbon dioxide and moisture to thrive and develop.

At sea level, the air contains approximately 21% oxygen. Studies have shown hatchability drops by about 6% for each 1% the oxygen content of the air drops below 21%.[14] As the embryo advances in age, its oxygen requirement increases and more carbon dioxide is given off. Each process is sped up approximately 100 times between the first and twenty-first day (17th day for quails) of incubation, as illustrated in Figure 3.[15] These are minimum values of oxygen necessary for the embryonic process.

Carbon dioxide in the atmosphere of the ADF is a natural by-product of the metabolic processes during embryonic development. The embryo requires low concentrations of carbon dioxide, but high concentrations are detrimental to hatchability. The tolerance limit has been established as 0.6%, and hatchability is reduced proportionately to any increases in this amount. Concentrations above 1.6 to 2.0% usually result in drastic hatch reductions.[10]

For the ADF, a small auxiliary system supplies the oxygen for normal embryo development. Carbon dioxide removal requirements of the ADF are minimal and are accommodated by a small system employing lithium hydroxide (LiOH) as the medium for removing carbon dioxide.

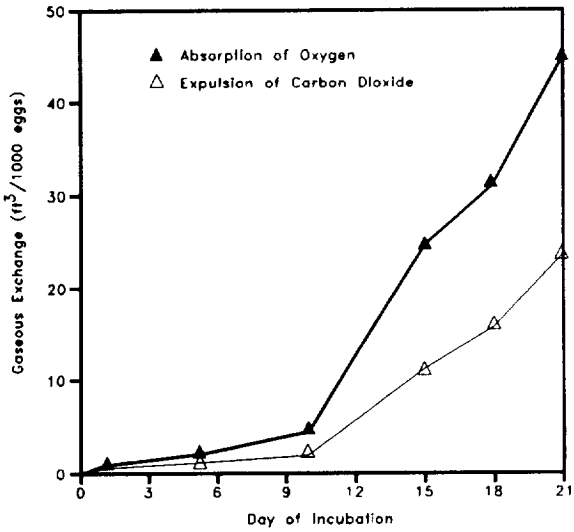

Figure 3. *Exchange of gaseous oxygen and carbon dioxide is critical to embryos during incubation. As embryo age increase, oxygen absorption and carbon dioxide expulsion increases at different rate.*

Humidity Control Subsystem- For an embryo to develop normally and transform into a fully developed chick, the egg water contents must evaporate at a controlled rate. When the egg contents dry too rapidly the chick will be smaller than normal; when they evaporate too slowly, the chick will be larger than normal. In either case the embryo is weakened, resulting in lower hatchability and a chick of poor health and reduced scientific value. To ensure proper dehydration of the egg contents, the relative humidity of the air in the ADF during the first 19 days (16 days for quail) must be maintained within rather narrow limits 66% RH ± 3%.[14] To regulate the evaporation of the egg contents, the amount of moisture in the surrounding air is controlled. High humidity reduces egg evaporation while low humidity increases it. Reducing the humidity lengthens the hatching period, while increasing humidity shortens it.[14]

The humidity control system of the ADF consists of two different subsystems. The automated misting system supplies additional moisture when the humidity level in the hardware becomes low. In contrast, the automated moisture removal system removes moisture when the humidity inside the hardware becomes excessive. These two subsystems have been designed to work together to maintain the proper humidity level in the ADF.

CONCLUSIONS

The ADF provides and continuously monitors all essential life-support systems for complete embryogenesis of quail and chicken eggs. The ADF builds upon the fundamental incubator design which was successfully flown on STS-29 in 1989. Habitat advancements now provides variable-G centrifugation, delayed incubation refrigeration, a viability detection system, and an automated injection system. These ADF features provide developmental biologists the hardware and an experimental model for study of complex vertebrate development.

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