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Translating Basic Research to Astronaut Health in Space: NASA Ames Rodent Specimen Biobanking for the Human Research Program

April E. Ronca[1,2], Alison J. French[3], and Jeffrey D. Smith[4]

[1]Space Biosciences Research Branch, NASA Ames Research Center, Moffett Field, CA; [2]Department of Obstetrics and Gynecology, Neurobiology and Anatomy, and Molecular Medicine and Translational Science, Wake Forest University School of Medicine, Winston-Salem, NC; [3]Life Sciences Data Archive, Space Biosciences Research Division, NASA Ames Research Center, Moffett Field, CA; [4]Division of Science and Technology Projects for Exploration, Kennedy Space Center, Cape Canaveral, FL

ABSTRACT

As an extension of NASA Ames' long history and sustaining international collaboration for sharing tissues acquired from one-off spaceflight experiments, we have recently established a new mobile operation for acquiring small animal biospecimens from ongoing ground-based studies supported by the NASA Human Research Program (HRP) organized at Johnson Space Center (JSC). Goals of Ames' Biospecimen Sharing Programs (BSPs) are to: (1) advance understanding of physiological responses and adaptations to the space environment utilizing animal models in support of fundamental space and gravitational biology research, and to promote human health in space and on Earth, (2) provide a repository of high-quality, well-preserved, and carefully archived and maintained biospecimens by applying modern approaches and established best practices in the biobanking field, and (3) establish a database for gathering broad and comprehensive scientific information corresponding to these samples, including cutting edge techniques for tracking and archiving of structural, descriptive, and administrative metadata. This program, modeled after contemporary human and animal biobanking initiatives, is yielding a rich archive of quality specimens that can be used to address a broad range of current and future scientific questions relevant to NASA Life Sciences, Exploration Medicine, and beyond.

Key words: Spaceflight; Biospecimen Sharing; Biobanking; Mammalian Models; Rodents

Correspondence to: April E. Ronca
Space Biosciences Research Division
NASA Ames Research Center
Moffett Field, CA 94035
Telephone: 650-604-3595
Fax: 650-604-3159
E-mail: April.E.Ronca@NASA.gov

ACRONYMS

ARC	Ames Research Center
BSP	Biospecimen Sharing Program
HHC	Human Health Countermeasures
HRP	Human Research Program
ISBER	International Society for Biological and Environmental Repositories
ISS	International Space Station
JSC	Johnson Space Center
KSC	Kennedy Space Center
LEO	Low Earth Orbit
NASA	National Aeronautics and Space Administration
NRA	NASA Research Announcement
STS	Space Transportation System
US/USSR	United States/Union of Soviet Socialist Republics

INTRODUCTION

Fifty years into the space age, human and animal studies have specified physiological responses and adaptations to spaceflights of up to 6 months in low Earth orbit (LEO), and up to 12 days beyond LEO. With the transition from the NASA Space Shuttle era to the ISS platform with future plans for long-duration missions (>6 months) to Mars or other destinations beyond LEO, it is clear that animal research in various biomedical fields on Earth and in space is critical to mission success. Since the 1960s, NASA Ames Research Center (ARC) has been leading Biospecimen Sharing for the NASA ARC Space Biosciences Division (formerly Life Sciences Division) to maximize utilization and scientific return from *rare, complex, and costly* spaceflight experiments (Ronca et al., 2016). In additional to advancing our knowledge of how the space environment influences basic processes of living systems, these space bioscience results are relevant to human health in space and on Earth. Here we describe a recent rodent biobanking program established at ARC to amplify the scientific impact of research funded by the HRP Human Health Countermeasures (HHC) Element at Johnson Space Center (JSC). This new HRP collaboration leverages Ames' expertise in biospecimen sharing and focuses on acquiring specimens from valuable HRP investigations being shared with qualified scientific experts through a NASA Research Announcement (NRA) mechanism. This effort further expands biospecimen sharing activities to ground-based studies utilizing analogue space platforms using rodents (e.g., hindlimb unloading, artificial gravity), thereby broadening the range of opportunities for NASA-strong research with translational relevance for human health protection in space (Alwood et al., in press). The application of contemporary biobanking approaches and best practices will preserve and archive high-quality tissues for future use. This program avoids waste of unused biospecimens that can be used to advance scientific understanding of physiological and molecular changes that occur while animals are tested, using time-honored ground-based gravitational and space research methodology. Retaining excess biospecimens using techniques that adhere to contemporary biobanking standards also follows one of the key guiding principles supporting the humane use of animals in scientific research, viz., "reducing the number of animals used to a minimum, to obtain information from fewer animals, or more information from the same number of animals" (National Research Council, 2011).

Ames Research Center Biospecimen Sharing: A Long History and Sustaining International Collaboration

The present effort builds upon more than 50 years of highly successful Ames collaborative investigations that have a proven track record for maximizing utilization and scientific return from unique animal specimens derived from NASA spaceflight research (Ronca et al., 2015). In the 1960s there were 21 biospecimen sharing experiments conducted on US Biosatellite I/II and III to determine whether basic biological processes (cell division, growth, and reproduction) occur in the absence of gravity.[1] During the period spanning 1975 to 1996, there were 9 Cosmos/Bion flights with approximately 200 collaborative experiments. Fundamental changes associated with exposure to the space environment included hematological changes, hormone changes, musculoskeletal atrophy, and vestibular and sensorimotor adaptation. The studies attempted to distinguish microgravity and/or radiation from spaceflight effects.

The Biospecimen Sharing Program (BSP) concept was originally pioneered during the early US/USSR collaborations conducted aboard unmanned Russian Cosmos flights. During these flights, small teams of dissectors worked in field laboratories at the landing site to expedite recovery of samples for return to multiple investigators in Moscow for subsequent analyses. Later, during the NASA Space Shuttle Era, there were eight missions with BSP activities on animals. The formal BSP began with the Spacelab 3 mission launched in 1985 from Kennedy Space Center (KSC) on the Space Transportation System (STS-51B). The program continued with three dedicated missions (1991-1998): Spacelab Life Sciences-1, Spacelab Life Sciences-2, and

[1]Biosatellite II was a repeat of Biosatellite I, which was never recovered

Neurolab, and involved a total of 48 collaborative experiments and principal investigators (PIs) from the US, Russia, Germany, France, and Japan. In 2005 and 2007, US and Russia collaborated again on the Foton M2 and Foton M3 flights. The US experiments on both were joint experiments, and although not a formal BSP, the overall objectives were essentially a BSP (i.e., multiple investigators sharing flight specimens). In the late Shuttle era (2010-2011), STS-131, STS-133, and STS-135 carried parent immunology and bone experiments with 10, 10, and 32 (respectively) secondary BSP investigations. The 30-day Bion-M1 flew in April 2013. Biospecimen Sharing involved 15 recovered mice and nine US investigations. New data on the impact of spaceflight on cerebral arteries, spinal cord, inner ear, and genetic processes are presently being analyzed. The 37/38-day Rodent Research-1 Validation Mission flown in September 2014 resulted in a highly successful, large scale post-flight BSP, in which flight mouse carcasses were euthanized and then fast-frozen on ISS (Choi and Ronca, 2015).

During the Shuttle era, as an extension of the BSP, flight and ground specimens not utilized by investigators were collected and subsequently stored and archived at the ARC Biospecimen Storage Facility. In a continued effort to maximize utilization of rare and valuable specimens, these spaceflight samples are available to the international science community. Since 1995, NASA has shipped samples to numerous investigators enabling new fundamental and translational insights on the regulatory effects of multiple mature and developing systems, including: skeletal, muscular, bone, cardiovascular circulatory, nervous, endocrine, reproductive, gastrointestinal and immune development, muscle, and regulatory physiology.

Practice, Assess, Refine, Practice ...

Large-scale, biospecimen sharing activities require a veritable orchestra of well-timed events. A single mistake can flow down to adversely affect multiple BSP experiments. However, all tissues and science achieved from BSP would be lost if sharing was not a part of these rare, complex, and costly experiments. For these reasons, BSP success requires intricate planning, careful coordination, and extensive, repeated practice. Once biospecimens for the primary, or parent, experiment are harvested, the secondary BSP dissection flow is initiated. A Central Dissector rapidly dissects the remaining biospecimens that are handed off, in turn, to 'runners' charged with the immediate transfer of biospecimens to specific dissection stations. Primary dissectors at each dissection station (ten or more) perform further sub-dissection and/or cleaning of specific organs, weighing samples, centrifuging samples, or otherwise preparing them to preserve optimal scientific quality (fresh, frozen, or chemical treatment). Depending upon the investigations selected for BSP, there may even be multiple investigators at various stations involved in processing a single organ or structure (e.g., distinct components of the inner ear). Postmortem decay is essential to avoid, particularly for tissues identified for molecular analyses that require RNA protection from degradation. Advance planning enables specimen quality verification before the protocol is used on spaceflown animals. Thus, repeated practice by the entire dissection team is required to insure that the flow operates smoothly, with sufficient speed and precision, and that operations at one dissection station don't interfere with those at another, or compromise tissue harvested and preserved later in the overall flow. The process of resolving these conflicts involves: Practice, Assess, Practice Refine, Practice, over and over until all of the scientific aims can be achieved as planned. Success is established when consensus on the activity is achieved among investigators utilizing diverse and potentially incongruent dissection requirements. There are many areas of possible adverse overlap, and most investigators have little to no experience sharing tissues from individual animals with multiple stakeholders. Thus, the overall process frequently involves a good deal of creativity and team problem solving.

One added benefit of BSP and this iterative process is the emergence of novel 'team science' efforts. One (of many) examples comes from the NIH Rodent (R)1 and (R)2 payloads flown on STS-66 and STS-72, respectively, that carried pregnant rats. Two investigative teams examining fetal development in space were interested post-flight in either behavioral responses or brain morphological changes. Because of the ongoing, cooperative efforts required for sharing the experimental subjects, the team members

(previously unknown to each other) were able to identify complementary aims in their research, resulting in two joint publications (Ronca et al., 2000; Ronca et al., 2008).

Biopreservation and Biobanking

NASA sharing of the animal cells and tissues and archival collections of rare products of animal flight studies was spearheaded by NASA ARC over 50 years ago. Since that time, advances in biopreservation techniques and 'omics' analyses, coupled with an increased clinical focus on personalized medicine, have led to the evolution of tissue archiving into the contemporary scientific discipline of biobanking. The term 'biobank' first appeared in the scientific literature in 1996, and for the next five years was used mainly to describe human population-based biobanks (Hewitt and Watson, 2013). The term is now applied to the growing numbers of biological collections of human, animal, plant, or microbial samples, specifically applied to sample archives with associated sample data (structural, descriptive, and administrative metadata), and to collections that are managed according to professional standards (International Society for Biological and Environmental Repositories (ISBER), 2013).

Most animal repositories are DNA banks for rare and endangered animal species (Ryder et al., 2000), although a few focus on animal health, e.g., the Morris Animal Foundation, and others provide insights into various human diseases derived from animal research, e.g., the NIA Aged Rodent Tissue Bank. The goal of the ARC BSP activities is to enhance the capacity and quality of research samples for space and gravitational biology. The program has recently been expanded to HRP supported research, the infrastructure augmented by adopting contemporary biobanking techniques for sample accrual, preservation, and archiving. In this way, we can establish common standards for spaceflight and ground-based studies across NASA programs, including Space Biology, GeneLab, and the HRP.

SUMMARY AND CONCLUSIONS

NASA ARC biospecimen sharing activities were initiated in the 1960s. The BSP was developed to ensure that valuable tissue samples, not used by the primary investigators, could be made available for distribution to and analysis by the scientific community. BSP was initially an extension of joint US/USSR studies conducted on Cosmos flights in order to share samples among agencies. Today, principal aims of the program are to: (1) maximize scientific return from the organisms flown in space and from ground-based analogue studies, (2) reduce overall animal usage, and (3) broaden participation by the research community. Recently we have established a mobile biospecimen sharing operation through which our dissection team travels to laboratory locations where NASA-funded, ground-based studies are being executed. We are presently supporting biospecimen sharing for an HRP-funded long-duration (90-day) rat hindlimb unloading study at University of California at Davis (PI: Charles Fuller, PhD). Thus, Ames' BSP is readily scalable to other NASA research projects as needed. Our program, modeled after contemporary human and animal biobanking efforts, is uniquely positioned to foster the establishment of high-fidelity translational linkages to human health in space and on Earth.

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