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Providing an Optimal Environment Utilizing the Avian Development Facility for Research in Microgravity

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ABSTRACT

Space Hardware Optimization Technology (SHOT), Inc. has developed an Avian Development Facility (ADF) to isolate the effects of microgravity on embryogenesis of Japanese quail embryos by initiating and preserving embryo development in weightlessness. The ADF will monitor embryogenesis during orbit by fixing specimens at various times and will shut down the experiment before leaving orbit. In effect, the ADF makes every attempt to minimize launch and re-entry effects in order to isolate and preserve the effects of the experimental variable(s) of the space environment. The ADF also allows for egg rotation (similar to turning in a natural environment) and provides separate carousel rotation to accommodate centrifugation controls (up to 1-G) in a microgravity environment. Although no non-avian applications of ADF have yet been implemented, application to several fields can be considered, such as cell science, plant science, invertebrate biology and aquatic biology. The ADF is currently scheduled to fly and be operated on the space shuttle mid-deck in November 2001 during the UF-1 flight to the International Space Station and back.

INTRODUCTION

Developing the technology that enables human life to be sustained in space and understanding the role of gravity in living systems are two major components of NASA's life science vision. Avian embryo development research will expand the field of knowledge in both areas. Unanswered is the question of whether an organism can go through all phases of development from fertilization to successful reproduction in microgravity.

Avian models are very well characterized and thus offer research opportunities structured for observing changes in cardiovascular, vestibular, musculoskeletal, immunological and neurological development in a microgravity environment [Hester et al., 1993; Hullinger, 1993 a, b; Jones et al., 1993; Vellinger and Deuser, 1990; Vellinger et al., 1996].

Space Hardware Optimization Technology (SHOT), Inc. has developed an Avian Development Facility (ADF) to isolate the effects of microgravity on embryogenesis of Japanese quail embryos by initiating and preserving embryo development in weightlessness. The ADF provides a "window" for study of embryogenesis in space. It will provide the facilities for investigators to better understand and then to mitigate or nullify the effects of altered gravity upon embryos. The ADF is currently scheduled to fly as a mid-deck experiment on the space shuttle in November 2001 on the UF-1 flight to the International Space Station and back.

The ADF provides a unique opportunity to exercise precise control of experimental environmental parameters, such as temperature, humidity, oxygen, carbon dioxide, centrifugation (utilizing side-by-side controls in microgravity), and refrigeration. No avian experiment hardware to date has provided this capability [Vellinger et al., 1994, 1995, 1996].

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ADF SYSTEM OVERVIEW

The Avian Development Facility (ADF) (see Figure 1 for exploded view) provides a test apparatus for supporting

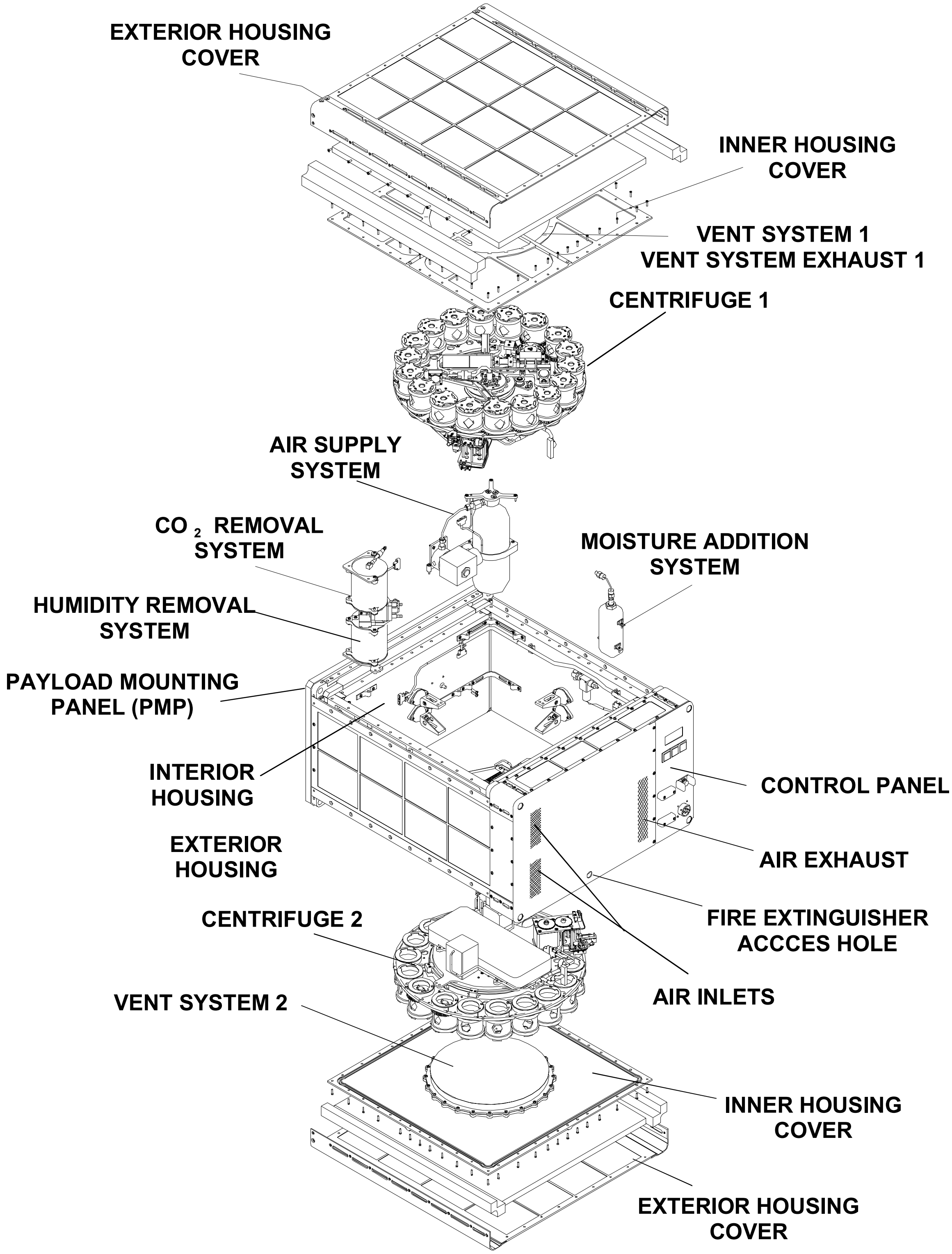

Figure 1 ADF Exploded View

microgravity experiments employing Japanese quail embryos. 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 one and/or two carousels to unit gravity.

The ADF is equipped with an independent automatic, programmable device for turning the eggs under either centrifuged or non-centrifuged conditions. Finally, the ADF has the capability to either chemically preserve or inject liquid agents (but not both during one flight increment) into any egg during the experiment. ADF incorporates vibration dampening in order to minimize launch and re-entry effects, isolate, and preserve the effects of the experimental variable(s) of the space environment.

The following sections describe the design of each system within the ADF and explain its capabilities.

ADF SUBSYSTEM DESIGN

Egg Holder Assemby - The ADF contains 36 egg holder assemblies within the specimen volume. Eighteen assemblies are mounted to each of two carousels.

The egg holder assembly provides all the interfaces with the egg. It restrains the egg while allowing vital gas exchange of the embryo across the eggshell and it also provides the first level of vibration dampening for the egg. The egg holders provide the eggs with a sealed environment when the eggs are injected with fixative at predetermined times throughout the incubation period. To promote normal embryo development, the egg holder assembly provides a means to turn the eggs while the embryos are exposed to 1-G from the centrifuge. Industry uses the rotation of the centrifuge to mimic the egg movements achieved in nature by the actions of the adult female birds.

Centrifuge System - The centrifuge system allows an investigator to expose egg specimens to microgravity, and/or provide side-by-side experiment controls within an atmospherically identical environment. Both sets of eggs are exposed to the same uncontrolled variables such as launch accelerations and landing vibrations. This produces an environment where microgravity is the only parameter different between the test and control egg specimens.

The centrifuge system is broken down into two subsystems: the carousel drive subsystem and the egg turning subsystem. The carousel drive subsystem provides a 1-G environment, while allowing for the positioning of the egg holders over the injection head for embryo fixation. The egg turning subsystem rotates the individual egg holders for optimum incubation.

Fixative Delivery - One of the innovative features of the ADF unit is its ability to chemically fix each quail embryo, preserving the tissue for post-mission evaluation and providing a snapshot of the embryo development at a specific growth stage. The ADF unit provides a precise study of the embryo development in microgravity through chemical fixation of the specimen, which eliminates post-termination decay of the embryological tissue. The chemical fixation occurs through a controlled fixative injection process that centers around the operation of the injection system. This injection system is a functioning chemical robot. It delivers approximately 30 ml of 4% paraformaldehyde fixative to an embryo sample on command via telemetry interface or pre-flight programmed timeline. The fixation preserves embryos prior to reentry.

Thermal Control System - There are two significant ADF thermal modes of operation, a chill mode and an incubation mode. A typical flight would consist of ADF being loaded with eggs on the ground and put in an “hibernation” cooling mode to keep the eggs cool enough to suspend development and minimize the impact of launch variables on embryo growth. ADF is loaded into the Shuttle Middeck while powered and remains active in the chill mode throughout the Shuttle launch. Once the Shuttle is on orbit, the ADF timeline and incubation mode is initiated. The incubation mode has a “planned” warm-up period to provide a thermal transition for the egg specimens. During egg incubation, ADF is in a heating mode to promote egg development. When in incubation mode ADF activates several subsystems that change the heat dissipation compared to the chill mode, such as rotating the egg carousel. The quality of temperature control during this phase is shown in Chart 1.

The ADF is unique in that is has two major operating modes (chill and incubation), it must meet all performance requirements in all environments.

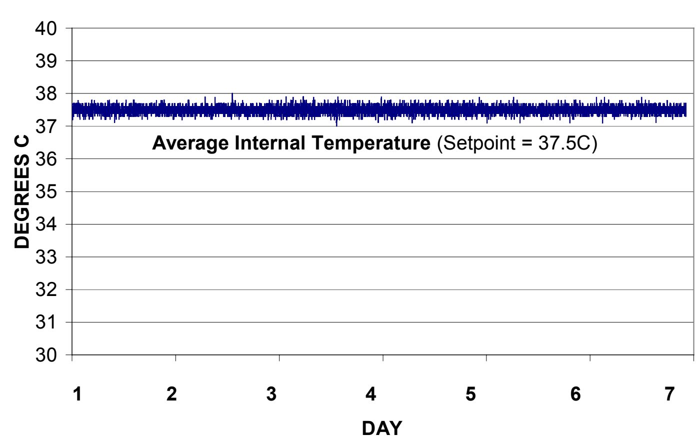

Chart 1 – Temperature Control & Monitoring (shown for incubation mode)

Humidity Control Subsystem - For an embryo to develop normally into a fully developed chick, the egg water content must evaporate at a controlled rate [9]. When the egg contents dry too rapidly, the chick is smaller than normal; when they evaporate too slowly; the chick is 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, studies have shown the relative humidity of the air during the first 16 days should be maintained at approximately 65% RH (North, 1984). To regulate the evaporation of the egg contents, the amount of moisture in the surrounding air is controlled.

The humidity control system consists of two different subsystems. The moisture addition system (Figure 1) supplies additional moisture when the humidity level in the hardware becomes low. In contrast, the automated humidity removal system (Figure 1) removes excessive moisture when the humidity inside the hardware becomes too high. These two subsystems are designed to work together to maintain the proper humidity level inside the ADF.

Gas Monitoring and Control - Permeation of oxygen and carbon dioxide through the eggshell is vital to the developing embryo [North, 1984]. Maintaining suitable concentrations of oxygen and carbon dioxide in the ADF specimen volume environment is critical to support normal quail embryo development. The gas monitoring and control system is composed of the Air System and the CO_2 Removal System, both of which ensure the environmental parameters within the ADF remain within acceptable limits. The two systems are mounted together a back corner of the specimen volume.

The air system maintains optimal oxygen levels within the ADF by supplying air (30% O_2 and 70% N_2) when the oxygen concentration falls below 3.087 psi (21% of 14.7 psi) within the specimen volume. The CO_2 removal system maintains optimal carbon dioxide levels by removing carbon dioxide from the system when the concentration rises above 0.0735 psi (0.5% of 14.7 psi) within the specimen volume. The ADF carries its own supply of air inside an air tank capable of holding 5000 psig. The tank will only be filled to approximately 3000 psig (30% Oxygen and 70% Nitrogen). The air system allows control of the level of O_2 independent of cabin air concentration. The quality of O_2 control is indicated in Chart 2.

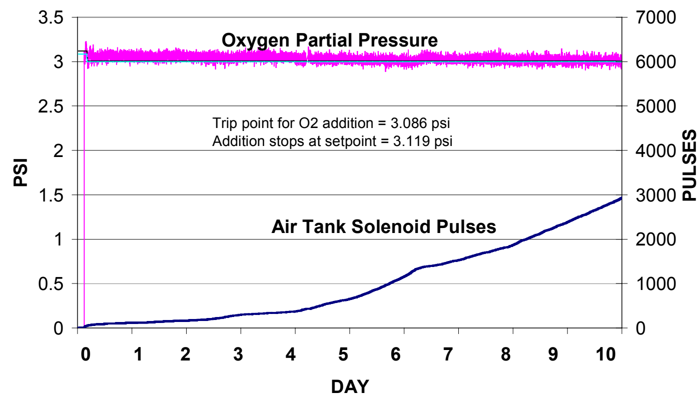

Chart 2 – Oxygen Monitoring and Control

CO_2 Removal System - Maintaining the appropriate carbon dioxide levels in the specimen volume is critical to normal embryo development. The ADF has the ability to remove carbon dioxide from the air inside the specimen volume. The CO_2 Removal System activates when the CO_2 partial pressure inside the specimen volume increase above 0.0735 psi (0.5% of 14.7 psi) removing CO_2 from the environment. The quality of CO_2 control is indicated in Chart 3.

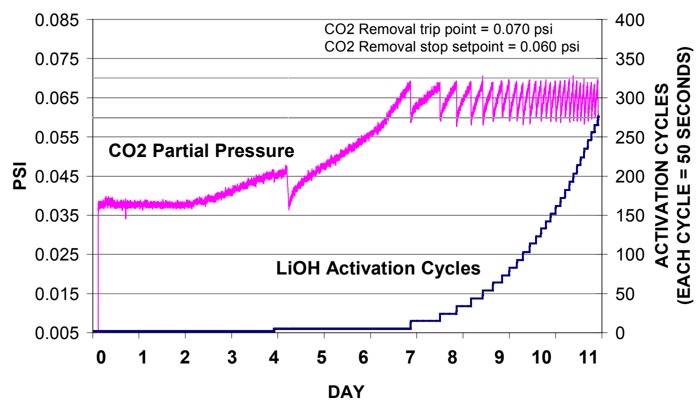

Chart 3 – Carbon Dioxide Monitoring and Control

Housing System - The ADF housing is a single middeck locker configuration, which allows the payload to interface with the shuttle Middeck. The housing consists of two subassemblies, an inner housing and an outer housing.

The outer housing provides the structural support, durability for the payload, protection, containment of the primary electronics, and the mounting interface from the ADF to the orbiter avionics wire tray.

The primary functions of the inner housing are to provide containment and structural support for the specimen volume. The void between the inner and outer housings is filled with the insulation system.

NON-AVIAN APPLICATIONS OF ADF

Considerable versatility has been built into the ADF design. The egg holder assemblies are easily replaced with other container assemblies, either closed or open to the controlled ADF internal environment. The centrifuge system is extremely desirable in a variety of life science and low-gravity fluid science applications in which variable inertial acceleration is a controlled parameter. The fixative delivery system is actually a generic chemical robot, rated for operation in the spaceflight environment.

Although no non-avian applications of ADF have yet been implemented, applications to the following examples can be considered:

- Cell science: Each egg holder assembly can accommodate a culture vial, up to 36 in number. The temperature and humidity environment for each culture is identical, and cultures can be subjected to selected levels of inertial acceleration in groups of 18. If desired, cells can be maintained in suspension prior to launch by rotating either the centrifuge rotors or the individual containers. The fixative delivery system will function as a generic chemical robot, delivering additives to each culture at a time specified by the experimenter.
- Plant science: Each egg holder assembly can accommodate a vial of germinating seeds, seedlings, or mature small plants (such as *Arabadopsis thaliana*) in up to 18 separate containers. The temperature and humidity environment for each specimen can be made identical, and vials can be subjected to selected levels of inertial acceleration in groups of 18. If desired, plants can be clinorotated at variable inertial accelerations by simultaneously rotating the centrifuge rotors and the individual containers. The fixative delivery system, as a generic chemical robot, delivers additives (water, hormones, and fixative) to each plant specimen on demand.
- Invertebrate biology: Each egg holder assembly can accommodate a vial containing small invertebrate specimens such as larvae and adult *Drosophila melanogaster* or developing nematodes *Caenorhabditis elegans* with hundreds of individuals in each of up to 36 separate containers without significant configuration changes of the ADF hardware. The temperature and humidity environment for each specimen can be made identical, and vials can be subjected to selected levels of inertial acceleration in groups of 18. If desired, specimens can be clinorotated at variable inertial accelerations by simultaneously rotating the centrifuge rotors and the individual containers. The fixative delivery system, as a generic chemical robot, can deliver additives to each vial on demand.
- Aquatic biology and ecosystems: Each egg holder assembly can accommodate a vial completely filled with liquid, which can contain, for example, a small number of small fish, such as *Medaka sp*. or model ecosystems that combine algae, small aquatic plants and minor invertebrate species (isopods, gastropods, etc.). The temperature environment for each small system can be made identical, and vials can be subjected to selected levels of inertial acceleration in groups of 18. If desired, specimens can be clinorotated at variable inertial accelerations by simultaneously rotating the centrifuge rotors and the individual containers. The fixative delivery system, as a generic chemical robot, can, with suitable modifications, deliver additives (water, food, acids and bases, fixative) to each vial of specimens on demand.

Thus the ADF is a versatile tool for gravitational research. Its application to fluid studies, although not mentioned here, is also feasible. The complete genomes of most of the species just mentioned have been sequenced making ADF an even more powerful tool for the study of gene expression and signal transduction. Modifications of ADF for these applications, while not trivial, are less difficult than building new and needlessly specialized flight lockers.

CONCLUSION

An experimental system for the study of avian development in modified gravity has been designed and built. This system consists of:

- A housing/chassis that fits the space shuttle mid-deck locker volume and isolates specimens via insulation between an inner and outer housing assembly.
- A number of egg-holder assemblies for the gentle containment and rotation of quail eggs with potential applications to other small containers of biological specimens or fluids.
- A pair of centrifuges with selectable inertial acceleration of the specimens between 0-G and 1-G or slightly higher.
- A fixative delivery mechanism that is a chemical robot under control of computer programs that run automatically or can be controlled by the investigator.
- An atmospheric control system that controls temperature, humidity, composition and flow rate of air in the specimen volume.

The ADF is useful for the study of the details of neuromuscular, musculoskeletal, vestibular, immunological, cognitive, and motor development by exploiting the simplicity of care provided by the embryonated avian egg. Such studies are enhanced by the option of returning fixed or live embryos. Finally, the ADF is a versatile tool for future application to a wide variety of gravitational experiments, especially those involving small specimens and varying levels of inertial acceleration.

ACKNOWLEDGEMENTS

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

ADF	Avian Development Facility
SHOT	Space Hardware Optimization Technology, Inc.
UF-1	Utilization Flight 1

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