Fertility Challenges on Spaceflight Missions

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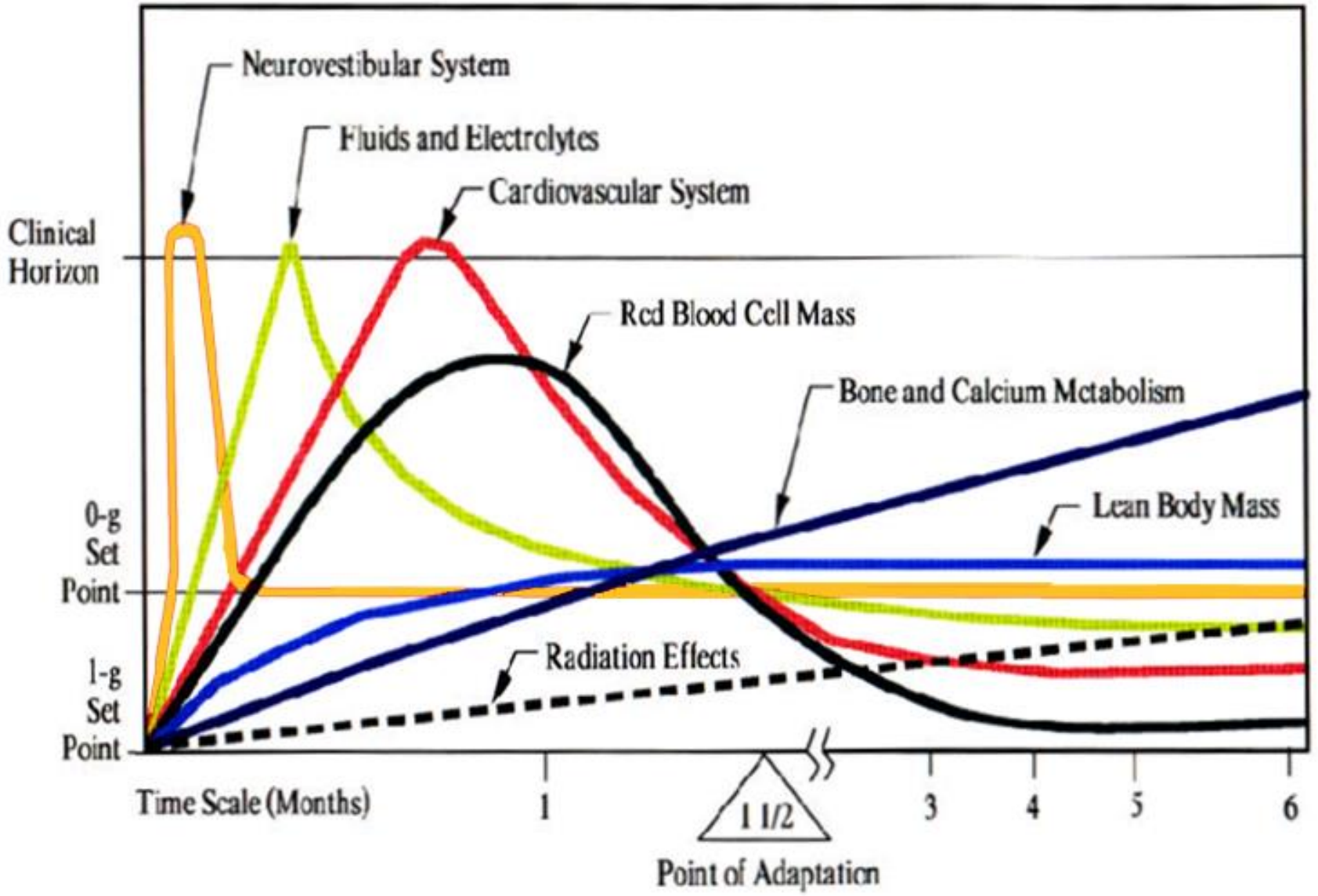
Translational Research Institute
Baylor College of Medicine

Reproductive tissues are sensitive to radiation

Single Dose (Gy)		Fractionated Dose (Gy)	
Ovary	2–6	Testes	1–2
Bone marrow	2–10	Ovary	6–10
Testes	2–10	Eye (lens)	6–12
Eye (lens)	2–10	Kidney	20–30
Mucosa	5–20	Thyroid	20–40
Gastrointestinal	5–10	Lung	23–28
Lung	7–10	Skin	30–40
Colorectal	10–20	Liver	35–40
Kidney	10–20	Bone marrow	40–50
Vasculoconnective tissue	10–20	Heart	43–50
Liver	15–20	Gastrointestinal	50–55
Skin	15–20	Vasculoconnective tissue	50–60
Peripheral nerve	15–20	Spinal cord	50–60
Spinal cord	15–20	Brain	55–70
Brain	15–25	Peripheral nerve	65–77
Heart	18–20	Mucosa	65–77
Bone and cartilage	>30	Bone and cartilage	>70
Muscle	>70	Muscle	>70

*From Rubin P. Law and order of radiation sensitivity: absolute versus relative. In: Vaeth JM, Meyer JL, eds. Frontiers of radiation therapy and oncology. Basel: Karger; 1989:7–40.

Many physiological systems are affected by spaceflight



Medical Complaints in Space

Based on ISS Missions:

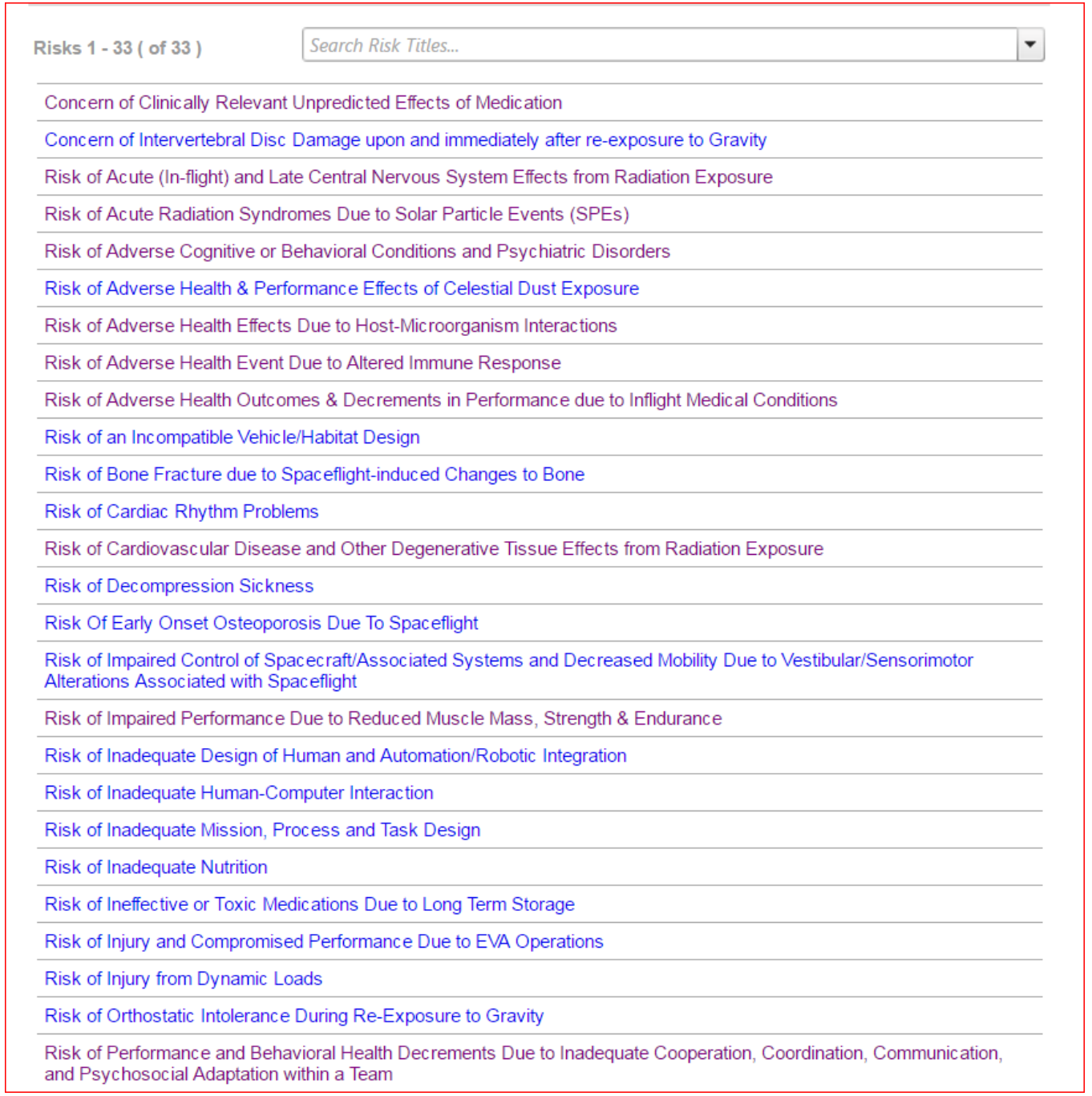
Anorexia
Space motion sickness
Fatigue
Insomnia
Dehydration
Dermatitis
Back pain
Upper respiratory infection
Conjunctival irritation
Subungual hemorrhage
Urinary tract infection
Cardiac arrhythmia
Headache
Muscle strain
Diarrhea
Constipation

From Clement, Fundamentals of Space Medicine, 2003

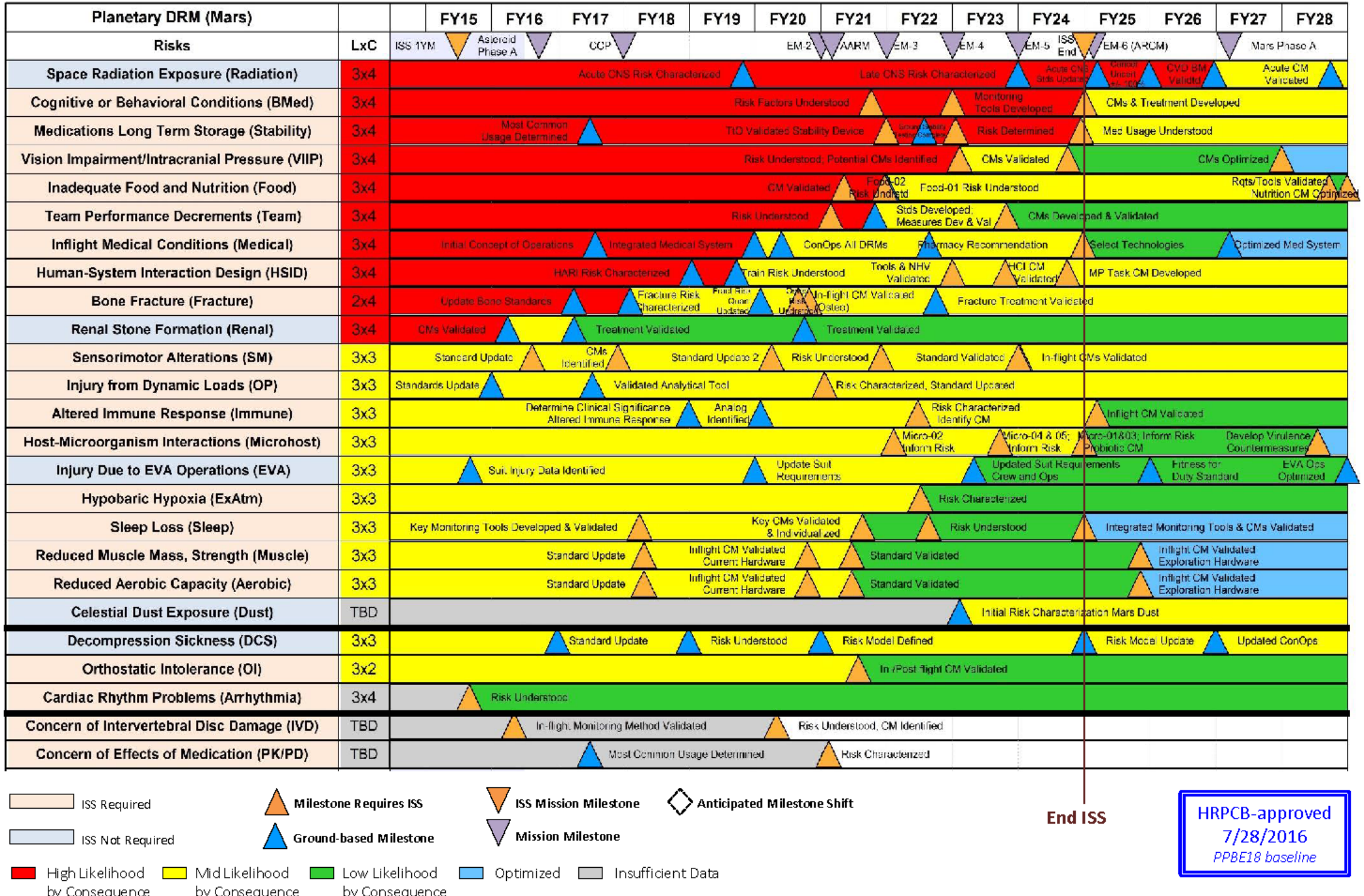
Based on Space Shuttle, 1988- 1995

Facial Fullness
Headache
Sinus congestion
Dry skin, irritation, rash
Eye irritation, dryness, redness
Foreign body in eye
Sneezing/coughing
Sensory changes
Upper respiratory infection
Back muscle pain
Leg/foot muscle pain
Cuts
Shoulder/trunk muscle pain
Hand/arm muscle pain
Anxiety/annoyance
Contusions
Ear problems (usu. Pain)
Neck muscle pain
Stress/tension
Muscle cramp
Abrasions
Fever, chills
Nosebleed
Psoriasis, folliculitis, seborrhea
Low heart rate
Myoclonic jerks

NASA's Human Research Program Risks to Human Spaceflight



https://humanresearchroadmap.nasa.gov/Risks/


Planetary DRM (Mars)
Risks
LxC
FY15
FY16
FY17
FY18
FY19
FY20
FY21
FY22
FY23
FY24
FY25
FY26
FY27
FY28
ISS 1YM
Asteroid Phase A
CCP
EM-2
AARM
EM-3
EM-4
EM-5
ISS End
EM-6 (ARCM)
Mars Phase A
Space Radiation Exposure (Radiation) 3x4
Acute CNS Risk Characterized
Late CNS Risk Characterized
Acute CNS Stds Updated
CVD BM Validtd
Acute CM Validated
Cognitive or Behavioral Conditions (BMed) 3x4
Risk Factors Understood
Monitoring Tools Developed
CMs & Treatment Developed
Medications Long Term Storage (Stability) 3x4
Most Common Usage Determined
TID Validated Stability Device
Risk Determined
Med Usage Understood
Vision Impairment/Intracranial Pressure (VIIP) 3x4
Risk Understood, Potential CMs Identified
CMs Validated
CMs Optimized
Inadequate Food and Nutrition (Food) 3x4
CM Validated
Food-02 Risk Undtstd
Food-01 Risk Understood
Rqts/Tools Validated
Nutrition CM Optimized
Team Performance Decrements (Team) 3x4
Risk Understood
Stds Developed; Measures Dev & Val
CMs Developed & Validated
Inflight Medical Conditions (Medical) 3x4
Initial Concept of Operations
Integrated Medical System
ConOps All DRMs
Pharmacy Recommendation
Select Technologies
Optimized Med System
Human-System Interaction Design (HSID) 3x4
HARI Risk Characterized
Train Risk Understood
Tools & NHV Validated
HCI CM Validated
MP Task CM Developed
Bone Fracture (Fracture) 2x4
Update Bone Standards
Fracture Risk Characterized
In-flight CM Validated (Osteo)
Fracture Treatment Validated
Renal Stone Formation (Renal) 3x4
CMs Validated
Treatment Validated
Treatment Validated
Sensorimotor Alterations (SM) 3x3
Standard Update
CMs Identified
Standard Update 2
Risk Understood
Standard Validated
In-flight CMs Validated
Injury from Dynamic Loads (OP) 3x3
Standards Update
Validated Analytical Tool
Risk Characterized, Standard Updated
Altered Immune Response (Immune) 3x3
Determine Clinical Significance Altered Immune Response
Analog Identified
Risk Characterized Identify CM
Inflight CM Validated
Host-Microorganism Interactions (Microhost) 3x3
Micro-02 Inform Risk
Micro-04 & 05; Inform Risk
Micro-01&03; Inform Risk Probiotic CM
Develop Virulence Countermeasures
Injury Due to EVA Operations (EVA) 3x3
Suit Injury Data Identified
Update Suit Requirements
Updated Suit Requirements Crew and Ops
Fitness for Duty Standard
EVA Ops Optimized
Hypobaric Hypoxia (ExAtm) 3x3
Risk Characterized
Sleep Loss (Sleep) 3x3
Key Monitoring Tools Developed & Validated
Key CMs Validated & Individualized
Risk Understood
Integrated Monitoring Tools & CMs Validated
Reduced Muscle Mass, Strength (Muscle) 3x3
Standard Update
Inflight CM Validated Current Hardware
Standard Validated
Inflight CM Validated Exploration Hardware
Reduced Aerobic Capacity (Aerobic) 3x3
Standard Update
Inflight CM Validated Current Hardware
Standard Validated
Inflight CM Validated Exploration Hardware
Celestial Dust Exposure (Dust) TBD
Initial Risk Characterization Mars Dust
Decompression Sickness (DCS) 3x3
Standard Update
Risk Understood
Risk Model Defined
Risk Model Update
Updated ConOps
Orthostatic Intolerance (OI) 3x2
In-/Post-flight CM Validated
Cardiac Rhythm Problems (Arrhythmia) 3x4
Risk Understood
Concern of Intervertebral Disc Damage (IVD) TBD
In-flight Monitoring Method Validated
Risk Understood, CM Identified
Concern of Effects of Medication (PK/PD) TBD
Most Common Usage Determined
Risk Characterized
ISS Required
ISS Not Required
Milestone Requires ISS
Ground-based Milestone
ISS Mission Milestone
Mission Milestone
Anticipated Milestone Shift
End ISS
High Likelihood by Consequence
Mid Likelihood by Consequence
Low Likelihood by Consequence
Optimized
Insufficient Data
HRPCB-approved
7/28/2016
PPBE18 baseline

Drosophila

Astronaut Jeffrey S. Ashby, pilot, works at the Space Tissue Loss-B experiment on Space Shuttle Columbia's middeck during a 5 day low Earth orbit mission. Just above and to the right of of his hands is the part of the Commercial Generic Bioprocessing Apparatus (CGBA) for the National Institute of Health (NIH-B experiment). It is an experiment designed to investigate the effects of space flight on neural development in Drosophila melanogaster (fruit fly) larvae. This information may help scientists understand how gravity affects nerve growth and development and how neural connections to muscle fibers work.

Pergamon

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CRICKETS IN SPACE

Eberhard Horn*, Sybille Böser*, Susanne Förster*, Pascal Riewe, Claudia Sebastian* and Hans Agricola*****

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****Department of Cell Biology, University of Jena, Erbertstr. 1, D-07*

"Crickets in Space" (CRISP) was a Neurolab experiment by which the balance between genetic programs and the gravitational environment for the development of a gravity sensitive neuronal system was studied. The model character of crickets was justified by their external gravity receptors, identified position-sensitive interneurons (PSI) and gravity-related compensatory head response, and by the specific relation of this behavior to neuronal activation systems. These advantages allowed us to study the impact of modified gravity on cellular processes in a complex organism. Eggs, 1st, 4th and 6th stage larvae of *Acheta domesticus* were used. Post-flight experiments revealed a low susceptibility of the behavior to microgravity (μg) and hypergravity (hg) while the physiology of the PSI was significantly affected. Immunocytological investigations revealed a stage-dependent sensitivity of thoracic GABAergic motoneurons to 3g-conditions concerning their soma sizes but not their topographical arrangement. Peptidergic neurons from cerebral sensorimotor centers revealed no significant modifications by microgravity. The contrary physiological and behavioral results indicate a facilitation of 1g-readaptation by accessory gravity, proprioceptive and visual sense organs. Absence of anatomical modifications point to an effective time window of μg- or hg-exposure related to the period of neuronal proliferation. © 2001 Elsevier Science Ltd. All rights reserved.

Biological Sciences in Space, Vol.12 No.4 (1998): 377-388

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Behavior and Reproduction of Invertebrate Animals During and After A Long-Term Microgravity: Space Experiments Using An Autonomous Biological System (ABS)

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229-8510, Japan
[5]Paragon Space Development Corporation, 810 E. 47th St., Suite 104, Tucson, Arizona 85713, USA

Fig.4 Ground and flight samples immediately after the first Mir experiment. Ground samples (the two cylindrical vessels at left), and the flight samples (two units at right) which were recovered after being exposed to microgravity for 4 months in Mir. Note the complete disappearance of water plant (hornwort) in the flight ABS units. The ABS unit shown here are, from left, GC8 and GC6 (the two ground units), FU10 and FU9 (the two flight units), respectively.

Fig.2 A space-flown water flea (Daphnia) carrying an embryo in its egg sac. The Daphnia was immobilized (kept in ice-cold water) when taken out from the ABS units after landing. The experiment was a 10-day flight in a Space-shuttle. Several embryos must have been shed from its egg sac while in space. The one embryo outside the egg sac shown here is the one shed at the cold treatment.

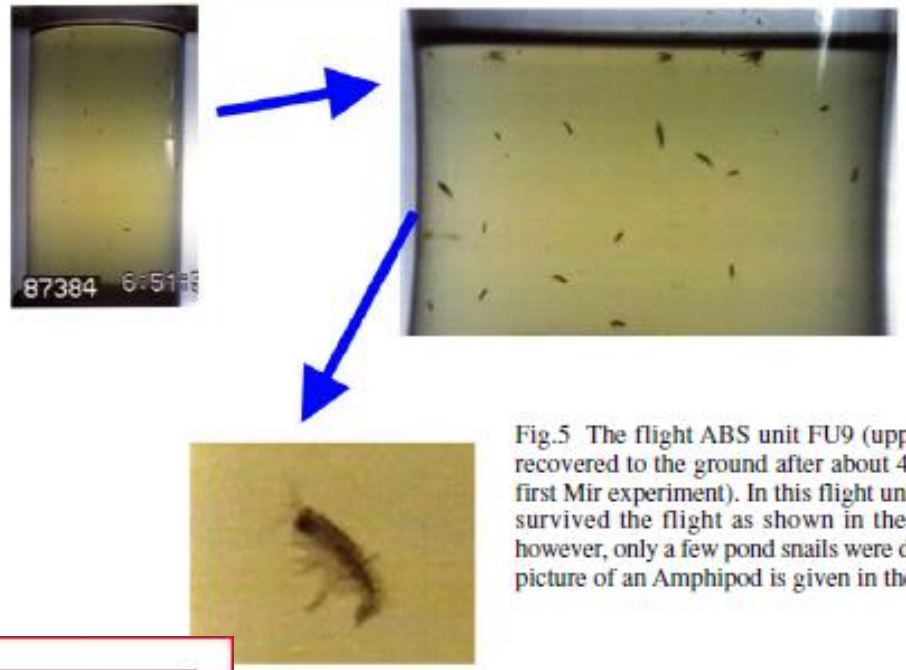

Fig.5 The flight ABS unit FU9 (upper-left panel) when recovered to the ground after about 4 months in Mir (the first Mir experiment). In this flight unit, many Amphipods survived the flight as shown in the upper-right panel; however, only a few pond snails were detected. An enlarged picture of an Amphipod is given in the bottom panel.

Fig.1 Returned flight ABS units (two cylindrical vessels at left) and one of the ground control units (one at right). The Space-shuttle experiment in May 1996.

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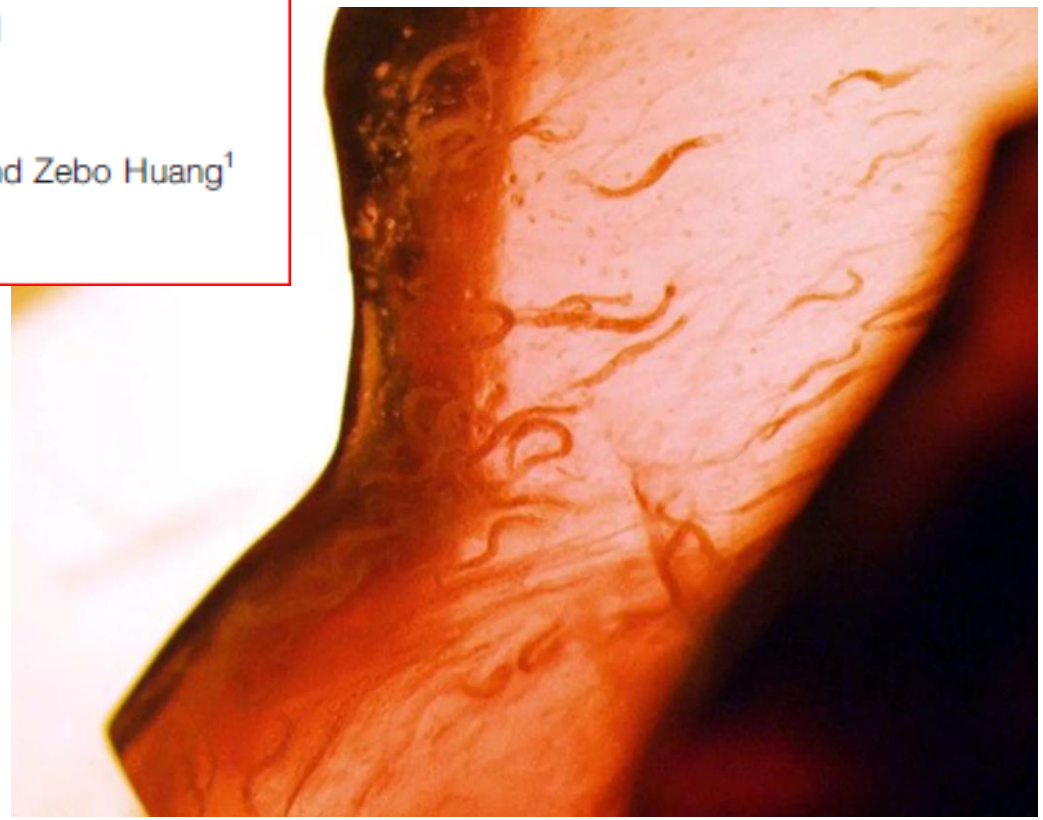
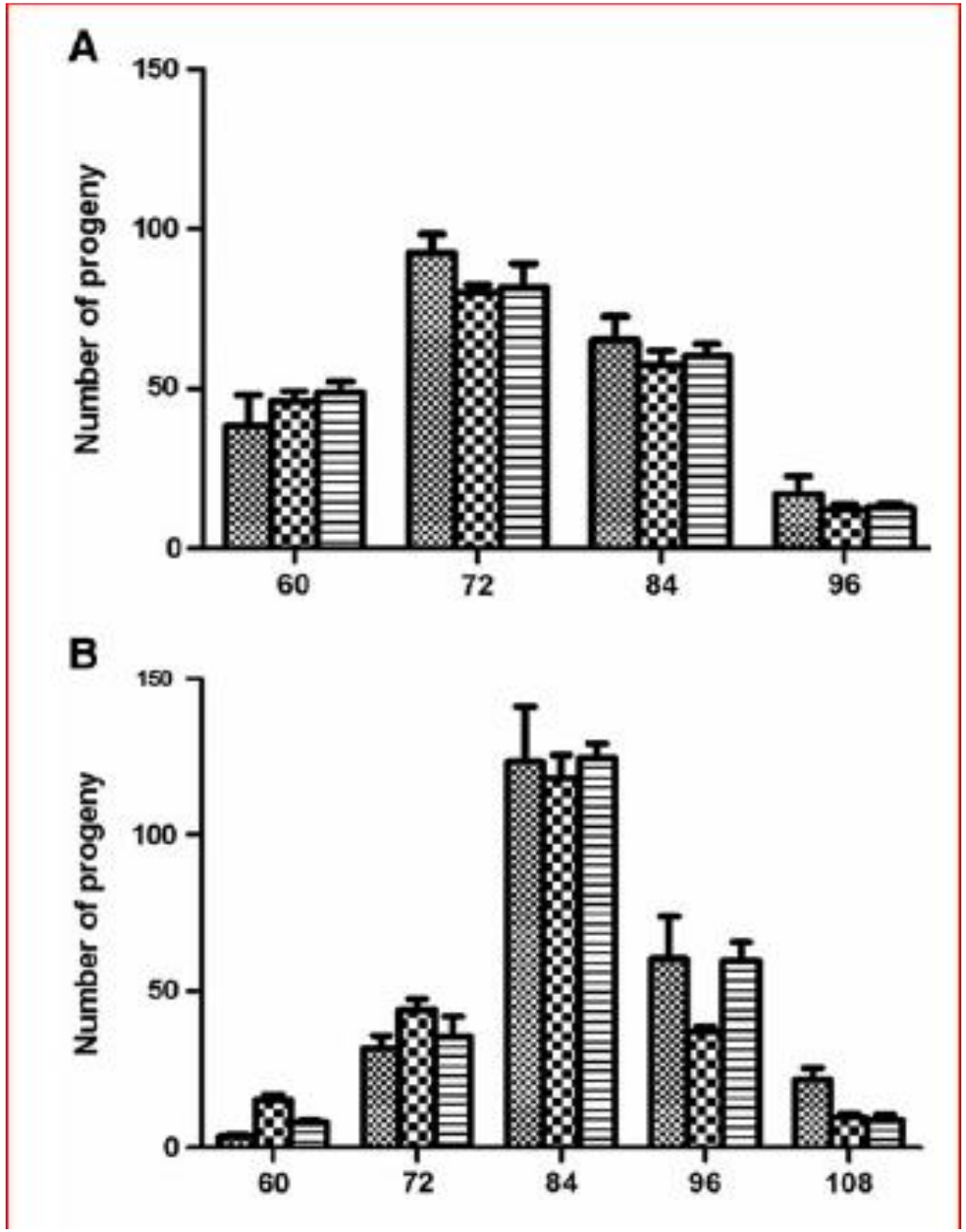
Reproductive and Locomotory Capacities of *Caenorhabditis elegans* Were Not Affected by Simulated Variable Gravities and Spaceflight During the Shenzhou-8 Mission

Liang Qiao,[1] Sang Luo,[1] Yongding Liu,[2] Xiaoyan Li,[2] Gaohong Wang,[2] and Zebo Huang[1]





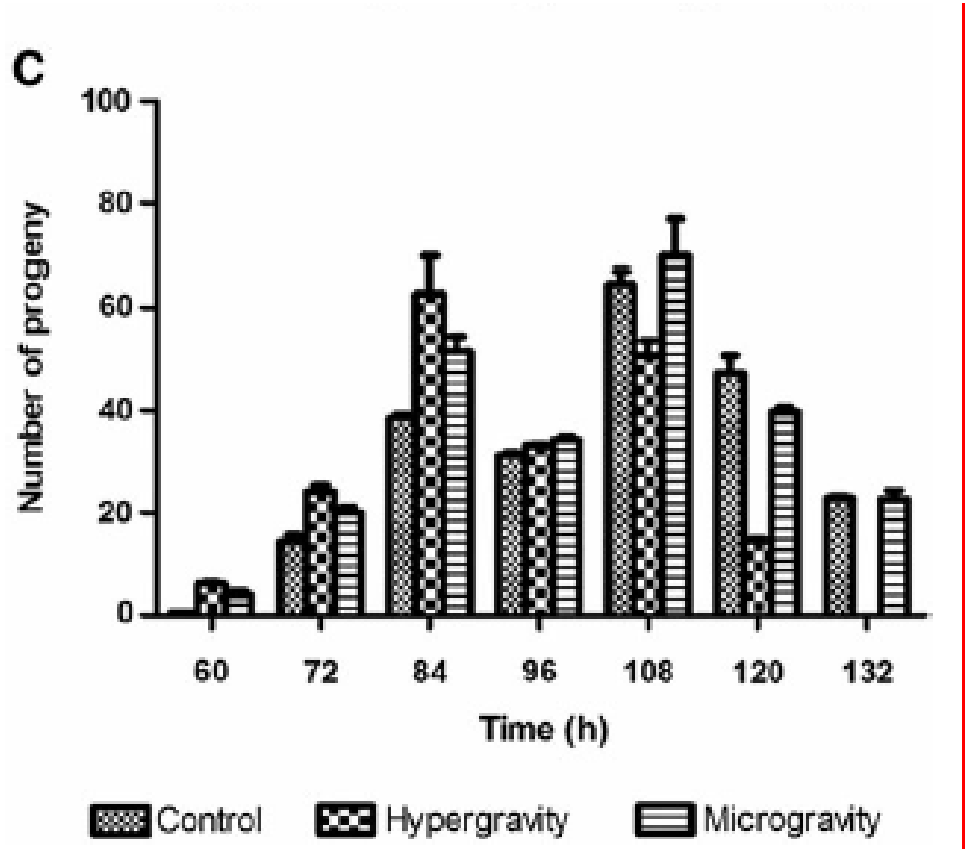
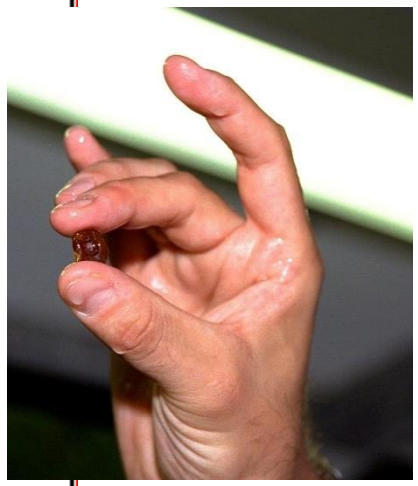


FIG. 2. Effect of centrifuge-produced hypergravity and clinostat-simulated microgravity on the brood size of *C. elegans*. Synchronized N2 (wild-type; **A**), HA759 (polyQ150 in sensory ASH neurons; **B**), and AM141 (polyQ40 in body wall muscle cells; **C**) nematodes in NGM plates containing OP50 bacteria were treated with hypergravity or microgravity from L1 to the end of egg production. After egg-laying started, the nematodes were transferred to fresh plates every 12 h, and the number of progenies were counted 2 days later in the original plates. Results are means ± standard error of the mean from three independent experiments.

J Appl Physiol
89: 1601–1607, 2000.

Influence of microgravity on crystal formation in biomineralization



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Received 25 April 2000; accepted in final form 3 July 2000

RESULTS

Nineteen adult snails and 298 embryos from STS-89 flight module and 19 adult snails and 176 embryos from the ground control module, respectively, were used for our experiments. From STS-90, we used 15 adult snails and 6 embryos from the flight module and 15 adult snails and 13 embryos from the ground control module.

Embryonic development. Microgravity does not influence shell building in snail embryos, when measured from the beginning of development, including during spawning and the first cleavages. The shell building occurs in a regular manner (4) (Fig. 1, *A–D*). The shell is formed with no differences shown between flight and

A water snail (Biomphalaria glabrata), like those that are part of the Neurolab payload on Space Shuttle Mission STS-90. The snails flew in a middeck locker-sized fresh water habitat, designed to allow the controlled incubation of aquatic species in a self-stabilizing, artifical ecosystem for up to three weeks under space conditions. Investigations during the Neurolab mission will focus on the effects of microgravity on the nervous system. The crew of STS-90, launched April 16 at 2:19 p.m. EDT, included Commander Richard Searfoss, Pilot Scott Altman, Mission Specialists Richard Linnehan, D.V.M., Dafydd (Dave) Williams, M.D., and Kathryn (Kay) Hire, and Payload Specialists Jay Buckey, M.D., and James Pawelczyk, Ph.D

Scanning Microscopy, Vol. 6, No. 4, 1992 (Pages 1129-1135) 0891-7035/92$5.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

SCANNING ELECTRON MICROSCOPE OBSERVATIONS OF BRINE SHRIMP LARVAE FROM SPACE SHUTTLE EXPERIMENTS

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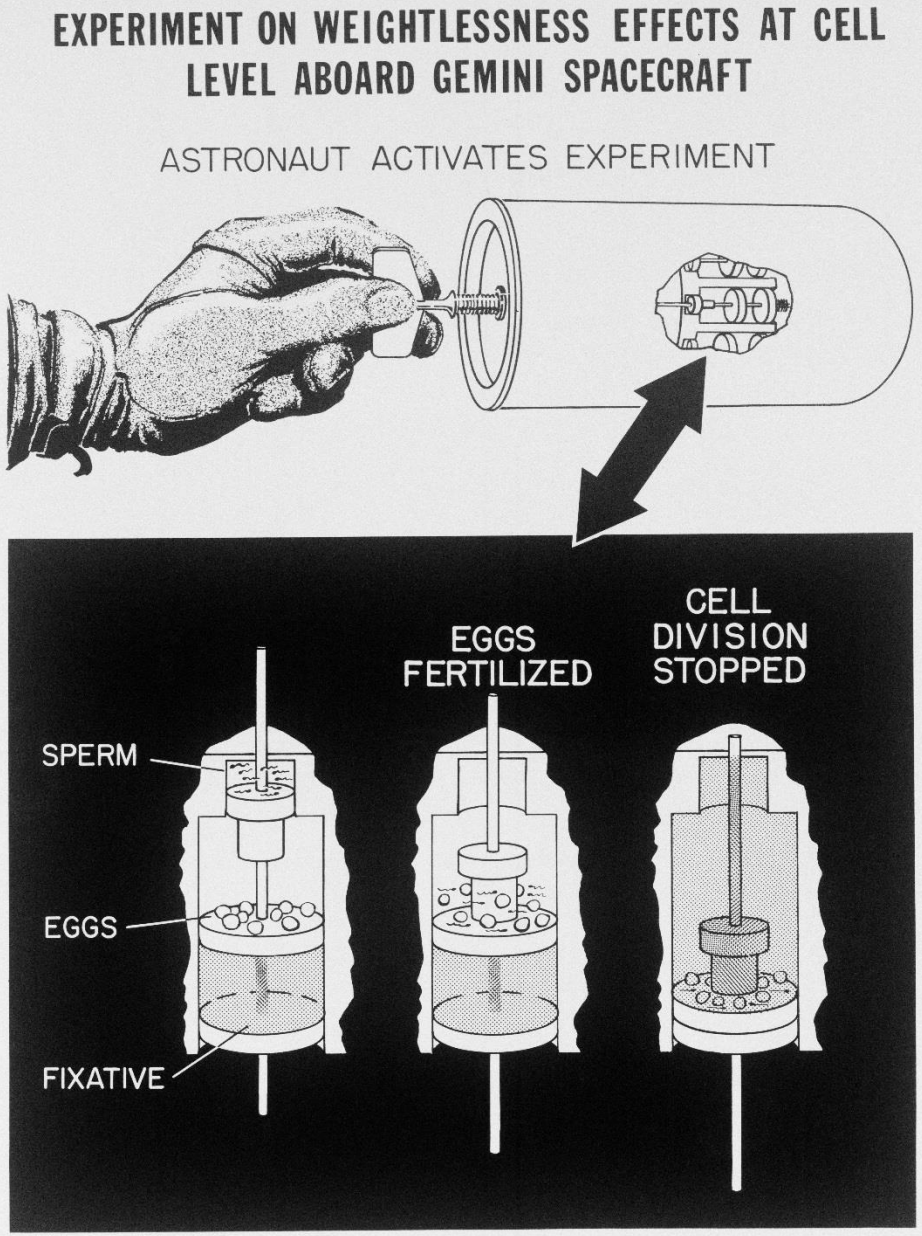
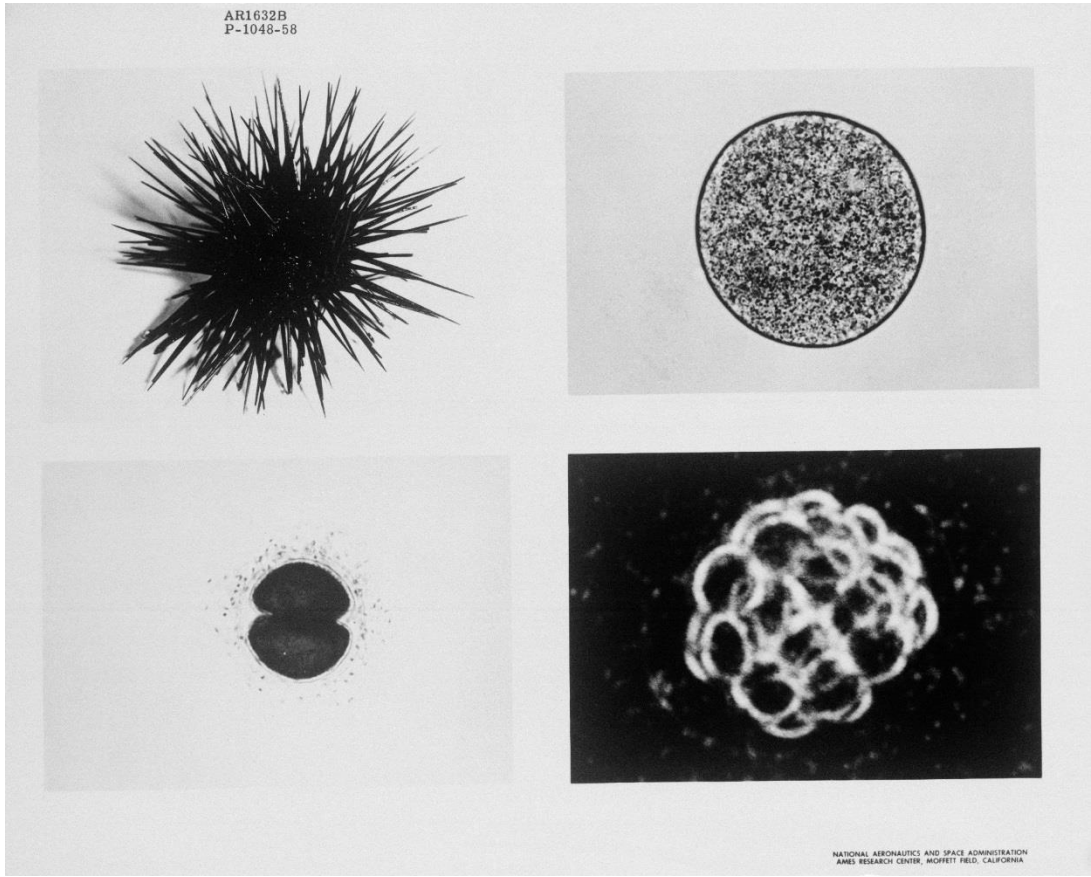
Brine shrimp cysts placed in 5 ml syringes were rehydrated with salt water and hatched during a 9 day space shuttle mission. Subsequent larvae developed to the 8th larval stage in the sealed syringes. We studied the morphogenesis of the brine shrimp larvae and found the larvae from the space shuttle experiments similar in rate of growth and extent of development, to larvae grown in sealed syringes on the ground. Extensive differentiation and development of embryos and larvae can occur in a microgravity environment.



S65-18766 (March 1965) --- Diagram of experiment planned for the Gemini-Titan 3 mission scheduled on March 23, 1965, to find out if there are effects of weightlessness on individual living cells. The round canister (top) shows the experiment package. It will contain eight identical chambers, each with sections of sperm, eggs and fixative. Cells are eggs of the spiny, black sea animal, the sea urchin. Bottom panel shows the three stages of each chamber. From left in the first stage, sperm, eggs and fixative are separated. By turning the handle, astronauts will fertilize a certain portion of the eggs, which will begin to divide. At 20 minutes after launch, further turns of the handle will force fixative into two chambers and stop cell division. At 70 minutes after launch, cell division in four more chambers will be stopped, and just prior to re-entry, growth of the remaining two chambers will be terminated by a turn of the handle. This system will allow study after the flight of how cells divided after various time periods in weightlessness. Abnormalities would suggest weightlessness effects on living tissue and possible hazard to prolonged manned spaceflight.

(March 1965) --- Effects of the weightless environment on cell division, the basic growth process for living tissue, will be studied during the Gemini-Titan 3 flight scheduled for March 23, 1965. A spiny black sea urchin (upper left) is stimulated by mild electric shock or potassium chloride. As a result it sheds many thousands of eggs. When fertilized, these eggs become actively dividing cells very similar in basic processes to cells of other animals, including humans. These pictures show stages of cell division. At upper right is a single cell; at lower right cell divisions have produced many cells. Cell photos are magnified about 700 times, and all cells shown are too small to be seen by the naked eye. (Photos at upper right and lower left are of sea urchin eggs. Group of cells at lower right are from a sand dollar, which like the sea urchin, is an Echinoderm. Its eggs are virtually identical and are used interchangeably with those of the sea urchin in NASA Ames Center weightlessness experiments.) The Gemini experiment will involve cell division like that shown here. This will take place during several hours of weightlessness aboard the Gemini spacecraft. The experiment will be flown back to laboratories at Cape Kennedy after spacecraft recovery. It has been designed so that any abnormal cell division found by postflight analysis should suggest that the weightless environment has effects on individual cells. This might mean hazards for prolonged periods of manned spaceflight.



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Journal of Biotechnology 47 (1996) 167–177

The sea urchin larva, a suitable model for biomineralisation studies in space (IML-2 ESA Biorack experiment '24-F urchin')

Hans-Jürg Marthy[a,*], Gilbert Gasset[b], René Tixador[b], Philippe Schatt[a], Brigitte Eche[b], Arnaud Dessommes[a], Thierry Giacomini[c], Gérard Tap[d], Dimitri Gorand[e]

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[e]*Service de Microscopie Electronique, Université Perpignan, France*

BIOLOGY OF REPRODUCTION **63**, 551–558 (2000)

Microgravity and Hypergravity Effects on Fertilization of the Salamander *Pleurodeles waltl* (Urodele Amphibian)[1]

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Centre de Biologie du Développement,[5] UMR 5547 CNRS, Université Paul Sabatier, Toulouse, France

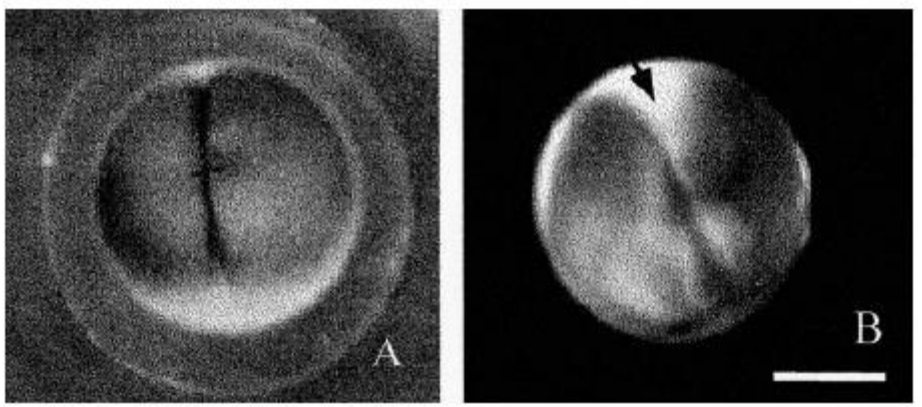

FIG. 2. Light micrographs of fertilized eggs photographed at the same time point. **A**) A 1G-egg (ground gravity). **B**) A μG-egg. Notice that the pigmentation concentrated around the animal pole and an unpigmented area covered a part of the animal hemisphere (arrow). Bar = 600 μm.

Astronaut Donald Thomas conducts the Fertilization and Embryonic Development of Japanese Newt in Space (AstroNewt) experiment at the Aquatic Animal Experiment Unit (AAEU) inside the International Microgravity Laboratory-2 (IML-2) science module. The AstroNewt experiment aims to know the effects of gravity on the early developmental process of fertilized eggs using a unique aquatic animal, the Japanese red-bellied newt. The newt egg is a large single cell at the begirning of development. The Japanese newt mates in spring and autumn. In late autumn, female newts enter hibernation with sperm in their body cavity and in spring lay eggs and fertilized them with the stored sperm. The experiment takes advantage of this feature of the newt. Groups of newts were sent to the Kennedy Space Center and kept in hibernation until the mission. The AAEU cassettes carried four newts aboard the Space Shuttle. Two newts in one cassette are treated by hormone injection on the ground to simulate egg laying. The other two newts are treated on orbit by the crew. The former group started maturization of eggs before launch. The effects of gravity on that early process were differentiated by comparison of the two groups. The IML-2 was the second in a series of Spacelab flights designed to conduct research by the international science community in a microgravity environment. Managed by the Marshall Space Flight Center, the IML-2 was launch on July 8, 1994 aboard the STS-65 Space Shuttle Orbiter Columbia mission.

Biological Sciences in Space, Vol.15 Suppl. (2001)

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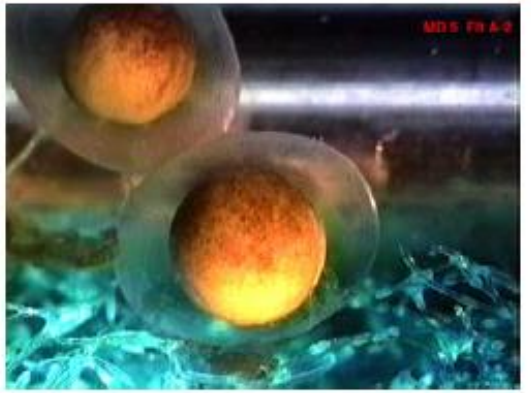
Japanese Red-Bellied Newts in Space - AstroNewt Experiment on Space Shuttle IML-2 and Space Flyer Unit

Masamichi Yamashita[1], Akemi Izumi-Kurotani[1], Mari Imamizo[2], Hajime Koike[3], Makoto Okuno[3], Carl J. Pfeiffer[4], Shinji Komazaki[5], Fumie Sasaki[6], Yoshinobu Ohira[7], Isamu Kashima[8], Sakae Kikuyama[9], Takeo Ohnishi[10], Yoshihiro Mogami[11], and Makoto Asashima[3]

1 Institute of Space and Astronautical Science; 2 Toho University; 3 University of Tokyo, 4 Virginia Polytechical Institute, State University; 5 Saitama Medical College; 6 Tsurumi University; 7 National Institute of Fitness and Sports; 8 Kanagawa Dental College; 9 Waseda University; 10 Nara Medical College; 11 Ochanomizu University

***Abstract*:** Biological effects of gravity was examined in embryonic development of Japanese red bellied newt. Two space newt missions were conducted in 1994 and 1995. The Second International Microgravity Laboratory was flown in 1994 as one of the SpaceLab missions. Space Flyer Unit, a Japanese space platform, was delivered to the earth orbit by the third launch of the H-II rocket and retrieved by Space Shuttle in 1996. Female newts were induced to lay eggs in orbit at these two space missions. Eggs were successfully obtained on both missions, and exposed to space environment from its early developmental stages. Morphology of the embryos was found not deviated from those developed on ground, as long as in the images taken in orbit or the examined specimen retrieved to ground. On the other hand, pathological changes were discovered in several organs of the adult newts that returned alive from their space flight.

a



b

c

Fig. 4 Embryos in Flight AAEU Cassettes
a; F A-2 on MD5, **b**; F A-3 on MD5, **c**; F A-3 on MD12
(MD: Mission Day; A-2 and A-3 are the name of the newt vessels)

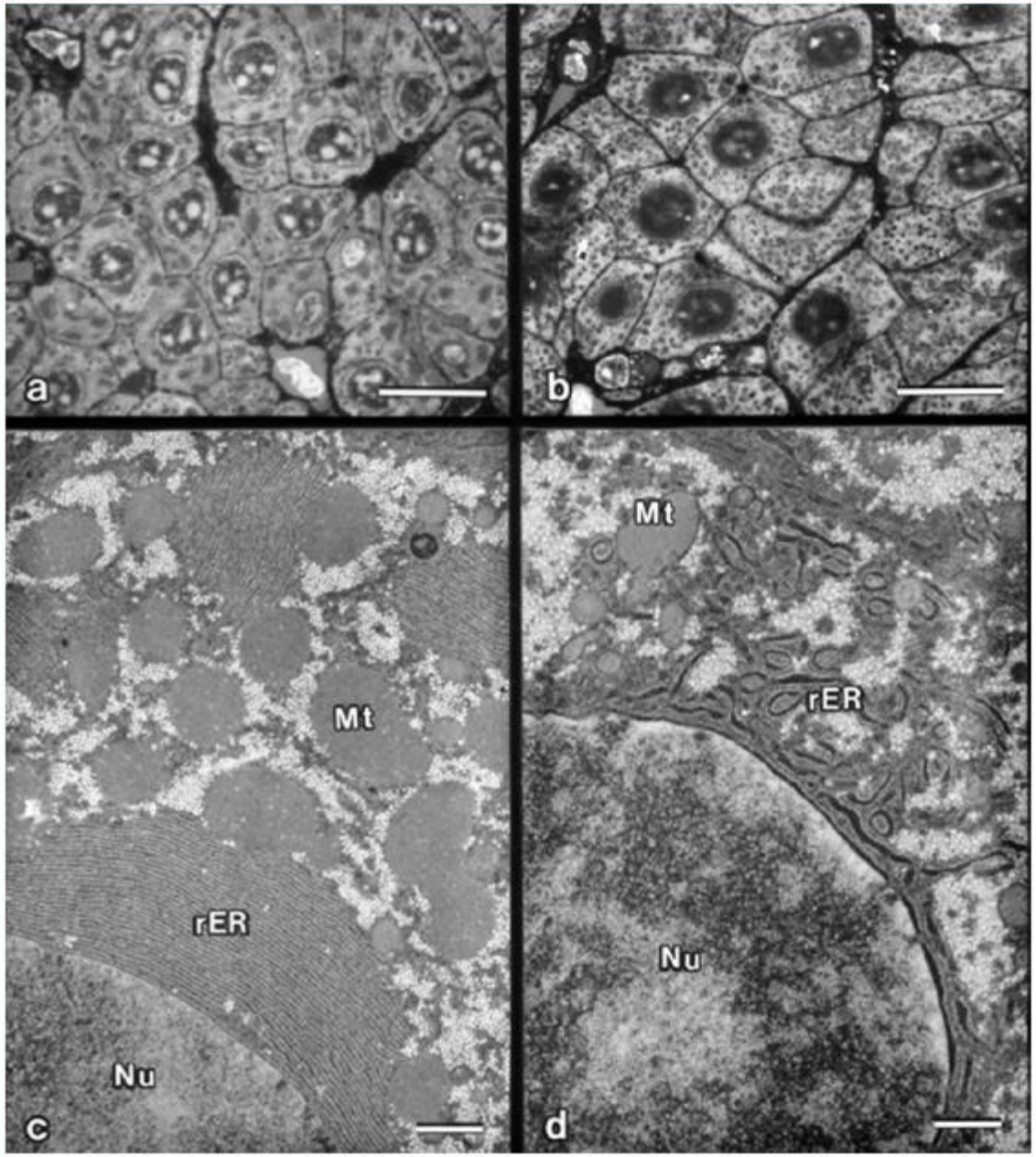


Fig. 6 Morphological changes in hepatic cells **a**, **c**; ground control, **b**, **d**; flight group retrieved in alive to ground. Compared to hepatic cells of ground control, the cells of flight group had smaller nucleolus. Rough ER of flight group was less in amount and showed distinct deformation. Morphological changes of mitochondria were also shown. Bar in **a** and **b**; 50 μm, **c** and **d**; 1 μm. Mt; Mitochondrion, Nu; Nucleus, rER; Rough Endoplasmic Reticurum.

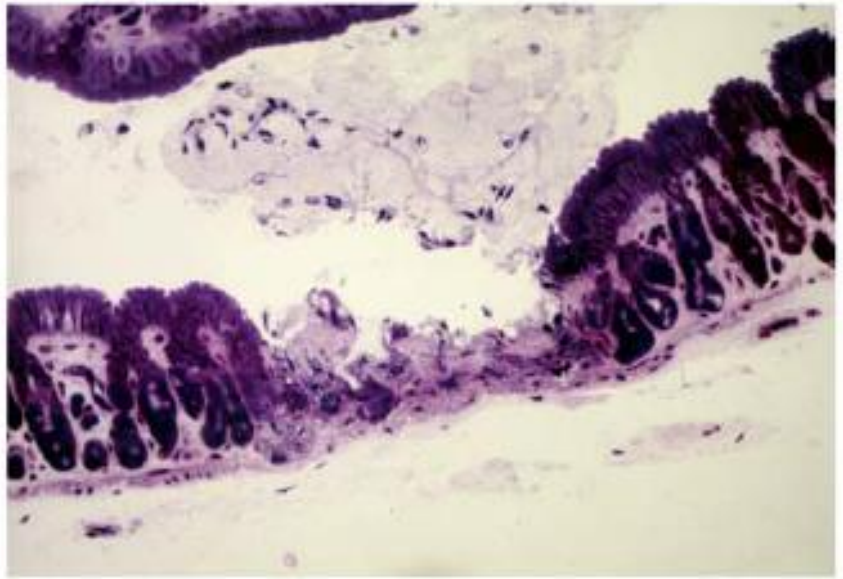


Fig. 7 Stomach of the newt, Flight A-3a
Deep ulcer in the center. Mucosa on right shows normal histology.

ELSEVIER

Hearing Research 88 (1995) 206–214

RESEARCH

Non-invasive assessment of otolith formation during development of the Japanese red-bellied newt, *Cynops pyrrhogaster*

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Received 1 August 1994; revised 9 May 1995; accepted 16 May 1995

Abstract

Pre-mated adult female newts and embryos have been flown on the International Microgravity Laboratory-2 (IML-2) Space Shuttle flight in 1994 (Wiederhold et al., 1992b). With the specimens available from this flight, the calcification of otoliths, ulna, radius and backbone of the flown larvae and adult newts were analyzed. The experiments presented here studied the development of the otoliths on the ground. Otoliths of living newts, from embryo to adult, were observed in situ with the application of a new X-ray and bio-imaging analyzer system. For the establishment of this method, newts at different developmental stages were used. An imaging plate temporarily stores the X-ray energy pattern at the bio-imaging analyzer. A latent image on the imaging plate was transformed into a digital time series signal with an image reader. Acquired digital information was computed with the image processor. The processed information was recorded on film with an image recorder, in order to visualize it on an enlargement computed radiograph. To analyze development of the otoliths, photo-stimulated luminescence level was detected by an image analyzer, using transmitted X-ray photons. A single clump of otoconia could first be seen at stage 33. Stage-36 embryos first have distinguishable otoliths, with the utricle in front and saccule behind. Our results show that this X-ray method detects the otoliths equally as well as sectioning. In the newt, the mandibular/maxillary bone formed before the spine. It is suspected that for the newt embryo, living in water, feeding becomes necessary prior to support of the body.

Develop. Growth Differ. (2001) **43**, 315–326

Effects of microgravity on the larval development, metamorphosis and reproduction of the urodele amphibian *Pleurodeles waltl*

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The FERTILE experiment was twice performed onboard the Mir space station during the Cassiopée and Pégase French space missions. The goal was to analyze the effects of microgravity on fertilization and embryonic development, and then on further development on the ground in the amphibian *Pleurodeles waltl*. The present paper reports development that occurred in the laboratory after landing. Recovered on the ground at the hatching stage, young larvae reared at room temperature underwent metamorphosis and became adults without obvious abnormalities. Of particular interest was the rearing temperature that induced a delayed metamorphosis for animals from the Cassiopée space mission, but not for animals from the Pégase mission. The rate of development and the morphology were analogous in these animals and in ground controls reared in a similar annual period. Analysis of offspring was performed using these animals. Males born in space were first mated with control ground-born females and then with females born in space. The mating gave progeny that developed normally. Depending on the methods used and on the limits of the analyses, the results clearly demonstrated that animals born in space were able to live and reproduce after return to the ground.

Key words: development, metamorphosis, microgravity, reproduction, urodele amphibian.

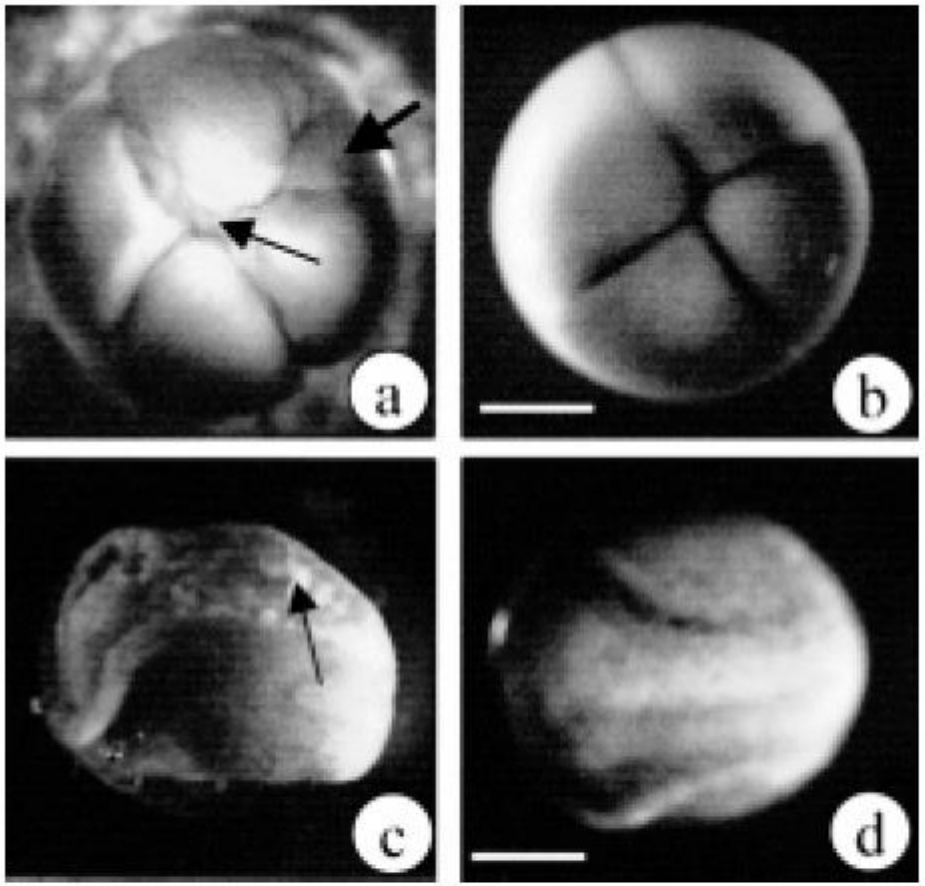

Fig. 1. Embryos during the cleavage period (a) in microgravity conditions (bold arrow, abnormal cleavage; thin arrow, separation between the top of the blastomeres) and (b) in the ground laboratory. Bar, 0.5 mm. Embryos during the neurula period (c) in microgravity conditions (arrow, isolated cells) and (d) in the ground laboratory. Bar, 0.6 mm.

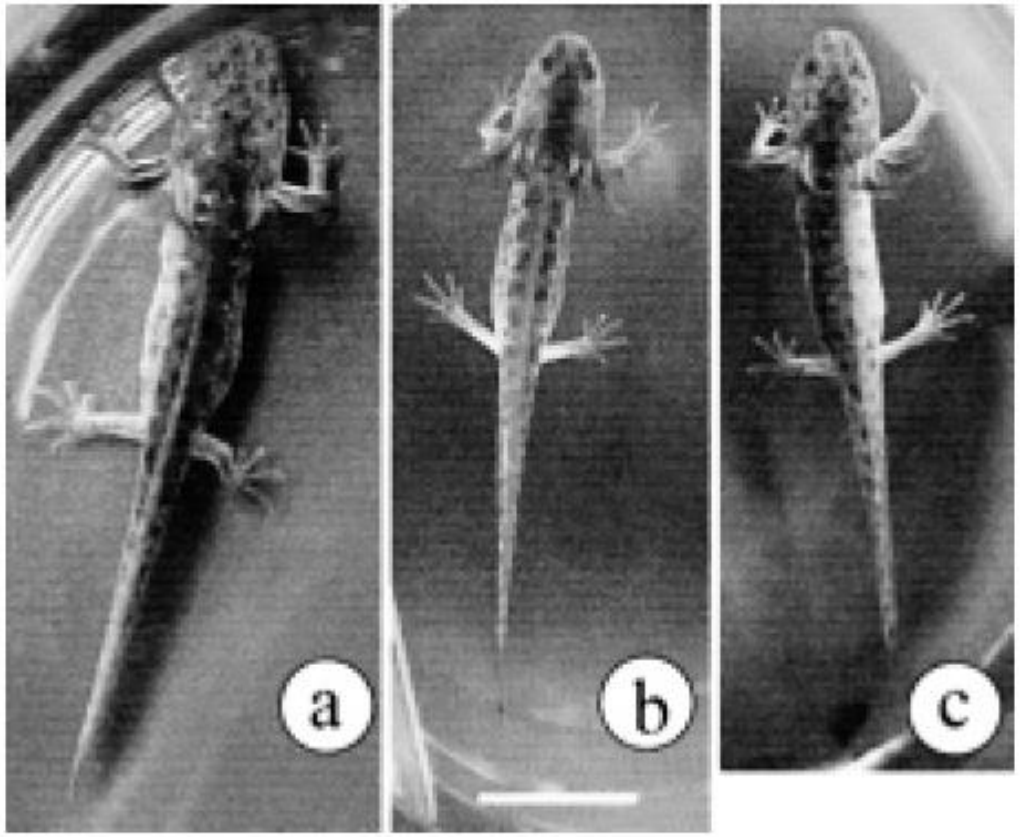

Fig. 4. Larvae at the beginning of metamorphosis (stage 55). Larvae born and reared up to the hatching stage onboard the Mir space station during the (a) Cassiopèe and (b) Pègase missions, then reared in laboratory conditions. (c) Ground control larva at the same developmental stage. Bar, 20 mm.

Table 2. Results of crosses between ground control animals and/or animals born and developed to hatching on board Mir during the Cassiopée mission

Crosses between: Females born in space		Males born in space		Ground	Ground	No.	F_2 offspring	% Fertiliz-	% Develop-	% Abnormal
1 *g*	µG	1 *g*	µG	females	males	eggs	ref.	ation	ment	embryos
		A96/301		β96/21		0				
		A96/303		β96/27		0				
			A96/304	β96/22		0				
			A96/304	β96/35		453	A-98	92	87	1
			A96/305	β96/25		0				
			A96/305	β96/29		0				
			A96/306	β96/18		4	B1a-98	100	100	0
			A96/306	β96/18		647	B1b-98	92	91	2
	β96/315		A96/306			604	B2-98	86	85	2
β96/302			A96/306			871	B3-98	91	89	27*
			A96/310	β96/31		997	C1-98	93	90	1
	β96/319		A96/310			399	C2-98	86	82	< 1
			A96/309	β96/24		201	D1-98	87	79	< 1
			A96/309			546	D2-98	93	93	< 1
			A96/312	β96/36		422 UF				
			A96/318	β96/14		0				
				Sd female	Sd male	279	c-97	89	87	< 1
				Sd female	Sd male	348	g-97	95	92	< 1
				Sd female	Sd male	199	h-97	93	91	< 1
				Sd female	Sd male	703	j-97	98	97	< 1
				Sd female	Sd male	1634	a-98	96	95	< 1
				Sd female	Sd male	425	b-98	91	89	< 1
				Sd female	Sd male	961	f-98	92	91	1
				Sd female	Sd male	1426	g-98	97	96	1
				Sd female	Sd male	165	h-98	88	87	18†
				Sd female	Sd male	793	j-98	98	97	< 1

ssed during cleavage stages and previously observed in standard progeny, †abnormalities expressed at tail-
rd animal; UF, unfertilized eggs; µG, microgravity.

In conclusion, the on-ground development of the animals born in space and the development of their progeny showed no differences with control *P. waltl* animals. Depending on the methods and limits of the analyses, the results clearly indicated that these amphibian animals born in space were able to live and reproduce after returning on earth. These results totally agree with those obtained with the fish *Oryzias latipes* (Ijiri 1997). Fish, and now amphibians, open the way for the colonization of space by man. Now, our present objective is to verify whether animals that are born in microgravity and reared up to sexual maturity on board a space station can reproduce without negative consequences after their return on earth.

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THE RESPONSE OF STRUCTURE AND FUNCTION OF THE GRAVIRECEPTOR IN A VERTEBRATE TO NEAR WEIGHTLESSNESS†

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(*Received* 9 *April* 1987)

Abstract—The paper sums up results of a 7-day space flight experiment (D-1-Mission-BW-STA 00-STATEX) using growing frog embryos and larvae (*Xenopus laevis*) as a model system.

Evaluation of photographs taken from the surface of sectioned deep-frozen objects, and micrographs using TEM and SEM show no aberrations in the shape, size, position, or respective electron density of the otolith membranes in larvae developed for 154 h under near-zero ***g***.

The further evaluation of the "weightless larvae" revealed a probably not yet described otolith-like formation below the dorsal wall of the vestibulum. In the weightless larvae this formation outnumbers, also qualitatively, strongly the 1-***g*** control samples.

The swimming behavior of the tadpoles which was observed about one hour after landing of the Space Shuttle showed a typical anomaly (loop swimming), which is known from larvae developed on the clinostat or from fish flown aboard Apollo capsules.

An extra result is the lack of striking effects of cosmic radiation on the embryonic development of the flown Xenopus eggs.

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1975–1978, March 1995
Developmental Biology

Amphibian development in the virtual absence of gravity

(embryonic axis/morphogenesis/swimming behavior)

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Communicated by John C. Gerhart, University of California, Berkeley, CA, December 2, 1994

ABSTRACT **To test whether gravity is required for normal amphibian development, *Xenopus laevis* females were induced to ovulate aboard the orbiting Space Shuttle. Eggs were fertilized *in vitro*, and although early embryonic stages showed some abnormalities, the embryos were able to regulate and produce nearly normal larvae. These results demonstrate that a vertebrate can ovulate in the virtual absence of gravity and that the eggs can develop to a free-living stage.**

NaCl/1.5 mM KCl/0.18 mM $MgCl_2$/0.75 mM $CaCl_2$/10 μM $ZnCl_2$/5 mM sodium Hepes, pH 7.4). Half of the chambers were incubated on the FEU centrifuge (rotating at 60 rpm to create a force of $1 \times g$) and half were incubated at microgravity (gravity levels ranged between 10^{-3} and $10^{-5} \times g$ in the Spacelab). The temperature difference between the $1 \times g$ centrifuge and the microgravity compartment was maintained within ±0.25°C. After the first 50 hr of the mission, the FEU

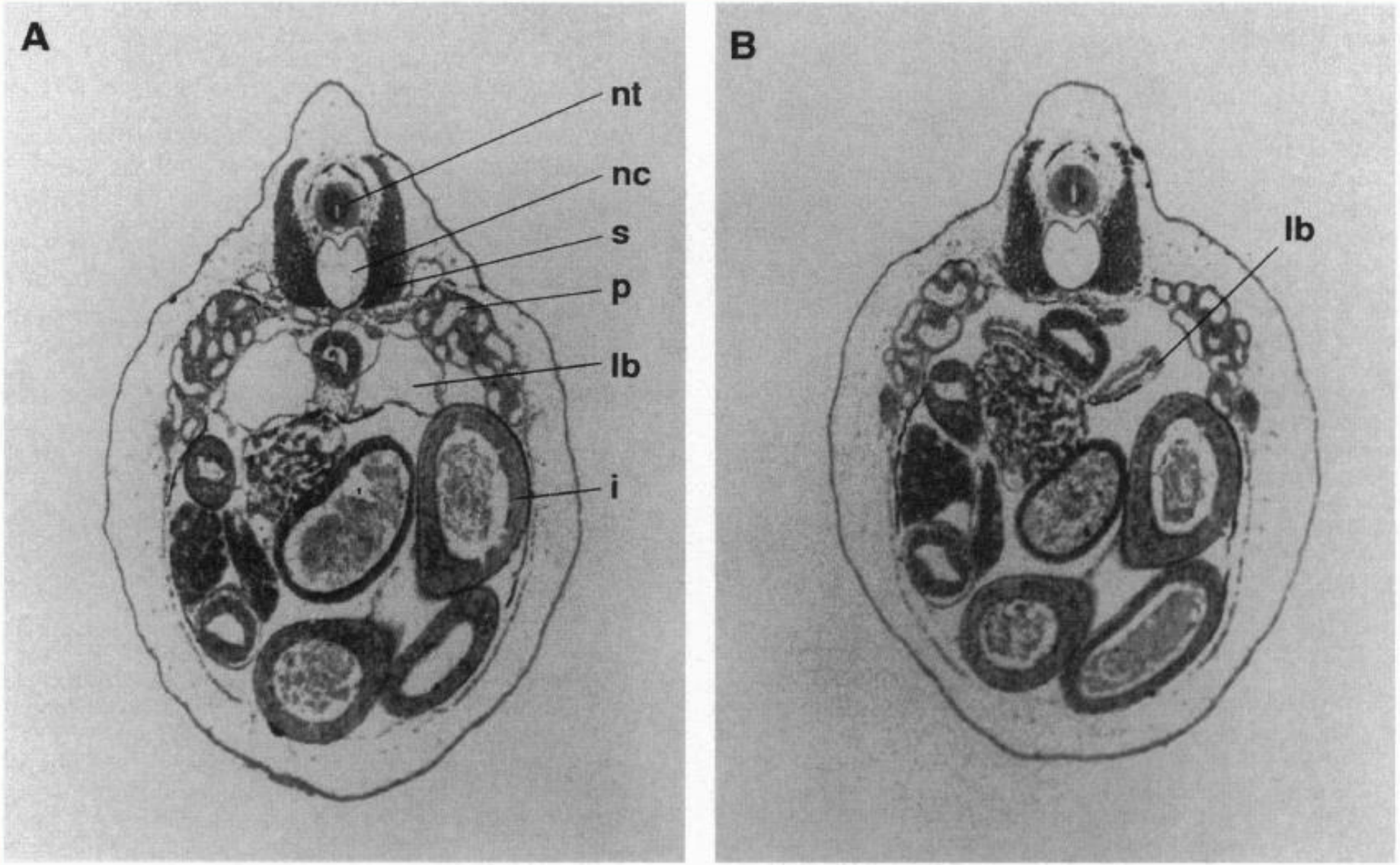

FIG. 2. The lung buds and tracheae of tadpoles developed in microgravity were generally not inflated. Transverse sections of tadpoles fixed inflight at stage 45 showed inflated lung buds in the 1 × *g* group (*A*) and uninflated lung buds in the microgravity group (*B*). Tadpoles were fixed inflight and stained with nuclear fast red, aniline blue, and orange G. Anatomical features noted are neural tube (nt), notochord (nc), somite (s), pronephros (p), lung bud (lb), and intestine (i). Six loops of the intestine are visible in this section.

Pergamon

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0273–1177(95)00637–0

REGULATIVE DEVELOPMENT OF *XENOPUS LAEVIS* IN MICROGRAVITY

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Is gravity required for normal embryonic development? Upon fertilization, most amphibian eggs rotate inside the fertilization membrane so that the animal-vegetal axis is aligned with gravity. This 'rotation of fertilization' /1/ is not a requirement for normal development, since eggs prevented from rotating can develop normally /2/. Nevertheless, the direction of the rotation of fertilization normally has a role in determining the polarity of the embryonic axis /1/, and eggs inclined with respect to gravity form the dorsal structures on the side of the egg uppermost in the gravitational field /3,4/. Thus, for over a century scientists have questioned whether gravity was required for amphibian embryogenesis /5,6/. Recent spaceflight experiments have successfully fertilized eggs of *Xenopus laevis* and reared the embryos to the gastrula stage. The morphology of the gastrulae was somewhat abnormal /7/. We report here that ovulation and subsequent development to a free-living stage can occur at microgravity ($<10^{-3}$ *g*). We also examined larval swimming behavior to determine if abnormal behaviors of *Xenopus* tadpoles previously observed during and following space flight /8/ would occur with larvae that began their lives in the virtual absence of gravity.

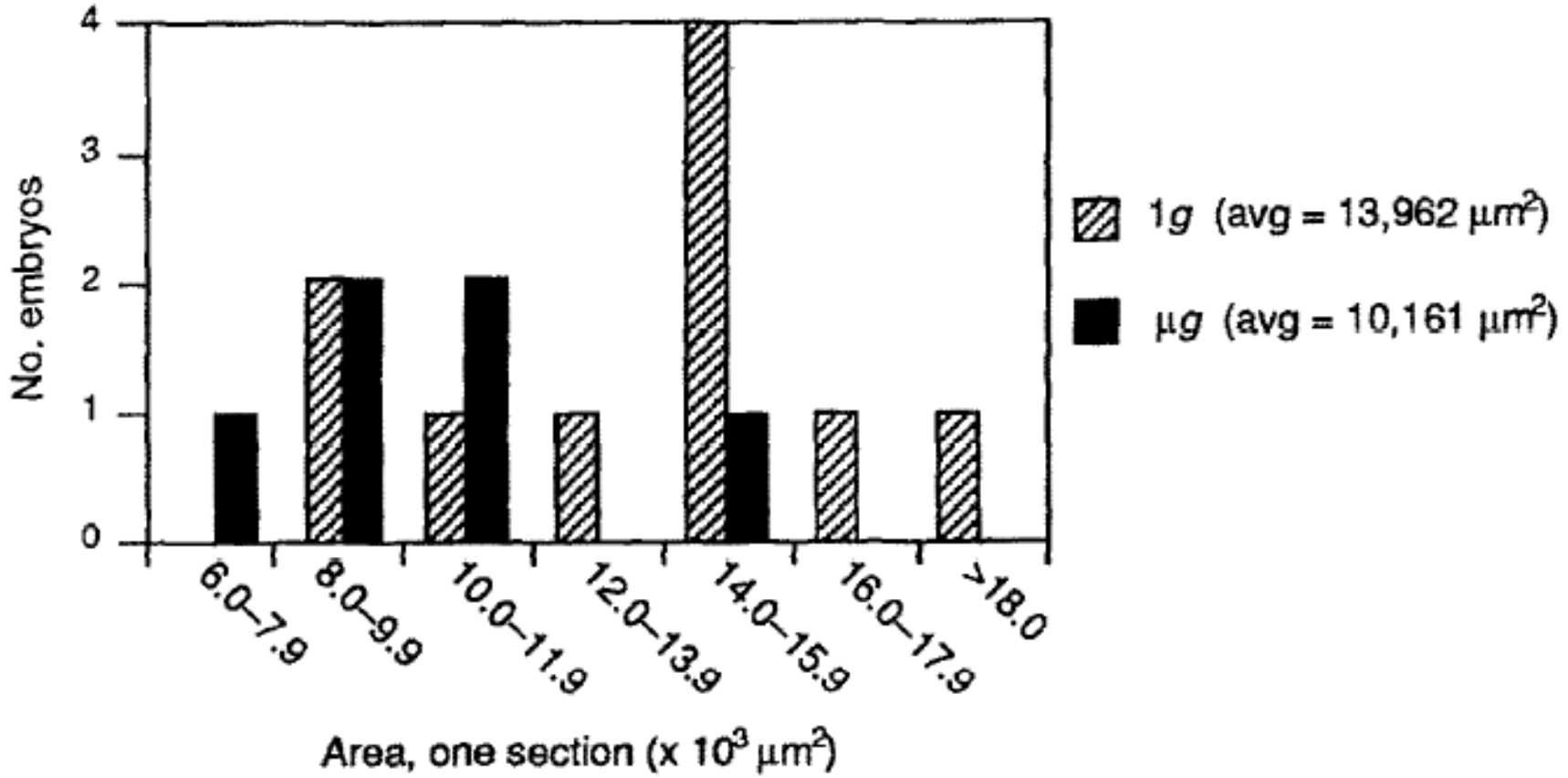

Fig. 3. The neural tube is slightly smaller in the µg sample ($p = 0.05$, Mann-Whitney U test). Thus, although the prospective neural tube region was ~ 40% thicker at the beginning of gastrulation (stage 101/4), this tissue thinned properly by the time of neurulation. Measurements were taken from one section at the level of the hindbrain, from embryos fixed in flight.

The post-flight behavior of the tadpoles raised in microgravity differed from those raised on the 1*g* centrifuge in a number of ways. Tadpoles reared at microgravity swam at a lower position in the water column than did the 1*g* controls. This positioning is consistent with a difference in lung volume. Indeed, serial sections of the lungs from tadpoles fixed shortly before landing (st. 45) reveal significantly smaller lungs in the microgravity tadpoles than in the 1*g* tadpoles (87,000 μm^2 vs. 197,000 μm^2, sum every tenth section, N= 40, p=0.023, Manr.-Whitney U test). Tadpoles in both groups were observed taking breaths of air during the first day after landing, and differences in swimming posture for the two groups diminished in the following days.

Tadpoles returned from spaceflight metamorphosed and matured normally. Reproductive function was unimpaired, and no abnormalities were found in the Earth-born F1 tadpoles.

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Spaceflight Effects on Cultured Embryonic Chick Bone Cells

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ABSTRACT

A model calcifying system of primary osteoblast cell cultures derived from normal embryonic chicken calvaria has been flown aboard the shuttle, Endeavour, during the National Aeronautics and Space Administration (NASA) mission STS-59 (April 9–20, 1994) to characterize unloading and other spaceflight effects on the bone cells. Aliquots of cells (~7×10^6) grown in Dulbecco's modified Eagle's medium (DMEM) + 10% fetal bovine serum (FBS) were mixed with microcarrier beads, inoculated into cartridge culture units of artificial hollow fiber capillaries, and carried on the shuttle. To promote cell differentiation, cartridge media were supplemented with 12.5 μg/ml ascorbate and 10 mM β-glycerophosphate for varying time periods before and during flight. Four cartridges contained cells from 17-day-old embryos grown for 5 days in the presence of ascorbate prior to launch (defined as flight cells committed to the osteoblastic lineage) and four cartridges supported cells from 14-day-old embryos grown for 10 days with ascorbate before launch (uncommitted flight cells). Eight cartridges prepared in the same manner were maintained under normal gravity throughout the flight (control cells) and four additional identical cartridges under normal gravity were terminated on the day of launch (basal cells). From shuttle launch to landing, all cartridges were contained in closed hardware units maintaining 5% CO_2, 37°C, and media delivery at a rate of ~1.5 ml/6 h. During day 3 and day 5 of flight, duplicate aliquots of conditioned media and accumulated cell products were collected in both the flight and the control hardware units. At the mission end, comparisons among flight, basal, and control samples were made in cell metabolism, gene expression for type I collagen and osteocalcin, and ultrastructure. Both committed and uncommitted flight cells were metabolically active, as measured by glucose uptake and lactate production, at approximately the same statistical levels as control counterparts. Flight cells elaborated a less extensive extracellular matrix, evidenced by a reduced collagen gene expression and collagen protein appearance compared with controls. Osteocalcin was expressed by all cells, a result indicating progressive differentiation of both flight and control osteoblasts, but its message levels also were reduced in flight cells compared with ground samples. This finding suggested that osteoblasts subjected to flight followed a slower progression toward a differentiated function. The summary of data indicates that spaceflight, including microgravity exposure, demonstrably affects bone cells by down-regulating type I collagen and osteocalcin gene expression and thereby inhibiting expression of the osteogenic phenotype notably by committed osteoblasts. The information is important for insight into the response of bone cells to changes of gravity and of force in general.
(J Bone Miner Res 2000;15:1099–1112)

STS029-01-001 (16 March 1989) --- Astronaut John E. Blaha, STS-29 pilot, checks an incubator on the mid deck of Earth-orbiting Discovery during Flight Day 4 activity. The incubator is part of a student involvement program experiment titled, "Chicken Embryo Development in Space." The student experimenter is John C. Vellinger. The experiment's sponsor is Kentucky Fried Chicken.

ELSEVIER

FEBS Letters 340 (1994) 34–38

FEBS 13681

FEBS LETTERS

The role of gravity in chick embryogenesis

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Abstract

Thirty fertilized chick eggs preincubated for 0, 7 and 10 days on earth (10 eggs each) were flown in the space shuttle 'Endeavour' and further incubated for 7 days under microgravity. Twenty out of thirty eggs (9/10 ten-day-old; 10/10 seven-day-old; 1/10 zero-day-old) were recovered alive after landing. The only living embryo of the zero-day-old group died 24 days after launch, and was comparable to a 16-day-old embryo. The high mortality of the 0-day-old eggs appeared to be related to the specific inner structure of the egg. Simulation experiments performed on earth indicated that when yolk stayed in the albumen for more than 2 days, most of the embryos died. The subtle difference in specific gravity between the yolk (1.029) and albumen (1.040) plays a critical role in early chick embryogenesis.

Histol Histopathol (1996) 11: 407-426

Histology and Histopathology

Microgravity in the STS-29 space shuttle discovery affected the vestibular system of chick embryos

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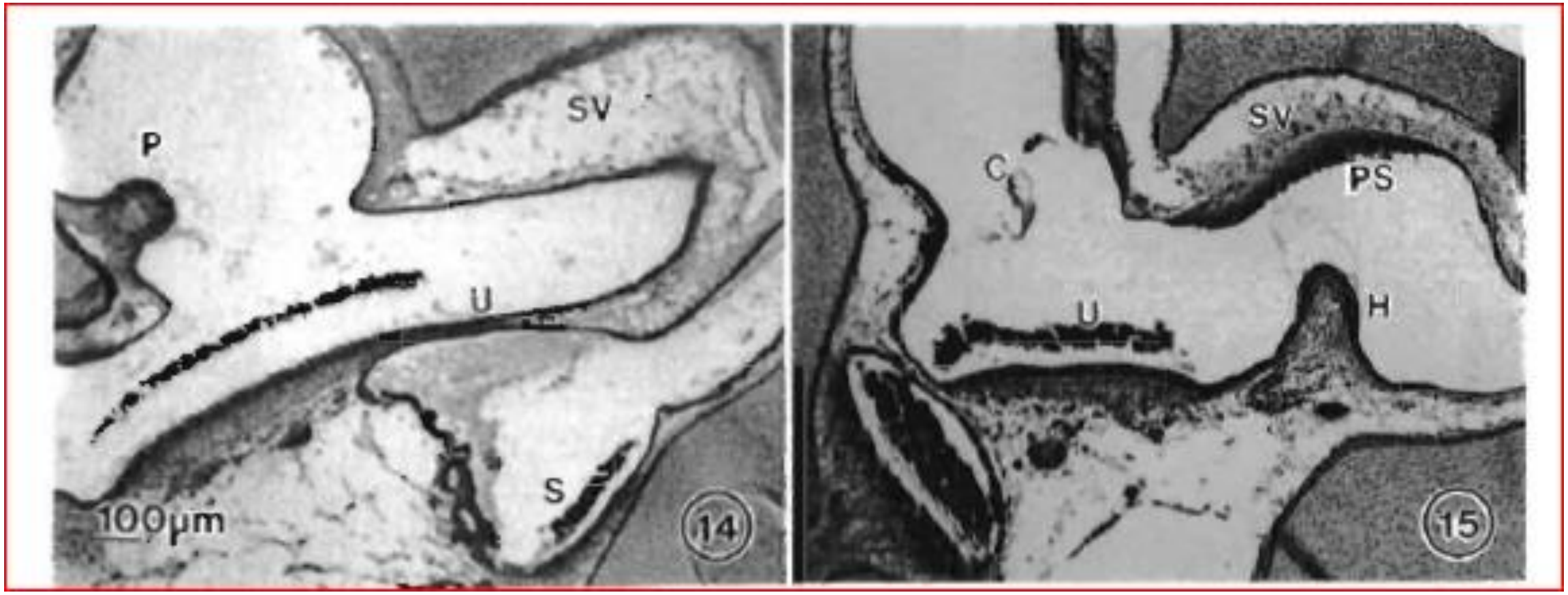
Flight

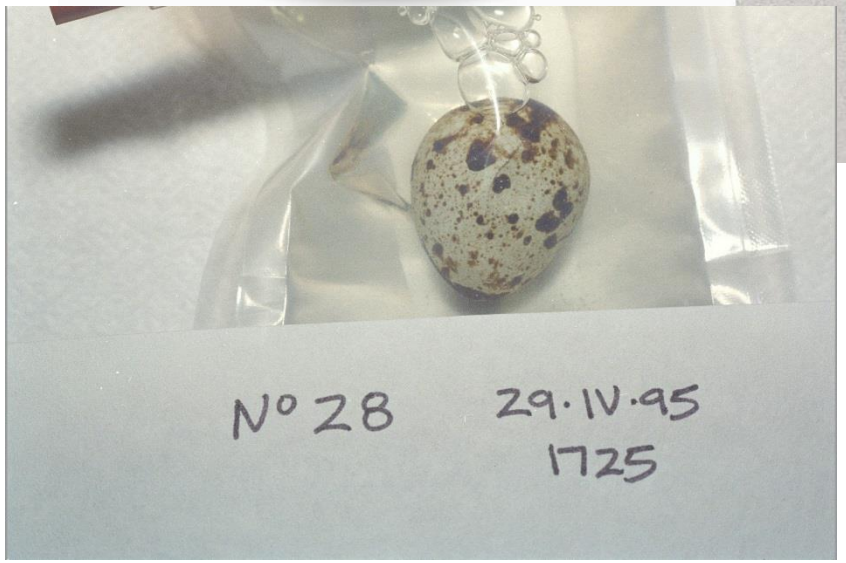
Ground Control

Cartilage cells stained for chondroitin sulfate; different colors were applied only to aid discrimination of different cells. No differences were seen between flight and control animals.



Left, Vestibule of a ground control chick at the level of the posterior (P) crista with the utricle (U) and the saccule (S). Structures appear normal at this magnification. Right, Vestibule of a flight chick at the level of the horizontal (H) canal. Structures appear normal at this magnification.

The Avian Development Facility (ADF) supports 36 eggs in two carousels, one of which rotates to provide a 1-g control for comparing to eggs grown in microgravity. The ADF was designed to incubate up to 36 Japanese quail eggs, 18 in microgravity and 18 in artificial gravity. The two sets of eggs were exposed to otherwise identical conditions, the first time this is been accomplished in space. Eggs are preserved at intervals to provide snapshots of their development for later analysis. Quails incubate in just 15 days, so they are an ideal species to be studied within the duration of space shuttle missions. Further, several investigators can use the same specimens to address different questions. The ADF originated in NASA's Shuttle Student Involvement program in the 1980s and was developed under the NASA Small Business Irnovation Research program. In late 2001, the ADF made its first flight and carried eggs used in two investigations.



SPACE SHUTTLE STS-71 (MIR 18)

Biological Sciences in Space, Vol.9 No.1(1995):p.3-16

Fish Mating Experiment in Space — What It Aimed at and How It Was Prepared

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Abstract The code name 'MEDAKA' was given to the fish experiment in the IML-2 (the second International Microgravity Laboratory), a Space-shuttle mission (STS-65) carried out in July 1994. Medaka is the Japanese name for a small fresh-water fish, *Oryzias latipes*. This experiment titled 'Mating behavior of the fish Medaka and development of their eggs in space' aimed to present data for designing the future fish-culture in space. The Medaka experiment accomplished its objectives to the point of 100 %. The fish mated, laid eggs in space, and these eggs developed normally to hatching (coming out as a baby fish) under microgravity. Its success totally depended on selection of the four fish sent to space. This paper describes the aims of the IML-2 Medaka fish experiment and how it was prepared, together with a brief report on what were achieved in space.

Key Words: IML-2, space biology, mating behavior, space-originated fry, fish, Medaka

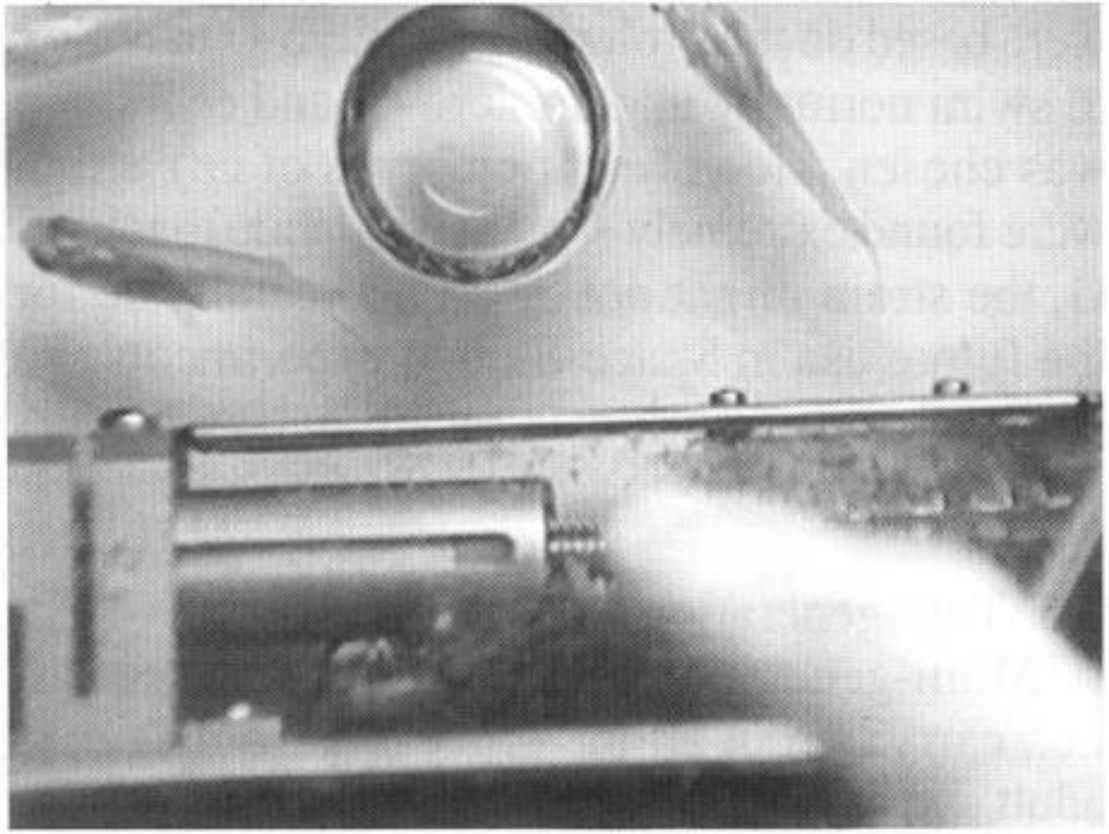

Fig.12 Separated area for embryos and fry (a picture taken in space). The Crew is pointing the newly-laid eggs.

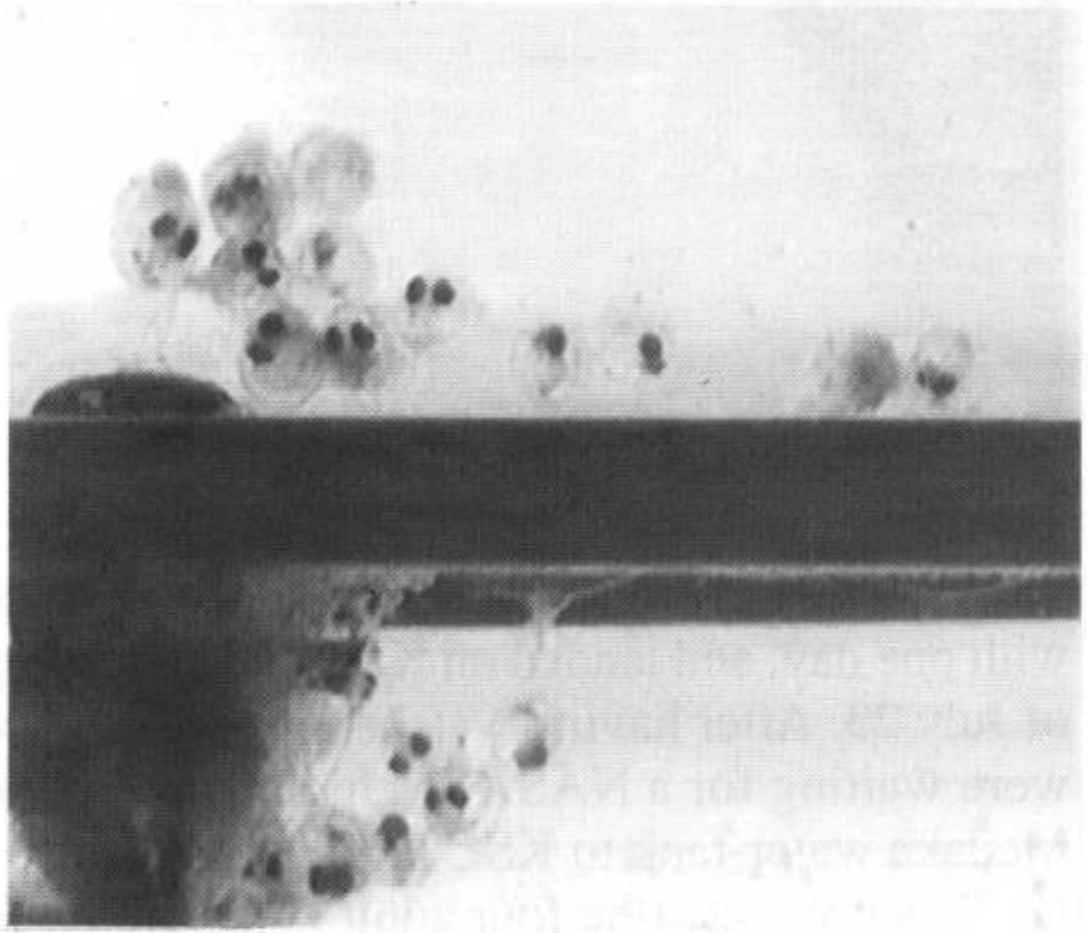

Fig.22 Many eggs laid in space have developed normally, showing two black-pigmented eyes.

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Research Article

Spaceflight Affects Postnatal Development of the Aortic Wall in Rats

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We investigated effect of microgravity environment during spaceflight on postnatal development of the rheological properties of the aorta in rats. The neonate rats were randomly divided at 7 days of age into the spaceflight, asynchronous ground control, and vivarium control groups (8 pups for one dam). The spaceflight group rats at 9 days of age were exposed to microgravity environment for 16 days. A longitudinal wall strip of the proximal descending thoracic aorta was subjected to stress-strain and stress-relaxation tests. Wall tensile force was significantly smaller in the spaceflight group than in the two control groups, whereas there were no significant differences in wall stress or incremental elastic modulus at each strain among the three groups. Wall thickness and number of smooth muscle fibers were significantly smaller in the spaceflight group than in the two control groups, but there were no significant differences in amounts of either the elastin or collagen fibers among the three groups. The decreased thickness was mainly caused by the decreased number of smooth muscle cells. Plastic deformation was observed only in the spaceflight group in the stress-strain test. A microgravity environment during spaceflight could affect postnatal development of the morphological and rheological properties of the aorta.

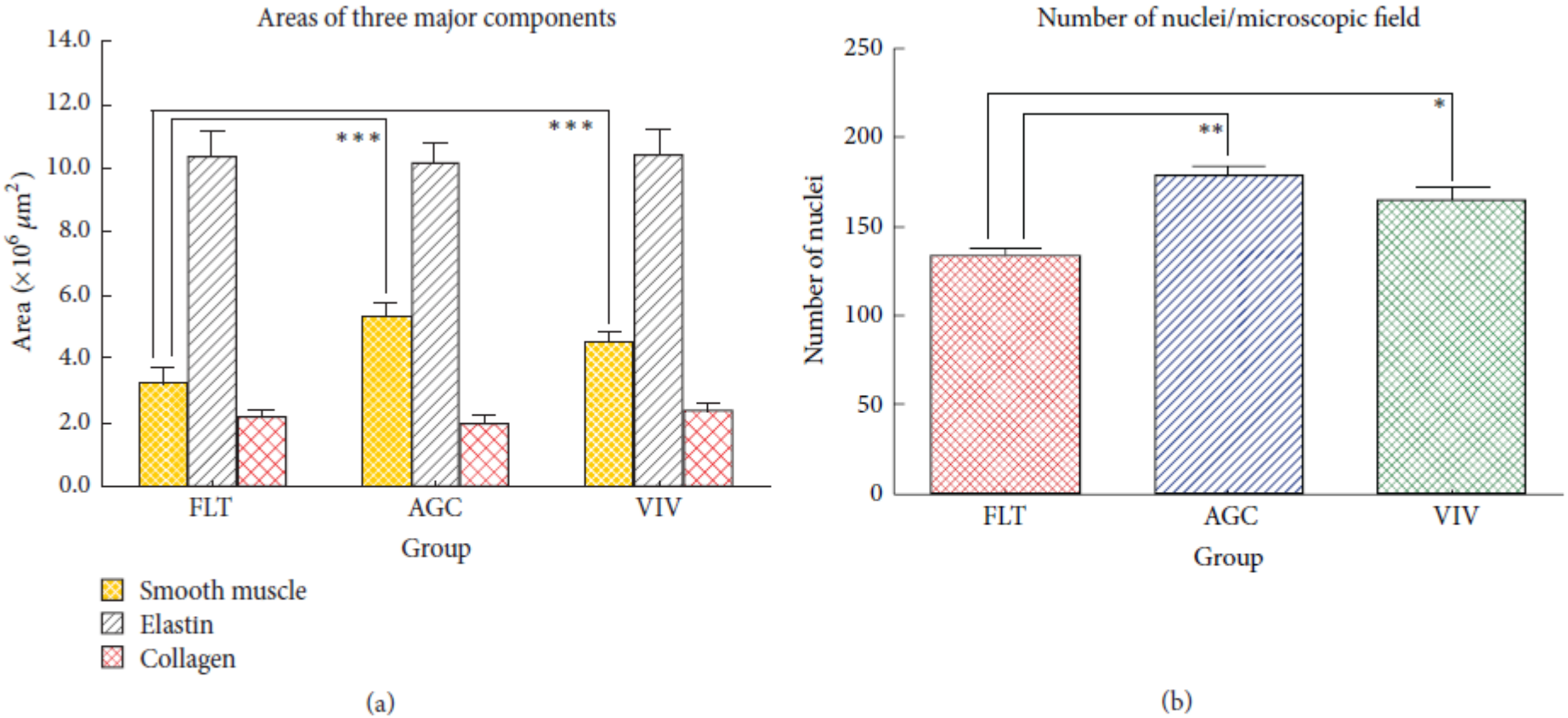

FIGURE 7: Area of the smooth muscle, elastin, and collagen fibers (a) in the longitudinal histological sections and number of nuclei of the smooth muscle cells (b) in the circumferential histological sections excised from the proximal thoracic aorta in the FLT, AGC, and VIV group rats. Values are mean ± SE. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. Area of each component was measured in three given microscopic fields for one histological section stained with EVG with an image analysis system and then averaged within each group. The number of nuclei was measured in three given microscopic fields for one section stained with HE with an image analysis system and then averaged within each rat group.

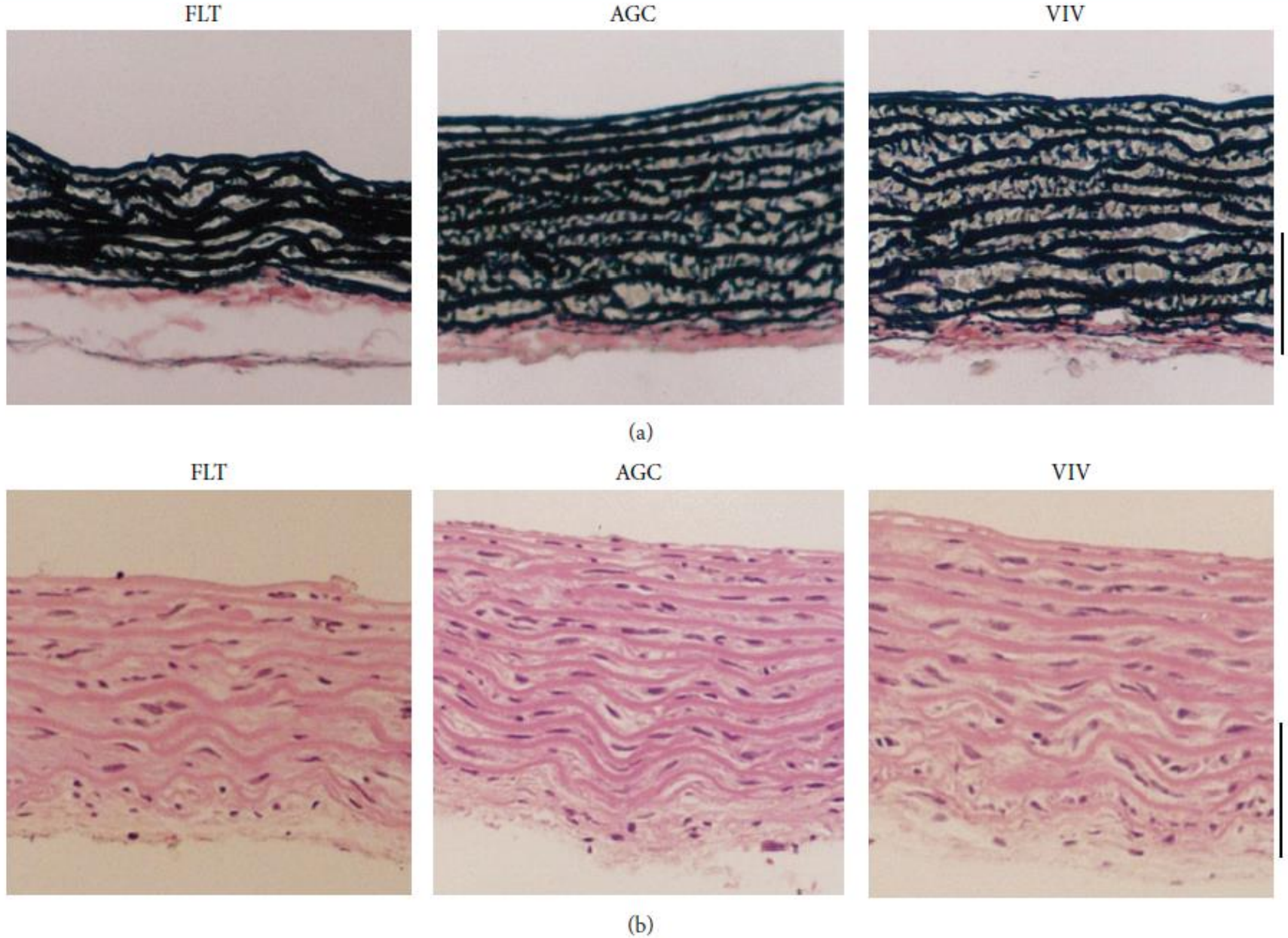

FIGURE 5: Photomicrographs of the longitudinal (a) and circumferential (b) histological sections of the proximal descending thoracic aorta stained with EVG (a) and HE (b) stains in the FLT, AGC, and VIV group rats. Perpendicular bar: 100 μm.

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Gynecological and Reproductive Issues for Women in Space: A Review

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The vast majority of women finalists for astronaut candidate selection have not had children. During the five selection cycles between 1989 and 1997, a total of 99 female finalist candidates were examined. Only 18 of the 99 were parous, and the total number of living children born to this group was 24. The delay in childbearing is often due to educational and career objectives that involve decisions about both marriage and children. Because of the training constraints that pregnancies cause, most of these individuals prefer to delay their first pregnancy until after completing one or two spaceflights. This has lead to deliveries at more advanced maternal age. The average maternal age at the time of delivery for the 7 children born to 6 U.S. women after spaceflight is 40. The mean age of the 6 that have spontaneously aborted after spaceflight is 41. At this time there are three on-going pregnancies in astronauts who have flown in space and the mean maternal age is 42 years. There has been considerable need for infertility services and assisted reproductive technology (ART) in older astronauts due primarily to gamete age. The success rates for ART in astronauts have been low, but comparable with other older ART patients. It is likely that the poor per-cycle fecundability relates to age alone and not spaceflight, but no study has been accomplished to assure that fact.

“Thirteen female astronauts have given birth to 18 children following spaceflight and have not experienced any increased pregnancy complications or increased assisted reproductive technology failures compared to the general population.”

E.S.Baker, unpublished data

More women are astronauts

NASA

The new class of trainees includes four women and four men. From left, Josh A. Cassada, Victor J. Glover, Tyler N. Hague, Christina M. Hammock, Nicole Aunapu Mann, Anne C. McClain, Jessica U. Meir and Andrew R. Morgan.

> 6100 applicants, including ~ 1000 women.

Five military pilots; 1 medical doctor; 2 Ph.D. level scientists

Women:

- 2 Pilots
- 2 Scientists