

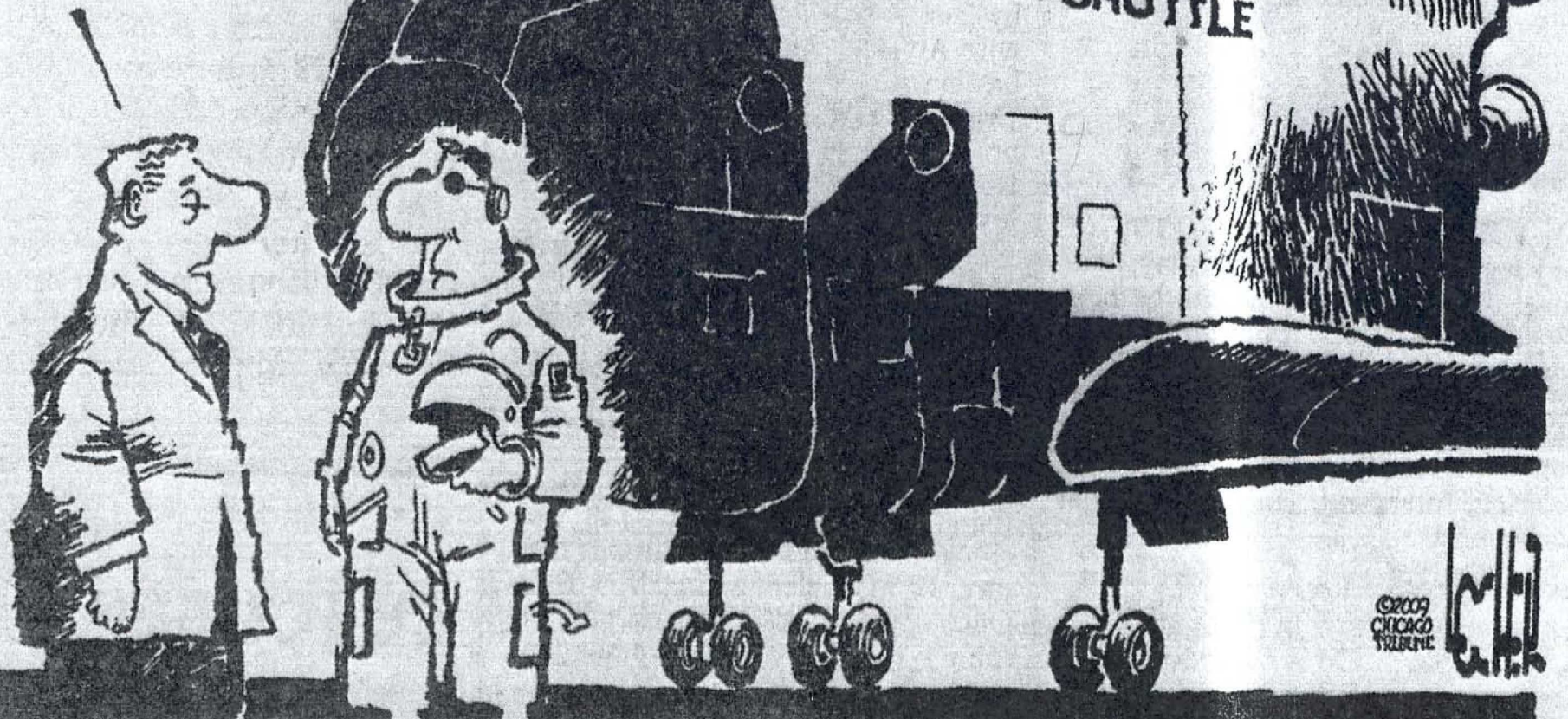
Infectious Disease Risk Associated With Space Flight

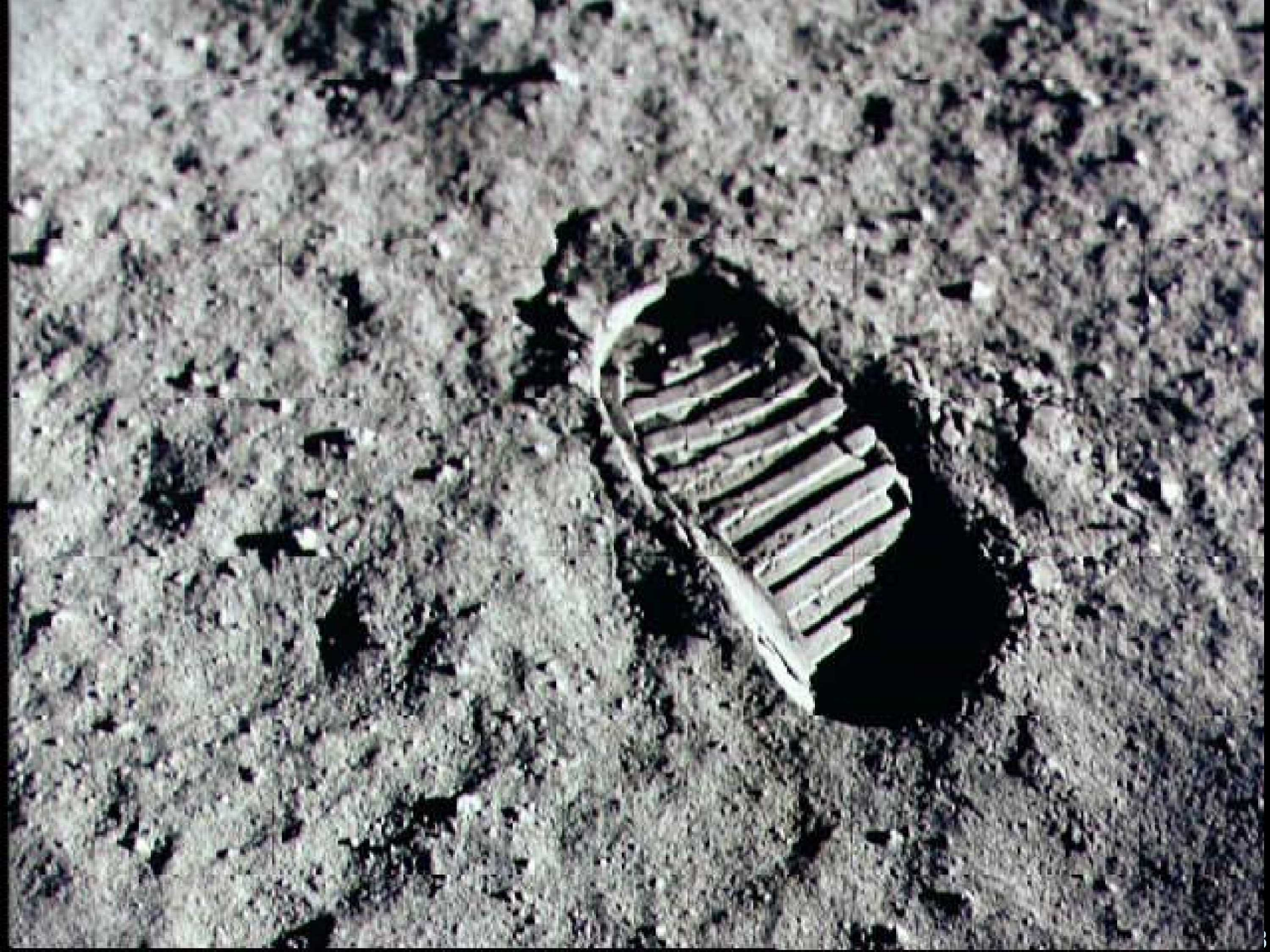


Human Exploration

Duane L. Pierson Ph.D.
NASA Johnson Space Center
April 2010

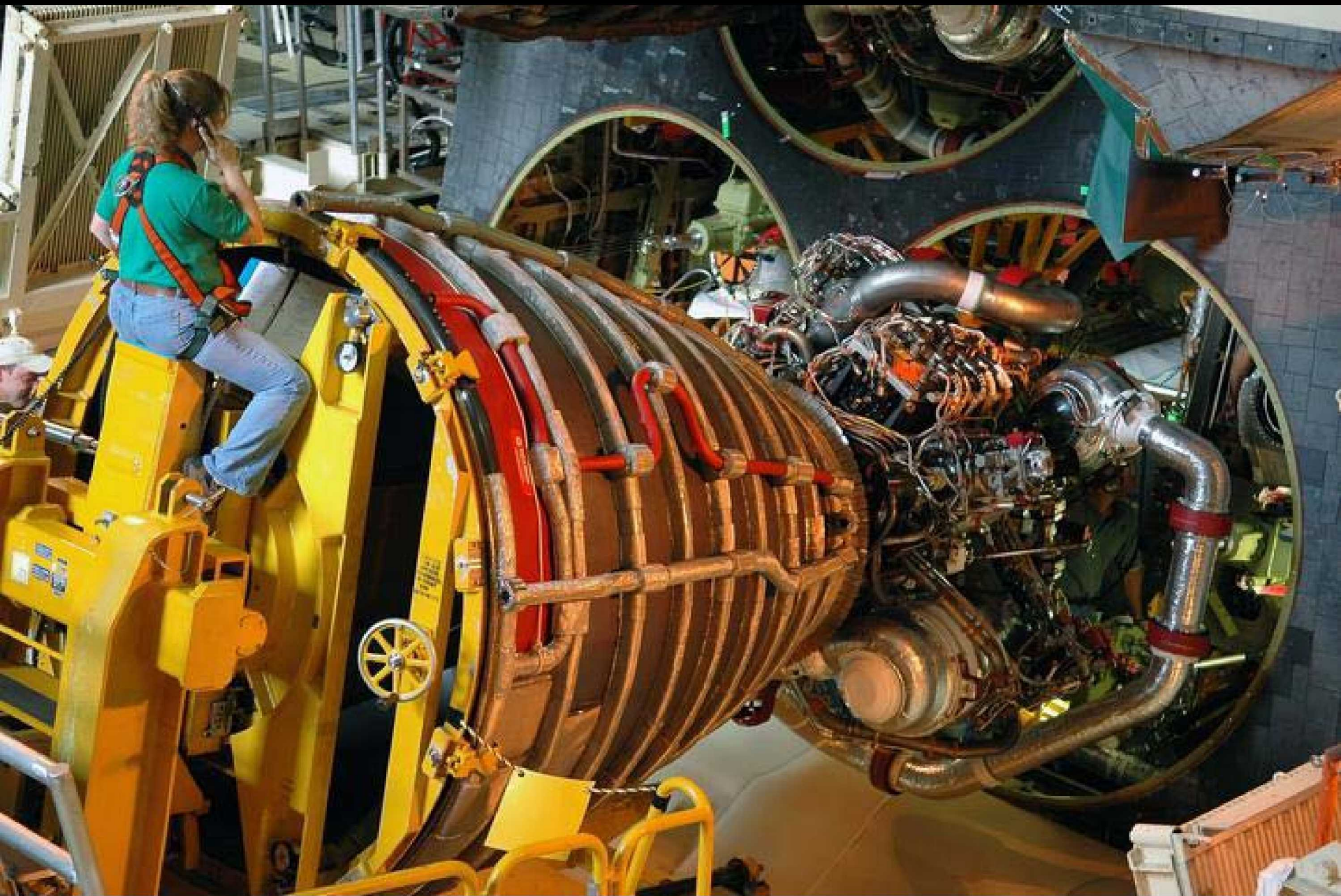
**SORRY, JUST BECAUSE YOU HAVE
20 MILLION MILES ON YOUR
ODOMETER AND A FEW LOOSE
TILES ON YOUR '74 VEHICLE, IT
DOESN'T MAKE YOU
ELIGIBLE FOR THE CASH
FOR CLUNKERS
PROGRAM.**







Shuttle In Sling Ready For Lift In VAB



**Shuttle Has Been
Moved To VAB And
And Will Be Attached
To External Tank**



















940207 185334 STS60 07 030

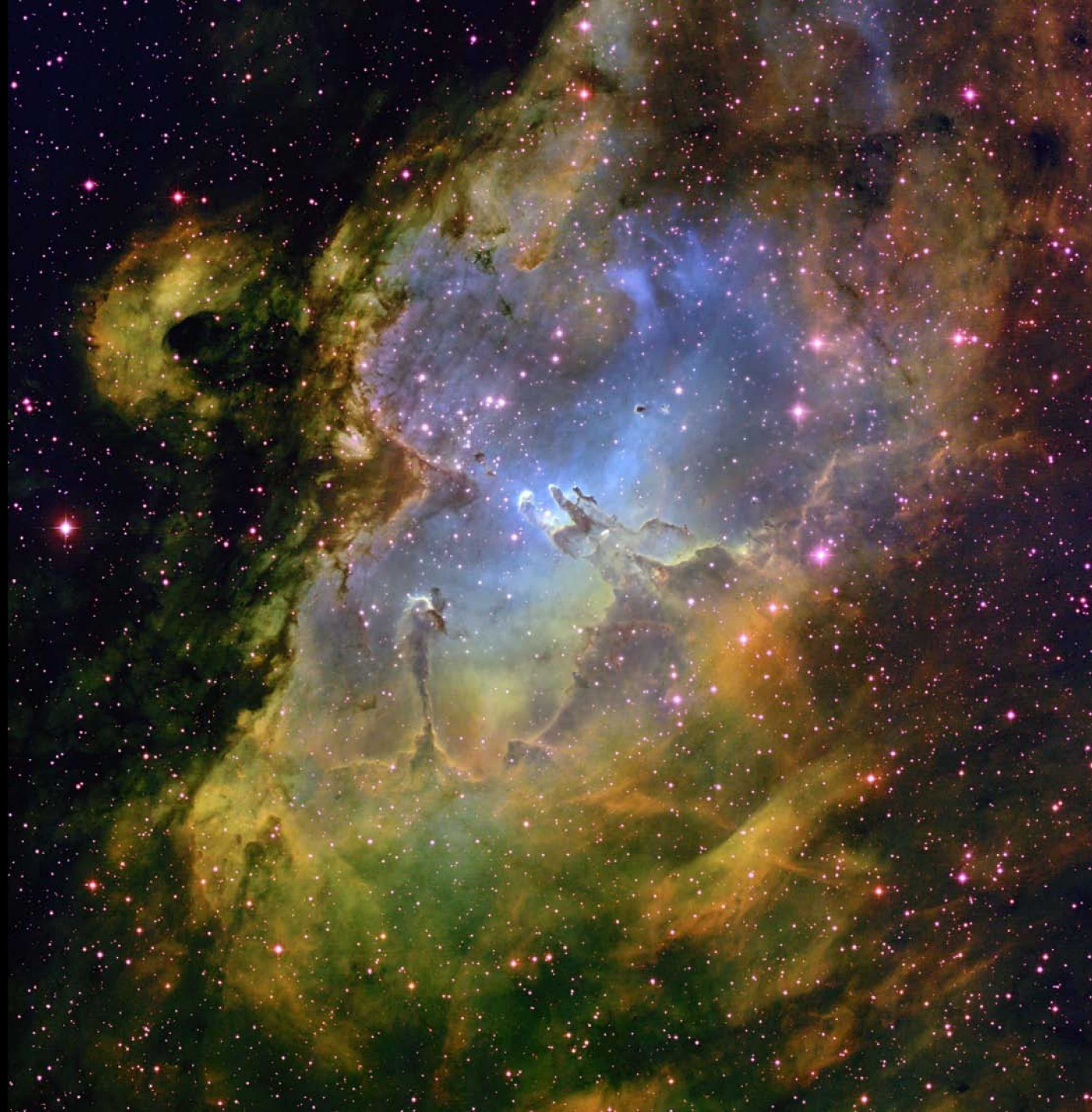


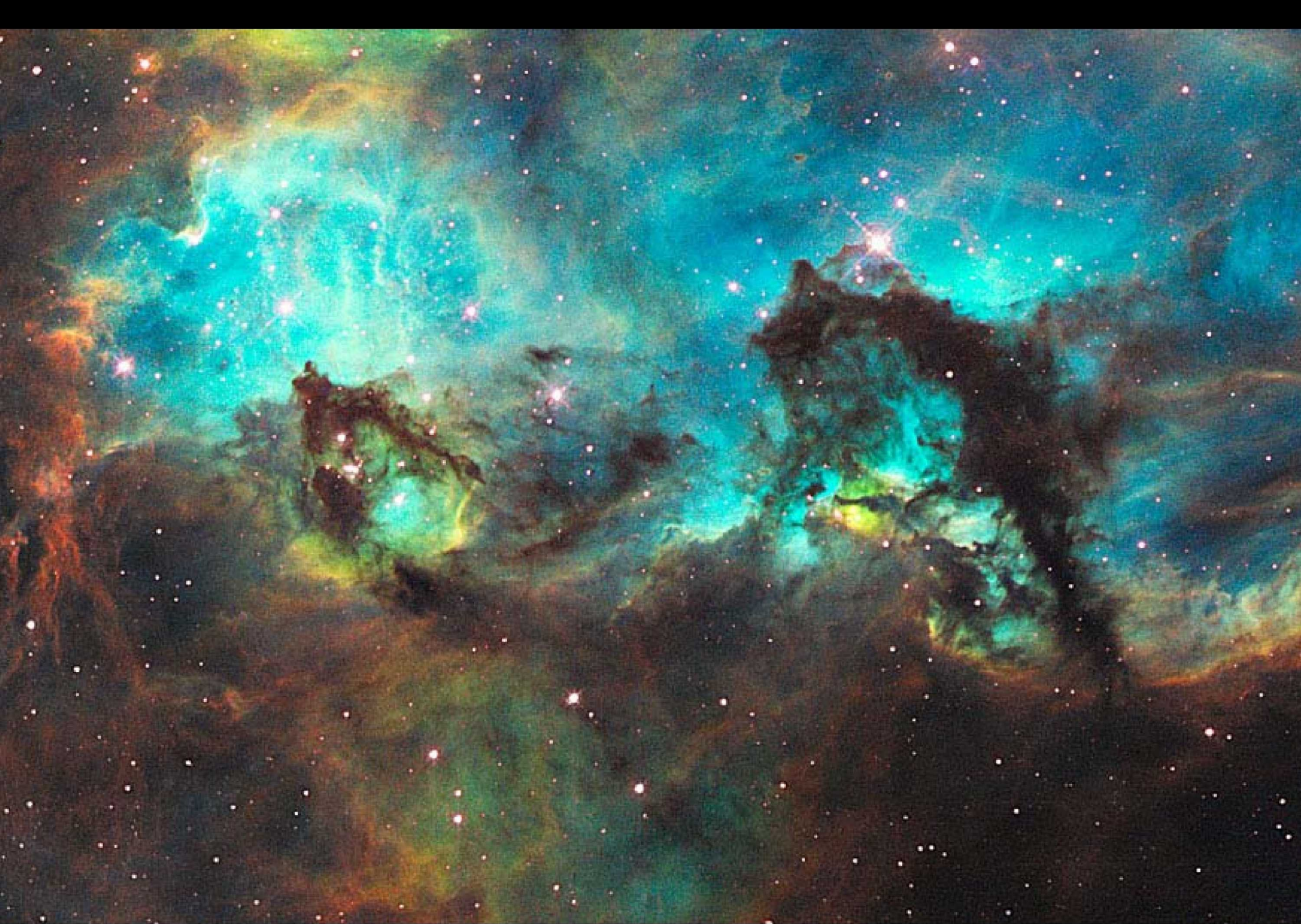










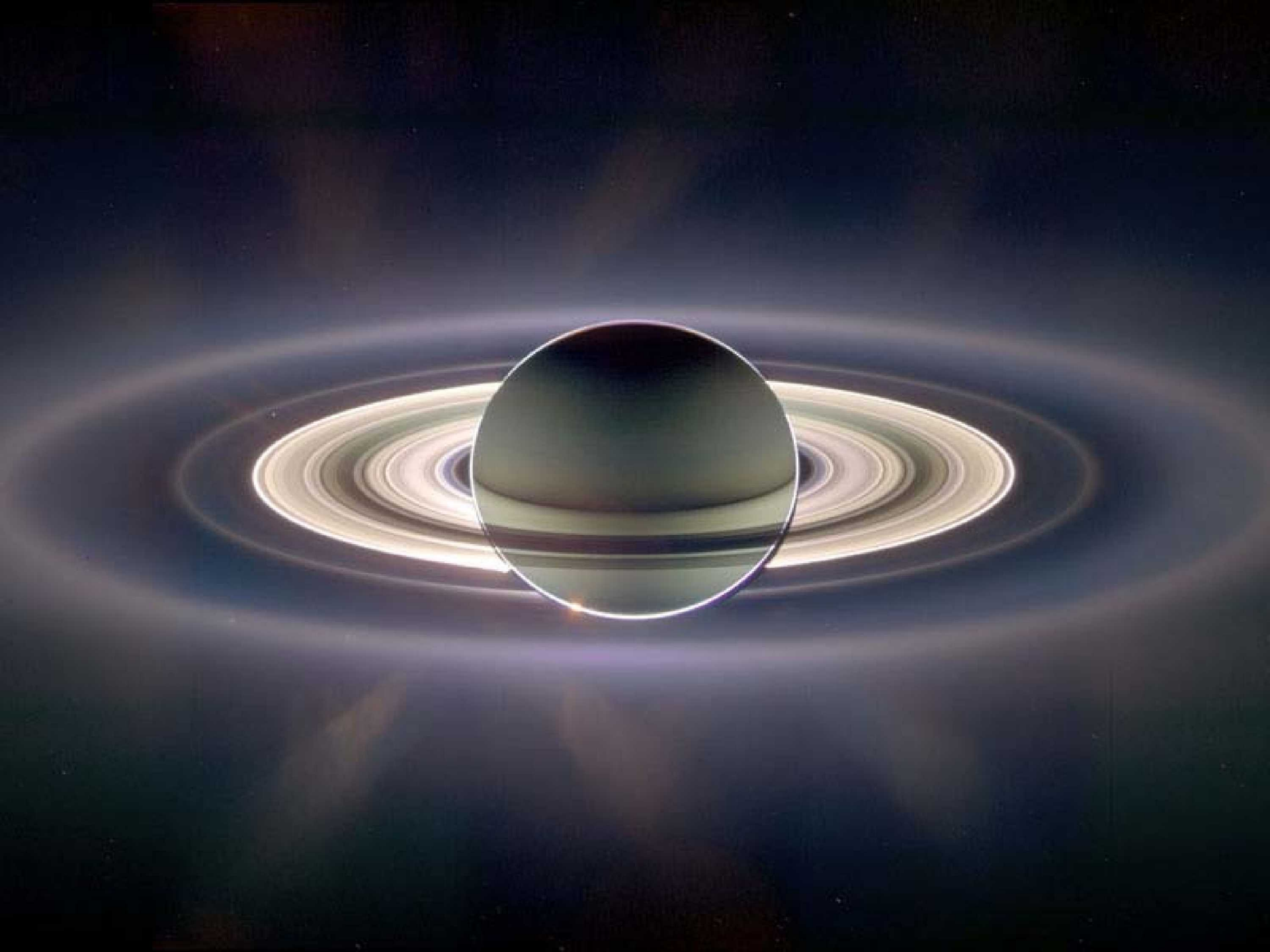
















MICROBIOLOGICAL RISKS

Sources

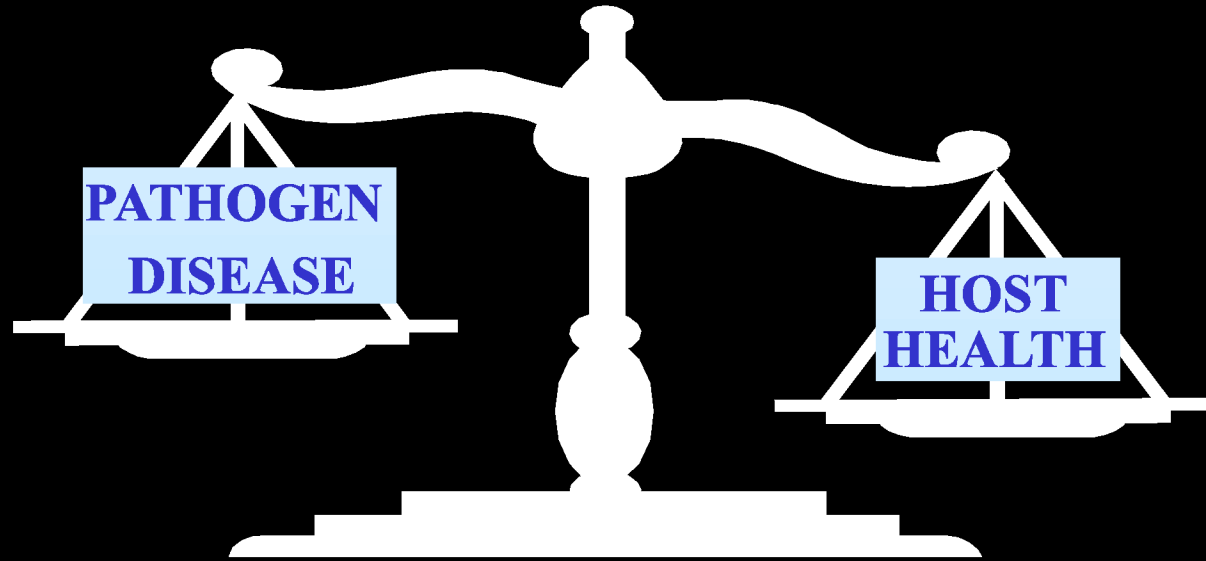
- Crewmembers
- Water
- Food
- Air
- Surfaces
- Payloads

Controls

- Preflight screening, quarantine, vaccination, antimicrobials, antivirals
- Preflight/inflight monitoring, biocides
- Preflight analyses
- Preflight/inflight monitoring, filtration
- Preflight/inflight monitoring, disinfection
- Preflight cleaning, biosafety assessment, disinfection



FACTORS INCREASING DISEASE RISK



- **Crowded living conditions**
- **Closed-loop environment (water/air)**
- **Reduced capability for personal hygiene**
- **Limited clean-up and disinfection capability**
- **Inability to isolate contagious crewmember**
- **Limited treatment capability and crew return**
- **Altered immune response**

SPACEFLIGHT FACTORS AFFECTING INFECTIOUS DISEASE RISK

Positive Factors

- Healthy well-conditioned crew
- Preflight exams & restricted access
- No exposure to many public health pathogens
- Diagnostic/treatment on board
- Earth to orbit medical consult

Negative Factors

- Isolated/enclosed environment
- Recycled air/water (urine, humidity condensate)
- Limited diagnostics/treatment on board
- Remote location/limited return pathogens (e.g. TB, HIV, Hep A/B/C)
- Uniquely stressful environment
- Diminished Immunity
- Increased virulence in bacteria

ADAPTATION TO SPACEFLIGHT

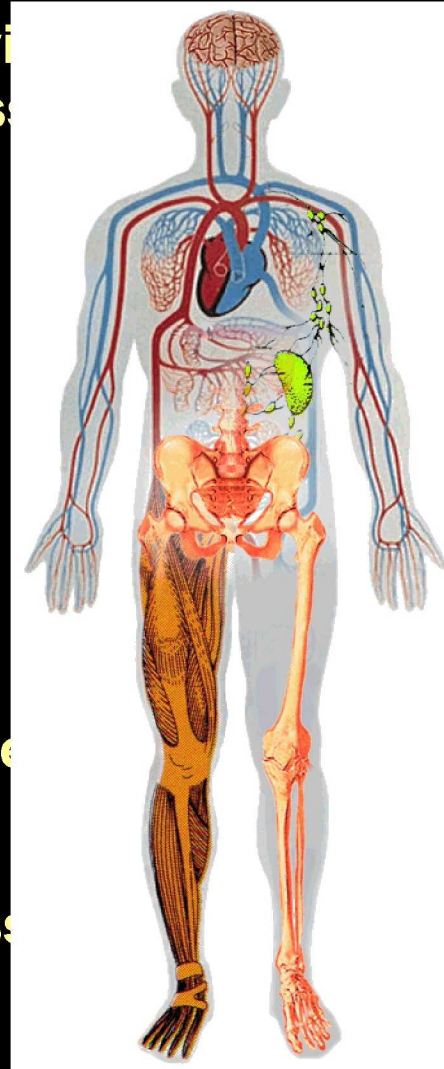
**Psychological/Behavioral
Performance issues**

**Taste and odor
sensitivity**

**Gastrointestinal
alterations**

**Fluid shifts,
hematological changes**

Muscle loss



Proprioceptive adaptations

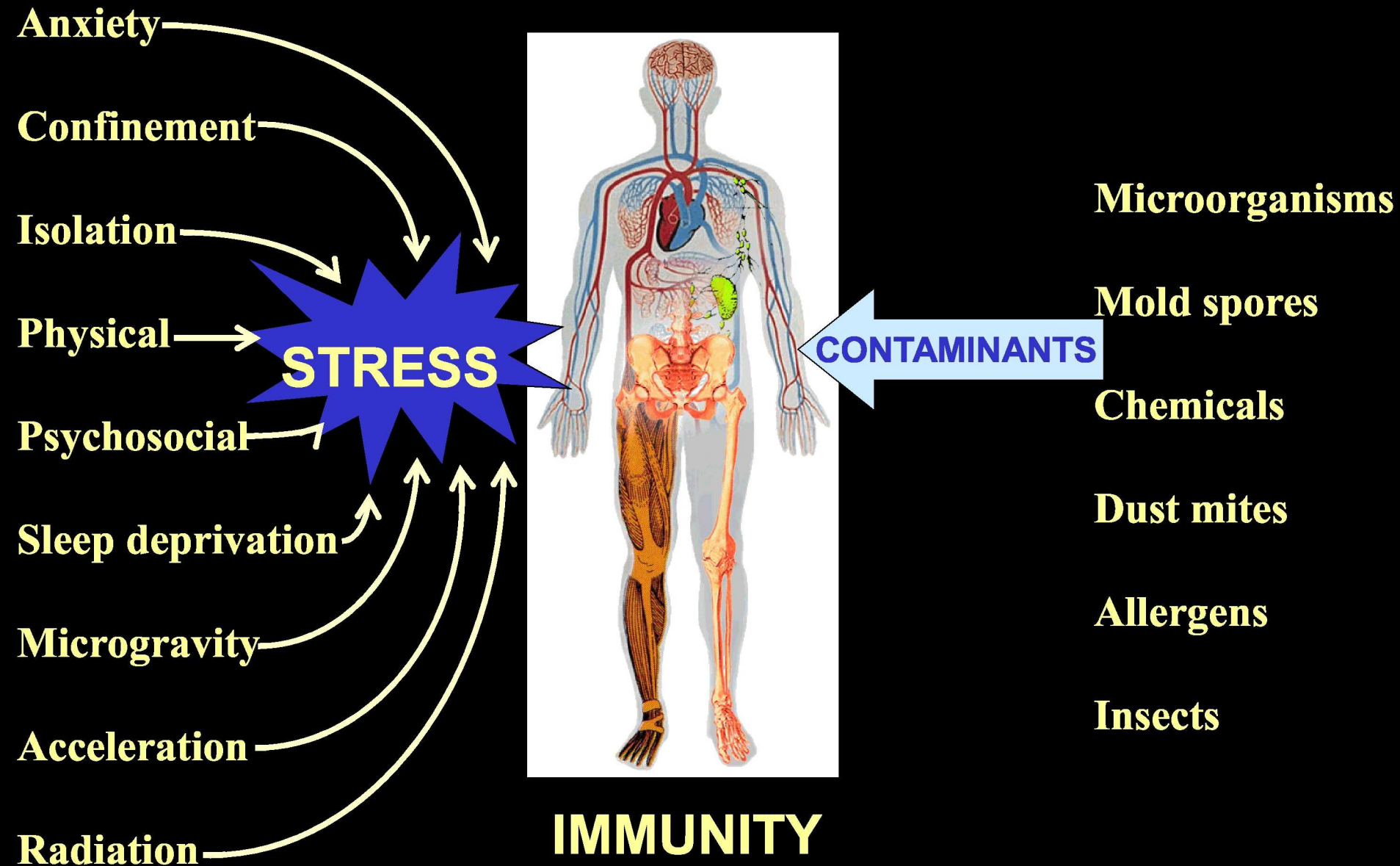
Cardiovascular adaptations

**Sleep and circadian
rhythm disturbances**

Bone loss

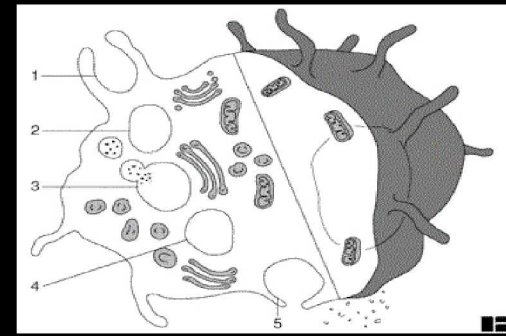
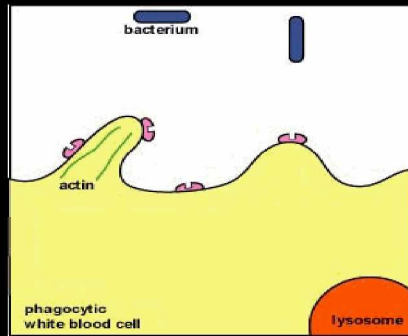
Immune changes

ENVIRONMENTAL FACTORS AFFECTING IMMUNITY



HUMAN SPACE FLIGHT IMMUNOLOGY

- White blood cell count Increased (neutrophils)
- Lymphocyte proliferative responses Decreased
- Cell mediated immunity Decreased
- Cytokine production Increased/Decreased
- Humoral factors No Change
- Specific antibody response No Change
- Neutrophil/Monocyte functions Decreased
- NK cell cytotoxicity Decreased
- Latent virus reactivation Increased



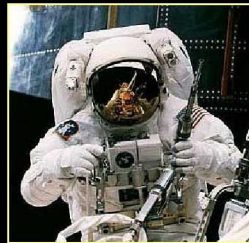
INFECTIOUS DISEASES IN ASTRONAUTS

STS-1 Through STS-108

- Fungal infections
- Flu-like syndrome
- Urinary tract infections
- Aphthous stomatitis
- Viral gastrointestinal disease
- Subcutaneous skin infections
- Viral reactivation
- URI (common cold, sore throat)
- Sty

IMMUNE SYMPTOMS

- Allergic rhinitis
- Hypersensitivity
- Coughing/Sneezing
- Rashes/Skin disorders
- Infectious of cuts
- Delayed wound healing



Stress Immune Response

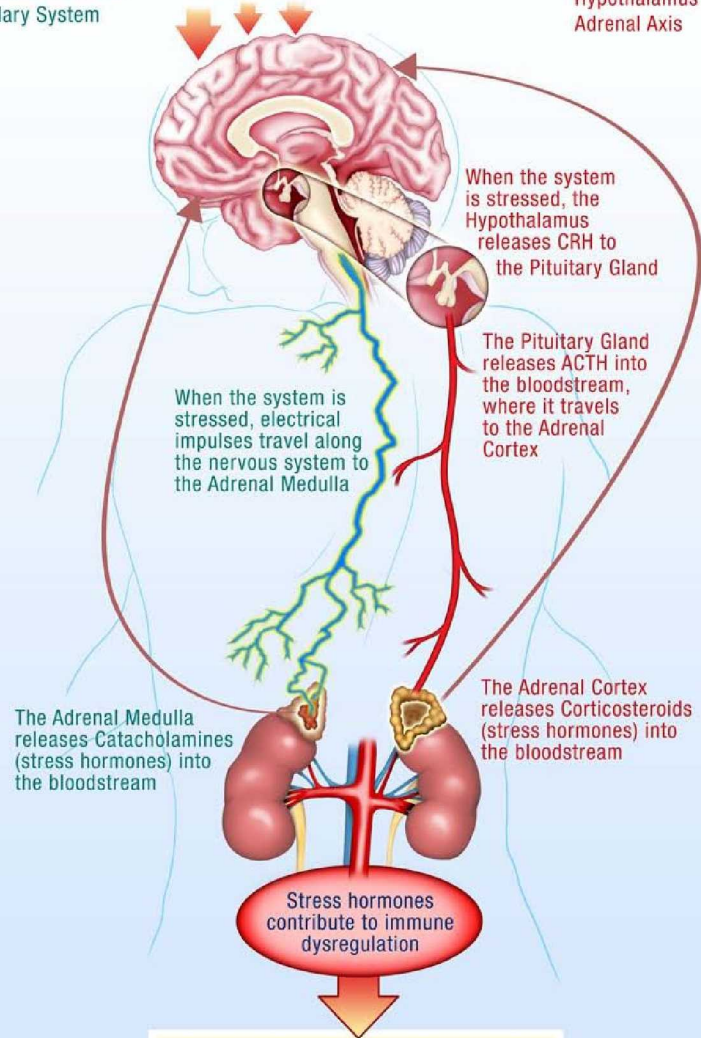
SAM

Sympathetic Adrenal Medullary System

SYSTEM STRESSORS

HPA Axis

Hypothalamus Pituitary Adrenal Axis



When the system is stressed, the Hypothalamus releases CRH to the Pituitary Gland

The Pituitary Gland releases ACTH into the bloodstream, where it travels to the Adrenal Cortex

When the system is stressed, electrical impulses travel along the nervous system to the Adrenal Medulla

The Adrenal Medulla releases Catecholamines (stress hormones) into the bloodstream

The Adrenal Cortex releases Corticosteroids (stress hormones) into the bloodstream

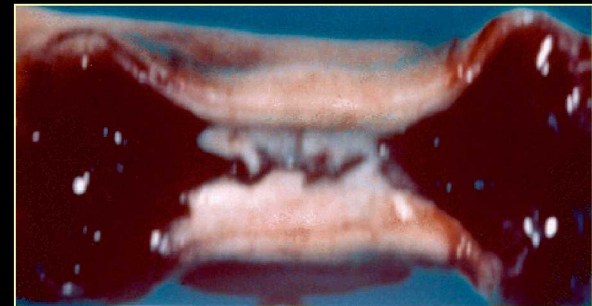
Stress hormones contribute to immune dysregulation

- Increased reactivation of herpes virus
- Allergies
- Others

Why Herpes viruses?

Herpesviruses are:

- 1. The most readily recognized latent viruses.**
- 2. Ubiquitous and represent important infectious disease risks with monogenic potential.**
- 3. Not mitigated by preflight quarantine. Space flight stress alters immune response.**
- 4. Diminished immunity results in reactivation & shedding of latent viruses**



Specific Application:

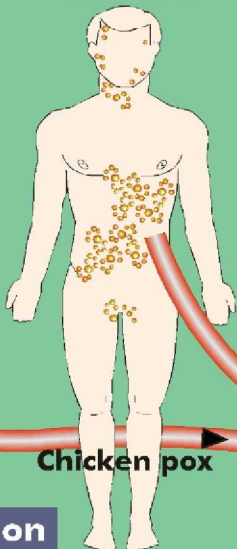
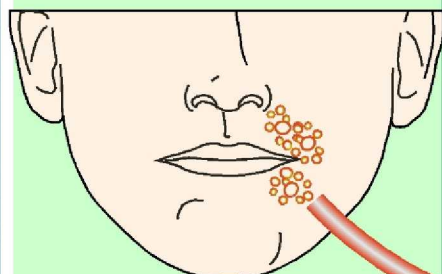
May be used as an early predictor of impending medically significant changes in the immune response.

LATENT VIRAL REACTIVATION

Herpes Simplex

Varicella

Latent virus



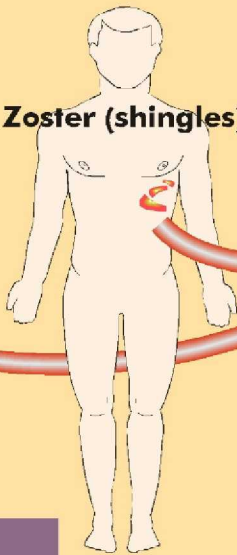
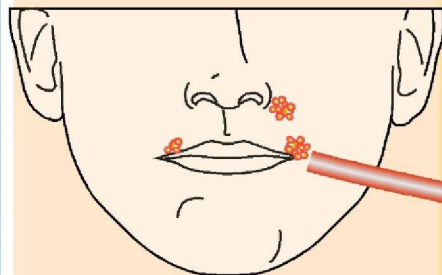
Virus transit up peripheral nerve

Sensory neuron in dorsal root ganglion

**Gingivostomatitis
Mild pharyngitis fever**

Chicken pox

Primary Infection



Virus transit down peripheral nerve

Spinal cord

Cold Sore

Zoster (shingles)

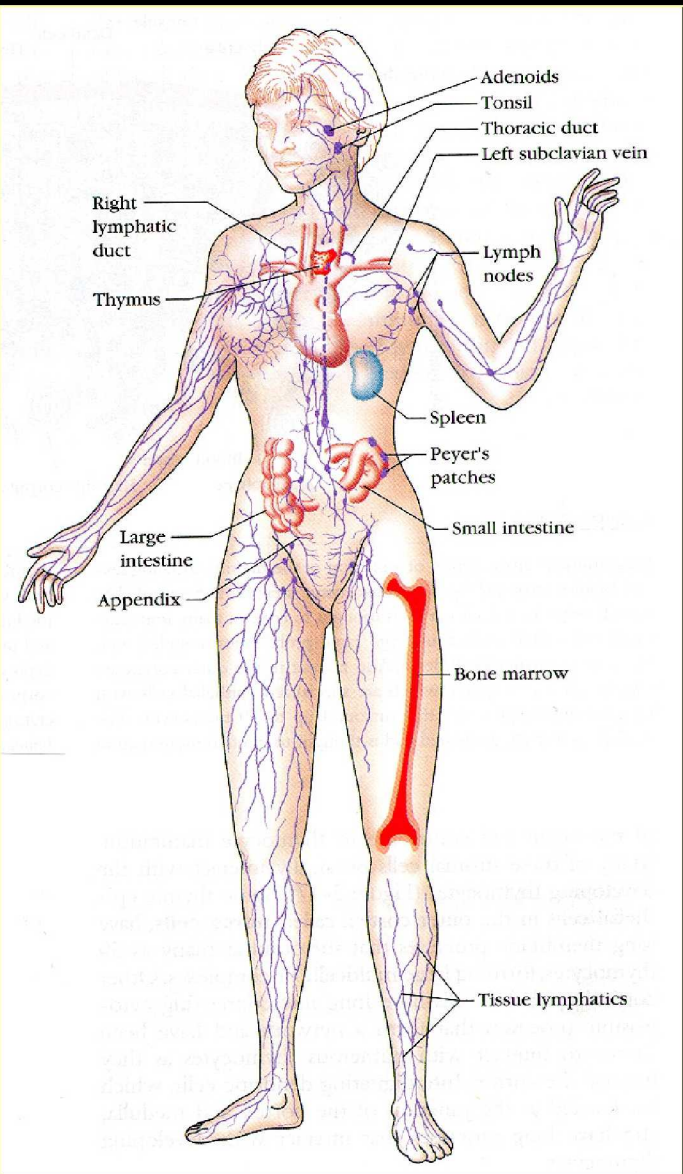
Stress →

Activation of virus in neuron

Recurrence

Herpes virus Infections

**4 of 8 herpes viruses reactivate
in response to spaceflight**



➤ **Herpes Simplex Virus (HSV-**

Ocular herpes, encephalitis

➤ **Varicella-zoster virus (VZV)**

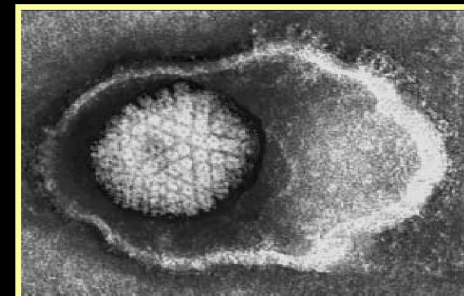
Chicken pox, shingles

➤ **Epstein-barr virus (EBV)**

Mononucleosis, tumors

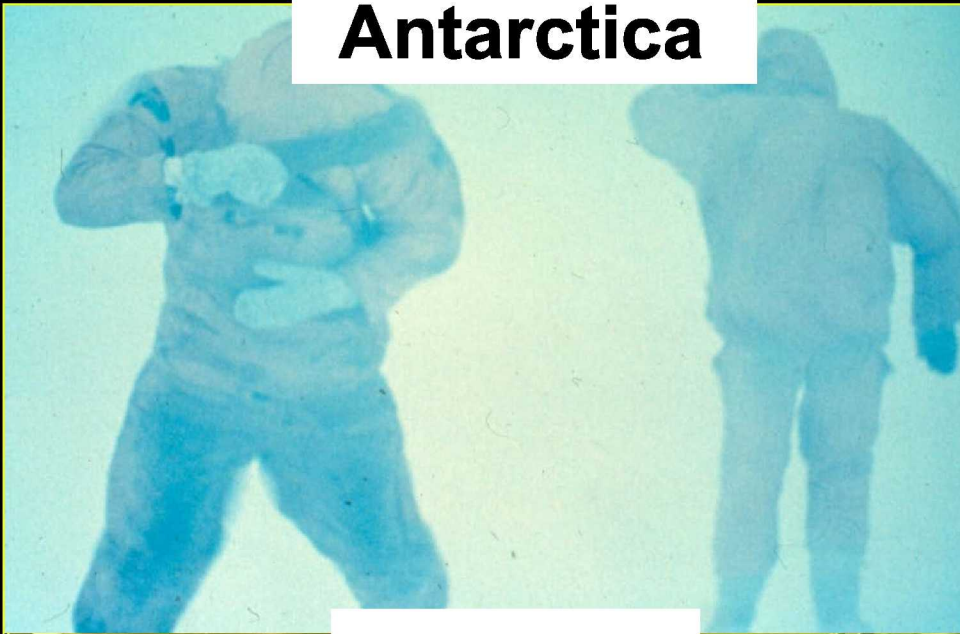
➤ **Cytomegalovirus (CMV)**

Mononucleosis, hepatitis



Space Analogs

Antarctica



AQUARIUS
THE WORLD'S ONLY
UNDERWATER LABORATORY

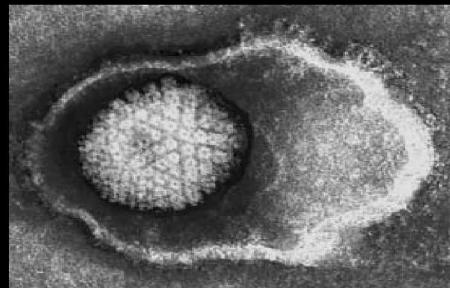


Closed Chamber

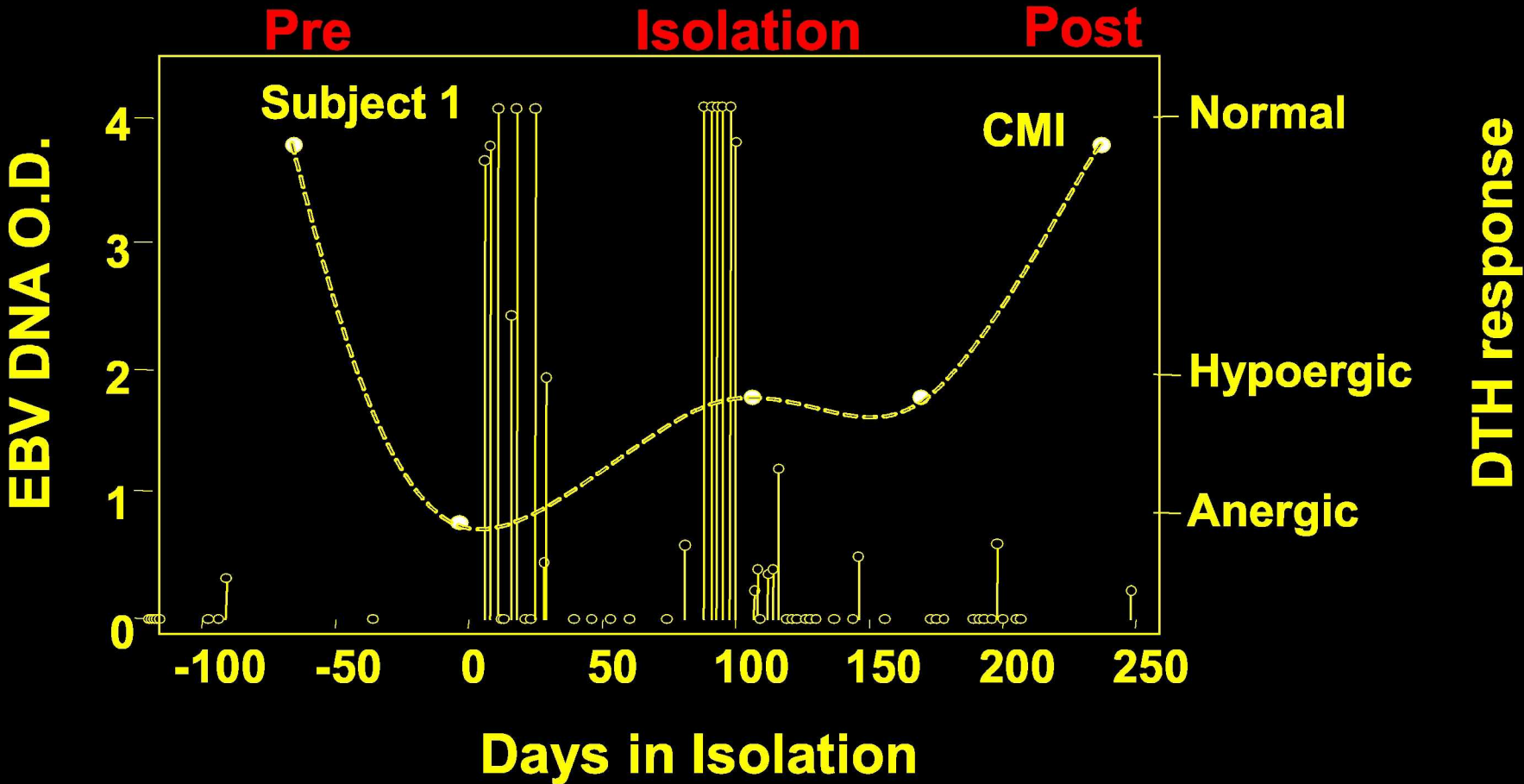


Bed Rest



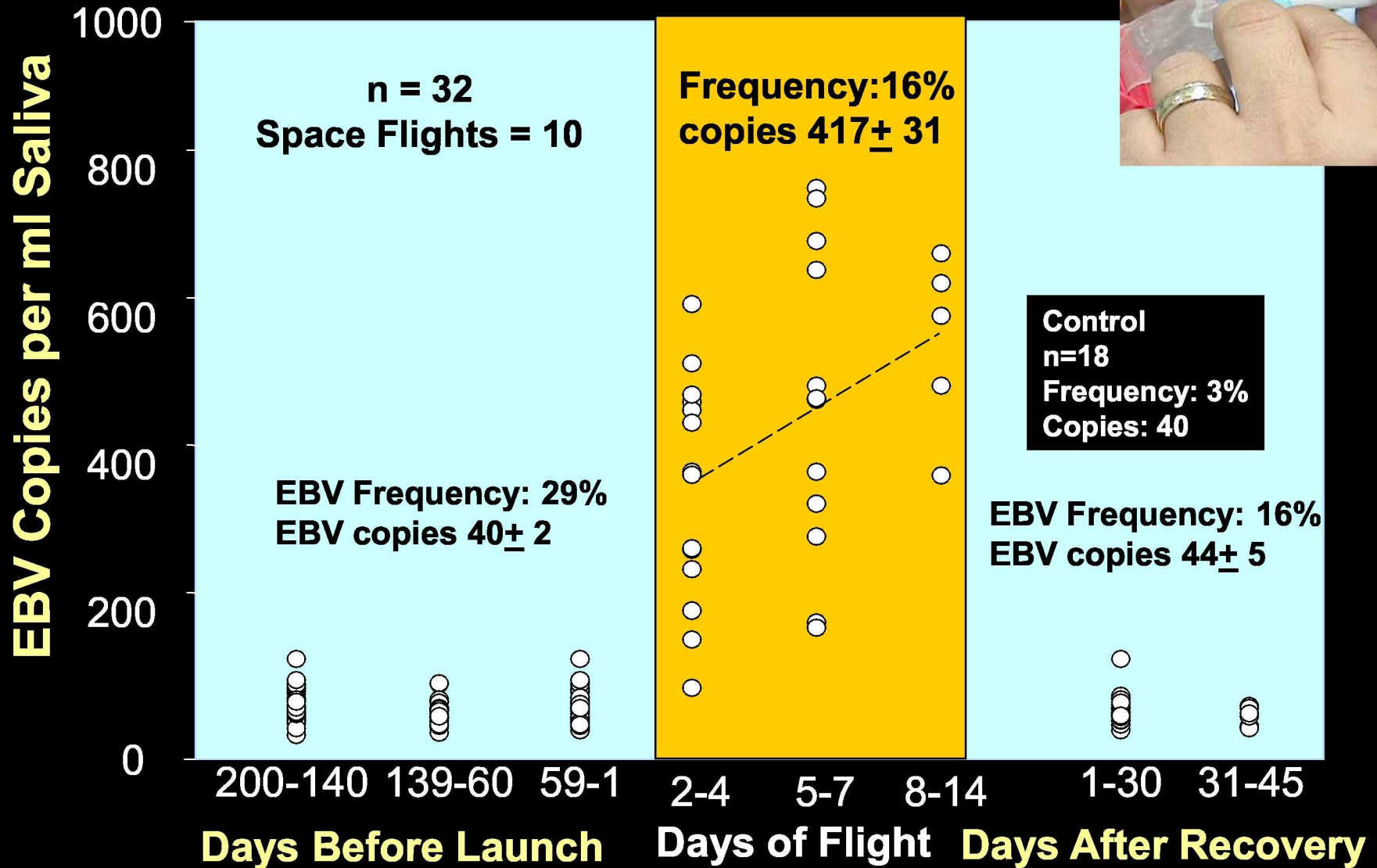


Antarctica: EBV

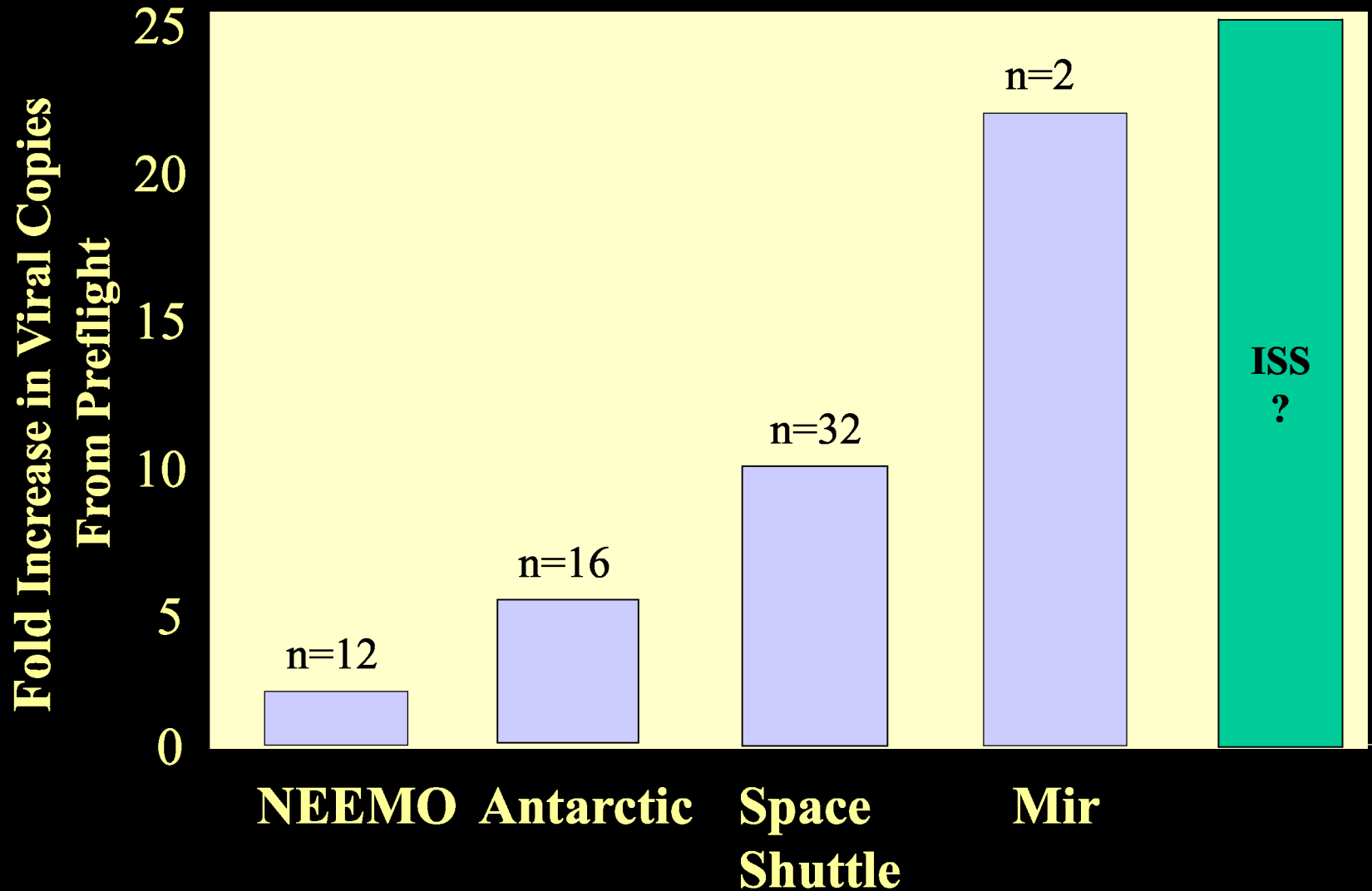


Mehta et al., J. Medical Virology 2000

Space Shuttle EBV Copies



Fold Increase In EBV Copy Numbers



Summary of Nested RT-PCR Analysis of EBV Gene Expression in Healthy Young Adults

Subject	Actin	EBER1	Latency I-III					IE/E ^a Replicative				Late Replicative	
			Qp	LMP2A	Cp/Wp	LMP1	EBNA2	BZLF1	BHRF1	SM	Fp	BALF5	gp220
1	+++												
2	+++	+++											
3	+	+											
4	+++	+											
5	+++												
6	+++	+++											
7	+++	+											
8	+	+											
9	+	+++											
10	+++												
11	+++	+						+					
12	+	+	+										
13	+++	+++	+										
14	+++	+				+							
15	+++	+											
16	+++	+											
17	+++	+++											
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23	+++	+++										+	
24	+++	+++	+									+	

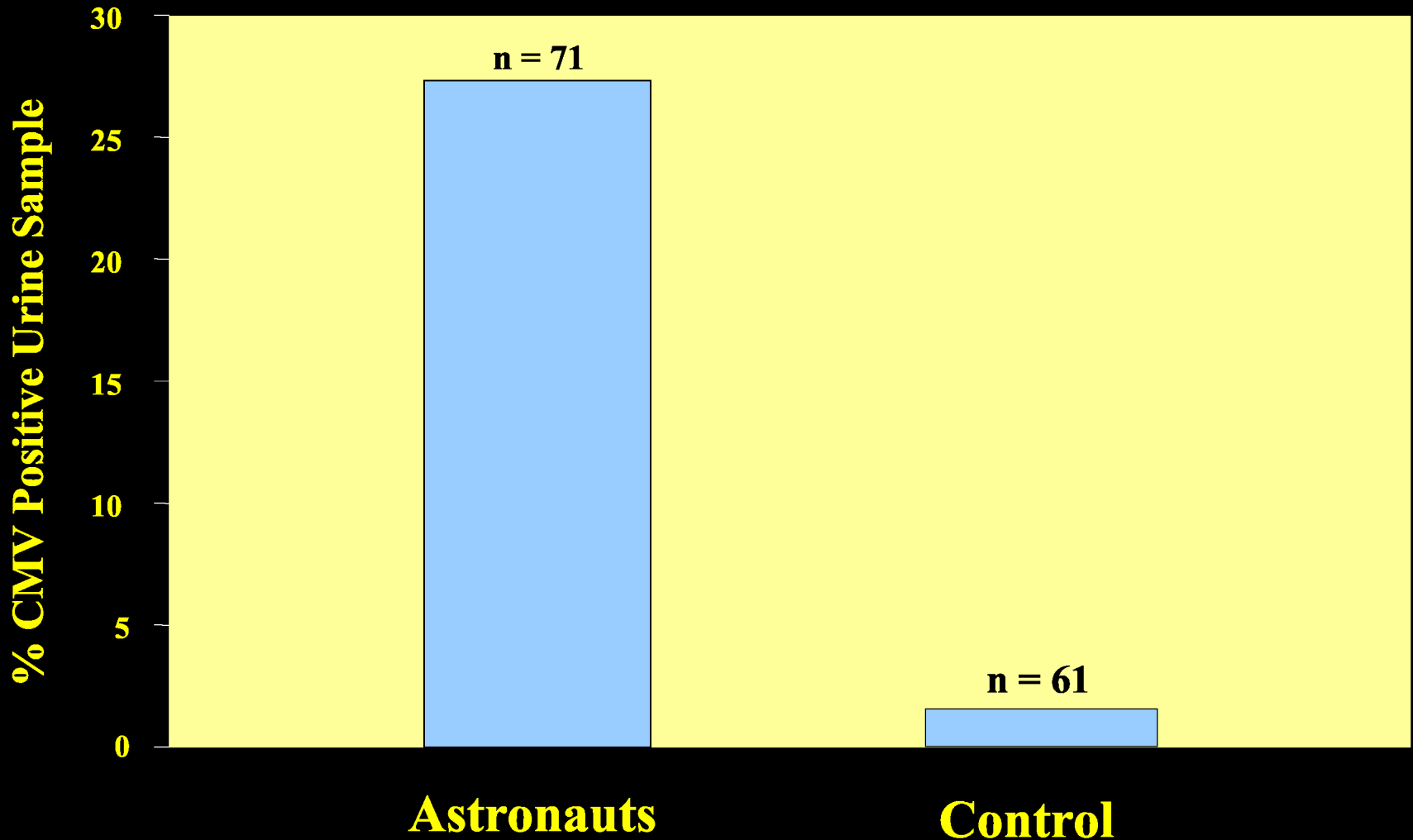
^aLegend (+++ = highly expressed; ++ = moderately expressed; + = low expression); ^bImmediate early/early.

Summary of Nested RT-PCR Analysis of EBV Gene Expression in Astronauts

Mission	Subject	Time ^b	EBER1	Latency I-III					IE/E ^c Replicative				Late Replicative	
				Qp	LMP2A	Cp/Wp	LMP1	EBNA2	BZLF1	BHRF1	SM	Fp	BALF5	gp220
Shuttle	1	L-10	+++	+			+				+			
		R+0	+++				+							
	2	L-10	+++								+			
		R+0	+++											
	3	L-10	+++							+				
		R+0	+++	+						+				
	4	L-10	+++					+						
		R+0	+							+	+++			
	5	L-10	+								++			
		R+0	+++								++			
	6	L-10	+++							+	+++			
		R+0	+++	++							+++			
ISS	1	L-10	+++	+			+				++			
		R+0	+++	+	+						+++	+++	++	+++
	2	L-10	+++											
		R+0	+++	+			+	+++			+++			
	3	L-10	+++									++		
		R+0	+++			+++					+++			
	4	L-10	+								+++	+++		
		R+0	+++					+++		+++	+++	+		+++
	5	L-10	+++	+							+++			
		R+0	+++	+			+++	+++		+++	+++	+	+++	+++
	6	L-10	+++		+	+++		+++			+++			
		R+0	+++	+						+	+++		+++	+++

^aLegend (+++ = highly expressed; ++ = moderately expressed; + = low expression). ^bCollection time: Launch minus 10-days (L-10); Recovery/landing day (R+0). Average Shuttle flight = 11 days; average ISS mission = 180 days; ^cImmediate early/early.

Space Shuttle: CMV Frequency



Incidence of Shingles and Post Herpetic Neuralgia (PHN)

Shingles: Reactivation of VZV producing blisters in dermatomal region

- Pain can be excruciating

PHN: Prolonged, sometimes incapacitating, lasting weeks, months, or years.

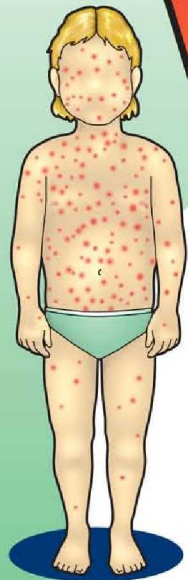
“In extreme cases, PHN can be worse than death.”

CDC

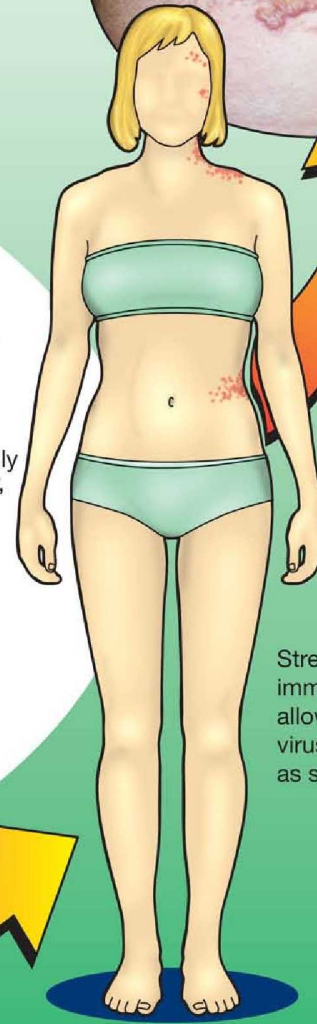
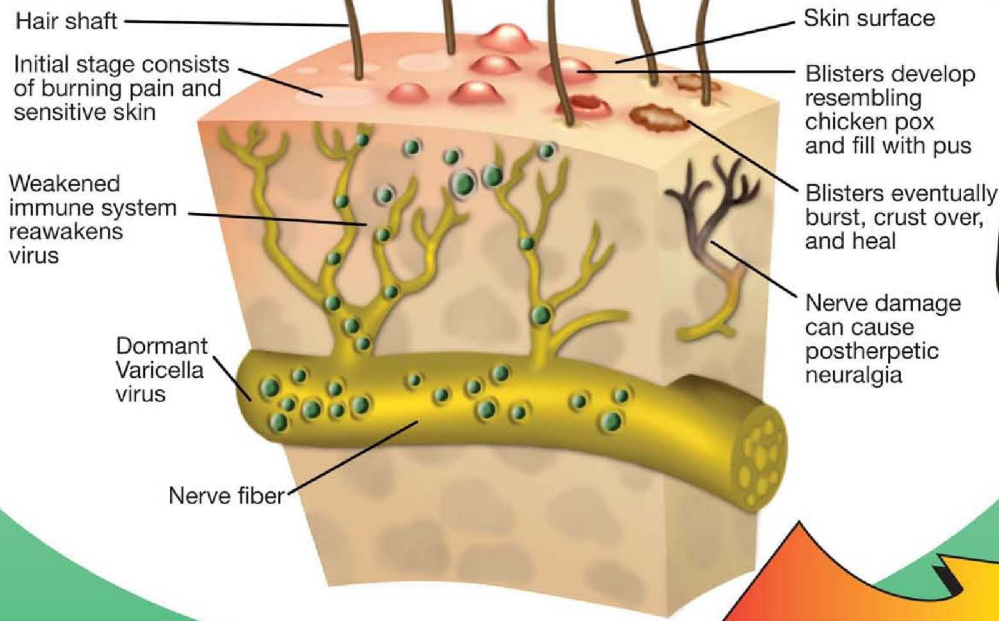
- One million cases of shingles per year
- Risk of shingles increases >10-fold with age
- Lifetime risk of developing zoster: 25-30%
- 100,000 to 200,000 cases of PHN per year



Childhood chicken pox becomes dormant in the nervous system



Primary Disease (Chicken Pox)



Reactivation (Shingles)

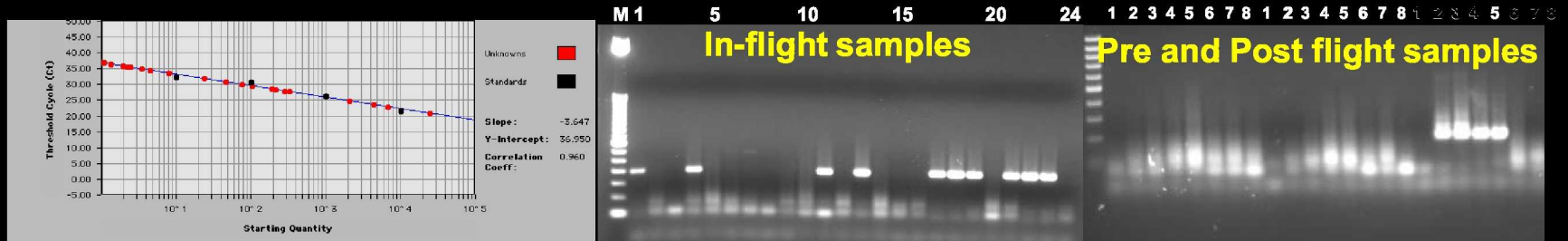
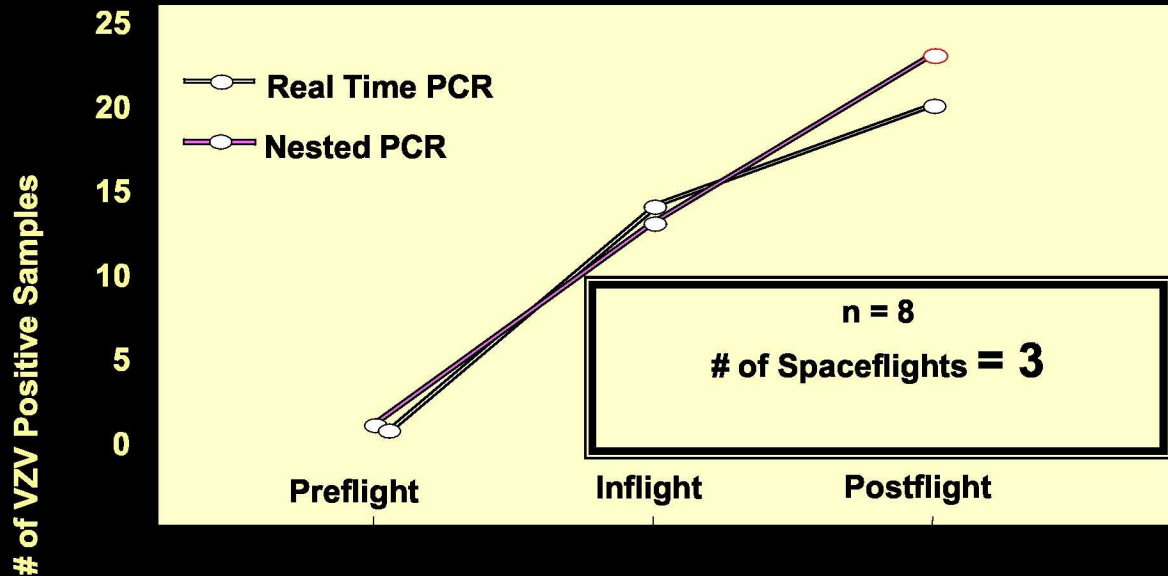


Shingles outbreak

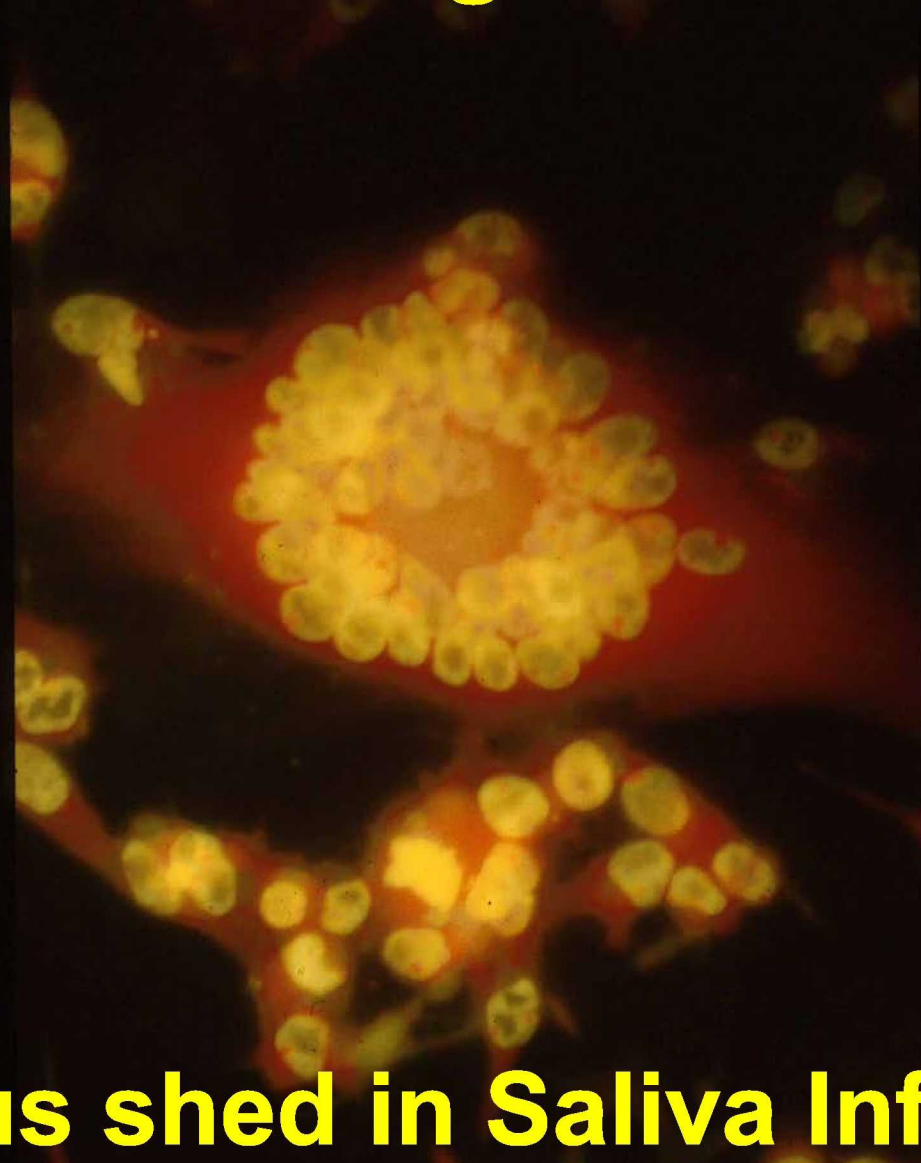
Stress on the immune system allows the latent virus to reactivate as shingles

First Report Of VZV DNA In Astronauts' Saliva

Mehta et al., *J Medical Virology*, 2004



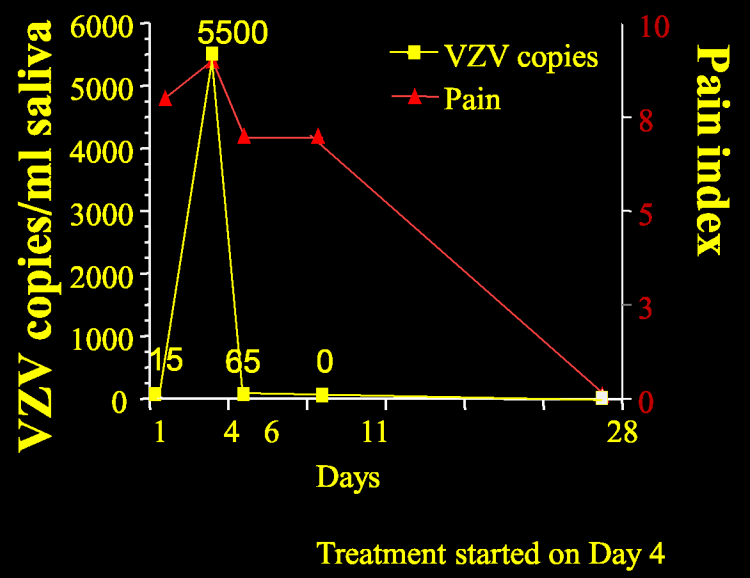
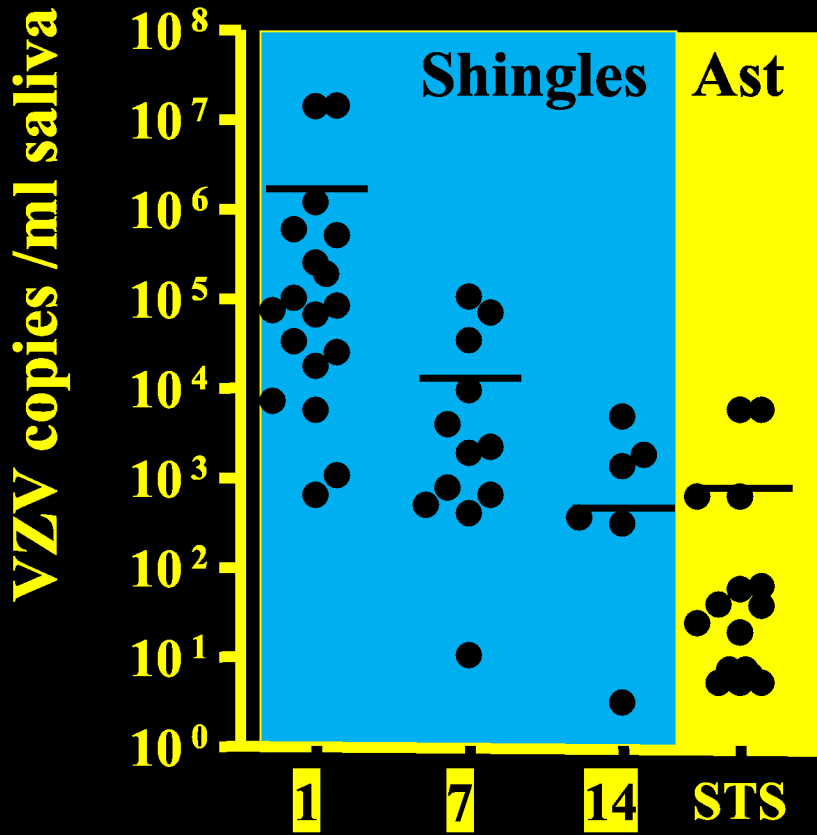
Clinical Significance?



Is the Virus shed in Saliva Infectious?

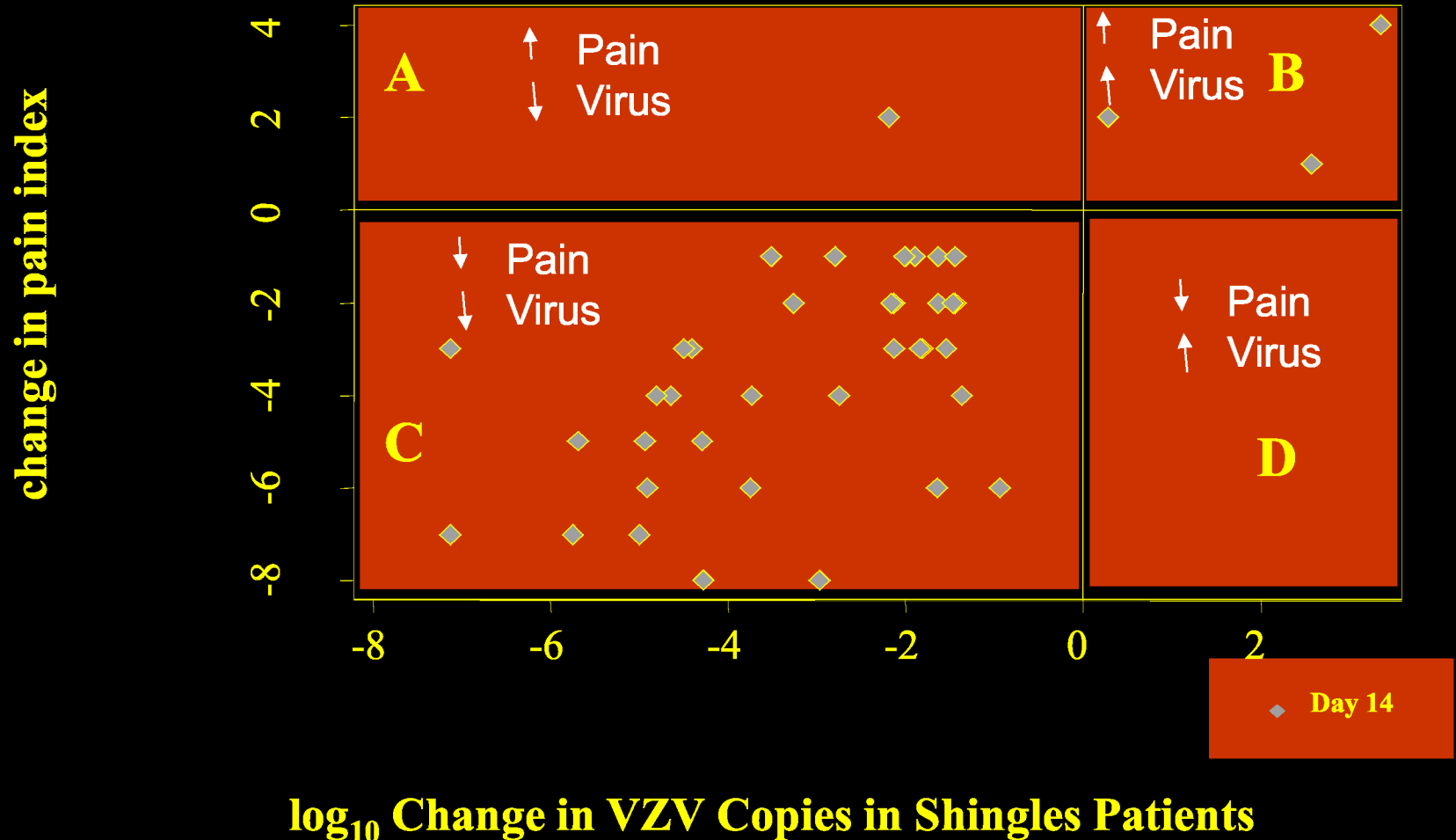
Salivary VZV In Shingles Patients & Astronauts

VZV copies in saliva of a 21 yr old patients with symptoms of Shingles.



Mehta et al., 2008; Journal of Infectious Diseases

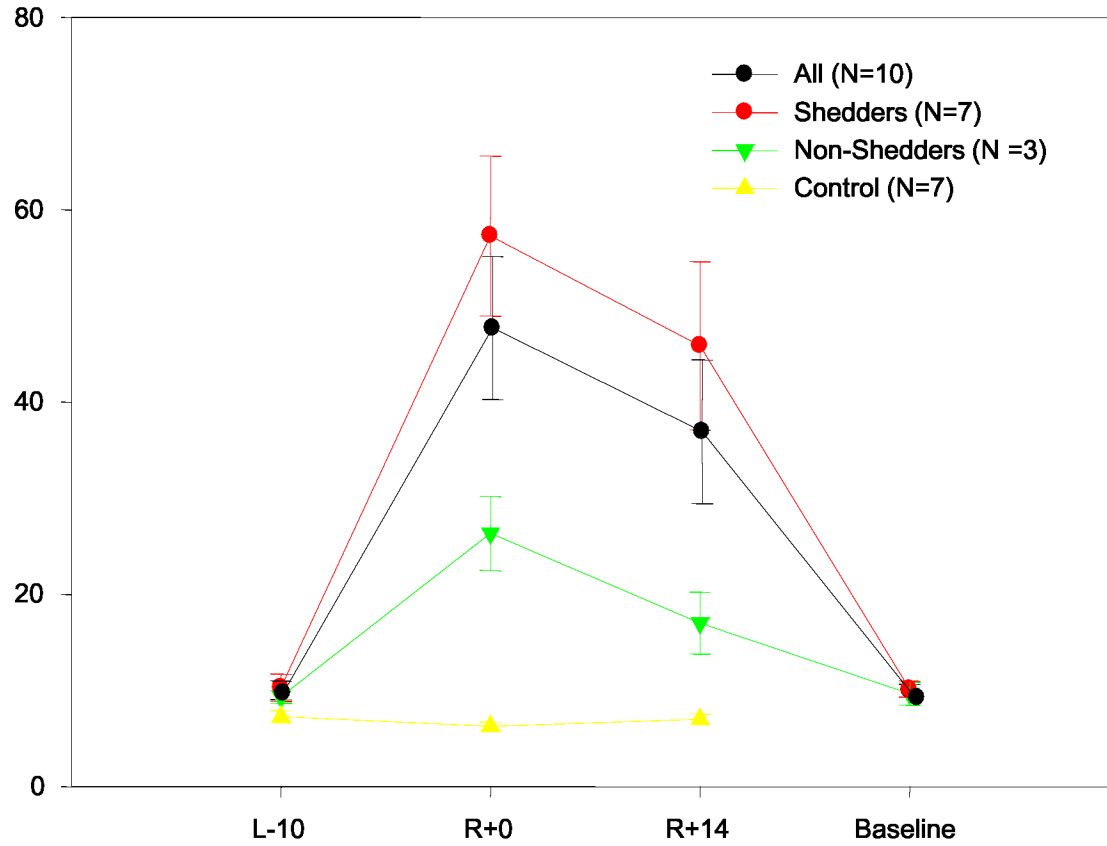
Change in Pain Index vs. Change VZV Copies After One Week of Treatment in Shingles Patients.



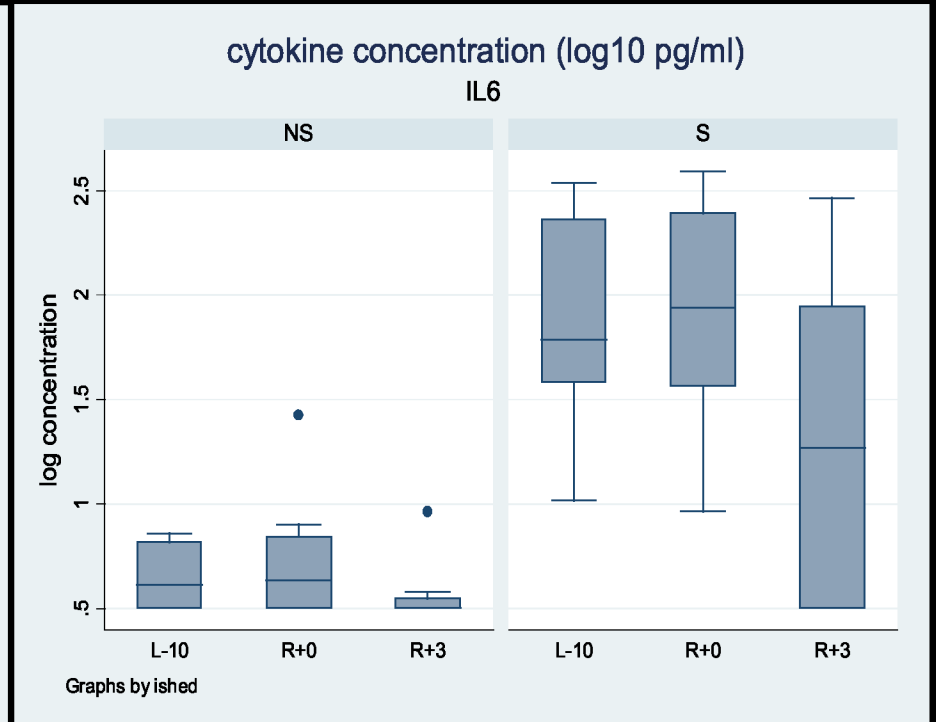
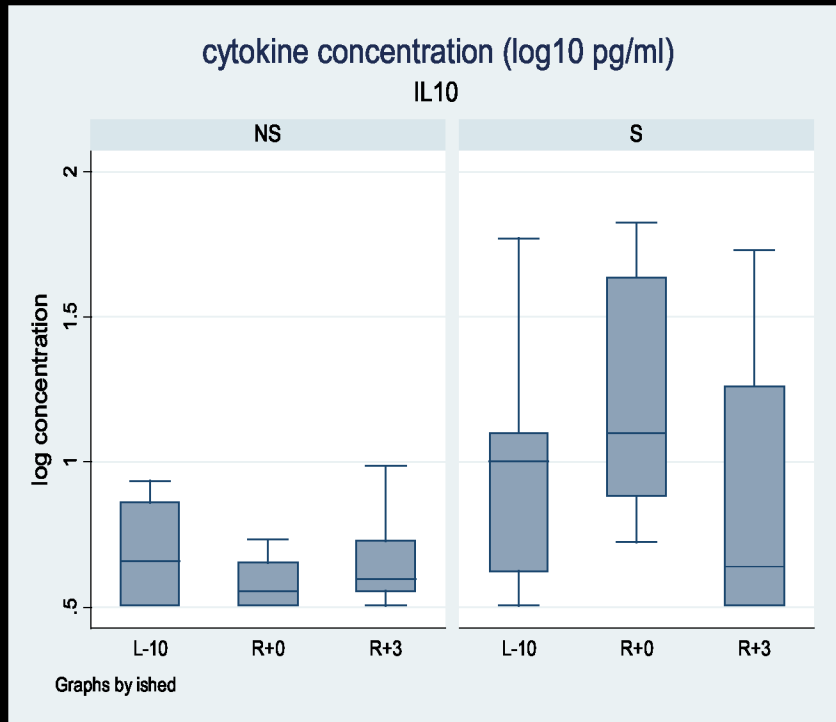
Note in almost every case, data points fell in quadrant C (both decreased)

NFKB in Astronauts

% of NF-kB nuclear positive cells (Mean +/- SE)



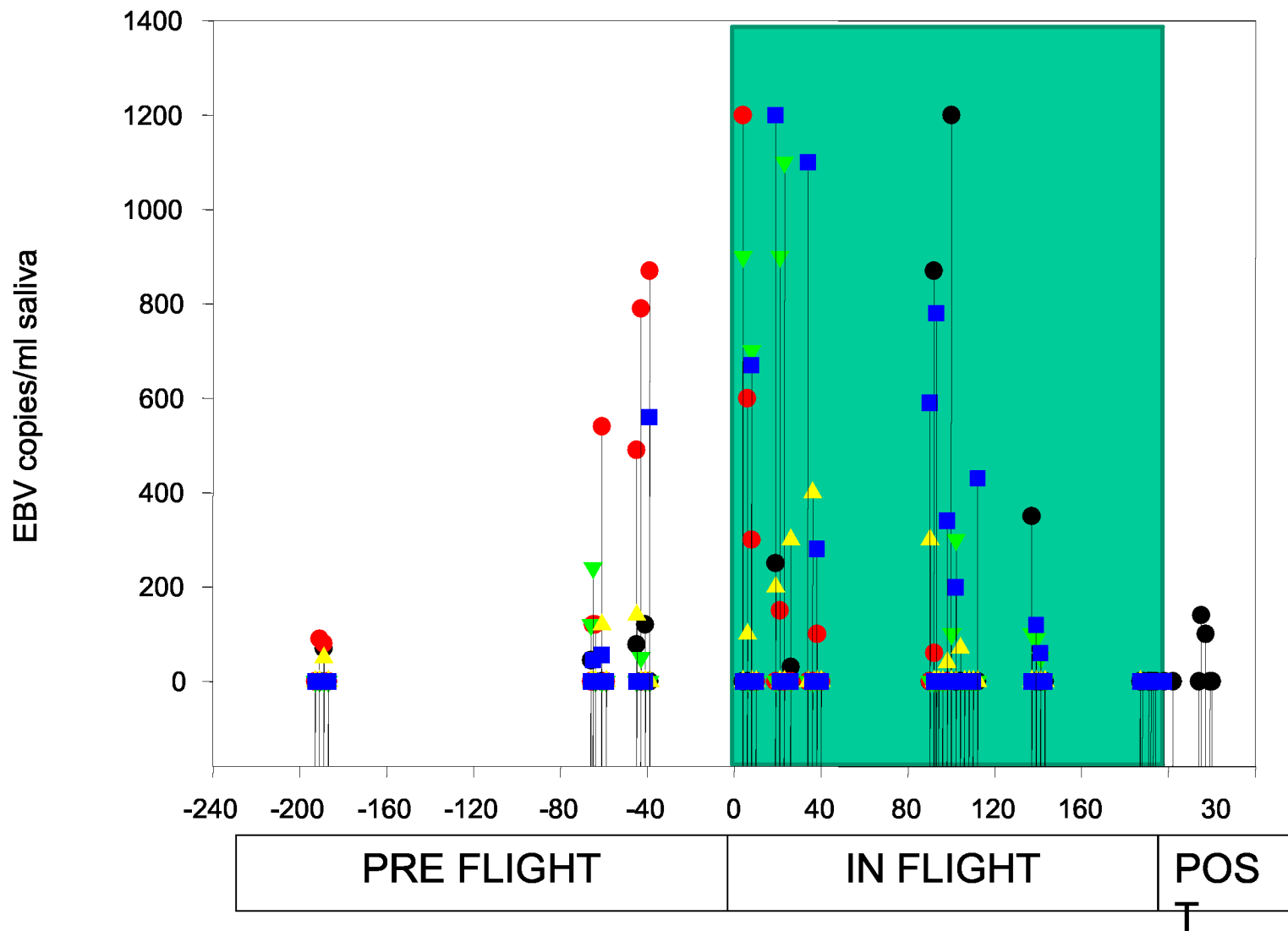
Cytokines

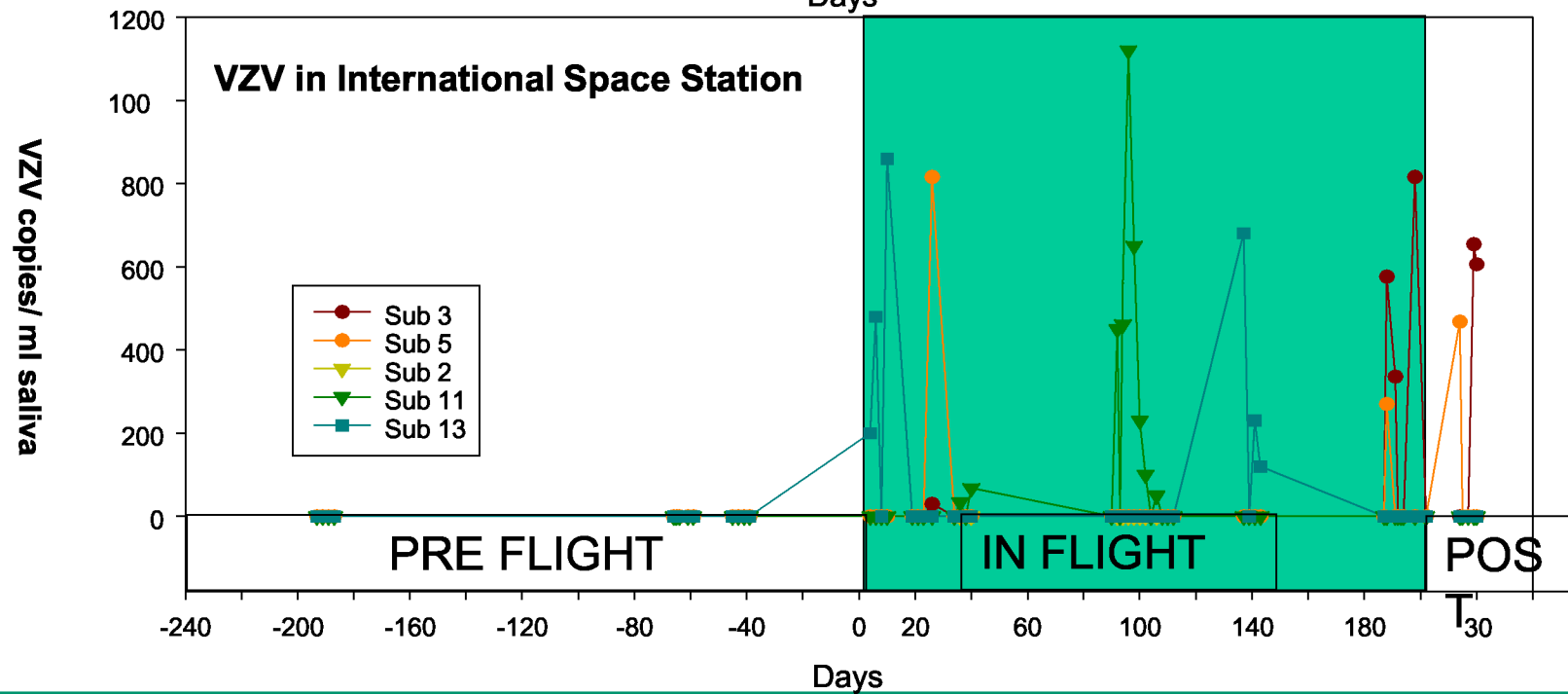
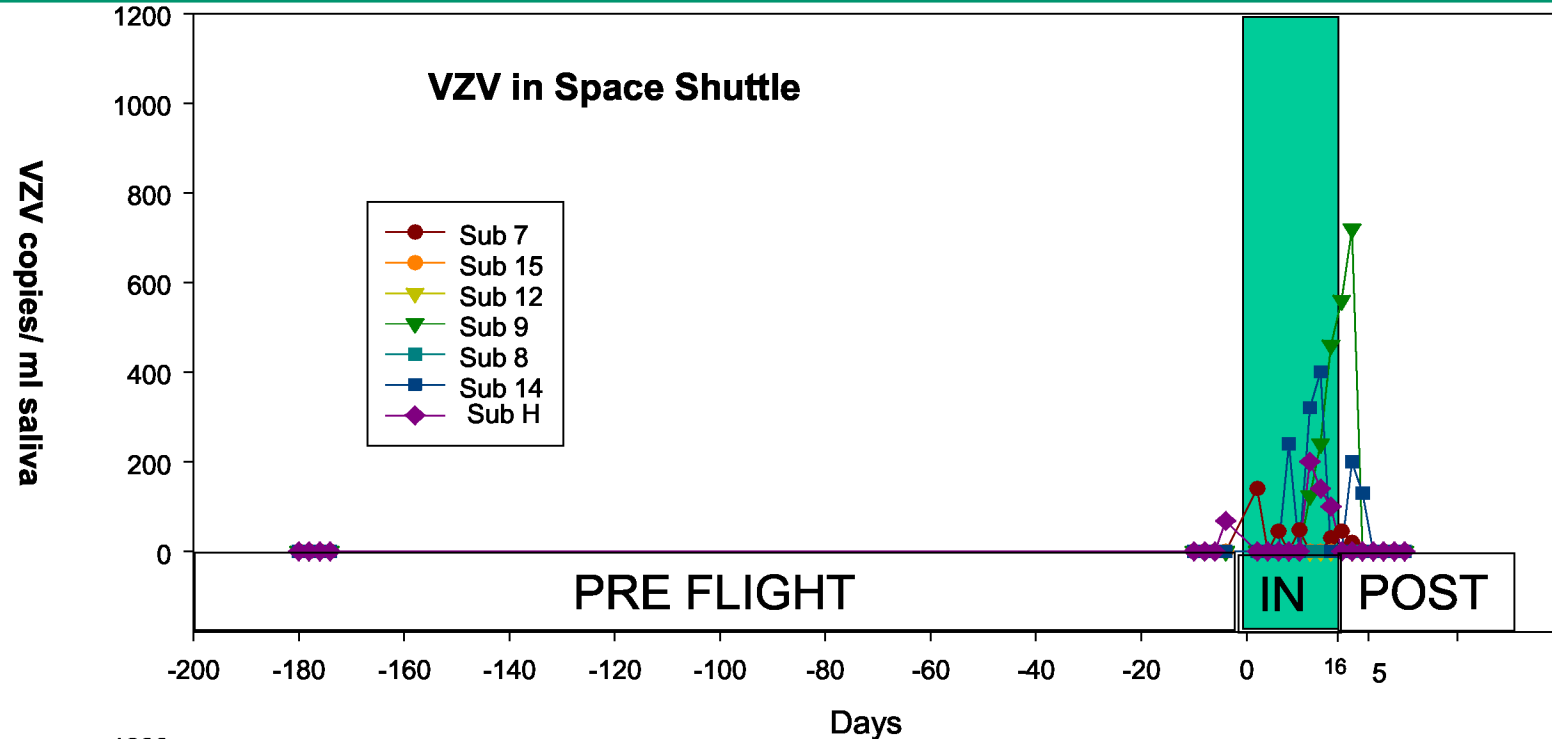




S130E012020

EBV in International Space Station





CONCLUSIONS

- **Space flight is a unique stress model.**
- **Antarctic Science Stations model many aspects of space flight.**
- **Stress associated with space flight results in increased reactivation of EBV, CMV, and VZV.**
- **Viral reactivation in astronauts appears to be linked to duration in space (stress/microgravity?).**
- **Space flight-associated stress manifested through the HPA-axis result in increased stress hormones, reduced CMI, and increased viral reactivation.**
- **Viral reactivation may be used as an early predictor of impending medically significant changes in the immune response.**

VZV can reactivate subclinically in healthy individuals after acute stress.

Changes in Microbial Pathogen Characteristics

Collaborative Studies

PI: Dr. Cheryl Nickerson, Arizona State University

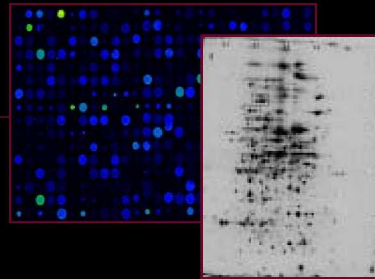
Salmonella typhimurium



Rotating Wall Vessel bioreactor reproduces aspects of microgravity (Low fluid shear, low mass diffusion)

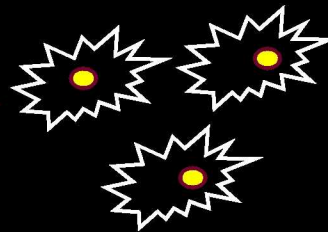


Salmonella grown in spaceflight analogues displayed increased virulence



Salmonella grown in spaceflight analogues altered their gene and protein expression

Classic virulence genes down-regulated
Ion response genes/pathways



Salmonella grown in spaceflight analogues altered their response to environmental stresses

Macrophage, acid, thermal, osmotic, oxidative

Nickerson et. al., 2000, *Infect. Immun.* 68:3147-3152; Wilson, et al., 2002, *Proc. Natl. Acad. Sci. USA.* 99:13807-13812; Wilson, et al., 2002, *Appl. Environ. Microbiol.* 68:5408-5416; Nickerson, et al., 2004, *Microbiol Mol Biol Rev.* 68:345-361.

MICROBE

Shuttle Atlantis, STS-115, launch Sept 9, 2006

Salmonella enterica Typhimurium experimental design and results

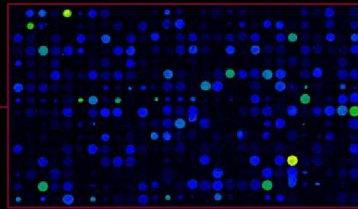


In-flight hardware



Salmonella grown during spaceflight displayed increased virulence in rich media

Killed mice faster and killed mice at lower doses than identical bacterial cultures grown on the ground
Virulence change dependent on the growth media



Salmonella grown during spaceflight altered their gene expression

167 genes differentially regulated
Ion response genes/pathways

Identification of the global molecular regulator, *hfq*, (“master switch”) of spaceflight induced cellular responses



Salmonella grown during spaceflight showed the presence of a material resembling a biofilm

Biofilms are important in disease causing potential and vehicle system failure

* *Synchronous ground controls maintained under identical conditions as those on-board Shuttle - ground and in-flight hardware loaded with same sample.*

MDRV

Shuttle Endeavour, STS-123, launch March 11, 2008
Experimental design and results



In-flight hardware



Confirmed the effect of spaceflight on *Salmonella* virulence observed in MICROBE
Demonstrated a “spaceflight response” regardless of culture media



Established a link between the spaceflight response and media composition

Ion levels can be modulated to control spaceflight-associated virulence response of *Salmonella*
Phosphate ion sufficient to alter related pathogenesis responses in spaceflight analogue model.



In combination with MICROBE results, MDRV is showing a common conserved response in many microorganisms

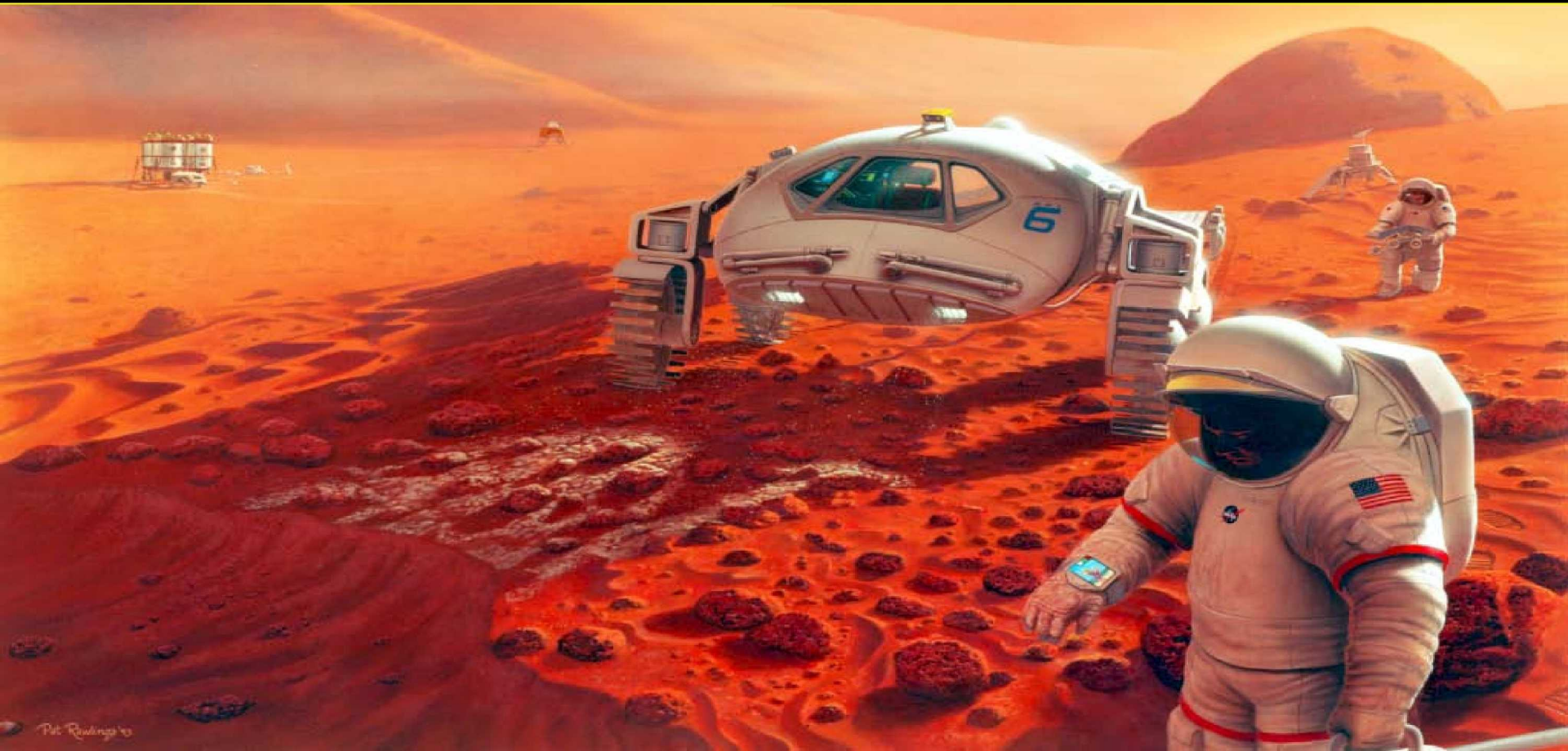
MICROBE and MDRV also evaluating organisms, such as *Pseudomonas aeruginosa* and *Candida albicans*

** Synchronous ground controls maintained under identical conditions as those on-board Shuttle - ground and in-flight hardware loaded with same sample.*

Overview

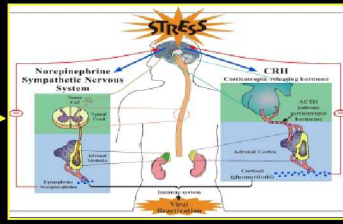


Increased Stress

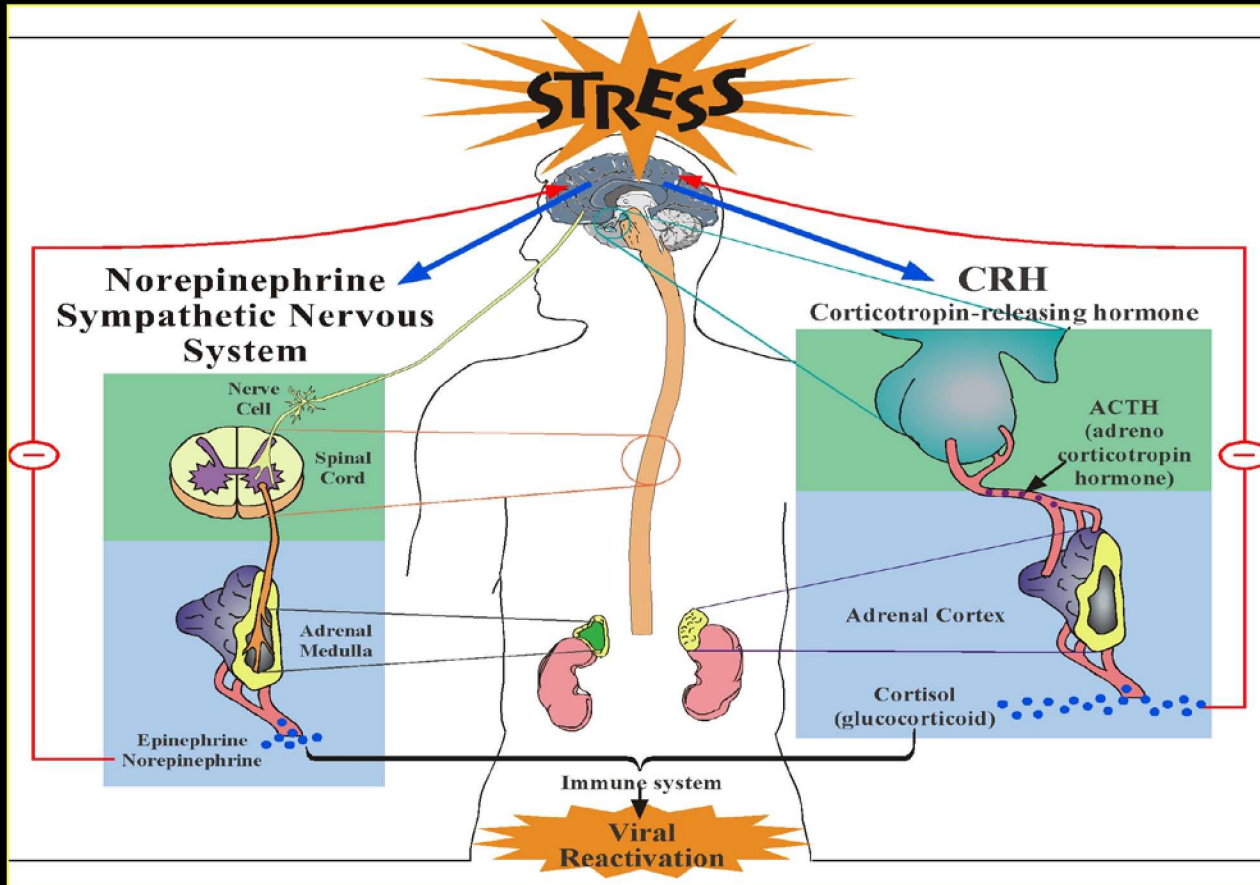




Increased Stress

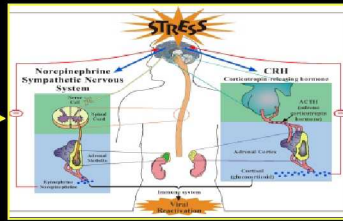


Increased Stress Hormones

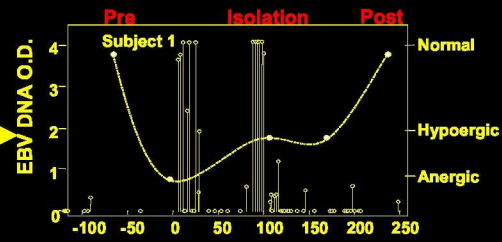




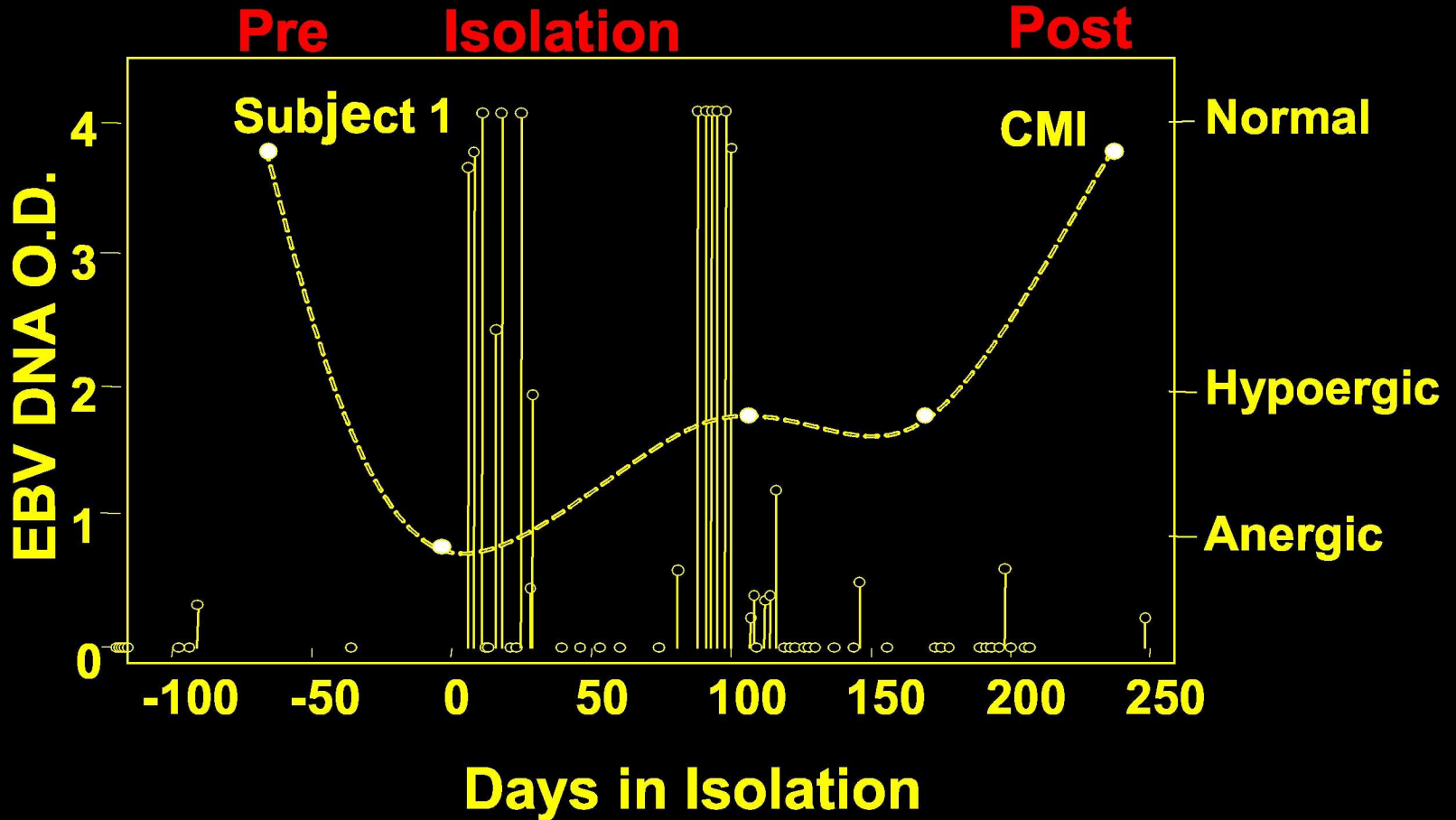
Increased Stress



Increased Stress Hormones

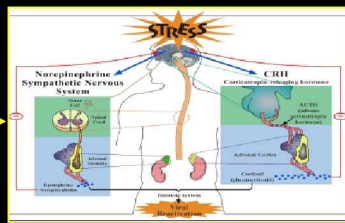


Decreased Immunity

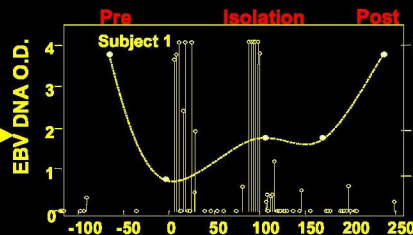




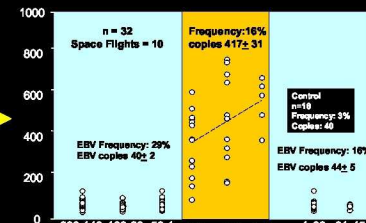
Increased Stress



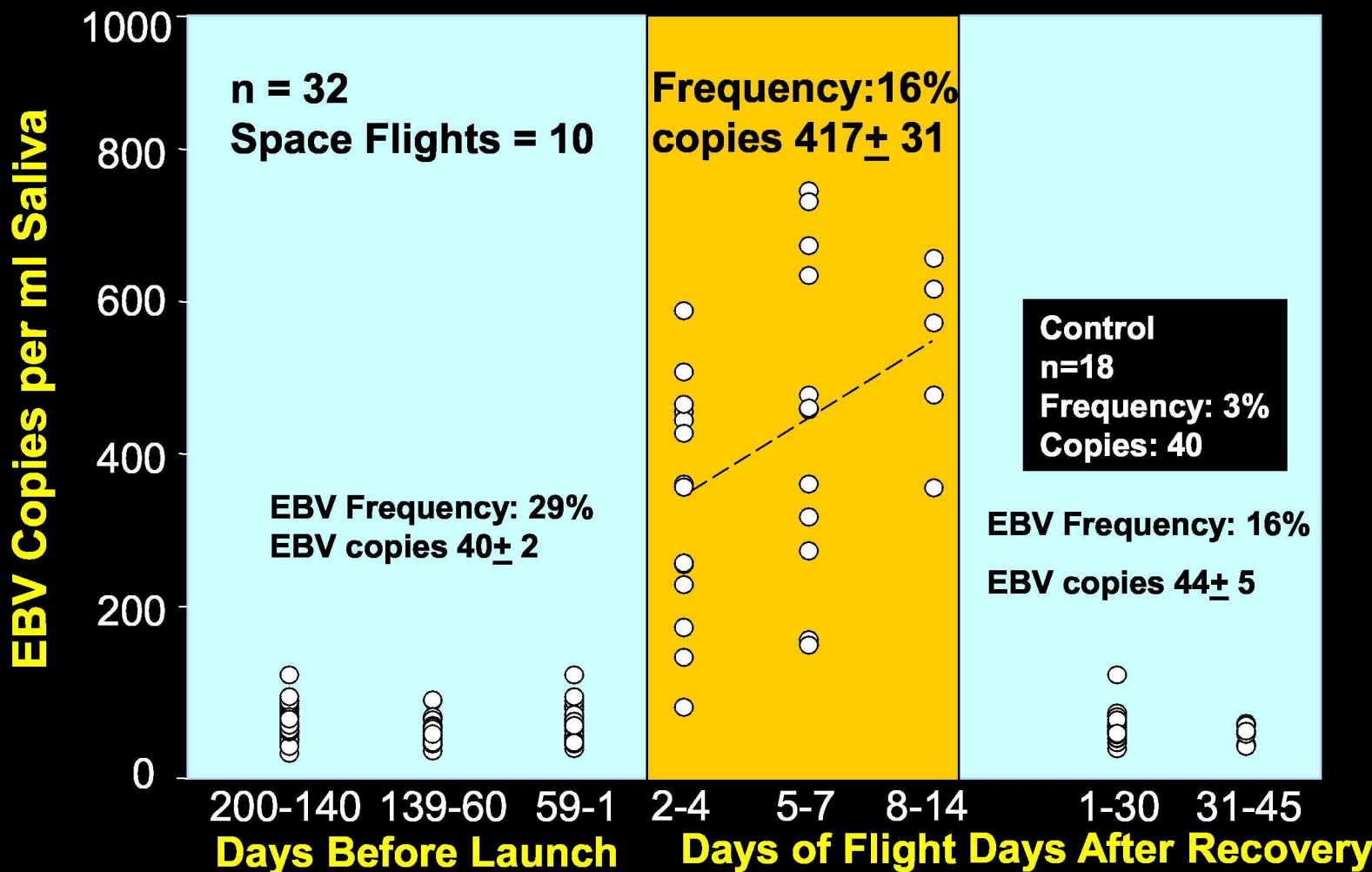
Increased Stress Hormones



Decreased Immunity

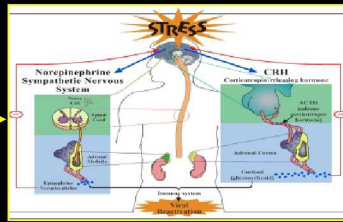


Increased Viral Reactivation

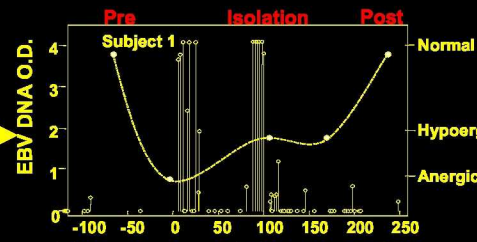




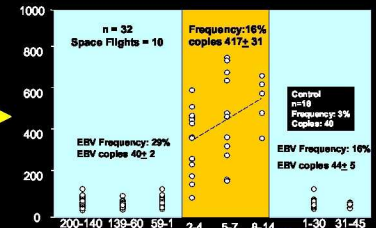
Increased Stress



Increased Stress Hormones



Decreased Immunity



Increased Viral Reactivation



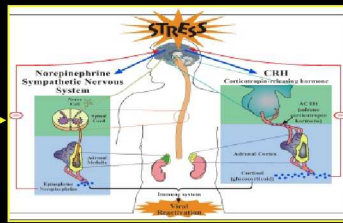
Disease Risks

- Shingles
- Ocular Herpes
- Hepatitis
- Tumors
- Mononucleosis
- Skin Lesions

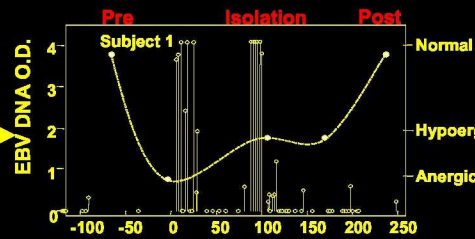




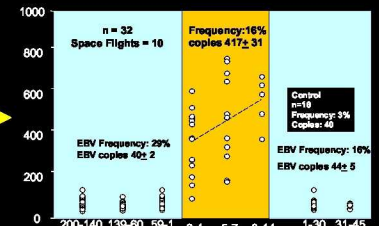
Increased Stress



Increased Stress Hormones

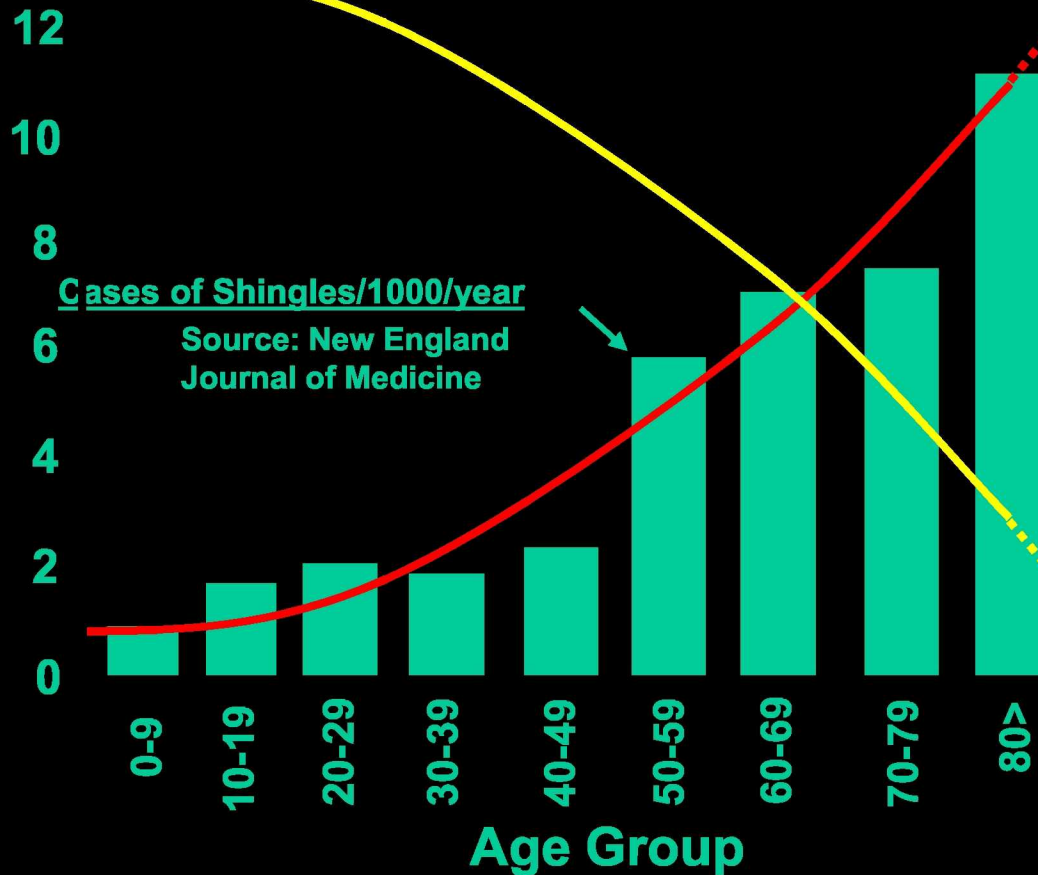


Decreased Immunity



Increased Viral Reactivation

Decreased Immunity



Increased Risk of Disease



Disease Risks

- Shingles
- Ocular Herpes
- Hepatitis
- Tumors
- Mononucleosis
- Skin Lesions

Collaborators

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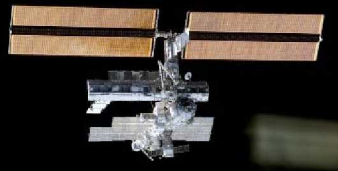
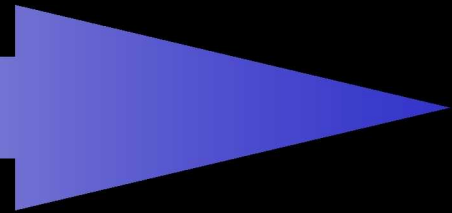
Questions?

IMPACT OF STRESS

Low

High

STRESS LEVELS AND MISSION IMPACT



Space Shuttle

ISS

Mir

Moon

Mars

NFκB

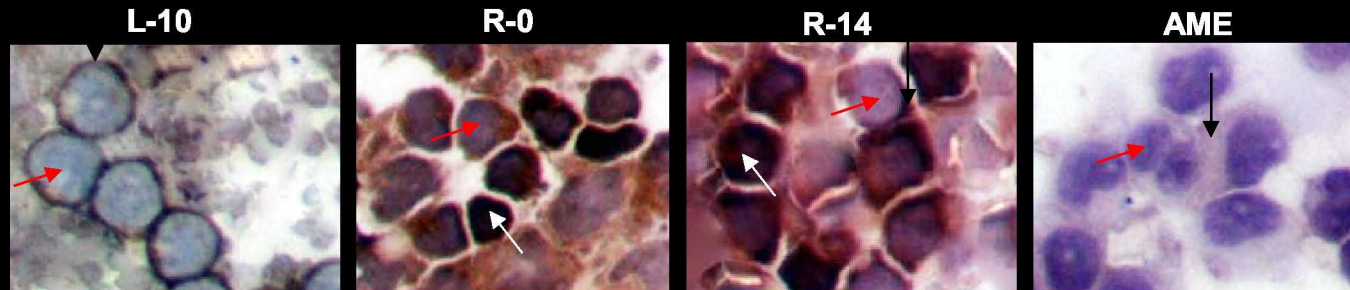
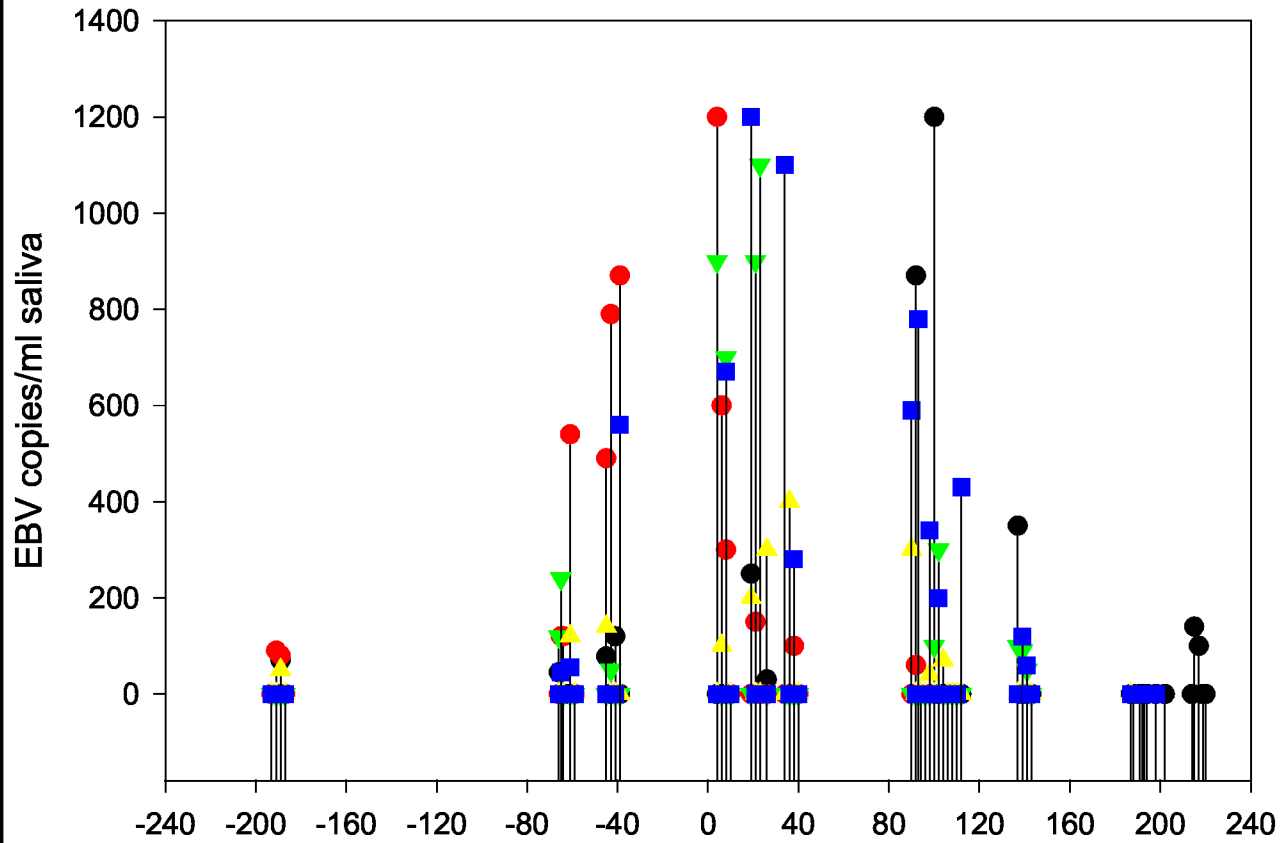


Figure 1: NF- κ B activation in the PBMC of astronauts at different time points. Cells from astronauts at different time points were collected were analyzed for nuclear p65 as described in Materials and Methods. In the cytoplasm (that is; in normal conditions), NF- κ B consists of a heterotrimer of p50, p65, and I κ B α . When it gets activated, that is; under stressed or diseased conditions I κ B α undergo phosphorylation and separated from the p65-p50 complex. Then the p65-p50 subunit translocated to the nucleus, attach to specific regions of DNA (that is; the promoters of some genes) and initiates gene transcription that are involved in inflammation and cancer. In this figure black arrow represents inactivated form of NF- κ B (in the cytoplasm), white arrow represents nuclear translocation of NF- κ B (p65-p50 complex) and red arrow represents hematoxylene staining in the nucleus of the cells that have inactivated form of NF- κ B.

EBV in 5 ISS



CMV In Space Shuttle And International Space Station Crewmembers

	# of Space Shuttle crewmembers shed CMV	# of International Space Station crewmembers shed CMV
<i>Before flight</i>		
180 d before Launch	0/7	0/5
45 d before Launch	0/7	0/5
10 d before Launch	3/7	not done
<i>After flight</i>		
At Landing	4/7	4/5
14 d after landing	4/7	not done
30 d after Landing	not done	4/5
Overall	4/7	4/5

Conclusions

1. Four of the eight herpes viruses reactivate in response to short term shuttle and long term ISS flights.
2. Reactivation and shedding of EBV, CMV, and VZV on ISS was more pronounced and shed for longer time post flight than short duration shuttle flights.
3. Effects of stressors associated with spaceflight are mediated through the HPA axis and the SAM axis resulting in diminished cellular immunity.
4. Changes on circadian rhythms of cortisol and DHEA occur both ISS and SS crewmembers.
5. Spaceflight developed PCR technology has been transferred to Physicians' laboratories for diagnosis of Shingles and post herpetic neuralgia.



Summary of Nested RT-PCR Analysis of EBV Gene Expression^a in Aging

Subject	EBER-1	Qp	Cp/Wp	LMP-1	EBNA-2	BZLF-1	SM	Fp	gp220
1	+++	+	+	+				+	+++
2	+++		+	+				+	++
3	+++	+	+	+				+	+++
4	+++	+		+		+		+	++
5	+++	+							+
6	+++	+	+	+				+	+++
7	+++			+				+	
8	+++	+							+++
9	+++			+				+	+
10	+++			+	+++		+++	+	+++
11	+++		+						

Note: accumulated data for multiple (2-3) timepoints for each elderly subject.

^aLegend (+++ = highly expressed; ++ = moderately expressed; + = low expression)

^b+ = EBV DNA present

CURRENT FOCUS: ON VZV



Unlike other neurotropic alphaherpesviruses in which primary infection is often asymptomatic, VZV (chickenpox) is characterized by malaise, fever, and an extensive vesicular rash.

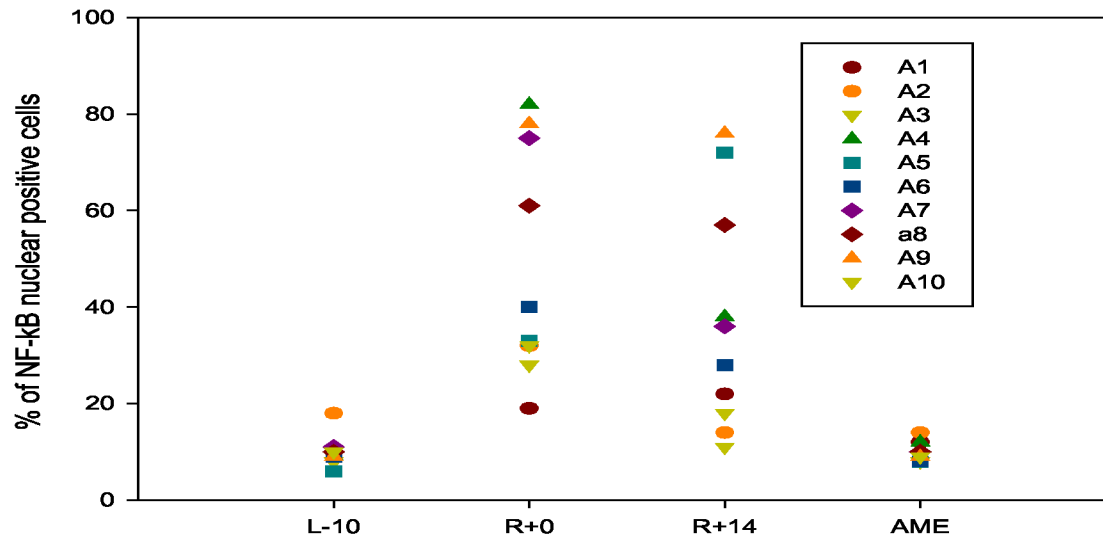
The occurrence of VZV 2 days before space flight in a 47 year-old healthy astronaut from a pool of 81 physically fit astronauts prompted our search for subclinical VZV reactivation during times of stress.



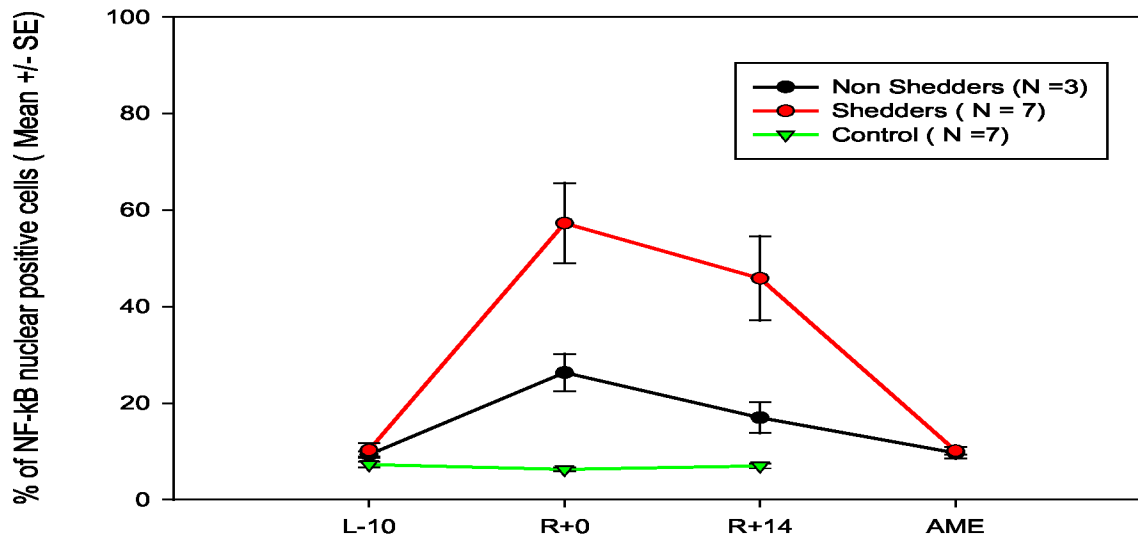




NFKB in 10 astronauts before and after space flight



Mean +/- SE



PATHOGENS

Public Health

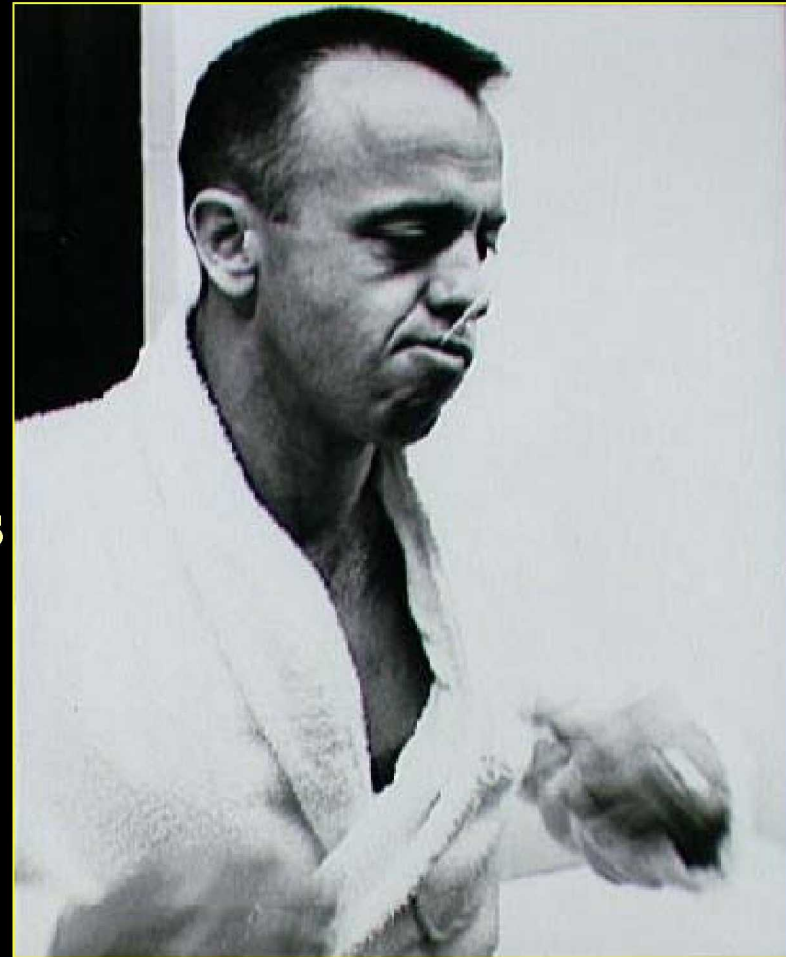
Mycobacterium tuberculosis
Helicobacter pylori
Staphylococcus aureus (MRSA)
Meningitis
STD's
Salmonella spp
Childhood diseases (e.g., measles)
Escherichia coli O157: H7
HIV
HAV, HBV, HCV
Herpes viruses
Influenza (respiratory viruses)

Space Flight

MRSA
Streptococci
Escherichia coli
Pseudomonas aeruginosa
Legionella pneumophila
Salmonella
Herpes viruses
Norovirus
Aspergillus
Penicillium
Candida
Giardia
Cryptosporidium

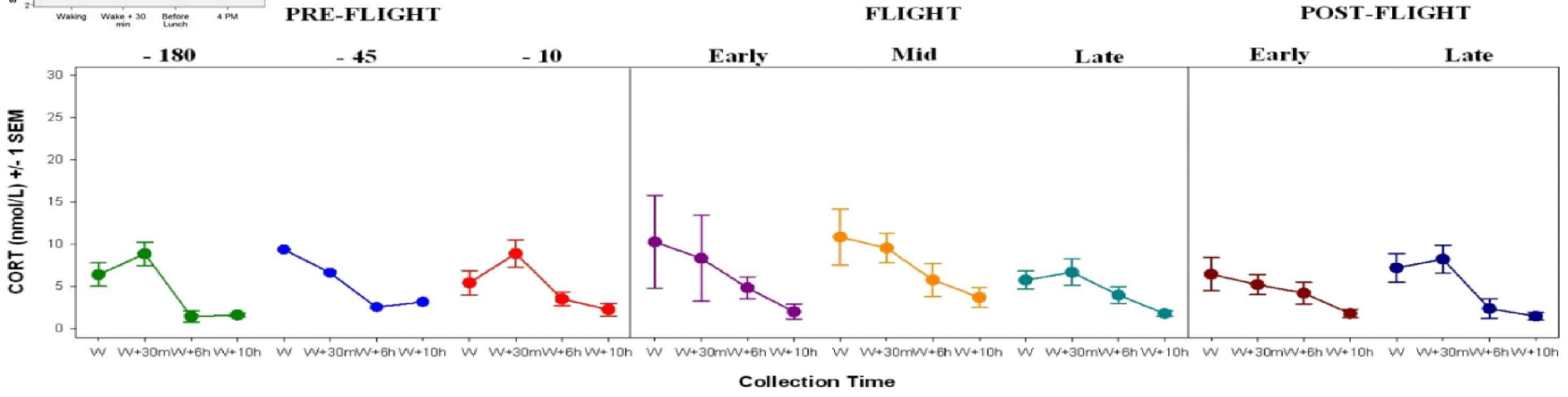
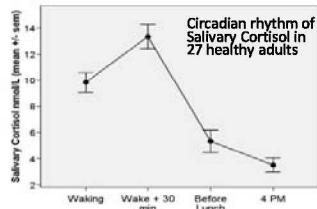
PREVENTIVE MEASURES

- **Crew Physical Examinations**
- **Immunization**
- **Health Stabilization Program**
- **Quarantine**
- **Preflight Food Testing**
- **Payload Biosafety Evaluation**
- **Establishment of Acceptability Limits**
- **Systems Design**
- **Environmental Monitoring**
- **In-Flight Housekeeping**
- **In-Flight Diagnostic Capabilities**
- **Antimicrobials**

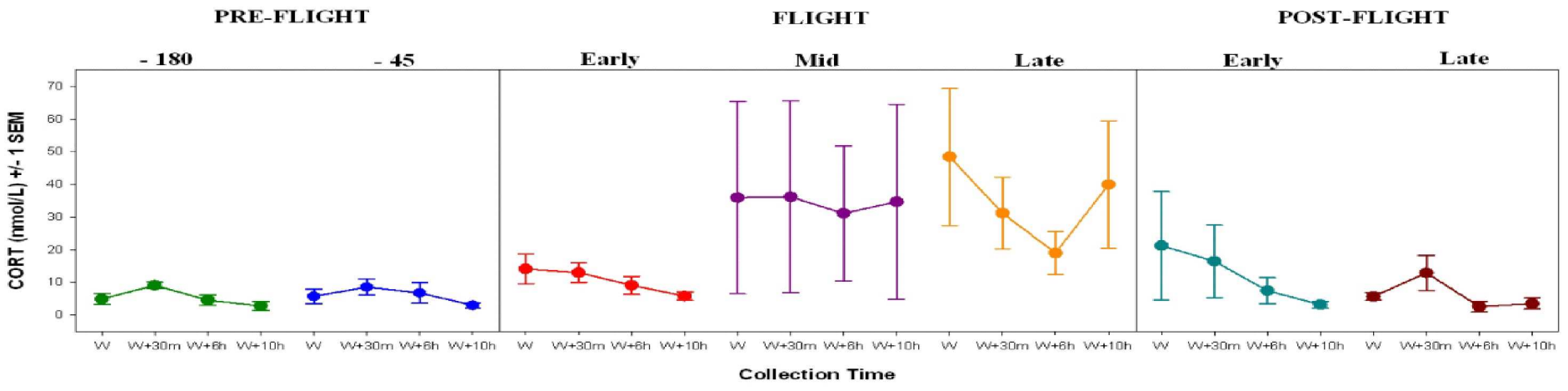


Salivary cortisol

Space Shuttle

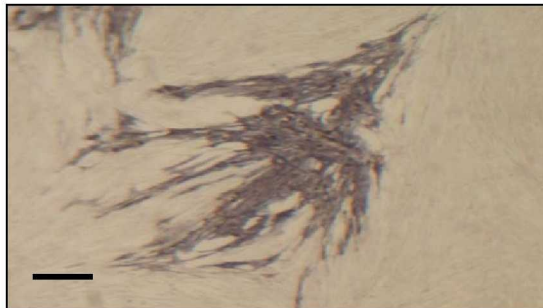


International Space Station

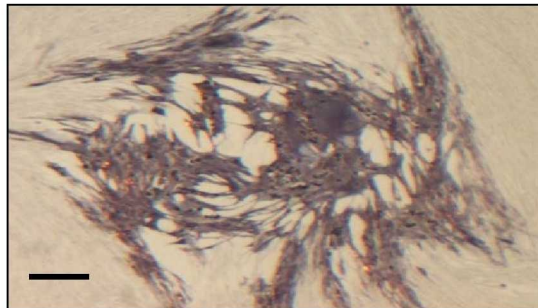


The decline in CMI to VZV associated with zoster led to the hypothesis that infectious VZV would also be present in the saliva of astronauts subjected to stress of spaceflight. Herein, not only was the detection of salivary VZV DNA associated with spaceflight validated, but also infectious virus was also detected in saliva. **This is the first demonstration of shed of infectious VZV in the absence of disease.**

subject 1



subject 2



subject 3



Recovery of infectious VZV from astronaut saliva. Human lung fibroblast cells cultures were inoculated with saliva from astronauts obtained on day 2 after landing. Typical herpes virus plaques were seen in cultures inoculated with saliva from subjects 1 and 2, but not with saliva from subject 3. The plaques stained with anti-VZV antibody but not with anti-HSV-1 antibody (not shown). magnification bar = 0.2 mm.